Contaminant Interactions and Biological Effects of Single-walled Carbon Nanotubes in a Benthic Estuarine System

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy
in Environment in the Graduate School
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ABSTRACT

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Abstract

Single-walled carbon nanotubes (SWNT) are filamentous nanocarbon structures. As their commercial and industrial use becomes more widespread, SWNT will enter the environment through waste streams and product degradation. Because of their highly hydrophobic nature, SWNT aggregate and settle out of aqueous environments, especially in saline environments such as estuaries. Therefore, sediments are a likely environmental sink for SWNT once released. It is important to understand how these materials will impact benthic estuarine systems since they are the probable target area for SWNT exposure in addition to containing many lower trophic level organisms whose survival and contaminant body burdens can have a large impact on the overall ecosystem. Disruptions in lower trophic level organism survival can have negative consequences for higher trophic levels, impacting the overall health of the ecosystem. It is also important to consider contaminant bioaccumulation, trophic transfer and biomagnification. If SWNT are taken up by benthic invertebrates, there is the possibility for trophic transfer, increasing the exposure of SWNT to higher trophic level organisms that otherwise would not have been exposed. If this type of transfer occurs in environmentally important species, the potential for human exposure may increase. My research aims to determine the magnitude of the toxicity and bioaccumulation of SWNT in benthic estuarine systems, as well as determine how they interact with other

contaminants in the environment. This research will contribute to the knowledge base necessary for performing environmental risk assessments by providing information on the effects of SWNT to benthic estuarine systems.

Before investigating the environmental effects of SWNT, it is imperative that a measurement method is established to detect and quantify SWNT once they enter the environment. This research utilized pristine, semiconducting SWNT to develop extraction and measurement methods to detect and quantify these specific materials in environmental media using near infrared fluorescence (NIRF) spectroscopy. Semiconducting SWNT fluoresce in the near infrared (NIR) spectrum when excited with visible-NIR light. This unique optical property can be used to selectively measure SWNT in complex media.

The fate, bioavailability, bioaccumulation and toxicity of SWNT have not been extensively studied to date. Pristine SWNT are highly hydrophobic and have been shown to strongly associate with natural particulate matter in aquatic environments. In light of this, I have focused my research to examine the influence of sediment and food exposure routes on bioavailability, bioaccumulation, and toxicity of structurally diverse SWNT in several ecologically-important marine invertebrate species. No significant mortality was observed in any organism at concentrations from 0.1 mg/kg to 1000 mg/kg. Evidence of biouptake after ingestion was observed for pristine semiconducting SWNT using NIRF spectroscopy and for oxidized ^{14}C-SWNT using liquid scintillation

counting. After a 24 hour depuration period, the pristine semiconducting SWNT were eliminated from organisms to below the method detection limit (5 μg/mL), and the ^{14}C-SWNT body burden was decreased by an order of magnitude to a bioaccumulation factor (BAF) of <0.01. Neither pristine SWNT nor oxidized ^{14}C-SWNT caused environmentally relevant toxicity or bioaccumulation in benthic invertebrates. Overall, the SWNT were not bioavailable and appear to associate with the sediment.

In addition to investigating the toxicity and bioaccumulation of SWNT as an independent toxicant, it is important to consider how they will interact with other contaminants in the environment (i.e. increase or decrease toxicity and bioaccumulation of co-contaminants, alter the environmental transport of co-contaminants, or induce degradation of co-contaminants). I wanted to investigate the effects of SWNT on a complex mixture of contaminants already present in a natural system. New Bedford Harbor (NBH) sediment, which is contaminated with polychlorinated biphenyls (PCBs), was amended with pristine SWNT to determine if the presence of SWNT would mitigate the toxicity and bioaccumulation of the PCBs in deposit-feeding invertebrates. A dilution series of the NBH sediment was created using uncontaminated Long Island Sound (LIS) sediment to test 25% NBH sediment, 50% NBH sediment, 75% NBH sediment, and 100% NBH sediment. The results of this work showed increased organism survival and decreased bioaccumulation of PCBs in treatments amended with SWNT, with the greatest reduction observed in the 25% NBH sediment treatment group

amended with 10 mg SWNT/g dry sediment. Polyethylene (PE) passive samplers indicated a reduction of interstitial water (ITW) PCB concentration of greater than 90% in the 25% NBH sediment + 10 mg SWNT/g dry sediment amendment. The ITW concentration was reduced because PCBs were not desorbing from the SWNT. Lower bioavailability leads to reduced potential for toxic effects, supporting the observation of increased survival and decreased bioaccumulation. Once in the sediment, not only are SWNT not bioavailable, they act as a highly sorptive phase, such as black carbon (BC), into which hydrophobic organic contaminants (HOCs), such as PCBS and polycyclic aromatic hydrocarbons (PAHs), can partition, thereby reducing the toxicity and bioavailability of co-occurring HOCs.

To more fully understand the long-term fate and impact of SWNT in this environment, their biodegradability also needs to be investigated. Biodegradation of SWNT could lead to release and/or transformation of sorbed HOCs as well as a change in the inherent transport, toxicity, and bioaccumulation of SWNT in the estuarine environment. Because the persistence of SWNT will be a primary determinant of the fate of these materials in the environment, I conducted experiments to determine if the fungus *Trametes versicolor,* the natural bacterial communities present in NBH sediment, and municipal wastewater treatment plant sludge could degrade or mineralize oxidized ^{14}C-SWNT. Over a six month time period, no significant degradation or mineralization was observed. In all treatments, approximately 99% of the ^{14}C-SWNT remained

associated with the solid phase, with only approximately 0.8% of added ^{14}C present as dissolved species and only 0.1% present as $^{14}CO_2$. These small pools of non-SWNT ^{14}C were likely due to trace impurities, as no differences in production were observed between treatments and abiotic (killed) controls.

Dedication

For the other Dr. Parks (MD), my academic inspiration and rhyming Valentine.

Contents

List of Tables

List of Figures

List of Abbreviations

AF^4	Asymmetric flow field flow fractionation
ANOVA	Analysis of variance
BAC	Benzalkonium chloride
BAF	Bioaccumulation factor
BBC	Bread and Butter Creek
BC	Black carbon
BCF	Bioconcentration factor
BET	Brunauer-Emmett-Teller theory
BSAF	Biota-sediment accumulation factor
CC	Coconut charcoal
CTAB	Cetyltrimethyl ammonium bromide
DCM	Dichloromethane
DI	Deionized
DO	Dissolved oxygen
DOM	Dissolved organic matter
GA	Gum arabic
HA	Humic acid
HOC	Hydrophobic organic contaminant
HPLC	High-performance liquid chromatography

HPLC-RD	High-performance liquid chromatography radiometric detection
HRP	Horseradish peroxidase
IS	Internal standard
ITW	Interstitial water
K_{OW}	Octanol-water partition coefficient
LIS	Long Island Sound
MDL	Method detection limit
MWNT	Multi-walled carbon nanotubes
NBH	New Bedford Harbor
NIR	Near infrared
NIRF	Near infrared fluorescence
NM	Nanomaterials
NOM	Natural organic matter
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PE	Polyethylene
ppm	part-per-million
PRC	Performance reference compound

PSF	Phagolysosomal stimulant fluid
QCM-D	Quartz microbalance
RSW	Reconstituted seawater
SC	Sodium cholate
SDBS	Sodium dodecyl benzene sulfonate
SDC	Sodium deoxycholate
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SRM	Standard reference material
SWNT	Single-walled carbon nanotubes
TEM	Transmission electron microscopy
TIE	Toxicity identification evaluation
TOC	Total organic carbon
U.S. EPA	United Stated Environmental Protection Agency
WHO	World Health Organization
XPS	X-ray photoelectron spectroscopy

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1. Introduction

1.1. Single-walled carbon nanotube physical-chemical properties

Single-walled carbon nanotubes (SWNT) are cylindrical carbon allotrope structures composed of sp^2 hybridized carbon, one carbon atom thick with diameters ranging from 1-2 nm and lengths up to hundreds of micrometers [1]. Each SWNT structure can be conceptualized as a rolled sheet of graphene with a unique diameter and chiral wrapping angle which are described by a set of integers *(n,m)* known as the chiral wrapping-angle index (Figure 1.1, reproduced from [2]) [2, 3]. These integers correspond to the start and end point in the graphene sheet lattice, which after "connecting" the two points, the length of the vector corresponds to the circumference of the resulting SWNT, and the angle relative to the top axis (zigzag) is the chiral wrapping angle. For example, in Figure 1.1, the *(8, 7)* SWNT in the bottom right consists of a starting point 8 cells over on the zigzag axis and 7 cells down on the armchair axis. This SWNT falls along the 1.0 nm diameter line which can be calculated from the SWNT circumference created by the distance between the two axes. The chiral wrapping angle for this SWNT is 30° as it lies along the armchair axis. The chiral wrapping angle can range from 0° (zigzag) to 30° (armchair) (Figure 1.2, reproduced from [4]) and the individual chiralties dictate the electronic properties of SWNT and classifies them as either semiconducting or metallic [1]. The distribution of physically possible SWNT isoforms is approximately 2:1 semiconducting:metallic, implying that 66% of SWNT

isoforms are semiconducting, and all current synthetic methods lead to more or less complex mixtures of both types [5]

Synthesis of isochiral SWNT materials is currently not possible, and extensive purification is required to prepare mixtures enriched in semiconductive or metallic species [6-8]. The electronic structure of SWNT is determined by the physical structure. When hypothetically rolling a sheet of graphene into a nanotube, the original wave vectors of the graphene become quantized along the tube circumference, whereas the wave vectors along the length of the tube remain constant [9]. The intersection of the quantized wave vectors and constant wave vectors make up the density of states (DOS) containing Van Hove singularities; therefore the combination of the diameter and chiral wrapping angle determine the electronic DOS for each SWNT (Figure 1.3, reproduced from [2]) [2, 9]. Semiconductive SWNT consist of two main van Hove transition states: E_{11} and E_{22} [1, 2, 10]. The E_{22} transition corresponds to the excitation of an electron from the ground state via absorption of a photon between 550 and 900 nm [1, 2, 10]. The E_{11} band corresponds to the band gap (800-1600 nm) and relaxation of the excited state electron back to the ground state through fluorescence [1, 2, 10].

1.2. Detection and characterization of SWNT

Several analytical techniques can be utilized to determine SWNT morphology, concentration, purity, and structure, including electron microscopy, Raman spectroscopy, UV-Vis spectroscopy, thermogravimetric analysis, quartz crystal

microbalance (QCM-D) with dissipation measurements, molecular tagging, and ^{14}C-labeling [11-16]. These methods are useful in gathering a range of information, but they are not specific to SWNT, are not ideal or applicable for many samples, and they have high detection limits (e.g., mg/L range for UV-Vis spectroscopy) with low selectivity, making them useful for characterization of SWNT in simple suspensions, but not more complex environmental matrices (e.g., sediment, soil, biota) [12, 16-23]. The addition of functional groups to SWNT allows molecular tracking, however these modifications may alter the behavior of SWNT by changing their surface chemistry, and therefore will not provide results comparable to those for non-functionalized SWNT [11]. Utilizing ^{14}C-labeled SWNT to track these materials in environmental fate studies is a useful quantitative technique but it involves the use of a radionuclide and is inappropriate for detection of "native" SWNT in environmental samples. Raman spectroscopy is limited in selectivity as it measures a change in the vibrational and rotational energy of molecules, which is not a property unique to SWNT. Also, Raman is not able to provide quantitative data as the signal intensity cannot currently be calibrated. Finally, electron and atomic force microscopy provide excellent qualitative and characterization information but the sample preparation process can lead to artifacts and these methods are not ideal for detecting or characterizing SWNT in environmental samples where the object of interest is carbon often in a background of carbon [24, 25]. In order to further investigate the environmental behavior, occurrence, fate, uptake and bioaccumulation of

SWNT in complex samples at environmentally relevant concentrations and facilitate a quantitative ecological risk assessment, selective and sensitive quantification and characterization methods are required [26].

As previously mentioned, SWNT have unique and tunable physical-chemical properties including their diameter and chiral wrapping angle which lead to differences in the electronic band gap [27, 28]. The band gap is inversely related to the SWNT diameter. This unique band gap leads to specific excitation-emission fluorescence in the near infrared region of the spectra for each semi-conducting SWNT, allowing for structural information to be obtained [2, 5, 10, 27]. When multiple excitation wavelengths are used and the fluorescence emission is scanned, various E_{11}-E_{22} transition pairs are detected, allowing for a bulk sample containing different (*n*,*m*) SWNT to be characterized [5]. Since there are few molecular or particulate carbon species that exhibit significant fluorescence in the near infrared region of the electromagnetic spectrum, this optical property provides a sensitive and specific measurement technique for semi-conducting SWNT. Thus, NIR fluorescence spectroscopy is potentially an ideal method for the detection of SWNT in complex environmental matrices (e.g., sediment and biota) [29, 30]. To date, NIRF microscopy has been used as a qualitative technique to visualize SWNT and determine their movements in phagocytic cells [29], HeLa cells [31], and in *Drosophila melanogaster* [30]. NIRF spectroscopy has also been used to measure the strain

of SWNT present in polymers that can be used as a coating for structural reinforcement [32].

Even though NIRF is a selective technique for semiconducting SWNT, the fluorescence of these materials can be quenched through various pathways such as aggregation and bundling, functionalization, and acidification. Therefore, SWNT must be pristine and individualized in order to obtain high quality, reliable data. The presence of functional groups on the surface of semiconducting SWNT provides an alternate relaxation pathway to an excited-state SWNT, thereby reducing the number of photon emissions resulting in fluorescence quenching [28]. High power sonication in the presence of an aqueous surfactant or polymer solution has been used successfully to individualize SWNT in solutions [10, 33-36]. In addition to the need for ultrasonication to suspend the SWNT, the specific suspension matrix (surfactant or polymer type) is an important factor that effects the fluorescence quantum yield of SWNT [5]. As previously mentioned, aggregates can quench fluorescence; therefore SWNT samples may need to be centrifuged after sonication to remove these aggregates and possible impurities [37]. Metal catalyst impurities have been shown to associate with aggregates and larger SWNT preferentially over individualized and smaller SWNT in an aqueous sodium dodecyl sulfate (SDS) solution, such that a centrifugation step aids in purifying the sample [33]. Although NIRF is a selective and sensitive measurement technique, the sample must be prepared to optimize conditions for fluorescence and minimize possible

quenching pathways. In order for a comparison between different types of samples to be made, they must be prepared using the same method.

1.3. Applications and environmental relevance

SWNT are considered an emerging contaminant of concern [38, 39], and it is imperative that research be performed to determine the fate, transport, toxicity and bioaccumulation of these materials before they are released with the potential to cause environmental damage. These materials are currently being used in a variety of products including lithium-ion batteries, textiles, chemical sensors, gene delivery vehicles, electronics, and structural composites [40-43], and have the potential for continued development for a wide range of industrial and commercial applications [44, 45]. The total carbon nanotube (both single- and multi-walled carbon nanotube, MWNT) production is estimated to range from 55-1100 metric tons per year in the United States alone [46]. Therefore, it is important to determine potential routes of SWNT release and environmental exposure in order to form a basis for environmental risk assessment and development of environmental regulations.

Because of their size and large surface area-to-mass ratio, SWNTs behave differently in aqueous solutions than do more well-studied dissolved or particulate-associated molecular contaminants [47, 48], and, like colloids [48], SWNT tend to aggregate and undergo sedimentation in the presence of organic matter, elevated ionic strength, and other particles [41, 49]. The unique physical and chemical properties of

SWNT along with the properties of the surrounding environment affect the ultimate fate and transport of SWNT in environmental systems [12, 50]. Some of the major potential mechanisms are abrasion, normal wear and tear, aging, improper use, disposal/recycling, spills, landfill leachate, industrial effluent, waste incineration, wastewater treatment, and atmospheric emissions [11, 41, 42, 51]. These routes of release will likely lead to accumulation of SWNT in terrestrial and aquatic soil and sediment as well as (potentially) food chains. Partly because of a lack of sensitive analytical methods, the occurrence and distribution of SWNT in the environment have not yet been reported. However, one model predicted that by 2012 the concentration of carbon nanotubes (all types) in contaminated sediment would reach approximately 0.5 μg/kg and the concentration in sludge-treated soils would reach approximately 0.4 μg/kg [52].

1.4. SWNT toxicity and bioaccumulation

A better understanding of the implications of unintentional releases into the environment for human and environmental health will require detailed studies of the toxicity and bioaccumulation of SWNT and other carbon nanomaterials such as MWNT and fullerenes in aquatic systems. This includes aqueous and sediment exposure pathways. Roberts et al. exposed the freshwater *Daphnia magna* to 20 mg/L lysophosphatidylcholine-wrapped SWNT over 96 hours and observed 100% mortality [53]. These authors were unable to determine an LC_{50}, but estimated it to be

approximately 10-20 mg/L. In another study, the rainbow trout (*Oncorhynchus mykiss*) was exposed to sodium dodecyl sulfate (SDS)-wrapped SWNT over 10 days [54]. SWNT precipitation was observed on the fish gills, and a dose-dependent increase in ventilation rate, gill pathologies, and mucus secretion was determined [54]. Cheng et al. (2007) observed a delay in hatching of zebrafish embryos at concentrations of SWNT above 120 mg/L, which was presumed to be due to the cobalt and molybdenum catalyst impurities [55].

Since benthic systems are a likely sink for SWNT, several toxicity studies have focused on benthic organisms. Templeton, et al. demonstrated that the deposit-feeding estuarine meiobenthic copepods (*Amphiascus tenuiremus*) did not display any adverse effects when exposed to purified, oxidized SWNT, with the exception of a one-day developmental rate delay for the 1.6 mg/L treatment [11]. Confocal microscopy allowed visualization of SWNT clusters present in the guts and fecal pellets of the copepods. The fluorescent nanocarbon impurities present in arc-discharge synthesized SWNT resulted in a significant increase in mortality, as well as reduced developmental success in copepods. The toxicity of the small byproducts could be a result of their size, and potentially higher bioavailability [11]. Another estuarine study exposed a meiobenthic copepod (*Amphiascus tenuiremus*) and a macrobenthic polychaete (*Streblospio benedicti*) to sediment amended with 5 mg ^{14}C-labeled SWNT/g dry sediment and found that there was no detectable uptake in the tissues, but the sediment and fecal pellets had similar

radioactivity suggesting the SWNT enter the gut and are then eliminated [12]. A similar study investigated the bioaccumulation of ^{14}C-labeled SWNT in the freshwater oligochaete *Lumbriculus variegates* from amended sediment [13]. It was reported that SWNT did not bioaccumulate in the organism's tissues and that the most probable explanation was that ingestion and subsequent depuration of SWNT-amended sediment occurred. Several additional studies have verified these findings and have shown that organisms ingest carbon nanomaterials, after which they are repackaged into fecal pellets and eliminated upon depuration with a control food source, without incorporation of the NMs into the gut lumen or tissues [13, 53, 56, 57].

When considering the toxic effects and bioaccumulation potential of SWNT materials, it is important to consider the type and extent of functionalization as well as the coating or surfactant used in the exposure. Aqueous exposures of multi-walled carbon nanotubes (MWNT) resulted in significant mortality in the freshwater daphnid *Ceriodaphnia dubia* from the pristine nanotubes, but not the oxidized materials [58]. It was hypothesized that the toxic effect of unfunctionalized, unpurified MWNT was due to aggregation and physical blocking of the gut and/or disruption in mobility of the organism, whereas the oxidized materials were less hydrophobic and more easily cleared [58]. However, pristine MWNT were less toxic to the estuarine amphipod *Leptocheirus plumulosus* and freshwater amphipod *Hyalella azteca* than were carbon black and activated carbon through a sediment exposure route [58]. The fate, transport,

toxicity and bioaccumulation of different carbon NMs in the environment depend on several environmental/experimental factors (i.e., sample preparation, experimental design, bioavailability, and organism tolerance) as well as specific nanomaterial composition [48, 59-62].

1.5. SWNT as black carbon in the environment

The introduction of SWNT to estuarine sediments could lead to environmental effects other than toxicity and bioaccumulation. Pristine SWNT are highly hydrophobic, and have been shown to form homo- and hetero-aggregates in natural systems such as estuarine waters [63]. These aggregates will eventually settle into sediments where they will likely interact with other hydrophobic contaminants that are present. Because SWNT are comprised entirely of sp^2 hybridized carbon (being essentially rolled sheets of graphene), they can be considered as a form of black carbon (BC). As a form of BC, SWNT have a high affinity to sediment and particulate organic matter, as well as a high sorptive capacity for HOCs such as polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbons (PAHs), and should be investigated as a possible geosorbent [18, 64-70]. The consequences of HOC-sorption to SWNT on the fate and bioavailability of these environmental contaminants are not well understood. Research on the adsorption of hydrophobic organic contaminants (HOCs) to other forms of black carbon (e.g., soot, char) has been extensively studied [71-77] and should provide insight on their basic sorbate-sorbent interactions with SWNT.

Both laboratory and field experiments have amended sediment with activated carbon or black carbon to test the utility of this amendment as an *in situ* remediation technique, and subsequently observed the reduction of interstitial water (ITW) concentrations and bioaccumulation of HOCs such as PCBs, PAHs, and various pesticides [52, 75, 78-84]. Highly hydrophobic HOCs such as PCBs and PAHs are known to adsorb to sediment organic carbon and form a thermodynamic equilibrium with the pore water in which the concentrations of the HOCs are negligible [85]. This equilibrium can be altered by the presence of other competing forms of carbon such as natural organic matter (NOM) [86, 87]. The physical characteristics of both the black carbon, such as type and structure [75, 77, 83, 88-91], and HOCs, such as log K_{OW} and planarity [72, 83, 89, 92], also dictate the adsorption efficacy. The degree of bioavailability is also related to sediment and HOC ageing as was demonstrated by Sundelin et al. when comparing the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in historically contaminated sediments versus laboratory amended sediments [92]. In addition to accounting for the physical characteristics of the BC and HOCs, their specific sorption behaviors need special consideration. When black carbon is present in an aqueous system, it is important to note that the two-phase equilibrium partitioning model does not sufficiently predict HOC partitioning behavior because it assumes sorption only occurs through absorption while ignoring any potential adsorption to other phases [76, 93]. This artifact will lead to an overestimation of uptake by organisms

[93]. In order to correct for the presence of adsorption to black carbon sources, non-linear sorption behavior must be considered.

The variables that impact HOC and BC interactions have also been observed in experiments using SWNT as the BC source [12, 65, 66, 68, 70]. The addition of humic acid (HA) to an aqueous suspension of SWNT and pyrene decreased the total amount of pyrene sorbed to SWNT, but the amount of pyrene sorbed to SWNT was 1.9 times higher than the amount sorbed to HA alone [66]. Ferguson et al. observed an organism-specific and black carbon-specific effect on HOC bioavailability [12]. When investigating the effect of SWNT and a standard reference material (SRM), 2975 diesel particulate matter, on HOC bioavailability to two different benthic invertebrates, the authors found that neither black carbon source decreased the HOC bioavailability for the meiobenthic copepod *Amphiascus tenuiremis*, but SWNT decreased the HOC bioavailability to the deposit/suspension feeding polychaete *Streblospio benedicti*, while diesel particulate matter increased the HOC bioavailability [12]. The difference in results between organisms may have been the result of differences in gut physiology between the organisms or of more selective feeding of the polychaetes [72, 90]. It is also important to account for metabolism and gastrointestinal chemistry of each species. Rust et al. investigated the metabolism and bioaccumulation potential of several benthic invertebrates on benzo[a]pyrene and observed variability between species from the same phylogenetic group [94]. In addition to metabolic activity, the gut fluid can lead to

partial dissolution and desorption of the HOCs from black carbon sources [71, 90]. As a type of black carbon and a material previously shown to act as a sorbate, it is important to investigate how the presence of SWNT in a natural system will impact the fate and bioavailability of co-contaminants. More work needs to be performed to assess the magnitude and mechanisms of effect SWNT has on native HOCs. Further, it is important to determine if SWNT behave differently from other black carbon sources such that there is a specific "nanotube" effect independent of its material property.

1.6. Biodegradation of carbon nanomaterials

Since sediments are a sink for SWNT, it would be beneficial to investigate what happens to SWNT once they enter this environment. The fate of carbon nanomaterials in the environment depends not only on their transport, but also on their transformations. Very few studies have investigated the environmental biodegradation of carbon nanomaterials, including SWNT. Hartmann et al. studied the effect of activated sludge on aged nC_{60} over a 48 day period [95]. Prior to incubation with activated sludge, these materials were aged for 36 months under indirect natural light conditions which induced minimal surface modification including oxidation [95]. At the conclusion of this study it was determined that the sludge was biologically active but the aged nC_{60} were not biodegradable under those conditions [95]. Another study investigated the ability of two white-rot basidiomycete fungi (*Phlebia tremellosa* and *Trametes versicolor*) to degrade C_{60} fullerol with and without the presence of wood [96]. A wood wafer was added to

approximate conditions in which plant material and other organic matter would likely be present, as well as stimulate the release of peroxidase enzymes excreted by the fungi to breakdown organic material [96]. The results suggested that, in the presence of both species of fungi, with and without the addition of wood, approximately 20-30% of the C_{60} fullerol was lost from the media system, presumably due to the complete mineralization of the C_{60} fullerol to CO_2, as well as the production of and incorporation into fungal hyphae [96]. However, release of C_{60} fullerol-derived CO_2 and fungal hyphae data were not confirmed quantitatively [96].

The remaining literature investigating the biodegradability of carbon nanomaterials utilize horseradish peroxidase (HRP) enzymes and phagolysosomal stimulant fluid (PSF) to simulate the enzymes released from fungi and the oxidative conditions of a lysosome in a cell, respectively. Liu et al. incubated SWNT for 90 days with a PSF/hydrogen peroxide (H_2O_2) mixture to simulate the environment that SWNT would encounter in a cell during phagocytosis [97]. The investigators studied how different functional groups, such as sulfonic acid and carboxylic acid, as well as a variable degree of functionalization would impact the biodegradability of the SWNT [97]. It was found that SWNT functionalized through aryl sulfonation, ozonolysis, or 15 minutes of acid-driven oxidation were not readily biodegradable, but SWNT that were oxidized for 1 hour or 3 hours underwent visible degradation as observed qualitatively by TEM and SEM [97]. Over the 90 day incubation, degradation of the highly oxidized

materials was qualitatively determined by a decrease in SWNT bundles and increase in shortened SWNT fragments. Dynamic light scattering (DLS) was used to quantitatively show the decrease in hydrodynamic diameter over time as a result of the degraded, shortened SWNT material [97]. The findings from Liu et al. are supported by other observations of biodegradation of oxidized carbon nanomaterials incubated with PSF/ H_2O_2 [98, 99] or HRP/H_2O_2 [98, 100-102]. Allen et al. determined that pristine SWNT could be degraded by HRP/H_2O_2 if an additional oxidative catalyst was present that could generate reactive oxygen species, which in turn oxidized the surface of the SWNT, rendering these materials completely degraded by 10 days [99]. These results suggest that an oxidizing environment is required for biodegradation to occur, and highly oxidized carbon nanomaterials are readily degraded but less oxidized and pristine carbon nanomaterials are not biodegradable.

1.7. Objectives

Given the pressing questions that surround the analysis, occurrence, fate, and effects of SWNT in the environment, I have structured my dissertation research to provide a comprehensive study on the effect of SWNT on and interactions with a benthic estuarine system. The first aim of this research was to develop a sensitive, selective, and quantitative method to extract, detect and quantify pristine, semiconducting SWNT in complex, environmental media (Chapter 2, [103]). The unique physical-chemical properties of these materials allowed for sensitive and selective

detection using NIRF spectroscopy while also providing more detailed characterization data than the previously available methods. Since each SWNT isoform has a unique excitation-emission profile, the relative abundance of each isoform in a mixture can be determined. First, the ideal suspension conditions were determined to validate the detection and quantification in a controlled, simple media. Once this method was established, spike recovery experiments in estuarine sediment were performed using pristine, semiconducting SWNT as well as ^{14}C-SWNT to validate the method.

After developing an analytical method, the toxicity and bioaccumulation of pristine, semiconducting SWNT in benthic estuarine invertebrates was evaluated (Chapter 3, [104]). Previously, no method was available to determine SWNT bioaccumulation without a covalently bound chemical tag or radiolabel. Experiments included both depurated and non-depurated treatments to determine if measured SWNT in the organisms were present in the tissue or the gut, along with sediment and food exposures to determine route of uptake.

The third aim of this research was to determine the interaction of SWNT with molecular co-contaminants such as hydrophobic organic contaminants (HOCs) (Chapter 4). I have studied how the presence of SWNT in sediment impacts the toxicity and bioavailability of polychlorinated biphenyls (PCBs) to estuarine benthic invertebrates in comparison to another, previously studied black carbon source (coconut charcoal, CC). I used New Bedford Harbor (NBH) sediment, a field collected sediment historically

contaminated with PCBs, to investigate the effect of SWNT on co-contaminants in an environmentally-relevant media. The change in interstitial water (ITW) concentrations as a product of black carbon addition was also monitored using polyethylene (PE) passive samplers.

The final aim of my research was to determine the biodegradability of ^{14}C-SWNT by the fungus, *Trametes versicolor*, as well as natural microbial cultures from NBH sediment and aerated wastewater treatment plant sludge (Chapter 5). Incubations were performed using sealed serum bottles with carbon dioxide traps, and time points were taken over a six month time period. Mineralization of ^{14}C-SWNT was quantified by measurement of $^{14}CO_2$ in the gaseous phase, whereas the aqueous and solid phases were separated by ultracentrifugation to determine the remaining intact ^{14}C-SWNT (solid phase) and any partially degraded ^{14}C-SWNT (aqueous phase).

A summary of the impact of the developed analytical method, its application in the field of nanoecotoxicology, and the information obtained about the toxicity, bioaccumulation, co-contaminant interactions, and transformation of SWNT is presented in Chapter 6. A discussion of how to further analyze the data from the degradation experiment and compile more definitive data to further expand knowledge in this field are also presented.

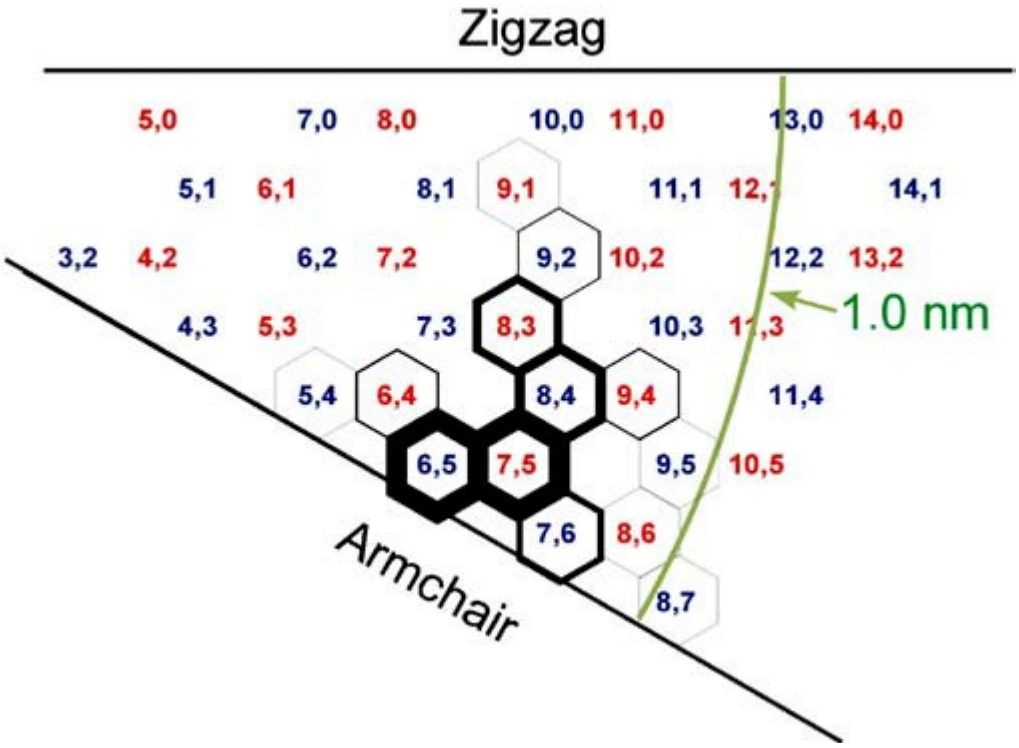


Figure 1.1. A graphene sheet illustrating various diameter and chiral wrapping angle pairs for several semiconducting and metallic SWNT species.
Adapted from Weisman RB, Bachilo SM, and Tsyboulski D. 2004. Fluorescence spectroscopy of single-walled carbon nanotubes in aqueous suspension. *Appl Phys A-Mater Sci Process* 78:1111-1116.

