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Dietary Influences on the Bioaccumulation of Pollutants by the Annelid, *Lumbriculus variegatus*: Experiments Comparing Artificial Particles and Natural Sediments

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The impact of sediment-associated contaminants upon the benthic fauna of aquatic ecosystems has assumed some prominence in recent years (Burton 1991). This is because of the potential hazards posed by the long-term release of pollutants from the sediments, where they accumulate, back into the water column. Such an eventuality could influence the ecology of the fauna and the re-entry of pollutants into associated food chains. These possibilities are difficult to quantify because the composition and properties of the sediment are infinitely variable and the routes of contaminant uptake almost as diverse (Luoma 1989). The most important pathways for the transfer of sediment embedded pollutants into benthic organisms include direct dermal contact, intermittent ingestion, contact with the overlying water column and interactions with pore water in the sediment system. A number of different approaches have been taken to the analysis of these systems. On the one hand it may be important to measure the flux of pollutants into the biota since toxic effects are likely to occur if the rate of uptake exceeds the ability of organisms to eliminate these pollutants and protect their sensitive metabolic pathways (Verrengia Guerrero et al. 2002). Alternatively if the aim of the study is to obtain an estimate of the environmental content of particular contaminants or to assess the pollutant load that benthic organisms might accumulate over time, an equilibrium partitioning approach is more desirable (Di Toro et al. 1991). Initial studies of these problems tended to concentrate upon the pollutant content of the pore and bulk water but it now appears that in some species direct ingestion of the sediment may be an additional route of uptake (Leppanen and Kukkonen 1998).

In order to study some of these problems we have used chemically well-defined components to produce a test system that is both consistent in its properties and sufficiently well characterised to enable predictions to be made on how natural sediments might react to a variety of pollutants. In pursuing this we have used a variety of commercially available hydrophobic chromatography resins as analogues of sediment particles. They have the advantages that they can be obtained with well-defined sizes, chemically specific surfaces and controlled interactions when used in an *in-vitro* assay (Davies et al. 1999a, 1999b). The current work used these artificial particles as part of a comparative study that

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compared these data with those from tests using a bulk sample of a natural sediment (Conrad 2000). The role of sediment ingestion was compared by using two different approaches to the use of feeding and non-feeding organisms (Conrad et al. 2000; Simkiss et al. 2000).

MATERIALS AND METHODS

The aquatic oligochaete worm *Lumbriculus variegatus* was obtained from Sciento, UK and cultured under standard conditions (Phipps et al. 1993). The animals were maintained in aquaria containing aerated dechlorinated tap water and shredded non-bleached paper towels. The cultures were reared at 20°C with a 16:8 hr light:dark photoperiod and fed on a commercial fish food (Tetrafin).

Artificial particles 40-65 μm in diameter were obtained from TosoHaas using the Toyopearl 650M series chromatography beads. These particles have a strong hydrophilic backbone derived from ethylene glycol and methyl methacrylate. The SP beads had an anionic surface containing sulphonyl propyl ligands ($O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_3$), the Phenyl surfaces were largely hydrophobic and contained the group ($O\text{-}C_6H_5$), while DEAE possessed the anionic di-ethyl-amino-ethyl groups ($O\text{-}CH_2\text{-}CH_2\text{-}N\text{-}(C_2H_3)_2$. Artificial sediments were made by mixing 75% acid washed sand sieved to provide particles in the range 100-300 μm (British Drug House) with 25% washed test material (w/w). A natural sediment was collected in bulk from a pond at the ARC Study Centre at Milton Keynes, UK. It was sieved to 1mm, homogenized and stored in separate aliquots at 4°C until used. It had a total organic carbon content of 1.7%. Over 70% of the particles were below 63 μm. It was substituted for the artificial particles in the sediment:sand ratio of 1:3. The test chemicals were all ^{14}C-radiolabelled. The biocide cyprocoazole was obtained from Novartis, cyfluthrin was provided by Bayer, dichlorophenol (2,4-DCP), trichlorophenol (2,4,5-TCP), and pentachlorophenol (PCP) together with pyrene were supplied by Sigma while trifluralin was provided by Dow Agrochemicals.

