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INFLUENCE OF BIOTURBATION BY THE AMPHIPOD *COROPHIUM VOLUTATOR* ON FLUORANTHENE UPTAKE IN THE MARINE POLYCHAETE *NEREIS VIRENS*

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Abstract—The uptake kinetics of fluoranthene in the polychaete worm *Nereis virens* were investigated in the presence and in the absence of amphipods to examine the effects of sediment bioturbation by the benthic amphipod *Corophium volutator* on the uptake in worms. Worms only and worms together with two different densities of amphipods were exposed to fluoranthene-spiked sediment for 12 d. Worms and overlying water samples for fluoranthene analyses were taken and total suspended solids in water column were measured after 1, 2, 5, 8, and 12 d. Results showed that in all treatments fluoranthene was rapidly accumulated by *N. virens* during the first two days and a steady state was reached within five days of exposure. Biota to sediment accumulation factors normalized to lipid concentration and to sediment organic carbon (BAF_{loc}) of worms exposed with the highest number of amphipods were significantly higher (two to three times) compared to worms exposed with fewer or without amphipods after one and two days of exposure. Bioconcentration factors (BCFs), calculated as the ratio between the uptake (k_1) and elimination (k_2) rate constants were not significantly different among treatments. When BCFs were calculated on the basis of dissolved fluoranthene concentrations (BCF_{diss}), values of the treatments where worms were exposed with 100 and 300 amphipods were slightly higher than those calculated on the basis of total (dissolved + particle-bound) aqueous fluoranthene (BCF_{tot}). However, the presence of fluoranthene bound to dissolved organic matter could have accounted for lower than expected BCF_{diss} values. The results suggest that bioturbation by amphipods affected the concentration of fluoranthene in the worms not by changing the worm to water partitioning (k_1/k_2) but by changing the worm to sediment partitioning (BAF_{loc}). In the treatments with worms a higher mortality of amphipods was found compared to those without worms. The presence of worms might have contributed to lower BCF values than expected.

Keywords—*Corophium volutator* Bioturbation Fluoranthene Toxicokinetics *Nereis virens*

INTRODUCTION

Soft-bottom marine environments in general have been shown to be strongly affected by bioturbation of benthic invertebrates [1]. Effects of benthic bioturbation on physical [2] and chemical characteristics of sediments [3,4], pore water, and overlying water [5,6] have been well documented.

Bioturbation by the amphipod *Corophium volutator* is known to change sediment permeability, water content, and sediment shear-strength [7,8]. *Corophium volutator* lives in U-shaped burrows that extend 3 to 4 cm into the sediment and that are continuously irrigated. By burrow construction and irrigation, total surface area for diffusive exchange of oxygen and nutrients at the sediment–water interface is increased and mineralization processes are stimulated [9,10]. Macroinvertebrate bioturbation also affects the fate and partitioning of sediment-bound contaminants in sediment profiles [11,12], pore water [13], and the water column [14]. Bioturbation is assumed to increase the rate of important physicochemical processes that occur at the sediment–water interface such as diffusion, desorption, degradation, and resuspension of organic and inorganic compounds [2,15]. However, studies quantifying and explaining the effects of bioturbation on these processes through dynamic models are scarce. Also, studies on the role of bioturbation in contaminant transport from sediment to water column and transfer to other trophic levels are lacking. A previous study showed that sediment bioturbation by *C. volutator* affected the total concentration of polycyclic aromatic hydrocarbons (PAHs) in overlying water and increased the concentration of fluoranthene in suspension-feeding mussels [16]. The increase was linearly related to the number of bioturbating amphipods in the sediment.

Based on the results of the previous investigation, the purpose of this study was to examine whether bioturbation by *C. volutator* also would exert an influence in fluoranthene uptake in the marine polychaete worm *Nereis virens*. *Nereis virens* constructs deep (8- to 10-cm), vertical well-irrigated, semipermanent burrows lined with mucus and is known as an omnivorous nonselective deposit feeder [17] that can also filter feed [18]. Previous studies on bioaccumulation suggest that the water phase is an important route of uptake for the accumulation of sediment contaminants in *Nereis diversicolor* [18,19]. *Nereis virens* and *C. volutator* are among the most common and abundant species in intertidal and shallow mudflats in northwestern Europe. The species coexist in the same habitat, reach high population densities during summer, and construct burrows at the sediment–water interface [20,21].