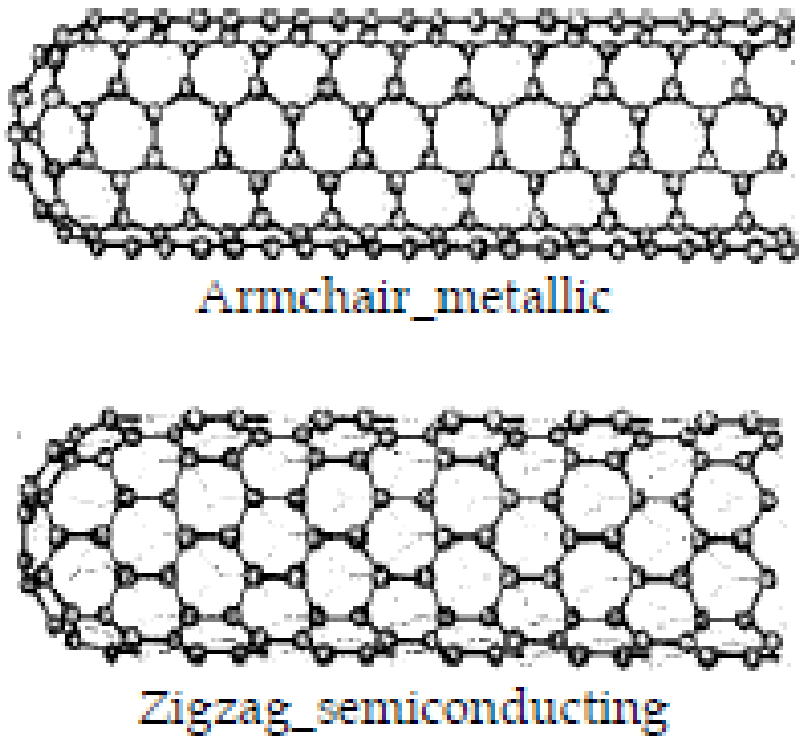


Figure 1.2. A cartoon illustrating the structure of a metallic and semiconducting single-walled carbon nanotube (SWNT).
Adapted from Fathi D and Forouzandeh B. 2010 *Interconnect Challenges and Carbon Nanotubes as Interconnect inNano VLSI Circuits*.

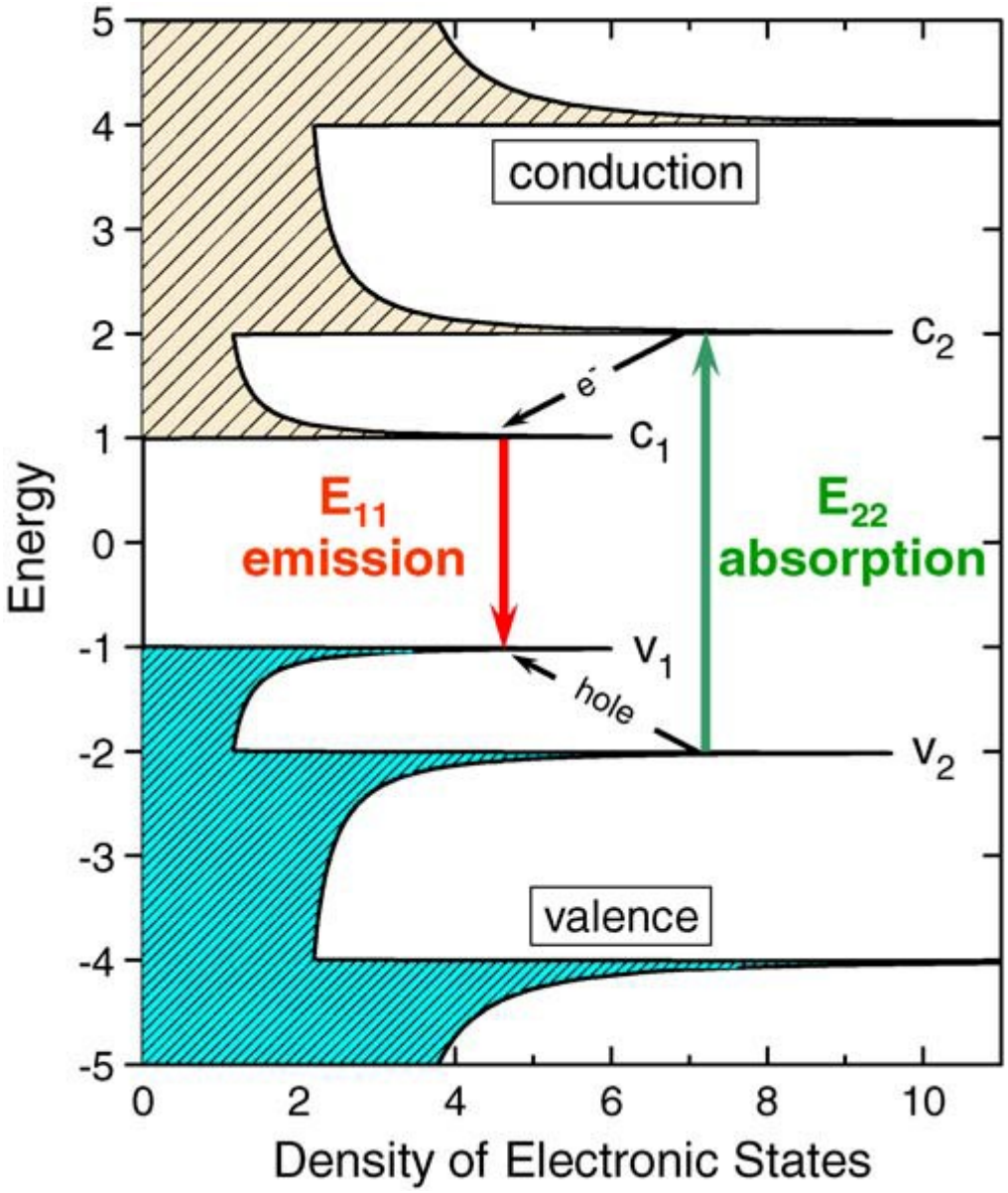


Figure 1.3. A representative density of states diagram of a semiconducting SWNT. Adapted from Weisman RB, Bachilo SM, and Tsyboulski D. 2004. Fluorescence spectroscopy of single-walled carbon nanotubes in aqueous suspension. *Appl Phys A-Mater Sci Process* 78:1111-1116.

2. Characterization and quantitative analysis of single-walled carbon nanotubes in the aquatic environment using near infrared fluorescence spectroscopy

2.1. Introduction

Single-walled carbon nanotubes (SWNT) have the potential for a wide range of industrial and commercial applications (including microelectronics and composite materials) due to their exceptional physical, mechanical, electronic and optical properties [44, 45]. The annual production volume is steadily increasing and more products containing SWNT are making their way into the marketplace [105]. With this increase in consumer usage and potential for environmental release, SWNT are considered a new class of emerging contaminants [38, 39]. In order to better understand the implications of unintentional releases into the environment, significant research has focused on characterizing the fate, transport and ecotoxicity of SWNT [11, 12, 17, 50, 53, 106-110]. According to the estimated production volume, product usage and waste disposal, the current concentration of SWNT in aquatic sediment is predicted to range up to 1 μg/kg [111, 112]. A critical limitation to the study of environmental behavior of SWNT in the aquatic environment and for sediment in particular is the lack of reliable detection methods in complex samples at environmentally relevant concentrations [26]. Sensitive, reliable and specific quantification and characterization methods are needed

when studying the occurrence, fate, uptake and bioaccumulation of SWNT in the environment, in addition to facilitating quantitative ecological risk assessment.

Several analytical methods have been applied to characterize SWNT under well-defined laboratory settings including TEM and Raman spectroscopy for qualitative analysis, as well as UV-Vis spectrometry, size exclusion chromatography, thermogravimetry-mass spectroscopy and quartz crystal microbalance (QCM-D) for quantitative analysis [14-16, 113]. These methods are limited by high detection limits (e.g., UV-Vis spectroscopy detection in the mg L^{-1} range) as well as matrix effects in natural samples – they are typically useful only for analysis of SWNT in pure form or in very simple solutions [12, 16-23].

SWNT can be classified as either semiconducting or metallic, depending on their electronic structure as described by the chiral wrapping-angle index *(n,m)* [23]. The distribution of allowed SWNT chiralities is approximately 2:1 semiconducting:metallic, that implies approximately 66% of SWNT species are semiconducting, and all current synthetic methods lead to more or less complex mixtures of both types [5]. Synthesis of isochiral SWNT materials is currently not possible, and extensive purification is required to prepare mixtures enriched in semiconductive or metallic species [6-8]. Individualized, semiconducting SWNT in aqueous surfactant suspension give distinct fluorescence emission in the near infrared (NIR) range when excited by visible/NIR light (600-800 nm); these unique spectral features can be used to determine structural information [2,

10]. Fluorescence has been observed directly across the band gap of semiconducting carbon nanotubes [5]. The energy of this transition is highly dependent on nanotube diameter [10]. Consequently, at different wavelengths, various distinct E_{xx} transitions will occur in a sample containing different *(n,m)* SWNT, allowing a characterization of the bulk SWNT sample [5].

Although the fluorescence of SWNT in aqueous suspensions is unique, it can be quenched by aggregation or bundling, chemical functionalization, or acidification. For reliable detection and quantification of SWNT by near-infrared fluorescence spectroscopy (NIRF), nanotubes need to be individualized in suspension. This can be achieved through high power sonication in the presence of surfactants, DNA or polymers [10, 33-36]. Importantly, the fluorescence quantum yield of SWNT can vary considerably depending on the suspension matrix [5]. A centrifugation step can be performed after sonication to remove larger agglomerates as well as impurities. Assuming that impurities are less well dispersed or have a significantly higher sedimentation rate in the surfactant solution than the SWNT, a decrease of the non-SWNT carbon fraction in a sonicated suspension is achieved through centrifugation [37]. Moore et al. showed that in an aqueous SDS solution metal catalyst impurities affiliate more with larger SWNT bundles than individualized or smaller SWNT bundles [33]. Consequently, bundles as well as metal catalyst residues can be effectively removed by centrifugation after sonication.

To date, NIR fluorescence microscopy has been applied to gain qualitative information of the *(n,m)* composition of mixed SWNT bulk samples, as well as to detect and image SWNT in biological samples such as macrophage cells and *Drosophila melanogaster* [29, 37]. Most biological and environmental samples show extremely weak or essentially no background photoluminescence in the NIR region. Consequently, NIR emission spectra from SWNT in biological samples can give very high signal-to-noise, enhancing sensitivity even in complex samples. In this paper I illustrate the utilization of the unique electronic properties of semiconductive SWNT to develop a method for extraction, characterization and quantification of these important manufactured nanomaterials from natural sediments using estuarine sediments as model sediments. To my knowledge, this is the first report of quantification of SWNT in sediments by extraction and NIRF measurement. As only semiconducting SWNT fluoresce, proximal metallic SWNT can act as a photon sink and might broaden the absorbance spectra, therefore it was crucial to develop methods for efficiently extracting and disaggregating SWNT mixtures prior to NIRF analysis [10]. SWNT used in this study show a low degree of functional groups, low metal content and high quality of semiconducting SWNT, all of which serve to minimize those interferences.

2.2. Experimental section

2.2.1. SWNT material

Four different types of CoMoCat SWNT (SouthWest NanoTechnologies Inc., SWeNT, OK, USA) were tested in this study: SG65 SWNT (lot-no. 000-0031), SG76 SWNT (lot-no. 000-0020), CG200 SWNT (lot-no. 400) and CG100 SWNT (lot-no. 000-0012). The SWNT were produced by chemical vapor deposition [114]. All materials had a carbon content > 90% by weight and relative purity of >90% [114]. All SWNT were used as received without any further purification. As indicated by the manufacturer, SG65 SWNT contains > 90% semiconducting tubes. SG65 SWNT were characterized by NIRF and optical absorption spectroscopy (diameter and chirality determination), SEM, Raman spectroscopy (SWNT quality) and XPS (surface functionalization); results of SWNT characterization are provided in Figure A1, Appendix A. The ^{14}C-labeled SWNT were received from Research Triangle Institute (RTI), NC. The as-produced ^{14}C-labeled SWNT material from RTI was purified by acid treatment and electrophoresis as described previously [115]. Compared to the CoMoCat SWNT, the ^{14}C-SWNT were oxidized and showed a much higher degree of dispersibility; characteristics of this ^{14}C-SWNT material have been previously reported [12].

All chemicals were analytical grade purchased from Acros (Deoxycholic acid sodium salt SDC, 99%; benzalkonium chloride BAC, and Sodium dodecyl benzene sulfonate SDBS, Sodium dodecyl sulfate SDS, Cetyltrimethyl ammonium bromide

CTAB), Sigma Aldrich (Pluronic-F127, Gum Arabic GA), Anatrace (Sodium cholate SC, sol-grade) and ICN Biomedicals, Inc. (TritonX-100). Natural estuarine sediment was collected from a pristine salt marsh estuary – Bread and Butter Creek (BBC) within the North Inlet Estuarine Research Reserve on the coast of South Carolina, USA. This sediment was well characterized (98% silt/clay, total organic carbon content f_{OC} = 2.6%, total black carbon content f_{BC} = 0.6%) and has been previously used for estuarine ecotoxicity bioassays and microcosm studies [12]. Subtidal sediment was collected from central Long Island Sound, NY (sand:silt:clay ratio 8:77:14, f_{OC} = 2.0%, f_{BC} = 0.26 ± 0.02%) [29, 75, 87].

2.2.2. Characterization of SWNT by NIRF spectroscopy

SWNT SG65 suspensions were prepared in 2% w/v surfactant in deionized water (c_0 = 1 mg SWNT/mL surfactant solution) by ultrasonication for 10 min at 50 Watt power input (microtip, Sonifier 450, Branson Ultrasonics) in a salt-water ice bath and centrifugation for 10 or 30 min at 17860 x g. The supernatant was diluted for spectroscopic characterization. The pH of all samples was determined to be neutral. NIR absorbance and fluorescence spectra of SWNT suspensions were measured using a Nanospectralyzer NS1 (Applied Nanofluorescence) with 3 discrete laser excitation wavelengths (wavelength/power: 638 nm/32 mW, 691 nm/31 mW, 782 nm/74 mW). The integration time was 100 ms with 100 spectral averages. Fluorescence emission spectra were scanned and recorded from 900 – 1300 nm after stepwise excitation by the three

lasers. These three excitation spectra can be used to create 3D excitation/emission contour plots (e.g. Figure A2) using a spectral modeling approach based on the known electronic transitions of individual semiconductive SWNT chiral species (discussed below) [23].

In an aqueous suspension of dispersed SWNT, fluorescence intensity is influenced both by SWNT *(n,m)* chirality and by and their respective concentrations. Individual SWNT *(n,m)* species give different fluorescence signal strengths even when present at equal mass concentrations. In order to interpret the data and determine the relative concentrations of each SWNT species, I have applied a set of correction factors that reflect the different intrinsic quantum yield of each species. These calibration factors were determined previously by photophysical measurements on individual SWNT in SDC and other surfactants [27] and were used in concert with the raw fluorescence spectra to calculate diameter and *(n,m)* distributions of SWNT species in samples. Quantitative calibration curves were developed based on total emission power and total quanta (peak area of each individual species). The measured NIR fluorescence intensity (emission power per wavenumber) was dependent on the optical excitation power, the SWNT concentration, sample matrix and the sample quality. I tested this parameter for quantitative purposes. Standards as well as samples were treated using the same preparation protocol (prepared with the same surfactant and batch of SWNT).

The concentration of SWNT stock solution was determined by UV-Vis spectroscopy (SpectraMax 5 Multimode Plate reader, Molecular Devices Inc.) comparing the absorbance at 775 nm before and after centrifugation [116]. No loss to settling of surfactant-wrapped SWNT was observed over a period of three months. Moreover, the stability of SWNT stock suspensions was studied by NIRF spectroscopy with no observed change in NIRF signal for more than three months indicating that SWNT dispersed in 2% w/v SDC were stable in suspension. For quantitative calibrations, serial dilutions of SWNT stock suspension in 2% w/v SDC or BBC control sediment extract were prepared from 25 ng/mL to 30 μg/mL in triplicates. The calibration curve for each of the three excitation lasers was constructed by plotting the total emission power as a function of SWNT concentration. Calibration curves for each *(n,m)* species present in the sample were derived from fitted spectra. The concentration of *(n,m)* species was then calculated based on relative abundance in the sample, thus permitting a quick determination of SWNT structure distribution in an extracted sample.

2.2.3. Extraction of SWNT from estuarine sediment and tissue

Various surfactants (TritionX-100, GA, SDS, SDBS, CTAB, SC and SDC) in combination with high power sonication were tested for their ability to extract SWNT from sediment. *Spiking step:* Natural sediment (1.5 g wet sediment: BBC [0.75 g dry weight] or LIS sediment [0.6 g dry weight]) was suspended in 8 mL of 30 practical salinity unit [psu] synthetic seawater (Instant Ocean®) simulating conditions in an

estuarine sediment. The sediment slurry was stirred vigorously during SWNT suspension addition: 13 μg (20,000 DPM) for ^{14}C-labeled SWNT or 0.1 - 50 μg for non-radiolabeled SWNT. The slurry was shaken for various times (24 h up to 30 days) on an orbital shaker or on a jar roller at 4° C and centrifuged 15 min at 1880 x g to remove the water phase. *Extraction step:* The extractant (surfactants dissolved in water, V = 3 mL, 1 – 10% w/v) was added to the sediment. This slurry was sonicated at high power (variable power input, 26 – 50 W) for 10 minutes followed by a centrifugation step (17,860 x g for 10 min). The supernatant was diluted for analysis by scintillation counting for ^{14}C-SWNT (cocktail: Ecoscint XR, National diagnostic, scintillation Counter: Tri-Carb 2800TR, Perkin Elmer) or NIRF spectroscopy for non-labeled SWNT. Subsequent extractions were performed on the sediment residue. *Concentration step (applied when necessary):* Four sequential sediment extracts were combined (2.5 mL each, total 10 mL) and transferred to an ultracentrifuge tube containing a 1 mL underlayer of 60% Iodixanol (OptiPrep Density Gradient Medium, Sigma Aldrich). The samples were ultracentrifuged at 207,000 x g for 12 h. Concentrated SWNT solution was collected from the interface between the surfactant solution and the Iodixanol density cushion (fraction size: 1 mL, representing a 10-fold concentration). Unspiked sediment samples were treated using the same procedure. Benthic organisms were extracted in 2 mL 2% w/v SDC by ultrasonication (40 Watts power input), centrifuged at 17,860 x g for 10 minutes at 22 °C and the supernatant measured and quantified by NIRF spectroscopy.

Analytical performance of the method was validated by analysis of precision among triplicate measurements, accuracy in measuring the spiked concentration (validated against recovery of ^{14}C-labeled SWNT), linear range, matrix effects (consistency of response between SWNT standards and standard-addition calibrations in sediments), and instrument/method detection limits. Data analyses, including analysis of variance (ANOVA), were conducted with an $\alpha = 0.05$.

2.3. Results and discussion

2.3.1. Dispersion of SWNT in aqueous solution

Disaggregation and individualization of SWNT in suspension was required prior to NIRF analysis. Several synthetic and natural surfactants were evaluated for their ability to disaggregate and individualize SG65 SWNT in aqueous suspension, including SDBS, GA, Pluronic-F127, SC and SDC. For this work, SG65 SWNT suspensions (c_0 = 1 mg mL^{-1}) were prepared by high power sonication and ultracentrifugation. The supernatant was removed and used for spectroscopy analysis (UV-Vis and NIRF).

For the tested surfactants (2% w/v) at different SWNT concentration, SDC-dispersed SWNT exhibited the most highly resolved NIRF spectral features (absorbance and emission) as well as the highest fluorescent yield relative to SDBS and SC (Figure A1 e, f). Additionally, 2% w/v SDC solutions proved to be the most effective dispersant for SWNT, as suspensions of SWNT in SDC were more resistant to ultracentrifugation. These results indicated that SDC can disperse SWNT more efficiently than other widely

used surfactants. These observations were in agreement with UV-Vis-NIR absorbance measurements of surfactant wrapped SWNT by [33]. The authors showed that UV-Vis-NIR absorbance spectra of SWNT in SDC showed the most resolved spectra compared to other widely used anionic, cationic and neutral surfactant implying a higher yield of individualized SWNT in suspension [33].

2.3.2. Extraction of SWNT from sediment

Prior to analysis of SWNT in sediments by NIRF spectroscopy, an effective method for extracting and stabilizing SWNT in aqueous suspension was developed. Various surfactants (BAC, TritionX-100, GA, SDS, SDBS, CTAB, SC and SDC) in combination with high power sonication were tested for their ability to extract SWNT from a sediment matrix (BBC sediment). Experiments were performed with both oxidized, radiolabeled (^{14}C-SWNT) and pristine (SG65) SWNT to evaluate analytical performance and to validate sample preparation methods. It was proposed that fluorescence intensity is influenced by sample preparation method [28, 117, 118]. By employing ^{14}C-labeled material, the extraction method can be evaluated independent of the optical properties of SWNT material. Since SWNT were transferred to an aqueous solution, sediment matrix effects on scintillation measurements were minimized. In contrast to the optical NIRF measurements, scintillation measurements were not influenced by the agglomeration state of SWNT.

The extraction efficiency for ^{14}C-labeled SWNT depended strongly on the surfactant, surfactant concentration and sonication procedure (Figure 2.1A). SWNT could not be extracted from the sediment by seawater, indicating a high affinity of SWNT to the sediment matrix [108, 119]. For surfactant concentrations of 5% w/v, the ability of each surfactant to extract SWNT from sediment from the least efficient to the most efficient was as follows: cationic (BAC and CTAB) << anion surfactants (SDBS and SDS) < neutral surfactant (Triton X-100). Moreover, results showed that comparable extraction efficiencies (> 65% with 5 sequential extractions) were achieved by using SC and SDC at lower concentration (2% w/v). Additionally, the results showed that sonication conditions had a strong influence on ^{14}C-SWNT recovery. SWNT-recovery increased from 35 ± 10% to 75 ± 4% by using high power (probe) compared to low power (sonic bath) sonication (Figure 2.1A). High power sonication was necessary to separate SWNT from sediment as well as individualize SWNT in suspension for NIRF-analysis (Table A1). The extraction efficiency for the different surfactants corresponded to their ability to stabilize SWNT in aqueous suspension. Aromatic surfactants such as SDBS and TritonX-100 stabilized SWNT better than conventional linear surfactants SDS (contain an alkyl chain). For Triton X-100, the stabilizing mechanism is postulated as the formation of hemi-micelles covering the SWNT surface with the benzene rings providing π- π stacking between the surfactant molecule and nanotube core [33]. Bile salts such as SC and SDC showed a high potential for individualizing and stabilizing SWNT in aqueous

suspensions [118]. It has been proposed that these surfactants can very effectively accommodate the curvature of the SWNT surface due to the slightly bent but rigid steroid ring [118].

Based on these results (recovery above 75% for ^{14}C-labeled SWNT), SDC and TritonX-100 were selected to further test their ability to extract SWNT from sediments prior to NIRF spectroscopic analysis. For NIRF measurements, spiked sediment extracts were measured relative to a sediment extract from non-spiked sediment. Cumulative recoveries for SG65 SWNT from BBC sediment obtained by 4 sequential extraction steps with 2% w/v SDC (81 ± 5%) were slightly higher compared to recoveries of ^{14}C-SWNT (75 ± 4%) using 2% w/v SDC (Figure 2.1B) and significantly higher compared to recoveries of SG65 (57 ± 10%) utilizing 10% w/v Triton X-100 (ANOVA, $\alpha = 0.05$,). Results demonstrated that 4 sequential extractions were sufficient to extract SG65 SWNT from the sediment (Figure 2.1B, C). After 5 extraction steps the solution did not yield recognizable characteristic spectral features of SWNT (Figure 2.1C). Based on the response for individual *(n,m)* SWNT species in SG65, it was possible to determine the recovery for *(n,m)* SWNT species (relative abundance > 1%). The recovery based on SWNT diameter is shown in Figure 2.1D, illustrating that recoveries of > 75% were yielded for all of the *(n,m)* SWNT species (except *(8,4)*, which was present in the SWNT mixture at only low abundance as shown in Figure A2). There was no evidence that extraction efficiency was systematically influenced by the diameter or electronic

structure of SWNT. Although recoveries varied among the individual SWNT species tested, there was no clear trend according to either diameter or chirality. Repeatability among the three replicate extractions was good (< 5%) for all SWNT species except for the *(6,5)*, which was the dominant species in the mixture. The cause of the higher variability in recovery of this species from sediment was not known.

NIRF spectra of SWNT extracted from sediment using 10% w/v Triton X-100 showed low intensity, peak broadening and limited peak resolution (Figure A3). For Triton X-100 the recovery was much lower than expected based on the results with ^{14}C-SWNT (Figure 2.1A). The lower apparent recovery of SG65 in Triton X-100 relative to SDC might be explained by the lower efficiency of this surfactant for disaggregating SWNT in suspension since agglomeration state plays a key role in NIRF response. Triton X-100 was a suitable surfactant for extraction of SWNT from sediment; however, it was not effective in disaggregating SWNT for NIRF analysis. Summarizing, my results revealed that SDC proved to be an excellent extractant for both the oxidized ^{14}C-SWNT as well as the pristine SG65 SWNT, which indicated its general utility for extracting nanotubes with varying surface chemistry from sediment.

2.3.3. Analytical method performance

Solutions containing only SDC (2% w/v) did not show any absorbance or emission in the NIR region. After sonication and a short centrifugation step, control sediment extracts displayed a yellowish to brownish color depending on the sediment

type and mass extracted. Optical absorbance spectra of these extracts revealed a significant optical density of these solutions in the region of maximum SWNT NIRF emission (See Figure A4). NIRF spectra of BBC control sediment extracts with no added SWNT showed indistinct features, with a generally rising baseline in each laser excitation channel in the region between 9500 – 11500 cm^{-1} (emission power value: from 0.000005 to 0.00003 nW/cm^{-1}). The emission peaks of several *(n,m)* species including the dominant species in SWNT: *(6,5)*; *(7,3)* and *(7,5)* fall within this region [27]. For quantification, NIRF spectra in sediment extracts were referenced against control sediment extracts containing no added SWNT. I attempted to matrix-match blank reference samples and samples of interest by quantitatively comparing optical densities in the range from 9500 to 11000 cm^{-1} to minimize matrix effects.

Figure 2.2 illustrates the quantitative response of the NIRF spectroscopy method for SG65 SWNT in sediment extract compared to 2% w/v SDC. Quantitation of SWNT in 2% w/v SDC suspension as well as in sediment extract was reliable and linear over more than three orders of magnitude (Figure 2.2). However, matrix effects decreased sensitivity somewhat in sediment relative to pure SDC solutions as the comparison of the NIRF spectra obtained at $C_{SWNT\ SG65}$ = 25 ng/mL in 2% w/v SDC and in sediment extract revealed (Figure 2.2B, C). NIRF spectra for the 638 nm and 782 nm excitation wavelengths yielded characteristic spectral features of SG65 SWNT, indicating the presence of these SWNT, whereas for the 691 nm excitation wavelength unique

identification of SWNT species was not possible. Instrument detection limits (IDL) were calculated as the SWNT concentration in 2% SDC solution that gave spectral peaks greater than 3x the amplitude of noise within the same spectral region in measured blank samples, based on data presented in Figure 2.2 ($n \geq 4$). IDL as determined to be 15 ng/mL in 2% w/v SDC.

Method detection limits (MDL) proved to be dependent on the recovery of SWNT from samples and on the level of interferences (e.g., internal filter and quenching effects by the components of the sediment matrix such as natural organic matter and colloids). $MDL_{sediment}$ was calculated using the same approach described above for IDLs, except in this case the measured spectra were obtained for SWNT in estuarine sediment extracts ($n = 3$) instead of 2% SDC solutions and the noise levels were determined from control (unspiked) sediment extracts. The initial $MDL_{sediment}$ was determined to be 525 ng/g in sediment after correction for recovery and sediment mass. In order to increase the sensitivity of SWNT analysis in sediment extracts, a concentration/clean-up step was developed, achieving a ten-fold concentration factor (Figure A5). With the concentration step the final $MDL_{sediment}$ was determined to be 62 ng/g in sediment. In addition to concentrating the SWNT sample, this ultracentrifugation step served as a matrix purification method, since dissolved substances (e.g, DOM and small organic molecules) were not sedimented by ultracentrifugation. This concentration step was included in the sample analysis method in cases where I found evidence for the presence of SWNT in

the samples based on observed spectral features (peaks), but where signals were below the initial MDL.

The work presented here was focused on estuarine sediments/water, however I have found that the method performs quite well in freshwater samples of sediment, water, and tissue. For example, I have also utilized my method to analyze waste water produced during commercial SWNT production, using ultracentrifugation to concentrate water samples from 24 to 4 mL. This method enabled us to achieve an MDL_{water} of 1 ng/L (in the waste water).

It is important to note that unlike thermogravimetric methods (e.g., CTO-375 and thermogravimetry-mass spectrometry), NIRF spectroscopy is uniquely suited to detect and quantify only carbon nanotubes in environmental samples. In particular, NIRF spectroscopy does not suffer from interferences caused by the presence of black carbon (BC) (e.g., soot and chars) in the samples. I have tested diesel soot as a model BC and have observed no emission in the NIR region. NIRF emission is a unique property of semiconducting SWNT, which is not shared by any other allotrope of black carbon.

2.3.4. NIRF spectra of SWNT in sediment extract

NIRF spectra of SG65 SWNT 2% w/v SDC and in sediment extracts illustrated that spectral features remained relatively constant after extraction from sediment (Figure A2). Fluorescence was most intense for these SWNT at 782 nm, although spectra at 638 nm show better resolution. In sediment extracts, slight peak broadening as well as a shift

in relative emission intensity for individual peaks were observed in comparison to SWNT in 2% w/v SDC (Figure A2A, B). NIRF spectra were also plotted as 3D contour plots for SWNT in 2% w/v SDC and in sediment extract (Figure A2C, D). These spectral maps were quantitatively quite similar; in both sediment extracts and 2% w/v SDC suspensions, *(6,5)* SWNT were identified as the main species (relative abundance: 60%) followed by *(7,5)* and *(7,3)* (both ~7%). Each distinct peak in Figure A2C and A2D corresponded to emission from the first band gap (E_{11}) of one semiconducting *(n,m)* SWNT, after excitation to the second band gap (E_{22}). The energy of this transition mainly depends on nanotube diameter. At different wavelengths various superpositions of distinct E_{11} transitions occur in a sample mix of different *(n,m)* SWNT [10]. The measured spectra were used to quantitatively model the *(n,m)* chirality distributions of SG65 SWNT in 2% w/v SDC and in sediment extract. Modeled histograms of diameter distribution for SG65 SWNT dispersed in sediment extract and in 2% w/v SDC (Figure A2E, F) indicated a population of SWNT with narrow diameters and low dispersion in both samples. A slight shift towards smaller diameters was observed in the sediment extracts compared to 2% w/v SDC, indicating a shift in the relative abundance of *(7,5)* towards *(6,5)* and *(7,3)* SWNT species [relative abundance: 5% *(7,5)*, 57% *(6,5)* and 11% *(7,3)*]. Since the emission peak of *(6,5)* and *(7,3)* are very close together in the NIRF spectra, the observed peak broadening might have influenced the histogram calculation.

It was also possible that interaction between sediment and SWNT might have influenced the detected SWNT diameter.

2.3.5. Determination of SWNT in estuarine sediment

My colleagues and I designed a series of experiments to evaluate matrix effects and determine quantitative performance for SWNT analysis by NIRF spectroscopy in different sediment samples spiked at a variety of concentrations with several SWNT types (*n,m* distributions) with different stabilizer coatings.

SWNT over a range of concentrations were spiked to natural sediments (BBC and LIS), equilibrated for 24 h and extracted by the method described above. For each sample set, control sediment was treated under the same conditions and was used as a blank in NIRF analysis. Recovery (shown as $C_{SWNT,quantified}$ in Figure 2.3) was quantified based on 4 sequential sediment extraction steps.

