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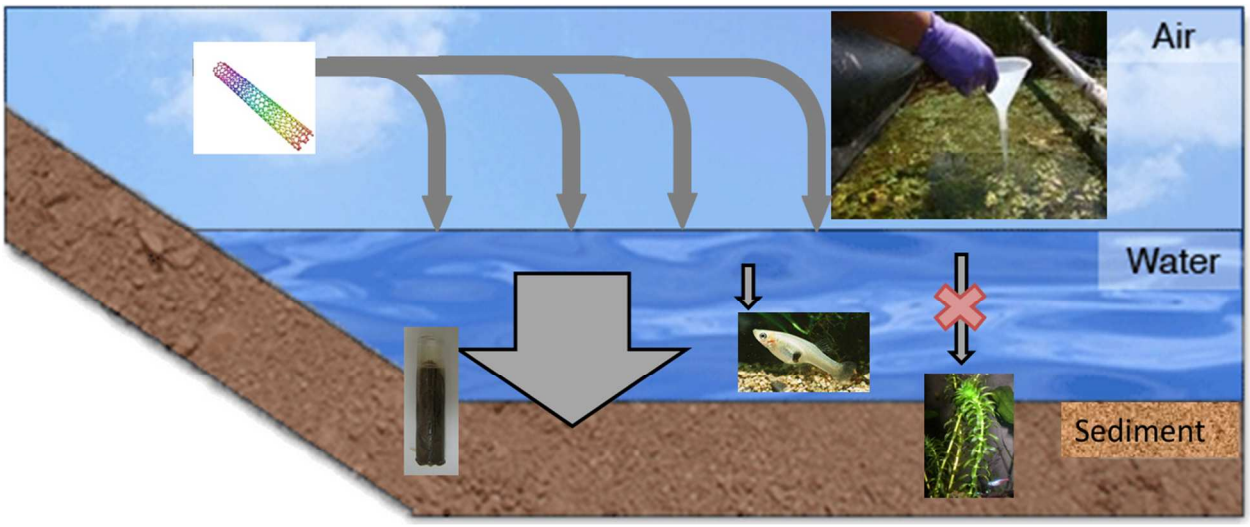
Fate of Single Walled Carbon Nanotubes in Wetland Ecosystems

Ariette Schierz[1,2], Benjamin Espinasse [1,2] , Mark R. Wiesner [1,2], Joseph H. Bisesi[3], Tara Sabo-Attwood[3], and P. Lee Ferguson[1,2,4,*]

[1]*Department of Civil and Environmental Engineering, Duke University, Durham, NC, USA.*
[2]*Center for the Environmental Implications of NanoTechnology (CEINT), Duke University, Durham, NC, USA.*
[3]*Department of Environmental and Global Health, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA.*
[4]*Nicholas School of the Environment, Duke University, Durham, NC, USA.*

[*]corresponding author: lee.ferguson@duke.edu



NanoImpact Statement

Single-walled carbon nanotubes (SWNTs) are one of the most important classes of engineered nanomaterials. While production and usage is steadily increasing, our current knowledge on their environmental fate and toxicity is still very limited. In order to properly assess the risk SWNTs might posses after an unintentional release into the environment, long-term behavior and distribution between different compartments must be evaluated in a relastic complex natural system. This is the first study evaluating the short and long-term behavior of SWNTs in a wetland ecosystem. We tracked the SWNT concentration in different environmental compartments over time after a pulse addition event into a outdoor wet-land mesoscosm simulating a spill into the aquatic environment. More than 99% of the dosed SWNTs were quickly removed from the water compartment where they were dosed. The major portion resided at the sediment surface. Little evidence was found to indicate uptake into mosquitofish and biota, respectively. The study shows that SWNTs are very persistent in natural systems and sediments act as major sinks for SWNTs. The distribution of SWNTs in the environment is mainly governed by their partioning towards sediments limiting its mobility and controlling its bioavailibity. Implications of this study are important mainly for near source emissions, spill situations and ecotoxicity tests.

Abstract

We report here the first studies addressing fate and transport of single walled carbon nanotubes (SWNTs) in aquatic mesocosms. The experimental design was structured to study the impact of nanomaterials within a tightly controlled and highly instrumented wetland ecosystems (aka mesocosm) and to address questions including fate and transport, effect on community structure, effects on biogeochemical function, and effects on productivity of the ecosystem. We added well-dispersed CoMoCat SWNTs ($c_{SWNT,0}$ = 2.5 mg L^{-1}) to the water column of a wetland mesocosm and examined the resulting phase distribution over time. Rapid settling of SWNTs from the water column was observed within a period of 2 days ($C_{w,t}/C_{w,0}$ < 0.01) after spiking. Samples from all mesocosm compartments (e.g. aquatic/semi aquatic plants, biofilm, mosquitofish and sediment) were analyzed to evaluate the transport and fate of SWNTs in the ecosystem. SWNTs were quantified in organism and sediment extracts using near-infrared fluorescence spectroscopy (NIRF). This technique can be used to quantitatively detect SWNTs in sediment and biotic matrices at environmentally relevant concentrations (MDL_{water} 5 µg L^{-1} $MDL_{sediment}$ 0.5 µg g^{-1} MDL_{biota} 5 µg g^{-1} wet weight) and qualitatively characterize SWNT samples before and after the studies. Results indicated that rapid aggregation and settling of SWNT resulted in accumulation of SWNT in surficial sediment. Sediment concentrations were spatially variable across the mesocosm, and thus estimates of SWNT mass balance within the mesocosm ranged from 7 – 48%. No bioaccumulation of SWNT in aquatic plants or vertebrates was observed over the 10-month incubation. However, NIRF imaging analysis suggested that mosquitofish ingested SWNT-laden particles but that burdens of SWNTs were confined to gut contents and may have been rapidly eliminated.

Keywords: Single-walled carbon nanotubes, bioaccumulation, mesocosm

Introduction

Single wall carbon nanotubes (SWNTs) possess exceptional physicochemical, optical and mechanical properties which results in a wide variety of potential applications including microelectronics, energy storage, drug delivery, environmental applications, and composite construction materials [1]. Recent developments are centered on developing new applications and products by using these unique properties of SWNTs. However, there are questions regarding the potential for SWNTs to exert human and environmental health effects if they are released, transported, and accumulated within the environment. With the increasing number of technological and commercial applications and steady production increase, emission of SWNTs into the environment via waste water discharge and/or point source emission from the manufacturing industry is likely [2, 3].

Laboratory studies of SWNT environmental impacts have focused on characterizing the fate, transport and ecotoxicity of SWNTs under well-controlled laboratory settings and within relatively short-time frames/scales up to months [4-8]. However, there is a clear need to assess the fate of SWNTs in realistic, field-relevant aquatic systems in order to inform risk-assessment of these materials, perform life-cycle analysis and design waste management [9,10].

