Belg. J. Zool. - Volume 129 (1999) - issue 1 - pages 269-284 - Brussels 1999

BIOAVAILABILITY OF CADMIUM AND ZINC TO MIDGE LARVAE UNDER NATURAL AND EXPERIMENTAL CONDITIONS: EFFECTS OF SOME ENVIRONMENTAL FACTORS

LIEVEN BERVOETS AND RONNY BLUST
Laboratory of Ecophysiology and Biochemistry,
Department of Biology, University of Antwerp (RUCA)
Groenenborgerlaan 171, B-2020 Antwerp, Belgium
e-mail: bervoets@ruca.ua.ac.be

Abstract. In this paper the effects of environmental factors on cadmium and zinc uptake by larvae of chironomids are discussed. The results of several laboratory experiments and field studies were pooled and analysed using uptake and accumulation models. In the field studies, the relationship between metal concentrations in larvae and sediment was studied on samples from several watercourses. The effect of different sediment characteristics on these relationships was investigated. In the laboratory experiments, larvae of *Chironomus riparius* were exposed to metals via the water, and the effects evaluated of three changing environmental factors, *i.e.* salinity, temperature, and pH. Non-linear regression models were constructed to determine the relative importance of the different environmental factors contributing to the variation in metal uptake or accumulation. For the field data, the amount of variation that could be explained by these models was limited. Only for zinc was a significant amount of variation (up to 66%) explained relating accumulated zinc to easily extractable zinc and considering total organic carbon (TOC) in the model. For the laboratory data, relating uptake levels to the metal ion activities explained no more than 6% and 24% of the total variation in respectively cadmium and zinc uptake. The integration of the different effects of the environmental factors in the models explained 67% of the total variation in cadmium uptake and 56% of the total variation in zinc uptake. Factors contributing most significantly to the explained variation were temperature, pH, and salinity of exposure, calcium ion activity and salinity of acclimation. The high, unexplained variation under field conditions is probably due to the large variation in exposure conditions in natural environments and a lack of knowledge concerning the relative importance of the different exposure routes under these circumstances.

Key words: metal uptake, environmental factors, Chironomidae, modelling.

INTRODUCTION

When trace metals are added to natural waters they are distributed over different compartments of the ecosystem *i.e.* the water column, the suspended matter, the sediment and the interstitial water (TESSIER & CAMPBELL, 1987; LUOMA, 1989; AMYOT *et al.*, 1994). Within each phase, metals will be partitioned among specific ligands, the so-called chemical speciation. In the sediment and the suspended solids, metals can interact with iron- or manganese oxides, adsorb to clay particles, bind to organic ligands, etc. In the water column and in the interstitial water, the trace metals will be present as hydrated ions, associated with dissolved inorganic or organic ligands, or adsorbed to inorganic and organic colloids. The distribution of a metal among the different components depends not only on the concentration but also on the nature of the compartments. Changes in the physical or chemical conditions such as pH, salinity, and alkalinity, influence the distribution between the phases (BORGMANN, 1983; SALOMONS & FÖRSTNER, 1984; LUOMA, 1989). The bioavailability of trace metals will largely depend on the chemical speciation. Thus, the total trace metal concentration in sediment or water should not be used as the only measure of metal contamination. Several studies have verified that metal availability from solutions is a function of the free metal ion activity (ALLEN *et al.*, 1980; DE LISLE & ROBERTS, 1988; BLUST *et al.*, 1992). Besides chemical processes, biological processes will influence the uptake of trace metals by organisms. Two major uptake routes have been identified, *i.e.* direct absorption of dissolved metal species from solutions, and via ingestion of particulate matter (including food) (LUOMA, 1989; HARE, 1992; RAINBOW & DALLINGER, 1993). Although mostly present at higher concentrations in food than in solution, trace metals associated with particles tend to be less available than are those in solution. Uptake mechanisms may be controlled physiologically by the organism. Control that could be exerted by the invertebrate with respect to these uptake mechanisms would be via some alteration to the number, or affinity of the membrane transport ligands (SIMKISS & TAYLOR, 1995).

In this paper the data of several studies that assessed the influence of environmental factors on the net uptake (in the laboratory) and accumulated levels (in the field) of trace metals by chironomid larvae, are pooled and analysed. In the field studies, the effect was assessed of the geochemical characteristics of sediments on the levels of trace metals accumulated by larvae of the midge *Chironomus riparius* (Meigen, 1804) (Diptera, Chironomidae). To relate metal levels in organisms with levels in sediments, trace metal partitioning among various sediment phases was determined and geochemical sediment characteristics were analysed (BERVOETS *et al.*, 1994, 1997, and 1998). In this study we analysed the pooled data of the three field studies for zinc and cadmium using accumulation models. In the laboratory experiments, the effects of some environmental factors on the net uptake of two trace metals, cadmium and zinc, by larvae of *Chironomus riparius* were studied (BERVOETS *et al.*, 1995, 1996a, 1996b; BERVOETS & BLUST, unpublished). Among the factors influencing uptake and accumulation of metals by aquatic organisms, salinity, temperature, pH, hardness, and the presence of organic ligands are the most important (PHILIPS, 1976; SIMKISS & TAYLOR, 1995). The separate and combined effects of the different components of these environmental factors were assessed by analysing the pooled data. The effects of changing environmental factors on the net metal uptake by fourth instar larvae were studied, in relation to the effects of the factors on the chemical speciation and on the physiological condition of the organism.

MATERIALS AND METHODS

Test organism

Midge larvae (Diptera, Chironomidae) are the central organisms in this study. Chironomid larvae were chosen because of their important position in freshwater ecosystems. Larval members of this family are found in almost every kind of fresh- and brackish water. Under certain conditions such as low levels of dissolved oxygen, larval chironomids may be the only insects present in benthic sediments (PINDER, 1986; WARWICK, 1990; CRANSTON, 1995). All larvae collected from natural waters for metal analysis in the field studies were identified as *Chironomus* gr. *thummi*. This species group contains at least 13 different species, which are morphologically very similar (WEBB & SCHOLL, 1985). Larvae used in the laboratory experiments were obtained from a controlled laboratory culture at the Royal Belgian Institute for Natural Sciences (KBIN, Brussels, Belgium). Larvae were cultured in 10 L plastic aquaria according to the methods of VERMEULEN *et al.* (1997). When the fourth larval stage (instar 4) was reached, the larvae were placed at 15°C in the dark and held in aquaria at high densities (1 larva per cm^2) to retard pupation while maintaining them in normal physiological state (INEICHEN *et al.*, 1979; BANGENTER & FISCHER, 1989).

