ELSEVIER

Aquatic Toxicology 71 (2005) 249–259

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Heavy metal toxicity and bioavailability of dissolved nutrients to a bacterivorous flagellate are linked to suspended particle physical properties

Jens Boenigk*, Anneliese Wiedlroither, Karin Pfandl

Institute for Limnology, Austrian Academy of Sciences, Mondseestr. 9, A-5310 Mondsee, Austria

Received 28 May 2004; received in revised form 15 November 2004; accepted 25 November 2004

Abstract

Many dissolved substances attach easily to sediment particles. In the presence of suspended sediments bioavailability of dissolved substances is therefore, usually reduced and clays are even applied to “wash” natural waters upon pollution. In organisms which feed on food organisms in the size range of these suspended sediment particles, however, bioavailability of such substances may even increase. For microorganisms the interaction with dissolved substances and suspended sediment particles so far has hardly been investigated. We specifically tested: (1) the importance of suspended particles as an uptake route for dissolved substances; and (2) the significance of particle surface properties, i.e. surface load and mineralogy. As a model system we used an axenically cultured strain of a widespread and often abundant flagellate (“*Spumella*-like” flagellate strain JBM10). We tested the toxicity of cadmium (II) and mercury (II) as well as availability of dissolved organic matter (DOM) in the absence as well as in the presence of different natural clays, i.e. a kaolinite, a montmorillonite, and a mixed clay, and of artificial silicate particles of different surface charge. When applied separately the presence of the heavy metals cadmium and mercury as well as of suspended particles negatively affected the investigated flagellate but nutritive organics supported growth of the investigated flagellate. Toxic stress response comprises behavioral changes including enhanced swimming activity and stress egestion of ingested particles and was generally similar for a variety of different flagellate species. In combination with suspended particles, the respective effect of trace metals and nutritive substances decreased. Regarding the particle quality, cadmium toxicity increased with increasingly negative surface charge, i.e. increasing surface density of silanol groups (Pearson’s product moment, $P = 0.005$). For mercury particle mineralogy still had a significant effect ($P < 0.001$) but surface load seems to play a minor role and for nutritive organics no significant effect of the investigated particle properties was found. We conclude that: (i) flagellates are as sensitive as higher animals to heavy metal pollution; (ii) suspended particles decrease bioavailability of dissolved substances and ingestion of suspended particles probably play a minor role as uptake route for dissolved substances; and (iii) suspended sediment particle properties, i.e. surface charge and mineralogy, are key factors for the interaction between microorganisms and dissolved substances in the presence of suspended sediments.

Keywords: Montmorillonite; Kaolinite; Chrysomonad; Spumella; Mercury; Cadmium; Surface charge; Mineralogy

* Corresponding author. Tel.: +43 6232 312529; fax: +43 6232 3578.
E-mail address: jens.beonigk@oeaw.ac.at (J. Boenigk).

0166-445X/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2004.11.009

1. Introduction

Suspended sediments are ubiquitary distributed in freshwater ecosystems: they are abundant in many lowland streams and rivers (Ganges, Yellow River: e.g. Li et al., 1998, 1999; Xu, 2000) and of general importance in all freshwater systems after precipitation or flood events (e.g. Lenzi and Marchi, 2000). Suspended sediment concentrations, i.e. especially sediments of the clay and silt fraction, span the range of less than 10 $\mu g\, l^{-1}$ (open sea) to several hundred $mg\, l^{-1}$ (coastal area, rivers, lakes) and up to several hundred grams per liter (Velegrakis et al., 1997; Xu, 2000, 2002; Brondson and Naden, 2000; Orton and Kineke, 2001). Depending on the freshwater body about 10% to >80% of the suspended sediment mass belongs to the clay fraction (<2 μm; for an overview see Walling and Moorehead, 1989). Clays differ in chemical and physical properties but show, in general, a comparatively high ion exchange capacity of 5 to >50 mequiv. per 100 g clay (montmorillonite > illite ≫ kaolinite) and charged substances attach easily to clay particles. Suspended sediments enter freshwater ecosystems mainly by resuspension and by surface flow from terrestrial ecosystems (Booth et al., 2000; Evans, 1994; Walling, 1990) and are often loaded with high concentrations of dissolved organic material as well as xenobiotics such as pesticides and heavy metals (Palanques, 1994; Yavuz et al., 2003; Tarasevich and Klimova, 2001; Clausen et al., 2001; Yu et al., 2000; Steegen et al., 2000; Friese et al., 1997). Heavy metals (e.g. Barbier et al., 2000; Dobrovol'skii, 1999), several organic toxins (e.g. microcystin: Morris et al., 2000), and nutritive biomolecules (e.g. peptides: Yu et al., 2000) are therefore often enriched in clays for a factor of 100 to more than 10,000 compared to the water.

Despite the central role of bacterivorous protists in aquatic ecology (e.g. Azam et al., 1983; Jumars et al., 1989) and the presumably high impact of suspended clay on protists (Boenigk and Novarino, 2004; England et al., 1993) the significance of suspended particles for availability of dissolved nutritive as well as harmful substances to bacterivorous flagellates has not been studied. The effect of (dissolved) heavy metals on protist communities is proven (e.g. Fernandez-Leborans and Herrero, 1999) and it has been hypothesized that loaded sediment particles are an important pathway for the uptake of these substances in protists (e.g. Sanders and Gilmour, 1994). Similarly, suspended sediments might be a pathway for the direct utilization of dissolved organic carbon by protists. In fact, even though protists select against clay particles during ingestion and digestion (Boenigk et al., 2001; Boenigk and Novarino, 2004), a considerable amount of suspended fine sediment particles is ingested. Chemical properties, i.e. the mineralogical composition of clays, have been hypothesized to affect aquatic organisms (e.g. Cuker and Hudson, 1992). It is not yet clear to what extent this finding is linked to surface-bound components, i.e. toxins as well as nutritive biomolecules. Suspended sediments may consequently either act as sink for dissolved substances and make them less available for protists or in contrast, make them even more available via ingestion of sediment particles (Weston et al., 2000; Stecko and Bendell-Young, 2000; Pollet and Bendell-Young, 1999).