Linear relationships were observed for extraction of several types of SWNT from two marine/estuarine sediments with different compositions over several orders of magnitude concentration (Figure 2.3). Recoveries were determined from the slope of the linear regression between SWNT added and SWNT quantified. Results revealed that higher recovery was observed for SG65 SWNT extracted from BBC sediment (103 ± 10%) relative to LIS sediment (66 ± 7%). The OC content of both sediment materials was comparable, whereas BBC sediment has a higher silt/clay fraction than LIS sediment. When extracting the same sediment wet weight the LIS control sediment extracts

exhibited a darker, yellowish color than the BBC extracts. The lower recovery from LIS sediment might be due to quenching effects from co-extracted colloidal sediment material, different organic material and/or a higher affinity of SWNT to LIS sediment matrix.

Furthermore, when measuring sediment extracts, it was found to be critical to use appropriate "blank" sediment reference samples, as spectra from various control sediment extracts were inconsistent, especially in the spectral range above 9500 cm^{-1} where an increase in the baseline was observed (See Figure A4). Attempts were made to matrix-match blank reference samples and samples of interest by quantitatively comparing optical densities in the range from 9500 to 11000 cm^{-1} to minimize matrix effects. As discussed above and illustrated in Figures 2.2B and 2.2C, the presence of internal filter effects and other matrix interferences altered the detection limits by a factor of seven and made accurate quantification of the samples difficult as SWNT concentrations approach the detection limit. The presence of matrix effects was therefore often the limiting factor in NIRF analysis of SWNT in sediments.

Tests of recoveries from sediment using SG65 SWNT with a variety of coatings were conducted due to the common use of these agents to amend SWNT to environmental samples in toxicity studies. Gum Arabic (GA) is an analog of natural organic matter and is commonly used as a nanoparticle coating in ecotoxicity studies, whereas Pluronic-F127 is often applied for human toxicology assessment tests [29, 34,

120-122]. High resolution and low resolution NIRF spectra were obtained in GA- and Pluronic-F127-coated SWNT, respectively. Whereas GA- and SDC-coated SG65 SWNT could be extracted from BBC sediment quantitatively (Figure 2.3), Pluronic-F127-coated SWNT could not be detected in sediment extracts by NIRF analysis even after concentration. Recoveries are reported in Table A1.

The low recovery of Pluronic-F127-coated SWNT from sediment might be related to their stronger tendency to form stable agglomerates prior to the sediment spiking (strong SWNT-SWNT interaction), and/or their higher affinity to the sediment matrix. It is also possible that the extracted SWNT are not detectable by NIRF analysis due to electronic quenching effects. There is evidence in the literature that Pluronic surfactants adsorb very strongly to multi-walled carbon nanotubes [33]. It is unclear what happens to the SWNT coating material when brought in contact with sediment matrix during the equilibrium phase. Depending on the nature of the coating, a fast or slow desorption, or/and an exchange of the coating material with components of the matrix is possible. Further investigations need to be conducted to study the interaction between the coating and SWNT (e.g., adsorption layer, strength, mechanism, desorption).

I note here that field-collected sediments from South San Francisco Bay, CA (collected in 2008) were extracted and analyzed for SWNT using my NIRF analytical method, and results indicated no SWNT contamination above the detection limit, even

in these wastewater-impacted sediments located in close proximity to potential industrial sources of nanomaterials (i.e., San Jose/Silicon Valley).

2.3.6. Evaluation of SWNT fate and bioaccumulation in sediment mesocosms

I have applied this method to track uptake and distribution of SWNT from estuarine sediment by benthic meiofauna, in an estuarine sediment microcosm study. A description of the microcosm study design has been reported previously [123]. In the current study, SG65 SWNT were spiked to a BBC sediment slurry to achieve a nominal SWNT concentration of 1 and 10 μg SWNT/g sediment. SG65 SWNT were dispersed in 2% w/v SDC by ultrasonication followed by a dialysis step (35 kDa cutoff) to remove unbound SDC. During the dialysis step binding of SWNT to the membrane was negligible (recovery > 90%). After a short resonication step, the resulting suspension was stable for the short time period needed for spiking. The sediment slurry was equilibrated for 7 days at 4 degree Celsius, and added as an overlayer (~3 cm) on top of the sediment cores. After incubation for 28 days, the meiofaunal community structure was characterized by counting of taxa and the uptake and the distribution of SWNT between meiofauna and sediment was evaluated by the NIRF method. In agreement with recent findings in my own and others' laboratories, little-to-no uptake of SWNT from the sediment into the tissues of any benthic organisms was observed [11, 17, 20, 104, 124]. NIRF spectra of different benthic organisms are presented in Figure A6. Because the sediment was allowed to incubate for the full 28 days of the experiment,

measurements of the SWNT at the beginning and end of the exposures were made to evaluate persistence of the each SWNT *(n,m)* species over this time frame in estuarine sediment (Figure 2.4). High recoveries were observed for the 10 μg SWNT g^{-1} spiked sediment before (measured total [SWNT] = 12.6 ± 2.3 μg/g) and after (measured total [SWNT] = 10.0 ± 0.5 μg/g) the 28 day incubation and for the 1 μg SWNT/g spiked sediment before incubation (measured total [SWNT] = 0.60 ± 0.02 μg/g, Table A2). Due to the method detection limit in sediments, limited amount of sediment material was available for extraction after the incubation time (28 mg compared to 470 mg at t = 0 days), and matrix effects, quantification of SWNT in the 1 μg SWNT/g spiked sediment at t = 28 days was not possible. However, SWNT were detected qualitatively in the sediment extract.

Results illustrated that SWNT concentration in the experimental sediment was homogenous and close to the expected nominal value. After the 28 day incubation, no significant decrease in SWNT concentration in the sediment was observed (Figure 2.4, ANOVA, $\alpha = 0.05$), revealing that even aged SWNT (that presumably became associated with sediment-NOM) were amenable to the presented analytical method. Furthermore, the results indicated that the biotransformation of SWNT was insignificant over a month time scale under aerobic conditions and high infaunal bioturbation activity.

Furthermore, no significant change was observed across the SWNT diameter and *(n,m)* distribution range in the sediment. In future studies, use of the developed

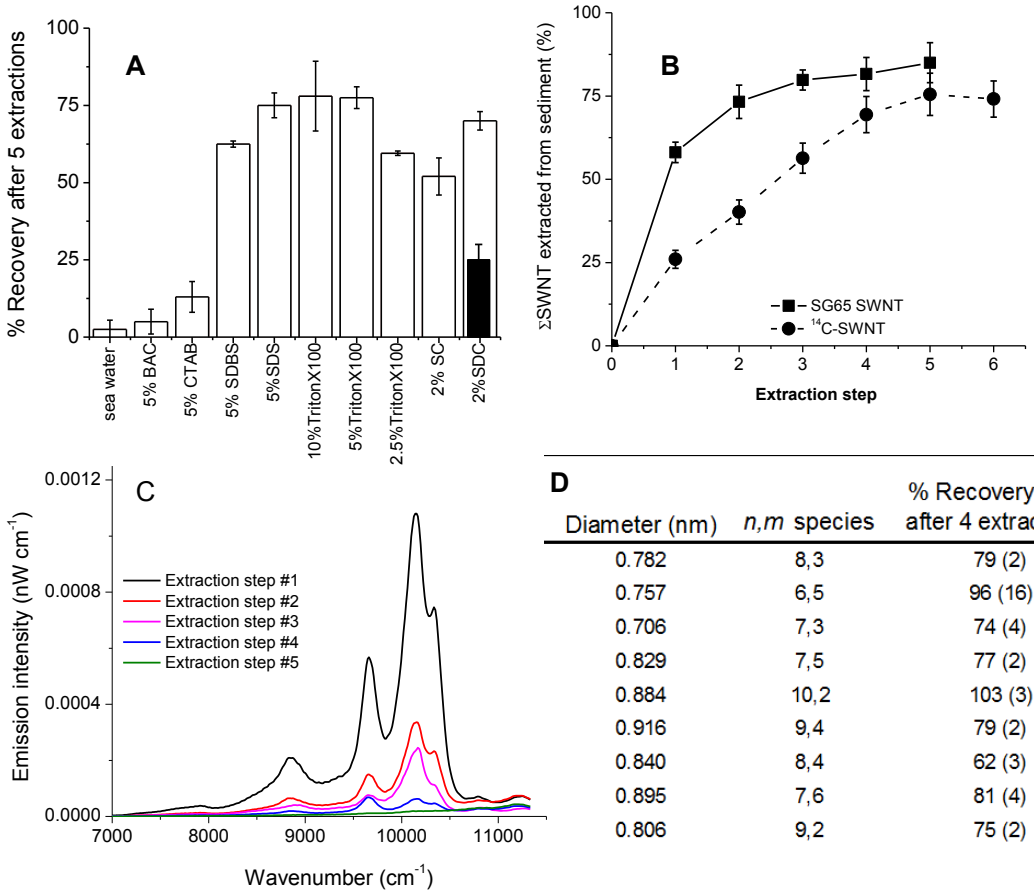
technique in parallel with toxicity test observations will allow researchers to gather more information regarding the environmental fate, biotransformation, and transport of SWNT in sediment environments. Results of the current work showed that SWNT have a low potential for biotransformation by meiobenthos through a sediment exposure route, and that these carbonaceous nanomaterials will persist in sediments over at least month time periods.

The high fidelity of NIRF spectroscopy in assessing the quantitative and qualitative properties of semiconducting SWNT in complex estuarine sediment and biota underscores the value of developing methods for analysis of engineered nanoparticles in the aquatic environment. The NIRF method is capable of the qualitative detection of any and all semiconducting SWNT present in environmental samples and achieves reliable detection without previous knowledge on chirality/diameter distribution. Quantitative information can be obtained using authentic standards.

The method reported here represents the most sensitive and selective technique currently available for analyzing SWNT in environmental samples, even in highly complex, particle-rich samples (e.g., sediment). It is clear that the high selectivity of the NIRF technique, based on SWNT-specific electronic transitions coupled to the generally low NIR fluorescent background and consequent high sensitivity in most biological and environmental samples allows this technique to serve as a useful method for conducting

both laboratory- and field-based fate, transport, and effects research on SWNT in the environment.

However, challenges remain in reducing potential optical matrix effects (e.g. internal filter effects) and novel instrumentation combinations such as NIRF with upstream asymmetric flow field flow fractionation (AF^4) may yield even more powerful analytical solutions for assessing the fate of SWNT in the aquatic environment.



Diameter (nm)	*n,m* species	% Recovery (SD) after 4 extractions
0.782	8,3	79 (2)
0.757	6,5	96 (16)
0.706	7,3	74 (4)
0.829	7,5	77 (2)
0.884	10,2	103 (3)
0.916	9,4	79 (2)
0.840	8,4	62 (3)
0.895	7,6	81 (4)
0.806	9,2	75 (2)

Figure 2.1. Validation of SWNT extraction and quantitative analysis from sediment. (A) Recoveries (mean ± s.d., n = 3) after ultrasonic extraction of ^{14}C-SWNT from BBC estuarine sediment using various surfactants for 10 min at 40 W (open bars) and 1 h using a low power ultrasonic bath (filled bars), determined by scintillation counting. (B) Cumulative extraction (mean ± s.d., n = 3) of ^{14}C-SWNT (●, scintillation counting) and SG65 SWNT (■, NIRF) from BBC estuarine sediment using 40 W ultrasonication in 2% SDC. (C) Representative NIRF spectra at 638 nm excitation wavelength after sequential extractions of SG65 SWNT from BBC estuarine sediment using 2% SDC and (D) Recovery of each SWNT electronic species from BBC sediment after extraction with 2% w/v SDC (n = 3) based on NIRF.

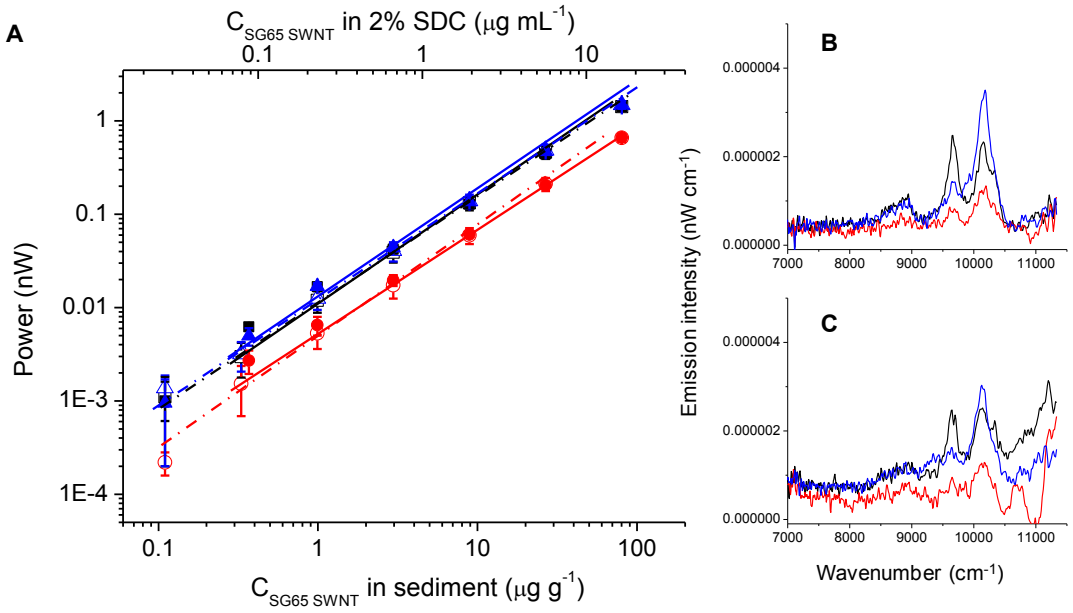


Figure 2.2. (A) NIRF quantitative response of SG65 SWNT in 2% w/v SDC (black open square, 638 nm; red open circle, 691 nm; filled blue triangle, 782 nm; solid lines: linear fits). Error bars represent one standard deviation about the mean (n = 3). Insets show NIRF spectra obtained at $C_{SG65,SWNT}$ = 25 ng/mL in 2% w/w SDC solution (B) and in BBC sediment extract (C). The spectra represent the emission spectra for each excitation wavelength: 638 nm (black), 691 nm (red), and 782 nm (blue).

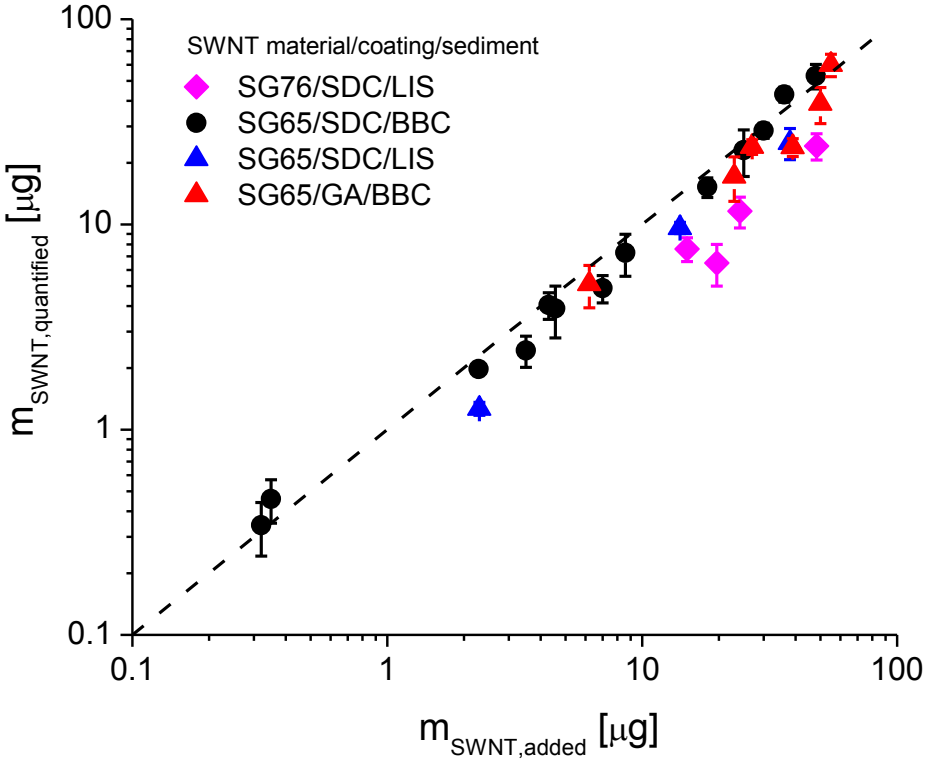


Figure 2.3. Quantitative measurement of two types of CoMoCat SWNT (SG65 and SG76) with different coatings (SDC and GA) in two estuarine sediments (BBC and LIS) over a wide range of amended concentrations ($C_{SWNT,\ added}$). Sediment extraction was performed after 24 h incubation. The dashed line represents a 1:1 relationship, $C_{SWNT,\ quantified}$ represents the cumulative SWNT concentration measured in extracts after four sequential extractions. The error bars represent one standard deviation about the mean (n = 3).

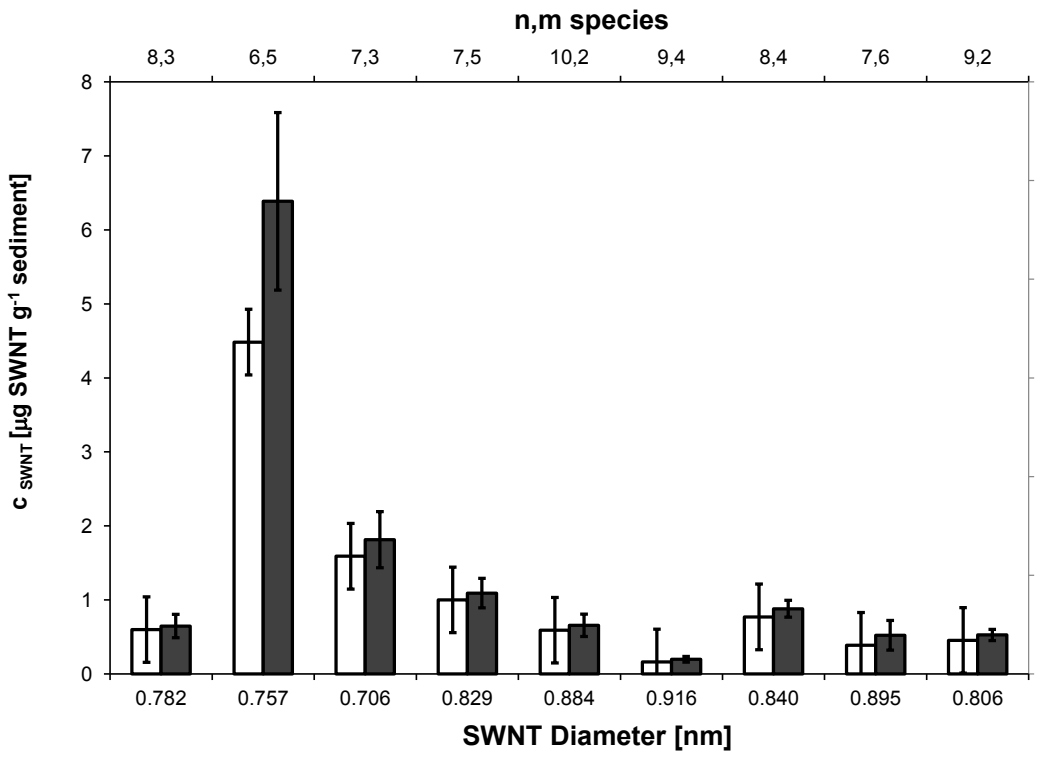


Figure 2.4. Extraction of SG65 SWNT from BBC estuarine sediments before (open bars) and after (filled bars) benthic meiofauna exposure for 28 days in sediment microcosms. SWNT-amended sediment had been equilibrated with sediment at 4 °C for 14 days before exposure. Extracted SWNT concentration (determined by NIRF) is plotted according to SWNT electronic species (top x-axis) and corresponding diameter (bottom x-axis). Error bars represent one standard deviation about the mean (n = 3).

3. Bioaccumulation and Toxicity of Single-walled Carbon Nanotubes (SWNT) to Benthic Organisms at the Base of the Marine Food Chain

3.1. Introduction

Single-walled carbon nanotubes (SWNT) are cylindrical carbon allotrope structures composed of one atom thick of sp^2 hybridized carbon, with diameters ranging from 1-2 nm and lengths up to hundreds of micrometers [1]. Each SWNT structure can be envisioned as a rolled sheet of graphene with a unique diameter and chiral wrapping angle corresponding to a set of roll-up vector integers (n, m) [1, 3]. These physical characteristics drive the electronic properties of the different SWNT isoforms [1]. The near infrared fluorescence spectra of SWNT suspensions can be measured to quantify and characterize (i.e., diameter distribution, chiral wrapping angle) the various pristine semiconducting SWNT in mixtures, although metallic SWNT (which constitute 1/3 of possible SWNT structures) are not detectable using this method [2, 5, 10]. In addition to the simple carbon backbone, SWNT can be modified by the addition of functional groups during synthesis to further alter their physical-chemical properties. As a new industrial material, SWNT are currently being used in a variety of products including lithium-ion batteries, textiles, chemical sensors, gene delivery vehicles, electronics, and structural composites [41, 42]. In the United States, total carbon nanotube (both single-

and multi-walled carbon nanotube, MWNT) production is estimated to range from 55-1100 metric tons year^{-1} [46].

With increased SWNT production, there is an increased risk of environmental release. Therefore, it is important to determine potential routes of SWNT environmental exposure to form a sound basis for performing environmental risk assessments as well as providing the necessary information for developing industrial controls and environmental regulations. SWNT can potentially be released from products by abrasion, normal wear and tear, aging, improper use, disposal/recycling, spills, landfill leachate, industrial effluent, waste incineration, wastewater treatment, and atmospheric emissions [11, 41, 42, 51]. These routes of release would likely lead to accumulation of SWNT in terrestrial and aquatic media (i.e., water, soil, sediments) and food chains. Current concentrations of SWNT in the environment have not yet been reported. However, one model predicts that by 2012 the concentration of carbon nanotubes (all types in sediment will reach approximately 0.5 μg/kg and the concentration in sludge-treated soils will reach approximately 0.4 μg/kg [52].

With the likely release of SWNT and other carbon nanomaterials (NMs) into the environment, understanding their potential adverse effects is critical. Research thus far has investigated the effects of pristine and functionalized carbon NMs with various coatings and utilizing different exposure media and organisms. Several studies have shown that organisms ingest carbon NMs, after which they are repackaged into fecal

pellets and eliminated upon depuration with a control food source, without incorporation of the NMs into the gut lumen or tissues [11, 53, 56-58, 125]. When investigating the effects of pristine versus functionalized carbon NMs, those effects depend on the type of carbon NM. Aqueous exposures of multi-walled carbon nanotubes (MWNT) resulted in significant mortality in the freshwater daphnid *Ceriodaphnia dubia* from the pristine nanotubes, but not the oxidized materials [58]. It was hypothesized that the toxic effect of the raw MWNT was due to aggregation and physical blocking of the gut and/or disruption in mobility of the organism, whereas the oxidized materials were less hydrophobic and more easily cleared [58]. However, pristine MWNT were less toxic to the estuarine amphipod *Leptocheirus plumulosus* and freshwater amphipod *Hyalella azteca* than were carbon black and activated carbon through a sediment exposure route [58]. In an earlier study from the Ferguson laboratory, purified SWNT did not induce toxicity, but the small nanocarbon impurities induced 36% mortality at 10 mg/l in the estuarine copepod *Amphiascus tenuiremis* [11]. The fate, transport, toxicity and bioaccumulation of different carbon NMs in the environment depend on several environmental/experimental factors (i.e., sample preparation, experimental design, bioavailability, and organism tolerance) as well as specific nanomaterial composition [48, 59-62].

Due to sample preparation and analytical detection challenges, very few ecotoxicological studies have examined the effects of pristine, non-functionalized SWNT

with minimal metal impurities. Also, sensitive and specific analytical methods have not previously been available for quantitatively measuring SWNT in environmental samples (e.g., sediments, tissues). However, the unique near infrared fluorescence (NIRF) of pristine, semiconducting SWNT has recently been applied to ecotoxicological studies, providing a new method for testing of lower and more environmentally relevant SWNT concentrations [103]. In the present study, in order to enhance the toxicological dataset on SWNT, I utilized several benthic organisms at the base of the marine food chain including the estuarine amphipod, *Ampelisca abdita*, and mysid shrimp, *Americamysis bahia*, to investigate the potential bioavailability, bioaccumulation and toxicity of four distinct SWNT materials through a sediment exposure route. Further, one SWNT material was used to explore the effect of exposure route (e.g., sediment and/or food source) on SWNT bioavailability, bioaccumulation and toxicity. Sediment and food exposures were selected rather than an aqueous route because SWNT are not very water soluble and are more likely to associate with particles including food and suspended sediment particles when in aqueous solution [18, 52, 58]. NIRF spectroscopy was utilized to determine the concentration and specific SWNT species (n,m) present in each environmental matrix (sediment, tissues). In addition to evaluating pristine semiconducting SWNT, ^{14}C-SWNT were used to analyze the potential bioavailability, bioaccumulation and toxicity of carboxylic acid-functionalized SWNT in the estuarine amphipod *L. plumulosus* via sediment and/or food exposures. Taken together, these

studies provide an indication of whether SWNT are likely to enter the marine food chain.

3.2. Materials and methods

3.2.1. Single-walled carbon nanotube materials

SWNT used in the current work were CoMoCAT nanotubes (SG65, SG76, CG100) from SouthWest NanoTechnologies, Inc (SWeNT), a proposed standard reference material SWNT from the Organization for Economic Co-operation and Development (OECD), and ^{14}C-SWNT produced by the arc-discharge method (Research Triangle Institute). Materials from SWeNT are well-characterized, high purity mixtures of SWNT with either narrow diameter distributions and specificity (SG65, SG76) or broad diameter distributions (CG100) [126-129]. For characterization of the SWNT stocks, stable suspensions of SWNT were prepared in 2% w/v sodium deoxycholate (SDC) using high power sonication at 50% amplitude for 10 minutes in a salt-water ice bath (Branson 450D Sonifier) followed by centrifugation at 17,860 g and 22°C for 30 minutes (accuSpin™ 1R, Fisher Scientific) to remove non-suspended SWNT bundles. Toxicity and bioaccumulation test suspensions of the unlabeled SWNT materials were prepared at 1 mg SWNT/mL 2% w/v SDC with no centrifugation step. Stock suspensions were resonicated prior to amendment to sediment or food sources if not used immediately after preparation.

The ^{14}C-SWNT were purified using nitric acid to introduce oxygen containing groups such as carboxyl to defect and end sites [11, 12, 115]. The ^{14}C-SWNT stock was 1.15 mg ^{14}C-SWNT/mL deionized water with an activity of 1.63 μCi/mL. This stock was resonicated for 10 minutes at 80-100 watts (Fisher Scientific Model 100 Sonic Dismembrator) prior to use.

3.2.2. Near infrared fluorescence spectroscopy

Stock SWNT suspensions and sample extracts (i.e., sediment, food and organism) were analyzed for near infrared fluorescence and absorbance as described previously using a NS1 Nanospectralyzer (Applied NanoFluorescence, LLC) [103]. Instrument and method detection limits were previously found to be 15 ng/mL (instrument), 62 ng/g (sediment), and 1 μg/l (water) [103]. This instrument employs three excitation lasers (638 nm, 691 nm, 782 nm) and detects fluorescence emission from 880-1580 nm with an InGaAs array detector cooled to -18 °C. The measurement parameters were as follows: 100 ms fluorescence integration time with 100 spectral averages for each excitation wavelength, and 60 ms absorbance integration time with one spectral average. Resulting NIRF emission spectra were analyzed with spectral fitting software to determine the specific SWNT chirality *(n,m)* species present. This determination is based on data collected by Weisman and Bachilo [27] demonstrating that each SWNT species has a unique diameter, chiral wrapping angle, and excitation-emission spectrum. The unique spectral signature allows for identification of individual species in SWNT mixtures.

NIRF and absorbance spectra were measured to obtain quantitative and qualitative characterization information for each SWNT stock suspension (Figure 3.1).

3.2.3. Extraction of SWNT from environmental media

My laboratory has found that 2% w/v SDC is useful in extracting and resuspending SWNT from sediment, water and biota samples [103]. Sediment extractions were performed using approximately 1.5g wet sediment. The sample was washed with 6 mL of deionized water twice by vortexing and centrifuging the sample for 10 minutes at 13,520 g and 20 °C (Eppendorf centrifuge 5804 R). Each wash was collected and saved. The final pellet was resupended in 2.5 mL 2% w/v SDC by ultrasonication for 10 minutes at 50% amplitude in a salt water ice bath. This sample was centrifuged for 15 minutes at 13,520 g and 20 °C and the supernatant was saved. The extraction was repeated two-three times. A known, equal volume of each extract was combined for analysis and quantitation by NIRF spectroscopy. The samples were measured against a sediment blank extract in order to account for interference in the spectra.

Brine shrimp and algae were extracted by centrifuging the sample at 13,520 g for 10 minutes at 22 °C and resuspending the pellet in 2 mL 2% w/v SDC by ultrasonication. The suspension was centrifuged at 17,860 g for 10 minutes at 22 °C and the supernatant was measured and quantified by NIRF spectroscopy. Amphipods and mysids were extracted using the same method without the initial centrifugation step. If SWNT was

not detected, the extracts were concentrated via ultracentrifugation for 2 hours at 369,734 g and 20 °C (SW 60 rotor, L8-80 Ultracentrifuge, Beckman Coulter) and re-measured by NIRF spectroscopy.

3.2.4. Sediment toxicity test

Seven day static toxicity tests with *A. bahia* (mysid) and *A. abdita* (amphipod) followed the methods described by Ho et al. [130]. Ten individuals of each organism type were added to test chambers containing 20 g wet sediment and 60 mL of 30‰ reconstituted seawater (RSW) prepared by diluting 100‰ brine with deionized water. Chambers were static and aerated in a 20 °C incubator with a light cycle of 16h:8h light:dark. Long Island Sound (LIS) reference sediment (1.8% organic carbon, 92% silt and clay-sized particles) was amended with SWNT [94]. Four SWNT were tested (SG65, SG76, CG100, and OECD) at three nominal concentrations each (0.1, 1, and 10 μg SWNT/g dry sediment), and toxicity in amended sediments was compared to toxicity in reference sediment and carrier controls (2% w/v SDC). Measured concentrations of SWNT in the sediment are reported (Table B1). Each treatment consisted of four replicate chambers. Three replicates per treatment were saved for NIRF analysis and one replicate for microscopy of the mysids and amphipods. Details of the sediment composition, SWNT amendment of sediment and food, and sample extraction methods are described in Appendix B. Mysids were fed approximately 100 μL of 0.15 g wet *Artemia salina*/mL RSW per chamber daily. On day 7, organisms were sieved (0.5 mm)

and counted to determine survival, missing organisms were considered mortalities. Dissolved oxygen (DO), pH, salinity and temperature were monitored throughout the test.

3.2.5. Bioaccumulation studies with *A. abdita* and *A. bahia* using SG65 SWNT

Seven day bioaccumulation studies were conducted as described for the toxicity tests, but utilized only the SG65 SWNT-amended LIS sediment. Treatments for the present study included i) unamended sediment, ii) 2% w/v SDC-amended sediment, iii) SWNT-amended sediment with unamended food, iv) unamended sediment with SWNT-amended food, and v) SWNT-amended sediment with SWNT-amended food. Each treatment consisted of six chambers per organism type for the unamended treatment (three each for depuration and non-depuration) while all other treatments consisted of eight chambers per organism type (three each for depuration and non-depuration NIRF spectroscopy analysis and one each for depuration and non-depuration microscopy analysis). Percent survival included data from all chambers per treatment for each organism type (six for unamended sediment and eight for all other treatments). The amended food sources for the amphipods and mysids were *Cyclotella meneghiniana* (algae) and *A. salina* (brine shrimp), respectively. Nominal concentrations were 10 μg SWNT/g dry sediment, 10 μg SWNT/mL algae culture, or 10 μg SWNT/g wet brine shrimp. Measured concentrations of SWNT in the sediment and food sources are reported (Table B2). Upon termination, the non-depurated replicates were sieved (0.5

mm), counted to determine survival, and saved for analysis. The replicates saved for depuration were sieved (0.5 mm), counted to determine survival, transferred to control reference sediment and water, fed control food, and left for 24 hours. After this depuration period, the organisms were sieved (0.5 mm) and saved for analysis. Sediment and food amendment methods as well as sample extraction are described in Appendix B.