The concentrations used for the bioaccumulation experiments were chosen to be well within the solubility level of the test chemical and outside the ranges associated with toxicity. The test system consisted of 0.5 g of particles with 1.5 g of sand and 20 mL dechlorinated tap water in glass vials in an incubator set at 20± 0.5°C. Particles were equilibrated with contaminants prior to use to give a final water concentration of 10^{-7} and 10^{-8} mol/L. Concentrations of contaminants were analysed using a Packard 2250CA Tricarb liquid scintillation spectrometer with corrections for chemical and colour quenching. The resulting Becquerels (Bq) were converted to pmols from specific activity data. Five animals were exposed to the chemicals for 48 hr in a static system in groups of 10 replicates. They were depurated by feeding in clean particles for 24 hr, killed, blotted dry and weighed before solubilizing in Soluene 350 and counting in Hionic-Fluor scintillation fluid (Packard).

Mass balance experiments were performed using the methods of Harkey et al. (1994) to provide concentrations of chemicals in the test solution, the depuration water, the vials and the particles by sonicating for 2 minutes and allowing them to stand for 24 hr in the scintillant before counting. Worms were killed, blotted dry, weighed, digested and analysed for radioactivity. Total quantities (pmol) of the chemicals that were added and subsequently recovered were used to derive a percentage recovery of chemicals in the experimental systems. Control experiments were performed using sand as the particle system. These particles were chosen to be in a size range (100-300 μm) which was too large to be ingested by the test organisms.

The bioaccumulation results for the controls (sand only) and experimental (sand plus particles) were analysed using a two tailed t-test (Minitab version 10.5 statistics package). Treatments that were different at $p<0.05$ were considered to be significant and feeding minus non-feeding accumulation was used to calculate the percentage uptake due to sediment ingestion.

RESULTS AND DISCUSSION

The recoveries of three typical contaminants from four different substrates using mass balance experiments are shown in Tables 1-3. The mean recovery in all the treatments involving artificial particles was 95% (range 85-101%). Mass balance results using sand as the control substrate also gave a recovery of 95% (range 91-100%). Similar data for the natural sediment was 65% (range 36-101%).

Table 1. Mass balance results for cyproconazole. All quantities are p*M*.

Particle	Total added	Water (48hr)	Depurated water (24hr)	Vial and particles	Worms	Total	Recovery %
Sand	27.8	21.1	1.7	4.7	0.4	27.8	100
Phenyl	61.1	12.4	1.1	46.3	0.5	60.3	98
SP	45.7	21.9	1	21.1	0.8	44.8	98
DEAE	58.3	30.7	2.7	24.6	0.9	58.9	101
Nat. Sed.	75.9	55.6	3.8	16.4	1.1	76.9	101

Table 2. Mass balance results for cyfluthrin. All quantities are p*M*.

Particle	Total added	Water (48hr)	Depurated water (24hr)	Vial and particles	Worms	Total	Recovery %
Sand	21.7	11.4	0.1	8.6	0.1	20.2	93
Phenyl	28.7	9.4	0.5	18.5	0	28.4	98
SP	22.9	9.5	0	13.7	0	23.2	101
DEAE	48.8	0.4	0	41.9	0	42.3	87
Nat.Sed.	52.5	16.1	0.1	2.8	0	19	36

Table 3. Mass balance results for 2,4,5-trichlorophenol. All quantities are in p*M*.

Particle	Total added	Water (48hr)	Depurated water (24hr)	Vial and particles	Worms	Total	Recovery %
Sand	74.6	29.8	0.1	1.5	37	68.4	91
Phenyl	359.1	112.2	0.1	132.6	74.5	319.4	89
SP	99.8	38.5	0.1	40.8	16.7	96.1	96
DEAE	10052	15	12.4	8474.3	33.3	8535	85
Nat. Sed.	99.8	30	0.1	3.1	23.5	56.6	57

The accumulations of radiolabelled contaminants in animal samples were converted to concentrations by dividing with the mass of the organisms. This was normalised against the concentration of chemical in the water samples at the end of the experiment to give a biological accumulation factor (BAF), showing the ratio of contaminant in the organism in relation to the concentration in the water.

Data for the total uptake of pollutant into the worms clearly involved that accumulated by the four routes of uptake (i.e. water column, pore water, sediment contact and sediment ingestion). In order to determine the contribution of dietary sources to this body load the control data from non-feeding worms was subtracted from the total body concentration of the feeding specimens. The dietary component was then expressed as a percentage of the total (Table 4) and only that which showed a change of 50% was taken to be a positive influence.

Table 4. Accumulation of pollutants (BAF) from a variety of natural and artificial sediments showing percentage of uptake due to dietary sources.