We investigated the effect of cadmium, mercury and dissolved nutritive organics on bacterivorous protists in the presence and absence of suspended sediment particles. This study aims at the understanding of the relative importance of suspended sediments in natural lake waters as an uptake route and for bioavailability of dissolved substances for protists. Specifically we tested to what extend suspended particles and surface charge of these particles change the positive/negative effect of dissolved harmful as well as nutritive substances on protists and whether the presence of suspended clay changes the toxicity of the heavy metals cadmium and mercury on bacterivorous flagellates. Both heavy metals are of quantitative importance in aquatic ecosystems, their binding properties to clays are comparatively good investigated (e.g. Auboiroux et al., 1998; Barbier et al., 2000), and general toxicity of these substances is well-known (e.g. Depledge et al., 1998).

2. Methods

2.1. Organisms, culture media, and clays

In most media and natural waters heavy metals applied at higher concentration will precipitate as salts. This is the case specifically for microbiological media as these are usually phosphate-buffered. We decided to use sterile filtrated lake water from Lake Mondsee (pH

8.1) instead of a phosphate buffered artificial medium. Due to the carbonate content of the natural water precipitation cannot be excluded for high concentrations of heavy metals but in contrast to the artificial media the chosen set-up mimics natural conditions and therefore allows for some conclusions on the field situation. After filtration through 0.2 μm the water was heated in the microwave two times (∼90 °C) to kill bacteria which may have passed filtration and to inactivate present viruses and afterwards the water was carefully examined for bacterial growth. This treatment caused a slight shift of pH, i.e., pH in the filtrated lake water increased to pH 8.4. The "*Spumella*-like" bacterivorous flagellate strain JBM10 was permanently cultured axenically in an inorganic basal medium (NSY medium: Hahn et al., 2003) fed with heatkilled bacteria (*Listonella pelagia* CB5). One week before the experiments were carried out, the flagellates were subcultured on sterile lake water originating from Lake Mondsee.

Several clays were used in this study: a kaolinite-dominated clay (84% kaolinite, 3% other clay minerals; zeta potential −47 mV) originating from a clay pit in the Eifel, Germany (cf. Boenigk and Novarino, 2004), a montmorillonite-dominated clay (IKOMONT WB90, IKO Minerals GmbH, Neuss, Germany: >85% montmorillonite, ∼10% other clay minerals; zeta potential −40 mV), and a kaolinite-montmorillonite clay (40% montmorillonite, ∼60% kaolinite; zeta potential −26 mV) originating from a clay pit in the Eifel, Germany.

In addition, artificial silicate particles (sicastar®, micromode: Ø 0.8 μm; zeta potential −61.5 mV) were used as standardized basis particles. Silicate beads possess a silanol-surface and therefore, show a strong negative surface charge as it is also the case in natural clays. In contrast to natural particles the surface layer of these particles and their chemical composition is defined. For testing the effect of surface charge, these particles were coated with acrylate yielding in a zeta potential of −38.4 mV (medium charge) and −6.2 mV (low charge), respectively. The response of "*Spumella*-like" flagellates to suspended particles has already been studied by Boenigk and Novarino (2004). In order to investigate the response to toxification we applied live observation techniques as well as growth experiments using the "*Spumella*-like" flagellate strain JBM10.

2.2. Behavioral response to exposure to heavy metals

High-resolution video microscopy was used for live observations of protist behaviour and feeding (10 replicates each). The use of an inverted microscope equipped with 100× oil immersion objectives has proved to be suitable for the investigation of several flagellate species (see Boenigk and Novarino, 2004). All observations were recorded on S-VHS tapes and have been analysed directly from the monitor. We followed the general set-up by Boenigk and Novarino (2004) and all experiments were carried out at room temperature (19–22 °C) at moderate illumination. After transfer to the petri dishes the organisms were adapted for at least 30 min. Hg^{2+} and Cd^{2+} were applied in concentrations of 0.01 mg l^{-1}, 0.1 mg l^{-1}, 1 mg l^{-1}, 10 mg l^{-1}, and 50 mg l^{-1} and 500 mg l^{-1} at the start of the experiments: Ten-fold concentrated stock solutions were prepared in distilled water and an aliquot was mixed with sterile lake water in a volume ratio of 1:10 immediately before the experiments were started. The time interval between the addition of toxic substances and the first visible reaction was noted for all observed individuals. In addition, cell death was noted when ever possible. In addition, toxic stress responses were investigated qualitatively for several protists of different taxonomic groups, including *Ochromonas* (strain DS), *Bodo saltans*, *Monosiga ovata* using mercury chloride as a model substance.

2.3. No observed effect concentration (NOEC) and LC_{50}

The median inhibitory dose (IC_{50}) was determined for the axenic strain JBM10 for the heavy metals mercury and cadmium. Experiments were carried out in 100 ml flasks using overhead shakers (Stuart Scientific STR 4) rotating at 10 rotations min^{-1}. Volume at the start of the experiment was 30 ml and heavy metals were added to final concentrations of 0.05 (0.75, 3, 12, 50, 200, 800, 2500, 10,000 and 40,000) μg l^{-1} for Hg^{2+} and of 0.039 (0.156, 0.625, 2.5, 10, 40, 160, 640, 2560, and 10,240) μg l^{-1} for Cd^{2+}, respectively. The effect of the kaolinite-dominated clay and of the standardized silicate particles on heavy metal toxicity was tested in parallel treatments following the same general set-up using additions of 10 mg l^{-1} kaolinite as well as additions of 10 mg l^{-1} silicate beads (Micromod). In addition, control treatments without heavy metals were run in triplicate.