Modeling approaches have estimated the expected carbon nanotube concentrations in the aquatic environment and sediments to be in the range of ng L^{-1} to perhaps low μg g^{-1} based on estimates of production, disposal, and persistence [2, 3, 11].There are many factors, which may control the fate, distribution, and bioavailability of SWNTs in the environment, including the location of release, biological and abiotic transformation, and transport dynamics. Modeling and experimental work also predict limited mobility of SWNTs in porous media due to homo- and heteroaggregation and sediments as the major long-term sink for SWNTs [12, 13 14 11 15, 16]. Several studies have reported little-to-no uptake of SWNTs into tissues of benthic organisms [8 4, 17, 18] or fish [19] exposed to SWNT amended sediments over short exposure periods up to one month,

however bioaccumulation studies have not been performed under realistic, field-mimetic conditions.

Knowledge about the behavior of SWNTs in terrestrial soil, the water column, and subaquatic sediment in fresh water mesocosm under realistic conditions or over longer time scales of several months to years is very limited [20]. A recent published study by Veleboer et al. evaluated the long term in situ effect on the bentic community composition of multi-walled carbon nanotubes (MWNTs) in sediments in the concentration range from 0.002-2 g kg^{-1} over a period of 15 months [20]. They observed differences between the benthic community structures exposed to MWNTs even at the lowest concentration of 0.002 g kg^{-1} and concluded the benthic community may have been more sensitive to MWNTs that to activated carbon. While this study evaluated long-term effects of MWNTs on a field-relevant benthic community structure, it did not consider/evaluate the fate and behavior of MWNTs in sediments.

Heretofore, detailed fate and transport studies of carbon nanotubes in natural systems has been limited by the lack of analytical methods available for detecting SWNTs and other carbon nanomaterials in complex environmental samples at environmentally relevant (e.g. < ppb) concentrations [21, 22]. Recently, we have developed and implemented NIRF spectroscopy-based methods to qualitatively and quantitatively characterize semi-conducting SWNTs in environmental matrices e.g. estuarine sediments, natural waters, and benthic organisms, and fish tissues [6, 8, 19]. These methods typically use a surfactant-assisted high-power sonication step to separate SWNTs from sample matrix as well as to exfoliate SWNT in suspension. Detection limits in the lower ng g^{-1} in sediments or μg L^{-1} in aqueous samples were achieved. Using these techniques, we have previously found that CoMoCat SWNTs could be extracted from estuarine sediments exposed to benthic organism after 28 days in laboratory studies [6].

In the present work, we have, for the first time, utilized a constructed wetland mesocosm to examine the fate of carbon nanotubes in the aquatic environment. Herein, we have utilized our

sensitive and selective NIRF-based analytical methods to perform the first comprehensive assessment of SWNT fate in a highly complex aquatic ecosystem. These studies were performed within the context of extensively instrumented wetland mesocosm experiments, the construction and design of which has been described in detail previously [23] [24]. The mesocosms we utilized consisted of a sloped bed that allows for the existence of both an aquatic/subaquatic environment and a terrestrial environment in order to mimic an emergent freshwater wetland. The system is open to weather and therefore allows for the volume of water and the terrestrial/soil compartment to vary with rainfall as may observed in natural wetland environments, and has been used extensively to study ecosystem responses to perturbations of environmental conditions. Two plant species *Elodea Canadensis* and *Lemna minor* as model species were placed representing typical species in emergent freshwater wetlands were planted in the mesocosm to determine the potential of these plants to take up SWNTs released into the environment. Our objectives in this work were to (1) assess the removal of SWNTs from the water column of the mesocosm after a single dosing event and subsequently to track the physical distribution of SWNTs in mesocosm compartments, and (2) to examine the potential for uptake and bioaccumulation of SWNTs in aquatic flora and fauna resident within the wetland mesocosm.

Experimental – Materials and Methods

Mesocosm design: Mesocosm construction, design, and operation have been described in detail previously [23]. Briefly, the mesocosms consisted of a rectangular box constructed of treated lumber and were located outdoors in a clearing of the Duke Forest in Durham, NC and were built in August 2009. The mesocosm box dimensions were 3.66 m (length) x 1.22 m (width) x 0.8 m (depth) and the bed was sloped at 13 degrees to simulate various humidity and redox gradients in the system. The interior of the mesocosm was laid out with a potable water-

grade quality geotextile (0.45 mm reinforced polypropylene, Firestone Specialty Products, US). The system was watertight and water levels therefore varied throught the experiment naturally due to input from rain events, losses from evaporation, transpiration, and sample drawing. A blend of soil (Soil&Sand, Durham, NC, USA) was designed to match soil specification with a sand content of 64%, clay 10% slit 26% and a Loss on Ignition of 5.1%. The mesocosm was filled with this blended soil forming a uniform 21 cm layer of soil along the mesocosm. Groundwater extracted from a well at the site in the Duke forest was used to establish an initial water level of 19 cm within the mesocosm, and then the water from rain events was allowed to maintain the water level in the boxes. Key water quality parameters are water hardness between 69 – 80 mg L^{-1} , DOC concentration between 11 -18 mg L^{-1} and pH of ~ 6.5-7.5.

The initial planting of flora within the mesocosm occurred 148 days prior to dosing, using terrestrial species ecologically relevant to the local ecosystem with live plugs (Mellow Marsh farm, Siller City,NC) of *Lobelia elongate, Carex Lurida, Panicum Virgatum Juncun effusus* and two aquatic species *Elodea Canadensis* and *Lemna minor*. Eastern mosquito fish *(Gambusia holbrooki)* were introduced at day 19 post dosing to avoid possible acute toxicity due to high initial SWNT concentrations in the aqueous phase. All experiments with *G. holbrooki* were conducted in a manner consistent with the ethical and legal guidelines of Duke University and the USA pertaining to the use of vertebrate animals in scientific research. The research protocol (#A214-10-08) was reviewed and approved by the Duke Institutional Animal Care and Use Committee (IACUC). The SWNT-dosing was performed after the ecosystem had reached stable conditions i.e.: when turbidity and pH (SI-Figure1) were stable with time (and rain events) and when all plants were well rooted and established.

SWNT material: The SWNT used in this study were CoMoCat SG65 SWNT (lot # 000-0032, SouthWest NanoTechnologies Inc. SWeNT, OK, USA). These SWNT were produced by chemical vapor deposition with a cobalt-molybdenum catalyst [25]. All materials had a carbon

content > 90% by weight and relative purity of >90% [25]. SWNT were used as received without any further purification. SG65 SWNTs were characterized by NIRF and optical absorption spectroscopy (diameter and chirality determination), SEM, Raman spectroscopy (SWNT quality) and XPS (surface functionalization); these information are provided in Schierz et al. [6]. SWNT SG65 suspensions were prepared at 1 g/L in 0.5% w/v Gum arabic by sonicating 100 mg SG65 SWNT in 100 ml 0.5% w/v Gum Arabic for 50 min at 50 Watt power input (0.5 inc. tip**,** Sonifier 450, Branson Ultrasonics, Danbury, CT, USA) in a salt-water ice bath. The day before exposure total of 10 batches were prepared and combined to provide a total volume of 1 L of suspension for dosing. One hour before dosing the combined suspension was retreated for 30 min by ultrasonication forming a SWNT suspension which was stable against sedimentation long enough for dosing the mesocosm as described below.

