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Selective scavenging of copper, zinc, lead, and arsenic by iron and manganese oxyhydroxide coatings on plankton in lakes polluted with mine and smelter wastes: results of energy dispersive X-ray micro-analysis

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Abstract

Energy dispersive X-ray micro-analyses of the remains of individual organisms in plankton samples, together with more conventional analyses of sediments and water, were performed for the purpose of investigating the accumulation of heavy metals by plankton in three Canadian Shield lakes polluted with Cu, Zn, Cd, Pb, As, and SO_4^{2-} from a base metal mine and smelter. The results showed that appreciable quantities of Cu and Zn were bound to crustacean exoskeletons, loricae of the rotifer *Keratella*, and loricae of the euglenophytes *Trachelomonas* and *Strombomonas*; euglenophyte loricae also contained As and Pb, but an associated chrysophyte cyst, though richer in Cu, Zn, and Pb, had no detectable As. In all specimens except the chrysophyte cyst, the trace elements were associated with Fe and Mn, implying that Fe and Mn oxyhydroxides deposited on the organisms' armour had scavenged them from the water. Euglenophyte loricae were richest in Fe and Mn and associated trace elements. Moreover, the inferred FeOOH and MnOOH phases discriminated between different elements, Zn being selectively bound by MnOOH whereas Cu and As were bound by FeOOH. Adsorbed Cu and Zn were associated with S to a significant degree only where the sediments were poorest in sulfide, suggesting that the metals were complexed by thiol compounds secreted by organisms as a defense against metal toxicity wherever environmental sulfide levels were too low to provide protection. The Fe, Mn, Zn, and Cu concentrations and Mn/Fe and Zn/Cu ratios of plankton hard parts decreased with the E_h of the sediments. Dissolved Fe and Mn released into the water column from anoxic but sulfide-poor sediments probably precipitated as FeOOH and MnOOH on surfaces of organisms wherever the water had sufficient dissolved O_2, the MnOOH/FeOOH ratio and hence the Zn/Cu ratio increasing with O_2 concentration; but strongly reducing conditions and H_2S production in sediments interfered with the formation of both FeOOH and MnOOH, Fe precipitating as FeS in the sediments whilst Mn remained in solution. Sulfides inhibited bio-accumulation of Cu and Zn by

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hindering the formation of FeOOH and MnOOH coatings and by suppressing the release of dissolved Cu and Zn (Cu in particular) from the sediments. The Cu, Zn, Fe, and Mn concentrations and Zn/Cu and Mn/Fe ratios of plankton hard parts and the concentrations of *bio-available* (DTPA-extractable) Cu and Zn in the sediments were lowest in a lake whose sediments had anomalously low E_h and high sulfide levels caused by sewage effluents, algal blooms, and SO_4^{2-}, even though these sediments had the highest *total* Cu and Zn content. Thus, bio-accumulation of metals depended on the bio-availability, not the total supply, of the metals.

The results of the research are consistent with the following generalisations: (1) FeOOH and MnOOH are commonly deposited on exposed surfaces of a wide range of plankton hard parts in different freshwater environments; (2) the FeOOH and MnOOH coatings scavenge heavy metals and metalloids from the water and are largely responsible for the passive accumulation of these elements by plankton; (3) FeOOH and MnOOH have different trace element affinities, thus affecting the proportions as well as the quantities of the elements bound to the plankton surfaces; (4) the accumulation of metals by plankton hard parts is controlled by environmental variables such as E_h and sulfide and O_2 levels, as these factors regulate the precipitation of FeOOH and MnOOH surface films, determine the MnOOH/FeOOH ratio, and control the speciation, solubility and bio-availability of metals stored in bottom sediments, whereas total metal concentrations in sediments may have no appreciable effect on the bio-accumulation of the metals; and (6) heavy metal bio-availability is limited mainly by sulfides generated in sediments; where sulfides are lacking, thiol compounds are released into the water by the plankton to prevent toxic effects of the metals. These conclusions have significant implications for the cycling and ecological effects of toxic and nutritional trace elements and for the monitoring, abatement, and prevention of water pollution due to mining, smelting, and other industrial activities.