The aim of this study was to compare the uptake kinetics of fluoranthene in *N. virens* in the absence of amphipods with that obtained in the presence of bioturbating amphipods. We tested the hypothesis that in a bioturbated system, desorption kinetics of sediment-bound contaminants in water would be increased and that accumulation levels of fluoranthene in worms would be consequently higher than in a nonbioturbated system. The role of amphipod bioturbation in fluoranthene bioavailability and the relationship between amphipod density, total suspended solids in overlying water, and fluoranthene body burdens in worms were also investigated.

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MATERIALS AND METHODS

Field sampling and animals

Sediment samples from the top 3 cm were collected from an intertidal mudflat (Oesterput) located in the Oosterschelde in the southeastern part of The Netherlands (51°36′N, 3°48′E). Sediment was wet-sieved through a 500-μm mesh sieve to remove indigeneous macroinvertebrates, transported to the laboratory, and stored at 4°C.

Amphipods were collected from an intertidal mudflat (Biezelingseham) located in the Westerschelde in the southwestern part of The Netherlands (51°27′N, 3°55′E). Samples of the surface sediment (top 3 cm) were taken and wet-sieved through a 500-μm mesh sieve; the amphipods were rinsed into polyethylene buckets containing freshly collected sea water. Amphipods were then transported to the laboratory and transferred to 10-L jars containing a 3-cm layer of (Oosterschelde) sediment filled with natural filtered seawater (i.e., sandbed-filtered seawater containing particles <10 μm) with a salinity of approximately 32 g/L. Organisms were acclimated to the same salinity, temperature, and light conditions as used in the experiments.

Polychaete worms, *N. virens* (Polychaeta: Nereidae), were obtained from a seabait farm, Topsy Baits (Wilhelminadorp, The Netherlands). Worms were acclimated and held in running seawater in tanks containing 2 to 3 cm of Oosterschelde sediment at a temperature of 15 ± 2°C for one week before commencement of the experiment.

Sediment spiking procedure

Sediment subsamples were taken for analyses of water content and organic matter. The water content was determined by drying the sediment at 70°C for 24 h to constant weight. The percentage organic matter was determined after combustion at 450°C for three hours. Physicochemical characteristics and background concentrations of total PAHs, polychlorinated biphenyls, metals, and organochlorine pesticides of the sediment used are described in Ciarelli et al. [22]; in this sediment the background concentration of fluoranthene was <0.25 μg/g dry weight (unpublished data).

Fluoranthene (log K_{ow} = 5.23) [23] was purchased from Aldrich Chemical Company (Steinheim, Germany; purity 98%) and dissolved in acetone (8 g/L). A calculated volume (66,375 ml) of the stock solution was added dropwise at a rate of 60 ml/h to 60 kg wet sediment slurry to achieve the required nominal concentration (10 μg/g dry weight), while the mixture was stirred for approximately four hours. The fluoranthene concentration was chosen on the basis of a previous study (unpublished), which showed no effect to the amphipods and the worms at this exposure concentration. After spiking, sediment was kept in a refrigerator at 4°C for 10 d to allow equilibration and partitioning of fluoranthene into the sediment. Before starting the experiment, sediment was again mixed homogeneously for approximately two hours. After settling, overlying water was decanted to remove most of the acetone carrier and two subsamples were taken for fluoranthene analysis.

Experimental set-up

The 3-L beakers were filled with 850 ml of spiked sediment corresponding to a 4-cm-thick sediment layer and 2,000 ml of overlying seawater. Sediment and water were allowed to equilibrate for 24 h before the addition of the organisms. The experiment involved the following three treatments: control without amphipods, low density of amphipods (n = 100), and high density of amphipods (n = 300). Low- and high-density treatments corresponded to 3,300 and 9,900 organisms/m^2, respectively. The number of amphipods used in this experiment was based on realistic environmental densities occurring in the field during wintertime [24]. Two worms ranging from 2.5 to 3.5 g wet weight were added to each vessel 24 h after addition of the amphipods. Three replicates were used for each treatment and exposure time. In addition to the three treatments with worms, a low- (100 amphipods) and high-density (300 amphipods) treatment without worms (three replicates each) were used to compare amphipod survival in the absence of worms. The exposure of amphipods alone lasted 13 d and water samples were taken only at the end of the exposure.