Mesocosm Dosing: The experiment was initiated on August 16, 2010 by dosing SG65 SWNT suspended in 0.5% gum arabic (Fisher Scientific Pittsburgh, PA) to the water column.

The water volume of the mesocosm was adjusted to 370 L prior to dosing in order to fill the box by half (i.e. to achieve roughly the same terrestrial and submerged surface area). SWNT suspension (920 ml) was dispersed evenly over 10 mins by slowly pouring the suspension into a funnel while maintaining the funnel tip under the water surface. A total SWNT mass of 920 mg were added in a single pulse at t_0, for a nominal concentration of 2.5 mg L^{-1} in the water column.

One control mesocosm without SWNT exposure was prepared and treated identically to the SWNT-dosed mesocosm, with the exception of the dose.

Monitoring of water pH, temperature, turbidity and water level were performed in the centre of the water column at variable time intervals throughout the experiment using a multiparameter probe (YSI556, YSI, Yellow Spring, Ohio) (Supporting information, Figure SI-1).

198 SWNT distribution in the different environmental compartments was analyzed by NIRF
199 spectroscopy. In this study we explore the capability of this novel analytical method NIRF
200 spectroscopy in identifying/characterizing qualitative and quantitative SWNT before and after
201 exposure. Also samples retrieved from the mesocosm were analyzed for SWNTs metal catalyst
202 impurities -Co and Mo- as a second fingerprint of CoMoCat SWNTs to complement
203 identification. Co and Mo concentration were determined after thermal treatment followed by an
204 acid digestion. Both methods take advantage of characteristic features of CoMoCat SWNTs.

205 **Determination of SWNT concentrations in the water column**

206 Water samples of 20 mL were withdrawn at approximately the middle of the mesocosm (~ 10
207 cm depth) at discrete time points throughout the experiment. Samples were stored at 4 C until
208 analysis. Water samples were subsampled for triplicate measurement and ultracentrifuged at
209 207,570 x g for 5.5 hours at 22°C to concentrate SWNTs in a pellet. The top 19.5 mL of
210 supernatant was removed carefully by pipetting and the absence of SWNT was verified by direct
211 NIRF analysis. The pellet (containing any SWNT materials present in the sample) was
212 suspended in 2% w/v% solution sodium deoxycholate (Acros, as sodium deoxycholate salt
213 SDC, 99) by ultrasonication (50 watts amplitude) for 10 minutes at 4°C. The surfactant-
214 dispersed sample was then centrifuged at 17,860 x g for 30 minutes at 22°C. The supernatant
215 was analysed for SWNTs as previously described by Schierz et al. using an NS1
216 NanoSpectralyzer® (Applied NanoFluorescence, Houston, TX) [6].

217 **SWNT in sediment traps and sediment cores**

218 Sediment traps (tubes $d_{opening}$ = 5 mm) were placed on the sediment surface prior to SWNT
219 dosing and subsequently removed at 1, 2 and 28 after SWNT dosage. Sample preparation prior
220 to NIRF analysis included ultracentrifugation for concentration, surfactant assistant
221 ultrasonication and pre-centrifugation as described above for water samples.

Sediment cores were collected after 8, 10 and 12 months. Three sampling locations were chosen randomly within the aquatic and terrestrial compartment. Figure 3 depicts sampling locations for the June 2011 sampling event (10 months). Cores were collected using polypropylene tubes with various diameters ($d_{i,1}$= 1.3 and $d_{i,2}$=2.6 cm) by pushing them into either the aquatic or the terrestrial compartment, the open side was closed with a plunger under water, the sample was removed and immediately flash frozen before transporting to the laboratory. Prior to analysis, the cores were extruded in the laboratory and sectioned into slices of 1 cm (water column, 0-1 cm, 1-2 cm, 2-3 cm and > 4 cm).

SG65 SWNT concentration in sediment was determined as described previously [6]. Briefly, whole slices were homogenized and washed twice with 8 ml DI water. Subsequently, the slurry was centrifuged 15 min at 1880 x g to remove the water phase. Then 3 mL 2 %w/v SDC were added to the sediment. This slurry was sonicated at high power (power input of 40 W) for 10 minutes followed by a centrifugation step (17,860 x g for 10 min). The supernatants of 4 sequential extraction steps were combined, measured and quantified by NIRF spectroscopy. Sediment samples from the control mesocosm treatment were treated using the same procedure and used as spectral references for NIRF analysis (after verifying absence of SWNT-derived signals from these samples through NIRF spectroscopy). Sediment dry weight was determined after drying a portion of sediment at 105º C for 48 h.

Sub-sets of sediment core samples were also acid-digested and extracts were analysed for Co and Mo impurities/metal catalyst residues of SG65 SWNTs by ICP-MS. These analyses were performed to provide further confirmation of the SWNT identification and quantitation in sediments performed using NIRF spectroscopy as described above. For this work, dried sediment samples were first combusted at 750º C for 12h. The residue then was acid digested (HNO_3:HCl=1:1, 1.5 ml) by heating for 45 min at 100º C. Immediately afterwards 6 mL DI water were added to the sample. The supernatant was then measured by ICP-MS (2% HNO_3) for Co

and Mo (Agilent Technologies USA, 7700 x ICP-MS). Residual Co and Mo in pristine, bulk SWNT SG65 were determined by combustion, acid-digestion and ICP-MS as described above. Measured recoveries using this technique were 81±8 % for Co and 85±9 % for Mo. Detection limits associated with the method are summarized in table SI-1.

Determination of SWNT in plants and biofilm

Aquatic plants were sampled manually, and rinsed with water to remove adhered soil, stored according to species in individual plastic bags at 4 ºC, and dried at 70 ºC for 48 hours. Stems and leaves were extracted in 2-10 mL 2% w/v SDC by ultrasonication (40 Watts power input), centrifuged at 17860 x g for 10 minutes at 22 °C and the supernatant was measured and quantified by NIRF spectroscopy. Biofilm samples were removed manually from mesocosm walls, dried at 70º C for 48 hours and analysed as described above. Cobalt and Mo impurities/metal catalyst residues of SG65 SWNTs in biota (plants, fish and biofilm) were determined by combustion, acid-digestion and ICP-MS.

Determination of SWNT in fish

Fish were collected by hand-net and flash frozen. For body burden SWNT determination, one fish were extracted in 2 mL 2% w/v SDC by ultrasonication (40 Watts power input), centrifuged at 17860 x g for 10 minutes at 22 °C and the supernatant measured and quantified by NIRF spectroscopy. At least 3 individual fishes were analysed from SWNT-treatment as well as from control.