1. Introduction

The scavenging of heavy metals by plankton and its nonliving products (such as loricae, moulted exoskeletons, and faecal pellets) in lakes and oceans is probably of considerable biogeochemical and ecological importance, and it has implications for the monitoring and control of pollution (Fowler, 1977; Jackson, 1978, 1988a, 1991; Subramian, 1978; Bistricki and Munawar, 1982; Lindahl et al., 1983; Suder and Wightman, 1983; Showell and Gaskin, 1992). However, the phenomenon is only superficially understood, as it is extremely complex and poses technical difficulties for those who would investigate it in depth. Plankton consists of many different kinds of organisms which may differ widely in their tendency to accumulate, exclude, or excrete particular metals, and its taxonomic makeup varies greatly through time and space in response to variations in environmental conditions; mechanisms of metal bio-accumulation vary, ranging from passive adsorption to active uptake and assimilation from both food and water; and factors such as nutrient levels, temperature, and light intensity, by controlling the organisms' growth, reproduction, metabolic rates, and biomass, may influence their rates of metal uptake and excretion and hence their metal content. The uptake and retention of a metal by an organism are also regulated by other phenomena, notably metal speciation, which varies with environmental factors; the binding

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of the metal by nonliving particles and complexing agents such as inorganic ions, humic matter, and biogenic chelators, which may impede *or* promote bio-accumulation of the metal; and synergistic and antagonistic interactions with other metals (including competition for binding sites).

There is indirect evidence that the ability of plankton to bind heavy metals depends largely on the species composition of the plankton (Briand et al., 1978; Jackson, 1978), but proof of such specificity appears to be lacking. The purpose of the present study was to seek direct evidence for preferential accumulation of particular heavy metals by specific members of plankton communities in metal-polluted lakes. As chemical analysis of bulk plankton samples by conventional methods is incapable of determining the composition of individual components of the plankton, the technique of energy dispersive X-ray microanalysis was used. Though subject to limitations, difficulties, and uncertainties of its own, this powerful, refined method offers the salient advantage of allowing different identifiable components of heterogeneous mixtures of microscopic organisms and particles to be analysed *individually* for a wide range of elements (Bistricki and Munawar, 1982; Lindahl et al., 1983). Information on environmental conditions and processes, contaminant levels, and metal speciation in the lakes — in bottom sediments in particular — was acquired by ordinary methods of analysis (Jackson, 1984). A brief account of our findings has been published elsewhere (Jackson and Bistricki, 1993); here the results are discussed more fully.

2. Field sites, materials, and methods

2.1. The field area

The samples were taken from Hamell Lake (HL), West Nesootao Lake (WNL), and the Northwest Arm of Schist Lake (NWSL), which are located in the Boreal forest zone of the Canadian Shield near a base metal mine and smelter at Flin Flon, Manitoba, Canada. For a map of the field area, see Fig. 14.1 of Jackson (1984).

The principal pollutants from the mine and smelter (in terms of gram atomic and gram molecular quantities) are Zn, Cu, and SO_4^{2-} along with smaller amounts of Cd, Pb, and As (Franzin, 1984). The lakes are polluted with smelter fallout, and NWSL also receives the outflow from a tailings pond (Flin Flon Lake) together with municipal sewage effluent, which gives rise to algal blooms. Unlike the other lakes, NWSL has bottom sediments characterised by strongly reducing conditions and copious production of sulfides owing to its ample supplies of decomposing organic matter and SO_4^{2-} (Jackson, 1978, 1984).

2.2. Field work

The field work was done during 24 and 25 July, 1979. The plankton was sampled by collecting epilimnion water from offshore sites and pouring it through a 10 μm net; HL and NWSL water was collected by lowering a flexible plastic tube to depths of 4 and 5 m respectively, but at the WNL site, where the water was only 1.5 m deep, a van Dorn bottle was used. At each sampling site the depth, pH, and conductivity of the water, and water temperatures at different depths, were recorded, and 3–5 replicate samples of fine-grained surficial bottom sediment were collected (to a depth of < ~ 10 cm) with an Ekman dredge.