Field studies

In the field studies midge larvae were collected at different sampling sites. Concentrations of Cu, Zn, Cd, Pb, Cr and Ni were measured in organisms and sediments. To identify trace metal partitioning among various geochemical phases, sediments were subjected to simultaneous extraction schemes. Four metal fractions were determined: (1) easily reducible (ER) metal (trace metals associated with Mn oxides); (2) reducible (R) metal (trace metals associated with Fe oxides); (3) metals bound to organic matter (ORG); (4) total metal (TOT), metals extracted using a mixture of HNO_3 and $HOCl_4$. In addition, several geochemical characteristics of the sediments were analysed; total organic carbon (TOC), Fe-oxides, Mn-oxides, and particle size distribution. For a detailed description of sampling, sediment handling, and analytical procedures, we refer to BERVOETS *et al.* (1994, 1997, and 1998). In this paper the analysis of the pooled data of cadmium and zinc is presented.

Laboratory experiments

In the laboratory experiments the effects of three environmental factors, *i.e.* salinity (0.24 to 10 ppt), temperature (5 to 25°C) and pH (5.5 to 10.0) on the net uptake of cadmium and zinc by larvae of the midge *Chironomus riparius* were studied using artificial river water. The composition of 1 L of this chemically-defined freshwater was 0.096 g $NaHCO_3$, 0.004 g KCl, 0.123 g $MgSO_4.7H_2O$ and 0.06 g $CaSO_4.2H_2O$, resulting in a salinity of 0.2 ppt and a pH of 7.8. The medium was prepared by dissolving the analytical grade products (Merck p.a.) in deionized water. Ionic stocks of cadmium and zinc, containing 100 μM Cd and 1,000 μM Zn respectively, were prepared. The radioisotopes ^{109}Cd and ^{65}Zn (Amersham International, UK) were used as tracers, 460 KBq/L of each tracer being added to the metal stock solutions. In all experimental exposure solutions, the resulting metal concentrations were 0.1 μM Cd and 1.3 μM Zn. These concentrations were chosen for their environmental relevance. The resulting radioactivity of both tracers was 0.46 KBq/L. For all experiments, 50 midge larvae of comparable size were placed in a series of plastic vessels containing 50 ml solution. For each experimental condition we used 6 to 8 replicates. These vessels were placed in a thermostatic water bath at the required temperature. Net uptakes of both cadmium and zinc were linear over time for at least 8 hours during exposure to a total concentration of 0.1 µM cadmium and 1.3µM zinc. Therefore accumulation was measured after 6 h of exposure. After exposure, the 50 individuals were collected on a 250 µm sieve and rinsed with 50 ml of deionized water. The influence of prior acclimation at the different environmental conditions on metal uptake was assessed. In addition the effect of the environmental factor on the chemical behaviour was added to uptake models. For a detailed description of the experimental set-up we refer to BERVOETS *et al.* (1995, 1996a,b).

The equilibrium concentrations of the chemical species considered were calculated using the computer program SOLUTION (BLUST *et al.*, unpublished), an adaptation of the program COMPLEX (GINZBURG, 1976). This speciation model allows the calculation of the composition of solutions in equilibrium with the atmosphere. Activity coefficients were calculated using the estimation method of Helgeson (BIRKETT *et al.*,1988). Results of the chemical speciation calculations were expressed on the molar concentration scale and multiplied by the appropriate activity coefficients to obtain species activities.

Modelling net uptake or concentration

To determine the relative importance of the different factors contributing to the variation in the net uptake of metals (in the laboratory experiments) or concentration (in the field studies), non-linear regression models were constructed (BLUST *et al.*, 1994, 1995; BERVOETS *et al.*, 1997). For the pooled data of the laboratory experiments, the net uptake was related to the terms that describe the change in the free metal ion activity, and different components of the environmental factors (*i.e.* salinity, temperature, and pH of exposure and acclimation) and the free calcium ion activity. In the field studies the metal concentration in the organisms was related to a term describing the variation in the metal concentration in the different sediment fractions (*i.e.* total, reducible, easily reducible metal concentrations or metals bound to organic matter) and to the different sediment characteristics (iron oxides, manganese oxides, TOC and particle size distribution). The relative importance of the different terms was determined by a forward selection procedure. This was done by starting with a single factor, the metal activity or concentration, and stepwise adding the other terms and evaluating their contribution to the total variation. A coefficient of proportionality (C_f) was introduced to relate the concentration in the environment to the metal concentration in the organisms.

RESULTS

Field studies

Total metal levels were always higher than reducible metal levels but important differences were observed between essential and non-essential metals. In this paper we discuss only the results for cadmium and zinc. For cadmium, total levels were only slightly higher than reducible levels. For zinc, however, the differences were much more pronounced (up to 5 times more total zinc compared to reducible zinc). This is illustrated in Fig. 1 for three different rivers.

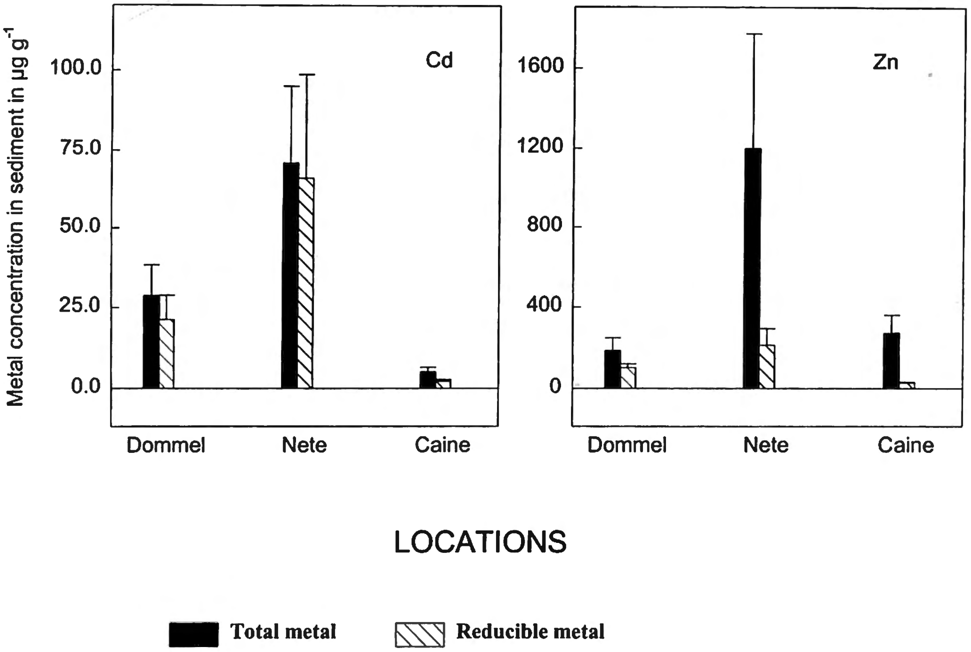

Fig. 1. – Results of metal partitioning in sediments from three rivers. Mean values with standard deviation are presented in µg g^{-1} dry weight.