Mesocosm fish were individually imaged using the NIRF imaging system and method previously described by our group [19]. Briefly, mesocosm fish were shipped live to the University of Florida for NIRF imaging. Upon receipt, fish were euthanized with buffered MS-222. Whole fish were excited by an 808 nm laser at 5 watts and emission above 1000 nm was captured with a

Princeton Instruments OMA V InGaAs 2 dimensional array detector (320 x 256 pixels). Fish were then dissected and individual organs were imaged to examine distribution of SWNT.

Results and discussion

With certain notable exceptions, nearly all studies of the fate, transport, and effects of nanoparticles in the environment have previously been conducted through laboratory-scale experiments. Realistic approximations of the natural environment for assessing nanoparticle fate have only been achieved in a few cases where aquatic mesocosms have been used to study the dynamics of nanoparticles within a simulated ecosystem [23, 24, 26-28]. For example, Buffet et al. have evaluated the fate and toxicity effects of CuO [27] and silver nanoparticles [28] on infaunal species over a short-term period of 21 days in a mesocosm under environmentally realistic conditions (outdoor). Ferry et al. tracked the distrubition and transfer of gold nanorods after a single dose exposure in laboratory constructed estuarine mesoscoms over 12 days [29]. Two studies by Lowry et al. and Colman et al. have evaluated the long-term behavior of silver nanoparticles with various coatings in ourdoor freshwater mesocosms simulating an emergent wet land environment [23, 24].

Short term SWNT behaviour (0-2 months post dosing)

SWNT concentration in the water column was followed hourly within the first 8 hours, and then in larger time intervals between 12-24 hours within the first week (Figure 1). NIRF-spectra of CoMoCat SWNT isolated from water samples at 0.5 h, 3 days and 7 days are presented as insets to Figure 1. SWNT characteristic NIRF features were found in all extracted water samples within the first 72 hours. After 0.5 h post spiking a SWNT concentration of 0.75 mg/L was found in water column. The SWNT concentration in the water column attenuated rapidly from the within a period of 2 days after amendment, as revealed by a decrease in SWNT concentration

of more than 99 % after 2 days ($C_{w,t}/C_{w,0}$ < 0.01,Figure 1). This decrease in concentration approximated a pseudo first order decay, resulting in a modeled half-life time for SWNT-particles present in the water column of 7.4 hours (Figure 1). Fast SWNT removal can be explained by aggregation of SWNTs and settlement of aggregates or/and by association of SWNTs with natural particulate shorten the residence time of SWNTs in the water column. Water quality parameters e.g. ionic strength, cation-composition and pH as well as the presence of NOM affect the aqueous stability of carbon nanotubes (CNT). Decreased stability of surfactant-facilitated MWCNTs spiked into natural waters as well as spiked into soil mineral slurries has been observed in laboratory studies [30] [31, 32] [12, 15, 33, 34]. It is likely that the gum arabic-suspended SWNTs were destabilized by cations and low dissolved organic carbon levels in water sampleand/or by fast release/desorption of the gum arabic coating. Average Ca^{2+} and Mg^{2+} water concentrations were between 21-29 mg L^{-1} and 1-5 mg L^{-1}, respectively, and DOC-concentration varied between 11-18 mg L^{-1}. It is known that pristine SWNTs show a strong tendency to agglomerate in water due to strong van-der-Waals interactions. Laboratory studies under well-defined conditions in artificial waters (single electrolyte solutions) have shown that CNTs could be dispersed and stabilized in water by natural organic matter (NOM) under vigorous agitation over one month [13, 31, 32] However the stability of CNTs in aquatic environment also depend the properties of the receiving water e.g. ionic composition, type and concentration of NOM as well as surface treatment-pre-coating on CNT surface [30]. These studies also allowed for a longer contact/mixing time between NOM and CNT. Our results showing rapid SWNT attenuation from water are in agreement with observation on stability of CTAB-stabilized MWNTs in natural waters (c_{TOC} = 2-28 mg L^{-1}) [35]. Lin at al. investigated the stability of surfactant-facilitated MWNTs in natural waters in laboratory studies [35]. Pristine and CTAB-stabilized MWNTs agglomerated quickly and were readily sedimentated from the water column, whereas SDBS- and TX100- stabilized MWNTs were found to be partially stable after mixing

with natural water. However, applied surfactant loading (ratio) in the study was higher than in the current work.

After one month, the frequency of water sampling was reduced to every two months. Over the time period of 12 months SWNT concentration in the water column remained below 5 µg L^{-1} (the detection limit of the NIRF method for aqueous samples).

Settling particulate material that accumulated in sediment traps were extracted and analysed by NIRF for presence of SWNTs. Results (Figure 2) showed detection of SWNT in sediment trap samples. NIRF spectra of SWNT extracted from sediment traps at day 1 and 30 days are shown inFigure 2. Increasing SWNT concentration in sediment traps (n=1) was observed over a period of 30 days. Material from the sediment trap consisted of a brown, organic-rich floc suggesting that SWNTs had become associated with natural particulate matter through heteroaggregation processes [12, 13, 36]. SWNT accumulation in sediment traps approximately mirrored the loss observed from the water column discussed above. In addition to the "initial" sediment trap deployment, a second set of sediment traps were placed in the mesocosm 9 months after SWNT-dosing and were extracted after 1-2 months exposure. SWNT concentration in extracts of the accumulated material in these sediment traps were below the NIRF method detection limits. Extracts did not show any evidence of SWNT presence (indicated by absence of SWNT-characteristic NIRF features) revealing that SWNT heteroaggregation and settling processes were most important during the first couple days after dosing, consistent with observed losses from the water column.

Long term behaviour (8 months after dosing)

Since results from water column and sediment trap analysis described above suggested that SWNTs were rapidly removed from the water column through aggregation and deposition, we analyzed SWNT concentration in sediment cores from aquatic (n_{Total}=16) and terrestrial compartment (n=3) at sampling periods 8, 10, and 12 months post spiking. Frozen cores were

sectioned into 1 cm slides up to a depth of 5 cm and were analyzed by NIRF spectroscopy [6]. The applied NIRF method has been shown to yield recoveries between 66 and 103% from estuarine sediment depending on SWNT type and coating [6]. Standard addition experiments using the soil material were performed to evaluate the NIRF method in the present study. We added pre-dispersed, GA-coated SWNT to the soil matrix (m_{SWNT}=5-20 µg; equal to 5-20 ug SWNT g^{-1} dry sediment), incubated the sediments for 24 hours, and extracted as described above. Recoveries were found to be 60±14 % for soil (mean ± one standard deviation, n=5 extractions). The lower recovery in the soil compared to estuarine sediments might be explained by matrix effects from increased background noise/absorbance in the mid-near infrared region (e.g. internal filter effects) or/and by a stronger interaction of SWNTs to the soil matrix leading to an incomplete removal. The applied soil material has a clay content of 10% and organic carbon of 5.1% [23]. Slightly lower recoveries in this case are consistent with observed high clay/organic content soils, as previous studies have shown that SWNTs have a strong affinity towards natural particulate such as clay particles or organic matter [37].