3.2.6. Bioaccumulation studies with *L. plumulosus* using ^{14}C-SWNT

L. plumulosus was exposed to ^{14}C-SWNT-amended *Isochrysis galbana* and/or reference sediment in a static-renewal design for 28 days. The reference sediment was from Bread and Butter Creek (BBC) in the North Inlet Estuarine Research Reserve near Georgetown, SC, USA (median grain size of 4 μm, 98% silt/clay, 2–4% organic carbon [131]). Each chamber contained 20 adult amphipods and 50 juvenile amphipods in 35 mL of sediment slurry (0.1106 g dry/mL slurry) and 150 mL of 15‰ aerated RSW held in a 25 °C incubator with a light cycle of 16h:8h light:dark. The treatments were as follows i) unamended sediment and algae, ii) unamended sediment and amended algae at 10 or 100 μg ^{14}C-SWNT/g dry algae, iii) unamended algae and amended sediment at 10 or 100 μg ^{14}C-SWNT/g dry sediment, and iv) amended sediment and amended algae at 10 or 100 μg ^{14}C-SWNT/g dry sediment or g dry algae. Each treatment included six chambers (three for depurated organisms and three for non-depurated organisms). Measured concentrations of ^{14}C-SWNT in the sediment at day 0 and day 28 as well as the average

in the amended algae are reported (Table B3). Amphipods were fed three times per week after a 50% water renewal (15‰ seawater filtered through a 0.2 μm filter). Physical measurements (i.e., DO, pH, salinity, and temperature) were monitored twice per week.

Upon termination of the test, half of the replicates were depurated and the other half was analyzed. Depuration was performed by transferring the organisms to control sediment and water where they were fed unamended algae for 24 hours before analysis. Overlying water, sediment, fecal pellets, and organisms were collected for analysis. Fecal pellets and organisms were collected by sieving the sediment of each replicate over a 250 μm sieve (organisms) stacked on top of a 125 μm sieve (fecal pellets). The organisms and pellets were rinsed with 15‰ filtered seawater to remove any remaining sediment. To analyze body burden, organisms were digested with 2 mL Scintigest Tissue Solubilizer (Fisher Scientific) and heated for two hours at 55 °C. Upon cooling to room temperature, Ecoscint XR (National Diagnostics) was added as the scintillation cocktail, and 250 μL of glacial acetic acid was added to quench chemiluminescence. Insta-Gel Plus (Perkin Elmer) was the scintillation cocktail for sediments and fecal pellets collected at test termination in order to better suspend these solids for analysis. Algae subsamples were bleached in a 2:1 algae:benzoyl peroxide solution, heated for 30 minutes at 55 °C and cooled to room temperature prior to addition of Ecoscint XR. All

samples were analyzed using a Tri-Carb 2800TR Liquid Scintillation Analyzer (Perkin Elmer).

3.2.7. Statistical analysis

Statistical analysis was performed using JMP® 9.0.0. When analyzing percent survival, a one-way analysis of variance (ANOVA, α=0.05) was performed on arcsine square root transformed data. Two-way analysis of variance (ANOVA, α=0.05) was performed on bioaccumulation data. Data were normalized by dry tissue mass and were $\log_{10}$ transformed if there was heterogeneous variance between treatments. For bioaccumulation data, upon determination of significant differences in ANOVA, Dunnett's t-test (α=0.05) was used to determine any treatment-specific significant differences from the controls. Unless otherwise noted, data are reported as average $\pm$ standard error. When analyzing diameter-specific uptake of SWNT in organisms and amended matrices, significance was determined by ANOVA followed by Tukey's Honestly Significant Difference multiple comparison test (α=0.05).

3.3. Results and discussion

3.3.1. SWNT sediment toxicity

For the four pristine SWNT evaluated at concentrations up to 10 μg SWNT/g dry sediment with the amphipod, *A. abdita*, and mysid, *A. bahia*, percent survival ranged from 85% to 100% (Figure B1). When the amphipod, *A. abdita*, and mysid, *A. bahia* were exposed to SWNT via sediment and food sources, percent survival ranged from 95% to

100% (Figure B2). One-way analysis of variance found no statistically significant differences from the controls for any organism, indicating the SWNT materials were not toxic in these exposures. These results are consistent with previous studies in my laboratory and others [11, 53, 56-58, 125]. Of the studies where toxicity was observed some speculate the mode of action to be gut impaction or impurities such as metal catalysts and nanocarbon byproducts [11, 58, 61, 132]. Adult *L. plumulosus* 28-day survival in amended treatments ranged from 87-98% of the control treatment survival, and procreation was evident in all treatments (Figure B3). An ANOVA was performed (α=0.05), but no statistically significant differences in survival from the controls were found.

3.3.2. SG65 SWNT bioaccumulation with *A. abdita* and *A. bahia*

Although SWNT did not lead to significant mortality in benthic organisms at the base of the food chain, bioaccumulation studies were performed to assess whether nanomaterials were bioavailable via sediment and/or food uptake routes. In NIRF analysis of the organisms, SWNT were not detected in either depurated or non-depurated mysid extracts or depurated amphipod extracts. However, SWNT were detected in extracts of sediment, spiked food sources (algae *C. meneghiniana* and artemia *A. salina*), and non-depurated amphipods exposed to amended algae (Figure 3.2, NIRF spectra in Figure 3.3). Since the algae culture was amended at 10 μg SWNT/mL, the concentration on a dry weight basis was much larger than the SWNT concentration in

the sediment and *A. salina*. Although this led to detectable SWNT body burdens in *A. abdita* prior to depuration, no SWNT were measured in the organisms after depuration. These results suggest that the SWNT were ingested primarily via amended algae and then immediately eliminated without crossing the gut lumen, lowering tissue concentration below that of the NIRF detection limit and are consistent with several other studies including both invertebrates and higher trophic level vertebrates [11, 53, 57, 58, 125, 133]. SWNT may not have been detected in mysid extracts due to their minimal tissue mass.

For the extracts that contained SWNT, further analysis was performed and the SWNT species distribution was determined. Each SWNT species was plotted as a ratio of relative abundance in the sample extracts to the relative abundance in the stock SWNT suspension (Figure 3.4). By comparing this ratio between extracts, I can determine if there was any preferential ingestion from the amended sediment and algae. For seven of the twelve SWNT species, no significant differences in relative abundance of the species among each type of extract (sediment, algae, and the two spiked-algae amphipod exposures) were found. For the species which were not significant, it is interesting to note that both the *(6,4)* and *(9,1)* species were not found in the sediment extracts but were present in the organism extracts. This is most likely due to matrix effects increasing noise in the mid-near infrared region of the spectrum and causing a false signal. Of the five SWNT species with significant differences in relative abundance

among extracts, three species [*(n,m) = (9,4)*, *(8,4)*, and *(9,2)*] have similar emission wavelengths and appear as one broad peak in the total emission profile. The variability in the spectral fitting software could be the cause of the differences in these low abundance species. In the case of the remaining two species [*(n,m) = (6,5)* and *(7,5)*], the only extract different from the others is the sediment matrix, which has a higher relative abundance of *(6,5)* and lower relative abundance of *(7,5)* compared to the other extracts. Although statistical analysis revealed differences in relative abundance of some SWNT species among extracts, there does not appear to have been any preferential ingestion of SWNT by organisms according to diameter or chiral wrapping angle.

3.3.3. ^{14}C-SWNT bioaccumulation studies with *L. plumulosus*

In the ^{14}C-SWNT bioaccumulation study, non-depurated *L. plumulosus* exposed to sediments and algae amended with ^{14}C-SWNT (Table B3) had significantly elevated body burdens with respect to the control treatment (control sediment + control algae; Figure 3.5). The presence of SWNT in the algae resulted in a significant increase of SWNT body burden by approximately a factor of five compared to the control treatment (non-depurated: 0.121 $\pm$ 0.012 μg ^{14}C-SWNT/g dry tissue; depurated: 0.104 $\pm$ 0.010 μg ^{14}C-SWNT/g dry tissue). However, it was the amended sediment exposures that had the most significant effect on SWNT body burden with a 50 fold increase in ^{14}C-SWNT signal relative to the control treatment. The presence of SWNT-amended algae did not further increase ^{14}C-SWNT uptake in amphipods in sediments previously amended with

SWNT (Figure 3.5). These findings suggest that the sediment-ingestion uptake route was more critical for SWNT accumulation in *L. plumulosus* than exposure via algae consumption. After a 24 hour depuration period, the body burdens of SWNT decreased and only the organisms from treatments which included the high ^{14}C-SWNT amended sediment (100 μg/g sediment + control algae, and 100 μg/g sediment + 100 μg/g algae) had significantly elevated body burdens (by about a factor of 5) (Figure 3.5). Although there were detectable levels of ^{14}C-SWNT in the amphipods, the overall bioaccumulation was low and, as noted above, treatments with SWNT amended algae were poor routes of exposure for SWNT. Bioaccumulation factors (BAF = [amphipod]/([sediment]+[algae]) were calculated for each treatment before and after depuration (Table 3.1). All calculated BAF values were below unity, and these decreased by about one order of magnitude after depuration. This data indicate that ^{14}C-SWNT were not bioaccumulative; instead, benthic organisms appear to take up SWNT through ingestion and then eliminate them promptly during depuration.

Evidence for ingestion and subsequent elimination of SWNT was obtained by the fecal pellet analysis. Fecal pellets in all ^{14}C-SWNT-amended treatments with the exception of the sediment + 100 μg/g algae treatment displayed significant radioactivity (Figure 3.6). This was expected since no measureable ^{14}C-SWNT body burden was detected before or after depuration in this treatment, and it is well established that *L. plumulosus* deposit feeds on sediments in addition to filter feeding on suspended

particulate matter [134-136]. Because the test was static, a 50% water renewal was performed on each feeding day (3x/week) prior to addition of algae. Measured subsamples indicated the presence of small amounts of ^{14}C-SWNT loss during the water renewals. The mass balance for ^{14}C-SWNT in the higher (100 ppm) sediment amendment treatments was approximately 40-65%. For four replicates, 8-15% was in the water, 34-54% was still in the sediment, and less than 1% was accounted for in the organism tissues and fecal pellets.

3.3.4. Summary

I have demonstrated that pristine SWNT and oxidized SWNT are not toxic to three important estuarine benthic invertebrates from the base of the marine food chain at the concentrations tested via sediment and food exposures. Further, once exposed to control sediment and food during depuration, any observed body burdens decreased significantly to BAFs well below unity, suggesting that SWNT that were ingested by invertebrates remained within the gut lumen and did not pass the gut epithelium. These results are consistent with other ecotoxicity studies evaluating the effects of carbon nanotubes on aquatic invertebrates [11, 53, 56-58, 125]. This suggests that once released into the environment, sediment-associated SWNT may be accumulated by benthic organisms but would be readily eliminated. Given these findings, SWNT appear to be bioaccessible from various environmental media but not truly bioavailable in that they do not appear to bioaccumulate and therefore do not cause adverse effects [137].

Furthermore, different organisms appear to have different uptake routes. I found *A. abdita* accumulated SWNT primarily via ingested algae whereas *L. plumulosus* accumulated SWNT via ingested sediment.

As I describe here, there was little evidence of the bioaccumulation of SWNT by organisms low in the benthic marine food chain. However, additional research is needed to investigate what occurs when such an organism with SWNT in its digestive tract is ingested by a predator. In one scenario, the SWNT may continue to behave inertly and be eliminated by the predator. Another scenario is that the predator's digestive tract is more effective at extracting the SWNT from the sediments and transferring them to the predator's tissues. The guts of some marine species have been shown to contain surfactants [138, 139] which may be similar to the surfactants used in this research (e.g., SDC, gum arabic) to extract SWNT from environmental media. In the second scenario, the presence of SWNT in the benthic environment could represent environmental risk to upper food chain organisms despite the lack of SWNT adverse effects and bioaccumulation at the base of the food chain. For example, this could have implications for human health if SWNT are capable of entering higher trophic levels that are relevant to human diets. No such evidence exists to date.

Table 3.1. Bioaccumulation factors (BAF) for single-walled carbon nanotubes (^{14}C-SWNT) in depurated and non-depurated *Leptocheirus plumulosus* tissues after a 28-day bioaccumulation study. BAF = [amphipod]/([sediment]+[algae]).

	Control sediment + 100 ppm algae	10 ppm sediment + 10 ppm algae	100 ppm sediment + control algae	100 ppm sediment + 100 ppm algae
Non-depurated BAF	0.013±0.002	0.054±0.005	0.068±0.016	0.051±0.005
Depurated BAF	0.0040±0.0008	0.0074±0.0012	0.0063±0.0004	0.0047±0.0008

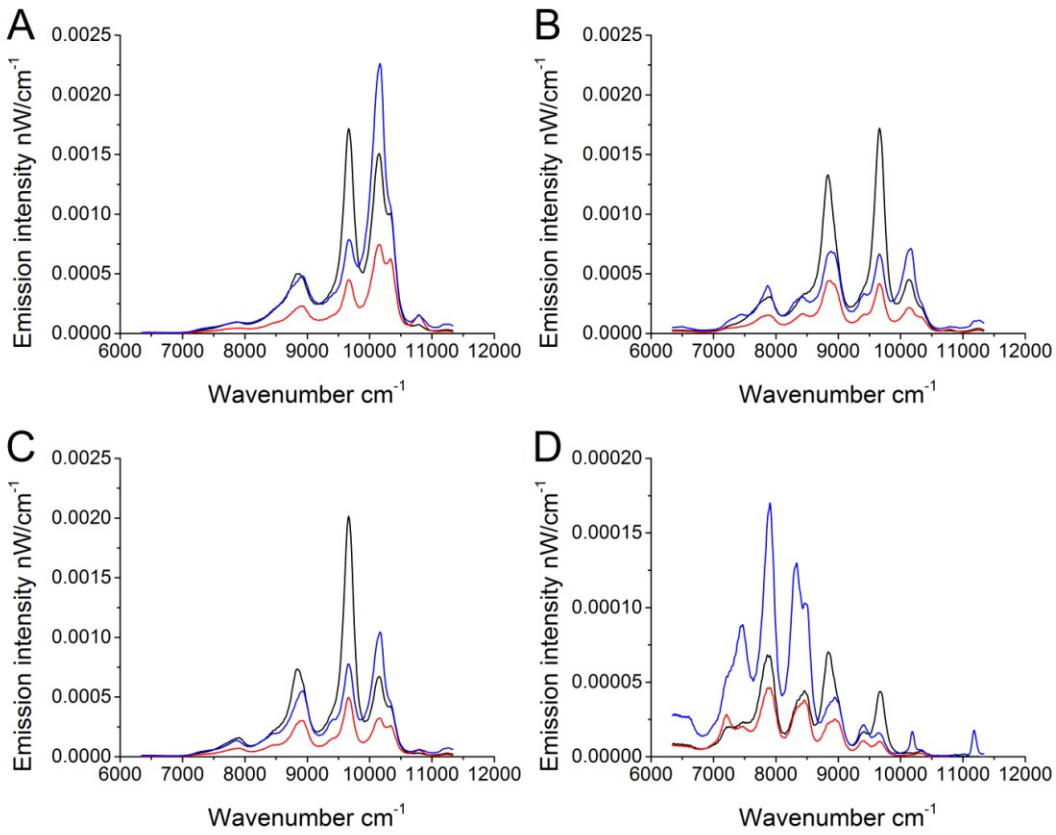


Figure 3.1. NIRF emission spectra for each single-walled carbon nanotube (SWNT) material used in the sediment toxicity testing: (a) SG65, (b) SG76, (c) CG100, and (d) OECD. The lines represent the emission spectra for each excitation wavelength: 638 nm (black), 691 nm (red), and 782 nm (blue).

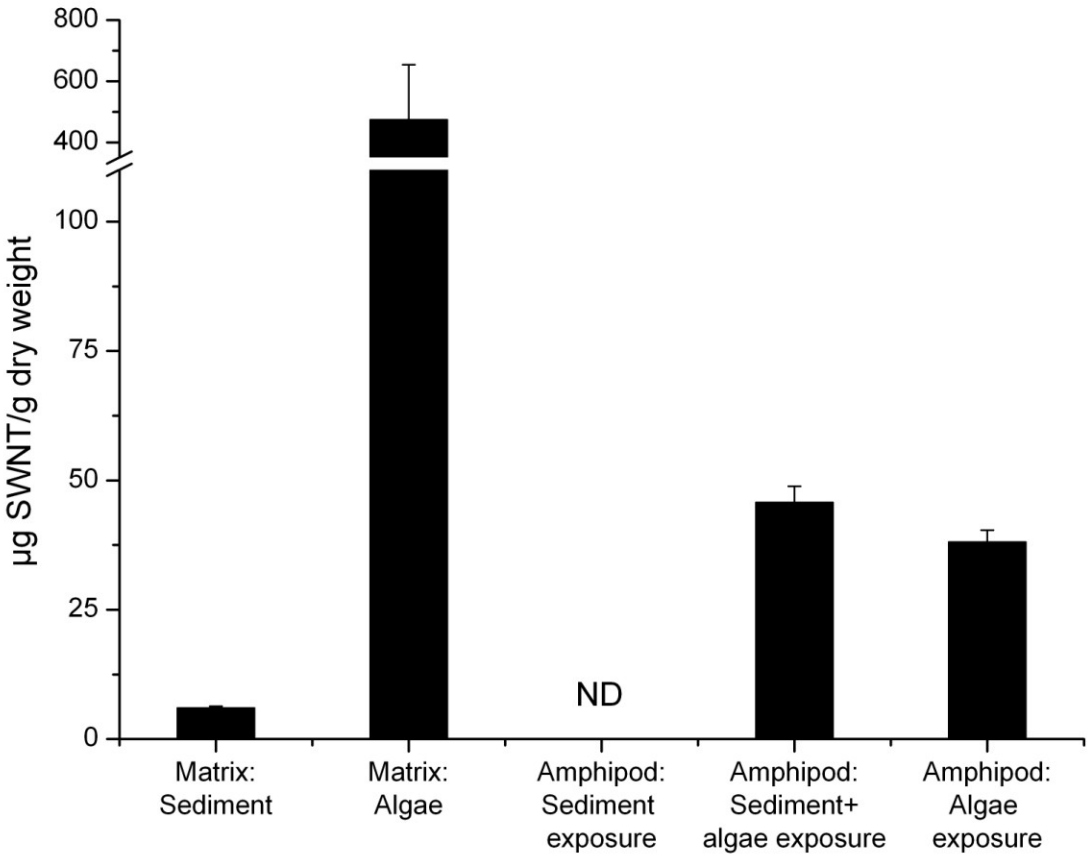


Figure 3.2. Measured concentration of SG65 single-walled carbon nanotube (SWNT) in the exposure matrices (sediment and algae), and in the non-depurated amphipods from each exposure (sediment, sediment+algae, and algae). All depurated amphipods were non-detects (ND). For each treatment and the sediment matrix, n=3. For the algae matrix, n=2.

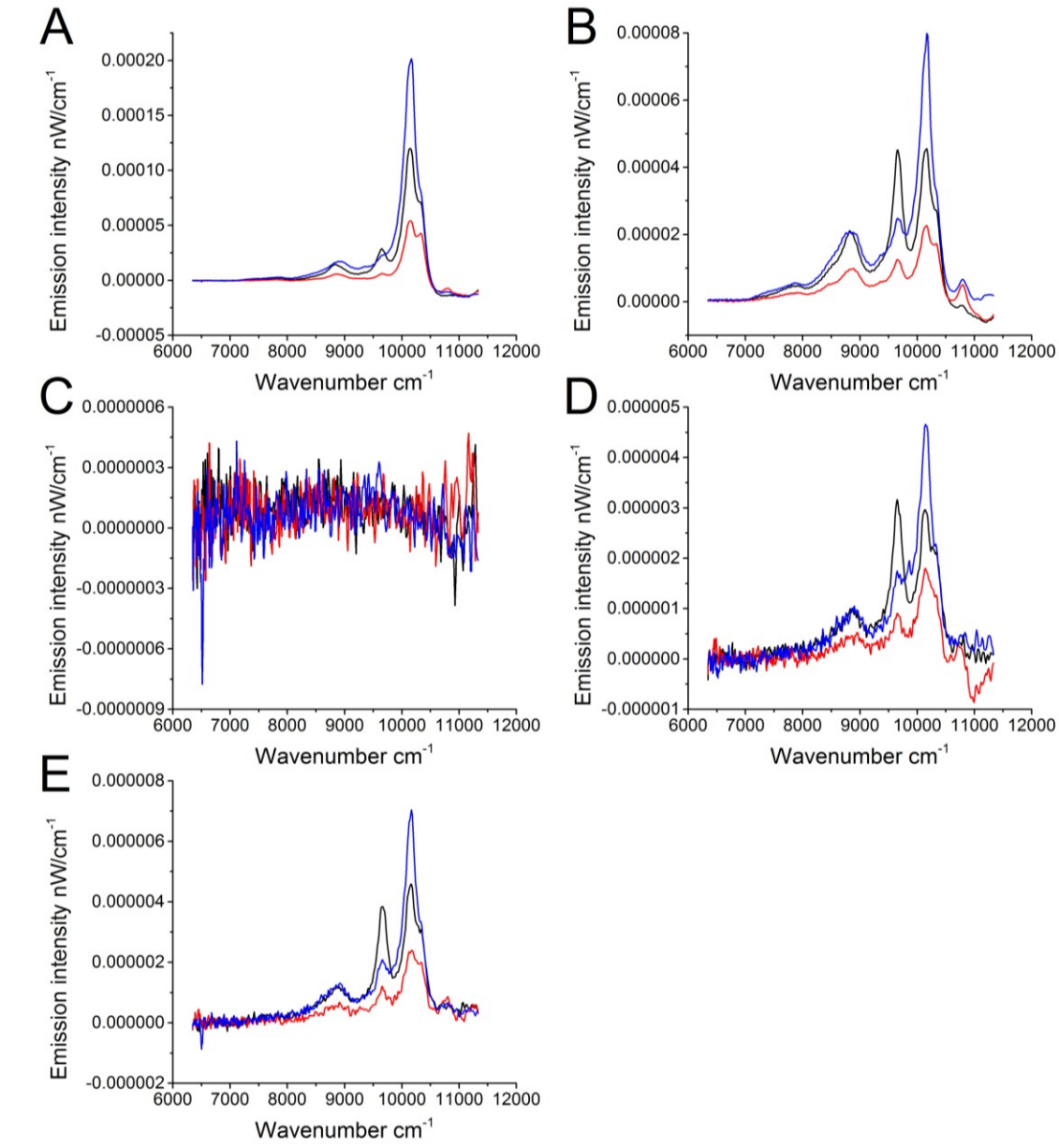


Figure 3.3. Representative NIRF emission spectra for single-walled carbon nanotube (SWNT) extracts of (a) sediment and (b) algae. Non-depurated amphipod extracts by exposure included (c) sediment exposure, (d) sediment and algae exposure, and (e) algae exposure. Analysis of all depurated amphipods in addition to all mysid samples resulted non-detects. The lines represent the emission spectra for each excitation wavelength: 638 nm (black), 691 nm (red), and 782 nm (blue).

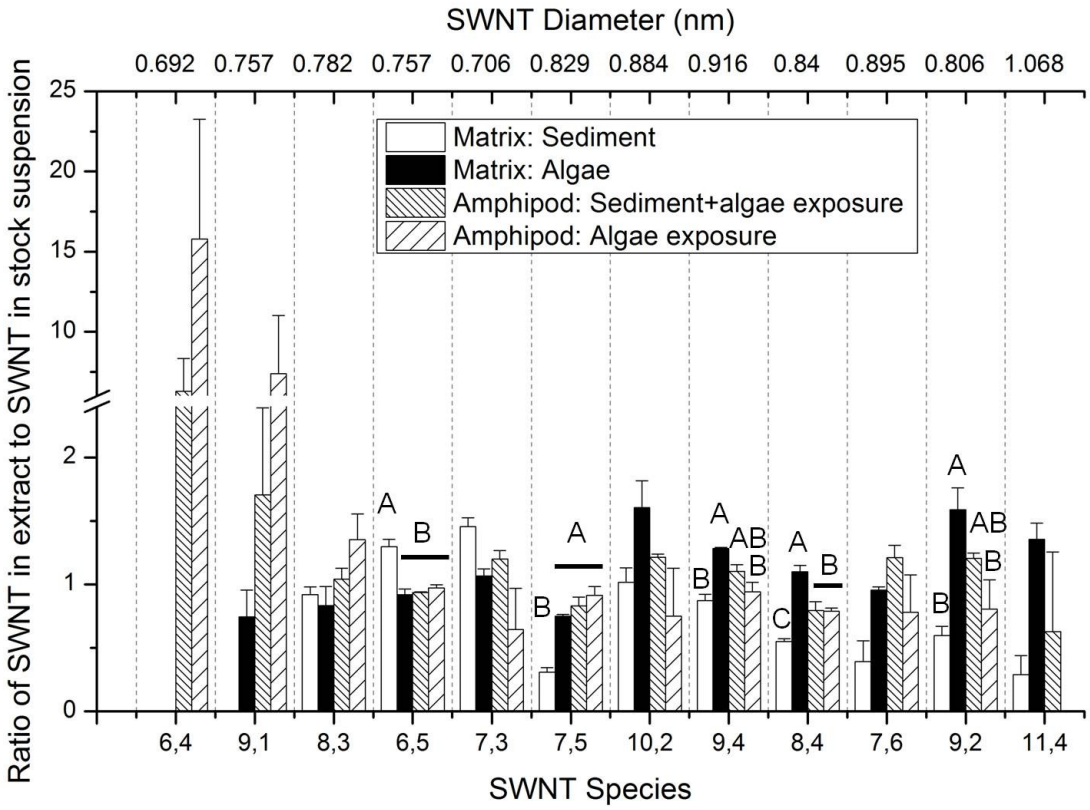


Figure 3.4. The ratio of the single-walled carbon nanotube (SWNT) species abundance (by SWNT diameter and chiral wrapping angle) in the respective matrix or organism extract to that in the SG65 SWNT suspension. Significant differences in relative abundance between extracts were determined within each SWNT species and are denoted by capital letters. Statistically different groups have a different letter. If a column does not appear it is because that SWNT species had a relative abundance less than 1% in that specific matrix and was not considered a major constituent of the mixture.

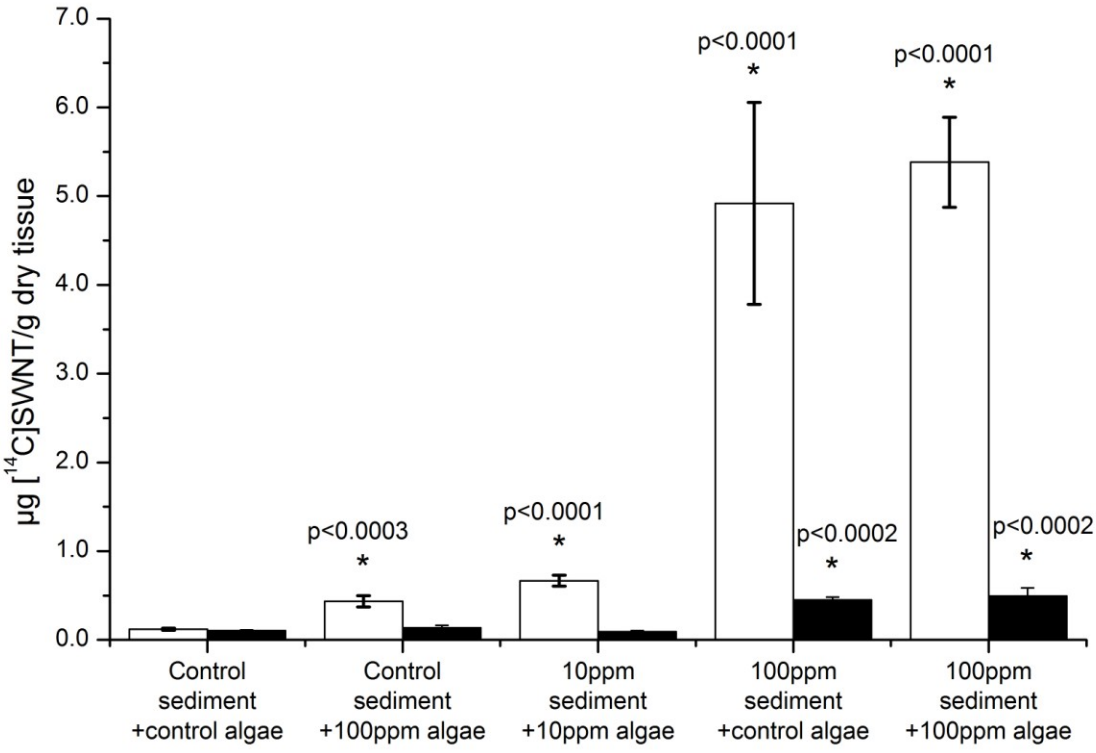


Figure 3.5. ^{14}C-Single-walled carbon nanotube (^{14}C-SWNT) body burdens in *L. plumulosus* at the end of a 28-day exposure in non-depurated organisms (open columns) and depurated organisms (filled columns). Each treatment has a sample size of n=3. P-values represent statistical differences (*) from the control sediment + control algae control.

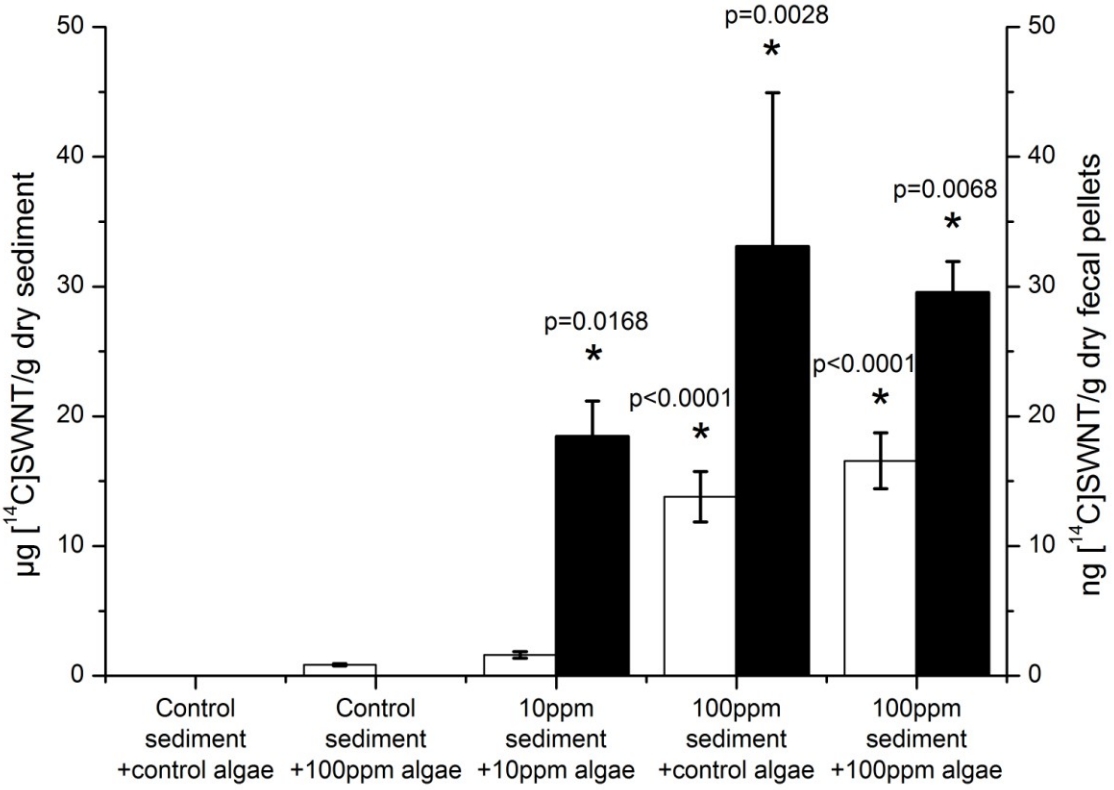


Figure 3.6. Concentration of ^{14}C-Single-walled carbon nanotube (^{14}C-SWNT) present in the sediment upon test completion (open columns, left y-axis) and in fecal pellets after a 24 hour depuration in control sediment (filled columns, right y-axis). Each treatment has a sample size of n=3. P-values represent statistical differences (*) from the control.

