REVIEW OF IMPACT OF HEAVY METALS ON STREAM INVERTEBRATES WITH SPECIAL EMPHASIS ON ACID CONDITIONS

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Abstract. Studies of the accumulation and toxicity of Cd, Zn, Fe, Pb and Cu under acid conditions to stream invertebrates are reviewed. The influence of pH on metal speciation decreases in the following order: Cu > Pb > Cd > Zn. The free metal ion is one of the most toxic species and is generally taken up directly from the water by organisms. The role of food in the uptake of metals depends mainly on feeding habits of the species, body size, life span and duration of the exposure. Surface adsorption can be regarded as a form of metal 'uptake' which increases at high pH. Biomagnification of metals along aquatic trophic food chains has not been proved for many metals. Toxicity of Cd, Fe, Zn and Pb increases at low pH, however not for all invertebrates. More knowledge is needed concerning sublethal effects of metals on invertebrates at different pH values and uptake, bioconcentration and biomagnification of metals at different pH values. Future studies should include experiments in artificial streams or in the field instead of short term tests and simple recording of field data.

1. Introduction

Acidification of surface waters has been an environmental problem in Europe and North America for several decades and new areas like in China and the developmental countries are threatened (Galloway, 1989). The present state of our knowledge about the origin and the effects of acidification on aquatic organisms, mainly fish and Crustacea has been summarized in several books and reviews, (e.g. Monitor, 1986; Morris *et al.*, 1989; Overrein *et al.*, 1988; Schindler *et al.*, 1988; Wren and Stephenson, 1991). Physiological studies were performed to explain the observed decrease in species diversity in acidified waters, revealing that ion regulation is disturbed by H^+ and Al^{3+} ions, which were elevated in acid water (e.g. Havas *et al.*, 1984; Havas and Likens, 1985; Herrmann, 1987). This led to the investigation of other metals in acidified lakes and a 'comeback' of metal toxicity studies, which so far had been mostly acute toxicity tests (LC_{50}) at neutral pH. Knowledge on the combined effects of low pH and metals on freshwater invertebrates is still very scarce, especially studies in running water ecosystems with stream invertebrates.

Several reviews summarize current knowledge on the effects of metals in freshwater. Luoma (1983) discussed the processes that control bioavailability on a theoretical basis. LaZerte (1986) reviewed studies about the sources and sinks of metals in lakes and soils with emphasis on the role of pH. Campbell and Stokes (1985) presented a comprehensive review on metal speciation, adsorption on biological surfaces and uptake in and toxicity to aquatic biota under acid and neutral conditions. Data

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on uptake and toxicity of 21 different metals were reviewed by Phillips and Russo (1978) regarding marine and freshwater species. This review is short in studies on stream invertebrates, but it contains some information on 'rare' metals like Ni and Cr. Some other reviews focussed on one metal and discussed bioavailability and toxicity of that particular metal more detailed than the above mentioned overviews (e.g. Cd: Ravera, 1984; Cu: Flemming and Trevors, 1989; Harrison and Bishop, 1983; Nor, 1987).

The aim of this review was to concentrate on summarizing current knowledge on the effects of pH on metal speciation, uptake by, adsorption on and toxicity to stream invertebrates. Only if information on stream invertebrates was lacking, other studies were discussed. Stream invertebrates were chosen because they are important food organisms for aquatic (Pisces, Coleoptera, Plecoptera) and terrestric (Aves, Odonata) predators and they are important decomposers, being responsible for the first steps of recycling of organic matter and energy. They often link the aquatic and terrestric environment as they need both environments to complete their life cycle. The knowledge on acidification and metals in lakes cannot be transferred to streams because these two ecosystems differ in fundamental characteristics like time for renewal of the waterbody, contact zones between the water and sediment and the water and air as well as the role of planktonic and benthic organisms in the ecosystem.

The following metals were chosen: Cd, Cu, Fe, Pb and Zn. They fullfill the criteria set up for this review: There is some information about (1) pH dependent availability in the water and (2) pH dependent toxicity to freshwater organisms. Al was not included because of the comprehensive information and reviews in the literature (Herrmann, 1987). For metals like Ni and Mn almost no information was available and metals like Hg are not likely to cause pH dependent changes in uptake and toxicity because they occur mostly as metalorganic compounds.

To obtain a complete survey of the published literature, a systematic online bibliography was set up from 1970 to 1989. The following data bases have been used: Biological Abstracts, Conference Proceedings Index, Dissertation Abstracts, Pollution Abstracts, Toxicology Abstracts, Ufordat and Ulidat. For the recent literature Current Contents for the Environmental Sciences was used. Using several synonymes, it was asked after streams, invertebrates (and different taxa), acidification and the above mentioned metals.

2. Metal Speciation

The term 'metal species' is used here as the molecular representation of a physicochemical form of an element (Bernhard *et al.*, 1986). Metal species can be grouped into different phases, e.g. the aqueous phase (free ionic species and soluble complexes), the solid phase (colloids, particles) and the biological phase (adsorbed to biological surfaces or incorporated in cells) (Flemming and Trevors, 1989). Metal speciation, partitioning and its bioavailability depend on several circumstances (Figure 1, Gerhardt, 1990b). These can be either physicochemical factors e.g. tem-

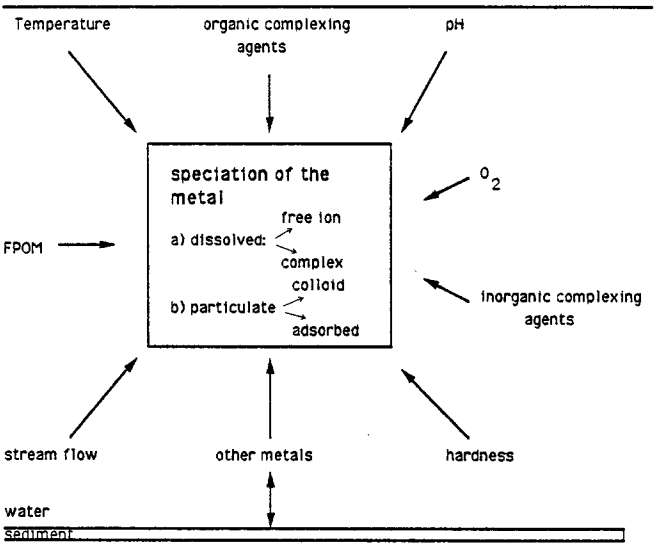

Fig. 1. Factors , which influence metal speciation.

perature, complexing agents, stream flow and pH or biological factors e.g. structure of cell surfaces, uptake and adsorption mechanisms. In this context, only those factors, which are supposed to interact with pH will be discussed.

The presence of complexing agents (humic acids, fine particulate organic matter, clay particles, calciumcarbonat) causes a decrease in free ionic metal species. Stackhouse and Benson (1989) found that *Daphnia magna* accumulated more Cd when 0.5 mg L^{-1} humic acid were added to the water, while metal accumulation was lowered at a higher concentration of humic acids (5 to 50 mg L^{-1}). Even if bioaccumulation depends on more factors than uptake, which should be a better measure for bioavailability, this study showed that the concentration of organic matter and also the exposure time affect metal availability for invertebrates. As the animals were rinsed in distilled water before the determination of Cd concentrations, the adsorbed Cd on the body should have been removed.

3. The Effects of pH on Speciation

pH can affect metal speciation by three different mechanisms (Campbell and Stokes, 1985). The first way is by changing the hydrolysis equilibrium and favoring the free aquo ion, which is supposed to be the most toxic species. Another way is by changing the complexation equilibria and competition between H^+ and metal ions at the binding sites of organic or inorganic ligands. Some metals (Al, Fe, Mn) are found in high concentrations in waters, rich in humic acids. Complexation with organic acids seems to increase at pH above 5.8 with the following order of stability: Fe, Pb, Cu > Cd > Zn (Campbell and Stokes, 1985).