All experiments were carried out at 16 °C at permanent illumination. Organisms were adapted to the experimental conditions, i.e. shaking and satiating food concentrations using heat-killed strain CB5, for 48 h before the experiment started. At the start of the experiment flagellate abundance was adjusted to 4000 flagellates ml^{-1} and food concentration was adjusted to 2×10^7 heat-killed bacteria ml^{-1}. Growth of the protists in the different treatments was then monitored for 24 h. Subsamples were stained with DAPI, filtered onto black nucleopore filters and checked under the epifluorescence microscope using filter set 01 for UV excitation (Zeiss no. 488001-0000).

2.4. Bioavailability of nutritive molecules

An artificial complex medium was used as standardized organic medium containing equal amounts of nutrient broth, soyotone, peptone, and yeast extract (NSY; all obtained from Difco). Flagellates were pre-cultured on heat-killed bacteria. 24 h before the experiments the flagellates were stepwise adapted to the NSY-concentrations used in the experiments and 6 h before the experiment the flagellates were transferred to the final concentrations of NSY and adapted to the experimental conditions, i.e. over head shaking at 16 °C in the light. Experiments were carried out in 100 ml flasks using overhead shakers (Stuart Scientific STR 4) rotating at 10 rotations min^{-1}. Volume at the start of the experiment was 30 ml. Total concentrations of NSY of 0.001 mg/ml (0.005, 0.02, 0.05, 0.2, 1, 5, 10, 20, 30, 40, 50, and 70 mg/ml) with and without 10 mg l^{-1} kaolinite-dominated clay were tested. Control treatments containing no additions of NSY as well as the treatments containing 30 and 40 mg ml^{-1} NSY were run in triplicate. Clay was added to the treatments at the start of the experiment and initial flagellate abundance was adjusted to 5000 flagellates ml^{-1}. Background concentration of heat-killed food bacteria was below 10^4 bacteria ml^{-1} and was therefore negligible. Growth of the flagellates was monitored over 24 h. Subsamples were stained with DAPI for 30 min, filtered onto black nuclepore filters and counted under the epifluorescence microscope.

2.5. Effect of clay mineralogy and particle surface charge

The effect of clay mineralogy on heavy metal toxicity and availability of organic molecules was tested using clay suspensions (10 mg l^{-1} final concentration) of a kaolinite, a montmorillonite and a mixed clay. Three sets with four different treatments each were run containing one of the three particles at a concentration of 10 mg l^{-1} or no particles (control). In the first set one of each treatments was supplemented with 0.1 mg ml^{-1} of organic NSY final concentration and control treatments were set up without addition of NSY. In the second and third set one of each treatment was supplemented with 1500 μg l^{-1} cadmium and 40 μg l^{-1} mercury, respectively. Controls were run without addition of dissolved substances. In the second and third set flagellates were fed with heat-killed food bacteria which were adjusted to a concentration of 2×10^7 bacteria ml^{-1}. All experiments were run in triplicate. The flagellates were adapted to the experimental conditions for 24 h and experiments were run at 16 °C in the light in 100 ml flasks using overhead shakers (Stuart Scientific STR 4) rotating at 10 rotations min^{-1}.

The effect of particle surface charge on heavy metal toxicity and availability of organic molecules was tested, in addition, using the same principle set-up but using artificial silicate beads with a surface charge of −61.5, −38.4 and −6.2 mV (zeta potential). Growth of the protists in the different treatments was then monitored for 24 h. Subsamples (0 h, 24 h: 5 ml) were stained with DAPI (2 ml), filtered onto black nucleopore filters and checked under the epifluorescence microscope. The effect of suspended particles and dissolved substances on growth was tested using two-way ANOVA. Further the effect of particle surface charge was tested by correlation analysis of the difference in growth rate (growth in the presence of dissolved substance − growth in the absence of dissolved substance) calculated for each particle type against surface charge of the respective particle type.

3. Results

3.1. Behavioral response to exposure to heavy metals

At undisturbed conditions most flagellates were attached to surfaces and feeding on bacterial which were propelled towards the cell body (see Boenigk and Arndt (2000) for a detailed description of the behaviour of undisturbed cells). For a Hg^{2+} concentration of 10 $\mu g\,l^{-1}$ as well as for Cd^{2+} concentrations of 100 $\mu g\,l^{-1}$ and below the flagellates showed no irritations and no stress reactions. However, cell divisions were only observed during the initial 30 min of an experiment. First visible reactions at non-lethal concentrations were irregularities in flagellar beating, i.e. short interruptions, and spining movements of the cell body which often resulted in detachment and swimming away of the cell. These behavioral responses do also occur in undisturbed cells (cf. Boenigk and Arndt, 2000) but the frequency of these responses increases and disturbance/toxic effects can therefore be identified on the population basis even though the behaviour of an individual cell may not be conspicuous. At concentrations of 100 $\mu g\,l^{-1}$ Hg^{2+} (1 mg Cd^{2+}) a concentration of visible food vacuoles at one side of the cell was often observed and after about 10 min stress egestions were observed, i.e., a simultaneous egestion of the contents of all visible food vacuoles including nearly undigested bacteria occurred (Fig. 1). The "normal" egestion could easily be distinguished from the stress egestion, as egestion of all the different food vacuoles does not occur simultaneously in healthy organisms. Despite this behavioral stress reaction, the flagellates survived for more than 24 h, i.e. no lethal effects were noted during the observation interval. For further increasing concentrations of toxic substances, the movement of the flagella became weaker and irregular after a while the cell shape became deformated and finally the cell died. For instance, at concentrations of 1 $mg\,l^{-1}$ Hg^{2+} (10 $mg\,l^{-1}$ Cd^{2+}) cells became irritated within minutes and started to swim away. Some cells burst, however, without showing the typical shrinkage (Fig. 1). At highest concentrations of toxic substances (above 10 $mg\,l^{-1}$ Hg^{2+}; above 50 $mg\,l^{-1}$ Cd^{2+}) the flagellates burst within minutes.