4. Effects of Single-walled Carbon Nanotubes on the Bioavailability of PCBs in Environmentally Contaminated Sediments

4.1. Introduction

Single-walled carbon nanotubes (SWNT) have unique physical, electronic, and optical properties allowing for a wide range of commercial applications such as nanoelectronics, structural composites, and drug delivery vehicles [42]. With continued research and development comes increased commercial production of SWNT and risk of their release into the environment. Pristine SWNT are highly hydrophobic, and have been shown to form homo- and hetero-aggregates in natural systems such as estuarine waters [63]. These aggregates eventually settle into sediments where they will likely remain and interact with other hydrophobic contaminants that may be present. In laboratory systems, research to date has shown that SWNT exhibit strong adsorption affinities to various hydrophobic organic contaminants (HOCs) [64-68, 70]. How SWNT will interact with these contaminants and affect their fate and bioavailability in the environment is not well understood.

Because pristine SWNT consist entirely of sp^2-hybridized carbon (being essentially rolled sheets of graphene), these materials represent a highly ordered and discrete form of black carbon. The extensive research on adsorption of organic contaminants to other forms of black carbon (e.g., soot, char) may provide mechanistic insight on the interactions of such compounds with SWNT. Previous studies have

shown strong sorption of HOCs to various forms of black carbon in both laboratory and field studies, resulting in reduced freely-dissolved concentrations and bioavailability of HOCs in aquatic organisms [75, 82, 83]. The degree of adsorption efficacy is dependent upon black carbon type as well as the physical characteristics of the HOCs such as log K_{OW} and planarity [75, 83, 88, 91]. Bioaccumulation of HOCs in the presence of black carbon is also dependent on the physiology of test organisms [12, 72] and other external factors such as aging (i.e., how long an HOC has been in the environment) and the presence of other competing forms of carbon such as natural organic matter (NOM) [86, 87]. The environmental factors that affect HOC bioaccumulation in the presence of traditional forms of black carbon were also observed to be a factor in similar experiments using SWNT as the black carbon source [12, 65, 66, 68, 70]. For example, Zhang et al. [66] found that the addition of humic acid (HA) to a suspension of SWNT and pyrene decreased the total amount of pyrene sorbed to SWNT, but the amount of pyrene sorbed to SWNT was nearly twice the amount sorbed to HA alone. This supports previous research illustrating that anthropogenic black carbon (i.e., from burning fossil fuels and biomass) is a more efficient sorbent than natural amorphous carbon sources [88]. As mentioned previously [72], similar organism-specific and black carbon-specific bioavailability of HOCs was observed for SWNT by Ferguson et al. [12]. When comparing the effect of SWNT and standard reference material (SRM) 2975 diesel particulate matter (National Institute of Standards and Technology) on the

bioavailability of HOCs to two different benthic invertebrates, these researchers found that neither black carbon source decreased the HOC bioavailability for the meiobenthic copepod *Amphiascus tenuiremis*, but SWNT decreased the HOC bioavailability to the deposit/suspension feeding polychaete *Streblospio benedicti*, while diesel soot increased bioavailability of HOCs [12].

The promise of black carbon as a media that reduces HOC bioavailability to terrestrial and aquatic deposit feeders has resulted in exploration of its use in environmental remediation as an *in situ* sediment amendment. Hilber and Bucheli [84] and Rakowska et al. [78] have reviewed the literature investigating the effectiveness of black carbon amendment to contaminated sediment *in situ* and studies [79-81, 140] have demonstrated the promise of black carbon amendment in certain cases for reducing bioavailability of HOCs in sediment. Results from these field studies suggest that the important factors for reducing the bioavailability of sediment-bound organic contaminants include the method of application and mixing, activated carbon dosage and properties (e.g. particle size, surface area, and pore size), sediment properties, and HOC properties [78, 84]. As a form of black carbon, SWNT may behave similarly with respect to contaminant sorption once released into the environment. Although the body of research on SWNT and HOCs is progressing, more work needs to be performed to assess the impact of SWNT as a co-contaminant and how its presence will affect the bioavailability of native HOCs to various benthic organisms. It is necessary to determine

how SWNT will interact with HOCs in environmental media compared to other black carbon sources (e.g., activated carbon) to determine if there will be a "nanotube" specific effect or if SWNT can be considered a general form of black carbon. Given these concerns, the objectives of my research were to determine if SWNT amendment to field-collected, HOC-contaminated New Bedford Harbor (NBH) sediment would decrease the bioavailability, bioaccumulation and toxicity of sediment-associated polychlorinated biphenyls (PCBs) to benthic invertebrates, and to compare the effects of SWNT as a sorbent versus activated carbon (i.e., coconut charcoal (CC)). Toxicity was assessed in two benthic marine invertebrates including the filter-feeding amphipod *Ampelisca abdita*, and the mysid *Americamysis bahia*. Further, bioaccumulation of PCBs was also evaluated using the polychaete, *Nereis virens*. The results generated by this study will be useful for performing environmental risk assessments of SWNT in the presence of HOCs and whether or not they will mitigate the toxicity and bioaccumulation of co-contaminants or act as a vector, increasing co-contaminant bioavailability.

4.2. Materials and methods

4.2.1. New Bedford Harbor sediment preparation and amendment

New Bedford Harbor (NBH) sediment was collected from the Superfund site in April 1994 (MA, USA). Dilutions of NBH sediment ranging from 0 to 100% were prepared using Long Island Sound (LIS) sediment (NY, USA) collected in February 1998 and April 2005 as "uncontaminated" diluents sediment. LIS reference sediment contains

1.8% organic carbon and 92% silt and clay-sized particles [94]. Two different black carbon sources were used for amendments: CoMoCAT single-walled carbon nanotubes (SG65, surface area = 630 m^2/g) from SouthWest NanoTechnologies, Inc (SWeNT) and Calgon powdered coconut charcoal (CC, surface area = 1100 m^2/g [141]). This CC has been commonly applied to toxicity identification evaluations (TIEs) to reduce the bioavailability of organic contaminants [142, 143]. Prior to use, powdered CC was deaerated in DI water under vacuum and then centrifuged to remove excess DI water [142]. Materials from SWeNT are well-characterized, high purity mixtures of SWNT [126-129], which I have previously characterized in great detail (Chapter 2 and 3). A 1 mg SWNT/mL suspension was prepared in 0.5% w/v gum arabic (GA) solution using high power sonication at 50% amplitude for 20 minutes (2x10minutes) in a salt-water ice bath (Branson 450D Sonifier). Both forms of black carbon were added to the sediment at 1 mg/g and 10 mg/g dry weight. Sediment was manually mixed upon amendment in 4-liter glass jars and allowed to mix on a rolling jar mill at 4 °C in the dark for fourteen days. After this time, the sediment was equilibrated at 4 °C in the dark for 11 months. An additional treatment containing 10 mg/g SG65 was added to the 25% NBH sediment group. This sediment was amended one month prior to the start of the test and stored at 4 °C in the dark. Prior to use, any extra overlying water was decanted.

4.2.2. Analysis of SWNT

The concentration of SWNT in the amended sediment was determined by near infrared fluorescence spectroscopy (NIRF) to verify nominal concentrations as described in Chapter 2 and 3 and published in the literature [103] and [104]. Sediment subsamples were washed with deionized water twice by vortexing and centrifuging the sample for 10 minutes at 13,520 x g and 20 °C (Eppendorf centrifuge 5804 R). The pellet was then sonicated (Branson 450D sonifier with a 3 mm tapered microtip) for 10 minutes at 50 % amplitude in 2.5 mL 2% w/v sodium deoxycholate (SDC). This step was repeated three times and each supernatant was collected. Equal aliquots of each supernatant were combined and the near infrared fluorescence was quantified using a NanoSpectralyzer 1 (NS1, Applied NanoFluorescence).

4.2.3. Sediment characterization

Total organic carbon (TOC) and black carbon (BC) content of the amended sediments was determined following the method described by Perron et al. [144-146]. Briefly, sediment samples were dried at 60 °C, ground, and stored in muffled glass vials. Subsamples for total organic carbon analysis were acidified with 1 M hydrochloric acid and dried at room temperature. For black carbon analysis, dried sediment was pressed through a 425 μm sieve, weighed into crucibles, and combusted at 375 °C for 24 hours. Subsamples of combusted sediment were weighed, acidified with 1 M hydrochloric acid,

and allowed to dry at room temperature. All TOC and BC samples were analyzed using a CHNS-O analyzer (Thermo Finnigan Flash EA 1112 Series).

4.2.4. Sediment toxicity testing

A seven day static toxicity test was performed following the methods described by Ho et al. [130] using test organisms *Americamysis bahia* (laboratory-cultured mysid) and *Ampelisca abdita* (field-collected amphipod). Test chambers contained 20 g wet sediment and 60 mL of 30‰ reconstituted seawater (RSW) prepared by diluting 100% brine with deionized water. Chambers were static and aerated in a 20 °C incubator with a light cycle of 16h:8h light:dark. All treatments were tested for toxicity. After 24 hours of equilibration, ten individuals of each organism (mysids and amphipods) were added to the test chambers and each treatment had three replicates with the exception of 100% NBH sediment (n = 2). On day 7, organisms were sieved (0.5 mm) from the sediment and counted to determine survival. Missing organisms were scored as mortalities. Dissolved oxygen (DO), pH, salinity and temperature were monitored during the test.

4.2.5. Passive sampler preparation

To determine the freely dissolved interstitial water (ITW) sediment concentrations of PCBs in the bioaccumulation study, passive sampling was employed. Passive sampling was performed using polyethylene (PE) [147, 148]. The PE sheeting was cut into one cm by one cm squares and the average mass determined. PE sampler thickness was 25 μm. Samplers were pre-extracted for 24 hours in hexane followed by 24

hours in methylene chloride. Samplers were soaked in Milli-Q water for 6-7 hours prior to transfer into new jars containing a performance reference compound (PRC) mixture. The PRC mixture contained the following deuterated polycylic aromatic hydrocarbons: d_{10}-phenanthrene, d_{12}-benzo[a]pyrene, and d_{14}-dibenz[a,h,]anthracene. Passive samplers were equilibrated with the PRC mixture in 80:20 methanol:Milli-Q water for 13 days on an orbital shaker. Following loading, PE were removed from the PRC solution, rinsed with nanopure water, dried with laboratory tissue, placed in a clean glass jar, and stored at 4ºC in the dark until deployment in the bioaccumulation study approximately 48 hours later.

4.2.6. Bioaccumulation study with *Nereis virens*

A 28-day flow through bioaccumulation study was conducted with the benthic polychaete *Nereis virens* (Aquatic Research Organisms) following guidance in U.S. EPA standard methods [149]. Exposures were performed in 1-L beakers which contained 200 mL sediment. A coil of mesh screening was placed inside the beaker's top edge to allow water to flow-out while preventing the polychaete from escaping the test chamber. The flow through setup included the use of a siphoning gravity diluter which distributed seawater into each chamber via an open-ended glass culture tube attached to the mesh screening. The average daily flow-rate over the course of the experiment was 6.5 mL/min for approximately 9.4 volumetric turnovers per day. The sediment in each chamber was equilibrated with the flow-through system for 7 days (day –7) prior to the

addition of organisms on day 0. One passive sampler (PE) was added to every chamber on day -1, and subsamples (n=2) were also collected. The passive samplers were inserted using forceps, and were placed approximately 3 cm from the side of the beaker to half the depth of the sediment (approximately 5-6 cm). Each treatment was conducted with four replicates with the exception of 100% NBH sediment and 100% NBH sediment+1mg CC/g dry sediment (n = 2). All chambers contained one polychaete with an initial mass of 1.5-8.8 g wet weight. Organisms were not fed during the exposure. Exposures were performed at 20 °C and 30 ‰ filtered Narragansett Bay seawater with a 18:6 h light:dark cycle and continuous aeration. At test initiation, a sediment subsample was taken from each treatment for SWNT and PCB quantification. After the 28-day exposure, organisms were removed by sieving through a 1 mm stainless steel sieve. Organisms were then placed in a 400 mL aerated, static depuration chamber (one organism per chamber) which contained 40 g LIS sediment and 150 mL of filtered Narragansett Bay seawater. After 24 hours, organisms were sieved out of the LIS sediment, rinsed with seawater, weighed and frozen at -4 °C until extracted for PCB and lipid content.

4.2.7. Sediment, tissue, and passive sampler extractions

The extraction method utilized in this research follows the method described by Friedman et al. [147]. Sediment was oven dried at 60 °C for 24 hours to determine the wet:dry mass ratio. Worm tissues were defrosted and one small section was removed for wet:dry mass ratio determination. Internal standard (IS; CB-198, 1.1 ng/μL) was added

to approximately 1 g of each dried sediment and the remaining worm tissue. For all tissue and sediment samples, 25 uL IS was added to control samples (LIS sediment Day 0 and LIS sediment with and without amendments), and 100 uL IS was added to NBH sediment samples. Sodium sulfate (baked for 4 hours at 700 °C) was added and the sediment or tissue homogenized with a glass rod. Samples were extracted three times with 1:1 acetone:hexane for a final volume of approximately 100 mL. After each addition of solvent, samples were sonicated for 20 minutes at 40 °C in a bath sonicator (Branson 3510) and centrifuged for 15 minutes at 800 x g and 4 °C (Thermo Scientific Sorvall RC 6+, F20S-6 x 100 rotor). Sodium sulfate was added to the composited extracts which were then stored in the dark at 4 °C for a minimum of 18 hours. Extracts underwent solvent exchange to hexane and the volume was reduced to 5 mL using a Zymark TurboVap II. For the worm extracts, a 0.5 mL subsample was taken for gravimetric lipid content determination. The remaining worm extract (~4.5 mL) and sediment extract (~5 mL) were run through silica cartridges (Waters Sep-Pak Vac Silica 6 mL). These columns were prepared by adding approximately one centimeter of sodium sulfate and activating with 9:1 hexane:dichloromethane (DCM) (3-5 mL). After activation, the extracts were loaded and eluted with three volumes of the same solvent mixture (12-15 mL total). The eluted extracts were solvent exchanged to hexane, volume reduced to 1 mL, and stored at -4 °C in the dark. Prior to analysis, samples were acidified with concentrated H_2SO_4 to oxidize any interfering organic matter. Sediment samples were

also treated with activated copper to remove sulfides. Based on the generated data, biota-sediment accumulation factors (BSAFs) were calculated as follows:

$$BSAF = \frac{C_L}{C_{OC}} \qquad [1]$$

where, C_L is the lipid normalized tissue concentration (μg/g lipid) of a given PCB congener and C_{OC} is the organic carbon normalized sediment concentration (μg/g OC) of a given PCB congener.

After removal from the sediments by sieving, the passive samplers were gently wiped to remove excess water or sediment and transferred to 20 mL glass scintillation vials for extraction. PAH IS (20 μL, 50 ng/μL) and CB-198 IS (25 μL to blanks, time 0, and LIS; 75 μL to NBH; 1.1 ng/μL) were added to each sampler. Samplers were extracted on an orbital shaker at room temperature in 10 mL hexane for 24 hours followed by 10 mL methylene chloride for 24 hours. Hexane and methylene chloride extracts were combined, solvent exchanged to hexane, volume reduced to 1 mL, and stored at -4 °C in the dark.

4.2.8. Analytical methods

PCB concentrations were determined in sediments, tissues, and passive samplers following the methods described in Friedman et al. [147]. An Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector operated in select ion monitoring mode was used for the PCB analysis of twenty-six PCB congeners (CBs 8,

18, 28, 44, 52, 66, 70, 77, 81, 99, 101, 105, 110, 114, 118, 123, 126, 138, 153, 156, 157, 169, 170, 180, 189, and 206). Performance reference compounds (PRCs) concentrations in passive samplers were determined using the same instrumentation. Tissue, sediment, and passive sampler data were corrected for Day 0 by subtraction of blank concentration values.

4.2.9. Analysis of passive sampler data to calculate freely dissolved PCB concentration

Exchange rate coefficients (k_e, day^{-1}) were determined for each PAH PRC in each treatment with the following equation:

$$k_e = \ln \frac{C_{PE,i}}{C_{PE,t}} \; x \; t^{-1} \qquad [2]$$

Where $C_{PE,i}$ is the concentration of the PRC in the initial passive samplers (ng/g passive sampler), t is the duration of the exposure in days (28), and $C_{PE,t}$ is the concentration of the PRC in the passive samplers collected from the sediment at the conclusion of the experiment, after 28 days of equilibration (ng/g passive sampler). $C_{PE,i}$ and $C_{PE,t}$ were corrected by subtraction for concentrations of each PRC in the passive samplers collected as blanks. Exchange rate coefficients were plotted against log K_{ow} (octanol-water partition coefficient; L/kg) [150, 151] for the three PRCs within a given treatment. Exponential trend line equations were then calculated for each treatment. The corresponding exchange rate coefficients for each target PCB congener within the treatment were calculated using the known log K_{ow} for that PCB congener. Using k_e for

each PCB congener in each treatment, equilibrium concentrations ($C_{PS,\infty}$; ng/g passive sampler) in all PE replicates were calculated with the following equation:

$$C_{PE,\infty} = \frac{C_{PE,t}}{1-e^{-k_e t}} \quad [3]$$

where $C_{PE,t}$ is the PCB congener concentration in the passive sampler at day t (t=28) (ng/g passive sampler), corrected for initial concentrations. Equilibrium concentrations in PE were used to calculate estimated freely dissolved interstitial water concentrations ($C_{free,PE}$; ng/L) in each replicate chamber with the following equation:

$$C_{free,PE} = \frac{C_{PE,\infty}}{K_{PE-w}} \quad [4]$$

with passive sampler-water partition coefficient values (K_{PE-w}) for PE for each PCB congener from Perron et al. (*submitted*) [148].

4.2.10. Statistical analysis

Statistical analysis was performed using JMP® Pro 10.0.0. One-way analysis of variance (ANOVA, α=0.05) was performed to determine statistically significant differences in toxicity, bioaccumulation and interstitial water concentration data. Toxicity data were transformed by arcsine square root, and bioaccumulation data were normalized by lipid mass and total organic carbon. Upon determination of significant differences in ANOVA, Dunnett's t-test (α=0.05) was used to determine any treatment-

specific significant differences from the controls. Unless otherwise noted, data are reported as average ± standard deviation for a sample of n = 3.

4.3. Results and discussion

4.3.1. Sediment amendment and characterization

Based on sediment extracts, the measured SWNT concentrations for all SWNT-amended sediments were approximately 50% of the nominal concentrations except the 75% NBH sediment amendment which was approximately 83% of the nominal concentration (Table 4.1). As expected, SWNT- and CC-amended sediments had a higher total organic carbon content than the unamended control with the exception of 75% NBH sediment + 1 mg/g SWNT, and 100% NBH sediment + 1 mg/g CC (Table 4.1). Black carbon content was higher in the black carbon-amended 25% NBH sediment treatments, but this trend was not seen in the 50% NBH sediment, 75% NBH sediment and 100% NBH sediment treatments (Table 4.1). Because of the elevated concentrations of BC already present in NBH sediments and limited sensitivity of the BC analysis, the addition of black carbon content by SWNT- and CC-amendment may not have been sufficient to statistically change the overall black carbon content of the higher % NBH sediment treatments.

4.3.2. Effects of carbon amendment on sediment toxicity

The effectiveness of the black carbon additions in reducing sediment toxicity varied with organism and % NBH sediment dilution (Figure 4.1). Overall, *A. bahia* was

more sensitive to the toxic effects of the NBH sediment than *A. abdita*. In the LIS sediment (0% NBH sediment) treatment, survival of *A. bahia* ranged from 75% to 100% and was significantly increased in the CC treatment (p=0.04). One LIS sediment replicate had low survival for both the mysid (20%) and the amphipod (40%), and was excluded based on its anomalous behavior. Previous TIE studies utilizing LIS sediment as the control sediment show consistently high survival in both organisms [130, 142, 144, 152]. For the 25% NBH sediment treatment, survival ranged from 13-83% with only 47% survival in the unamended control and the highest survival in the 1 mg SG65/g dry sediment and 10 mg SG65/g dry sediment (73±15% and 83±12%, respectively). The 50% NBH sediment treatment led to 0% survival, but when amended with 1 mg SG65/g dry sediment, survival significantly increased to 50% (p=0.0015). There was no survival with *A bahia* in either of the 75% NBH sediment and 100% NBH sediment treatment groups even with the black carbon amendments.

Amphipod *A. abdita* survival was 100% in the LIS sediment treatment (Figure 4.1). The effect of CC in the LIS sediment treatment group was opposite for *A. abdita* compared with *A. bahia*. A significant reduction in survival (p=0.04, 80±10% survival) was observed. A similar trend in survival between organisms was observed for the 25% NBH sediment group ranging from 43-100% with survival in the unamended control at 77% and the highest survival in the 1 mg SG65/g dry sediment amendment (p=0.05, 100±0% survival). No significant differences were found in the 50% NBH sediment and

75% NBH sediment treatment groups due to high variability; however, there was an increased survival trend in the SWNT amended sediment as compared to the non-amended control; for example, the presence of SWNT increased survival from 50% to 80% in the 50% NBH sediment treatment group, and increased survival from 10% to 50% in the 75% NBH sediment treatment group (Figure 4.1B). There was no survival in the 100% NBH sediment treatments, regardless of black carbon amendment. The trend of improved survival by both organisms when black carbon is present, specifically SWNT, is consistent with prior findings using other forms of black carbon [142]. The mechanism of action for reduced toxicity is likely driven by the decreased bioavailability of the PCBs as they sorb to SWNT or CC [71, 72, 75, 77, 142, 152, 153]. This hypothesis was further investigated by assessing the bioaccumulation of PCBs in both a sediment-dwelling organism and PE passive sampler.

4.3.3. Effects of carbon amendment on PCB bioaccumulation

The survival of the marine polychaete, *N. virens*, over a 28 day period was not negatively impacted by the presence of carbon amendment. In the LIS sediment treatments, survival was 100%. For the 25%, 50% and 100% NBH sediment treatments, survival ranged from 50-100%, while the survival was 75% for both 75% NBH sediment with and without SWNT present. In some cases, the presence of SWNT appeared to improve survival (e.g., 100% NBH sediment + 1 mg/g SWNT) and in others seemed to reduce survival (e.g., 25% + 10 mg/g SWNT). Because of the design of the polychaete

exposures (focusing on a bioaccumulation assay and not a toxicity assessment), further analysis of treatment effects on survival was not viable.

PCB bioaccumulation in *N. virens* was normalized to sediment PCB concentration and is represented as the biota-sediment accumulation factor (BSAF). For most treatments, total PCB BSAFs were reduced in the presence of SWNT relative to treatments that were not amended with any black carbon (Figure 4.2). The addition of 10 mg SWNT/g dry sediment to 25% NBH sediment led to the most dramatic reduction in total PCB bioaccumulation of 26 congeners (Figure 4.2A) while the presence of 1 mg SWNT/g dry sediment in 25%, 50% and 100% NBH sediment treatments led to decreased PCB BSAF, but the effect between sediment treatments was not of the same magnitude, most likely related to the difference in mass of SWNT amended to the sediment. Unexpectedly, this reduction trend was not observed in the 75% NBH sediment treatments. The same overall trends in total PCB BSAF (Figure 4.2A) is reflected in the sum of 11 (out of 12) dioxin-like PCBs identified by the World Health Organization (WHO) as PCBs of concern to human health (i.e., CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 169, and 189) (Figure 4.2B), and the three non-ortho substituted dioxin-like (toxic) PCBs [154](CBs 77, 126, and 169) (Figure 4.2C). The effect of CC addition to the various NBH sediment dilutions on PCB BSAF (i.e. total, WHO and toxic) is also illustrated in Figure 4.2. Total PCB BSAF is decreased in the presence of 10 mg/g CC but the effect was not significant. WHO and toxic PCB BSAF did not change

with addition of 10 mg/g CC to 25% NBH sediment. However, in the 100% NBH sediment treatment group, the 1 mg/g CC addition led to a lower BSAF for total, WHO and toxic PCBs, but this relationship could not be statistically tested due to low sample size (n=1).

For a given concentration of SWNT or CC, SWNT was generally more effective at reducing the BSAF (e.g. 25% NBH sediment + 10 mg/g SWNT or CC; Figure 4.3 and 4.4). To illustrate the variability in amendment effect across congeners, Figure 4.3 illustrates the observed BSAF values for representative PCB congeners according to chlorine substitution for the 25% NBH sediment treatment. No significant differences were determined for the 50% NBH sediment and 75% NBH sediment treatments, whereas significance could not be tested in the 100% NBH sediment treatment due to a survival-limited sample size (n = 1 for 100% NBH sediment and 100% NBH sediment + 1mg/g CC). A significant difference across the 25% NBH sediment treatment was determined for CB-8 (ANOVA, p=0.0424, Figure 4.3A) and CB-189 (ANOVA, p=0.0344, Figure C1B), but the high variability masked any specific difference in amendment from control. However, the BSAF for CB-18 was significantly reduced for both the 10 mg/g SWNT (p=0.0194, Figure 4.3B) and 10 mg/g CC amendment (p=0.0056, Figure 4.3B) while the BSAF for CB-28 was significantly reduced for only the 10 mg/g CC amendment (p=0.0490, Figure C1A). In addition to reduced bioavailability, the 10 mg/g CC amendment significantly increased the BSAF for CB-206 (p=0.0112, Figure C1C).

For all PCB congeners, 10 mg/g SWNT reduced the average BSAF compared to the unamended treatment (Figure 4.4). The 1 mg/g SWNT reduced average BSAF in approximately 68% of congeners. Due to high variability, there are few significant differences for each congener in the treatments compared to control, but the overall trend is clearly represented by the average values (Figure 4.4). Overall, the 1mg/g SWNT did not effectively reduce PCB bioavailability, but the 10 mg/g SWNT amendment was much more effective.

The observed decrease in PCB bioaccumulation was consistent across the full range of PCB Log K_{OW} in the 10 mg/g SWNT amendment treatment, suggesting that SWNT effectively reduced PCB bioavailability across all congeners (Figure 4.4); whereas the 1 mg/g SWNT amendment did not alter the bioaccumulation of PCBs in polychaetes, regardless of PCB Log K_{OW} (Figure 4.4B). In contrast, the 10 mg/g CC amendment behaved differently from either SWNT amendment. As the PCB K_{OW} increased, the effectiveness of the CC to reduce the bioavailability diminished and eventually led to slightly higher PCB bioaccumulation than in the unamended sediment (Figure 4.3 D-F and 4.4B). It is possible that the CC did not bind all the PCB congeners to the same extent, thereby increasing the rate at which the higher K_{OW} congeners partition into the interstitial water compared to the PCBs in the SWNT amendment. It is also conceivable that organism-specific factors such as selective ingestion or gut physiology may have influenced the BSAFs measured in the present study, thus convoluting interpretation of

BC amendment to sediment on bioavailability of PCBs. These results are similar to previous work in my laboratory where the bioavailability of PAHs to the polychaete *Streblospio benedicti* was reduced by SWNT but increased by soot [12]. In order to more completely understand the impact of SWNT- and CC-amendment to contaminated sediments, I have used sorptive sediment interstitial water (ITW) passive samplers to selectively sample and predict concentrations of PCBs in ITW.

4.3.4. Effects of carbon amendment on interstitial water PCB concentrations

Polyethylene passive samplers were used to determine the freely dissolved concentrations of PCBs in the ITW of the system, providing an estimate for the bioavailable concentration [147, 148]. ITW PCB concentrations were reduced in the presence of both SWNT and CC for all treatments except CB-170, which was significantly increased ($p<0.05$) in the 25% NBH sediment + 1mg/g SWNT and 25% NBH sediment + 10mg/g CC amendments, and CB-28, -52, -66, -99, -110, and -118 75% NBH in the 75% NBH sediment + 1mg/g SWNT amendment (Table 4.2, Table C1). At 25% NBH sediment, both SWNT and CC reduce ITW concentration by 92% and 95%, respectively. However, at 100% NBH sediment, SWNT reduces the ITW concentration by a factor of two greater than CC at 49% and 25%, respectively (Table 4.2). This data suggests that SWNT is more efficient than CC at adsorbing PCBs at higher sediment concentrations. As mentioned previously, the black carbon characteristics such as surface area, pore size,

and particle size play an important role in its ability to reduce organic contaminant bioavailability [78, 84].

The trends observed in ITW PCB concentration reduction are consistent with the trends in PCB bioaccumulation by *N. virens*. There was an overall trend of decreasing bioaccumulation with SWNT present, with the exception of 75% NBH sediment. The largest reduction in ITW PCB concentration by SWNT occurred in the 25% NBH sediment + 10 mg/g SWNT treatment and corresponds with the notable decrease in PCB BSAF by *N. virens* (Figure 4.2A). The PCB concentrations measured in the PE passive samplers support the observed trends in PCB bioaccumulation and may be a suitable technique for field testing the effectiveness of activated carbon applied in the field for remediation as also shown by Oen et al. [86].

When the PCB bioaccumulation in *N. virens* is normalized to the ITW concentration (bioconcentration factor, BCF), it is apparent that the mechanism of reduced bioaccumulation by SWNT amendment is due solely to reduced ITW concentration (Figure 4.5). This conclusion is illustrated by the same pattern of data in unamended sediment and sediment amended with both 1 mg/g SWNT and 10 mg/g SWNT (Figure 4.5). However, the data from sediment amended with 10 mg/g CC deviates from the pattern. The BCF for PCB congeners in the 25% NBH sediment + 10 mg/g CC is larger than the control and SWNT amended sediments (Figure 4.5, green diamonds) suggesting CC interacts with the PCBs in NBH sediment by a different

mechanism than SWNT. This could be due in part to differences in black carbon surface area. The surface area of the CC was approximately two times that of the SWNT. It is possible that CC interacts with sediment differently than SWNT, and, along with the higher surface area of CC, the equilibrium partitioning of the PCBs was affected such that a higher concentration was equilibrated onto the CC and subsequently into the ITW, accounting for the higher PCB ITW concentration in the 100% NBH sediment + 1 mg/g CC treatment. The surface area was determined using Brunauer-Emmett-Teller (BET) theory which is based on the adsorption of gas molecules to the surface of the material. This method could be further improved to better simulate the available surface area to specific molecules of interest by changing which gas is used. By using larger gas molecules, the surface area that is not available to many contaminants would also be unavailable and not detected. In addition to surface area, iIt is also possible that the surface chemistry, such as surface oxidation, led to the difference in contaminant sequestration onto the CC compared to the SWNT. The CC was readily dispersed into deionized water suggesting it is more hydrophilic than SWNT, which can only be temporarily dispersed in DI water after high power sonication. This surface oxidation likely present on the CC would cause weaker bonds to the PCBs, thereby reducing the ability of CC to sequester PCBs. This would be especially important when steric hindrance effects occur simultaneously, as was the case for the PCBs with higher

chlorine substitution (e.g. larger molecule), and the resulting increase in bioaccumulation by *N. virens*.

4.3.5. Summary

I have conducted a detailed study of the interaction of SWNT and organic contaminants in a complex, field-contaminated estuarine system. My results show that a 10 mg/g amendment of SWNT or CC to PCB-amended sediment greatly reduces (>90%) the ITW PCB concentration as well as significantly reduces PCB bioaccumulation by *N. virens*, but that there is also a difference in response of the two black carbon types with respect to individual PCB congeners sorted by their log K_{ow} (Figure 4.5). More studies need to be performed to determine if this effect is generally applicable to HOC-contaminated sediments. In my experiments, I used relatively low (i.e., 1 mg/g to 10 mg/g SWNT) mass black carbon amendment in order to mimic approximate maximum carbon nanoparticle burdens that might be encountered in the most heavily-contaminated sediments. It would be beneficial to test carbon nanoparticle amendments to sediment in the range of 2-4% mass, as this was the range Rakowska et al. [78] determined activated carbon to be efficient at contaminant sequestration with minimal negative side effects on the benthic community. The ability of SWNT to reduce PCB ITW concentration and subsequent PCB toxicity bioaccumulation in benthic invertebrates is a positive, indirect effect of SWNT addition in a contaminated estuarine sediment system. These results, in addition to the lack of toxicity and bioaccumulation potential of SWNT

themselves suggest that the materials I tested are not of great concern to the environmental health of a benthic, estuarine ecosystem.

Table 4.1. Sediment characterization including measured SWNT concentration (mg/g dry), total organic carbon (TOC %), black carbon (BC %), and total PCB concentration (ng/g dry sediment and ng/g TOC). Values represent mean (standard deviation).