The third mechanism is that pH can also affect soprtion processes (coprecipitation

of metals with metal hydroxides, adsorption to biological surfaces). Adsorption of Cd, Pb, Cu and Zn to inorganic or organic surfaces increases with increasing pH. The tendency of adsorption of metals to $Fe(OH)_3$ gel increases in the following order: Pb < Cu < Zn < Cd (Nelson and Campbell, 1991). The mechanisms behind these associations range from weak van der Waals forces to strong covalent bonding (Flemming and Trevors, 1989), e.g. causing allosteric conformational changes at the binding site (Campbell and Stokes, 1985). Fine particulate organic matter play an important role for the adsorption of metals (Ravera, 1984; Stephenson and Mackie, 1988), more than inorganic clay and silica (Gardiner, 1974). The fraction of the total amount of the metals Cd, Cu, Fe, Pb and Zn associated with particulate matter increases rapidly at pH levels above 3.5 and increases with pH in the order Fe, Pb > Cu, Cd > Zn. As an increase in pH causes elevated adsorption and coprecipitation of metals, the metal concentrations in the water decrease, which may lead to less bioavailability from the water. This is confirmed by field studies revealing enhanced sedimentation of Zn, Cd and Mn at levels above 5.5 and for Fe at pH above 4.8 (Borg *et al.*, 1989). In concordance with this, a decrease in pH caused increased levels of Cd, Cu, Fe, Pb, Mn and Zn in the water (Allard *et al.*, 1987; Borg, 1983; Monitor, 1987). Pb release from sediments does not occur above pH 3 (Nelson and Campbell, 1991). The concentration of Cd in the water, for example, was negatively correlated with the pH of the lakes investigated by Borg (1983). This was supported by Stephenson and Mackie (1988), who found that at pH 5 about 10 times more Cd could be measured in the water than at pH 7. Förstner (1984) reported that lakes with a pH of 4.1 to 5.3 exhibited 5 to 10 times higher Cd concentrations than lakes with a pH of 6.0 to 7.8. These results from field surveys could be supported in laboratory tests, where after addition of 0.2 mg L^{-1} Cd to stream water, ca 50% of the Cd_{tot} adsorbed onto the sediment and the container walls at pH 7 during the first day, while the concentration remained constant in the pH 5 water (Gerhardt, 1990a).

The elevated metal concentration in stream water at low pH can be caused by enhanced input from the soil, remobilization from the sediment or a decreased sedimentation rate from the waterbody. In acid soils with low organic content the mobility of Cd can increase 100 fold resulting in a washout into streams (Christensen, 1989). At pH above 6, however, Cd seems to be immobilized in the soil as it was not taken up by plants (Sverdrup and Warfvinge, 1990). All these studies indicate a higher bioavailability of Cd below pH 5, although Cusimano *et al.* (1986) postulate that between pH 4 and 7 no significant changes in the speciation of Cd occur, Cd^{2+} being always the dominant (> 90%) species. This result was, however, taken from a model calculation based on thermodynamic data and stability constants under the assumption that no organic ligands and no adsorbing surfaces were present, which is far from reality. Many studies reveal that the sediment pore water is not sufficiently acidic allowing for metal remobilization to occur (Borg *et al.*, 1989; Dillon *et al.*, 1988). Thus decreased sedimentation seems to account for the increased metal concentrations in acidic waters instead of increased remobilization.

4. Uptake of Metals by Invertebrates

In this chapter, uptake ('intake') is defined as the rate of incorporation of a metal into the organism. The uptake rate is a natural measurement of bioavailability, which has mostly been evaluated indirect from toxicity data or bioconcentration data (Pärt, 1987). Uptake always occurs at a membrane, e.g. the gill or the gut, depending on the source of the metal, from the water or food, respectively.

Luoma (1983) described different mechanisms of uptake for metals. Fe, for example, can be taken up and transported through the membrane either with special carrier molecules, siderochromes, or via endocytosis. Apolar metal species or lipid soluble forms can pass through the membrane by diffusion. Some essential trace metals are transported together with nutrients (co-transport). For many metals the mechanisms of uptake of different metal species into the organism are not sufficiently known, e.g. the relative importance of carrier faciliated transport and endocytosis, the nature of metal interactions with carrier molecules and the type of particles transported by endocytosis (Luoma, 1983).

5. Effects of pH on Metal Uptake

Acidity can influence the mechanisms of uptake. At low pH (pH 4.7) carriers are often inefficient in their metal transport and probably H^+ out compete metal ions at their binding sites (Yan *et al.*, 1989). This can compensate the elevated availability of metals at low pH. Bioavailability of a metal species for the organism is not proportional to the availability of that species in the water. Furthermore, H^+ ions can cause allosteric alterations in macromolecules in the membrane, which can result in changes in membrane permeability. This can affect metal transport by diffusion. pH also affects the membrane potential, which can change the transport of polar species. However, in this field we only have theoretical knowledge.

Young and Harvey (1988) found in a field study that chironomids from acid oxygen deficient lakes (pH 5) contained less Cu and Zn than those from neutral lakes. The opposite was found by Lewis and McIntosh (1986) who exposed *Asellus aquaticus* for 20 days to sediment containing 266 to 367 μg Pb g^{-1} and 352 to 772 μg Zn g^{-1} at pH 4.5, 5.5 and 7.5. In spite of a significant release of Zn from the sediment at pH 4.5 and 5.5, the animals did not increase their accumulation of Zn. The accumulation of Pb was however enhanced at low pH. Pynnonen (1991) studied the accumulation kinetics of Cd in freshwater bivalves. In acidified water (pH 4 to 5) Cd accumulation decreased, probably as a result of shell closure which might serve as an avoidance mechanism.

Even if in many studies a decrease in pH resulted in an increased uptake of a metal because of changes in the bioavailability of the metal for the organism, there are some studies showing that in acid lakes (pH 4.4) the benthic fauna does not accumulate more metal than at pH 5.4 (Broberg and Lindgren, 1987). On the contrary, the authors found that tubificid worms accumulated 5 times more Cd

and 1.6 times more Cu under neutral conditions than at pH 5. There may be several possible explanations for this phenomen:

1) At low pH H^+ and the metal ion compete for the binding sites at the gut- and gill mebranes and/or the sensitivity of the carrier molecules for some metals may be lowered.

2) At higher pH, the metals may be adsorbed or coprecipitated and therefore less bioavailable.

3) Surface adsorbtion onto the animals may account for the increased metal levels at neutral pH.

4) The animals used for the experiment were from a site with pH neutral water, thus the lowering of the pH can have caused stress and less food ingestion by the animals. Indeed, bacterial biomass was decreased at low pH, which represents a great deal of tubificid food.

The uptake of metals is even influenced by other abiotic and biotic factors, some of them interacting with pH (Figure 2). Bjerregaard (1991) measured the uptake of Cd in a marine crab in relation to its 'physiological condition', a summation parameter for parameters like water content, Na^+, K^+, Ca^{2+}-concentrations in different tissues and osmolality in the haemolymph. No significant differences in uptake of Cd in crabs with 'good' or 'bad' conditions were found. However, correlations between the single parameters and metal concentration in the animals were given, but in my opinion they were not convincing. Probably other improtant factors were not taken into account. It is difficult in experimental work to investigate all factors that may affect metal uptake and then evaluate the most important ones. Therefore another way of solving this problem seems to be the use of multiple regression models including abiotic and biotic factors for the prediction of metal levels in animals. van Hattum *et al.* (1991) performed a field survey of several metals (Cd, Pb, Cu, Zn), where their concentrations in the water and animals were measured. Simultaneously they applied a model including the following biotic and abiotic factors: metal concentration in the species, size, body weight, haemocyanin present or not, trophic level, metal concentration in the water and sediment, dissolved organic C and Ca^{2+} concentration. The choice of these factors was not further discussed in the article. For Zn and Cu this model showed better correlation with the field data than the model that only used abiotic factors, which seemed to be sufficient for the prediction of Cd values in the organisms. The correlation factors were not very strong, around 0.6. This indicates that even this multifactor analysis does not include all important factors. After the above discussion on the effects of pH on speciation and bioavailability, this factor should be added to the model. On the other hand, it is necessary to add the life stages to the model, as size it not sufficient. For example, the last larval stages of mayflies often decrease food intake because of morphological changes in the mouthparts before emergence (Kjellberg, 1972). This in turn, affects size and metal uptake from the food.

6. Uptake from Water or Food

Bioconcentration was defined as the amount of a metal accumulated by an organism in relation to its concentration in the abiotic environment (Kosalwat and Knight 1987). This, however, was called *bioaccumulation* by Dallinger *et al.* (1987). Taylor (1983) distinguished between bioconcentration and bioaccumulation, stressing that the latter term should be used for a life-time bioconcentration of a pollutant from the abiotic environment.

Biomagnification refers to the accumulation of a substance from food organisms leading to a higher concentration in organisms of a higher trophic level. If there is only a transfer of the metal through the foodweb, without increasing concentrations in the top predators, then the term *food chain effect* should be used (Dallinger *et al.*, 1987).