The qualitative observations of other flagellates exposed to mercury showed that, in general, this stress reaction was similar for other flagellates as well. The general toxic stress response of *Ochromonas* was the same as for the "*Spumella*-likëflagellate, but due to the larger cell size an addition of toxic substances was survived for a longer time. Behaviour of *Bodo saltans* also was generally comparable to *Spumella*, but the cells did not swim away. In contrast they became irritated and jerked at high frequencies. This led often to detachment of the flagellates (resulting in swimming away) or even to loss of the flagellum. Stress egestion was also noted. For *Monosiga ovata* increased movements of the cell compartments after addition of toxic substances within the cell were observed. After several minutes the flagellum contracted irregularly and repeatedly stopped beating for about 0.5 s. Finally the flagellates died and the flagellum was straight visible. Stress egestion seem to occur, i.e. cells which did not contain any visible food

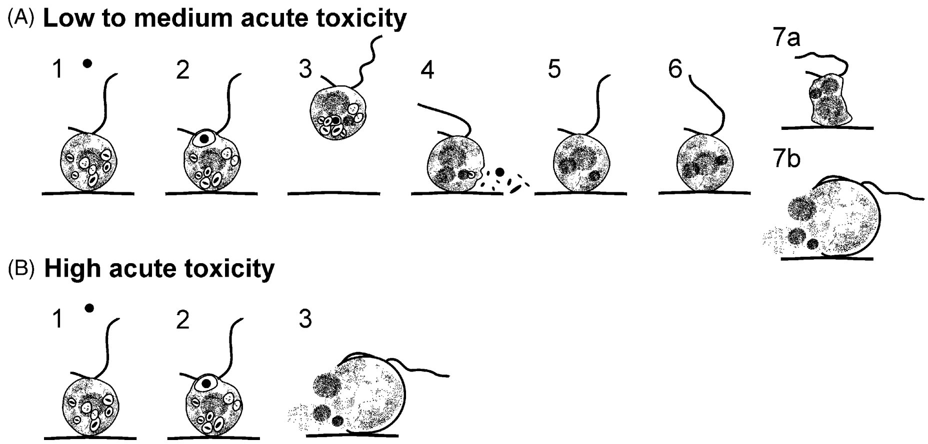

Fig. 1. Behavioral response of *Spumella* sp. to toxic stress. At low toxicity (A) the cells usually do not swim away but stress egestions (A4) are observed. At medium toxicity activity of the cells increases and the cells usually swim away (A3), stress egestions (A4) are also frequently observed. Finally the cells die and either shrinkage or bursting of the cells is observed. At high toxicity (B) the cells are irritated within minutes and the cells burst after a while.

particles were observed in clouds of small particles which may be the remains of formerly ingested particles. The egestion process itself, however, could not yet be identified.

3.2. No observed effect concentration (NOEC) and LC_{50}

Heavy metal toxicity increased with concentration both in the presence of suspended particles and in the absence of suspended particles. Growth rates were significantly negatively correlated with heavy metal concentrations for mercury (Spearman rank correlation: −0.893, $P < 0.001$; −0.75, $P = 0.038$; −0.821, $P = 0.014$ without particles, with beads, and with clay, repectively) and for cadmium (Spearman rank correlation: −0.652, $P = 0.014$; −0.398, $P = 0.173$; −0.862, $P < 0.001$ without particles, with beads, and with clay, respectively). Only for the cadmium-bead treatment the correlation was not significant for the tested range of cadmium concentrations. No observed effect concentration (NOEC) for mercury was 10 $\mu g\,l^{-1}$ independent of the presence of silicate beads and clay, respectively. LC_{50} (24 h) for mercury was 182 $\mu g\,l^{-1}$; and 232 $\mu g\,l^{-1}$ and 174 $\mu g\,l^{-1}$ in the presence of clay and silicate beads, respectively. In these treatments a strong negative effect of the suspended particles was observed (when corrected for that, an increase in LC_{50} is observed also in the presence of beads). For mercury concentrations above 800 $\mu g\,l^{-1}$ no living cells were detected after 24 h in any of the treatments. Regarding cadmium no observed effect concentration (NOEC) was 600 $\mu g\,l^{-1}$ independent of the presence of silicate beads and clay, respectively. LC_{50} (24 h) was 5500 $\mu g\,l^{-1}$ and decreased to 3900 $\mu g\,l^{-1}$ and 3500 $\mu g\,l^{-1}$ in the presence of clay and silicate beads, respectively.

3.3. Bioavailability of nutritive molecules

Regarding the dissolved organics, the effect of suspended particle addition (Δr = growth without organics − growth with organics in the presence of the respective particle) was significantly positively correlated with the concentration of organics (Spearman rank correlation for log transformed Δr: 0.621, $P = 0.022$). For organics concentrations below 5 $g\,l^{-1}$ the suspended clay had a negative effect on growth rate but a positive effect at concentrations above 5 $g\,l^{-1}$. Positive growth was observed for organics concentrations of up to 50 $g\,l^{-1}$ and only in the 70 $g\,l^{-1}$ treatment the flagellates died back.

3.4. Effect of clay mineralogy and particle surface charge

3.4.1. Effects in the absence of dissolved substances

In general, the presence of particles, natural clays as well as artificial silicate beads, decreased significantly the flagellates growth rates (ANOVA, $P = 0.03$). This finding was independent on the presence or absence of dissolved substances. Particle surface charge did not significantly modify flagellate growth rate in the absence of dissolved substances. This was consistently found in treatments with low (Pearson's product moment, correlation coefficient = −0.241, $P = 0.335$) and high abundances of food bacteria (Pearson's product moment, correlation coefficient = −0.374, $P = 0.126$).

3.4.2. Effects in the presence of dissolved organics

The presence of dissolved organics, in general, significantly increased growth rates (two-way ANOVA, $P = 0.003$). For the tested concentration of organics (0.1 $mg\,l^{-1}$) there was no significant general impact of suspended sediments and further there was no significant difference in the effect of dissolved organics additions between the different particle types (two-way ANOVA, $P = 0.087$); regarding the investigated particle properties, i.e. surface load, no consistent trend was found (Pearson's correlation, correlation coefficient = −0.145, $P = 0.399$; Fig. 2).