2.3. *Laboratory analyses*

Analysis of sediments and interstitial water

The sediments were stored at 4°C and then subjected to detailed analysis. Sediment pH and E_h were measured with a pH meter (employing Pt and calomel electrodes for the E_h determinations). Total Cu, Zn, Cd, Pb, Fe, and Mn concentrations were determined by atomic absorption spectrophotometry (AAS) performed with a Perkin-Elmer AAS analyser (a model 5000 for determination of Pb and a model 403 for all other metals) following digestion with hot $HNO_3/HClO_4$, and organic C and total N analyses were done with a Carlo-Erba analyser. The "free" (volatile) sulfide content of the sediment was estimated by purging sediment–water mixtures with a stream of N_2, collecting the entrained gaseous sulfide (presumably H_2S) by precipitating it as ZnS in pairs of traps containing Zn acetate solution, solubilising the ZnS with *p*-phenylenediamine and $FeCl_3$, and measuring the resulting complex colorimetrically (Strickland and Parsons, 1968; Stainton et al., 1977). The "bound" (nonvolatile) sulfide fraction was volatilised by digestion of sediment with hot (90°C) 6 M HCl and then determined by the methods employed for free sulfide. The interstitial water of the sediment was separated from the solid components by centrifugation at 10,000 rpm for 15 min and analysed for SO_4^{2-} using the technique of Stainton et al. (1977); portions of the water were also digested with hot HNO_3, further acidified with HCl, and analysed for Mn and Fe by AAS. After removal of interstitial water, the spun-down sediment was rinsed twice with N_2-purged water, whereupon various operationally defined metal fractions were extracted with N_2-purged solvents. "Bio-available" metal species adsorbed to sediment particles, bound by complexing agents, or occluded in labile oxyhydroxides, were extracted with the chelating agent DTPA (Lindsay and Norvell, 1978), and readily reduced "amorphous" Mn and Fe oxyhydroxides and oxides (hereafter designated as MnOOH and FeOOH for convenience) were extracted from a separate set of centrifuged sediment samples by treatment with 0.1 M $NH_2OH \cdot HCl$/0.1 M HNO_3 (pH 1.7) (Chao, 1972; Gupta and Chen, 1975) after weakly adsorbed metal species had been leached out with 1 M Ca acetate and 0.5 M $CaCl_2$ solutions. Following digestion with hot HNO_3 and addition of HCl, all extracts were analysed for metals by AAS. Analytical data expressed as concentrations in the sediment were calculated on the basis of oven-dry (105°C) weight. Note that the solvents used to extract metal fractions are not highly selective and probably extract mixtures of metal species. For instance, $NH_2OH \cdot HCl/HNO_3$ may extract Fe from humic–Fe complexes and FeS as well as FeOOH. Nevertheless, past experience as well as the results of the present study suggests that the fractionation schemes employed are based on generally valid assumptions and provide information which is very useful in the comparative study of biogeochemical pathways of metals in different freshwater environments (Jackson, 1984, 1986, 1988a, 1988b, 1989, 1993; Jackson et al., 1993).

Microscopic examination and micro-analysis of plankton

The plankton samples were frozen in 99% ethanol at −40°C, freeze-dried, and stored at room temperature. Energy dispersive X-ray micro-analyses were done during the period 1980–1982. Freeze-dried specimens were dispersed in water, collected on Nuclepore filters of various pore sizes down to 0.45 μm, air-dried at room temperature, coated with C in a vacuum evaporator, and then examined and analysed using an AMR-1000 scanning electron microscope and a Tracor-Northern TN-11 analytical system (Bistricki and Munawar, 1982). The electron beam was focused on a selected spot on the surface of each specimen, the resulting X-ray spectrum was printed as a series of peaks on chart paper, and element abundances were estimated by measurement of peak heights with respect to the baseline, the values being expressed as counts/sec normalised to a 240 sec lifetime. A few analyses were also performed with a Philips-505 scanning electron microscope and EDAX 9100/60 X-ray micro-analysis system, which estimated the elemental composition automatically. A 20 kV electron beam was used in all analyses.