Bioconcentration includes metal uptake from the water and sediment. However, as many detritus feeders ingest sediment particles containing microalgae, bacteria and fungi no clear difference is possible between the uptake from the abiotic or biotic (food) environment. Only for predators it may be reasonable to distinguish these two ways of uptake. Because of the great volume of water passing the gills, which also have a greater surface and thus contact area to the medium than the gut, uptake via water should be the dominant pathway for aquatic organisms. Andersson and Gabring (1988) reported that 50% of the Cd taken up via the gills was found in the internal organs of fish, while only 3% of the Cd in the gut was transported to internal organs. Herwig *et al.*, (1989) investigated the uptake of Cd by *Dreissena polymorpha* into different organs. After one week of exposure, Cd was found in all epithelia which had direct contact with the contaminated water. After 3 weeks, Cd was found in the digestive tract and finally in the kidney, gonads and neural tissue.

Van Hattum *et al.*, (1989) showed that direct uptake of Cd from the water was the dominant pathway for *Asellus aquaticus* at low concentrations of dissolved Cd (0.1 to 0.3 mg L^{-1}). Direct uptake from the water accounted for 50 to 98% of the body burden of the isopods after 30 d of exposure. Abel and Bärlocher (1988) found for another crustacean, *Gammarus fossarum*, that under continuous exposure uptake of Cd from the water was the dominant pathway, while uptake from food might become more important after a short exposure, because then the metal would be 'conserved' in the food, while the metal concentration in the water might decrease fast after the pollutant 'pulse'. It should, however, be asked, if the food can take up the metal during a short exposure. As adsorption processes are fast, detritus particles may take up the metal during several hours, while for algae and invertebrates a longer time of exposure should be required. Thus, the role of the uptake of a metal from food during a short exposure depends mainly on the feeding habit of the species.

Kosalwat and Knight (1987) showed for the detritus feeding chironomid larvae *Chironomus decorus* that the relationship between the concentration of Cu in the water and the midges was exponential, while that between the concentration in the sediment and the midges was only linear. Cu was given in concentrations ranging from 0.05 to 1.0 mg L^{-} for 48 hr.

Baetis rhodani, a detritus feeding and grazing mayfly was exposed either to Cd in the food (150 $\mu g\ g^{-1}$) or water (0.07 $\mu g\ g^{-1}$) for about 30 d (Gerhardt, unpublished). At pH 7 Cd concentrations in the animals, which received Cd from the water were 5 times higher than in those ones fed with Cd-food (detritus). There was, however, no clear tendency at pH 5. The concentration factors were below 10. Similar concentration factors were observed by van Hattum *et al.* (1989) for the shredder *Asellus aquaticus* when exposed to 1 $\mu g\ g^{-1}$ Cd in the sediment for 30 days. Timmermans *et al.* (1989), however, found concentration factors of ca. 2000 in predatory invertebrates after a 30 d exposure of Cd in the water (0.1 $\mu g\ g^{-1}$) or in the food (algae: 300 to 600 $\mu g\ g^{-1}$). Predatory organisms seem to take up Cd faster and to a greater extent than detritus feeders. Only for the water mite, the uptake from food was 3 times higher than that from water at the end of the exposure after 30 d. This reveals that species specific uptake rates may be more important than the source of the metal for the metal concentration in the animal.

Hackstein (1988) exposed *Gammarus tigrinus* to Cd in the food (7.5 $\mu g\ g^{-1}$) for 9 mo and compared the uptake by the animals with that in the control group, being exposed to 0.5 to 1 $\mu g\ g^{-1}$ Cd L^{-1} in the water. In each size class. *g. tigrinus* took up only 10% more Cd from the food than from the water. However, parameters of toxicity showed that Cd in the water was more toxic than Cd in the food. The femals layed more eggs to compensate for elevated mortality and the whole generation time was longer when the animals were exposed to Cd in the water compared to Cd in food.

Another factor that influences the way of uptake of a metal can be tolerance. Brown (1977) showed in a 12 d experiment that intolerant speciemens of *Assellus meridianus* accumulated up to 6.8 μg Cu g^{-1} and 28 μg Pb g^{-1} from a solution containing 0.5 mg L^{-1} of both metals. Tolerant animals however accumulated these metals to a higher extent from the food which contained 2.6 mg Cu g^{-1} and 6.7 mg Pb g^{-1} than from the solution.

Merlini and Pozzi (1977) compared the uptake of Zn from food and water by the fish *Lepomis gibbosus*. Moreover they distinguished between the natural food source, the snail *Viviparus ater*, and an artificially prepared diet. Uptake from the artificial diet was 6 times higher than from the snail after 25 days of feeding. The kind of diet influenced the uptake from the water. Fish fed uncontaminated artificial diet accumulated 3 times more Zn from the water than the organisms fed by the natural diet. This reveals that laboratory studies with artificial diet can lead to wrong conclusions about the role of food in the uptake of metals. Moreover, in nature, a species can often choose between different kinds of food, while in the laboratory the animals are often fed uniformly and food is more available, which may result in an over estimation of the food as source for metal accumulation (Dallinger *et al.*, 1987).

7. Biomagnification

Taylor (1983) reviewed about 40 laboratory studies about biomagnification of metals

in marine and freshwater trophic webs including data on molluscs, crustaceans and fish. He plotted all the values for metal concentrations in the animals and the corresponding concentrations in the water from all studies in one graph showing no tendency to higher concentrations in predators. As the concentration factors for invertebrates (<100) were higher than for vertebrates (<20) no biomagnification was indicated. Some other more recent field studies reported metal concentrations in invertebrates of different feeding habits. van Hattum *et al.* (1991) found for Zn that the concentrations in the animals decreased in the following order: predator > filter feeder > deposit feeder. Timmermans *et al.* (1989) found a similar order for that metal: predator > deposit feeder > filter feeder. However, for Pb, Cu and Cd they did not find higher metal concentrations in predators than in species with other feeding habits. In these field studies metal concentrations in the animals were only measured at one time in a year, ignoring the different life stages and sizes of the investigated species. These are, however, important factors as some species (e.g. Hydropsychidae) switch from detritus food to small invertebrates in their last larval stages.

Even if there is no biomagnification in a foodweb, a metal transfer to predators is possible as some of the above mentioned studies showed. This transfer may lead to harmful levels in tissues and cause toxic effects (Dallinger *et al.*, 1987). This is supported by a study of Ferard *et al.* (1983) in an artificial food chain containing algae (exposed to Cd of 0 to 250 $\mu g\ g^{-1}$ for 20 d), daphnids (exposed to Cd in algae of 4.5 to 370 $\mu g\ g^{-1}$ for 14 d) and fish (exposed to Cd in daphnids of 1.3 to 259 $\mu g\ g^{-1}$ for 4 d). It could be shown that Cd was transferred to the fish via food but without being biomagnified. However, in this study the exposure time for the fish was too short, as equilibrium conditions for Cd in fish seem to take about 80 d (Canton and Slooff, 1982).

8. Surface Adsorption

Adsorption processes include different kinds of mechanisms: 1) Physical adsorption is a rapid, reversible and pH dependent mechanism, caused by non-specific forces of attraction (van der Waal forces), e.g. Cd on $Fe_2O_3 \times H_2O$.2) electrostatic adsorption is a reversible exchange of ions. 3). Specific adsorption (chemisorption) is pH dependent and partly reversible and is due to surface bonds on specific sites.

While sorption phenomena on inorganic surfaces have been studied to some degree, the mechanisms of adsorption onto biological surfaces are scarcely known (Campbell and Stokes, 1985).

In some of the cited experiments on metal accumulation, no difference was made between uptake into the organism (intake) and onto the organism (adsorption). Only for some metals it is known that adsorbed metals onto the body can cause detrimental effects in the animals. Layers of Al, Fe and Mn hydroxides have been observed on invertebrate species and on the sediment of some streams (Gerhardt, 1992a; McKnight and Feder, 1984). Even Zn (Xu and Pascoe, 1991) and Pb (Hare *et al.*, 1991) were associated with such layers. If the gills are covered with metal

hydroxide layers, respiration will be affected. On the sediment, such layers prevent algal growth, which is the main food source for grazers; thus typical grazing invertebrates were not found in the stream (McKnight and Feder, 1984). Hare *et al.* (1991) radio labelled Cd, Zn and Pb in a sediment, which was given to the mayfly *Hexagenia rigida*. The animals adsorbed Pb onto the body, especially when skin age increased, which was measured by the extent of covering with Fe-hydroxides. Probably Pb was coprecipitated with Fe hydroxides onto the skin of the mayfly.