3.4.3. Effects in the presence of heavy metals

The addition of mercury significantly decreased growth rates (two-way ANOVA, $P < 0.001$) and the effect of mercury addition depended significantly on the type of particle (two-way ANOVA, $P < 0.001$; Fig. 2). However, again surface load was not correlated with mercury toxicity (Pearson's correlation, correlation coefficient = −0.088, $P = 0.868$; Fig. 2).

Presence of cadmium, in general, significantly decreased the flagellates growth rates in the presence as well as in the absence of suspended particles (two-way ANOVA, $P < 0.001$). In contrast to mercury and dis-

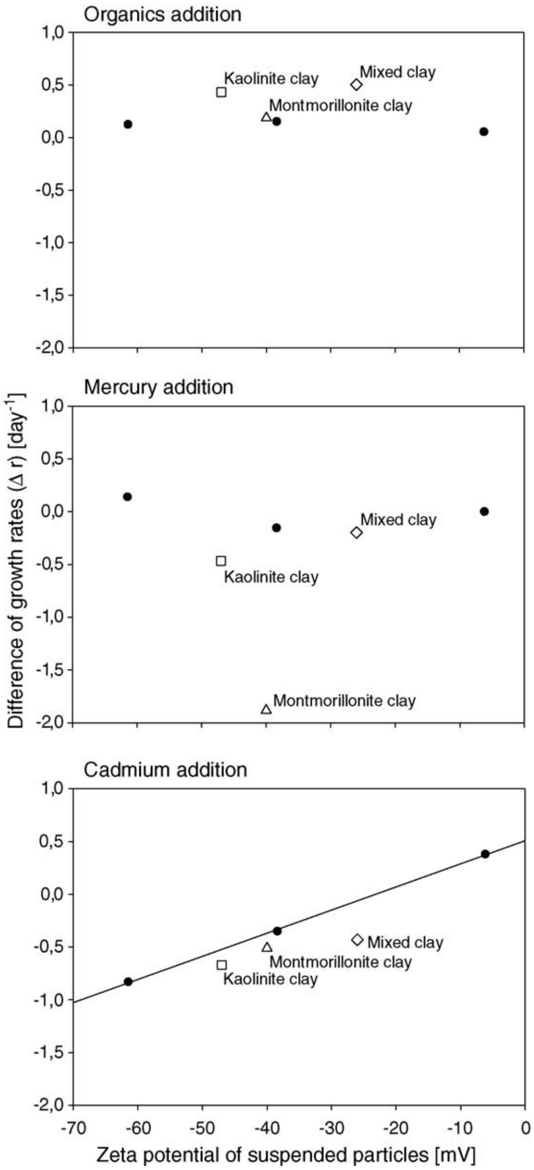

Fig. 2. Significance of suspended particle surface charge (zeta potential) for availability of dissolved substances, i.e. dissolved organics, mercury(II), and cadmium(II). The effect of suspended particles on availability of suspended substances is indicated by the difference of growth rates in the absence and in the presence of the respective substance for each of the different particles. Negative values indicate a decreased growth rate in the presence of the dissolved substance for a given particle whereas positive values indicate an increased growth rate in the presence of the dissolved substance. Black symbols represent charged silicate beads, white symbols represent natural clays, i.e. kaolinite (square), montmorillonite (triangle), and mixed clay (diamond).

solved organics, cadmium toxicity was correlated with suspended particle surface charge and the type of suspended particles had a significant effect on the flagellates growth rates (ANOVA, $P < 0.001$). The investigated particle character, i.e. particle surface load, was significantly correlated with cadmium toxicity: With increasingly negative surface load (in terms of zeta-potential) the effect of cadmium addition was increasingly negative (Pearson's product moment, correlation coefficient −0.937, $P = 0.0056$; Fig. 2).

4. Discussion

4.1. The sensitivity of "Spumella-like" flagellates to mercury and cadmium is comparable to those of other freshwater organisms

Mercury and cadmium are important environmental pollutants due to their highly toxic nature and widespread occurrence in aquatic systems (Tsui and Wang, 2004; Tchounwou et al., 2003; Koch, 1995). No observed effect concentration in the growth experiments correspond well with the results of the live observations (∼10–100 $\mu g\,l^{-1}$ Hg^{2+}; 0.1–1 $mg\,l^{-1}$ Cd^{2+}). This implies that the decreasing growth rate at low concentrations of heavy metals is due to behavioural changes (less efficient feeding behaviour). For the investigated flagellate the 24 h LC_{50} was 174–232 $\mu g\,l^{-1}$ for Hg^{2+} and 3500–5500 $\mu g\,l^{-1}$ for Cd^{2+}. These observed lethal concentrations are in the range reported for other organisms even though somewhat higher than the lowest reported values. For instance, the 24h EC_{50} for *Daphnia* has been reported to be in the range of 15 $\mu g\,l^{-1}$ for mercury (II) (Bringmann and Kühn, 1982) and around 160 $\mu g\,l^{-1}$ for cadmium (Bellavere and Gorbi, 1981). In general, mercury (II) concentrations of 5–100 $\mu g\,l^{-1}$ are toxic to freshwater invertebrates and fish but cadmium concentrations of up to several $mg\,l^{-1}$ are often tolerated without observing toxic effects (Koch, 1995). Studies on microbial species are, however, rare. For freshwaters, the 24 h LC_{50} for different ciliate species exposed to mercury was in the range of 4.3 to 190 $\mu g\,l^{-1}$ Hg^{2+} with *Uronema nigricans* as the most sensitive and *Euplotes affinis* the least sensitive organism (Madoni et al., 1992). The 24 h LC_{50} values of cadmium for several ciliate species have been reported to be in the range of 205–2650 $\mu g\,l^{-1}$ at pH 7.4

with *Colpidium campylum* the most sensitive and *Euplotes patella* the least sensitive (Madoni et al., 1992).