The relationships between total metal concentration in sediments and midge larvae for the pooled data are shown in Fig. 2. For both metals only poor relationships were found. To determine the relative importance of the different sediment factors we used non-linear regression models. The metal concentration in the organisms was related to a term describing the variation in the metal concentration in the different fractions (ER, R, ORG, TOT) and a term that described the variation in metal concentration in the pore water. The different sediment factors considered in the models were the manganese (Mn), the iron (Fe) and the organic matter (TOC) content, and grain fraction < 63 µm. However, it was not possible to model the accumulated metal levels using the pooled data because we did not measure the same characteristics in the three studies. In the separate studies, only for zinc was it possible to explain 48 and 66 % respectively of the variation, when organic matter was included in the accumulation model for zinc. The models that explained most of the variation were: $Zn_{CHI} = F*Zn_{TOT}*TOC^k$, in a first study (BERVOETS *et al.* 1997) and $Zn_{CHI} = F*ZnR*TOC^k$ in a second study (BERVOETS *et al.*, 1998).

For cadmium it was not possible to construct a model that explained a significant amount of variation in levels accumulated by the larvae.

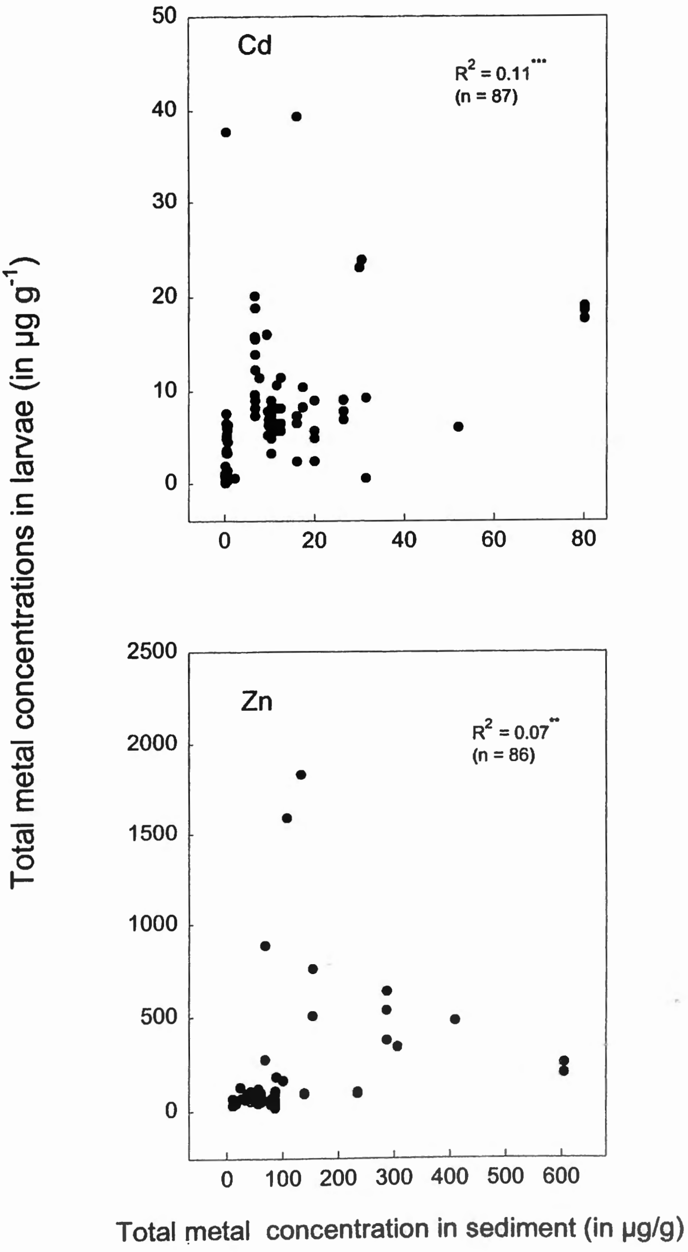

Fig. 2. – Relationships between trace metal levels in sediments and in chironomid larvae in µg g^{-1} dry weight for cadmium and zinc.

Laboratory studies

In Fig. 3 the results of the calculations of the chemical speciation model in defined solutions are summarised. The effects of salinity, temperature and pH on free metal ion activity are presented for cadmium and zinc. In the case of salinity the contribution of the free cadmium ion activity decreased from 72 % at a salinity of 0.24 ppt to 6 % at a salinity of 10 ppt, and the contribution of the free zinc ion activity decreased from 64 % to 31 % over the same salinity range. In the case of pH the contribution of the free cadmium ion activity decreased from 71.6 % at a pH of 5.5 ppt to 0.19 % at a pH of 10, and the contribution of the free zinc ion activity decreased from 69.5 % to 0.03 % over the same range. Temperature had only a small effect on the free metal ion activity over the tested range.