Worms and amphipods were exposed to fluoranthene-spiked sediment for 12 and 13 d, respectively. The experiment, consisting of a total of 51 beakers, was conducted at 15°C and the overlying water was not replaced during the exposure; temperature and dissolved oxygen were checked at the beginning, after five days, and at the end of the experiment, in the overlying water. The organisms were not fed during the exposure because it was considered that the addition of organic material would alter the partitioning and availability of fluoranthene.

Water samples for total suspended solids (TSS) were taken after 2, 3, 6, 9, and 13 d after the addition of the amphipods. Water samples (1 L) for total fluoranthene (dissolved + particle bound) analysis were taken after 1, 5, and 12 d after the addition of worms. Tissue samples for fluoranthene analysis were taken from unexposed amphipods and worms before the experiment and from exposed organisms after 1, 2, 5, 8, and 12 d. At the end of each exposure time worms were placed in clean seawater for four hours to allow gut depuration. The water was changed twice during depuration time and the mucous–sediment mass excreted by the worms and sticking to their bodies was discarded. Tissue samples were then immediately frozen in liquid nitrogen and stored at −20°C. Amphipods were placed in clean seawater and rinsed with deionized water and frozen at −20°C. Worms and amphipods were freeze-dried before fluoranthene analysis.

Analytical methods

Concentrations of fluoranthene were determined in sediment, overlying water, worms, and amphipods. The analytical methods used for the sediment and amphipods are identical to those described in Ciarelli et al. [16].

For total fluoranthene analysis in overlying water, water samples were adjusted to pH = 2 using 33% nitric acid before extraction. After addition of 50 ml of hexane the sample was shaken for one minute in a separation funnel. The water fraction was collected into the sample bottle and the pH was adjusted to 9 using 5 M sodium hydroxide. Subsequently, hexane (100 ml) was added in the water fraction and the mixture was shaken for one minute in a separation funnel. Both hexane fractions were combined and subsequently dried over an anhydrous sodium sulfate column. The dried hexane extract was evaporated under nitrogen in a turbovap apparatus and further reduced to 1 ml after addition of 3.5 ml of a 2% ethanediol solution in methanol.

Total suspended solids in overlying water were determined gravimetrically in the low- and high-density treatments. A similar method was used as that described in Ciarelli et al. [16].

Briefly, TSS were determined gravimetrically after filtration of 200 and 100 ml of water, in the low- and high-density treatments, respectively. Preweighed glass fiber filters (Type GF/C Whatman, Clifton, NJ, USA; 1-μm nominal pore size) were dried at 50°C and weighed after 24 h. The concentration was expressed as mg/L by calculating the difference between the total and initial weight and dividing by the volume of water filtered.

Worm samples were Soxtec®-extracted (Tecator AB, Hoganas, Sweden) for 1.5 h with 70 ml of a hexane:acetone mixture (1:1, v/v). Worm extracts were evaporated under nitrogen in a block heater (40°C) and residues were dissolved in 15 ml of hexane. The extracts were concentrated and reduced to approximately 1 ml in a turbovap apparatus (30°C) and after addition of 3.5 ml of a 2% ethanediol solution in methanol, extracts were further reduced to 1 ml.

For lipid content analyses in the worms, extracts were cleaned-up over a 15-g aluminum oxide (6% H_2O) column and eluted with 200 ml of hexane to remove lipids. The lipid weight of the samples was determined by evaporating a fixed amount (10%) of the total Soxtec extract and weighing the residue.

Analyses of the PAH compounds were performed by injecting 20 μl of methanol extracts into a high-performance liquid chromatograph (HPLC-system; Separations, Amsterdam, The Netherlands). The PAH compounds were separated over a LC-PAH C_{18} reverse-phase column, using a 56 to 100% acetonitrile gradient (0.7 ml/min). The PAHs were detected by compound-specific fluorescence detection (Jasko 785A, Tokyo, Japan).

All the PAH analyses were carried out by OMEGAM (Amsterdam, The Netherlands), accredited as a testing laboratory by the Laboratory Accreditation Board of The Netherlands. Each set of samples was analyzed under quality assurance–quality control protocols, which included procedural blanks, replicate analyses, and control materials. Identification and quantification of fluoranthene were performed by comparing retention times and peak areas with those of certified standards (Standard Reference Material and Schmidt, Amsterdam, The Netherlands).

