Arch. Environ. Contam. Toxicol. 39, 315–323 (2000)
DOI: 10.1007/s002440010110

ARCHIVES OF
Environmental
Contamination
and Toxicology
© 2000 Springer-Verlag New York Inc.

Ecotoxicity of Contaminated Suspended Solids for Filter Feeders (*Daphnia magna*)

R. Weltens, R. Goossens, S. Van Puymbroeck

VITO—Flemish Institute for Technological Research, Department of Environmental Toxicology, Boeretang 200, B2400 MOL, Belgium

Received: 21 September 1999/Accepted: 21 March 2000

Abstract. It is generally assumed that the dissolved fraction of a toxic substance in surface water is mainly responsible for toxicity to aquatic organisms. However, toxic compounds are often adsorbed or chemically bound to suspended particles in the water column, depending upon the physico-chemical conditions. In the present study potential adverse effects to filter feeding organisms by metal contaminated particles were investigated. In our hypotheses the adsorbed metals might desorb in the gastrointestinal tract—due to different physico-chemical conditions—and exert toxic effects. Clay and sand particles, algae and organic material (peat) were artificially contaminated with cadmium and zinc. The contaminated materials were resuspended in standard conditions and toxicity was measured for the water flea *Daphnia magna* (mortality at 48 hours). As a reference, supernatant solutions were used containing the same concentration of dissolved metal as the suspensions. It was also established that the test concentrations of solid material (250 and 500 mg/l uncontaminated particles) did not cause any mortality within 48 hours. Daphnids are filter feeders: they filtrate large amounts of surrounding water, redrawing particles as a food source. Results strongly indicate that contaminated particles threaten the health of these particle-feeding organisms. Compared to the reference severe acute toxic effects were seen and cadmium accumulation was increased when contaminated solid material was present. Results were essentially the same for the different materials used in the experiments, except for sand contaminated with cadmium. This shows that mineral as well as organic materials can contribute to the particle bound toxicity. Different results were obtained when a static set up was used instead of a flow through set up, illustrating that the route of administration is important to make particles available and thus to evaluate their toxicity. Contaminated particles clearly have toxic potency, not only because they are a continuous source of dissolved xenobiotics, but also because the particle bound fraction can become available within the body of particle feeding organisms. This could lead to unexpected high tissue concentrations. More insight is needed to predict the bioavailability of adsorbed pollutants. Results of this study already indicate that suspended solids should be considered as a separate compartment in risk evaluation of chemicals, effluents or natural surface waters.

Correspondence to: R. Weltens

Natural surface waters contain suspended solids in the water column. By definition the total of solid material that can be extracted from the water column is called "suspended solids." They are a mixed collection of different materials: resuspended sediment material, erosion material of the surrounding soils, shells, colloids, phyto- and zooplankton, fecal material, organic decomposition materials, etc.

In the water column of contaminated natural surface waters many pollutants adsorb to the suspended solids (Rodgers *et al*. 1987). Although many physicochemical parameters are involved (Admiraal *et al*. 1995), the adsorption behavior for organics can be predicted roughly from the compound's log K_{ow} (oil/water partitioning coefficient). Moreover particles mainly exhibit a negative surface charge due to adsorption of organic matter. Positively charged molecules (*e.g*., heavy metals) therefore are attracted from the water by electrostatic forces (Allen *et al*. 1995).

Organic molecules with high K_{ow} values and positively charged molecules concentrate on suspended solids and indeed very high concentrations can be measured in the solid material collected from contaminated natural waters (VMM 1998). Since aquatic organisms are exposed to these highly concentrated micropollutants, it is necessary to investigate their toxicological impact.

Suspended solids can decrease toxicity of many chemicals for some aquatic organisms by decreasing the free concentration of the molecules (Kördel *et al*. 1997), but at the same time they can increase the bioavailability for other organisms (Farag *et al*. 1994; Carvalho *et al*. 1995; Pentinnen *et al*. 1995). Toxic effects due to particle-bound contaminants can indeed be expected in aquatic organisms at different trophic levels.

Particle-feeding organisms are important inhabitants of the aquatic ecosystem. These organisms feed on particles and contaminated particles might in these organisms end up in the gastrointestinal tract and exert toxic effects. McCarthy (1983) calculated that PAHs, adsorbed on yeast particles, were assimilated by daphnids at the same rate and efficiency as the carbon

(20–30%). Since the physicochemical conditions in the digestive tract can be very different from the surrounding water, contaminants might desorb within the gut. Because the amount of toxic substance per particle can be very high, toxic effects could be caused even after digestion of a limited amount of particles.

In risk assessment studies the dissolved concentrations of compounds are used to predict bioaccumulation. When desorption of molecules from suspended solids *in the body* becomes prominent, however, tissue concentrations in the organisms could be unexpectedly high. Because particle-feeding organisms are an important food source, situated at the beginning of the food chain, this phenomenon could lead to biomagnification.

Another possible effect of contaminated particles on particle feeding organisms like daphnids, is a drastic reduction in food intake causing serious chronic effects on the population level. It has been shown many times that toxic products reduce food uptake in daphnids. Contaminated food particles affect food uptake even more than dissolved contaminants (Allen *et al.* 1995).

Given these arguments, it seemed worthwhile to investigate potential toxic effects of artificially contaminated materials in particle feeding water fleas (*Daphnia magna*).