3. Results and discussion

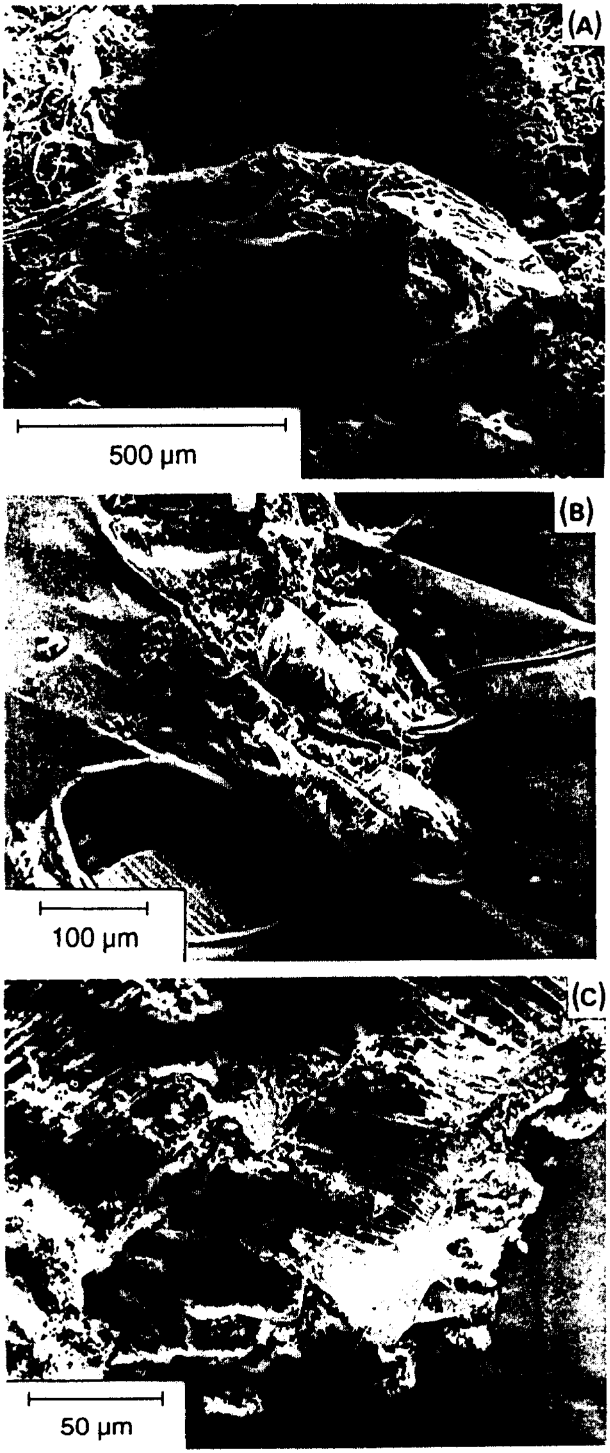
3.1. Characteristics of the plankton and its physicochemical environment

Selected analytical data and observations that serve to describe the waters and sediments of the three lakes are presented in Tables 1–3. Obviously the environmental conditions in

Table 1

Some characteristics of the water, sediments, and biota at the sampling sites. Abbreviations: Cond. = conductivity. The pH and conductivity values are medians of three replicate measurements

Lake	Depth of water (m)	Surface water		Water temp. (°C)		Water stratified (+) or unstratified (−)	Description of sediments and overlying water
		pH	Cond. (μS/cm)	surface	bottom		
Hamell	7	7.05	68	20.2	19.9	−	Brown bottom mud; no H_2S odour. FeOOH-encrusted stone was found at depth of 3–4 m. The most common phytoplankton species were chlorophytes.
West Nesootao	1.5	7.20	40	19.7	19.5	−	Brown bottom mud with plants and plant fragments; no H_2S odour. Abundant aquatic vascular plants throughout the lake. The most common phytoplankton species were chlorophytes.
Schist	15	7.20	580	20.3	11.5	+	Foul black bottom mud with H_2S odour. Plankton more abundant than in the other lakes; dominant phytoplankton species were cyanobacteria and chlorophytes. Plankton blooms occur here because of sewage effluent.