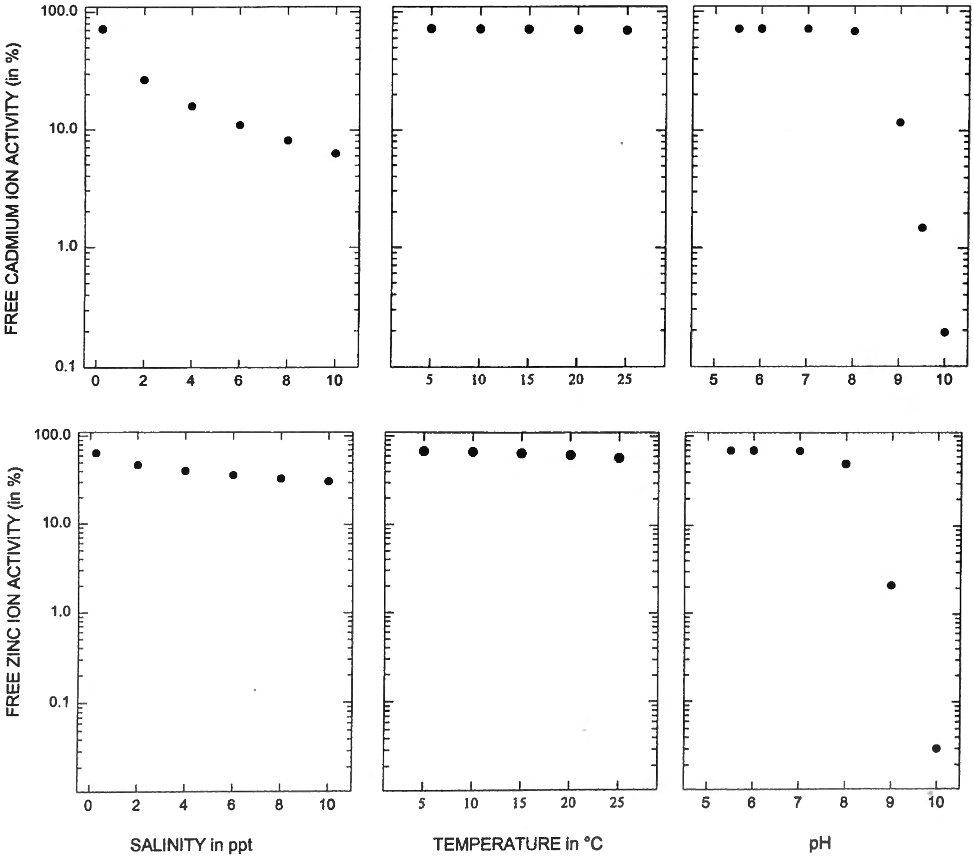

Fig. 3. – Relative contribution of the free metal ions (in %) in function of the environmental factors salinity, temperature, and pH.

In Fig. 4 net uptake as a function of the environmental factors is illustrated for cadmium and zinc. The results of uptake by larvae acclimated at their culture conditions are presented. For all environmental factors an effect was observed on net metal uptake within each acclimation group (BERVOETS *et al.*, 1995, 1996a, 1996b; BERVOETS & BLUST, unpublished). The net uptake of cadmium and zinc decreased with increasing salinity but increased with increasing temperature. For pH, net uptake increased with increasing pH between 5.5 and 9.0 but decreased between pH 9.0 and 10.0. In most cases, prior acclimation to different environmental conditions had a significant effect on the net uptake but this effect was not always consistent.

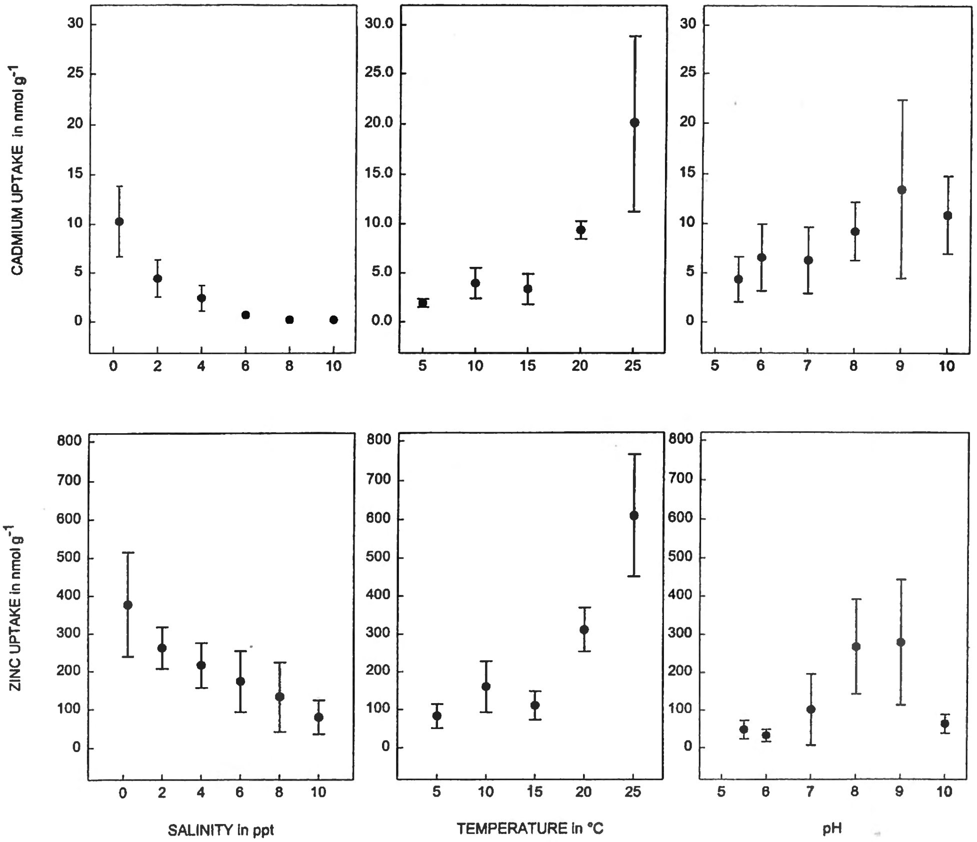

Fig. 4. – Uptake of cadmium and zinc (in nmol. g^{-1}) in function of the environmental factors, for larvae acclimated at their culture conditions.

Non-linear models were constructed for the pooled data (n= 580 for Cd and n=628 for Zn) to determine the relative importance of the different environmental factors and components of those factors on uptake of cadmium and zinc. Factors that were considered in the model were the free metal ion activity, salinity, temperature, and pH of exposure and acclimation, diffusion rate, and calcium ion activity. This was done by starting with a single factor, the metal ion activity, and stepwise adding the other terms, evaluating their contribution to the total variation. Tables 1 and 2 give the results of the non-linear regression analysis. As shown in this table, the free metal ion activity explained 6.2 % and 24 % of the variation in metal uptake for respectively cadmium and zinc (Fig. 5). The model that explained most of the variation in net cadmium uptake was:

$$Cd_{midge}=C_f*(Cd^k_{act}*t^l_{exp}*pH^m_{exp}*sal^n_{exp}*Cd_{diff}^p*Ca^q*sal^r_{accl}*t^s_{accl})$$