Heavy metal toxicity is difficult to extrapolate because presence of organic matter and suspended matter can decrease their toxicity, and fluctuations in nutrient levels may affect heavy metal toxicity (Fernandez-Leborans and Novillo, 1995). Depending on the water chemistry toxicity of the investigated heavy metals for flagellates must be assumed to be potentially higher than observed in our study. For instance, highest toxicity of cadmium has been reported for pH around 7 (Skowronski et al., 1991) and is therefore expected to be lower at the pH realized in the lake water used in our study (pH 8.4). Furthermore, Lake Mondsee is located at the border of the northern limestone Alps and consequently the lake water contained high amounts of carbonate ions. Only a minor fraction of cadmium and mercury can therefore, be assumed to be dissolved and toxicity may consequently be higher in freshwater bodies situated in basement rock.

4.2. Suspended sediments reduce heavy metal toxicity and availability of nutritive molecules for protists

Suspended sediments have been shown to affect growth of protists negatively, even though these effects are weaker as in metazoans (Jack and Gilbert, 1993; Boenigk and Novarino, 2004; Pfandl et al. in preparation). Suspended sediments also play a crucial role in the transport of xenobiotics, e.g. heavy metals, as well as of nutritive organic molecules (Nakhone, 1997; Echeverria et al., 2002; Sarkar et al., 2000; Takahashi et al., 1999; Coles and Yong, 2002; Jensen and Bro-Rasmussen, 1992). Dissolved substances attach to suspended sediment particles and in the presence of suspended clays a high fraction of dissolved substances is therefore removed from the water column (Yavuz and Aslan, 2002; Sarkar et al., 2000) and concentrated in the particulate fraction (Barbier et al., 2000; Morris et al., 2000; Yu et al., 2000; Friese et al., 1997). The sorption characteristics of suspended sediments are even specifically applied to 'wash' natural waters after pollution and to remove pollutants from the water column (Yavuz et al., 2003; Yavuz and Aslan, 2002). These substances are thus less available from solution but availability may increase by ingestion of these loaded particles. Toxicity therefore, depends on the main uptake route. Main uptake of heavy metals directly from the water (e.g. in the mussel *Mytilus galloprovincialis*; Fisher et al., 1996), as well as main uptake from particulate matter (e.g. from food in the crustacean *Hyalella azteca*: Stephenson and Turner, 1993) has been demonstrated.

As expected, both suspended fine sediments as well as heavy metals had a significantly negative effect on the flagellates growth rate but dissolved nutritive organic molecules supported flagellate growth. In combination with suspended particles, the respective effect of trace metals and nutritive substances generally decreased. Regarding the heavy metals NOEC was not significantly affected by sediment addition but LC_{50} increased for mercury indicating a positive effect of suspended sediments. In contrast, LC_{50} decreased for cadmium when suspended sediments were present indicating a higher mortality of the flagellates. However, the negative effect of cadmium, i.e. the decrease in growth rate relative to treatments without cadmium addition, was generally lower in the presence of suspended sediments compared to treatments without suspended sediments. Thus, the generally negative effect of suspended sediments on flagellate growth may overlay its positive effects, i.e., heavy metal sorption.

Regarding the organics, only for high concentration of dissolved nutritive organics the suspended sediments had a positive effect. Binding of organics to clay may decrease the concentration of freely dissolved organics and consequently still allow for high growth rates of the flagellates at total concentrations exceeding the optimal concentration. At concentrations realised in the field, i.e. below $100\,mg\,l^{-1}$, the stimulating effect of dissolved organics is reduced in the presence of suspended sediments. This finding is in contrast to a stimulating effect of suspended sediments on flagellate growth in the presence of live bacteria by Boenigk and Novarino (2004). The different findings may be due to an indirect effect, i.e., bacterial stimulation in the study of Boenigk and Novarino (2004) whereas in the current study axenic cultures were used and such indirect effects therefore did not occur.

It can be concluded that suspended fine sediments significantly decrease toxicity of mercury and cadmium and decrease availability of dissolved organic carbon for the investigated flagellate. Thus, direct uptake of dissolved substances seems generally to be a more important uptake route compared to ingestion of particle attached substances.

4.3. Surface charge and mineralogy are key components for protist-suspended sediment–dissolved substance interactions

In general, heavy metal ions are sorbed by cation exchange (Barbier et al., 2000) onto clay minerals. Sorption of heavy metals on clays has been studied for montmorillonite (Barbier et al., 2000), illite (Echeverria et al., 2002) and kaolinite (Coles and Yong, 2002; Sarkar et al., 2000). Sarkar et al. (2000) suggested that silanol groups are responsible for the bulk of mercury retention in kaolinite and the charged silicate beads used as artificial clay particles are therefore assumed to be suitable model particles. We investigated the effect of mineral characteristics, especially that of surface charge, using different natural clays as well as artificial silicate beads with a silanol surface.

Montmorillonite shows generally a higher sorption of heavy metals than kaolinite (Barbier et al., 2000). We expected therefore montmorillonite to reduce availability of dissolved heavy metals stronger than kaolinite. This was, however, only observed for the cadmium treatment. In the organic treatment particle type had no significant effect; mercury toxicity was even least reduced in the montmorillonite treatment. The silanol groups or the surface charge in terms of zeta potential may therefore be of a minor importance regarding the availability of these substances. Only in the cadmium treatment we found a significant correlation with particle surface charge (zeta potential). Surprisingly, we found the strongest negative effect of cadmium addition for suspended sediment particles with a high surface charge. This indicates on the one hand that for cadmium availability surface charge is an important parameter. On the other hand the density of surface silanol groups may also be not that important for cadmium retention as in that case we would expect an opposite trend, i.e. a low negative effect of cadmium to be related with high negative surface charges of the particles.