Representative NIRF spectra of extracted sediment core sections in the aquatic and terrestrial compartment over the depth of 4 cm are presented in the supporting information (Figure SI-2). For the aquatic compartment, SWNT-characteristic NIRF features were observed in all extracts from the surficial sediment fraction (depth: 0- 1cm, n=16) qualitatively identifying the presence of SWNTs in these samples. Only 4 out of the 16 analyzed aquatic cores showed evidence for SWNT presences in the depth fraction 1 to 2 cm ($MDL_{sediment}$ 0.5 µg g^{-1}). No SWNT were detected in below the depth of 2 cm. No SWNT-characteristic NIRF spectral features were observed in any samples collected from the terrestrial soil samples from the SWNT-dosed mesocosm, consistent with the aquatic dosing strategy and lack of mechanisms for transport of SWNTs from the water to the terrestrial section of the mesocosm. Terrestrial soils were not

368 analyzed for Co or Mo. In addition, samples collected from the control (non-SWNT-dosed)
369 mesocosm also showed no SWNT-specific NIRF spectral features, as expected.

370 Quantitative analysis of SWNT in aquatic sediment was based on calibrated NIRF
371 spectroscopy. Figure 3 summarizes the SWNT loading in the surficial sediment (depth: 0-1 cm)
372 at different sampling events and sampling locations within the SWNT-dosed mesocosm. SWNT
373 loading in the surficial sediment showed very high variability among sampling locations (Figure
374 3) and ranged from 1.5 ± 0.6 to 61.2 ± 8.8 µg g^{-1} dry sediment. This high variability was
375 consistent across multiple sampling dates, such that no temporal trend in SWNT concentrations
376 within the surficial sediment was apparent after 8 (mean: 9.3 +/- 4 µg SWNT g^{-1} dry sediment,
377 median: 9.4 µg SWNT g^{-1} dry, n=3), 10 (mean: 14.9 ± 3.6 µg SWNT dry, median: 6.2 µg SWNT
378 g^{-1} dry, n=8), and 12 months (mean: 10.4 ± 10.5 µg SWNT g^{-1} dry, median: 6.2 µg SWNT g^{-1}
379 dry, n=5) (Table SI-2). As reported previously for shorter-term (30 day) SWNT incubations in
380 estuarine sediment [6], NIRF feautures of SWNT extracted from sediment over the 8-month
381 experimental duration were qualitatively and quantitatively constant, suggesting that SWNTs
382 experienced very little degradation or structural modification during this time. This observation is
383 consistent with predictions of very high stability of SWNTs in the environment [6]. NIRF
384 spectroscopy is, in fact, a sensitive indicator of surface modifications on pristine SWNTs, since
385 oxidation/defects in the SWNT tend to quench and broaden the fluorescence of these materials
386 in the near infrared region [38, 39].

387 Several investigators have explored the application of metal impurities within carbon nanotubes
388 for use in analytical detection of these materials in environmental samples [40, 41]. The trace metal
389 elements Molybdenum and Cobalt are used as catalysts in CoMoCat SWNT production and
390 remain residual after purification due to embedment or encapsulation into SWNT structure
391 during the growth process [25]. The CoMoCat SWNTs used in this study were found to have a
392 metal content of 0.9 ± 0.2 % Co and 2.1± 0.6% Mo determined by thermal combustion followed

by acid digestion and analysis by ICP-MS, consistent with values provided by the manufacturer. In the present work, we have evaluated the measurement of Mo:Co ratio in sediment cores as a fingerprint/characteristic marker of SWNT presence in sediment cores retrieved after 10 months. To this end, selected cores were sectioned in 1 cm slices and subsequently split. One half was analysed using the NIRF method and the other half was digested for Co and Mo analysis. As shown in Figure 4b, sediment from the SWNT-dosed mesocosm showed elevated Mo levels in the top two centimeters of the cores, mirroring data for SWNT concentrations measured by NIRF spectroscopy (Figure 4a). Mo levels in the two centimeters were significantly higher in the SWNT treatment compared to the control (t-test two tails, $p = 0.00005$). At depth below 2 cm, Mo concentrations reached baseline levels, comparable to those measured in the control. Profiles of the ratio Mo:Co showed similar behaviour, confirming the presence of SWNTs in the top layer sediments (Figure 4c). Assuming a Mo content of 2.1% in bulk CoMoCat SWNT (as measured and reported above), the Mo concentration in the top sediment fraction would be equivalent to a SWNT concentration of 10 ± 1.2 µg SWNT g^{-1} sediment and 4.9 ± 1.7 µg g^{-1} sediment in the fraction 0-1 cm and 1-2 cm, respectively. For comparison, SWNT concentration determined by NIRF in the top 1 cm of the core was 8.1 ± 2 µg g^{-1} (Figure 4a). The close correlation between SWNT measurements made by the NIRF-method and the metal residue method can be seen as complementary lines of evidence for the presences of SWNTs in the sediment top layer over a period of 10 months. The good agreement of NIRF and metal residue analysis results for SWNTs in sediments measured here have implications for analysis of SWNTs in field-contaminated sediments. It can be envisioned that the application of both techniques concurrently will lead to enhanced confidence in the reporting of SWNT occurrence in samples collected from suspected contaminated sites, especially where much is known (e.g. metal content) about the SWNT expected to occur in the samples. Further, the promising results for detection of SWNTs by rare-metal residue analysis in sediments suggests that this approach

might be viable for analysis of SWNT (or MWNT) that are not intrinsically fluorescent (such as covalently modified or metallic SWNT) in the environment.

Taken together, the results of our water column, sediment trap, surficial sediment, and core-profiling analyses support the hypothesis that sediment can be considered as an important sink for SWNT after release into the aquatic environment. The first centimetres of sediment layer (0-5 cm) are biologically active and relevant and are subject to homogenous mixing due to bioturbation. It has been reported in literature that the state of carbon nanotube agglomeration affects CNT toxicity [42-44]. Other studies observed little to no uptake of SWNTs from the sediment into tissues of various benthic organism after controlled spiking/dosing studies [6, 8, 18, 45], indicating very little bioaccumulation potential of sediment-natural particular associate SWNT. However in more realistic aquatic ecosystems, SWNTs that become associated with natural particulates and settle into the surficial sediment layer might be at higher risk for remobilization than artificially-spiked samples. Residence time of SWNTs in this sediment layer should be controlled by the bioturbation activity, sediment formation rate as well as geochemical/physical factors controlling remobilization. No benthic organisms were introduced into the mesocosms of the present study, therefore further work in this area is needed to assess the differences in bioavailability of sediment-associated SWNTs in naturally-settled vs. artificially-spiked sediments.