Uptake kinetics and bioaccumulation factors

The kinetics of the accumulation of fluoranthene in the worms were determined by fitting the data to a first-order one-compartment toxicokinetic model assuming that the overlying water was the main route of uptake of fluoranthene for the worms and assuming the internal concentration to be zero at $t = 0$

$$C_a(t) = \frac{k_1}{k_2} C_w(1 - e^{-k_2 t})$$

where $C_a(t)$ = fluoranthene concentration in worms at time t (μg/g), k_1 = uptake rate constant defined as the amount of fluoranthene accumulated from water per mass of organism per unit of time (ml/g/d), k_2 = elimination rate constant of fluoranthene (d^{-1}), C_w = fluoranthene concentration in water column (mg/L), and t = time (days).

The uptake (k_1) and elimination (k_2) rate constants were estimated by fitting the models to the data using nonlinear curve fitting with Systat® 5.0 (SPSS, Chicago, IL, USA, 1990–1994). The uptake rate constant (k_1) was estimated for each treatment, whereas the elimination rate constant (k_2) was estimated for all treatments together. To know whether the particle-bound fraction of fluoranthene would contribute to the uptake in worms, the uptake (k_1) and elimination (k_2) rate constants were estimated by using the dissolved fraction of fluoranthene as well as the total aqueous fluoranthene concentration (i.e., dissolved + TSS-bound) were used. The dissolved fraction was not directly measured in this study but was derived by subtracting the fraction of particle-bound fluoranthene, measured in the previous study [16] where analagous treatments (i.e., with 100 and 300 amphipods) were used, from total concentration, which was measured in this study. The fractions dissolved and particle-bound were assumed to be approximately constant in the low- and high-density treatments with similar numbers of organisms. Results of the former study showed that 78 and 60% of total fluoranthene in the water column was present as dissolved in the low- and high-density treatments, respectively, after 10 d of exposure. For all treatments, the fluoranthene concentrations at each sampling time were pooled and the averages were used for the estimation of the uptake (k_1) and elimination (k_2) rate constants. The following equations were used to determine the bioconcentration factors (BCF_{diss} and BCF_{tot}), and the lipid ($_{lip}$)- and sediment organic carbon ($_{oc}$)-normalized bioaccumulation factor (BAF_{loc}):

$$BCF_{diss} = k_1/k_2 \quad \text{(based on dissolved fluoranthene)}$$

$$BCF_{tot} = k_1/k_2 \quad \text{(based on total aqueous fluoranthene)}$$

$$BAF_{loc} = [(\text{tissue}/f_{lip})/(\text{sed}_{oc})]$$

where k_1 (ml/g/d) and k_2 (d^{-1}) are the uptake and elimination rate constants as defined above, tissue/f_{lip} is the lipid-normalized fluoranthene body burden (μg/g dry weight), and sed_{oc} is the sediment organic carbon-normalized fluoranthene (μg/g dry weight).

Statistical analyses

Significant differences between means were tested with one-way analysis of variance followed by pairwise comparisons among treatments (Tukey's honestly significant difference test). The TSS and BAF_{loc} data were checked for normality (Shapiro–Wilks test) and for homogeneity of variances (Bartlett's test) before performing analyses of variances. Differences were considered significant when $p < 0.05$.

RESULTS

Sediment

Mean percentage dry matter of the fluoranthene-spiked sediment was 58.7 (±3.3). Mean percentage organic matter was 4.25; percentage organic carbon was 2.53 (±0.5) and was derived as follows: $f_{oc} = 0.6 \times f_{om}$ [25]. Mean measured fluoranthene concentration in sediment at $t = 0$ was 8.3 (±2.26) μg/g dry weight.

Percentage recovery of amphipods and fluoranthene uptake

Recovery of amphipods gradually decreased during the exposure time of 13 d. Mean percentage recovery of amphipods after one day ranged from 90.7 (±4.0) to 81.1 (±6.9) in the low- and high-density treatment, respectively. After 13 d, mean percentage recovery declined to 54 (±2.9) and to 34.8 (±6.7) in the low- and high-density treatment, respectively. In the treatments without worms, mean recovery remained high at 92.3% after 13 d of exposure. This suggests a negative impact of worms on the survival of amphipods (Table 1).