On the other hand, elevated metal levels of the skin can also be caused by an active excretion of the metals from the internal organs into the skin just before molting and emergence. Meyer *et al.* (1991) found that Pb and Cd in the freshwater crustacean *Astacus astacus* were located in the mesocuticula and not in the outermost layer of the cuticula as should be expected if caused by adsorption. Also Hackstein (1988) concluded from her experiments with *Gammarus tigrinus* that Cd was actively excreted into the cuticula. She found that at high temperature (27 °C) the concentration of Cd in the animals was lower than at low temperature (7 °C). As the intake of Cd should increase with the temperature because of an increasing metabolism another explanation should be behind the observed phenomenon. Hackstein assumed that the growth rate and the molting frequency were increased by high temperature. As the larvae change their skin more often they also can get rid of higher metal levels than the animals at low temperature. It is a pity that these speculations could not be supported by data on the molting frequency. However, a similar argumentation was made by van Weers (1975), who studied Zn accumulation by the marine crustacean *Crangon crangon*. Increasing water temperature increased the frequency of molting and thereby the rate of Zn elimination.

Many studies, however, only describe metal losses during molting and emergence, without differing between externally adsorbed and excreted metals (Gerhardt, 1990a; Krantzberg and Stokes, 1988; Timmermans and Walker, 1989). As the metal losses in these studies were higher at neutral than at low pH, mainly adsorption onto the skin should have accounted for the metal concentration of the skin. Further evidence for surface adsorption of metals is provided by experiments involving transplantation of chironomids from lakes with neutral pH to those with low pH. Rapid losses of Al, Cd, Fe and Zn were observed (Krantzberg and Stokes, 1988).

9. Toxicity of Metals to Stream Invertebrates

Uptake and bioaccumulation of a metal does not necessarily cause toxic effects. A metal can be stored in a non-toxic species or bound to metallothionein. For example, molluscs can accumulate metals to orders of magnitude above the ambient concentration in the water without visible adverse effects (Harrison and Bishop, 1983). Only a few studies deal with the simultaneous effect of metals on uptake and toxicity (Sedlacek *et al.*, 1983; Winner, 1984; Winner and Gauss, 1986).

Toxicity depends on abiotic (e.g. pH, temperature, hardness) and biotic (e.g. size, weight, life stage, tolerance, competition) factors (Figure 2; Gerhardt, 1990b).

Lithner (1989) postulated that the toxicity of a metal should be negatively correlated to its natural occurrence in the earth crust and its background levels in the water. This may be true for Fe and Al, the most frequent metals, at neutral pH. Acidification and other changes in environmental factors may however increase metal toxicity. Temperature increases usually cause increases in the toxicity of metals (Cr, Zn, Cd) in fish and invertebrates, probably because of increasing uptake (Wang, 1987). Hardness is an important factor ameliorating metal toxicity for aquatic organisms. For example, the competition of Ca^{2+} and Cd^{2+} at the binding sites of membranes may lead to a decreased uptake of Cd in the presence of Ca^{2+}. Other metals form unavailable complexes with $CaCO_3$. The oxidation stage and the ionic charge of a metal species affects its toxicity, e.g. the anionic form CrO_4^{2-} has a higher mobility and long-term toxicity than the cationic form (Cr^{3+}).

pH affects metal toxicity in the range of pH 4 to 7, because of changes in metal speciation (Campbell and Stokes, 1985). Two types of reaction of the metals were separated: (1) Metals, which cause a decreased biological response with decreasing pH (Cd, Cu, Zn), probably because of the competition of H^+ and the metal ion at the binding sites. (2) Metals, which cause an increased biological response while pH decreases (Pb), probably because of changes in speciation and bioavailability. Mackie (1986), however, found that these two types of behavior of a metal may depend on the animal species investigated. Moreover, he found a third type of behavior, where below a certain pH value a switch from one of the two types to the other one occurs (Cd toxicity in the gastropod *Amnicola limosa*).

One important biotic factor affecting the toxicity of a pollutant is the stage in the life cycle of an organism. Toxicity of Cu was reported to decrease in the freshwater clam *Corbicula manilensis* and the midge *Chironomus tetans* in successive developmental stages (Wang, 1987).

Moreover, some populations of a species may be more tolerant to a metal than other populations, becuase of mechanisms like decreased uptake, increased excretion or induced metallothionein production. Tolerance can be genetically based (adaptation), when the populations have lived in a contaminated environment for several generations. Tolerance can also be induced by acclimation, as some studies on preexposure to low metal concentrations reveal. For example, fish preexposed to Cd showed a higher Cd retention in the gut and less uptake than non-preexposed animals (Andersson and Gabring, 1988).

Species specific differences in the tolerance to metals are indicated by a wide range of LC_{50} values, eg. for Cd: Crustacea: 0.04 mg L^{-1} (Thorp and Lake, 1974), Hydropsychidae (Insecta): 200 mg L^{-1} (Williams *et al.*, 1987), Leptoceridae (Insecta): 2000 mg L^{-1} (Thorp and Lake, 1974) and Polycentropodidae (Insecta): $>$ 2500 mg L^{-1} (Gerhardt, unpublished). The highest values were reached by predators, which corresponds to the high bioaccumulation of metals in predators (see above).

Interaction between species, like competition or parasitism, can be an additional stress factor and may lead to higher biological response to a pollutant. Kraak *et al.* (1991) investigated the effect of a trematode parasite on the heavy metal concen-

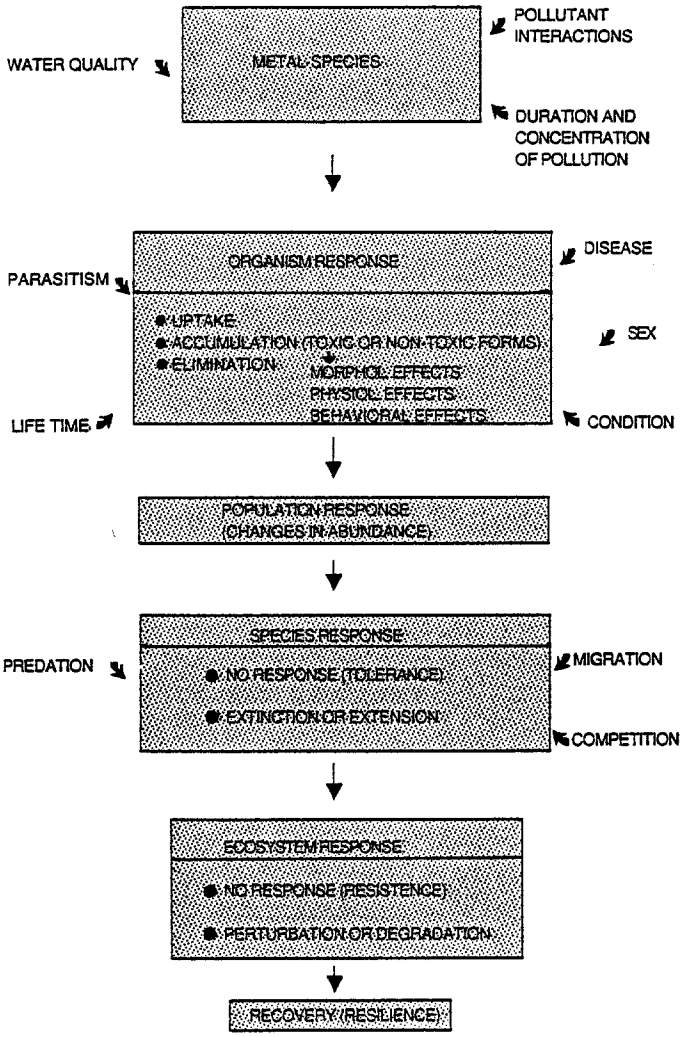

Fig. 2. Factors affecting organisms, species and ecosystems (according to McCahon & Pascoe (1990), changed)

trations in *Dreissena polymorpha*. Infested and non-infested mussels taken from the field were analysed for Cu, Cd, Zn and Pb. The concentrations of all metals in soft tissues were higher in the animals with the parasite than in the healthy animals. This study indicates that biotic stress can change metal uptake and toxicity. More laboratory studies with several interacting species together should be performed.