	Natural sediment		Toyopearl Phenyl		Toyopearl SP		Toyopearl DEAE	
Chemical	Total uptake	Dietary %	Total uptake	Dietary %	Total uptake	Dietary %	Total uptake	Dietary %
2,4-DCP	24	0	101	0	17	0	126	0
2,4,5-TCP	227	0	210	0	116	0	358	0
PCP	453	72	334	62	305	59	40	0
Fenprop.	153	0	165	0	42	0	165	0
Trifluralin	107	0	44	0	60	0	160	65
Cyprocon.	7	0	16	0	14	41	11	30
Cyfluthrin	8	0	3	0	4	0	54	76
Pyrene	1175	85	678	75	337	50	970	82

The worm *L. variegatus* normally reproduces by architomy, a procedure whereby new individuals bud off from the anterior end of the parent that is left in a non-feeding state for a period of up to a week (Leppanen and Kukkonen 1998). By

selecting headless individuals these workers were able to use these animals as controls for the normal feeding individuals, thereby obtaining data on the importance of ingestion in the accumulation of pollutants. Conrad et al. (2000) used a similar approach by surgically de-heading the worms rather than by sorting reproducing adults. By using this technique Conrad (2000) was able to study 22 compounds from 17 different chemical families to investigate the relationships between their physiochemical properties. She concluded that the dissociation potential, the presence of ionic quaternary nitrogen groups, the hydrophobicity and molecular weight could all influence the bioavailability of chemicals in contaminated sediments.

The present work was a parallel study to that of Conrad (2000) in that it used the same test organism and some of the same compounds but with two other variables based upon manipulating the size and the surface properties of artificial sediments (Davies et al. 1999a, 1999b; Simkiss et al. 2000). Thus the difference between feeding and non-feeding states was induced by varying the size of the components of the sediment particles and their surface properties were varied by using synthetic resin beads.

The validity of these approaches was determined using mass balance experiments that measured the recovery of three contaminants from four different substrates in the test system. The 3 radiolabelled chemicals consisted of cyproconazole, a triazol with a pK_a of 10.3; cyfluthrin, a pyrethroid with no acid/base properties; and 2,4,5-trichlorophenol with a pK_a of 6.2. The recovery of these three very different chemicals averaged 95% for the sand, 95% for the resins but only 65% (range 36-101%) for the sediment. There are two possible explanations for the low recovery from the natural sediment. First, extracting the radioisotopes from this source resulted in a strongly coloured solution that was difficult to correct accurately for quench in the scintillation analyses. Second, the complex sediment probably contained deep, slowly equilibrating ligands from which it was difficult to extract the chemicals with the method used. The elimination of such inaccessible sites is seen as one of the benefits of the resin particle assay since anything in a natural sediment that has an equilibrium time that is measured in periods of days or weeks will have little effect on absorption in a worm that has a gut transit time of around 12 hours (Brooke et al. 1996; Leppanen and Kukkonen 1998; Davies et al. 1999b).

If the worm *L. variegatus* is exposed to contaminants in a water-only test the animals clump together and show abnormal behaviour patterns that modify their exposure to the water column. For this reason we used sand particles into which the worms could burrow but which were too large to ingest as non-feeding controls. By subtracting this value from the results obtained from animals that were exposed to sand containing natural sediment or artificial resin particles it was possible to identify what contribution ingestion had on the accumulation of contaminants from the substrate. The results in table 4 show that of the pollutants tested only two showed significant uptake attributable to intestinal absorption. These were PCP and pyrene. Furthermore the hydrophobic resin particles

(Toyopearl Phenyl) that were used as analogues of the organic carbon content of sediment produced very similar values to those obtained with natural samples (Table 4). Both these results confirmed the conclusions reached by Conrad (2000). In addition it was clear from our data that if the sediment contained charged ligands this could further influence the bioavailability of charged contaminants. When a negative charge was introduced into the artificial resin particles (Toyopearl SP) the contribution of the dietary intake of pyrene was reduced and when a positive charge was added (Toyopearl DEAE) the intestinal uptake of cyfluthrin was increased and that of PCP was abolished.

These results indicate that tests using standardized resin particles can be used to investigate whether a pollutant is likely to be accumulated by sediment-feeding organisms. They also provide an opportunity to examine how electrostatic charges in the sediment are likely to influence the absorption of contaminants by the biota.

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