The aim of this study was to test whether metal molecules, adsorbed to suspended solids of different types, possibly desorb from the particle's surface when they enter the new physicochemical environment of the gastrointestinal tract of the particle feeding organisms. It is important to gain insight in these phenomena since at present most risk assessment is based on the dissolved concentrations of xenobiotics. When the particle bound fraction of a contaminant is potentially available to this important trophic level of particle feeding organisms, toxic effects and bioaccumulation and magnification cannot be predicted properly from dissolved concentrations only.

This study was intended to test the hypotheses that metal molecules, attached to solid material in the water column of surface water, provide an extra source of metal toxicity to filter feeding organisms. Therefore the water-only effect values were measured for two metal salts solutions (cadmium chloride and zinc chloride) in the acute toxicity test on *D. magna*. This baseline dose-response curve (mortality as a function of dissolved metal concentration) was established in both the setups that were further to be used in the study. Again, in both setups the effects of uncontaminated solid materials (clay, sand, peat, algae) were tested, to establish their background toxicity.

Finally the artificially contaminated materials were tested (suspensions and supernatants), and effects were compared to both the background toxicity and to the baseline toxicity as expected from the dissolved metal concentration. Dissolved metal concentrations were measured at the start and at the end of the experiments.

In a separate experiment the tissue concentrations of organisms exposed to a suspension of contaminated solid material were compared to those of organisms that were exposed to the supernatant of the same suspension, as a measure for metal uptake.

Materials and Methods

Contamination of Materials

Natural suspended solids are composed of both mineral and organic materials. In this study different materials (clay, sand, algae, and peat) were separately used in the experiments and artificially contaminated. Clay (60% < 2 μ) and sand particles (60–600 μ) are a substantial component in many natural rivers in Flanders; unicellular algae are an important food source for water fleas in natural surface waters; and peat (12 mm, sieved to 1 mm) was used as a reflection of degrading natural organic materials. Cadmium and zinc were used to contaminate the solid particles because toxicity of these metals for daphnids is well documented and analyses of these elements is relatively simple in different matrices.

Contamination of Peat, Clay, and Sand. Materials are commercially available: Caoline clay (Kaldic SP20; < 20 μ), peat (Argofina 12 mm), and sand (Sibelco M34; 0.06–0.6 mm). The composition of these materials is summarised in Table 1.

Ten grams per liter of solid material is suspended in a volume of 1 L of a metal solution (concentration y g/L). This suspension is stirred overnight, allowed to settle, and a sample of the overlying fluid is filtered over a Corning Millipore filter (0.22 μm) for metal analyses (z g/L; plasma emission spectrophotometry ICP OES Jarrell Ash Atomcomp 750). The suspension is filtered over a Corning Millipore filter (0.22 μm), and the solid material is collected from the filter and washed two times with ethanol (90%) to remove all water. After each washing procedure the solid material is centrifuged (4,000 rpm; 10 min) and finally dried in an oven (105°C). Dried materials are afterward kept in a tightly closed glass vessel at room temperature until use.

Adsorbed concentrations can be estimated:

$$\text{bound metal/10 g} = (y - z)/10 \text{ g} \qquad (\text{g/g})$$

Table 2 shows the estimated concentrations of the spiked materials used in this study. These estimated values are not completely correct because small amounts of metal molecules could have desorbed during the washing procedure. The actual metal concentrations on the particles were not measured, since this study did not intend to quantify the relationship between particle load and effect.

For each experiment dried material is resuspended in *Daphnia* medium at the different test concentrations and allowed to equilibrate for 1 night to obtain a stable suspension. Then the dissolved concentration—due to desorption of previously adsorbed metal molecules—is measured and the remaining amount of adsorbed material is calculated. Samples of the solutions were again taken at the end of the experiments to evaluate the free metal concentration in the suspension due to desorption during the experiment. Samples for analyses were centrifuged at 4,000 rpm for 10 min, subsequently filtered over a 0.22-μm membrane filter, and acidified with HNO_3 (ultrapure, Merck, Darmstadt, Germany).

Contamination of Algae. Algal cultures were renewed once a year (CCAP, Widesmere Laboratory, Ambleside, Cumbria, UK) and grown in the lab in Jaworski medium (16 mg/L $Ca(NO_3)_2.4H_2O$, 10 mg/L KH_2PO_4, 42 mg/L $MgSO_4.7H_2O$, 13.25 mg/L $NaHCO_3$, 1.9 mg/L EDTA-FeNa, 1.9 mg/L EDTA-Na_2, 2 mg/L H_3BO_3, 1.15 mg/L $MnCl_2.4H_2O$, 0.8 mg/L $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.033 mg/L vitamin B_{12}, 0.033 mg/L vitamin B_1, 0.033 mg/L biotin, 67 mg/L $NaNO_3$, 30 mg/L $Na_2HPO_4.12H_2O$, 42 mg/L $C_2H_4O_2.Na.3H_2O$, 42 mg/L oxoidl29, 83 mg/L tryptone, 83 mg/L yeast extract, 833 mg/L $CaCl_2$).