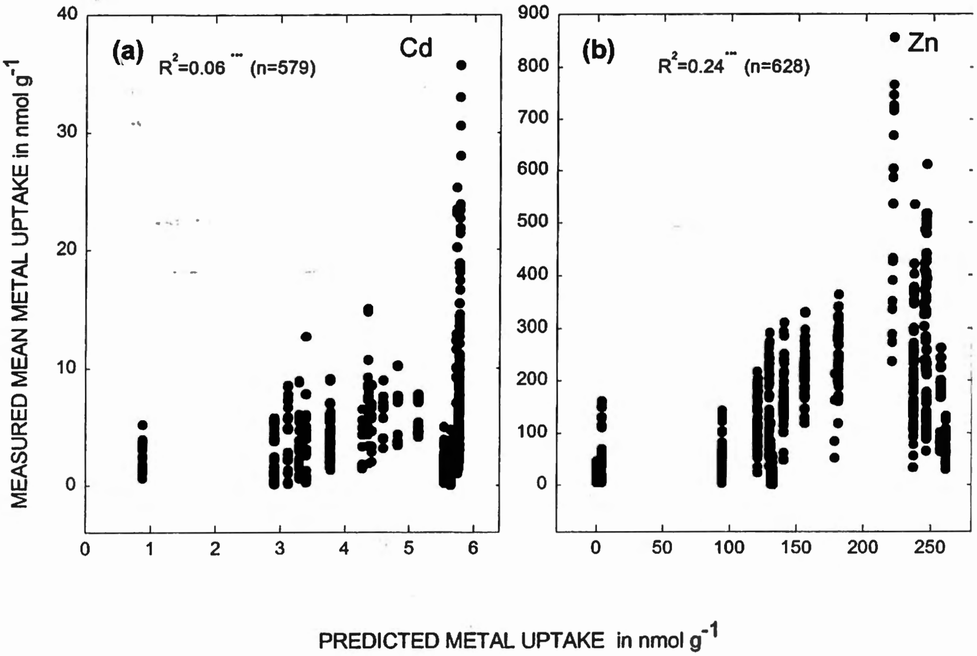

Fig. 5. – Predicted *versus* measured cadmium and zinc uptake by larvae of *Chironomus riparius*, considering only the free metal ion activities.(a) $Cd_{upt} = C_f*(Cd_{act}^k)$ ($R^2 = 0.062**$, n = 579); (b) $Zn_{upt} = C_f*(Zn_{act}^k)$ ($R^2 = 0.237**$, n = 628).

The model that explained most of the variation in net zinc uptake was:

$$Zn_{midge}=C_f*(Zn^k_{act}*t^l_{exp}*sal^m_{exp}*pH^n_{exp}*Ca^p*sal^p_{accl})$$

where Cd_{act} and Zn_{act} are the metal ion activities, t_{exp}, pH_{exp}, sal_{exp} are the exposure temperature, pH, and salinity, t_{accl}, pH_{accl}, sal_{accl} are the acclimation conditions, and Cd_{diff} is the diffusional rates of the free metal ion. In the case of net cadmium uptake, the factors that accounted for the effect of temperature, salinity, and calcium were negative, indicating that cadmium uptake increased with a decrease in these factors. The models explained 67% and 56% of the variation in uptake by midge larvae for respectively cadmium and zinc (Fig. 6).

TABLES 1 and 2

Cadmium uptake (top) and zinc uptake (bottom) by Chironomus riparius*: non-linear regression model for the pooled data.*

*B: partial regression coefficients; SE: Standard Error for partial regression coefficients; L1, L2: confidence limits for partial regression coefficients. Cadmium uptake in midge larvae in nmol/g. ns Not significant; * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p \leq 0.001$*

Variable	*B*	*SE*	*L1*	*L2*
(1) $Cd_{upt} = C_f{*}(Cd_{act}^{k})$ ($R^2 = 0.062^{**}$, n = 579)				
Coefficient	1.74***	0.394	1.35	2.13
k-exponent	0.281***	0.057	0.224	0.338
(2) $Cd_{midge} = C_f{*}(Cd^{k}_{act}{*}t^{l}_{exp}{*}pH^{m}_{exp}{*}sal^{n}_{exp}{*}Cd_{diff}^{p}{*}Ca^{q}{*}sal^{r}_{accl}{*}t^{s}_{accl})$ ($R^2 = 0.67^{***}$, n = 579)				
Coefficient	1.62^{-09}	ns		
k-exponent	0.132***	0.044	0.088	0.176
l-exponent	-3.50***	0. 489	-3.99	-2.84
m-exponent	2.84***	0.723	2.12	3.56
n-exponent	-0.245***	0.026	-0.271	-0.219
p-exponent	14.2***	1.18	13.0	15.4
q-exponent	-0.072***	0.011	-0.083	-0.144
r-exponent	0.316***	0.024	0.292	0.340
s-exponent	0.376***	0.055	0.321	0.431
(1) $Zn_{upt} = C_f{*}(Zna_{act}^{k})$ ($R^2 = 0.237^{**}$, n = 628)				
Coefficient	0.263^{ns}			
k-exponent	0.978***	0.093	0.885	1.071
(2) $Zn_{midge} = C_f{*}(Zn^{k}_{act}{*}t^{l}_{exp}{*}sal^{m}_{exp}{*}pH^{n}_{exp}{*}Ca_{p}{*}sal^{p}_{accl})$ ($R^2 = 0.559^{**}$, n = 628)				
Coefficient	5.67^{-10}	ns		
k-exponent	1.63***	0.261	1.37	1.89
l-exponent	1.46***	0.118	1.34	1.58
m-exponent	0.137***	0.049	0.088	0.186
n-exponent	5.76*	2.44	3.32	8.20
p-exponent	-0.040***	0.007	-0.047	-0.033
q-exponent	0.116***	0.015	0.101	0.131

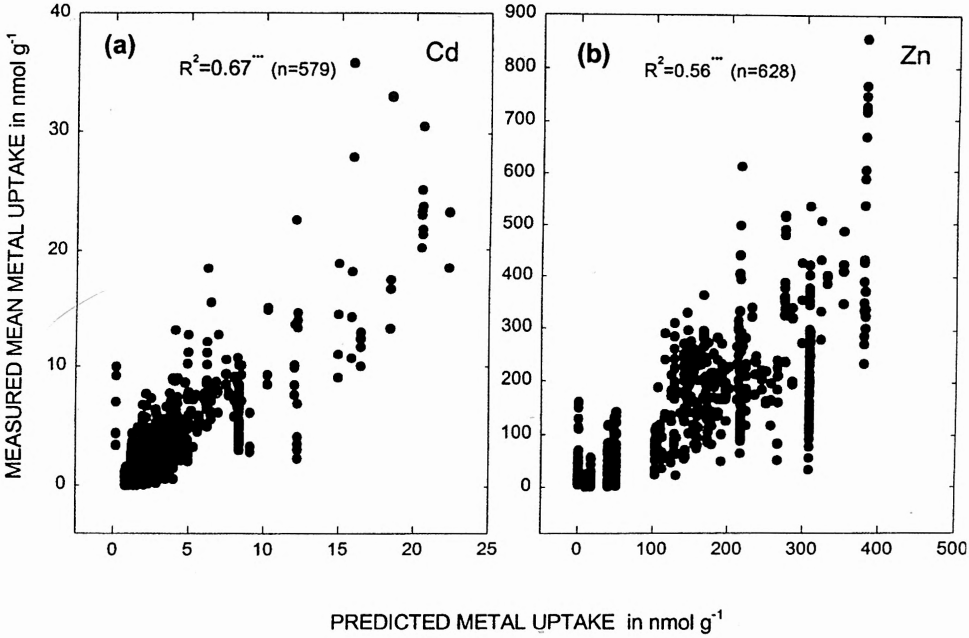

Fig. 6. – Predicted versus measured cadmium and zinc uptake by larvae of *Chironomus riparius*, for the uptake models.(a) $Cd_{midge}=C_f*(Cd^k_{act}*t^l_{exp}*pH^m_{exp}*sal^n_{exp}*Cd_{diff}^p*Ca^q*sal^r_{accl}*t^s_{accl})$ (R^2 = 0.67***, n = 579); (b) $Zn_{midge}=C_f*(Zn^k_{act}*t^l_{exp}*sal^m_{exp}*pH^n_{exp}*Cap*sal^p_{accl})$ (R^2 = 0.559**, n = 628).