Sediment	Treatment	mg SWNT / g dry[a]	TOC %[a]	BC %[a]	Total PCB[b] (μg/g dry)	Total PCB[b] (μg/g TOC)
25% NBH	Unamended control	--	2.67 (0.12)	0.51 (0.04)	60.1 (17.5)	2250 (658)
	1 mg/g SWNT	0.54 (0.04)	3.22 (0.40)	0.62 (0.08)	70.8 (5.2)	2200 (161)
	10 mg/g SWNT	4.89 (0.62)	3.36 (1.49)	0.73 (0.03)	58.6 (23.3)	1740 (692)
	10 mg/g coconut charcoal	--	3.24 (0.33)	0.69 (0.17)[b]	53.4 (12.3)	1650 (380)
50% NBH	Unamended control	--	4.75 (0.23)	0.92 (0.11)	105 (3)	2210 (71)
	1 mg/g SWNT	0.47 (0.04)	5.74 (0.25)	0.88 (0.10)	171 (91)	2970 (1580)
75% NBH	Unamended control	--	7.35 (0.13)	1.33 (0.11)	219 (68)	2980 (931)
	1 mg/g SWNT	0.83 (0.02)	7.19 (0.56)	1.23 (0.14)	164[c]	2280[c]
100% NBH	Unamended control	--	9.28 (0.78)	1.62 (0.28)	285 (21)	3070 (221)
	1 mg/g SWNT	0.52 (0.04)	9.42 (0.25)	1.58 (0.12)	316 (101)	3350 (1070)
	1 mg/g coconut charcoal	--	8.81 (0.08)	1.57 (0.25)	417 (36)	4740 (406)

Sample size: [a]n=3, [b]n=2, and [c]n=1

Table 4.2. Average freely dissolved interstitial water (ITW) concentration of polychlorinated biphenyls (PCBs) and their percent decrease by SWNT or CC amendment for each treatment.

Sediment	Treatment	Total ITW PCB concentration (μg/L)	Percent decrease in ITW PCB concentration
25% NBH	Control	9.24 (0.52)	--
	1 mg/g SWNT	5.71 (1.35)	38
	10 mg/g SWNT	0.77 (0.06)	92
	10 mg/g coconut charcoal	0.46 (0.03)	95
50% NBH	Control	12.04 (1.60)	--
	1 mg/g SWNT	8.80 (0.53)	27
75% NBH	Control	12.53 (1.42)	--
	1 mg/g SWNT	14.22 (1.18)	-14
100% NBH	Control	16.29	--
	1 mg/g SWNT	8.31 (0.94)	49
	1 mg/g coconut charcoal	11.86 (2.05)	27

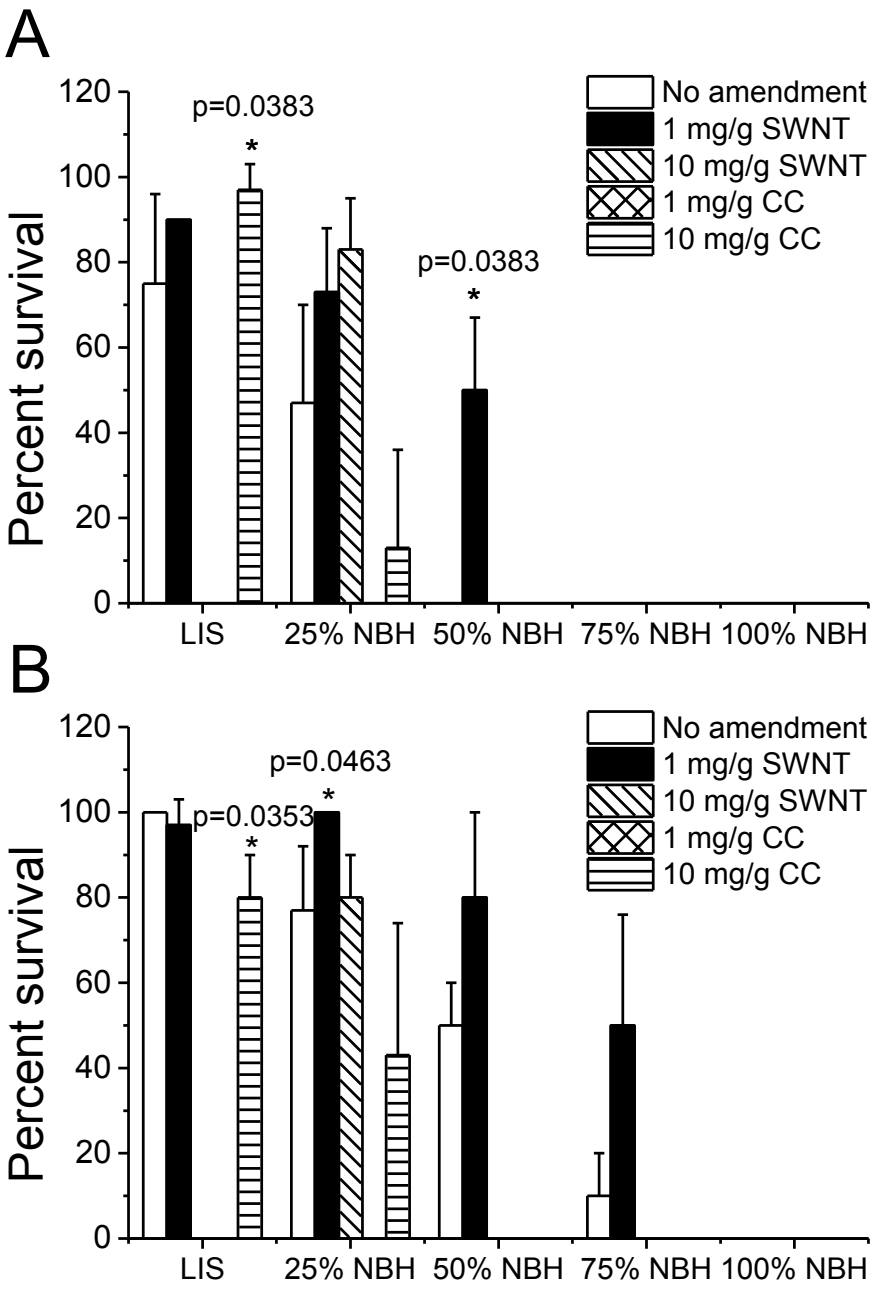


Figure 4.1. Percent survival of *Americamysis bahia* (A) and *Ampelisca abdita* (B) during the toxicity experiments. Data are expressed as average + standard deviation (n=3).

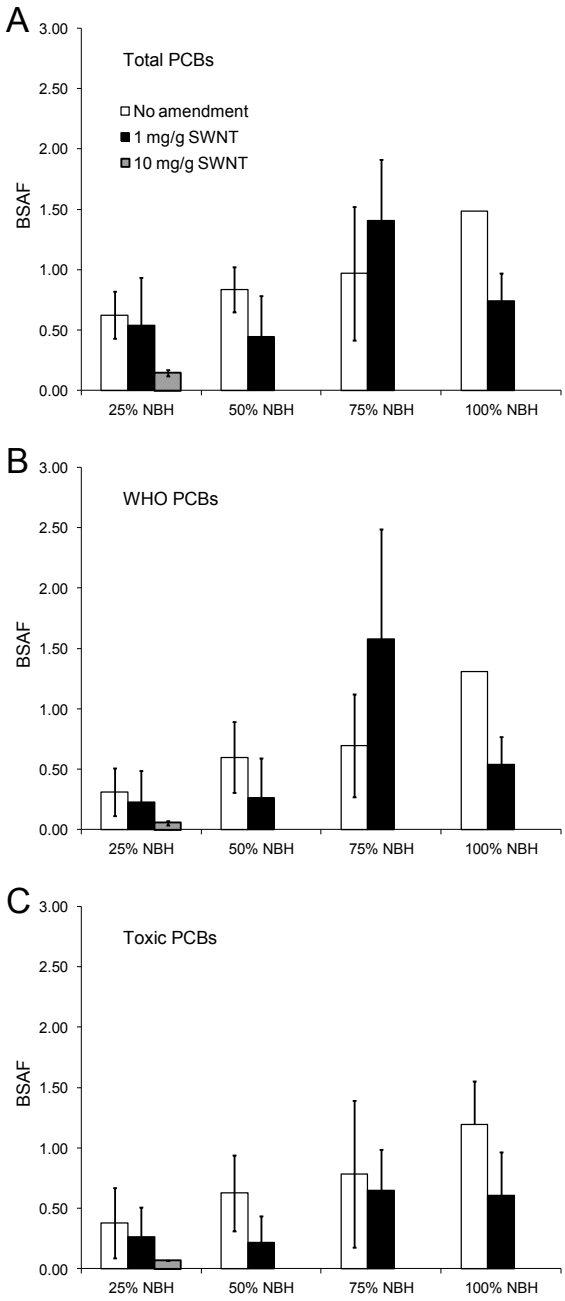


Figure 4.2. PCB BSAF in *Nereis virens* as a result of NBH sediment exposure.
The total (A), World Health Organization (WHO) (CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 169, and 189) (B), and toxic (CBs 77, 126, and 169) (C) polychlorinated biphenyl (PCB) biota-sediment accumulation factor (BSAF) in *Nereis virens* exposed to New Bedford Harbor (NBH) sediment with and without single-walled carbon nanotube (SWNT) amendment.

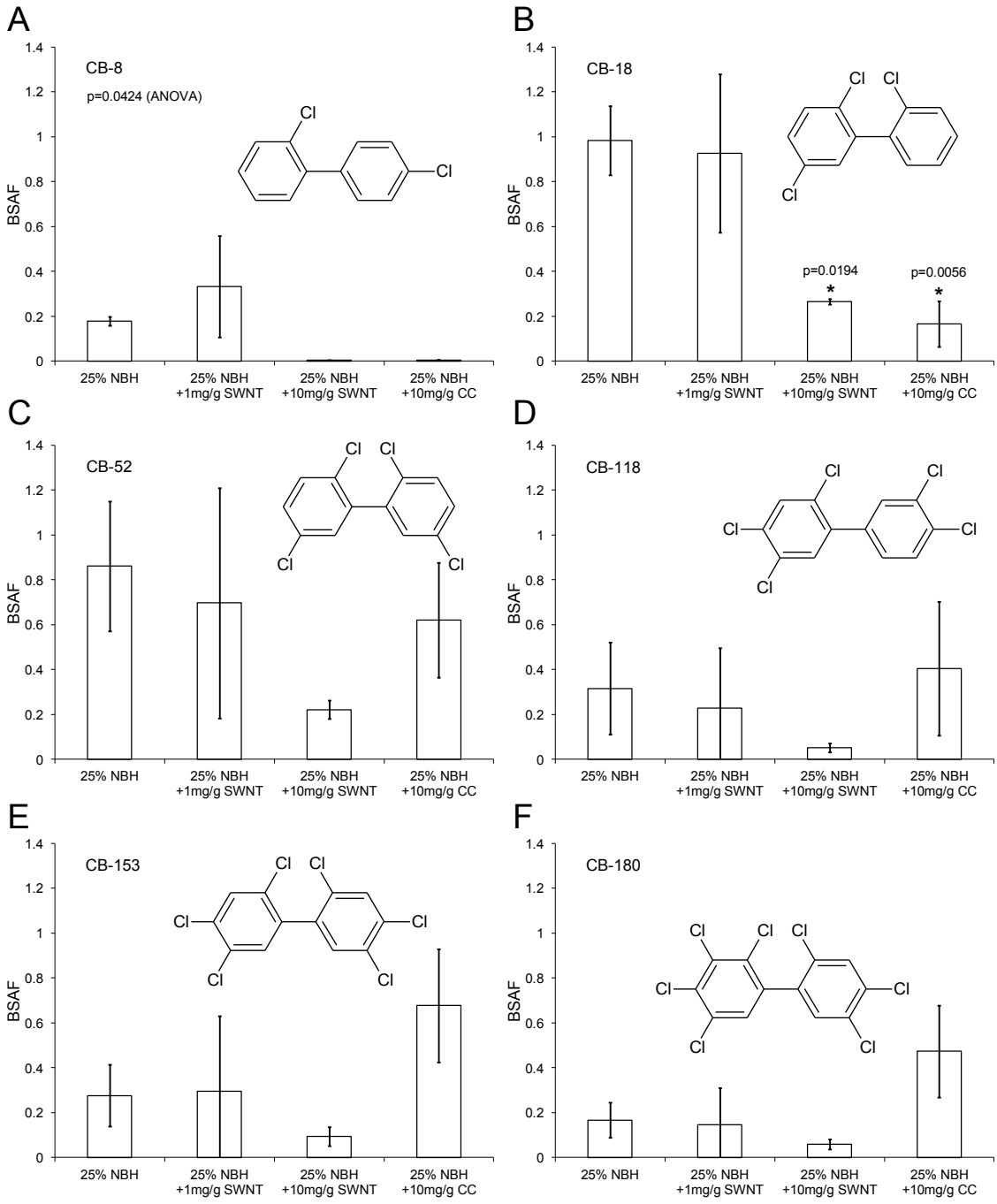


Figure 4.3. Biota-sediment accumulation factors (BSAFs) for representative polychlorinated biphenyls (PCBs) with increasing chlorination from A-F in the 25% New Bedford Harbor (NBH) sediment exposure.

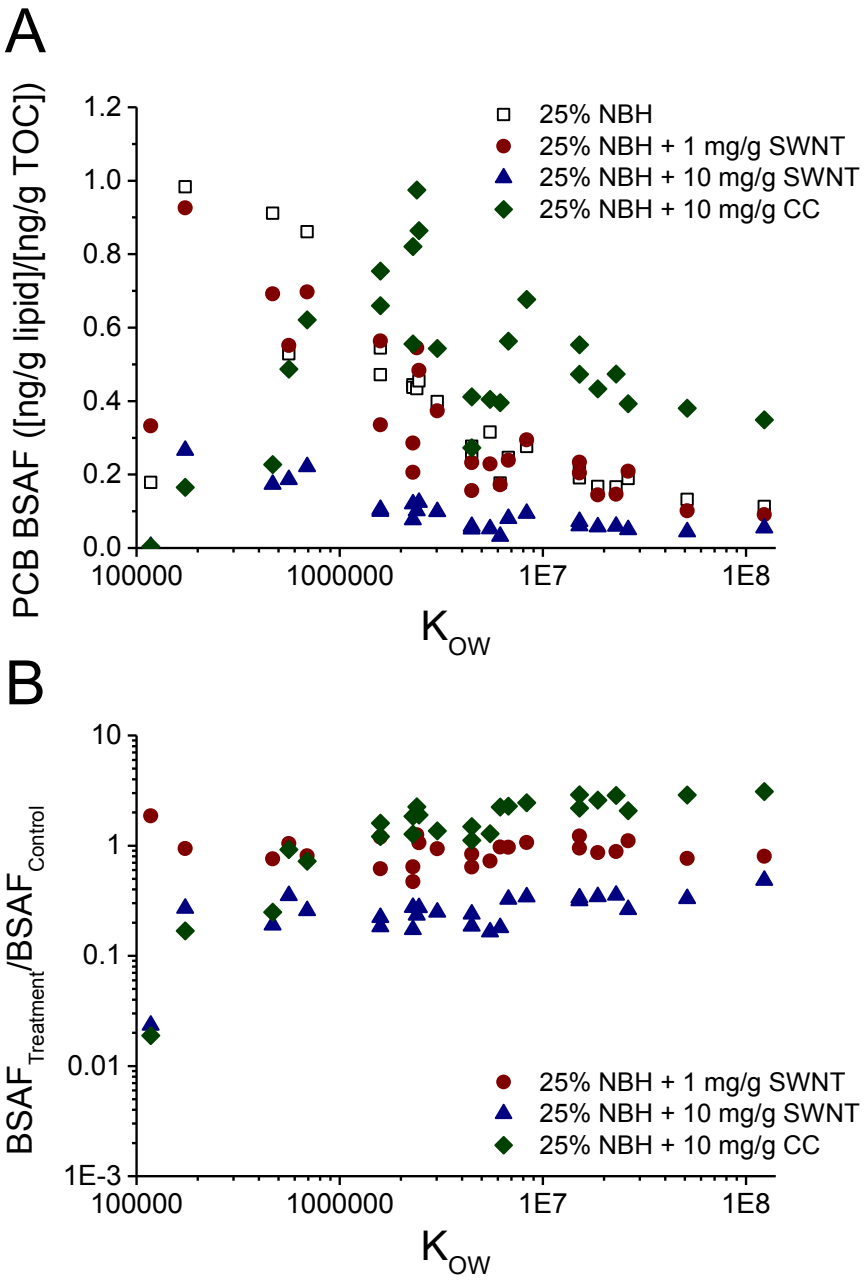


Figure 4.4. Biota-sediment accumulation factors (BSAFs) as a function of PCB K_{OW} for the 25% New Bedford Harbor (NBH) sediment exposure (A), and the ratio of $BSAF_{Treatment}$ to $BSAF_{Control}$ versus PCB K_{OW} (B).

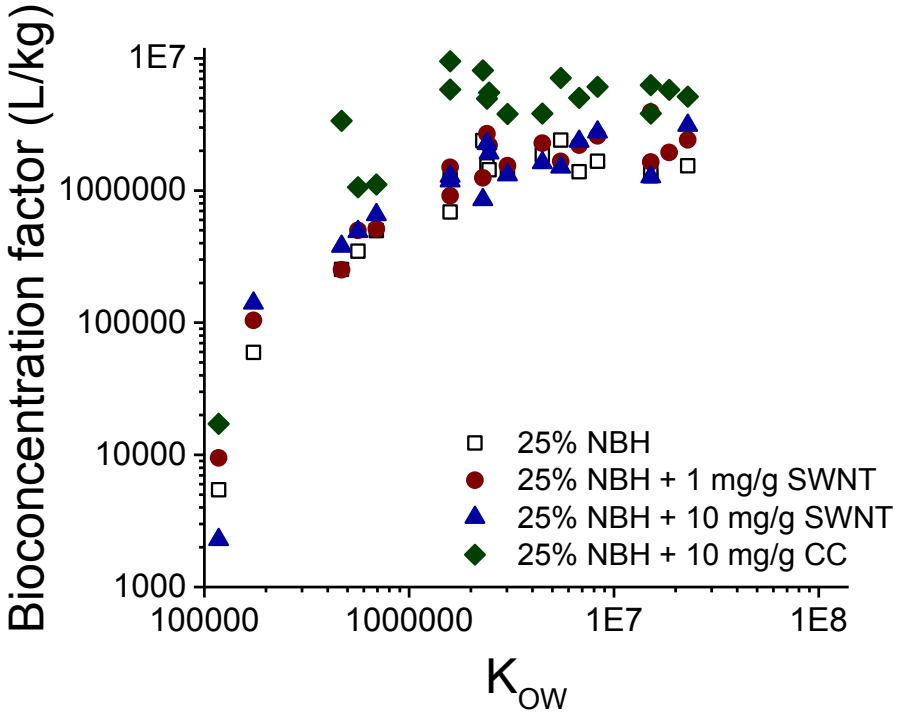


Figure 4.5. The bioconcentration factor (bioaccumulation/interstitial water concentration) versus PCB K_{OW} illustrating that SWNT affect PCB bioaccumulation through reduced bioavailability in the ITW.

5. ^{14}C-SWNT Biodegradability by *Trametes versicolor* and Natural Microbial Cultures found in New Bedford Harbor Sediment and Aerated Wastewater Treatment Plant Sludge

5.1. Introduction

As the field of nanotechnology continues to progress, the number of potential commercial applications increases, which leads to increased probability of their presence in the environment [44-46, 52, 111]. Once present in the environment, it is important to understand how these materials will behave, what effects they might have on the environment, and what effects the environment may have on them (i.e., their environmental fate, transport, toxicity, and especially their transformation/degradation). These mechanisms are dependent upon the characteristics of the environment in addition to the characteristics of the nanomaterial [41, 60, 61, 155]. Properties such as nanomaterial type (i.e., carbon and metal), and surface chemistry (i.e., surface coating, covalently bound functional groups, and defect sites) strongly affect not only the initial incorporation into the environment, but also the long-term fate and transport of the material throughout the environment [41, 60, 61, 155].

Several studies have investigated the toxicity of carbon nanomaterials in the environment [14, 41, 43, 47, 48, 51, 54, 55, 59, 60, 133]; however, little work has been conducted to investigate their biodegradation under environmental conditions. Hartmann et al. found that aged nC_{60} incubated in activated sludge was not biodegraded

after 48 days [95]. These materials were aged under indirect natural light for 36 months leading to minimal oxidation and surface modification [95]. After the initial 28 days of incubation, sodium acetate was added to confirm bioactivity of the sludge and was fully mineralized within a few days of addition, suggesting that the aged nC_{60} were not biodegradable under those conditions [95]. Another study investigated the ability of two white-rot basidiomycete fungi (*Phlebia tremellosa* and *Trametes versicolor*) to degrade C_{60} fullerol [96]. The data presented by Schreiner et al. suggests that in the presence of both species of fungi, with and without the addition of wood to stimulate release of enzymes, approximately 20-30% of the C_{60} fullerol was lost from the media system [96]. Complete mineralization of the C_{60} fullerol to CO_2, as well as the production of and incorporation into fungal hyphae was assumed to account for the 20-30% loss of C_{60} fullerol. However, these data were not verified by quantitative measurement of evolved $^{13}CO_2$ or incorporation of ^{13}C into the fungal biomass, and therefore the mechanism of loss could not be confirmed [96].

The contrasting data presented from the previous two studies is further complicated by the findings of Liu et al. [97]. When determining the biodegradability of SWNT, these authors used various pre-treatments to introduce different functional groups to the SWNT as well as to vary the extent of functionalization [97]. The authors functionalized SWNT by ozonolysis (incubation with 0.24 wt. % ozone for 20 minutes), aryl sulfonation (incubation with sulfanilic acid and sodium nitrite), and carboxylation

(incubation with 3:1, 98% H_2SO_4:70% HNO_3 for either 15 minutes, 1 hour, or 3 hours), and subsequently incubated them in phagolysosomal stimulant fluid (PSF) in the presence of hydrogen peroxide (H_2O_2) over 90 days [97]. Transmission electron microscopy (TEM) and field-emission scanning electron microscopy (FE-SEM) were used to determine if the incubations led to degradation, which was characterized by the disaggregation of SWNT and an increase in shortened SWNT over time [97]. Incubation of SWNT with no functionalization as well as those functionalized through aryl sulfonation, ozonolysis or a 15 minute carboxylation treatment in PSF/H_2O_2 resulted in no biodegradation of SWNT [97]. However, discernible biodegradation was observed when SWNT underwent carboxylation treatment for one hour and for three hours prior to incubation with PSF/H_2O_2 [97]. Their findings suggest that the specific initial functional groups as well as the amount dictate whether or not a material is biodegradable [97]. These data support other observations that oxidized carbon nanomaterials will more readily undergo biodegradation when incubated in PSF/ H_2O_2 [98, 99] as well as horseradish peroxidase HRP/H_2O_2 [98, 100-102] but similar pristine materials will not unless an additional oxidative catalyst is added to form oxygen-containing functional groups on the surface of the material [99].

My previous work and that of my colleagues has focused on the environmental detection [103], toxicity and bioaccumulation [104], and effects on co-contaminants of SWNT in benthic estuarine systems. In the current study, I aimed to determine the

biodegradability of SWNT in natural systems. The focus was on the white-rot basidiomycete fungi, *Trametes versicolor*, as well as the naturally occurring microbial communities present in field-collected estuarine sediment from New Bedford Harbor (NBH, MA, USA) and aerated wastewater treatment plant sludge. The ^{14}C-SWNT material used in this study was previously oxidized [12]. These functional groups should increase the likelihood of biodegradation but the exact extent of carboxylation required for degradation to occur is not yet known and cannot be directly determined from the work reported by Liu et al [97].

5.2. Materials and methods

5.2.1. Biological media

Trametes versicolor cultures were maintained and routinely prepared as needed on 2% dextrose agar plates at room temperature. The 2% dextrose media contained 20 g/L agar, 10 mg/L streptomycin sulfate, and 130 mg/L yeast nitrogen base without amino acids. Mycelial suspensions were obtained by homogenizing plugs of the fungal growth zone from the plates in a minimal volume of sterile aqueous 2% dextrose media and subsequent inoculation in Erlenmeyer flasks containing the same media. Incubations were kept in the dark at 25 °C on an orbital shaker (90 rpm) for 7 days. The resulting fungal pellets were removed and stored in sterile saline solution (0.85% NaCl) at 4 °C until use [156].

New Bedford Harbor (NBH) sediment was collected from the Superfund site in April 1994 (MA, USA). The sediment slurry was prepared by adding 40 g wet NBH sediment per L of 30‰ reconstituted seawater (RSW) prepared by diluting 100‰ brine with deionized water and aerating for 5 days prior to use. Aerated sewage sludge was collected from the North Durham Water Reclamation Facility (Durham, NC, USA) in June 2012. The sludge was concentrated by allowing the solids to settle and decanting the overlying water. This was repeated until the concentration reached 0.02 g/mL dry weight (d.w.). The slurry was aerated for 5 days prior to use.

5.2.2. Single-walled carbon nanotube material

^{14}C-SWNT produced by the arc-discharge method (Research Triangle Institute) were used in the current work. This material underwent nitric acid purification to remove amorphous carbon and metal catalyst impurities, which introduced oxygen containing groups such as carboxyl to defect and end sites [11, 12, 115]. The ^{14}C-SWNT stock contained 1.15 mg ^{14}C-SWNT/mL deionized water with an activity of 1.63 μCi/mL. This stock was sonicated for 10 minutes at 50% amplitude (approximately 30 watts; Branson 450D sonifier with a 3 mm tapered microtip) prior to use. A SWNT suspension of P3-SWNT (Carbon Solutions, Inc.) was made by dispersing 1 mg SWNT/mL deionized water by the sonication method mentioned previously. These materials have similar oxidation as the ^{14}C-SWNT materials and are a comparable non-radiolabeled source.

5.2.3. SWNT degradation experiment

The degradation experiment was performed using 125 mL serum bottles sealed with Teflon-coated grey butyl rubber stoppers and aluminum crimp caps. A hanging carbon dioxide base trap with filter was fitted to each bottle by inserting it into but not through the bottom of the stopper. Each replicate contained either 0.03 g dry *T. versicolor* pellets in 10 mL 2% dextrose media (3g/L d.w.), 0.2 g dry sediment in 10 mL NBH sediment slurry (20 g/L d.w.), or 0.2 g dry sludge in 10 mL sludge slurry (20 g/L d.w.). Killed controls were prepared by amending 1% $HgCl_2$ to the culture media. ^{14}C-SWNT (55 μg, 0.078 μCi) was added to each bottle, which was pre-sparged with oxygen for one minute prior to sealing with the stopper and crimp cap. Bottles were incubated in the dark at 25 °C on an orbital shaker (90 rpm) for a total of 168 days with sampling time points taken at time zero, seven days, fourteen days, 28 days, 56 days, 112 days, and 168 days. Each time point included three killed controls (except time zero) and three experimental replicates for each treatment. Three bottles per media were set up as oxygen controls and contained the same media and SWNT addition as well as 2 μL resazurin dye for a final concentration of 2 μg/mL. As oxygen is depleted from the system, the dye is reduced and changes from pink to clear [157]. This observation was only possible in the fungus treatment as the sediment and sludge slurries were too turbid to observe the initial color.

Upon completion of each time point, replicate bottles were sacrificed for analysis. Evolved ^{14}C-CO_2 was trapped by using syringes to add 0.5 mL of 4 N NaOH to the hanging trap filter of each bottle followed by the addition of 0.1 mL of 2 N HCl to the media through the butyl stopper. This volume of acid was determined to sufficiently lower the pH of the media below pH 2 and subsequently drive any ^{14}C-CO_2 into the trap. Bottles were then placed back onto the orbital shaker for three hours to allow the carbon dioxide to equilibrate fully into the base trap. After this time period, the bottles were sampled one at a time. The aluminum crimp cap and butyl stopper were removed and the carbon dioxide trap was carefully separated. The filter and remaining NaOH were transferred to a scintillation vial, and the trap was rinsed with approximately 100 µL of 4 N NaOH into the vial. Twenty milliliters of Ecoscint XR scintillation cocktail was then added to the scintillation vial. The media bottles were recapped with the same butyl stopper, sealed with a new aluminum crimp cap, and frozen at -20 °C until further analysis could be performed.

5.2.4. Analysis of sample media

The media were sampled to measure the aqueous phase for any partially degraded, dissolved ^{14}C-SWNT transformation products. The samples were thawed and quantitatively transferred to Ultra-Clear™ thinwall tubes (Beckman Coulter) for ultracentrifugation (5.5 hours, 25 °C, 288,000 x g) using a SW 41 Ti rotor in a L8-80M Ultracentrifuge (Beckman Coulter). The supernatant was removed and each remaining

pellet (together with the centrifuge tube) was stored in a 50 mL polypropylene screw cap centrifuge tube at -20 °C until further analysis could be performed. A 3 mL subsample of the supernatant was transferred to a scintillation vial and 15 mL of Ecoscint XR scintillation cocktail was added. The remaining supernatant was stored in a 15 mL polypropylene screw cap centrifuge tube at -20 °C. These samples will be further analyzed by high performance liquid chromatography-radiometric detection (HPLC-RD) to determine if the activity present in the aqueous phase was due to partial degradation of the ^{14}C-SWNT over time or if the same ^{14}C-compound was present over time due to an impurity in the ^{14}C-SWNT stock.

Pellets were analyzed using a Biological Oxidizer OX600 (R.J. Harvey Instrument Corporation). Only two of the three killed and sample replicates were analyzed on the biological oxidizer for each media and each time point. Each sample was transferred to a ceramic boat which was then inserted into the instrument. A carbon dioxide trap containing 10 mL of carbon-14 cocktail (R.J. Harvey Instruments) was mounted at the instrument outlet port. Once the sample was combusted, the trap was removed and the cocktail transferred to a scintillation vial. The trap was rinsed into the vial twice with approximately 2-3 mL of cocktail each time. Methanol was used to rinse the trap three times between samples. D-mannitol-1-^{14}C (Sigma-aldrich) was used as the calibration/recovery standard. All samples were analyzed using a Tri-Carb 2100TR Liquid Scintillation Analyzer (Packard).

5.3. Results and discussion

Over the course of the experiment, the oxygen control bottles containing resazurin were observed for the loss of color indicative of oxygen depletion. No resazurin dye was observed in the oxygen control bottles at the sampling performed on day 56, suggesting depletion of oxygen occurred between 28 and 56 days switching from an aerobic system to an anaerobic system. Schreiner et al. performed a degradation experiment in 50 mL jars with the same fungal species and observed continued growth when the jars were opened every three weeks to replenish the oxygen supply [96]. In the current experiment, the increased headspace (approximately 3 times compared to that used by Schreiner et al [96]) and initial sparging with pure oxygen should have provided aerobic conditions for approximately 9 weeks. This time limit is in agreement with the observed change in the oxygen controls.

The average percent recovery of ^{14}C in each media over the 168 day experiment was 103 $\pm$ 5%, 101 $\pm$ 5%, and 96 $\pm$ 9% in the fungus, sediment, and sludge, respectively. The data confirms that the full ^{14}C-SWNT activity was accounted for in the system with no significant loss from the sealed bottles over time (Figure 5.1). By measuring the ^{14}C activity in each compartment (i.e., gas, aqueous, and solid phases), the mineralization process could be monitored. The ^{14}C activity measured in the carbon dioxide trap was indicative of ^{14}C-SWNT that has been completely mineralized, while the ^{14}C activity measured in the acidified aqueous phase was indicative of ^{14}C-SWNT degradation

intermediates. Any ^{14}C activity remaining in the solid phase is assumed to be intact ^{14}C-SWNT.

The percent of total ^{14}C-SWNT activity in each sample compartment over 168 days is summarized in Figure 5.1 for each media type. None of the treatments, *T. versicolor* (Figure 5.2A), natural bacterial cultures found in NBH sediment (Figure 5.2B), and wastewater sludge (Figure 5.2C) showed any significant degradation of ^{14}C-SWNT over the entire experiment. The results for the samples (closed symbols, Figure 5.2) were not different from the killed controls (open symbols, Figure 5.2). Approximately 99% of the total activity remained in the solid phase (red symbols, Figure 5.2), 0.8% in the aqueous phase (black symbols, Figure 5.2), and less than 0.1% (blue symbols, Figure 5.2) in the gas phase, independent of time and media.

The lack of degradation from these media is consistent with other studies performed on SWNT and fullerenes (nC_{60}) [95, 97]. Hartmann et al. investigated the ability of activated sludge to degrade aged nC_{60} over 48 days [95]. After the first 28 days, no degradation was observed so 5 mg/L sodium acetate was added to test the bioactivity of the sludge. A few days after the addition, all of the sodium acetate was mineralized, confirming the bioactivity of the sludge. At the end of the next 20 days, no biodegradation of nC_{60} was also observed, and it was concluded that these aged nC_{60} were not readily biodegradable [95]. Since the current experiment was sealed until time points were sampled, it was not possible to check the bioactivity of the media over time.