The internal concentrations of fluoranthene in *C. volutator* ranged from 58 (±33) to 90 (±56) and from 93 (±5) to 135 (±50) μg/g, in the amphipods of the low- and high-density

Table 1. Percentage recovery of *Corophium volutator* and concentration of fluoranthene in amphipods and total suspended solids (TSS) in water column at different densities and at different times of exposure (means ± SD)

Time (days)	Amphipod density	Amphipod recovery (%)	Fluoranthene concn. in *C. volutator* (μg/g dry wt)	TSS (mg/L)[a]
2	100	91 (±4.0)	58 (±33)	38 (±2.0)A
	300	81 (±7.9)	135 (±49)	100 (±17)B
3	100	78 (±2.9)	90 (±56)	29 (±6.6)A
	300	75 (±1.7)	130 (±14)	71 (±1.4)B
6	100	73 (±1.5)	63 (±5.7)	34 (±3.6)A
	300	64 (±0.9)	93 (±4.9)	64 (±8.6)B
9	100	64 (±3.1)	ND[b]	26 (±2.3)A
	300	51 (±1.8)	ND	66 (±6.4)B
13	100	54 (±2.9)	ND	35 (±6.7)A
	300	35 (±6.7)	58[c]	39 (±4.0)A
	100[d]	92 (±4.0)	—	70 (±4.6)A
	300[d]	92 (±1.2)	—	177 (±7.3)B

[a] Means of treatments that do not share the same capital letter are significantly different from each other at $p < 0.05$.
[b] ND = not determined.
[c] Based on one value.
[d] Treatments without worms.

treatments, respectively. However, because of the few data and the high standard deviation, the differences between the two treatments were not statistically significant. Based on these results, steady state seemed to be reached already after two days of exposure in both densities and after 13 d, fluoranthene concentration in the amphipods of the high-density treatment seemed to decline (Table 1).

Overlying water

Two days after addition of the amphipods to the test chambers, TSS concentrations in the low- and high-density treatments with worms were 38 (±2) and 100 (±17) mg/L, respectively. After 13 d, TSS values declined to 35 (±6.7) and to 39 (±4) mg/L in the low- and high-density treatments, respectively. A substantial decrease in time (almost a factor three) was found in the high-density treatment, which coincided with a low recovery of amphipods. Nevertheless, TSS concentrations in the high-density treatments were significantly higher than in the low-density treatment for each sampling time with the exception of $t = 13$ d. In the treatments without worms, TSS values were almost a factor 2 to 4.5 higher (70 ± 4.6 and 177 ± 7 mg/L in the low- and high-density treatments, respectively) after 13 d (Table 1). This was due to a better survival of the amphipods exposed without worms compared to those exposed with worms.

At $t = 1$ day, total average fluoranthene concentrations in the water column ranged from 5.8 μg/L in the treatment without amphipods to 15 μg/L, in the high-density treatment. After 13 d, mean concentrations of fluoranthene decreased to 9.9 and 9.2 μg/L in the low- and high-density treatments, respectively, and were not significantly different among treatments except for the values at $t = 1$ day (data not shown).

Fluoranthene uptake in N. virens

Fluoranthene was rapidly accumulated by *N. virens* during the first two days of exposure in all treatments. Steady-state body burdens were reached after five days in all treatments. Fluoranthene concentrations in the worms exposed with 300 amphipods were significantly higher ($p < 0.05$) than those in worms exposed without amphipods at $t = 1$, 2, and 12 d (Fig. 1a to c). After one and two days, accumulation was also significantly higher compared to worms of the treatment with 100 amphipods. Worms that were exposed with 100 amphipods did not accumulated significantly more fluoranthene than those exposed without amphipods. Bioconcentration factors, calculated as the ratio between the uptake (k_1) and the elimination (k_2) rate constants obtained using the dissolved fluoranthene concentrations (BCF_{diss}), ranged from 4.68 to 4.87 (log basis) in the treatment without and with 300 amphipods, respectively (Table 2). The BCF_{diss} values of the treatment with 100 and 300 amphipods were slightly higher compared to bioconcentration factors based on total fluoranthene concentrations (BCF_{tot}). Because 95% confidence limits of the uptake (k_1) rate constants estimated with both methods overlapped, BCF_{diss} and BCF_{tot} were probably not significantly different from each other and were also not significantly different among treatments. However, although total aqueous fluoranthene concentration was increased and worms were significantly affected by bioturbation, the uptake kinetics and bioconcentrations factors were not influenced.

The BAF_{loc} biota to sediment concentrations normalized to total tissue lipids and to total sediment organic carbon were calculated at each time and reported in Table 3. The BAF_{loc} values in worms exposed with amphipods ranged from 0.1 to 1.6 during the exposure. According to Table 3, the worms in the treatment with 300 amphipods were significantly different from the treatment without amphipods and from that with 100 amphipods on days one and two. After 12 d, a significant difference was found only between the treatment without amphipods and that with 300 amphipods.