DISCUSSION

Field studies

Using the pooled data of three studies, we related total cadmium and zinc levels measured in sediment to metal levels in chironomid larvae. For both metals only a poor relationship was found. Total metal concentrations in sediments have been proved to be poor predictors of metal levels in sediment dwelling invertebrates (*e.g.* BENDELL-YOUNG & HARVEY, 1991 ; TESSIER *et al.*, 1993). The absence of a clear relationship is mainly due to changes in bioavailability of metals with changing geochemical characteristics of the sediments. When geochemical sediment fractions were considered as well as sediment characteristics, it was possible to improve the relationship for zinc. For the other metals it was not possible to increase the explained variation in accumulated levels.

The determination of trace metal availability from solid phases is often difficult, even in laboratory experiments. Besides physical and chemical factors, biological processes will affect metal bioavailability, *e.g.* : both animals and plants may alter the metal form prior to bioaccumulation ; feeding strategy influences bioaccumulation. The relatively poor prediction of metal concentrations in the larvae for most metals may have been caused by the large differences in sediment composition. The unexplained variation could be due to numerous environmental factors that were not taken into account, such as sediment pH and redox potential. Moreover we know little about the relative importance of the different exposure routes under field conditions. Chironomids however, are in close contact with both the sediment and the overlying water (BENDELL-YOUNG & HARVEY, 1991) and metal concentrations in pore water will not necessarily be the same as in the water column (LUOMA, 1989). In one of the studies (BERVOETS *et al.*, 1997) accumulation was compared between chironomids and tubificid worms (Tubificidae). For all metals, the accumulation models for tubificids explained more of the variation than they did for chironomids. This is possibly due to the fact that tubificid worms are less exposed to the overlying water than chironomids. The guts of the larvae were not purged prior to analysis. This may be an additional reason for the high amount of unexplained variation. Trace metals in the gut of aquatic animals can represent a high percentage of the total quantity (GOWER & DARLINGTON, 1990; SAGER & PUSCO, 1991; BROOKE *et al.*, 1996).

Although the explained variation did not exceed 70 % in any of the cases, our results indicate that knowledge of the geochemistry of river sediments is important for predicting metal availability to chironomids. This is supported by other studies where consideration of sediment characteristics allowed a better explanation of accumulated metal levels (GUNN *et al.* 1989; BABUKUTTY & CHACKO, 1995; GONZALES *et al.*, 1995).

Laboratory experiments

Generally, the free metal ion is considered as the available metal species. However, relating net metal uptake to the free metal ion activity for the pooled data only explained a small part of the variation; 6 % for cadmium uptake and 24 % for zinc uptake. In the case of salinity and pH, the environmental factor had a marked effect on the contribution of the free ion activity for both metals over the tested ranges. When only salinity was changed, free ion activity explained 52 % of net cadmium uptake (BERVOETS *et al.*, 1995) and 59 % of zinc uptake (BERVOETS *et al.*, 1996a). In the case of temperature, the effect on speciation was rather small. Nevertheless temperature of exposure contributed significantly to the explained variation in net uptake of both metals. In the uptake model of the pooled data, the temperature of exposure contributed significantly to the explained variation. The effect of temperature is expected to be the result of the combined effects on the chemical behaviour (speciation and diffusion) and on the physiology of the organism (*e.g.* COSSINS & BOWLER, 1987; BLUST *et al.*, 1994; MOLLER *et al.*, 1994). Several authors have observed an increase in respiration by larvae of *Chironomus sp.* with increasing temperature (*e.g.* JOHNSON & BRINKHURST, 1971; HAMBURGER & DALL, 1990). Also salinity and pH of exposure significantly contributed to the uptake model, supplementary to the effect of these factors on speciation. For salinity this was probably due to a physiological effect (*e.g.* respiration, osmoregulation). For pH, however, net uptake increased with increasing salinity whereas the contribution of the free metal ion decreased. A hypothesis put forward in the literature is that the free metal ions (*i.e.* Cd^{2+} and Zn^{2+}) are in competition with the hydrogen ions at the membrane level and therefore restrict net uptake under acid conditions (CAMPBELL, 1995). In addition, pH also will have a physiological effect.

For the calcium ion activity also, an increase resulted in a decreased net uptake for both cadmium and zinc. As for the hydrogen ion activity, this is probably due to competition between calcium and metals for the same uptake sites (Spry & Wood, 1989; Markish & Jeffree, 1994; Hogstrand *et al.*, 1995). In the case of acclimation, only for salinity was an important and consistent effect observed on net cadmium uptake, resulting in an increased uptake with increasing salinity of acclimation.

Although all experiments took place under controlled conditions (*i.e.* chemically defined water, and controlled environment) the integration of the chemical and biological effects of changes in environmental conditions explained, for the pooled data, no more than 67 and 59% of the variation in respectively cadmium and zinc uptake. A possible explanation is that not all effects were considered. The relatively high unexplained variation can also be partially due to the natural variation in metal uptake by midge larvae (Seidman *et al.*, 1986; Timmermans *et al.*, 1992). This is demonstrated when the same non-linear uptake models are constructed with the mean uptake values. With those models, 85% of the net cadmium uptake and 68% of the net zinc uptake could be explained (Fig. 7). A last possible explanation for the unexplained variation is that some environmental factors do not have a consistent effect on metal uptake. This was true for both temperature and pH of acclimation.