10. Mechanisms of Metal Toxicity

Metals can exert adverse effects at several sites:

(1) the cell membrane: Transport mechanisms can be disrupted, e.g. by blocking

carrier molecules or replacing essential metals by a toxic metal ion, which can change membrane stability. Cd^{2+} may replace Ca^{2+}, which changes the permeability of the gill membrane in fish. There is much work on ion regulation in fish as affected by pH. Al and Cu seem to cause Na^+ losses, while Cd, Mn and Zn do not cause any effects in fish. The Ca^{2+} flux, however, is affected by several metals: Cd > Cu > Zn > Mn (McDonald *et al.*, 1989).

(2) the DNA: Zn can cause cross linking within the DNA-molecule leading to undesired aggregations of nucleic acids, which can inhibit the transcription process.

(3) the nervous system: Cu can depress the electrical response of nervous cells in fish.

(4) enzymes: Cu can inhibit enzymes like Acethylcholinesterase by binding to the SH groups of the enzyme (Flemming and Trevors, 1989).

The basic biochemical reactions of the above described mechanisms of metal toxicity are:

(1) the competitive blockade of a functional group of a macromolecule.

(2) the displacements of essential ions by toxic ions.

(3) conformational change in proteins (e.g. oxidation of SH-groups) (McDonald *et al.*, 1989).

11. Toxicity of Certain Metals

Cd

LC_{50}-tests/EC_{50}-tests

Most studies have been short term toxicity tests with high, often ecologically irrelevant concentrations and mortality as endpoint. Crustacea have been the most used invertebrates. The obtained LC_{50} values vary from 0.04 mg L^{-1} to > 2000 mg L^{-1} (Gerhardt, 1990b). In the last years, other parameters than mortality have also been used for acute toxicity testing. Grebe and Schaeffer (1991) exposed the planarian *Dugesia dorotocephala* for 1 hr to 25 to 100 mg L^{-1}. They observed changes in locomotory behavior and morbidity. The extremely high concentrations and the short exposure time however make the ecological relevance of this study doubtful. Pascoe *et al.* (1991) used the feeding rate and the time used for separation of a praecopula in *Gammarus* sp. as behavioral parameters for Cd-toxicity. Feeding rate was measured by reduction in standard leaf discs, serving as food for *Gammarus* sp. during 24 hr. Heinis *et al.* (1990) noted in a 96 hr test deviations in feeding behavior of the chironomid *Glyptotendipes pallens* at Cd concentration of above 0.1 mg L^{-1}. At 5 mg Cd L^{-1} long periods of inactivity were observed.

Acute Toxicity Studies Regarding pH Changes

Bauer *et al.* (1988) investigated the survival of *Gammarus fossarum* at pH 3.3, 4.5 and 5.5 and low metal concentrations (Cd: 1 μg L^{-1}, Pb: 5 μg L^{-1}, Zn: 200 μg L^{-1}). In all experiments survival decreased at low pH. However, the metal levels were very low, so that the low pH alone may have accounted for the observed

TABLE I

pH dependent toxicity of Cd in freshwater organisms

Test/Parameter	Species	Experimental	Results	Ref.
LC_{50} (96/168 hr) morality	*Salmo gairdneri* (Pisces)	CF, r = 2, n = 10 14–18 °C, a,	pH 4.7:28 μg L^{-1} pH 5.7:0.7 μg L^{-1} pH 7.0:0.5 μg L^{-1}	[1]
LC_{50} (96 hr) mortality Cd (5 μg L^{-1} to 8 mg L^{-1})	*Hyalella azteca* (Crustacea) *Enallagma* sp. (Odonata) *P. compressum* (Bivalvia) *Amnicola limosa* (Gastropoda)	ST,, r = 3, n = 10 20–25 °C pH: 2 to 6	pH 5.0:0.01 mg L^{-1} pH 5.5:0.02 mg L^{-1} pH 6.0:0.03 mg L^{-1} pH 3.5:7.10 mg L^{-1} pH 4.0:8.70 mg L^{-1} pH 4.5:10.7 mg L^{-1} pH 3.5:2.10 mg L^{-1} pH 4.0:0.70 mg L^{-1} pH 4.5:0.40 mg L^{-1} pH 3.5:6.40 mg L^{-1} pH 4.0:3.80 mg L^{-1} pH 4.5:2.70 mg L^{-1}	[2]
field test (96 hr) mortality (Cd, Zn, Pb, Al, Mn, Fe) 10 d exposure	*Leptophlebia cupida* (Ephemeroptera) *Prosimulium hirtipes* (Diptera) *L. cupida*	r = 8, transplants	pH 6 to 4.2: less Cd body burden, no mortality pH 4.2: 82% mortality pH 4.2: 20% death	[3]
LC_{50} (7 d) (1 μg L^{-1})	*Gammarus fossarum* (Crustacea)	N.D.	pH 3.5: 100% (1 d) pH 4.5: 50% (4 d) pH 5.5: <50% (7 d)	[4]
LC_{50}(120 hr)	*Leptophlebia marginata* (Ephemeroptera) *Baetis rhodani* (Ephemeroptera)	ST: r = 1, n = 15 and CF: r = 2, n = 15 "	pH 5: 5.0 mg L^{-1} pH 7: 5.0 mg L^{-1} pH 5: 3.0 mg L^{-1} pH 7: 2.3 mg L^{-1}	[5]
16 d El-survival mortality hatching	*Brachydanio rerio* (Pisces)	(1–1000 μg L^{-1}) r = 0, a, 25 °C	less survival at pH<5.5 and increasing Cd. 4.1 mg L^{-1}: 100% (6 d). delayed at pH < 6 stimulated at pH 6–7.	[6]
field/lakes	benthic invertebrates	taxa in an acid (pH 3), alkaline (pH 7.9) and control (pH 5.8) pond	pH 7.9: Chironomidae > Crustacea > Insecta + Annelida pH 5.8: Crustacea > Chironomidae > Insecta + Annelida pH 3.2: Chironomidae >>> Insecta	[7]

[1] Cusimano *et al.* (1986), [2] Mackie (1986), [3] Hall *et al.* (1988), [4] Bauer *et al.* (1988), [5] Gerhardt (1992b), [6] Dave (1985), [7] Wickham *et al.* (1987).
CF: continuous flow system, ST: static bioassay, r: replicates, n: numbers of animals per treatment, a: artificial water, N.D.: no details, EL: embryolarvae-tests, F1 and F2: 2 generations tested.

survival rates. Experiments at such low metal concentrations should be performed for more than 7 d in order to see effects. For example, Canton and Slooff (1982) found an increased mortality in *Daphnia magna* at Cd concentrations > 0.67 μg L^{-1} in a 32 d experiment. Mackie (1986) found that the toxicity of Cd increased with decreasing pH for *Hyallela azteca* in a LC_{50}-test, while it decreased for *Pisidium compressum* and *Amnicola limosa*. The same was observed for *Salmo gairdneri* (Cusimano *et al.*, 1986).

Studies on Sublethal Effects of Cd and pH

Long-term studies with lower, ecologically realistic metal concentrations are rare in the literature, especially with regard to the combined effects of pH and metals (Gerhardt, 1990b). Ravera (1984) reported a reduction in population density and biomass of *Daphnia galeata* at Cd > 10 μg L^{-1}. Changes in species composition in lake ecosystems have been found at Cd levels of 2 μg L^{-1}. Wickham *et al.* (1987) compared the invertebrate species composition of an acid (pH 3.2), and alkaline (pH 7.9) and a control (pH 5.8) pond. In the acid pond metal concentrations of Al, Cu, Cd, Fe, Zn and Pb were elevated. This pond was dominated by chironomids, while in the other ponds also other taxa were abundant. In such field studies the key factor for the observed changes in species composition cannot be revealed. In Wickhams study, e.g. the Al concentrations were twice as high as those known to cause fish death. However, Fe was the most accumulated metal in the organisms. Moreover, the pH in the acid pond was so low, that this factor alone may have caused the observed differences. From other field studies the pH limits for survival of aquatic organisms are known and summarized in Figure 3 according to findings by Otto and Svensson (1983) and Ökland and Ökland (1986) (Gerhardt, 1990b). Laboratory studies under controlled conditions and with variation of single factors one after the other are necessary to reveal the most important factors causing toxicity. Dave (1985) studied the survival of embryolarvae and the hatching of *Brachydanio rerio* during 16 d with Cd concentrations up to 4 mg L^{-1}. At pH < 5.5, the embryolarvae survived less than at neutral pH. Between 0.03 to 4 mg L^{-1} survival was less at neutral than at acid pH. A pH < 6 caused delayed hatching.