DISCUSSION

Toxicokinetics of fluoranthene in N. virens

Polychaete worms have been extensively used in several sediment toxicity studies [26,27] and in bioaccumulation studies [19,20,28,29] to assess effects of contaminated sediments. Most of these studies suggested that feeding habits of infaunal invertebrates and hydrophobicity of the compounds are important factors that influence the route of uptake of sediment-bound contaminants. Under nonequilibrium conditions the mode of exposure and the route of contaminants uptake can have a major influence on tissue concentration and on uptake kinetics in the organisms [30]. However, this study is one of

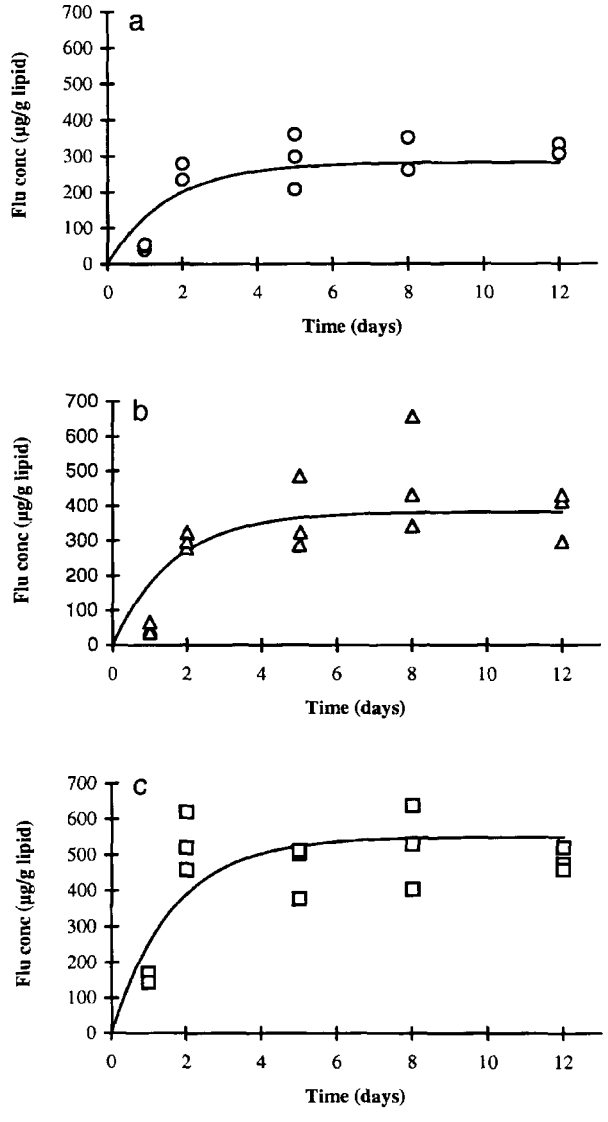

Fig. 1. (a) Uptake kinetics curve of fluoranthene in the worm *Nereis virens* as a function of time in the absence of amphipods. (b) Uptake kinetics curve of fluoranthene in the worm *N. virens* as a function of time in the presence of 100 amphipods. (c) Uptake kinetics curve of fluoranthene in the worm *N. virens* as a function of time in the presence of 300 amphipods.

the few in which toxicokinetics in worms was investigated in two different modes of exposure (i.e., in the presence and in the absence of sediment resuspension by bioturbating amphipods) to assess the importance of bioturbation in the bioavailability of fluoranthene for the worm *N. virens*. The results confirmed the hypothesis tested, that is, that the worms' body burdens would be higher in the presence of bioturbating amphipods than in their absence. However, the uptake (k_1) and elimination (k_2) rate constants and bioconcentration factors were not affected by bioturbating amphipods. Fluoranthene resuspension in the water column, because of bioturbation, increased its bioavailability for the worms, suggesting that aqueous fluoranthene is an important source for uptake for *N. virens*. This finding was consistent with results of other studies that implied that *N. virens* is able to accumulate contaminants from sediment via the water phase. Ray et al. [18], for example, found that uptake rates of cadmium from water by bottom-dwelling activities were 16 to 39 times higher than the uptake rates of cadmium from sediment. McElroy et al. [17] also showed that benz[*a*]anthracene added to the water column was more bioavailable for uptake by worms than benz[*a*]anthracene added to the sediment reservoir. Fowler et al. [19] found that uptake of polychlorinated biphenyl from water in the closely related polychaete *N. diversicolor* was more rapid compared to that from sediment; concentration factors were two to three orders of magnitude higher than those based on uptake from sediment.