Although benthic deposit feeders were not investigated for accumulation of SWNTs in the present work, several other varieties of biota from the mesocosms were analysed for SWNT uptake/accumulation. Specifically, samples of the plants *Elodea Canadensis* and *Lemna minor*, the biofilm growing on the hard surfaces of the mesocosm walls, and the mosquito fish *Gambusia holbrookis* were retrieved 10 months post-spiking and subsequently analysed using both the NIRF method and ICP-MS (for Mo and Co residue). NIRF spectra of the different organism are shown in the supporting information. Limits of quantification (concentrations giving

443 analytical signals 3 × blank measurements) for plants, biofim and fish were 1140 ng g^{-1}, 250 ng
444 g^{-1} and 780 ng g^{-1} (based on wet weight), respectively. NIRF spectra of plants (Figure SI-3)
445 samples showed an elevated background signal possibly due to internal filter effects or
446 interference from photopigments as described previously for other sample types [6]. At the 10
447 month sampling time, the SWNT concentration in biofilm, plants and mosquito fish were below
448 the NIRF detection limits revealing no-or very little uptake of SWNTs into mesocosm biota.
449 Mesocosm biota samples were also analyzed for Co and Mo by ICP-MS. Analysis did not show
450 any evidence for elevated Mo or Co levels with MDL_{Co} & MDL_{Mo} for fish, plant and biofilm of 30
451 & 630 ng g^{-1} wet weight, 16 & 340 ng g^{-1} wet weight and 7 & 140 ng g^{-1} wet weight, respectively.

452 Although quantitative analysis of SWNTs in mosquitofish suggested limited uptake/accumulation
453 by these organisms from SWNT-spiked mesocosm water at the 10-month sampling point, we
454 applied a secondary, more sensitive (~ 5 ng total mass-basis SWNT detection limit) but semi-
455 quantitative NIRF-based imaging analysis technique in order to attempt detection of trace
456 SWNTs that may have been ingested by the fish[19]. This technique uses a high-power (5 watt)
457 NIR laser (808 nm) in concert with a cryogenically-cooled InGaS 2D-array detector for
458 ultrasensitive (< 5 ng total SWNT/sample) detection, imaging, and mapping of SWNT within the
459 tissues of fish without the need for extraction[19]. We used this method to image SWNTs in
460 several fish collected from the control and SWNT-dosed mesocosms. Results (Figure 5) show
461 no or very little measureable fluorescence in fish collected from the control mesocosm, while
462 several fish collected from the SWNT-dosed mesocosm exhibited bright fluorescence
463 associated with the gut cavity after illumination with 808 nm laser light. Localization of the
464 fluorescence to the intestine was confirmed by carefully dissecting the gut cavity and imaging all
465 isolated organs as well as the remaining (gutted) carcass of the mosquitofish as described
466 previously [19]. Results showed that in all cases, NIRF signal was confined to the intestinal tract,
467 with no measureable fluorescence above background attributable to other organs or to the

carcass. It should be noted that the observation of NIRF signal in the intestines of fish from the SWNT-dosed mesocosm was highly variable, i.e. not all fish exhibited fluorescence. This result, together with the observation of fluorescence confinement to the gut tract of fish, suggests that the mosquitofish may have ingested SWNT-laden particles either after resuspension from the sediment or from residual SWNTs in the water-column through feeding activities. Lack of movement past the gut tract indicates that while fish may have ingested SWNTs following exposure, no appreciable uptake into tissues occurred. These results are consistent with our previous findings of limited uptake of SWNTs in fathead minnows after gavage dosing [19]. It should be noted that confirmation of SWNT identity within intestines of fish by NIRF spectral acquisition was not possible in the current study, as no spectrometer was available for interfacing with the imaging system employed for this purpose.

Conclusion

We have conducted the first comprehensive assessment of SWNT fate in an aquatic ecosystem through careful dosing of a wetland mesocosm system and subsequent analysis. Our observations indicate that SWNTs heteroaggregate readily with natural particles in the aquatic environment, and that they attenuate rapidly from the water column, consistent with laboratory reports of their instability in the presence of ionic and non-ionic components of natural waters. The results of our sediment trap and surficial sediment analyses indicate that particle settling and incorporation into bedded sediment is the most important process determining the fate of SWNTs in aquatic systems. No appreciable uptake and accumulation of SWNTs in any of the biotic compartments of the wetland mesocosms was observed, indicating that SWNTs were relatively inert to bioaccumulation under the studied conditions, and this finding is again very consistent with results previously reported for laboratory studies of these carbon nanoparticles under controlled conditions. Findings of possible ingestion of SWNT-laden particles by fish living in the mesocosm, while intriguing from an analytical standpoint, were unimportant to the overall

fate of SWNTs in the mesocosm. The very high spatial variability observed for SWNT concentrations in surficial sediments after 12 months reflected considerable heterogeneity in the deposition of SWNT to sediments within the mesocosm, possibly due to mesoscale variability in transport dynamics and particle settling/bioturbation. Within the 25 to 75 percentile interval, SWNT concentrations ranged from 1.9 to 13.5 µg SWNT g^{-1} dry sediment (n=17) with a mean of 11.1 +/- 3.6 µg SWNT g^{-1} dry sediment and median of 6.2 µg SWNT g^{-1} dry sediment). Calculation of SWNT mass balance based on these sediment inventories and the initial dosing quantity of SWNT resulted in estimates accounting for between 7% (25^{th} percentile concentration assumption) and 48% (75^{th} percentile concentration assumption) of the initially added SWNT. While it is possible some of the added SWNTs may have been lost to analytically "obscured" compartments such as plant – associated detritus, we assess that uncertainties in the calculation due to the high spatial variability of SWNTs in sediments were the dominant contributor to the incomplete mass balance achieved in the present case.

Taken together, our results paint a picture of heteroaggregation, very limited mobility, extensive persistence in aquatic sediments, and low bioavailability for SWNT in aquatic systems. These data should be invaluable for informing risk assessments of carbon based nanoparticles in the context of contaminated aquatic systems. Further, our novel combination of NIRF spectroscopy and imaging with residual metal catalyst analysis by ICP-MS illustrates the utility of this complementary approach to SWNT metrology in complex environmental samples. More work is needed to assess the sensitivity and robustness of this technique for detection and quantitation of SWNT s and other carbon based nanoparticles in aquatic sediments.

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528

529 **Figure Captions**

530 **Figure 1.** SWNT concentrations (± one standard deviation) within the water column (blue)
531 followed over 1 month after SWNT amendment. Insets show near-infrared fluorescence
532 emission spectra (NIRF spectra) for SWNTs extracted from water samples at 0.5 h (A), 3 days
533 (B) and 7 days [c < DL; 5 µg/L] (C) after spiking, legend:– 638 nm (black), - 691 nm (red) and –
534 782 nm (blue) excitation wavelength. (D) Fit of SWNT water column concentration data to a
535 first-order decay model results in a calculated half life of $t_{1/2}$ ~7.4 hours (r^2 = 0.76)

536 **Figure 2.** SWNT accumulation in sediment traps in the SWNT-dosed mesocosm one month
537 following SWNT amendment. The m_{SWNT} mass (n = 1) is normalized to the total surface area of
538 the sediment traps. Insets show representative near-infrared fluorescence emission spectra
539 (NIRF spectra) for of CoMoCat SWNT extracted from sediment traps after 1 day (A) and 30
540 days (B).