In general particle mineralogy is a significant factor determining toxicity of heavy metals but surface charge only partly explain for the observed effects. Particularly, the density of surface silanol groups played a lesser role than expected. The different trends probably are related to the different retention characteristics of cadmium, mercury and organic substances onto different clays (Barbier et al., 2000; Echeverria et al., 2002; Coles and Yong, 2002; Sarkar et al., 2000) and this aspect has to be further investigated. However, particle characteristics such as mineralogy and surface charge significantly alter the availability of dissolved components to a bacterivorous flagellate.

5. Conclusions

Our results indicate that: (i) the sensitivity of the investigated flagellate, i.e. *Spumella* sp. to heavy metal pollution is in the range reported for other organisms; (ii) particle associated substances are less available than dissolved substances for *Spumella* sp. and suspended particles act as a sink for dissolved substances with regard to the investigated flagellate; and (iii) particle properties, i.e. surface charge and mineralogy, significantly affect bioavailability of dissolved substances.

Acknowledgements

We thank the Austrian Science Fund (FWF) for financial support of this study (project P15940) and IKO Minerals for providing one of the clays used in this study.

References

Auboiroux, M., Baillif, P., Touray, J.C., Bergaya, F., 1998. XPS analysis at constant ionic strength of Ca–Cd and Ca–Pb exchanges on a Ca–montmorillonite. CR AC S IIA 327, 727–730.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257–263.

Barbier, F., Duc, G., Petit-Ramel, M., 2000. Adsorption of lead and cadmium ions from aqueous solution to the montmorillonite/water interface. Coll. Surf. A 166, 153–159.

Bellavere, C., Gorbi, G., 1981. A comparative analysis of acute toxicity of chromium, copper and cadmium to *Daphnia magna*, *Biomphalaria glabrata*, and *Brachydanio rerio*. Envrion. Technol. Lett. 2, 119–128.

Boenigk, J., Arndt, H., 2000. Particle handling during interception feeding by four species of heterotrophic nanoflagellates. J. Eukaryot. Microbiol. 47, 350–358.

Boenigk, J., Matz, C., Jürgens, K., Arndt, H., 2001. Confusing selective feeding with differential digestion in bacterivorous nanoflagellates. J. Eukaryot. Microbiol. 48, 425–432.

Boenigk, J., Novarino, G., 2004. Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates. Aquat. Microb. Ecol. 34, 181–192.

Booth, J.G., Miller, R.L., McKee, B.A., Leathers, R.A., 2000. Wind-induced bottom sediment resuspension in a microtidal coastal environment. Cont. Shelf Res. 20, 785–806.

Bringmann, G., Kühn, R., 1982. Results of toxic action of water pollutants on *Daphnia magna* tested by an improved standardized procedure. Z. Wasser Abwasser Forsch. 15, 1–6.

Brondson, R.K., Naden, P.S., 2000. Suspended sediment in the river Tweed and Teviot. Sci. Total Environ. 251 (252), 95–113.

Clausen, L., Fabricius, I., Madsen, L., 2001. Adsorption of pesticides onto quartz, calcite, kaolinite, and alpha-alumina. J. Environ. Qual. 30, 846–857.

Coles, C.A., Yong, R.N., 2002. Aspects of kaolinite characterization and retention of Pb and Cd. Appl. Clay Sci. 22, 39–45.

Cuker, B.E., Hudson, L., 1992. Type of suspended clay influences zooplankton response to phosphorus loading. Limnol. Oceanogr. 37, 566–576.

Depledge, M.H., Weeks, J.M., Bjerregaard, P., 1998. Heavy metals. In: Calow, P. (Ed.), Handbook of Ecotoxicology. Blackwell Science Ltd., Oxford, pp. 543–569.

Dobrovol'skii, V.V., 1999. Highly dispersed soil particles as a factor of heavy metal mass transfer in the biosphere. Euras. Soil Sci. 32, 1169–1176.

Echeverria, J.C., Churio, E., Garrido, J.J., 2002. Retention mechanisms of Cd on illite. Clays Clay Min. 50, 614–623.

England, L.S., Lee, H., Trevors, J.T., 1993. Bacterial survival in soil: effect of clays and protozoa. Soil Biol. Biochem. 25, 525–531.

Evans, R.D., 1994. Empirical evidence of the importance of sediment resuspension in lakes. Hydrobiologia 284, 5–12.

Fernandez-Leborans, G., Herrero, Y.O., 1999. Toxicity and bioaccumulation of Cadmium in marine protozoa communities. Ecotox. Environ. Safety 43, 292–300.

Fernandez-Leborans, G., Novillo, A., 1995. The effects of cadmium on the sucessional stages of a freshwater protozoa community. Ecotox. Environ. Safety 31, 29–36.

Fisher, N.S., Teyssier, J.-L., Fowler, S.W., Wang, W.-X., 1996. Accumulation and retention of metals in mussels from food and water: a comparison under field and laboratory conditions. Environ. Sci. Technol. 30, 3232–3242.

Friese, K., Mages, M., Wendt-Potthoff, K., Neu, T.R., 1997. Determination of heavy metals in biofilms from the River Elbe by total-reflection X-ray fluorescence spectrometry. Spect. Acta B 52, 1019–1025.

Hahn, M.W., Lünsdorf, H., Wu, Q., Schauer, M., Höfle, M.G., Boenigk, J., Stadler, P., 2003. Isolation of novel ultramicrobacteria classified as actinobacteria from five freshwater habitats in Europe and Asia. Appl. Environ. Microbiol. 69, 1442–1451.

Jack, J.D., Gilbert, J.J., 1993. The effect of suspended clay on ciliate population-growth rates. Freshw. Biol. 29, 385–394.

Jensen, A., Bro-Rasmussen, F., 1992. Environmental cadmium in Europe. Rev. Environ. Contam. Toxicol. 125, 101–174.

Jumars, P.A., Penry, J.A., Baross, M.J., Perry, M.J., Frost, B.W., 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. Deep-Sea Res. I 36, 483–495.

Koch, R., 1995. Umweltchemikalien. VCH–Weinheim.

Lenzi, M.A., Marchi, L., 2000. Suspended sediment load during floods in a small stream of the Dolomites (northeastern Italy). Catena 39, 267–282.