The overlying water of treatments without amphipods was very clear, suggesting that the presence of fluoranthene in the water column and the uptake by the worms were the consequences of diffusive exchanges between sediment and water. In the treatments with amphipods, the uptake by the worms was increased by the presence of burrows, which can significantly increase total sediment–water surface [6,31,32].

The hypothesis that accumulation of PAH from overlying water can play an important role in the uptake of fluoranthene is also based on the fact that most of the members of the family Nereidae are omnivorous, burrowing in the sediment in search of food, and do not necessarily obtain their food by ingesting sediment. Studies of Fauchald and Jumars [33] and Smith et al. [34], for example, showed that *N. diversicolor* may be a scavenger, suspension feeder, or surface deposit feeder depending on the tide conditions and the availability of food. Because most of the burrowing organisms have been shown to ventilate the burrows for respiratory purposes by pumping oxygenated overlying water in their burrows [35], overlying water enriched with resuspended fluoranthene by bioturbating amphipods is likely to have led to higher body burdens in the worms exposed with the amphipods. The importance of this phenomen was already shown in a similar study performed previously with mussels in the overlying water [16]. The uptake of fluoranthene in mussels from the overlying water increased linearly with time and with amhipod density, whereas fluoranthene concentration and water turbidity remained more or less constant. Moreover, because the particle-bound fluoranthene was shown to increase with amphipod density and with time, mussels were concluded to accumulate probably also the particle-bound fraction of fluoranthene by continuous filter-feeding the sediment-resuspended particles in the water. This was the main difference with the present study, where the dissolved fraction of fluoranthene seemed to be the most important for uptake in the worms, as was shown by the BCF_{diss} values.

However, the BCF values calculated in our study, even those based on dissolved fluoranthene concentrations, may be underestimated for two different reasons. The first reason is that only the freely dissolved fraction of fluoranthene is probably mostly available for uptake in the worms. This is on the basis of a study carried out by Meador et al. [28], who reported higher BCF values (approx. a factor of five), for the amphipod *Rhepoxynius abronius* when based on free interstitial PAH concentrations (BCF_{free}) than on total ones (BCF_{tot}). The BCF_{free} were also closer to the predicted ones than BCF_{tot}. Although the differences were tiny, our BCF_{diss} values were also higher than those calculated on the basis of total aqueous fluoranthene, suggesting enhanced uptake of the dissolved fraction. The second reason for underestimated BCF values is the de-

Table 2. Measured fluoranthene concentrations in the water column (total and dissolved), calculated uptake and elimination rate constants (k_1 and k_2), and (log basis) bioconcentration factors (BCF) in the different amphipod densities. Standard deviations (±SD) and 95% confidence limits are given in parentheses

	Amphipod density		
Parameters[a]	0	100	300
C_{wtot} (μg/L)	5.85 (±0.5)	11 (±1.3)	12.5 (±2.9)
C_{wdiss} (μg/L)	5.85 (±0.5)	8.57 (±1.0)	7.49 (±1.7)
k_{2tot} (d^{-1})	0.60 (0.37–0.82)	0.60 (0.37–0.82)	0.60 (0.37–0.82)
k_{2diss} (d^{-1})	0.62 (0.38–0.86)	0.62 (0.38–0.86)	0.62 (0.38–0.86)
k_{1tot} (ml/g/d)	28,900 (18,400–39,400)	23,000 (14,800–31,000)	25,000 (16,000–34,000)
k_{1diss} (ml/g/d)	29,700 (18,700–41,000)	27,500 (18,100–37,000)	45,500 (29,000–62,000)
$LogBCF_{tot}$ (ml/g)[b]	4.68	4.59	4.62
$LogBCF_{diss}$ (ml/g)[c]	4.68	4.65	4.87

[a] Parameters are estimated on the basis of dissolved (C_{wdiss}) and total (C_{wtot}) (particle-bound + dissolved) fractions of fluoranthene in the water column; the values here reported are based on the average of concentrations obtained at each time (i.e., $n = 2$ and $n = 6$, for the treatments without and with amphipods, respectively).
[b] BCF_{tot} = calculated as the ratio between k_{1tot} and k_{2tot}, obtained on the basis of total fluoranthene.
[c] BCF_{diss} = calculated as the ratio between k_{1diss} and k_{2diss}, obtained on the basis of dissolved fluoranthene.

clining number of amphipods and concentration of TSS and likely of total fluoranthene in overlying water (see further in the Discussion).