A concentrated algal suspension (mixture of *Chlorella vulgaris*, *Chlamydomonas* sp., *Raphidocelis subcapitata*, and *Scenedesmus subspicatus*) is added to a metal solution (1 L, y g/L) and stirred for 1

Table 1. Composition of the commercially available particles used for spiking

Sand (Sibelco M34)	Caoline Clay (Kaldic SP20)	Peat (Argofina)
Natural white quartz sand (Mol (B))	Natural Clay (Bretagne (Fr))	Natural degraded organic material (NL)
Composition: >98.5% SiO_2	Composition 33.4% Al_2O_3 2.55% K_2O 50% SiO_2 0.4 MgO	
Granulometry:	Granulometry	12 mm (sieved at 2 mm)
mean = 160 μ	98% < 20 μ	
σ: 15 μ	92% < 10 μ	
min: 63 μ	78.5% < 5 μ	
max: 600 μ	59.4% < 2 μ	
	mean: 1.2 μ	
pH: 7–8	pH: 6.5 ± 0.5	
Hardness: 7 mohs		

Table 2. Calculated load concentrations for different materials after artificial spiking with $CdCl_2$ or $ZnCl_2$

Type of Solid Material	Amount of Solid Material Used in the Spiking Procedure	Concentrations of the Spiking Solutions (mg/L)	Estimated Load on (dry°) Solid Material
Clay	10 g/L	27.3 $CdCl_2$	0.71 mg Cd/g
	10 g/L	45.5 $CdCl_2$	1.28 mg Cd/g
	10 g/L	22.8 $ZnCl_2$	0.79 mg Zn/g
Sand	10 g/L	98.28 $CdCl_2$	1.60 mg Cd/g
	10 g/L	95.2 $ZnCl_2$	0.74 mg Zn/g
Peat	10 g/L	45.5 $CdCl_2$	1.90 mg Cd/g
	10 g/L	25.5 $CdCl_2$	1.10 mg Cd/g
	10 g/L	33.75 $ZnCl_2$	1.10 mg Zn/g
	10 g/L	19.8 $ZnCl_2$	0.65 mg Zn/g
Algae	0.2×10^9 cells/L	18.2 $CdCl_2$	1.2 mg Cd/10^9 cells
	2×10^9 cells/L	12.8 $ZnCl_2$	1.8 mg Zn/10^9 cells

° Except for algae.

night. Cell number is counted (× cells/L; Coulter counter: Coulter electronics model D) and then the suspension is centrifuged (2,000 rpm, 10 min). A sample of the supernatant is taken for metal analyses (z g/L; Plasma emission spectrophotometry ICP OES Jarrell Ash Atomcomp 750), and supernatant is carefully removed. The pellet is resuspended in *Daphnia* medium at a preset cell concentration and used immediately in the experimental setup.

Adsorbed concentrations can be estimated:

$$\text{bound metal/x} = (y - z)/x \qquad (\text{g}/10^9 \text{ cells})$$

Table 2 shows the estimated concentrations of the spiked algae used in this study.

Samples of the solutions were also taken at the end of the experiments to measure the free metal concentration in the suspension due to desorption during the experiment. Samples for analyses were centrifuged (2,000 rpm, 10 min) and taken from the supernatant solution.

Stability Test on Spiked Materials. Clay material spiked with cadmium was used to prepare test concentrations with different amounts of suspended solids (125, 250, 500, 750, and 1,000 mg/L) in *Daphnia* medium and allowed to equilibrate while stirring for 1 night (as for normal experiments). Dissolved cadmium concentrations were measured, and the test solutions were then used in static or perfusion set up (see below). After an exposure period of 48 h the dissolved concentrations were measured again for both setups in the supernatant after centrifugation and filtration (0.22 μm).

Daphnia Bioassays

Static Acute Toxicity Test. This test is based on OECD guideline 202 (1984), except that adult organisms are used instead of neonates.

In short: adult organisms (*D. magna*, bred in the lab since 1994) are exposed to a dilution series of test substance/particles and mortality is assessed after 48 h at room temperature. Survival in control (OECD) daphnia medium should at least be 90%. For each test concentration four replicates with each five organisms are tested in glass test vessels containing 20 ml of solution.

This setup was used to test both pure metal solutions (LC_{50} for $CdCl_2$ and $ZnCl_2$) and to evaluate the effect of suspended solids in a set up where the material could settle down.

Test concentrations for pure solutions are listed in Table 3. Test concentrations for solids were 250 and 500 mg/L for clay, sand, and peat, and 0.5 and 1×10^9 cells/L for the algae (Table 4).

Flow-through Acute Toxicity Test. To administer the suspended solids in a more realistic way, a flow-through setup was build which allowed to expose the organisms to the solids when they are actually in suspension (Figure 1).

Four exposure chambers each containing 20 adult organisms in approximately 25 ml of solution, were simultaneously perfused via a multiway pump in the one direction and four chambers in the other direction. The source solutions were continuously stirred by magnetic stirrer, keeping the solids in suspension. For each chamber the effluent solution was collected in a vessel that served again as source solution for the reverse direction, allowing recirculation of the test solutions. Mortality was assessed after 48 h: per concentration the animals were transferred in groups of five to a small beaker and mobility was assessed. Four test conditions could be tested simultaneously (2 vessels/condition).

In one series of experiments again the LC50 values of $CdCl_2$ and $ZnCl_2$ solutions were measured to evaluate the possible effect of the perfusion (extra oxygen supply) on the sensitivity of the organisms. In another series the effect of suspended solids was tested: *Daphnia* medium, two concentrations of suspended solids, and the supernatant of the most concentrated suspension were tested. These effects were measured both with contaminated and noncontaminated materials to measure background effects due to the physical presence of suspended solids.

Test concentrations for pure solutions are listed in Table 3. Test concentrations for solids were 250 and 500 mg/L for clay, sand, and peat, and 0.5 and 1×10^9 cells/L for algae (Table 4).