Zn

LC_{50}-tests/EC_{50}-tests with Respect to pH

Willis (1989) reported an 96 hr LC_{50} for the leech *Erpobdella octoculata* of 8.8 mg L^{-1}, when 1 yr old animals were tested and 2.05 mg L^{-1} for newly hatched animals. Cusimano *et al.* (1986) found that the LC_{50}2-96 hr for fish decreased with increasing pH (Table II). However, Wang (1987) concluded from his review that the toxicity of Zn, Cd and Cu increased with increasing pH. Campbell and Stokes (1985) summarized studies on algae, fungi, crayfish and fish. Most studies indicated less toxicity (measured as growth inhibition, mortality) at pH values below 6. For acid tolerant algae however the opposite was found. Zn can form precipitations

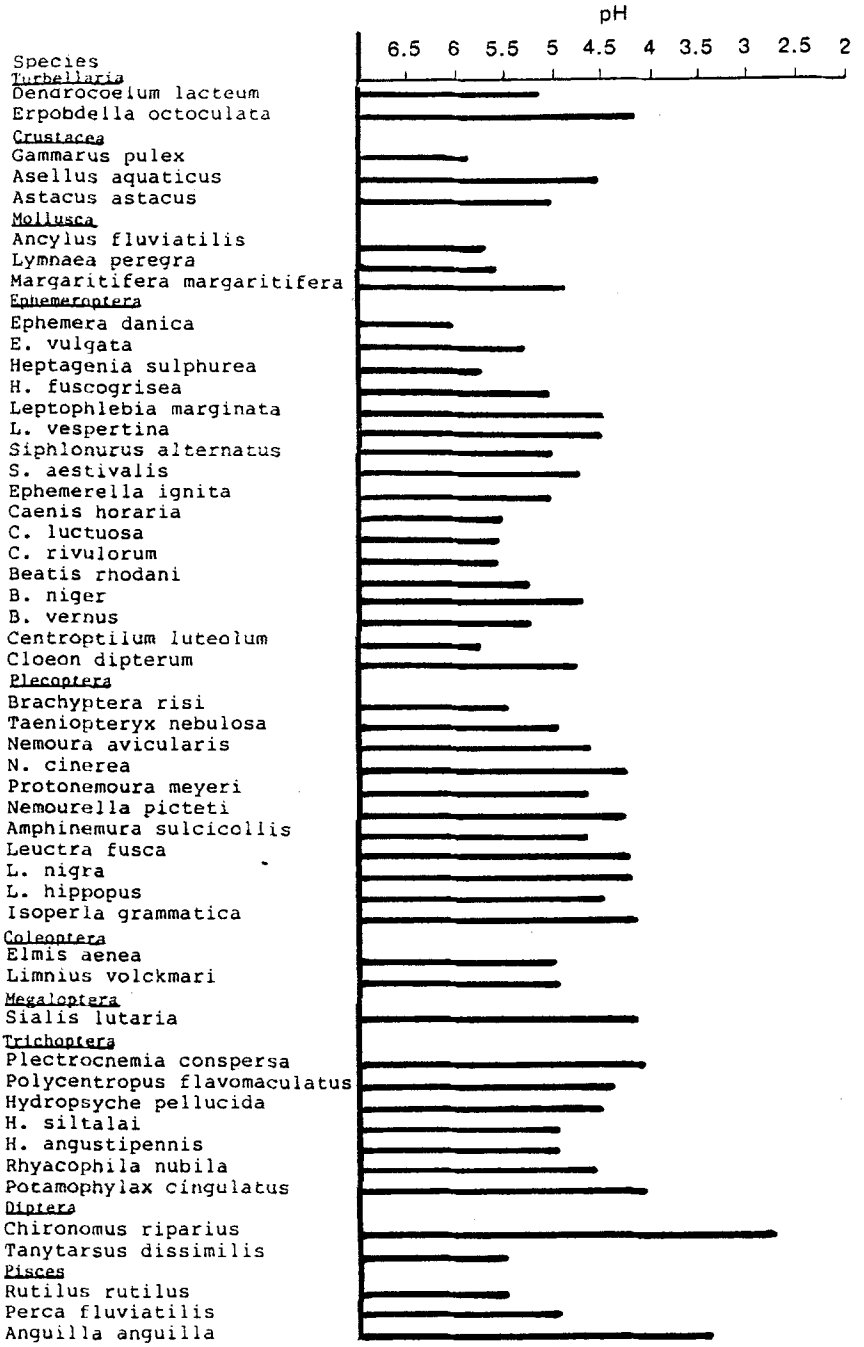

Fig. 3. pH tolerances of aquatic invertebrates and fishes (according to Otto & Svensson 1983 & Ökland & Ökland 1986).

TABLE II

pH dependent toxicity of Zn, Fe, Pb and Cu for freshwater animals

Test/Parameter	Species	Experimental	Results	Ref.
Zn				
LC_{50} (96 hr)	*Salmo gairdneri* (Pisces)	see before	pH 4.7:671 μg L^{-1} pH 5.7:97 μg L^{-1} pH 7.0:66 μg L^{-1}	[1]
natural metal mixture (nm) mortality	*Jordanella floridae* (Pisces)	CF, 2 generations 50% nm, pH 5.8	100% death after 10 d (1.gen.) after 2 d (2. gen.).	[11]
size at maturity reproduction		30% nm, pH 5.8 "	smaller than pH 7. spawning 6 d delayed	
Fe				
LC_{50} (55 hr)	*Asellus aquaticus* (Crustacea)	St, r = 3, n = 10 15 °C, a, buffer	pH 4.5: 400 mg L^{-1} pH 6.5: 600 mg L^{-1}	[7]
field acid coal waste: (Al: 4.0 mg L^{-1}) (Fe: 0.7 mg L^{-1}	plants/invertebrates	invertebr. samples (1) pH 3–4 (2) pH 6.5–8	acid: many species neutral: few species (Fe-hydroxide layers)	[8]
field (Fe: 0.5 – 3.3 mg L^{-1})	invertebrates	field samples	pH 3–4: no Ephemeroptera, few Plecoptera and Trchioptera. pH 7: reduced abundance	[9]
Pb				
LC_{50} (96 hr) (pH 3.5 – 6) Pb: 0.005 – 80 mg L^{-1}	*P. ccompressum* (Bivalvia) *Amnicola limosa* (Gastropoda) *Hyalella azteca* (Crustacea) *Enallagma* sp. (Odonata)	see before	pH 3.5: 38.0 mg L^{-1} pH 4.0: 21.0 mg L^{-1} pH 4.5: 11.0 mg L^{-1} pH 3.5: 10.0 mg L^{-1} pH 4.0: 21.0 mg L^{-1} pH 4.5: 10.0 mg L^{-1} pH 5.0: 0.01 mg L^{-1} pH 5.5: 0.02 mg L^{-1} pH 6.0: 0.02 mg L^{-1} pH 3.5 to 4.5 >60 mg L^{-1}	[2]
Cu				
LC_{50} *(48 hr)* *mortality* LC_{50} (72 hr) mortality hatching larval growth adult emergence	*Chironomus decorus* (Diptera)	pH 7–7.8 Cu in sediment (4000 mg kg^{-1}) 15 d, pH 7, Cu: 1–5 mg L^{-1} Cd: 2 g kg^{-1} "	0.74 mg L^{-1} 5.80 mg kg^{-1} less hatching at 5 mg L^{-1} growth decreased emergence decreased	[10]

[1] Cusimano *et al.* (1986), [2] Mackie (1986), [3] Hall *et al.* (1988), [7] Maltby *et al.* (1987), [8] McKnight and Feder (1984), [9] Scullion and Edwards (1980), [10] Kosalwat and Knight (1987), [11] Hutchinson and Sprague (1986).
Legend: see Table I.

on the body and with the mucous onto the gills of *Gammarus pulex*, which may cause more toxic effects than the free Zn ion in the water (Xu and Pascoe, 1991).

Studies on Sublethal Effects of Zn and pH

Munzinger and Guarducei (1988) exposed the snail *Biomphalaria glabrata* to elevated Zn concentrations at pH 7.3 for 33 d. Fecundity and growth rate were reduced at 0.5 mg L^{-1} and hatching of the embryos was delayed at 1 mg L^{-1}. By the end of the experiment 60% of the juvenile snails and 20% of the adults had died at 1.5 mg L^{-1}. Nebeker *et al.* (1984) found a no effect level of 5.2 μg L^{-1} for all life stages of the caddisfly *Clistoronia magnifica*.