541 **Figure 3.** Plan view of mesocosm showing sediment/soil sampling locations at 10 months post-
542 dosing (circle) and SWNT concentrations (mean µg SWNT g^{-1} dry sediment ± one standard
543 deviation) measured using NIRF spectroscopy in surficial soil/sediment in the aquatic (A-series)
544 and terrestrial (T-series) compartments [depth: 0- 1 cm] (n=8 aquatic and n=3 terrestrial
545 samples). At sampling locations marked with an asterisk (*), NIRF spectra indicated the
546 presence of SWNT, but the concentration was below limit of quantification MDL< 0.5 µg SWNT
547 g^{-1} dry sediment. Sampling locations were assigned randomly except for triplicate samples from
548 location A5, which were retrieved within 10 cm radius.

549 **Figure 4.** Distribution of CoMoCat SWNT in an aquatic sediment core (n=3) after 10 months. A)
550 Samples from SWNT-dosed mesocosm analysed by NIRF spectroscopy. B) Solid-phase
551 molybdenum concentration and C) Ratio of Mo:Co concentration in sediment cores from SWNT-

552 dosed and control mesocosms showing elevated molybdenum levels in the surficial sediment of
553 only SWNT-dosed mesocosms. Error bars represent ± one standard deviation.

554 **Figure 5** Near infrared fluorescence imaging of mosquitofish collected from control (A, B) and
555 SWNT-dosed (C, D) mesocosms under visible light (A, C) and 808 nm laser illumination (5 watt
556 power). Bright fluorescence observed in SWNT-exposed fish under NIR laser irradiation (D) was
557 suggestive of SWNT burdens in the gut of the fish. Dissection of intestines and subsequent
558 imaging confirmed that fluorescence was confined to the gut of the fish.

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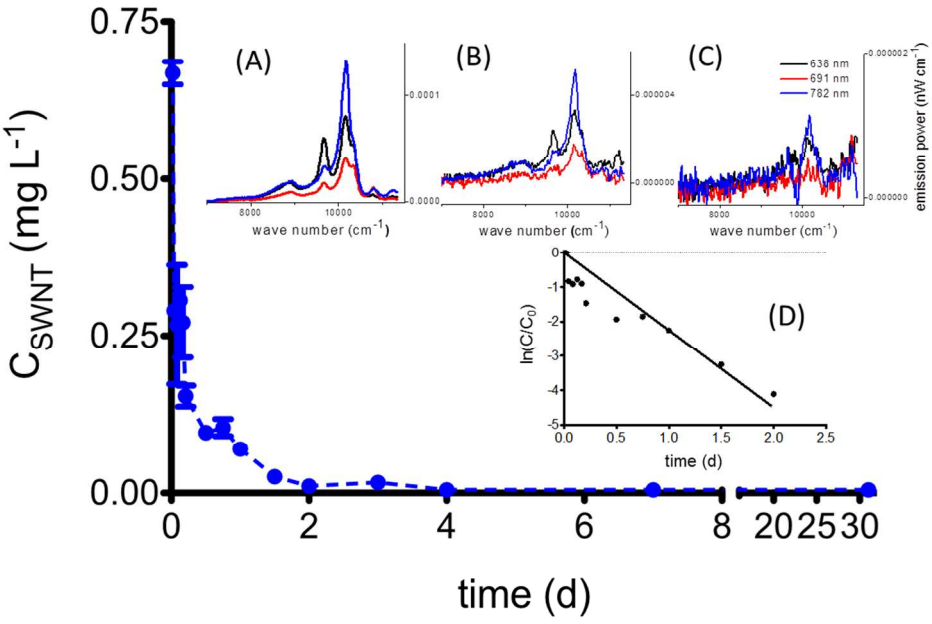

Figure 1. SWNT concentrations (± one standard deviation) within the water column (blue) followed over 1 month after SWNT amendment. Insets show near-infrared fluorescence emission spectra (NIRF spectra) for SWNTs extracted from water samples at 0.5 h (A), 3 days (B) and 7 days [c < DL; 5 µg/L] (C) after spiking, legend:– 638 nm (black), - 691 nm (red) and – 782 nm (blue) excitation wavelength. (D) Fit of SWNT water column concentration data to a first-order decay model results in a calculated half life of $t_{1/2}$ ~7.4 hours (r^2 = 0.76)
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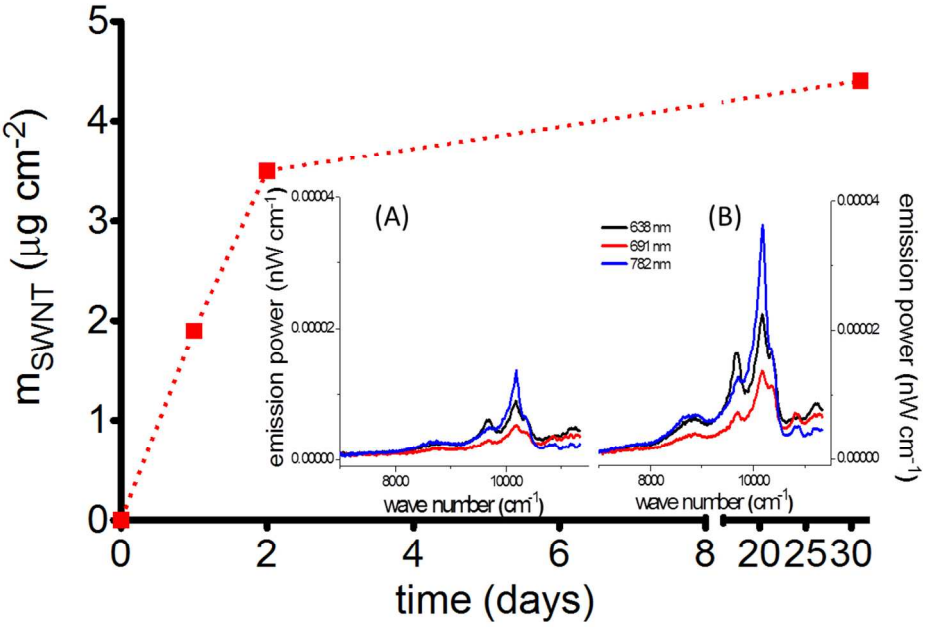