The suspended solids concentration was not measured during or after the experiments. Each experiment was started with a well-known concentration of suspended solids (250 and 500 mg/L solids as stated above). The flow-through setup was build in translucent materials. Since suspensions were continuously stirred, settlement of particles in the system could only take place during the transfer in the catheters or in the exposure chambers. By using small-diameter transfer tubes, the hydrostatic pressure built up in these narrow tubes does not allow any sedimentation within the tubing. A minimal amount of sedimentation could be noticed at the outlet of the chambers, indicating that the

Table 3. Water-only tests: Test concentrations and (48-h) LC50 (mg/L) values for adult *Daphnia* in static and flow-through setup (n = number of experiments)

	Test Concentrations (mg/L)					n	LC50 ± SD (mg/L)
Static setup							
$ZnCl_2$	20.25	11.2	6.2	3.5	1.9	2	5.4 ± 0.162
$CdCl_2$	1.8	1	0.56	0.25	0.18	1	1.44
Flow-through setup							
$ZnCl_2$	20.25	10.12	5	1.35		2	6.2 ± 0.405
$CdCl_2$	1.8	1.3	0.91	0.45		2	1.47 ± 0.18

Table 4. Cadmium concentration (mg/g) in whole *Daphnia* tissue after exposure of adult organisms to suspension or to the supernatant of this suspension after centrifugation. Both contained 246 μg/L dissolved Cd and the suspension contained additionally 47 mg/L solids loaded with 360 mg Cd/g. Samples were taken immediately after exposure (t = 0) and after 1 (t = 1) and 2 h (t = 2) in cadmium-free *Daphnia* medium

t	Supernatant (mg Cd/g tissue)	Suspension (mg Cd/g tissue)
0	172	463
	164	430
1	158	291
	160	312
2	153	252
	138	291

Results from two replicates are shown for each condition (each replicate is a pool of 20 organisms).

suspended solids concentration decreased by a very small amount by the end of the experiment.

This decrease in suspended solids concentration was not quantified because the (visible) small loss of material would not have any consequences for the conclusions. This study did not intend to quantify the relation between the amount of particulate material (or rather particle surface) and the measured effect.

Bioaccumulation Experiment. Twenty organisms per well were exposed in 24 well plates for 19 h to the following conditions (18 replicates per condition):

- supernatant: 246 μg/L dissolved $CdCl_2$
- suspended solids solution: 246 μg/L dissolved $CdCl_2$, 47 mg/L suspended solids (caoline clay, 360 mg Cd/g)

After exposure, organisms from four replicates (for each test condition) were pooled for metal analyses (see below). Organisms from 10 replicates were pooled and placed in *Daphnia* medium to enable them to void their intestinal gut contents (transit time is approximately 45 min). From this pool 80 organisms were collected for analyses after 1 h and another batch of 80 animals were collected after 2 h in *Daphnia* medium. Four replicates were left to measure mortality after 48 h of exposure.

Organisms collected for analyses were thoroughly rinsed with distilled water and dried at 70°C in an oven overnight, and dry weight was measured. They were then mineralized in HNO_3 and diluted with demineralized water for cadmium analyses with ICP.

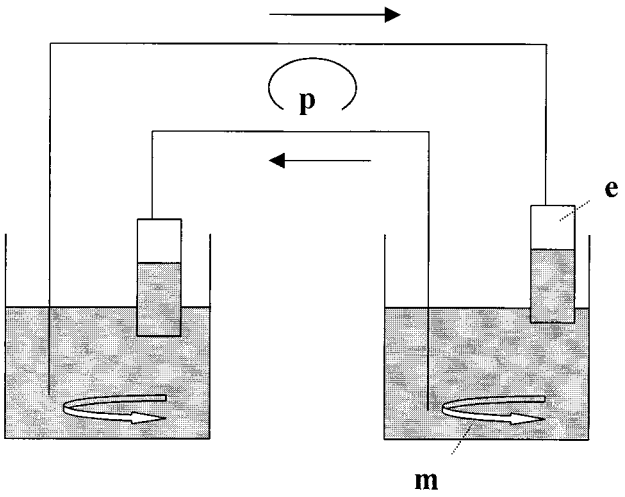

Fig. 1. Scheme of the flow-through setup. m = magnetic stirrer, p = pump, e = exposure chamber. Constant mixing of the solutions in the beakers provides solids in suspension. A (multivalve) pump transfers the suspension through the exposure chamber into the opposite beaker where it is mixed again and transferred in the reverse direction to the second exposure chamber (recirculation of fluid for 48 h). No medium refreshment was provided during 48 h of exposure. Four parallel solutions can be tested in this system (4 × 2)

Results

LC50 Values for $CdCl_2$ and $ZnCl_2$

Table 3 summarizes the results from water-only acute toxicity tests (48-h exposure) in the static and flow-through setups. It is clear that perfusion does not affect the toxicity results for these metals in the *Daphnia* bioassay.

Effect of Uncontaminated Suspended Solids

To interpret further results it is important to determine the background effects caused by the physical presence of suspended solids. In these experiments concentrations of clay, sand, and peat of 500 mg/L were tested for 48 h in the flow-through setup, and mortality was assessed. No significant effects were noticed, indicating that the test concentrations are not causing significant background mortality within this exposure time (respectively 27 ± 17, 7.5 ± 10.6, and 12.5 ± 17.7% mortality in the perfusion set up and 14 ± 10.6, 6 ± 2.8 and 12 ± 5.6% mortality in static conditions; mean ± SD; n = 2).

Algae were not separately tested since experience with *Daphnia* breeding learned that the test concentrations are well below the normal algal cell concentrations that are used as food in the breeding compartments without any adverse effect.

Spiking and Stability of Spiked Materials

Adsorbed metal concentrations were estimated from the loss of metal in the spiking solution (see methods). On resuspension a

new equilibrium will be achieved after a short time—due to desorption of limited amounts of metals—which is stable during the 96-h test period in fish medium (Van den Belt *et al*. 1999).