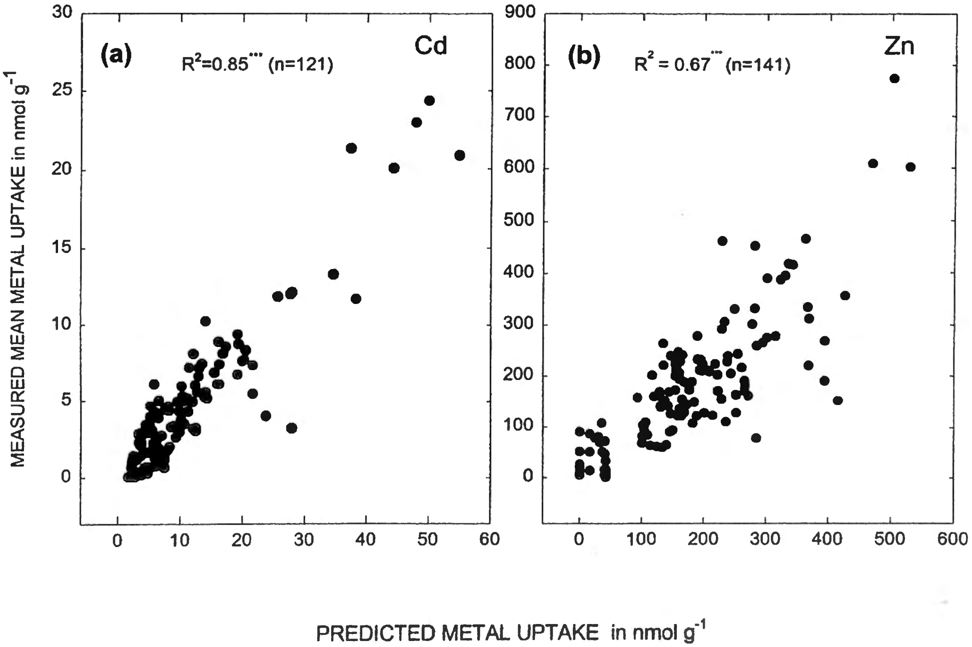

Fig. 7. – Predicted *versus* measured cadmium and zinc uptake by larvae of *Chironomus riparius*, for the uptake models, considering the mean uptake values.
(a) $Cd_{midge}=C_f*(Cd^k_{act}*t^l_{exp}*pH^m_{exp}*sal^n_{exp}*Cd_{diff}{}^p*Ca^q*sal^r_{accl}*t^s_{accl})$ ($R^2 = 0.850^{***}$, n = 121);
(b) $Zn_{midge}=C_f*(Zn^k_{act}*t^l_{exp}*sal^m_{exp}*pH^n_{exp}*Ca_p*sal^p_{accl})$ ($R^2 = 0.671^{**}$, n = 144).

From this study it was obvious that both the bioavailability of metals and the physiology of the exposed organisms need to be considered jointly to explain effects of environmental factors on net metal uptake and accumulated levels. When we consider all possible factors contributing to metal bioavailability and when the relative contribution of the different exposure routes is known, it will be possible to explain or even predict metal accumulation by midge larvae under natural exposure conditions.

ACKNOWLEDGEMENTS

L. Bervoets is a Postdoctoral Fellow and R. Blust is a Research Associate of the Fund for Scientific Research-Flanders (FWO-Vlaanderen).

REFERENCES

ALLEN, H.E., R.H. HALL & T.D. BRISBIN (1980) – Metal speciation. Effects on aquatic toxicity. *Environ. Sci. Technol.,* **14**: 441-443.

AMYOT, M., B. PINEL-ALLOUL & P.G.C. CAMPBELL (1994) – Abiotic and seasonal factors influencing trace metal levels (Cd, Cu, Ni, Pb and Zn) in the freshwater amphipod *Gammarus fasciatus* in two fluvial lakes of the St.Lawrence river. *Can. J. Fish. Aquat. Sci.*, **51**: 2003-2016.

BABUKUTTY, Y. & J. CHACKO (1995) – Chemical partitioning and bioavailability of lead and nickel in an estuarine system. *Environ. Toxicol. Chem.*, **14**: 427-434.

BANGENTER, B.R. & J. FISHER (1989) – Different dormancy response in the sympatric *Chironomus* species, *Ch. plumosus* and *Ch. muditarsus*. *Zool. Jb. Syst.*, **116**: 145-150.

BENDELL-YOUNG, L. & H.H. HARVEY (1991) – Metal concentrations in chironomids in relation to the geochemical characteristics of surficial sediments. *Arch. Environ. Contam. Toxicol.,* **21**: 202-211.

BERVOETS, L., R. BLUST, M. DE WIT & R. VERHEYEN (1997) – Relationships between river sediment characteristics and trace metal concentrations in tubificid worms and chironomid larvae. *Env. Pollut.,* **95**: 345-356.

BERVOETS, L., R. BLUST & R. VERHEYEN (1995) – The uptake of cadmium by the midge larvae *Chironomus riparius* as a function of salinity. *Aquat. Toxicol.*, **33**: 227-243.

BERVOETS, L., R. BLUST, & R. VERHEYEN (1996a) – Uptake of zinc by the midge larvae *Chironomus riparius* at different salinity's: role of speciation, acclimation and calcium. *Environ. Toxicol. Chem.* , **15**: 1423-1428.

BERVOETS, L., R. BLUST, & R. VERHEYEN (1996b) – Effect of temperature on cadmium and zinc uptake by the midge larvae *Chironomus riparius. Arch. Environ. Contam. Toxicol.*, **31**: 502-511.

BERVOETS, L., L. INT PANIS & R. VERHEYEN (1994) Trace metal levels in water, sediments and *Chironomus gr. thummi*, from different water courses in Flanders (Belgium). *Chemosphere*, **29**: 1591-1601.

BERVOETS, L., D. SOLIS, A-M. ROMERO, P. VAN DAMME & F. OLLEVIER (1998) Trace metal levels in chironomid larvae Chironomidae, Diptera) and sediments from a Bolivian River: impact of mining activities. *Ecotoxicol. Environ. Saf.*, **41**: 275-283.

BIRKETT, J.D., I. BODEK, A.E. GLAZER, C.F. GRAIN, D. HAYES, A. LERLMAN, D.B. LINDSAY, C.P. LORETI & J.H. ONG (1988) – Description of individual processes. In: *Environmental inorganic chemistry. Properties, Processes, and Estimation Methods.* BODEK, I., W.J. LYMAN, W.F. REEHL & D.H. ROSENBLATT (Eds.). Setac Special Publication Series, Pergamon Press, New York, Chapter 2.

BLUST, R., M. BAILLIEUL & W. DECLEIR (1995) – Effect of total cadmium and organic complexing on the uptake of cadmium by the brine shrimp *Artemia franciscana. Mar. Biol.*, **123** : 65-73.

BLUST, R., E. KOCKELBERGH & M. BAILLIEUL (1992) – Effect of salinity on the uptake of cadmium by the brine shrimp *Artemia franciscana. Mar. Ecol. Prog. Ser.*, **84** : 245-254.

BLUST, R., L. VAN GINNEKEN & W. DECLEIR (1994) – Effect of temperature on the uptake of copper by the brine shrimp *Artemia franciscana. Aquat. Toxicol.*, **30** : 343-356.

BORGMANN, U. (1983) – Metal speciation and toxicity of free metal ions to aquatic biota. In : *Aquatic Toxicology* . NRIAGU, J.O. (Ed.). John Wiley and Sons, New York : 47-71.