Based on the redox dye used (resazurin), the experiment likely went anoxic between 28 and 56 days, thus switching from an aerobic system to an anaerobic system. The amount of evolved $^{14}CO_2$ beyond this time point did not change, suggesting that no anaerobic degradation was occurring under those conditions as well.

A study investigating the ability of phagolysosomal stimulant fluid (PSF) to degrade one SWNT material before and after various transformation treatments concluded that the type and extent of functionalization was a critical factor in SWNT biodegradation [97]. Although PSF is not relevant to the external environment, it is important in internal cellular processes as the phagolysosomes are likely compartments for SWNT once they are in a cell and targeted for removal via phagocytosis [97]. Liu et al. found that pristine SWNT and those functionalized by ozonolysis and aryl sulfonation were not significantly biodegraded after 90 days [97]. However, carboxylation led to visible biodegradation once the SWNT reached a certain level of oxidation (e.g., presence of carboxylic acid groups) [97]. The total oxygen content was not reported, but observed degradation depended on the length of time the SWNT were pre-treated with a nitric and sulfuric acid mixture such that a 15 minute pre-treatment did not lead to visible degradation after 90 days, but a one hour and three hour pre-treatment led to visible degradation after 90 days in PSF [97]. Several other studies support the observed degradation of oxidized SWNT, MWNT, and fullerol in both cellular [97, 98, 101, 158] and simulated environmental media [96, 98-100, 102]. It is

likely the ^{14}C-SWNT material used for this study did contain oxidized functional groups such as carboxylic acid resulting from the acid purification process [11, 12, 115], but the exact extent of oxidation was not reported. Further, it is possible that the SWNT used here were more similar to the material that was acid-treated for only 15 minutes by Liu et al. [97], supporting the conclusion that the ^{14}C-SWNT were not biodegradable under those conditions.

It is important to note that in addition to high surface oxidation, the presence of a strong oxidizing agent such as hydrogen peroxide (H_2O_2) is required to catalyze the degradation [100]. When carboxylated SWNT were exposed to horseradish peroxidase (HRP) over 16 weeks, degradation only occurred when H_2O_2 was present [100]. For pristine SWNT, a Fenton catalysis reaction (addition of $FeCl_3$) was required to cause degradation [99]. The additional catalyst led to the production of hydroxyl and hydroperoxyl radicals through homolytic cleavage of H_2O_2, which in turn oxidized the SWNT surface, allowing for interaction with the active sites of the HRP and subsequent degradation [99].

In order to better test the aerobic degradation of ^{14}C-SWNT over time, the current experiment could be redesigned such that three replicate sealed flasks with each media (fungus, sediment and sludge) would be setup with $^{14}CO_2$ trap flasks. The system would be sparged weekly to collect $^{14}CO_2$ followed by a subsample of the liquid culture medium for aqueous and solid phase analysis and then re-sealed to continue incubation.

Mercuric chloride killed controls and media-only controls would also be added to determine any abiotic degradation and background signal. Finally, it would be interesting to test liquid fungal culture and an agar media fungal culture simultaneously to determine any differential effect based on substrate.

5.3.1. Summary

It is very important to investigate the biodegradability of environmental contaminants, including nanomaterials to get a complete picture of their environmental fate and transport as well as their toxic potential. Previous work by myself and others in the Ferguson laboratory has shown a lack of toxicity and bioaccumulation of pristine SWNT and oxidized ^{14}C-SWNT to benthic estuarine invertebrates [104]. Because of their relative persistence in the sediment, it is important to understand what possible transformations they could undergo that would lead to more hydrophilic degradation products that are more readily transported and have greater toxicological effects. The results from this study and prior studies conclude that pristine and minimally oxidized carbon nanomaterials are not readily biodegradable under environmental conditions such as exposure to fungus, and the bacteria present in sediment and aerated sludge [95].

However, the data does suggest that strongly oxidizing conditions such as those in phagolysosomes may lead to degradation of SWNT present in cells [97, 98, 101, 102]. This application is more suited for the drug delivery of SWNT and should be taken into

account as a purposefully engineered mechanism of biodegradation once the drug has been delivered. It would be beneficial to test the biodegradation potential of SWNT with a quantitatively determined range of oxidation in similar environmentally relevant media as well as the HRP/H_2O_2 mixture in order to more specifically determine how much oxidation supports natural biodegradation of these materials. While aerobic degradation is generally considered most important for degrading carbon molecules, another area of interest is to determine if SWNT can be degraded under environmental anaerobic conditions such as with anaerobic microbial communities in anoxic sediment. Degradation in this media could facilitate increased hydrophilicity which could in turn lead to increased mobility in the environment.

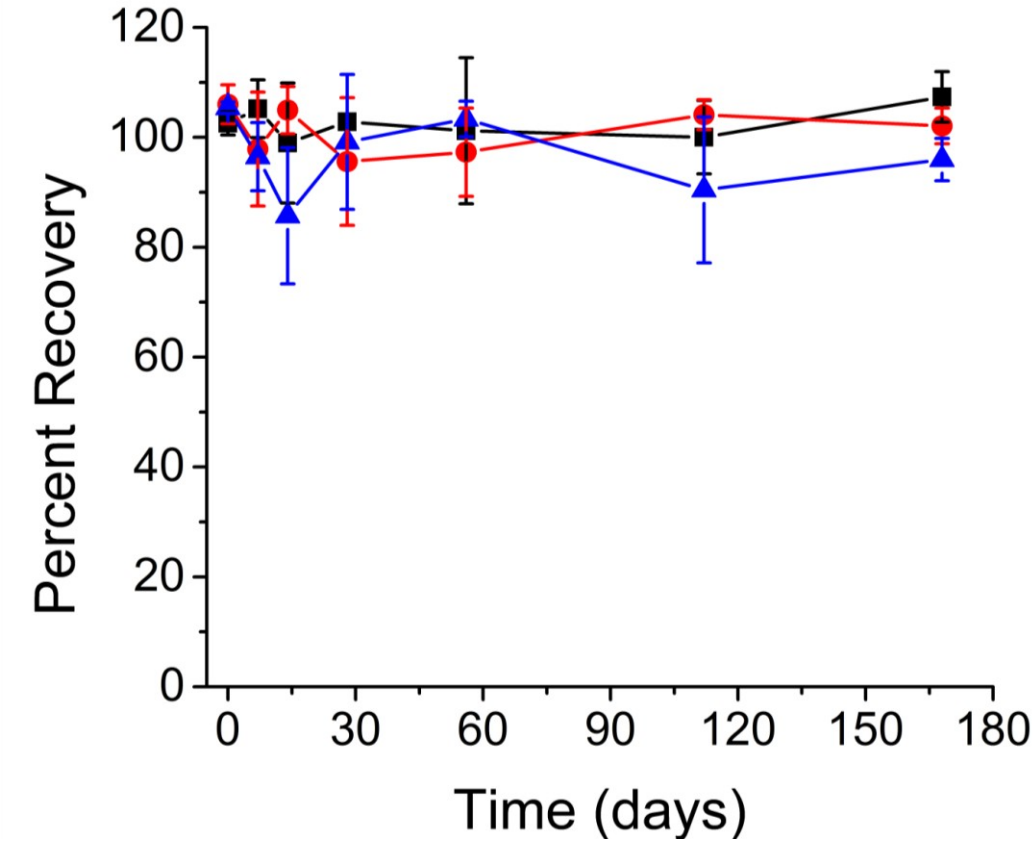


Figure 5.1. Percent recovery (^{14}C-SWNT) over time remains constant at approximately 100% for each test media: fungus (black squares), NBH sediment (red circles), and blue triangles (sludge). Data is presented as average $\pm$ standard deviation (n=6).

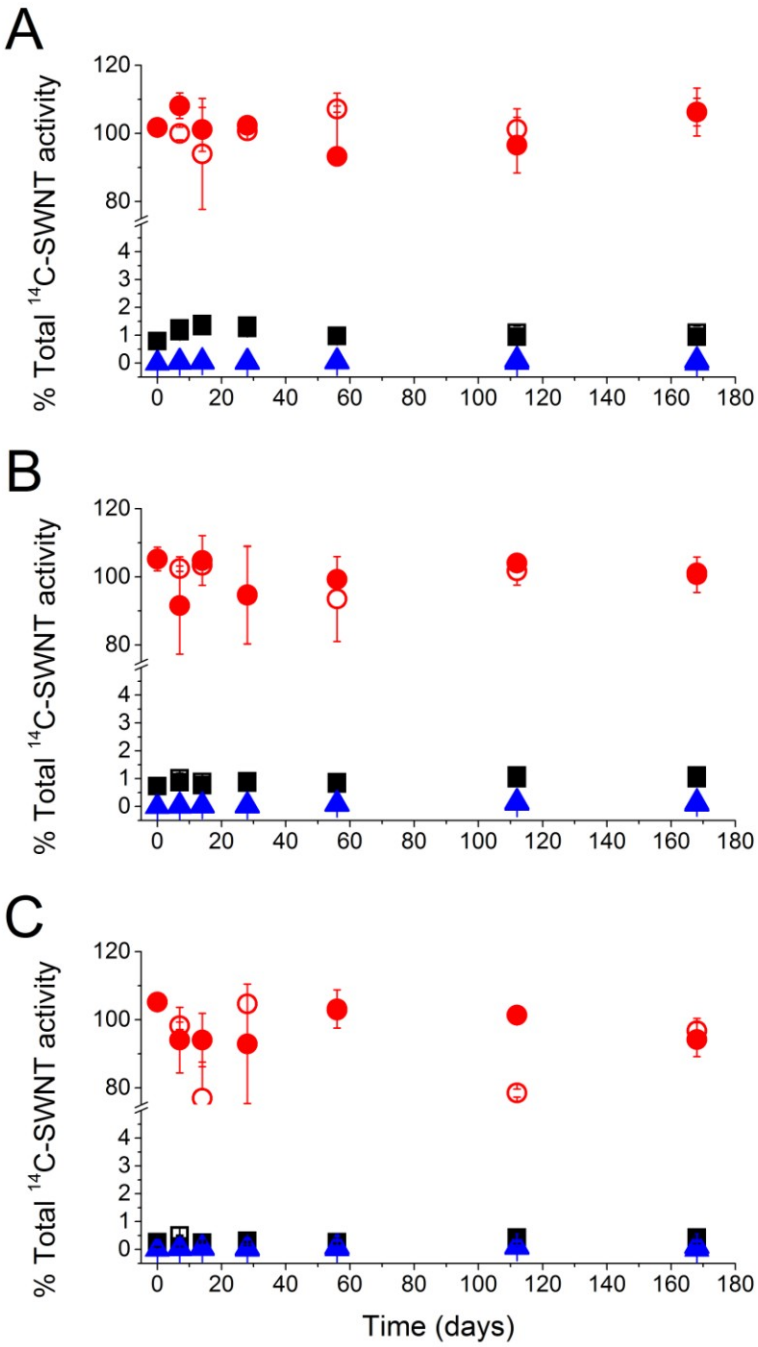


Figure 5.2. The percent total ^{14}C-SWNT activity in each phase (solid phase: red circles, aqueous phase: black squares, and gas phase: blue triangles) measured over time in the fungus (A), NBH sediment (B), and wastewater treatment plant sludge (C) degradation experiments. Open symbols repesent killed controls and closed symbols represent samples. Data is presented as average $\pm$ standard deviation (n=3).

6. Conclusions

With the ever-growing field of nanotechnology research and product development, nanomaterials will very likely enter the environment, and their discharge will increase with the increasing number of applications [42, 44-46, 52, 111]. Because of this phenomenon, it is essential to learn and understand how nanomaterials enter the environment, where they go, what they impact, and what impacts them. Their fate, transport, transformation, toxicity, and bioaccumulation need to be understood and well-researched so the risk can be properly assessed such that any needed environmental regulations can be put into place and enforced.

The focus of this research was to provide that information for semiconducting single-walled carbon nanotubes in a benthic estuarine environment. Near infrared fluorescence (NIRF) spectroscopy was an ideal measurement technique to utilize for complex media. The selective and sensitive nature of the method allows for minimal sample processing and efficient analysis, while providing a full characterization of the various SWNT isoforms in a mixture including quantitative analysis [5, 10, 27, 28]. This method was validated with the measurement of ^{14}C-SWNT by liquid scintillation counting, an independent and well-established detection method. The NIRF spectroscopy method presented in this dissertation is currently the most sensitive and selective measurement method available for analyzing unfunctionalized, semicondictive SWNT in complex environmental media, whether from the laboratory or field. However,

further improvements can be made by trying to reduce the few optical matrix effects I have observed in low SWNT signal samples, as well as adding more sophisticated analytical instruments upstream such as asymmetric flow field flow fractionation (AF^4), which can separate SWNT by length and potentially reduce matrix-interferences prior to NIRF analysis.

Once the quantitative NIRF method was developed and validated, I could move forward with testing the toxicity and bioaccumulation of the semiconducting SWNT. Since these materials are highly hydrophobic and associate with organic matter and sediment, these media are the most likely route of exposure in the estuarine environment. None of the SWNT material tested (i.e., pristine, semiconducting and oxidized) were toxic to three important benthic invertebrates at the base of the marine food chain within a range of SWNT exposure from 0.1 μg/g to 100 μg/g. The organisms ingested the SWNT amended sediment or algae (depending on the organism feeding preferences), but significantly eliminated the SWNT after depuration suggesting the SWNT are bioaccessible but not bioavailable once they are ingested. Some higher level benthic organisms have surfactants in their gut [138, 139] that could possibly exfoliate SWNT from the surface of the sediment or algae, potentially increasing their bioavailability. It could be beneficial to investigate this further as SWNT may be more bioavailable to these organisms and therefore have the potential to bioaccumulate in the tissues. This gut surfactant could be collected and used to extract SWNT-amended

sediment with a possible comparison of ultrasonication extraction opposite a gentler, more natural slow mixing [139].

In addition to the direct effects of SWNT on benthic organisms, it is equally important to determine their indirect effects and interactions with co-contaminants as natural systems are not simple one-contaminant exposure systems. I utilized New Bedford Harbor (NBH) sediment, containing polychlorinated biphenyls (PCBs), to provide a more complex, environmental media compared to laboratory-spiked sediment employed for the toxicity and bioaccumulation study. The presence of SWNT in the 25% NBH sediment treatment led to increased survival, decreased bioaccumulation of PCBs in *Nereis virens*, and approximately 90% reduction in total PCB interstitial water (ITW) concentrations. Although most of the differences were not significant, the typical amendment for *in situ* remediation using activated carbon is 2-4% by mass [78], and the highest amendment concentration of SWNT I used was only 1% by mass. SWNT are still expensive to produce and are not yet sustainable as a remediation amendment [159], but having the knowledge of their interactions with other contaminants in the environment is beneficial.

Since I have observed that SWNT do not cause toxicity, do not bioaccumulate, act as a sorbent, and overall, are not highly mobile once in the sediment, it was imperative to assess whether these materials were biodegradable. If they can undergo biodegradation, the intermediate degradation products would be more oxidized and

therefore more hydrophilic. This transformation could significantly alter the fate and transport of the new materials. In my exposures, I used T*rametes versicolor* because white-rot fungi are more aggressive in breaking down carbon-based materials and this species was one of the two shown by Schreiner et al. to degrade fullerol [96]. I also wanted to test more environmentally relevant media such as NBH sediment and aerated waste-water treatment plant sludge. The results over six months of incubation illustrate that oxidized ^{14}C-SWNT material was not biodegradable by any of the three microbial consortia/cultures. This finding is consistent with the limited results reported by others for SWNT degradation [95, 97]. For example, Hartmann et al. did not observe degradation of aged nC_{60} over 48 days in sludge, which could be due to the minimal oxidation of the material that occurred as a result of aging by indirect natural light [95].

Although less environmentally relevant, the study by Liu et al. concludes that the amount and type of functionalization affects the biodegradability of the nanomaterial, as observed in phagolysosomal stimulant fluid (PSF); however, the exact degree of carboxylation for each material tested was not reported [97]. Continuing this work to investigate the carboxylation threshold where you find degradation would provide insight into which SWNT materials will be biodegradable once in the environment. This could be used in reverse engineering as a way to decrease the persistence of SWNT in the environment. However, it is imperative that the degradation

products are tested for toxicity and bioaccumulation so the nanotechnology community is aware of any potential, unexpected adverse effects.

Overall, semiconducting SWNT have unique properties that make them candidates for NIRF spectroscopy analysis, which has opened up the field of environmental nanotechnology and nanoecotoxicology to learn more about this specific class of materials in a complex environment. My results show that once released, neither the pristine, semiconducting SWNT nor oxidized SWNT are likely to cause severe toxic effects and will not be appreciably bioaccumulated by benthic deposit-feeding organisms. SWNT may be bioaccessible via ingestion but they seem to be freely eliminated through the gut in the presence of a clean environment and clean food. In my experiments, SWNT behaved as a sorbent and reduced the bioaccumulation of PCBs in organism tissue and sediment interstitial water. If SWNT enter an esturarine sediment system they would remain sorbed to the sediment (with or without co-contaminants), and it is not likely they would be biodegraded unless they were in a highly oxidizing environment or the nanomaterial was already highly oxidized.

Appendix A. Characterization and quantitative analysis of single-walled carbon nanotubes in the aquatic environment using near infrared fluorescence spectroscopy

A.1. Optical and spectroscopic characterization of SG65 SWNT

SEM data were acquired by FEI XL 30ESEM. XPS spectra were acquired at 15 kV and 150 W (10 mA) with a take-off angle of 90 degree from the sample normal (Kratos Axis Ultra, Kratos Anlalytical Ltd., Tokyo, Japan) and date fitting was performed using CasaXPS software (C 1S peak was at 284.5 eV). Dry powdered SWNT were measured on copper tape. Raman spectra were recorded using dry SWNT samples using a LabRam confocal Raman spectrophotometer (JY Horiba) equipped with a liquid nitrogen-cooled, charged coupled device detector and a He/Ne (632.817 nm) laser for excitation. Each spectrum represents the average of at least 5 scans with integration times of 120 seconds each. Raman peak identification was based on [160]. The characteristic G and D bands occurred at 1539/1591 cm^{-1} and 1309 cm^{-1}, respectively.

A.2. Evaluation of SWNT dispersion

UV-Vis-NIR absorbance spectra (Figure A.1E) and NIRF spectra (Figure A.1F) of SG65 SWNT in different dispersions (sodium benzylsulfate-SDBS, sodium cholate SC and sodium deoxycholate SDC). NIRF emission spectra (Figure A.1F, 782 excitation

wavelength) and UV-Vis-NIR absorbance spectra (Figure A.1E) of surfactant wrapped SG65, c_0 = 1 mg/ml in 2% w/v surfactant, high power sonication and ultracentrifugation. Sodium deoxycholate gave sharp optical absorbance peaks for SG65 SWNT and very high quality suspensions, which also corresponded to very high sensitivity in NIR fluorescence emission. Within the tested surfactants sodium deoxycholate is the most effective surfactant for maximizing the concentration of suspended, individual SWNT in solution prior to NIRF analysis.

Table A.1. Extraction of SWNT from BBC estuarine sediments followed by NIRF investigating the influence of SWNT coating and sonication conditions (Recovery after 4 extractions). Error is one standard derivation about the mean (n=3). Results reveal optimal sonication conditions: t_{sonic} = 10 min at 40 Watts. Increased sonication time or power did not improve the SWNT recovery, likely due to the increase in matrix component extraction (i.e. higher optical density of the sediment extracts). In this case the recovery is overestimated. When applying lower sonication power, the recovery showed a higher degree of variability (e.g. 30 Watts, 90 ± 15% recovery).

SWNT coating	**power input (Watts)**	**% Recovery** t_{sonic} = 10 min	**% Recovery** t_{sonic} = 60 min
SDC	55	141 ± 11	135 ± 12
SDC	36	90 ± 15	85 ± 14
SDC	39	81 ± 5	73 ± 2
SDC	22	59 ± 7	n.d.
GA	39	82 ± 21	n.d.
Pluronic-F127	39	Below DL	n.d.

n.d.=not determined

Table A.2. Extraction of SWNT from various estuarine sediments followed by NIRF analysis investigating the influence of sediment material, SWNT coating and SWNT material. Sediments were mixed for at least 14 days after SWNT spiking. The method was also used to verify the spiked SWNT concentration in two natural sediments prior to uptake experiments. In this studies SWNT with different coatings and different SWNT types were used. For different types of SWNT materials (except SG76) the spiked SWNT concentration could be verified, implying that the applied sediment spiking leads to a homogenous distribution of SWNT in the test sediment. As shown in standard addition experiments, recoveries for the extraction of SG76 SWNT from sediment were lower than for SG65 SWNT. Possible explanations include an inefficient spiking and/or higher affinity towards the sediment matrix.

Sediment	**SWNT type**	**Coating**	$m_{SWNT,spiked,sed}$ **(μg g^{-1})**	$m_{SWNT,extracted,sed}$ **(μg g^{-1})**	**% Recovery**
LIS	SG65	SDC	1	4.89 ± 0.9	489
LIS	SG65	SDC	10	6.02 ± 0.7	60
LIS	SG65	GA	10	8.1 ± 2.1	81
LIS	SG76	SDC	10	2.7 ± 0.8	27
LIS	CG100	SDC	10	6.9 ± 2.7	69
BBC	SG65	n/a [2]	1	0.6 ± 0.02	60
BBC	SG65	n/a [2]	10	12.6 ± 2.3	126
BBC[1]	SG65	n/a [2]	10	10 ± 0.5	100
BBC	CG200	n/a [2]	10	5.4 ± 0.8	54

[1] SWNT spiked sediment was exposed to benthic organism for 28 days.
[2] SG65 SWNT were dispersed in 2 w/v% SDC by ultrasonication and a dialysis step [35kDa cutoff] to remove unbounded SDC were done. After a short resonication step the resulting suspension is stable for the short time period of spiking.

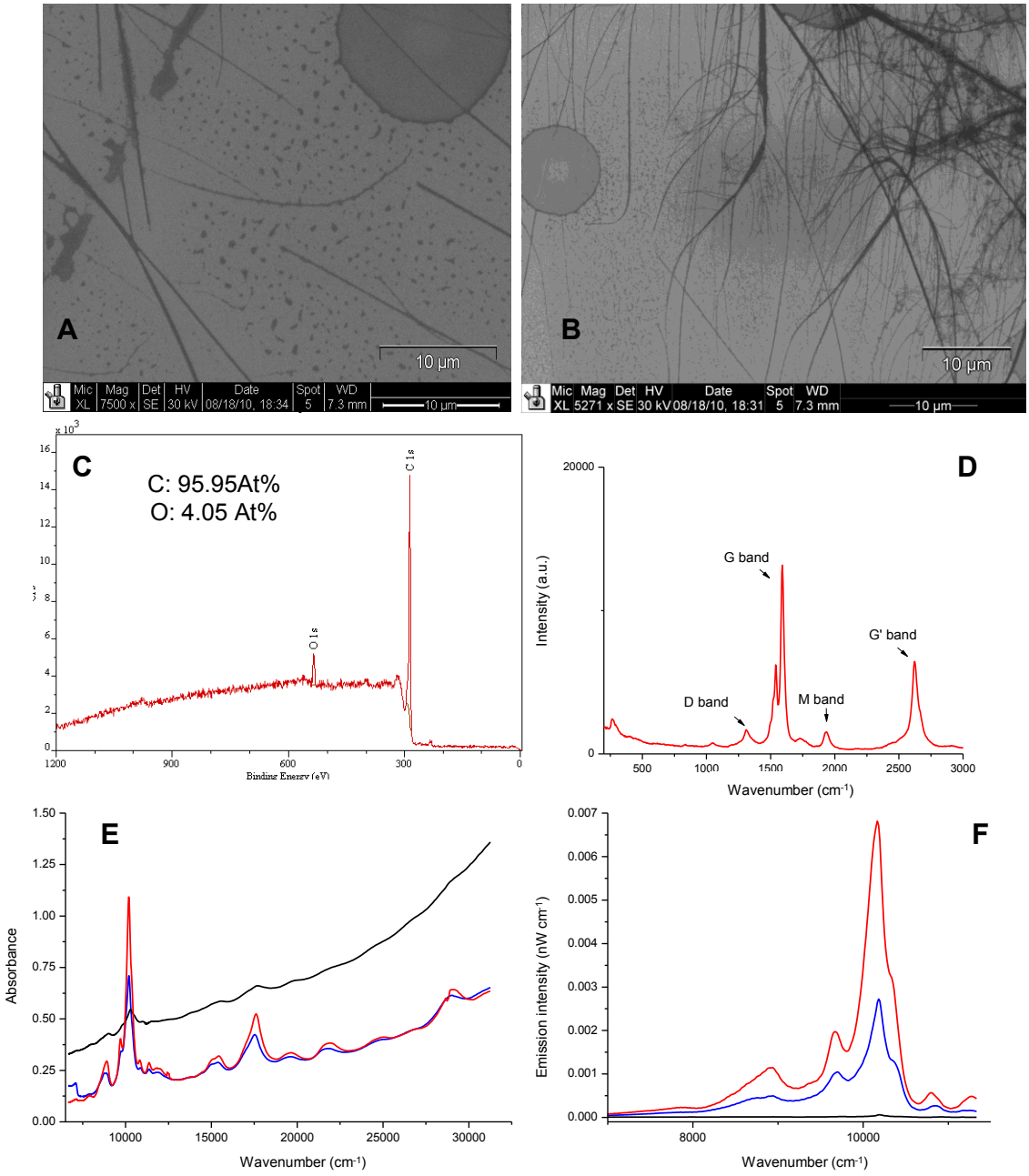


Figure A.1. Optical and spectroscopic characterization of SG65 SWNT.
SWNT SG65 characterization: SEM image (A,B) , XPS spectra (C) and Raman spectra at 632 nm (D). The characteristic G and D bands occurred at 1588 and 1321 cm-1. UV-Vis-NIRF absorbance spectra (E) and NIRF emission spectra (F) of SG65 SWNT in different dispersions (sodium benzylsulfate-SDBS (black), sodium cholate SC (blue) and sodium deoxycholate SDC (red)).

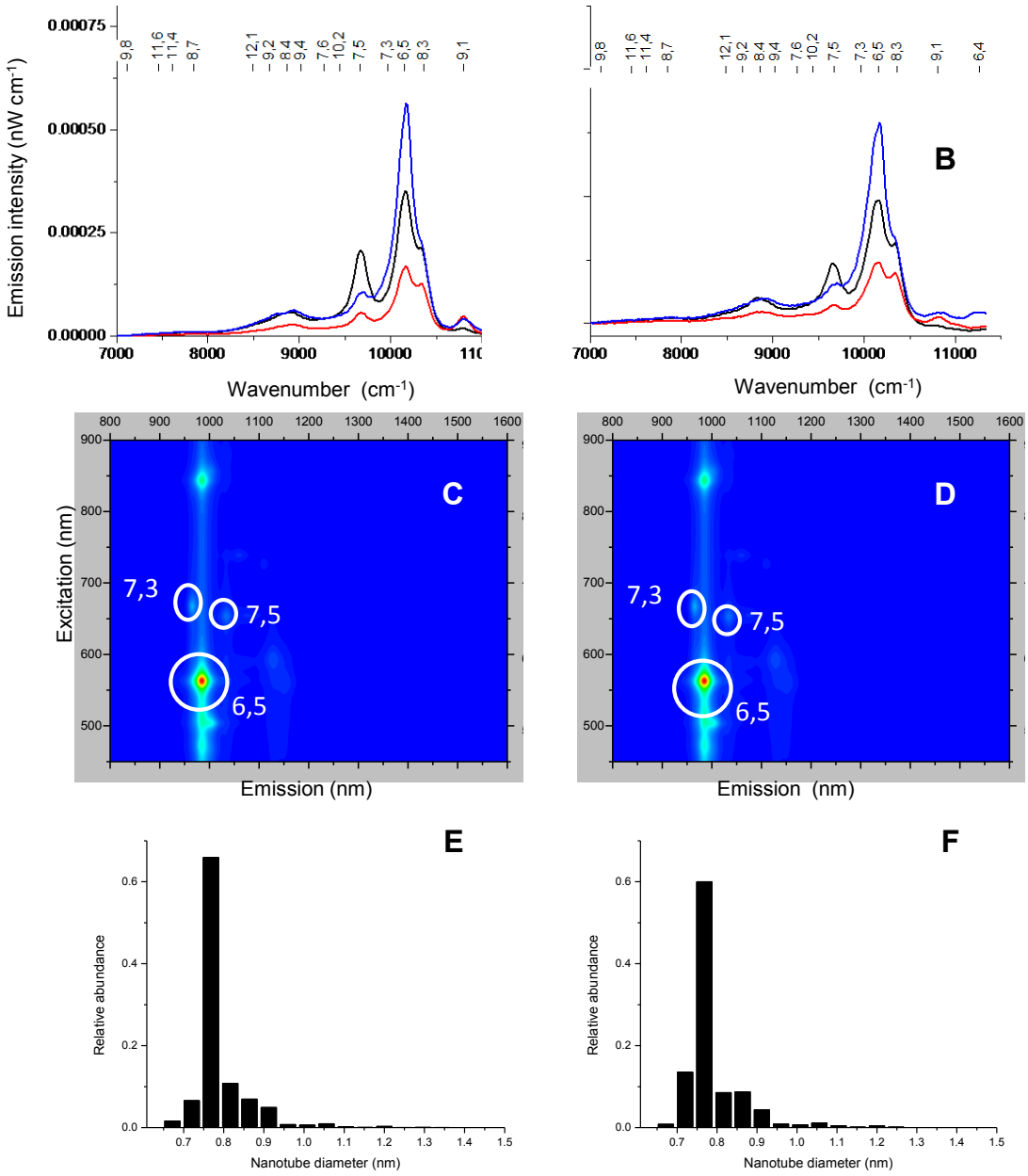


Figure A.2. Representative NIRF data from SG65 SWNT extracted from sediment samples. NIRF spectra (A, B) measured at three individual excitation wavelengths (638nm (black), 691nm (red), 782nm (blue), y-axis are on the same scale, 2nd x-axis: (n,m) species), excitation-emission contour plots (C, D, axis: excitation wavelength vs. emission wavelength) and diameter distribution histograms (E, F) of SG65 SWNT in 2% w/w SDC (1st column: A, C, E) and in BBC sediment extract (surfactant: 2% w/w SDC, 2nd column: B, D, F).

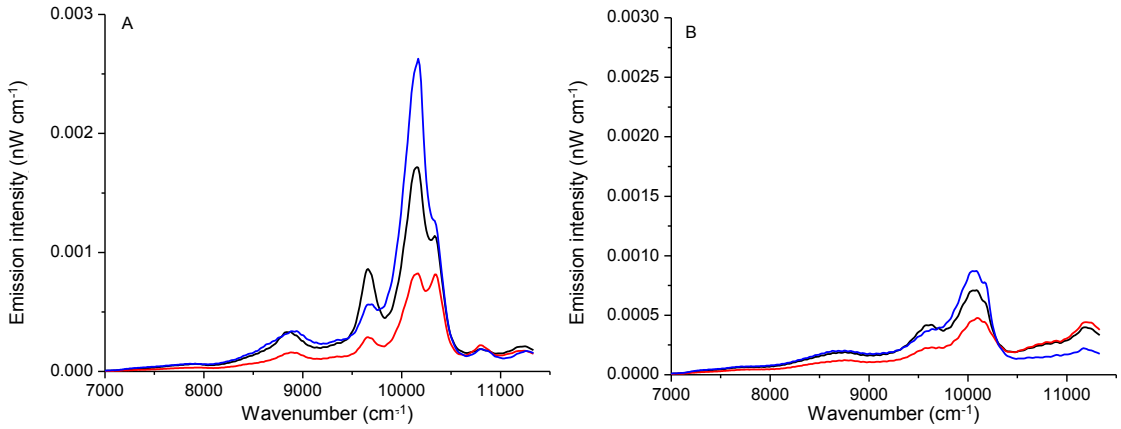


Figure A.3. Comparison of two surfactants on their ability to extract SWNT from estuarine sediment and stabilize SWNT in sediment extract.
Extraction of SG65 SWNT from BBC estuarine sediment with 2% w/v SDC (A) and 10% w/v TritonX-100 (B). NIRF spectra show the emission for each excitation wavelength (638 nm (black), 691 nm (red), 782 nm (blue) excitation wavelength) of the combined extracts: extracts 1 - 4 ($m_{SG65\ SWNT}$ = 20 μg). NIRF signal show highly resolved peaks in SDC, whereas peak broadening is observed in TritonX-100.