Figure 2 shows results of a cadmium on clay spiking experiment performed in our lab for *Daphnia* medium. Different concentrations of spiked material (125, 250, 500, 750, and 1,000 mg/L) were used in both setups, and organisms were exposed to the suspensions for 48 h. The dissolved metal concentrations were measured both at the beginning and at the end of the exposure period. Results clearly show that the suspension remained stable during a 48-h exposure period in both flow-through and static setups, and that the concentrations were completely comparable in both setups.

Table 5 resumes different spike experiments performed at our lab (including those of the present study) to illustrate the stability of cadmium and zinc suspensions in both flow-through and static setups, and for different materials. Samples were taken at the start and the end of the 48-h experiment. These results show that the equilibrium period of 1 night before use, is suitable to obtain a stable suspension. Even for algae, which were used immediately after resuspension (see methods), only limited changes in dissolved concentrations were measured. These slight changes were comparable between static and flow-through systems. Only for cadmium-algae deviating results were seen in the static setup where a notable decrease in the dissolved concentration was measured.

These results (Table 5) ensure that the dissolved metal concentrations are comparable for both types of setup, and therefore mortality results in function of dissolved concentration can be compared between static and flow-through experiments.

These results also show that the dissolved concentrations of cadmium and zinc were in any case well below the LC50 values (see Table 3) and therefore are suitable for further experiments.

Effect of Contaminated Suspended Solids

Figure 3 shows the mortality data in the different conditions. In these figures the dose-response for water-only experiments with $CdCl_2$ and $ZnCl_2$ are shown (bullets and trend lines), both in static (open) and flow-through (filled) setups. Mortality in the presence of contaminated solid materials (algae, sand, peat, and clay; squares) are shown in function of the dissolved metal concentration for static (open squares) and flow-through systems (filled squares). If the hypotheses were true that only the dissolved concentration of a xenobiotic were responsible for toxicity, all conditions would fit the water-only dose-response curve. This is true for the static suspensions (except clay; see below), but clearly not for the flow-through conditions. Often 100% mortality is seen in the flow-through condition, even with low dissolved metal concentrations. Since dissolved concentrations are comparable for both setups, these results strongly indicate that the adsorbed fraction exerts a significant contribution to toxicity on condition that the particles are available to the organisms.

These results can indeed be explained in terms of bioavailability. Because daphnids are filter feeders they continuously filter the surrounding water. In the case of the flow-through setup, particles are directly available to the organisms, while in the static setup the particles will settle at the bottom of the vessel and will not be directly administered to the organisms. Due to the small size of the clay particles (60% of the particles < 2 μm), however, this material is kept in suspension spontaneously by the movement of the swimming daphnids and is therefore available even in the static condition. The deviant behavior of sand contaminated with cadmium will be discussed later.

Bioaccumulation Experiments

Clay was used in a static setup, and organisms were exposed for 19 h to cadmium-containing solution. Due to the small particle size and the large number of organisms in a small volume, availability of the particles during exposure was guaranteed.

Table 4 summarizes the results of this experiment. Cadmium concentrations in organisms exposed to the suspension are clearly much higher than in organisms exposed to the supernatant. This can be explained by the presence of contaminated particles in the gut, but the normal transit time of food in *Daphnia* is about 45–60 minutes (Espiritu 1994). Therefore the elevated levels after 2 h indicate that desorbed cadmium taken up in the gut, is responsible for these higher concentrations. The concentrations used in this experiment caused no mortality in the supernatant but 24% (±12 SD, four replicates) in the suspension after 48 h. This confirms the chemical results: an elevated uptake of cadmium causes an increase in toxic response.

Discussion

Effect of Uncontaminated Suspended Solids

The experiments showed that uncontaminated solids used at the maximum test concentration of 500 mg/L caused no significant mortality within the 48 h of exposure. Study on fish with exposure to clay particles up to concentrations of 2,000 mg/L during 144 h also revealed no mortality (Van den Belt *et al*. 1999). Sublethal and chronic effects however have been described. Hughes (1976) described increased respiration rate, morphological changes in gill tissue, and decoloration of the skin in fish at chronic exposure to low concentrations (90 mg/L) of suspended solids in a flow-through setup. Scott and Redmond (1989) studied effects of chronic exposure of *Ampelisca* to 40–50 mg/L of contaminated dredged material and showed that populations were affected: reproduction success decreased and survival of newborn organisms was drastically reduced.

Depending on geography and urban activity, the composition and amount of suspended solids in surface waters can be very different. Values higher than 4,000 mg/L have been reported (Helawell 1986) and in effluents even values up to 100 g/L can be measured. In Flanders, mean values in surface waters are about 20 to 50 mg/L, with some excep-