BROOKE, L.T., G.T. ANKLEY, D.J. CALL & P.M. COOK (1996) – Gut content weight and clearance rate for three species of freshwater invertebrates. *Environ. Toxicol. Chem.*, **15** : 223-228.

CAMPBELL, P.G.S. (1995) – Interactions between trace metals and aquatic organisms : A critique of the free-ion activity model. In : *Metal speciation and bioavailability in aquatic systems.* TESSIER, A. & D.R. TURNER (Eds.). John Wiley & Sons, New York : 45-102.

COSSINS, A.R. & K. BOWLER (1987) – Temperature biology of animals. Chapman & Hall, London.

CRANSTON, P.S. (1995) – Morphology. In : *The Chironomidae. The biology and ecology of non-biting midges.* ARMITAGE, P., P.S. CRANSTON & L.C.V. PINDER (Eds.). Chapman & Hall, New York : 11-30.

DE LISLE, P.F. & M.H. ROBERTS JR. (1988) – The effect of salinity on cadmium toxicity to the estuarine mysid *Mysidopsis bahia* : Role of chemical speciation. *Aquat. Toxicol.*, **12** : 357-370.

GINZBURG, G. (1976) – Calculation of all equilibrium concentrations in a system of competing complexation. *Talanta*, **23** : 142-149.

GONZALES, M.J., L. RAMOS & L.M. HERNANDEZ (1995) – Distribution of trace metals in sediments and the relationship with their accumulation in earthworms. *Intern. J. Environ. Anal. Chem.*, **57** : 135-150.

GOWER, A.M. & S.T. DARLINGTON (1990) – Relationships between copper concentrations in larvae of *Plectrocnemia conspersa* (Curtis) (Trichoptera) and in mine drainage streams. *Environ. Pollut.*, **65** : 155-168.

GUNN, A.M., D.T.E. HUNT & D.A. WINNARD (1989) – The effect of heavy metal speciation in sediment on bioavailability to tubificid worms. *Hydrobiologia,* **188/189** : 487-496.

HAMBURGER, K. & P.C. DALL (1990) – The respiration of common benthic invertebrate species from the shallow littoral zone of Lake Esrom, Denmark. *Hydrobiologia*, **199 :** 117-130.

HARE, L. (1992) – Aquatic insects and trace metals : bioavailability, bioaccumulation, and toxicity. *Critical Rev. in Toxicol.,* **22 :** 327-369.

HOGSTRAND, C., R.W. WILSON, D. POLGAR & C. WOOD (1994) – Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. *J. Exp. Biol.*, **186 :** 55-73.

INEICHEN, H., U. RIESEN-WILLI & J. FISHER (1979) – Experimental contribution to the ecology of Chironomidae (Diptera). II. Influence of the photoperiod on the development of *C. plumosus* in the 4th larval Instar. *Oecologia*, **39** : 161-183.

JOHNSON, M.G. & R.O. BRINKHURST (1971) – Production of benthic macroinvertebrates of Bay of Quinte and Lake Ontario. *J. Fish. Res. Board Can.*, **28** : 1699-1714.

LUOMA, S.N. (1989) – Can we determine the biological availability of sediment-bound trace elements? *Hydrobiologia*, **176/177 :** 379-396.

MARKISH, S.J. & R.A. JEFFREE (1994) – Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves : an explanation of how water hardness reduces metal toxicity. *Aquat. Toxicol.*, **29** : 257-290.

MOLLER, V., V.E. FORBES & M.H. DEPLEDGE (1994) – Influence of acclimation and exposure temperature on the acute toxicity of cadmium to the freshwater snail *Potamopyrgus antipodarum* (Hydrobiidae). *Environ. Toxicol. Chem.*, **13**: 1519-1524

PHILLIPS, D.J.H. (1976) – The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. *Mar. Biol.*, **38**: 59-69.

PINDER , L.C.V. (1986) – Biology of freshwater chironomidae. *Ann. Rev. Entomol.*, **31** : 1-23.

RAINBOW, P.S. & R. DALLINGER (1993) – Metal uptake, regulation and excretion in freshwater invertebrates. In: *Ecotoxicology of metals in invertebrates.* DALLINGER, R. & P.S. RAINBOW (Eds.). Lewis Publishers, Chelsea, USA: 119-132.

SAGER, M. & R. PUSCO (1991) – Trace element concentrations of oligochaetes and relations to sediment characteristics in the reservoir at Altenwörth/Austria. *Hydrobiologia* **226**: 39-50.

SALOMONS, W. & U. FÖRSTNER (1984) – *Metals in the hydrocycle.* Springer Verlag, NY.

SEIDMAN, L.A., G. BERGSTROM, D.J. GINGRICH & C.C. REMSEN (1986) – Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi. Tissue and Cell*, **18**: 395-405.

SIMKISS, K. & M.G. TAYLOR (1995) – Transport of metals across membranes. In: *Metal speciation and bioavailability in aquatic systems.* TESSIER, A. & D.R. TURNER (Eds.). John Wliey & Sons, New York: 1-44.

SPRY, D.C. & C.M. WOOD (1989) –. A kinetic method for the measurement of zinc influx *in vivo* in the rainbow trout, and the effects of waterborne calcium on flux rates. *J. Exp. Biol.*, **142**: 425-446

TESSIER, A & P.G.C. CAMPBELL (1987) – Partitioning of trace metals in sediments: Relationships with bioavailability. *Hydrobiologia,* **149**: 43-52.

TESSIER A., Y. COUILLARD, P.G.C. CAMPBELL & J.C. AUCLAIR (1993) – Modelling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis. Limnol. Oceanogr.*, **38**: 1-17.

TIMMERMANS, K.R., E. SPIJKERMAN, M. TONKES & H. GOVERS (1992) – Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. *Can. J. Fish. Aquat. Sci.,* **49**: 655-660.

VERMEULEN, A.C., G. LIBEROO, M. BOYEN, P. DUMONT, F. OLLEVIER & B. GODDEERIS (1997) – Improving the culture technique of *Chironomus riparius* (Diptera, Chironomidae): substrate use and incubation of eggs and larvulae; abstract of the 13th International Symposium on Chironomidae, Freiburg, Germany.

WARWICK, W.F. (1990) – Morphological deformities in chironomidae (Diptera) larvae from the lac St-Louis and Laprairie basins of the St. Lawrence river. *J. Great Lakes Res.*, **16**: 185-208.

WEBB, C.J. & A. SCHOLL (1985) – Identification of larvae of European species of *Chironomus* Meigen (Diptera: Chironomidae) by morphological characters. *Syst. Ent.*, **10**: 353-372.