Maltby and Naylor (1990) proposed the monitoring of growth data and reproduction parameters like size and number of offspring as appropriate parameters for indicating sublethal stress in stream invertebrates. These parameters are ecologically meaningful because the observed responses can be related to 'higher-level-effects' (e.g. population, community). 0.3 mg Zn L^{-1} caused significant decrease in the adsorbed energy and size of the offspring of *Gammarus pulex*, when the animals were exposed to 0.1, 0.3 and 0.5 mg L^{-1} $ZnSO_4 \cdot 7\ H_2O$ in artificial pond water for 6 d (scope of growth) or 4 w (reproduction experiments). The level of Zn causing growth reduction in brooding females in the short time experiment had also long-term effects on their reproduction, e.g. the reduction of offspring size and the increase in abortion rate. Smaller offspring would take longer to maturity and might reproduce at a smaller size, which could mean reduced fecundity and a decreased intrinsic rate of increase of the population (Maltby and Naylor, 1990).

Some field studies reveal changes in the species composition at elevated Zn levels in the water (Clements *et al.*, 1988; Norris *et al.*, 1982). Clements *et al.* (1988) compared the effects of Zn and Cu in outdoor experimental streams and in the field at pH 8 and high hardness of the water. Exposure to 15 to 27 μg L^{-1} Zn and 12 to 20 μg L^{-1} Cu reduced the number of taxa and individuals within 4 d. After 10 d, the contaminated streams were dominated by *Hydropsychidae* and *Ortocladiini* instead of Ephemeroptera and *Tanytarsini*, which were dominant in the control streams. This reveals that chironomids are metal tolerant.

Fe

LC_{50}/EC_{50} Studies with Respect to pH

Studies on the toxicity of Fe seem to be rare because it is an essential metal, very frequent in the earth crust and the organisms seem to have developed regulatory mechanisms. In most of the early studies on Fe toxicity to fish the effects of pH and Fe were not possible to separate (reviewed in Douderhoff and Katz, 1953). The LC_{50}–96 hr values between the studies depending on the test species and methods (Gerhardt, 1990b), e.g.: Salmonidae, 20 mg $FeSO_4L^{-1}$ (Weigelt, 1900), *Asellus aquaticus*, 400 mg $Fe^{+}L^{-1}$ (Maltby *et al.*, 1987) or 3 mg Fe^{2+} L^{-1} (Walter, 1966).

Maltby *et al.* (1987) regarded the *effect of pH* on acute Fe toxicity. They used

animals from sites with high (2.48 mg Fe L^{-1}) and low (0.88 mg Fe L^{-1}) pollution. Both groups of invertebrates tolerated more Fe at pH 6.0 than at pH 4.5. The authors concluded that Fe may be less toxic at high pH, especially after the addition of a buffer, which may have complexed Fe ions making them less available. Maltby and Naylor (1991) found that younger life stages of *A. aquaticus* were more sensitive to Fe stress than older stages and that stressed females invested more energy in reproduction and their offspring was of less size. Dave (1984) found in a short term experiment with larvae of the zebra fish that the median survival times of embryolarvae at pH 4 were significantly reduced at Fe^{3+} above 2 mg L^{-1}, while there were no effects at pH 7 and concentrations of up to 32 mg L^{-1}.

Studies on Sublethal Effects of Fe and pH

Field studies in streams with iron pollution showed that the periphyton and the benthic invertebrate community was affected by iron hydroxide precipitations on the streambed at neutral pH, e.g. the diversity of the species decreased (McKnight and Feder, 1984) and oligochaetes and chironomids became dominant (Greenfield and Ireland, 1978). Rasmussen and Lindegaard (1988) investigated the invertebrate fauna in a stream within a pH range from 6.7 to 8.8 and Fe levels varying from 0 to 32 mg L^{-1}. At Fe^{2+} concentrations below 0.2 mg L^{-1}, the community was undisturbed with Ephemeroptera and Plecoptera being dominant. An increase in the iron concentration from 0.2 to 0.3 mg L^{-1} led to a decrease in 14 taxa. The species which tolerated the highest Fe concentrations (10 mg L^{-1}) were those being abundant also in highly organic pollutant streams.

Laboratory tests on chronic Fe toxicity at different pH values are very scarce. An online literature research revealed only a few papers, none about Fe toxicity in streams. Gerhardt (1992a) exposed the mayfly *Leptophlebia marginata* to 0, 10, 20 and 50 mg Fe_{tot} L^{-1} for about 30 d in a static test design containing stream water at pH 4.5 and 7.0. At pH 7.0, no lethal or sublethal effects of Fe could be found. At pH 4.5, however, the animals became more inactive and stopped food consumption, while having a constipated gut. Moreover they were covered with Fe-coatings.

Pb

LC_{50}-tests/EC_{50}-tests with Respect to pH

The knowledge about Pb in stream ecosystems has concentrated on accumulation and to some degree also on toxicity tests. The concentration factors, calculated from field measurements reveal that Pb is strongly accumulated ($>$ 4000 fold) and that accumulation is dependent on the species (Nehring *et al.*, 1979; Besser and Rabeni, 1987). Pb accumulation is also influenced by other metals. In chironomids, Pb decreases in the presence of Fe-hydroxides (Krantzberg and Stokes, 1988).

Acute toxic tests showed that Pb caused a pH independent mortality in the predator *Enallagma* sp. and *Hyalella azteca*, while mortality increased with decreasing pH in the filter feeder *Pisidium casertanum* (Mackie, 1986). At low pH, toxicity of

Pb was found to be greater for the sediment feeder *Tubifex tubifex* and *Limnodrillus hoffmeisteri* than for fish. It can be concluded that particle feeding species are more stressed by Pb, probably because Pb has a tendency to adsorb to surfaces at neutral pH. In a short term field experiment Hall *et al.* (1988) showed that 80% of *Prosimulium hirtipes* died at pH 4.2 and elevated Pb concentrations, while the survival of *Leptophlebia cupida* was 100%. Campbell and Stokes (1985) reviewed studies on pH dependent toxicity of Pb, indicating increased uptake and toxicity at low pH for algae and fungi. The authors concluded that the pH dependent change in speciation may be more important than any effect of competition between H^+ and Pb ions. Mackie (1986), however, found no effect of pH on Pb toxicity (Table II).

Studies on Sublethal Effects of Pb and pH

Nyman (1981) investigated foraging behavior of the zebra fish (*Brachydanio rerio*) exposed to 100 μg Pb L^{-1} for 14 d. Reaction distances and handling time were recorded for every fish receiving a prey (*Dahnia magna*) after an acclimation period of 30 min. The reaction distance was significant lower and the handling time was higher for the exposed fish than for the controls. During the handling of a prey exposed fish lost 60% of their prey while the controls lost only 30%. Oladimeji and Offem (1989) investigated acute and chronic toxicity as well as uptake and elimination of Pb in fish (*Clarias lazera* and *Oreochromis niloticus*) and invertebrates (*Chironomus tetans* and *Benacus* sp.). The animals response to Pb was dependent on the dose and time of exposure, spiral movements and respiratory (opercular) movements increased. In another chronic study, freshwater crayfish (*Orconectes nais*) and midges (*Chironomus riparius*) were exposed for 120 respective 30 d to leachates from mine tailings. These leachates had elevated metal concentrations. Pb was 10 times higher and Cd was 5 times higher than in the control water. Survival of the crayfish and the midge was reduced after 30 d and the growth of the midge was decreased, with no pupation occurring in the contaminated water (Besser and Rabeni, 1987).

Cu

LC_{50} tests/EC_{50} tests with Respect to pH

Copper is one of the best investigated metals considering its uptake by and toxicity to aquatic organisms, probably because it has been used as algicide and molluscicid (Flemming and Trevors, 1989; Nor, 1987). However, there is a relative paucity of studies on pH dependent toxicity to invertebrates and the response of the aquatic community as a whole (Nor, 1987). LC_{50} tests show that Gastropoda, Crustacea and Oligochaeta are sensitive to Cu, while Trichoptera and Odonata are more tolerant with LC_{50} (48 hr) values varying from 5 μg L^{-1} (*Daphnia* sp.) to 64 000 μg L^{-1} (Trichoptera) (Flemming and Trevors, 1989; Rehwoldt *et al.* 1973). Cusimano *et al.* (1986) reported acute toxicity data on fish with respect to pH, revealing that Cu is more toxic at neutral pH than at low pH. Campbell and Stokes (1985) reviewed studies on the toxicity of Cu at different pH values on algae, fungi, crayfish and

fish. Toxicity increased with increasing pH. Clements *et al.* (1988) performed an acute toxicity test with Cu on a whole benthic stream community, which had colonized some artificial substrate traps in a stream. Then these traps were taken into the lab and exposed to different Cu levels in the water. After 96 hr the number of individuals and taxa was reduced in the contaminated water and the response was dose dependent. The response was also dependent on the season, as the traps were colonised by different species. The authors concluded from this and earlier experiments, that the species in community tests were more sensitive to Cu than in single species tests.