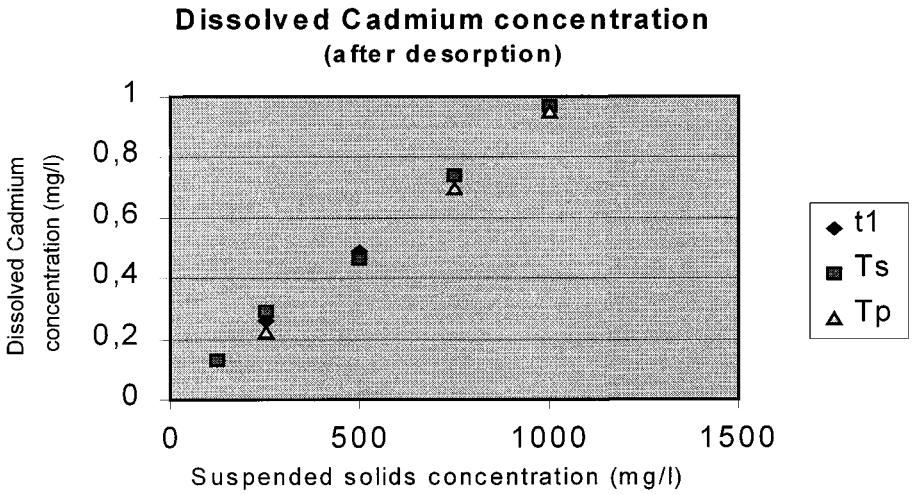

Fig. 2. Desorption of cadmium and zinc from contaminated particulate material at t1 (start of the experiment), Ts (end of the static experiments), and Tp (end of the perfusion experiments)

Table 5. Concentrations of dissolved cadmium and zinc (mg/L) in the suspensions after resuspension of contaminated solid materials in *Daphnia* medium at the start of the experiment (t1) and at the end of the experiment (Ts after use in a static set up, Tp after use in a perfusion set up.)

Original Metal Load	Material	Concentration of Suspended Solids	t1	Ts	Tp
Cadmium					
1.28 mg/g	clay	125 mg/L	0.13	0.13	
			0.14		
		250 mg/L	0.26	0.29	0.22
			0.25		
		500 mg/L	0.49	0.46	
				0.57	
		750 mg/L		0.74	0.7
		1,000 mg/L		0.97	0.95
0.71 mg/g°	clay°	250 mg/L°	0.09		
		500 mg/L°	0.117		
1.6 mg/g°	sand°	250 mg/L°	0.06	0.07	0.07
		500 mg/L°	0.13	0.15	0.12
1.1 mg/g°	peat°	250 mg/L°	0.15	0.18	0.16
		500 mg/L°	0.16	0.18	0.18
1.9 mg/g°	peat°	250 mg/L°	0.19		
		500 mg/L°	0.24		
1.2 mg/10^9 cells°	algae°	0.5 $*$ 10^9 cells/L°	0.18	0.07	0.16
		1 $*$ 10^9 cells/L°	0.26	0.16	0.25
Zinc					
0.79 mg/g°	clay°	250 mg/L°	0.11		
		500 mg/L°	0.18		
0.74 mg/g°	sand	250 mg/L			
		500 mg/L	0.19	0.19	0.19
0.65 mg/g°	peat	250 mg/L	0.09	0.1	
		500 mg/L	0.12	0.11	0.13
1.1 mg/g°	peat	250 mg/L	0.16		
		500 mg/L	0.21		
1.8 mg/10^9 cells°	algae	0.5 $\times$ 10^9 cells/L	0.16	0.1	0.1
		1 $\times$ 10^9 cells/L	0.25	0.16	0.16

° Used in the present study.

tions going up to about 100–200 mg/L (VMM 1998). The regulatory rules in Flanders recommend a maximum of 50 mg/L of suspended solids in surface water and 60 mg/L in industrial effluents that end up in surface waters (with exceptions for some industrial activities).

Effect of Contaminated Solids

Results demonstrate that *suspended* contaminated particles exert a toxic effect that cannot be explained by the dissolved concentration of the heavy metals or their physical presence.