Calculated BAF_{loc} values in all treatments varied from 0.14 to 1.6, which were included in the range found by Meador et al. [28] for *Armanda brevis* (a nonselective deposit-feeding polychaete) and *R. abronius* (a nondeposit-feeding amphipod). In this study, where bioaccumulation of PAHs by the two different infaunal invertebrates was compared, the authors found that tissue body burdens of high-molecular PAHs were significantly higher in the polychaete compared to the amphipod. Sediment ingestion was suggested to be the dominant route of exposure for the polychaete, *A. brevis*. However, fluoranthene and pyrene seemed to behave more like low-molecular PAHs, because the two species acquired nearly identical body burdens. The authors concluded that, for the low-molecular PAHs and for fluoranthene and pyrene, the contribution of tissue burden by sediment ingestion was insignificant for the polychaete and that the interstitial water was the main route of uptake for both organisms. This observation, in conjunction with the BAF_{loc} data, which were comparable to our results, support the hypothesis of aqueous fluoranthene uptake by *N. virens*.

Table 3. Lipid content and biota to sediment accumulation factors normalized to lipid concentration (μg/g lipid) and to organic carbon concentration (μg/g fluoranthene/g organic carbon) (BAF_{loc}) in *Nereis virens* in different treatments at different times

Time (days)	Number of amphipods	BAF_{loc} (SD)[a]
1	0	0.14 (0.02)A
	100	0.15 (0.05)A
	300	0.48 (0.06)B
2	0	0.80 (0.08)A
	100	0.91 (0.07)A
	300	1.63 (0.25)B
5	0	0.88 (0.23)A
	100	1.11 (0.32)A
	300	1.42 (0.23)A
8	0	0.93 (0.15)A
	100	1.45 (0.49)A
	300	1.60 (0.36)A
12	0	0.97 (0.06)A
	100	1.16 (0.22)A,B
	300	1.48 (0.09)B

[a] Means of treatments that do not share the same capital letter are significantly different from each other at $p < 0.05$ (Tukey's test).

Biotic interactions between N. virens *and* C. volutator

Results on percentage recovery of amphipods indicated that mortality was higher in the presence than in the absence of worms, suggesting that *N. virens* had a negative effect on *C. volutator*. Percentage recovery in the treatment with 100 amphipods was approximately 10% higher than the treatment with 300 amphipods. What type of interaction occurred between the amphipods and the worms in our experiment is not clear. The relationships between *C. volutator* and the closely related polychaete *N. diversicolor* have been extensively studied. Some authors found that the two species may coexist without having negative effects on each other [24,36], whereas others recorded negative correlations between their densities and reported that *N. diversicolor* may cause disturbance in amphipod recruitment and induce their migration after destroying *Corophium* burrows and forcing them to construct new burrows [21]. Smith et al. [34] observed interspecific competition for food between the two species when they coexisted under natural conditions and fed on the same epipelic diatoms species in laboratory experiments. Some authors [33,34] also reported the ability of *Nereis* species to act as predators. In our experimental system, we think that competition for food (diatoms) and the presence of mucus secretions produced by the worms might have been the most important factors that caused stress and mortality of the amphipods, rather than predation. If the amphipods were a real component in the diet of the worms, we would not have been able to see the dead amphipods in the sediment at the beginning of the exposure (after one, two, and five days) as we did. Because of the negative impact of the worms on the amphipods, TSS concentration, total fluoranthene concentration in overlying water, and BCF and BAF values may be underestimated.

CONCLUSIONS

The results of this study showed that the presence of bioturbating amphipods increased bioavailability of aqueous fluoranthene and consequently accumulation by the worm *N. virens*. The enhanced uptake of fluoranthene by worms is due to amphipod-mediated increase of the dissolved fraction. However, the uptake and elimination rate constants of fluoranthene

in the worms and bioconcentration factors were not significantly affected by the bioturbating amphipods. The presence of worms caused disturbance and rapid mortality of the amphipods, which is one of the reasons that BCF and BAF_{loc} values may be underestimated.

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