Figure 2. SWNT accumulation in sediment traps in the SWNT-dosed mesocosm one month following SWNT amendment. The m_{SWNT} mass (n = 1) is normalized to the total surface area of the sediment traps. Insets show representative near-infrared fluorescence emission spectra (NIRF spectra) for of CoMoCat SWNT extracted from sediment traps after 1 day (A) and 30 days (B).
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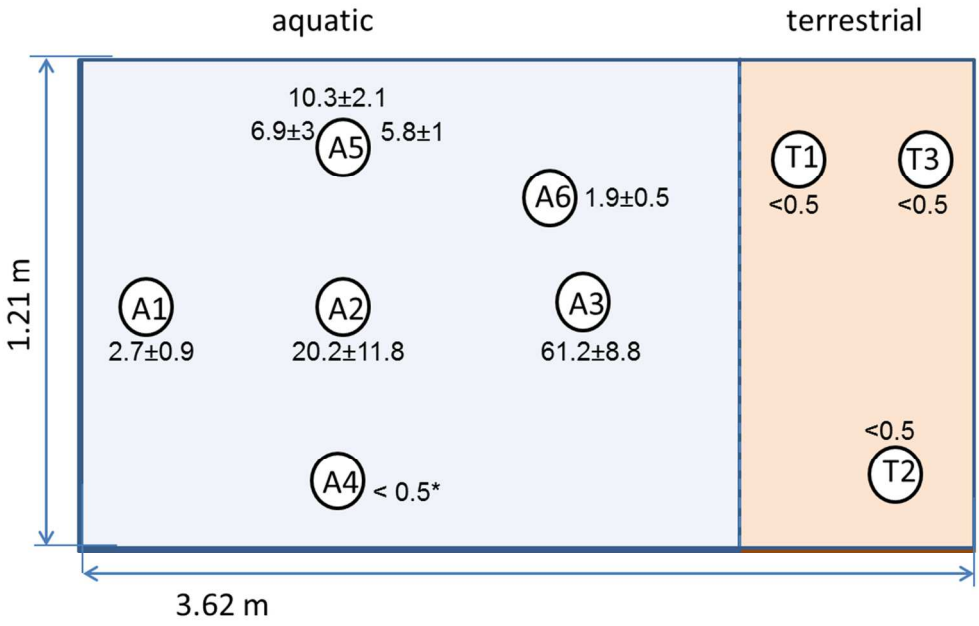

Figure 3. Plan view of mesocosm showing sediment/soil sampling locations at 10 months post-dosing (circle) and SWNT concentrations (mean µg SWNT g^{-1} dry sediment ± one standard deviation) measured using NIRF spectroscopy in surficial soil/sediment in the aquatic (A-series) and terrestrial (T-series) compartments [depth: 0- 1 cm] (n=8 aquatic and n=3 terrestrial samples). At sampling locations marked with an asterisk (*), NIRF spectra indicated the presence of SWNT, but the concentration was below limit of quantification MDL< 0.5 µg SWNT g^{-1} dry sediment. Sampling locations were assigned randomly except for triplicate samples from location A5, which were retrieved within 10 cm radius.
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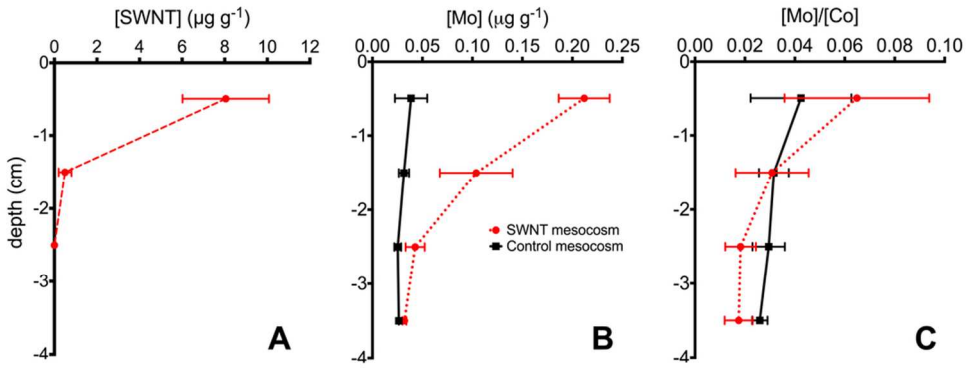

Figure 4. Distribution of CoMoCat SWNT in an aquatic sediment core (n=3) after 10 months. A) Samples from SWNT-dosed mesocosm analysed by NIRF spectroscopy. B) Solid-phase molybdenum concentration and C) Ratio of Mo:Co concentration in sediment cores from SWNT-dosed and control mesocosms showing elevated molybdenum levels in the surficial sediment of only SWNT-dosed mesocosms. Error bars represent ± one standard deviation.
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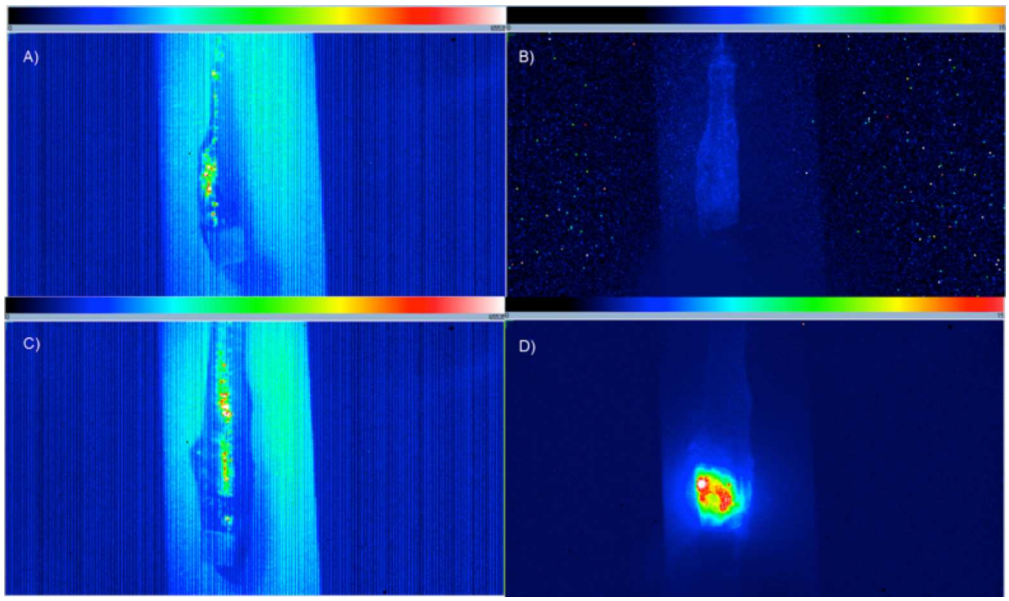

Figure 5 Near infrared fluorescence imaging of mosquitofish collected from control (A, B) and SWNT-dosed (C, D) mesocosms under visible light (A, C) and 808 nm laser illumination (5 watt power). Bright fluorescence observed in SWNT-exposed fish under NIR laser irradiation (D) was suggestive of SWNT burdens in the gut of the fish. Dissection of intestines and subsequent imaging confirmed that fluorescence was confined to the gut of the fish.
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