Studies on Sublethal Effetc of Cu and pH

Other parameters of toxicity than mortality reveal that adverse effects of Cu contamination occur even at low concentrations. The NOEC (no effect concentration) for freshwater molluscs and Crustacea were 8 to 14.8 $\mu g\ L^{-1}$ with survival, growth and feeding being measured in a 6 weeks experiment (Arthur and Leonard, 1970). Maund *et al.* (1991) found a similar LOEC (lowest effect concentration) for *Gammarus pseudolimnaeus* (14.6 $\mu g\ L^{-1}$). They further reported that 23 $\mu g\ L^{-1}$ reduced the population size and caused broader size classes in the test species. Concentrations of 2 mg kg^{-1} DW in the sediment caused reduced growth and delayed emergence in chironomids as well as deformities in the mouth parts (Kosalwat and Knight, 1987). These authors also found chironomids to be more sensitive to Cu than damselflies, caddisflies and stoneflies, probably because of the thin cuticula of the chironomids. 0.2 to 0.6 mg Cu L^{-1} caused decreased activity in *Dugesia tigrina* (See 1976). Sublethal levels of Cu inhibited growth in molluscs (15 $\mu g\ L^{-1}$) and reproduction in crayfish (35 $\mu g\ L^{-1}$) (Harrison and Bishop, 1983). Clements *et al.* (1990) exposed substrate traps, after having been colonized by a benthic invertebrate community for 30 d to 12, 25 and 50 $\mu g\ L^{-1}$ for 10 d in model streams and simultaneously in the field. They observed a change in the species composition in the Cu contaminated treatments. The laboratory streams had different species (e.g. Elmidae were dominant) and were more sensitive to Cu contamination than the *in situ* exposed communities. The authors argue that perhaps food was short in the laboratory streams, which received tap water, in comparison to the field experiment, where the stream water contained enough particles. In spite of this criticism, such a test design seems to be ideal for ecotoxicological tests, because a whole community can be tested and laboratory and field experiments are combined. Its a pity that there are no similar studies regarding ther effects of pH on metal toxicity.

12. Interaction of Metals

In nature, metal pollution always occurs as a mixture of different metals. Laboratory tests, however, have mostly regarded only one single metal. As metals interact with each other, studies about the toxicity of a single metal may not be sufficient in order to evaluate the metals effect in the ecosystem. There are three possible types of interaction:

(1) Additivity: The effect of two metals together is the sum of the effects of the single metal.

(2) Synergism: The metal mixture causes an effect being stronger than the sum of the single metals.

(3) Antagonism: The metals exhibit an effect being smaller than the summation of the effects of the single metals.

The mechanisms behind these interactions are 1) competition for the binding sites on cell surfaces (antagonism), 2) affinity change for one ion in consequence of the binding of another ion at another site of the same molecule (antagonism or synergism), 3) change in membrane permeability (antagonism or synergism) (Vranken *et al.*, 1988).

Wang (1987) reviewed some studies on metal-metal interactions in aquatic freshwater organisms. The most studied pair was Cd and Zn and the oberved effects varied from antagonism to synergism depending on the test species. There is no information about how the concentrations of the applied metal mixtures were chosen. The concentration of the single metal in a mixture is however important for the result of the test, because the metals differ in their toxicity. Meyer *et al.* (1991) exposed the crayfish *Astacus astacus* to 2 $\mu g\ L^{-1}$ Cd and 20 $\mu g\ L^{-1}$ Pb alone as well as together for 10 weeks. The metals were accumulated in the midgut gland to a lower extend than when exposed together.

Vranken *et al.* (1988) reviewed several models, which can be used for the study of metal mixtures. If the metals exert an additive effect the *simple similar action model* may be appropriate. Here the metals are assumed to react similar by producing parallel slopes for the individual toxicants. Another model (the *concept of equitoxic mixtures*) describes a 'toxic unit' as an equal fraction of both EC_{50} values for the two metals in a mixture. This concept assumes that the components contribute in a similar way to the biological response caused by the mixture, that means it is a redefinition of the simple similar action model. The *independent dissimilar action model* assumes that the organism will only react to the mixture of metals, if one is present at a concentration higher than the incipient lethal level. This model describes also synergistic and antagonistic interactions.

Studies based on this theoretical background are very scarce. Toussaint *et al.* (1990) tested the effects of Cu and Zn in equitoxic mixtures on the filtration rate of *Dreissena polymorpha*. They found that both metals were accumulated more in the mixture than alone. Cu was 40 times more toxic than Zn. The mixture had a less than additive effect on the filtration rate of the test species. Vranken *et al.* (1988) tested the toxicity of a mixture of Hg, Zn, Cu and Ni according to the toxic unit concept for a marine nematode. While the pair Cu and Hg exerted an antagonistic effect on the acute toxicity (mortality) in the nematode, the chronic toxicity (developmental inhibition) was synergistically affected by the mixture. Such contradictory results show that short term as well as long term tests with metal mixtures in different concentrations are necessary. No studies on the effect of pH on the toxicity of metal mixtures have been found in the literature.

13. Summary and Conclusions

This review concentrates on the current knowledge about the effects of pH on speciation, uptake, adsorption and toxicity of the metals Cd, Cu, Fe, Pb and Zn in stream invertebrates.

Metal speciation is affected by several physiochemical and biological factors, pH being one of the most important, because it affects sorption processes, complexation and solubility of metals.

Metals can be taken up by different mechanisms and this depends on the metal species. At low pH the availability of the free metal ions increases, however the efficiency of carrier mediated transport decreases because of the competition between metal ions and H^+. Thus, the actual bioavailability is not identical with the chemical availability of a metal species. In general, a decrease in pH implicates increased metal uptake, as laboratory studies often revealed. Field studies, however, could not support this observation. Neither experiments (field, laboratory) nor models deliver acurate predictions of the metal concentrations in aquatic organisms.

Another important factor for metal uptake is the source of the metal, being abiotic (from water or sediment) or biotic (from food organisms). For detritus feeders, however, this is impossible to separate. In general, uptake from the water seems to be the most important pathway of contamination, while uptake from food seems to be a complex phenomenon, depending mainly on the feeding habit in different life stages of the animals, species specific factors like tolerance, and the time of exposure to the contaminant.

Field surveys and laboratory tests indicate that except for Hg, no biomagnification should occur. However, these studies ignored several important aspects: 1. biomagnification experiments should be performed at equilibrium conditions, which are highly species dependent; 2. whole lifetime exposure to the natural diet should be used because the animals often switch to other food items according to season and/or life stage.

The role of surface adsorption in metal uptake and toxicity is not clear, because direct studies are lacking. Adsorption is only indirectly concluded from experiments, where metal concentrations before and after molting or emergence have been determined. However, the loss of metals with the skin can have two reasons, 1. the metals have been passively adsorbed onto the body, 2. the metals have been actively eliminated from internal organs into the cuticula.

Toxicity of metals depends on numerous abiotic (e.g. pH, °C) and biotic (e.g. uptake, tolerance, competition) factors. Most studies in the past have however been short term toxicity tests with high, ecological irrelevant metal concentrations, mortality as endpoint and no additional factors have been considered. There is a trend to acute toxicity studies, considering temeprature, hardness and pH as varying factors and measuring other parameters than mortality, e.g. behavior, enzyme inhibition. Another trend in toxicology is the long term exposure to low metal concentrations and effect parameters like fecundity, hatching, larval growth or

behavior. These parameters are more ecological relevant than mortality. However chronic toxicity experiments of metals with respect to pH and metal speciation are still very scarce. Multispecies designs should be preferred to single species tests in chronic toxicity experiments, because biotic stress like competition or parasitism can affect metal uptake and toxicity.

This review reveals the following gaps in toxicological research concerning metals and acidification in streams:

(1) uptake of different metal species at different pH values.
(2) the role of surface adsorption for uptake and toxicity of metals.
(3) toxicity of different metal species.
(4) toxicity of metal mixtures at varying pH values.
(5) sublethal effects of metals and acidification in long term exposure to invertebrates in multispecies designs.
(6) the role of adaptation for the metal pollution problem.
(7) field validation of laboratory results from acute and chronic tests.

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