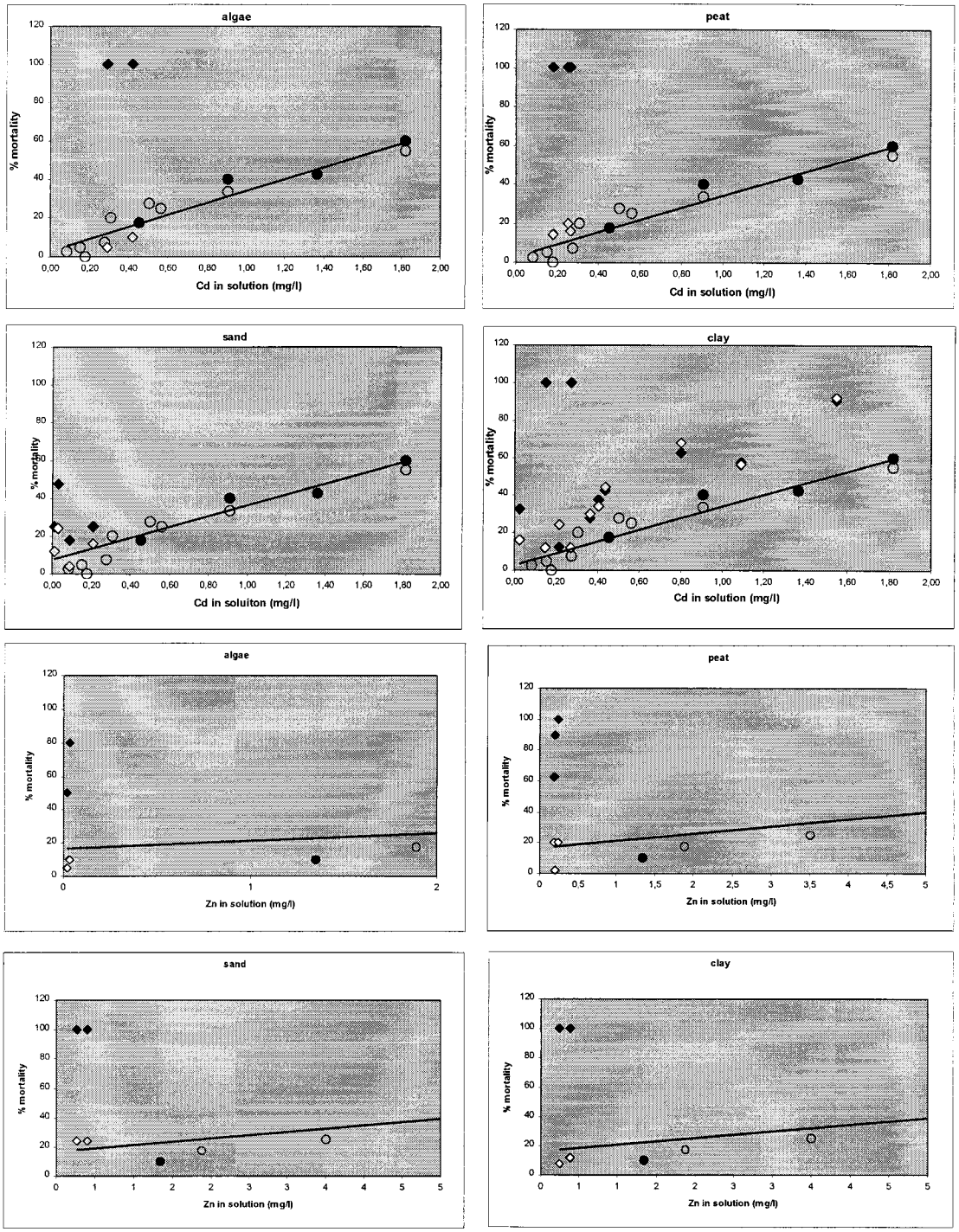

Fig. 3. Mortality data in the different experiments. a: experiments with cadmium contamination; b: experiments with zinc contamination. Mortality is shown in terms of the dissolved metal concentration to illustrate the superimposed impact of particle-bound contaminant for different types of material (algae, peat, sand, and clay). For each metal are shown: (a) dose-response curves for dissolved Cd and Zn salts in a static (○) or a flow-through setup (●) and (b) mortality in the presence of contaminated solids in a static (◇) or a flow-through setup (◆)

In some conditions 100% mortality was caused when contaminated particles were present and suspended, although the dissolved concentration was not toxic in water-only or in sedimented exposure (Figure 3). Except for sand contaminated with cadmium, in all other cases mortality was much higher in the presence of the contaminated suspended solids than in conditions without solids (supernatant or water only experiments) or when solids were allowed to settle (static setup, which is actually comparable to supernatant except for clay). This can only be explained when the particle-bound fraction of

the contaminant can enter the body and tissues to exert their lethal effect.

The deviant behavior of sand, *i.e.*, *not* causing a notable increase in mortality when contaminated particles were present, could not be explained in terms of low adsorbed pollutant concentration. Actually the residual load on the sand particles for cadmium was rather high (1.34–1.36 mg/g) when compared to other materials. Possibly these high loads interfere with normal food uptake mechanisms (Allen *et al*. 1995).

The bioaccumulation experiment confirms the hypotheses that the metal becomes bioavailable within the body. Desorption in the gut is probably due to different physicochemical conditions in the gastrointestinal tract. Although pH is neutral in *Daphnia* gut (Pennak 1978), enzymatic conditions can be in favor of desorption processes.

In a parallel study on trout, lethal effects were also measured when exposed to clay particles loaded with cadmium. Toxic effects could be due to the microenvironment of the gill, but more research on this subject is needed (Qiao and Farell 1996; Van den Belt 1999).

Effect of the Composition of the Particulate Matter

Composition of suspended solids in surface waters is variable, depending on geography and urban activity. *Daphnia* food uptake mechanism is described by Pennak (1978). Complex movements of thoracic legs produce a constant current of water that serves to filter food particles from the water and collect them in a median ventral groove at the base of the legs. This stream of food is fed to the mouth parts where the particles may be ground between the surfaces of the mandibles before being taken into the mouth. It is well known that organic detritus of all kinds, as well as bacteria and algae, are very important food sources. All organic particles of suitable size are ingested without any selective mechanism. Undesirable material or large tangled masses are introduced between the mandibles and removed by spines at the base of the first legs and kicked out.

From this description it could be expected that clay and sand would not be accepted as food particles by the organisms, and therefore no severe acute effects would be expected within the short exposure time. Surprisingly, effects were comparable for organic and inorganic materials used: in almost any case the suspended solids increased toxicity drastically. This might indicate that food uptake is completely nonselective and the inorganic sand or clay particles are not rejected. The bioaccumulation experiment confirms this for clay particles—immediately after exposure the gut contains particles loaded with cadmium.

Many bioassays with *Daphnia* are based on the reduction in food uptake rate as a toxic signal (Allen *et al*. 1995; Taylor *et al*. 1998). Ingestion rates were not measured in this study, but due to the presence of the toxicants and their interference with food uptake, the uptake of the particles was probably limited. Nevertheless, drastic lethal effects were measured in their presence, indicating that even small amounts of contaminated materials are dangerous for these invertebrates.

Route of Administration

The different results obtained in the different setups illustrate the importance of the route of administration in ecotoxicity tests. For both hazard and risk assessment, information on exposure has to be evaluated. The bioavailable fraction of the contaminants has to be determined for each specific exposure route. To evaluate the possible risk for filter feeders an administration via a flow-through system is needed to evaluate the effect of adsorbed molecules correctly.