Li, B.G., Eisma, D., Xie, Q.C., Kalf, J., Li, Y., Xia, X., 1999. Concentration, clay mineral composition and Coulter counter size distribution of suspended sediment in the turbidity maximum of the Jiaojiang river estuary, Zhejiang. China J. Sea Res. 42, 105–116.

Li, G.X., Wei, H.L., Han, Y.S., Chen, Y.J., 1998. Sedimentation in the Yellow River Delta Part I: Flow and suspended sediment structure in the upper distributary and the estuary. Mar. Geol. 149, 93–111.

Madoni, P., Esteban, G., Gorbi, G., 1992. Acute toxicity of cadmium, copper, mercury, and zinc to ciliates from activated-sludge plants. Bull. Environ. Contamin. Toxicol. 49, 900–905.

Morris, R.J., Williams, D.E., Luu, H.A., Holmes, C.F.B., Andersen, R.J., Calvert, S.E., 2000. The adsorption of microcystin-Lr by natural clay particles. Toxicon 38, 303–308.

Nakhone, L.N., 1997. Cadmium adsorption and desorption on humic acid, montmorillonite, goethite, kaolinite and calcareous clay. Dis. Innovat. 9, 53–71.

Orton, P.M., Kineke, G.C., 2001. Comparing calculated and observed vertical suspended-sediment distributions from a Hudson River estuary turbidity maximum. Estuar. Coast. Shelf Sci. 52, 401–410.

Palanques, A., 1994. Distribution and heavy metal pollution of the suspended particulate matter on the Barcelona continental shelf (North-western Mediterranean). Environ. Pollut., 85.

Pollet, I., Bendell-Young, L.I., 1999. Uptake of ^{109}Cd from natural sediments by the blue mussel *Mytilus trossulus* in relation to sediment nutritional and geochemical composition. Arch. Environ. Contamin. Toxicol. 36, 288–294.

Sanders, R.W., Gilmour, C.C., 1994. Accumulation of selenium in a model freshwater microbial food web. Appl. Environ. Microbiol. 60, 2677–2683.

Sarkar, D., Essington, M.E., Misra, K.C., 2002. Adsorption of mercury (II) by kaolinite. Soil Sci. Soc. Am. J. 64, 1968–1975.

Skowronski, T., Szubinska, S., Pawlik, B., Jakobowski, M., Bilewicz, R., Cukrowska, E., 1991. The influence of pH on cadmium toxicity to the green alga *Stichococcus bacillaris* and on the cadmium forms present in the culture medium. Environ. Pollut. 74, 89–100.

Stecko, J.R.P., Bendell-Young, L.I., 2000. Uptake of ^{109}Cd from sediments by the bivalves *Macoma balthica* and *Protothaca staminea*. Aquat. Toxicol. 47, 147–159.

Steegen, A., Govers, G., Nachtergaele, J., Takken, I., Beuselinck, L., Poesen, J., 2000. Sediment export by water from an agricultural catchment in the Loam Belt of central Belgium. Geomorphology 33, 25–36.

Stephenson, M., Turner, M.A., 1993. A field study of cadmium dynamics in periphyton and in *Hyalella azteca* (Crustacea: Amphipoda). Water Air Soil Pollut. 68, 341–361.

Takahashi, Y., Minai, Y., Ambe, S., Makide, Y., Ambe, F., 1999. Comparison of adsorption behavior of multiple inorganic ions on kaolinite and silica in the presence of humic acid using the multitracer technique. Geoch. Cos. A 63, 815–836.

Tarasevich, Y.I., Klimova, G.M., 2001. Complex-forming adsorbents based on kaolinite, aluminium oxide and polyphosphates for the extraction and concentration of heavy metal ions from water solutions. Appl. Clay Sci. 19, 95–101.

Tchounwou, P.B., Ayensu, W.K., Ninashvili, N., Sutton, D., 2003. Environmental exposure to mercury and its toxicopathologic implications for public health. Environ. Toxicol. 18, 149–175.

Tsui, M.T.K., Wang, W.X., 2004. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. Environ. Sci. Technol. 38, 808–816.

Velegrakis, A.F., Gao, S., Lafite, R., Dupont, J.P., Huault, M.F., Nash, L.A., Collins, M.B., 1997. Resuspension and advection processes affecting suspended particulate matter concentrations in the central English Channel. J. Sea Res. 38, 17–34.

Walling, D.E., 1990. Linking the field to the river: sediment delivery from agricultural land. In: Boardman, J., Foster, I.D.L., Dearing, J.A. (Eds.), Soil Erosion on Agricultural Land. Wiley, West Sussex, pp. 129–152.

Walling, D.E., Moorehead, P.W., 1989. The particle size characteristics of fluvial suspended sediment: an overview. Hydrobiologia 176, 125–149.

Weston, D.P., Penry, D.L., Gulmann, L.K., 2000. The role of ingestion as a route of contaminant bioaccumulation in a deposit-feeding polychaete. Arch. Environ. Contamin. Toxicol. 38, 446–454.

Xu, J., 2002. Implication of relationships among suspended sediment size, water discharge and suspended sediment concentration: the Yellow River Basin, China. Catena 49, 289–307.

Xu, J.X., 2000. Grain-size characteristics of suspended sediment in the Yellow River China. Catena 38, 243–263.

Yavuz, O., Altunkaynak, Y., Guzel, F., 2003. Removal of copper, nickel, cobalt and manganese from aqueous solution by kaolinite. Water Res. 37, 948–952.

Yavuz, O., Aslan, H., 2002. Removal of cadmium from aqueous solution by natural and thermally activated kaolinite. Freshw. Environ. Bull. 11, 194–197.

Yu, C.H., Norman, M.A., Newton, S.Q., Miller, D.M., Teppen, B.J., Schafer, L., 2000. Molecular dynamics simulations of the adsorption of proteins on clay mineral surfaces. J. Mol. Struct. 556, 95–103.