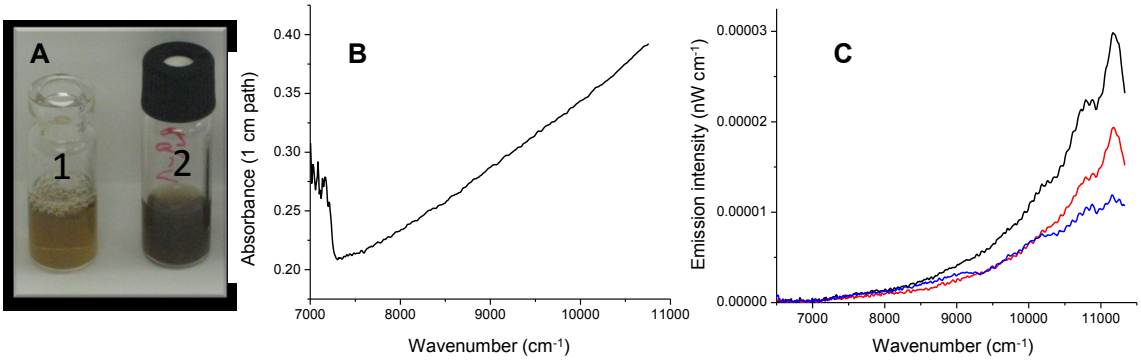


Figure A.4. Sediment extracts of BBC (A,1) and LIS (A,2) estuarine sediment in 2% w/v SDC were optically dense and opaque. NIR spectra of sediment extracts are characterized by an increase in absorbance (B) and fluorescence signal (C, 638 nm (black), 691 nm (red), 782 nm (blue) excitation wavelength) with increasing wavenumber when measured against 2% w/v SDC. Interferences due to internal filter effects are observed and revealed by an increase in NIRF signal above 9500 cm-1.

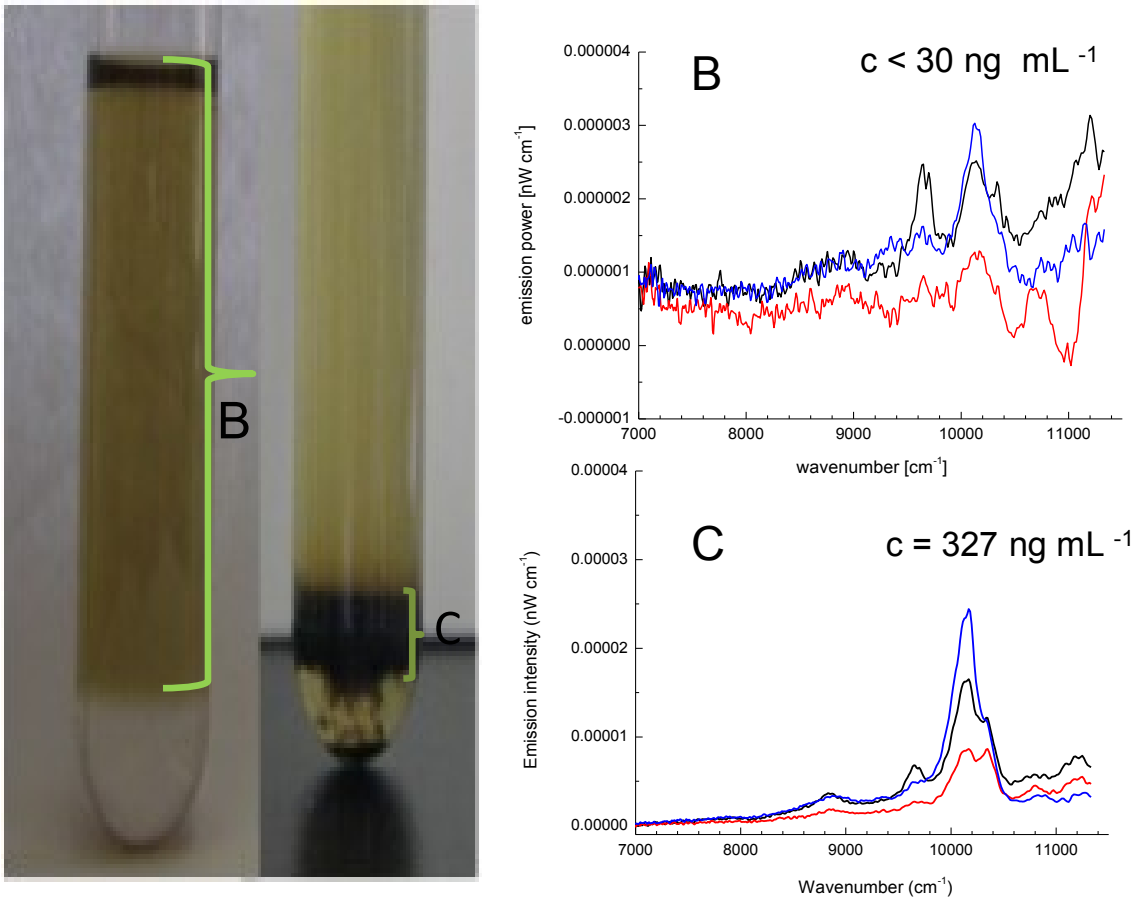


Figure A.5. SWNT sediment extract cleanup by ultracentrifugation.
The combined sediment extracts were transferred into an ultracentrifuge tube on a cushion of 60% (w/v) iodixanol (A). Results revealed that SDC-wrapped SWNT were separated from the sediment extract and concentrated in the layer above the iodixanol cushion by ultracentrifugation. NIRF-spectra of SG65 SWNT in combined extracts were measured before (B) and after the ultracentrifugation concentration step (C). Comparison of both NIRF spectra illustrated that the NIRF signal of SWNT in extracts can be improved by a factor of 10 after ultracentrifugation. Moreover, NIRF-signal of individual peaks is quantitatively enhanced and highly resolved indicating the increase in sensitivity.

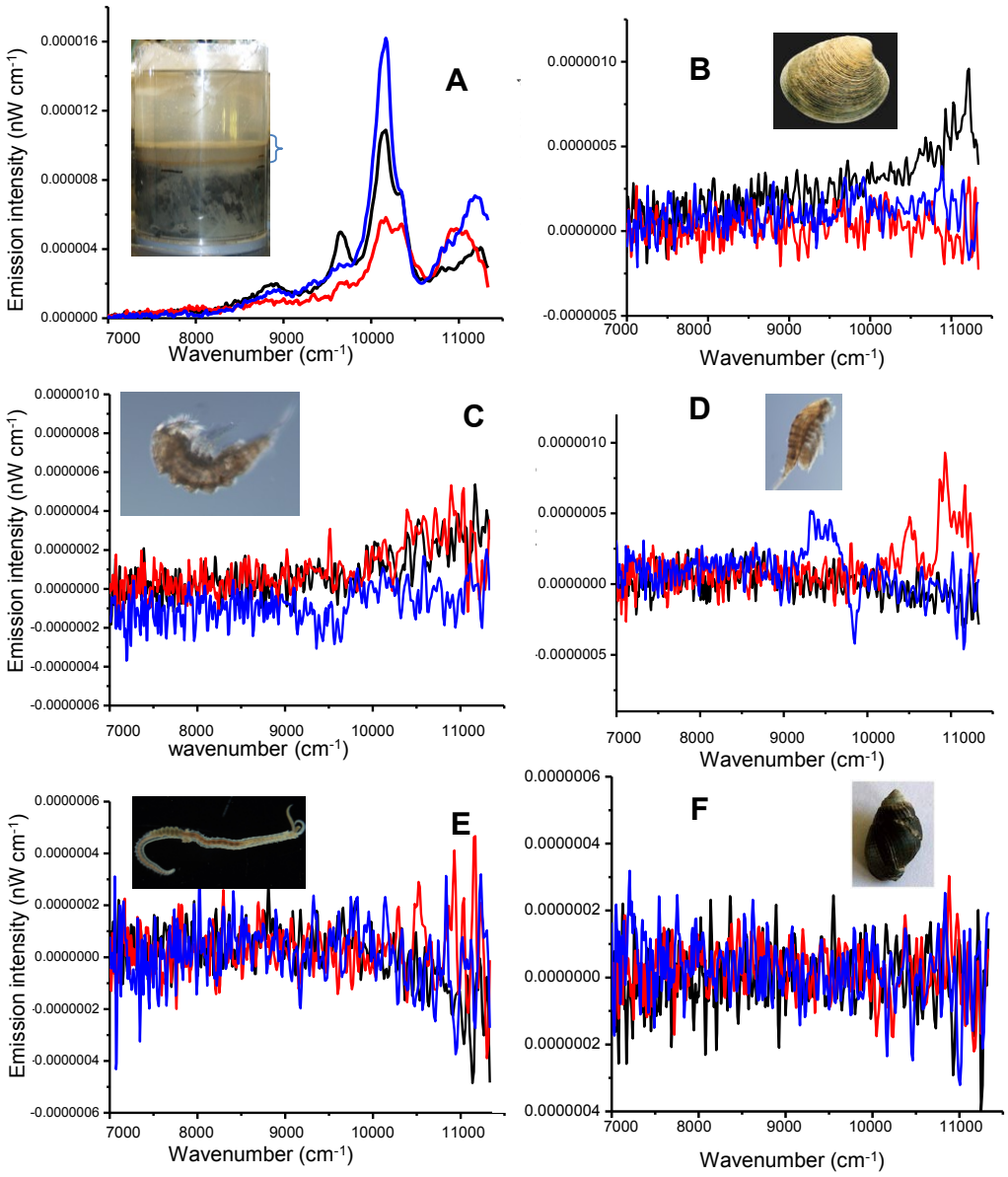


Figure A.6. Analysis and detection of SG65 SWNT in BBC sediments and organisms at the end of a 28 days exposure. Figures show the NIRF spectra for each excitation wavelength (638 nm (black), 691nm (red), 782 nm (blue) excitation wavelength) of the analyzed sediment and organism tissues. SWNT were detectable and quantifiable in spiked sediment layers by NIRF (A). No SWNT were detected in organism tissue. NIRF spectra of organism tissues do not show any SWNT characteristic features [clams *Mercenaria mercenaria* (B), copepod *Enhydrosoma propinquum* (C), copepod *Pseudobradya pulchella* (D), polychaete worm *Streblospio benedicti* (E) and snails *Nassarius obsoleta* (F)]

Appendix B. Bioaccumulation and Toxicity of Single walled Carbon Nanotubes (SWNT) to Benthic Organisms at the Base of the Marine Food Chain

B.1. Sediment and food amendment with SWNT

Deionized (DI) water, SWNT suspended in the carrier solution or the carrier solution alone (2% w/v SDC) was added directly to LIS sediment (0.26 g dry/g wet) and mixed manually. Bread and Butter Creek (BBC) sediment slurry was made using 1 g wet sediment for every 100 mL of 30‰ reconstituted seawater (RSW, concentrated 100‰ Narragansett Bay seawater + DI). Slurries were mixed on a stir plate overnight prior to addition of ^{14}C-SWNT. Sediments were amended by adding the ^{14}C-SWNT into the vortex and mixing for 30 minutes. All amended sediment samples were allowed to equilibrate on a roller mill at 4 °C in the dark for at least fourteen days prior to usage.

SWNT-amended algae was made fresh prior to each feeding. The volume of SWNT suspension needed was based on the algae culture cell count which can be correlated to the algae dry weight. The algae was mixed on a stir plate while the SWNT suspension or carrier control was added into the vortex and equilibrated for thirty minutes. *Artemia salina* was amended by measuring out the appropriate wet weight for feeding (approximately 0.7 mg wet weight/mysid) and adding 30‰ RSW and the appropriate volume of SWNT or carrier control solution.

Table B.1. Measured concentration of SWNT in the toxicity test sediments.

Nominal Concentration	SWNT-spiked sediment: measured concentration (ppm) ± standard error			
	SG65	SG76	CG100	OECD
0 ppm	ND	ND	ND	ND
0.1 ppm	ND	ND	ND	ND
1 ppm	4.89±0.54	NQ	NQ	ND
10 ppm	6.02±0.40	2.73±0.46	6.89±1.55	46.38±7.42

ND=Not detectable, NQ=Not quantifiable

Table B.2. Measured concentration of SWNT in the bioaccumulation test sediment and food sources.

Nominal Concentration	Measured concentration (ppm) ± standard error		
	SG65-spiked sediment	SG65-spiked *C. meneghiniana*	SG65-spiked *A. salina*
0 ppm	ND	ND	ND
0.1 ppm	ND	--	--
1 ppm	4.89±0.54	--	--
10 ppm	6.02±0.40	473.9±179.6	5.22±0.73

ND=Not detectable, --=not applicable

Table B.3. Concentration of ^{14}C-SWNT in spiked sediment and algae.

Treatment	Time (days)	Matrix	Average µg [^{14}C]SWNT/ g dry	Standard Error
Control	0	Sediment	0.044	0.044
10 ppm	0	Sediment	8.4	0.1
100 ppm	0	Sediment	72	2
Control	3 days/ week	Algae	0	0
10 ppm	3 days/ week	Algae	3.88	0.47
100 ppm	3 days/ week	Algae	33.9	4.4
Control sediment+ control algae	28	Sediment	0.00	0.00
Control sediment+ 100 ppm algae	28	Sediment	0.85	0.08
10 ppm sediment+ 10 ppm algae	28	Sediment	1.6	0.3
100 ppm sediment+ control algae	28	Sediment	13.8	1.9
100 ppm sediment+ 100 ppm algae	28	Sediment	16.6	2.2

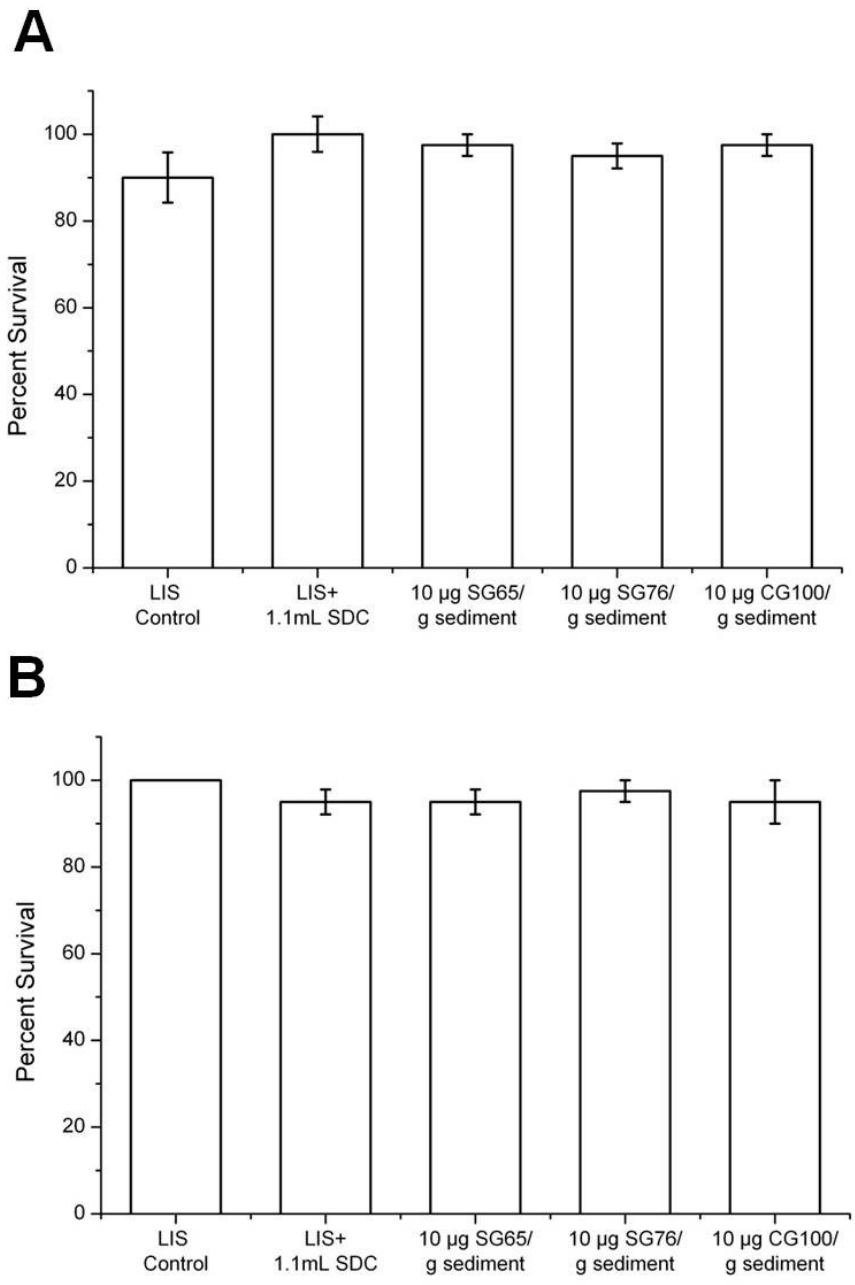


Figure B.1. Percent survival of mysid, *A. bahia* (A), and amphipod, *A. abdita* (B), at the highest concentration (10 µg/g) of SWNT amendment to sediment. No significant mortality was observed at any concentration tested (0.1 µg/g, 1 µg/g, 10 µg/g). Averages and standard error are presented with a sample size of n=6.

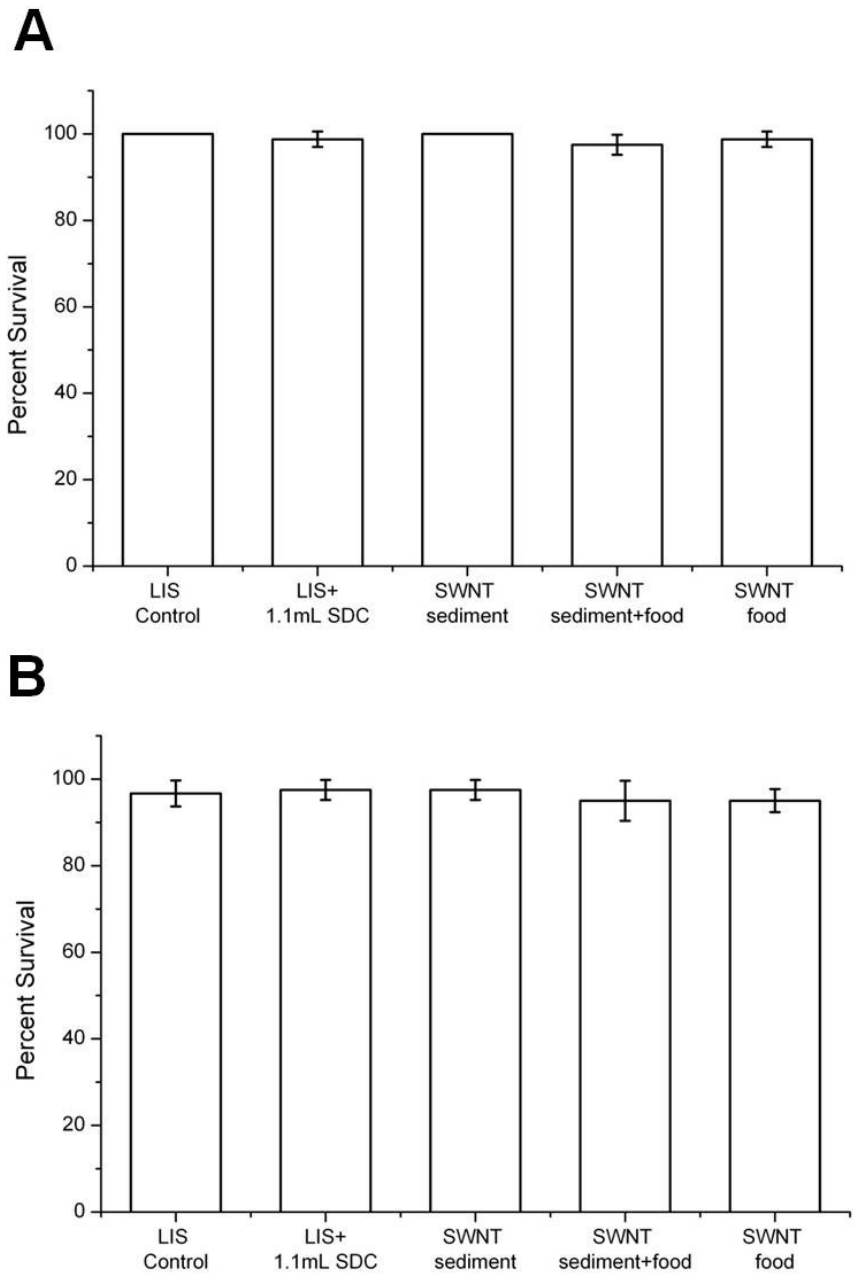


Figure B.2. Percent survival of mysid, *A. bahia* (A), and amphipod, *A. abdita* (B), at a concentration of 10 μg SG65/g sediment and/or food. No significant mortality was observed. Averages and standard error are presented with a sample size of n=6.

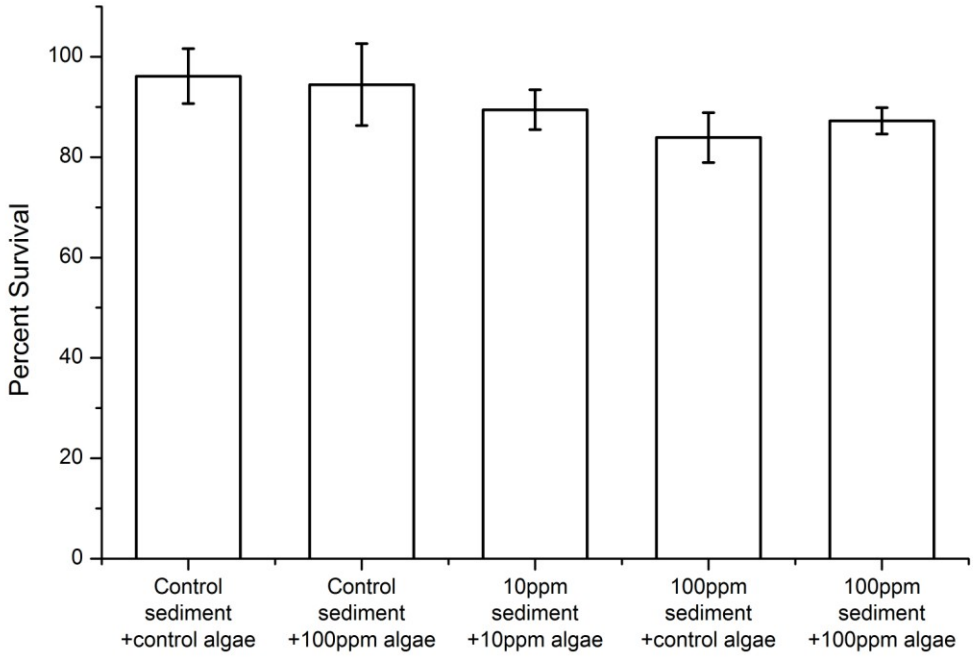


Figure B.3. Percent survival of *Leptocheirus plumulosus* after a 28-day exposure to 10 μg SWNT/g or 100 μg SWNT/g sediment and/or food. No significant mortality was observed. Averages and standard error are presented with a sample size of n=6.

Appendix C. Effects of Single-walled Carbon Nanotubes on the Bioavailability of PCBs in Environmentally Contaminated Sediments

Table C.1. Average freely dissolved interstitial water (ITW) polychlorinated biphenyl (PCB) concentrations (ng/L) measured by polyethylene (PE) passive samplers in each New Bedford Harbor (NBH) sediment treatment with and without single-walled carbon nanotube (SWNT) or coconut charcoal (CC) amendment. n = 1-4, *p<0.05 compared to unamended control from the same treatment.

Average PCB concent-ration (ng/L)	Treatment											
		25% NBH				50% NBH		75% NBH		100% NBH		
	LIS + Gum Arabic	Un-amended control	+ 1 mg/g SWNT	+ 10 mg/g SWNT	+ 10 mg/g CC	Un-amended control	+ 1 mg/g SWNT	Un-amended control	+ 1 mg/g SWNT	Un-amended control	+ 1 mg/g SWNT	+ 1 mg/g CC
8	55.2 (44.9)	2960 (391)	1670 (546)*	114 (6)*	14.9 (1.2)*	2640 (1130)	2990 (468)	4340 (365)	4130 (909)	5500	2650 (259)	1840 (741)
18	61.4 (35.2)	3390 (627)	1940 (685)*	339 (21)*	178 (30)*	4960 (479)	2750 (153)*	4210 (1020)	5580 (408)	5260	2940 (815)	5440 (1490)
28	16.3 (17.7)	1950 (321)	1400 (145)*	169 (19)*	26.6 (4.1)*	2980 (622)	2030 (19)	2630 (83)	2960 (148)*	3610	1790 (149)	2900 (128)
52	4.71 (4.65)	512 (103)	380 (42)	81.0 (8.0)*	125 (9)*	810 (205)	570 (28)	741 (22)	807 (22)*	1030	485 (33)	916 (36)
44	1.80 (2.02)	152 (16)	110 (13)*	27.0 (3.5)*	33.3 (5.3)*	230 (29)	153 (10)*	214 (25)	253 (7)	265	162 (10)	279 (47)
70	0.56 (0.23)	47.1 (9.2)	27.6 (7.6)*	5.10 (1.03)*	5.85 (1.06)*	67.9 (15.6)	47.7 (4.3)	59.9 (8.1)	69.9 (3.8)	116	45.9 (5.0)	86.6 (13.6)
66	0.15	50.5	41.9	6.09	5.24	81.5	51.9	64.4	78.4	108	47.2	80.9

	(0.21)	(9.6)	(6.9)	(0.80)*	(0.56)*	(29.5)	(3.2)	(2.5)	(4.0)*		(2.2)	(4.2)
101	0.48 (0.42)	31.5 (0.7)	22.4 (3.6)*	4.55 (1.87)*	14.9 (3.0)*	40.9 (14.4)	32.4 (3.0)	56.9 (3.9)	63.7 (13.5)	86.4	41.6 (3.1)	49.7 (39.9)
99	0.39 (0.36)	34.6 (9.1)	23.9 (1.8)	6.23 (0.54)*	11.9 (1.2)*	52.6 (14.4)	38.1 (4.4)	49.1 (3.1)	62.8 (7.1)*	74.8	36.2 (2.6)	63.5 (2.2)
81												
77		1.80 (0.31)	2.29 (0.56)	0.49 (0.11)*	0.51 (0.11)*	3.25 (1.86)	2.48 (0.57)	2.63 (0.12)	3.14 (1.37)	5.37	2.39 (0.51)	4.97 (0.25)
110	0.62 (0.45)	52.5 (11.3)	45.3 (4.8)	11.1 (0.89)*	18.9 (1.8)*	83.9 (28.8)	61.4 (4.9)	75.8 (2.64)	98.0 (7.6)*	107	52.7 (2.1)	93.0 (2.1)
123									13.6 (23.5)			
118	0.16 (0.23)	25.8 (5.1)	26.7 (2.5)	4.94 (0.40)*	7.23 (0.76)*	43.8 (24.2)	27.6 (2.9)	34.9 (2.5)	45.8 (5.5)*	53.7	23.9 (1.1)	47.1 (1.8)
114												
153	0.14 (0.19)	17.3 (5.6)	11.9 (1.2)	2.98 (0.76)*	8.11 (0.51)*	22.8 (8.1)	19.3 (3.3)	24.7 (4.2)	33.3 (7.7)	35.4	15.5 (1.2)	27.6 (0.9)
105		1.08 (0.23)	0.83 (0.02)	0.21 (0.07)*	0.40 (0.04)*	1.44 (0.63)	1.06 (0.09)	1.27 (0.16)	1.78 (0.47)	2.19	0.93 (0.14)	1.36 (0.11)
138		11.1 (4.0)	8.07 (0.92)	1.94 (0.18)*	5.42 (0.83)*	15.6 (4.7)	13.2 (1.9)	15.7 (2.4)	20.0 (4.7)	22.6	9.44 (0.44)	20.3 (1.2)
126												
156		1.16 (0.10)	0.91 (0.19)	0.31 (0.06)*	0.58 (0.06)*	1.42 (0.46)	1.15 (0.11)	1.89 (0.26)	2.00 (0.41)	2.17	1.04 (0.15)	1.78 (0.01)
157		1.02 (0.88)	0.48 (0.06)		0.45 (0.04)	1.26 (0.39)	1.09 (0.06)	1.48 (0.17)	2.08 (0.53)	2.15	1.04 (0.13)	1.58 (0.10)
180		1.74 (1.55)	1.01 (0.89)	0.26 (0.37)	1.06 (0.26)	2.58 (1.12)	1.85 (0.02)	2.45 (0.44)	20.94 (0.27)	3.39	2.32 (0.33)	2.81 (0.46)

169												
170			0.99 (0.40)*		0.66 (0.15)*	1.24 (0.84)	1.36 (0.66)	2.71 (0.92)	2.17 (1.12)	2.20	1.45 (0.33)	2.23 (0.22)
189												
206												
Total	1410 (1070)	9240 (520)	5710 (1350)*	774 (61)*	459 (32)*	12000 (1600)	8800 (535)	12500 (1420)	14200 (1180)	16300	8310 (943)	11900 (2050)

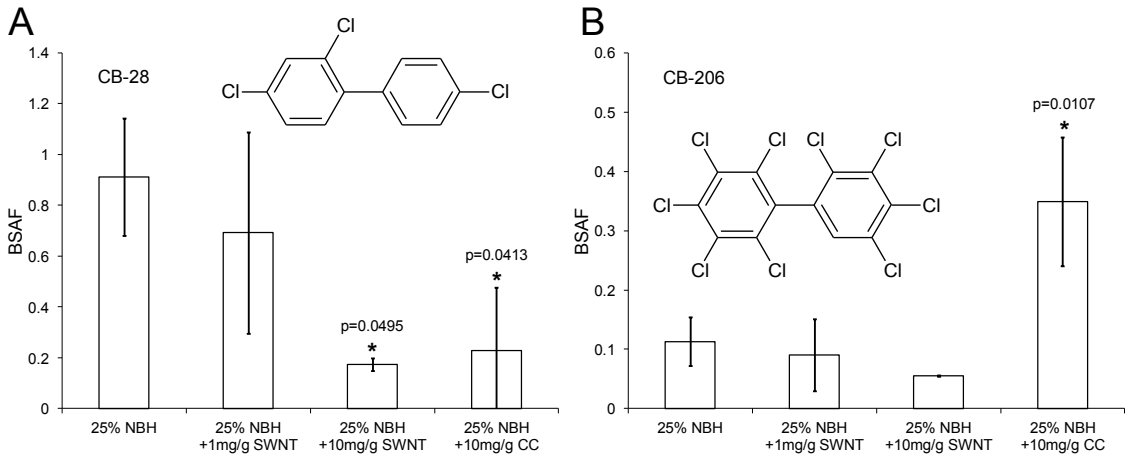


Figure C.1. Statistically significant ($p<0.05$) biota-sediment accumulation factors (BSAFs) for congeners (A) CB-28 and (B) CB-206 in the 25% NBH sediment treatment not yet illustrated.

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Biography

Ashley Nicole Parks was born in Tarzana, California on March 21st, 1986 to Keith and Denice Parks. She has one younger sister, Heather Parks. Ashley attended the University of San Diego, where she received her Bachelor of Arts degree in Biochemistry in May 2008. She started graduate school in August 2008 in the Chemistry and Biochemistry Department at the University of South Carolina, where she joined the laboratory of Dr. P. Lee Ferguson. In the summer of 2009, the Ferguson group moved to Duke University, and Ashley continued in the Nicholas School of the Environment and received a certificate from the Integrated Toxicology & Environmental Health Program.

Publications

Parks AN, Portis LM, Schierz A, Washburn KM, Perron MM, Burgess RM, Ho KT, Chandler GT, Ferguson PL. 2013. Bioaccumulation and toxicity of single walled carbon nanotubles (SWNT) to benthic organisms at the base of the marine food chain. *Environmental Toxicology and Chemistry*

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Yang S, **Parks AN**, Saba SA, Ferguson PL, Liu J. 2011. Photoluminescence from Inner Walls in Double-Walled Carbon Nanotubes: Some Do, Some Do Not. *Nano Lett* 11:4405-4410.

Honors and Awards

Student Travel Award, ICEEN, September 2012, Banff, AB, Canada
Student Services Contract, U.S. Environmental Protection Agency
EP11D000011: Nanomaterials Analytical Support, Oct 2010-2012
EP09D000098: Nanotechnology Research Support, Jan 2009-Aug 2010
Student Platform Presentation Award, 3rd place, and Student Travel Award, ICEEN, August 2010, Clemson, SC, USA