The effect of contaminants adsorbed to natural suspended solids is often studied on extracts (Santiago *et al*. 1993), but the ecotoxicological relevance of extracts of particulate matter is far from fully evaluated (Broman *et al*. 1994). Shuytema *et al*. (1995) compared different setups (flow-through, static, and elutriate tests) and came to the conclusion that in 25% results were not comparable for the different setups if the sediments were contaminated with metals, and in the case of organic contaminated sediments even more diversity was noticed.

Contaminated Solids and Risk Assessment

To perform a sound risk assessment good information and estimation of the target organism and the route of exposure is absolutely necessary. Analytical determination of the total pollutant content is not sufficient; also, the bioavailable fraction has to be determined. Bioavailability describes the portion of a contaminant that can be taken up by the organism from its environment and food and is subsequently transported, distributed, and metabolized by the organism (Kördel *et al*. 1997). Risk and hazard assessment for chemicals in water is often based on dissolved concentrations. Although regulatory prescribed tests are designed to mimic the possible environmental effects of new chemicals, suspended solids are not assessed. Positively charged and hydrophobic molecules, however, will only be present at very low dissolved concentrations, but they can become a substantial component of the solid fraction in the water column, being bioavailable to specific organisms.

These experiments with artificially contaminated materials strongly indicate that toxic effects of contaminated solid materials in the water column cannot be neglected and should be examined into more detail. Metal molecules like cadmium and zinc, adsorbed to organic or mineral materials, are clearly bioavailable to filter-feeding water fleas. This situation can be expected in industrial or sewer effluents, or when cleaning or dumping contaminated dredged sediments. In these cases the suspended material compartment may be an important toxicological component, causing acute toxicity in higher organisms.

References

Admiraal, Tubbing, Breebaart (1995) Effects of phytoplankton on metal partitioning in the lower river rhine. Water Res 29:3941–3946

Allen, Calow, Baird (1995) A mechanistic model of contaminant induced feeding inhibition in *Daphnia magna*. Env Toxicol Chem 149:1625–1630

Broman, Näf, Rannug (1994) Mutagenic effect of extracts from particulate matter collected with sediment traps in the archipelago of

Stockholm and the open northern baltic. Bull Env Cont Tox 53:669–674

Carvalho, Guilhermino, Ribeiro, Gonçalves, Soares (1995) METIER II: ecotoxicity of poorly water soluble compounds: concentration versus dose. Arch Env Cont Tox 29:431–434

Espiritu E (1994) Development of an enzymatic toxicity test with selected *Phyllopod* species. PhD thesis, University of Ghent, Faculty of Agricultural Sciences and Biology, Ghent, Belgium.

Farag, Boese, Woodward, Bergman (1994) Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ Toxicol Chem 13(12):2021–2029

Helawell (1986) Biological indicator of freshwater pollution and environmental management. Elsevier Applied Science Publishers, Barking, Essex, pp 100–120

Janssen, Persoone (1993) Rapid toxicity screening tests for aquatic biota. 1. Methodology and experiments with *Daphnia magna*. Env Toxicol Chem 12:711–717

Kördel, Dassenakis, Lintelmann, Padberg (1997) The importance of natural organic material for environmental processes in waters and soils. Pure Appl Chem 69(7):1571–1600

McCarthy (1983) Role of particulate organic matter in decreasing accumulation of poly nuclear aromatic hydrocarbons by *Daphnia magna*. Arch Env Cont Tox 12:559–568

OECD (1984) Guidelines 202, *Daphnia* sp. acute immobilisation test and reproduction test. OECD guidelines for testing chemicals

Qiao, Farrell (1996) Uptake of hydrophobic xenobiotics in water loaded with sediments from the Frase River. Env Tox Chem 15(9):1555–1563

Pennak (1978) Cladocera (water fleas). *In* Fresh water invertebrates of the United States. John Wiley & Sons, New York

Pentinnen, Kukkonen, Oikari (1995) The kinetics of cadmium in *Daphnia magna* as affected by humic substances and water hardness. Ecotoxicol Environ Saf 30:72–76

Rodgers, Dickson, Saleh, Staples (1987) Bioavailability of sediment bound chemicals to aquatic organisms—some theory, evidence and research needs. *In* Dickson, Maki, Brungs (eds) Fate and effects of sediment bound chemicals in aquatic systems

Santiago, Thomas, Larbaigh, Rossel, Echevarina, Tarradellas, Loizea, McCarthy, Mayfield, Corvi (1993) Comparative ecotoxicity of suspended sediment in the lower Rhone river using algal fractionation, Microtox and *Daphnia magna* bioassays. Hydrobiologia 252:231–244

Schuytema, Nebeker, Cairns (1996) Comparison of recirculating, static and elutriate aquatic sediment bioassay procedures. Bull Environ Contam Toxicol 56:742–749

Scott, Redmond (1989) The effect of a contaminated dredged material on laboratory populations of the tubicolous Amphipod *Ampelisca abdita*. *In* Cowgill, Williams (eds) Aquatic toxicology and hazard assessment, 12th volume. ASTM STP 1027, American Society for Testing and Materials, Philadelphia, pp 289–303

Taylor G, Baird DJ, Soares AMVM (1998) Surface binding of contaminants by algae: Consequences for lethal toxicity and feeding to *Daphnia magna strauss*. Env Tox Chem 17(3):412–419

Van den Belt, Van Puymbroeck, Witters (1999) Toxicity of cadmium contaminated clay to the zebra fish *Danio rerio*. Arch Env Cont Tox 38(2):191–196

VMM (1998) Zwevende stof in Vlaamse waterlopen: verwerking meetgegevens campagne 1994–1995 (Suspended solids in Flemish surface water: analyses and evaluation of the results for the material collected during 1994–1995). Flemish Environmental Agency