(A)
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(B)
100 µm
(C)
50 µm

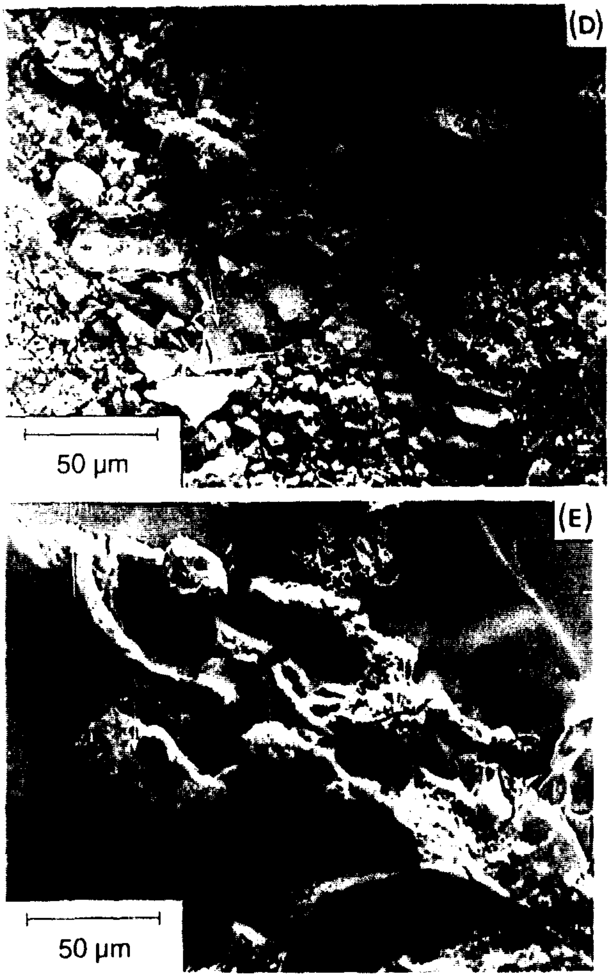

Fig. 1. Electron micrographs of the remains of crustacean exoskeletons from Schist Lake (A, C, D), Hamell Lake (B), and West Nesootao Lake (E). The arrows indicate spots where energy dispersive X-ray micro-analyses were done. The microscopy was performed with the AMR-1000 scanning electron microscope.

NWSL are profoundly different from those found in WNL and HL. The most strikingly anomalous features of NWSL include surface water with elevated concentrations of dissolved salts (Table 1) and bottom sediments with extremely low E_h values, abundant sulfide and SO_4^{2-}, and Mn that is largely in solution (Tables 2, 3). The highly reducing bottom conditions in NWSL are intensified by thermal stratification, which is stabilised by the lake's relatively great depth (Table 1). Note that although the total Cu concentrations in sediments are an order of magnitude higher in NWSL than in WNL and HL, the DTPA-extractable Cu content is lower owing to immobilisation of Cu by sulfide. Cu is immobilised more efficiently than Zn by reaction with sulfide (Table 2), probably because CuS is more stable than ZnS in this particular environment owing to its higher standard entropy (Jackson, 1978, Jackson, 1979).

The plankton specimens analysed comprised (1) chitinous exoskeletons of crustaceans and particles identified with a greater or lesser degree of confidence as fragments thereof

Table 2
The total Cu, Zn, Pb, free and bound sulfide, organic C, and N content and DTPA-extractable Cu and Zn content of sediments from Hamell Lake, West Nesootao Lake, and Schist Lake (medians of 3 or 5 replicate values, with ranges in parentheses). Number of replicate samples analysed: Hamell Lake, 5 (4 for Pb analyses); West Nesootao Lake, 3 (2 for Pb analyses); Schist Lake, 5. Abbreviations: conc. = concentration

Lake	Total metal conc. (μmol/g) and Zn/Cu ratio (mol/mol)				DTPA-extractable metal conc. (μmol/g) and Zn/Cu ratio of extract (mol/mol)			Sulfide (μmol/g)		Organic C (mmol/g)	N (mmol/g)
	Cu	Zn	Pb	Zn/Cu	Cu	Zn	Zn/Cu	Free	Bound		
Hamell	2.45 (1.24–25.1)	8.52 (4.11–23.3)	0.433 (0.204–0.917)	3.25 (0.929–9.98)	0.417 (0.236–4.88)	1.85 (1.76–2.17)	4.38 (0.395–7.645)	0.110 (0.0843–0.481)	0.219 (0.0702–0.507)	14.4 (11.3–18.0)	2.00 (1.69–2.68)
West Nesootao	5.32 (5.04–6.69)	15.6 (13.3–15.9)	3.11 (2.97–3.06)	2.51 (2.37–3.09)	0.840 (0.773–0.913)	1.33 (1.22–1.33)	1.46 (1.45–1.72)	0.221 (0.0794–0.223)	0.232 (<0.011–0.420)	9.22 (8.73–11.7)	0.978 (0.842–1.42)
Schist	91.8 (76.3–115)	18.9 (14.8–23.5)	2.70 (1.65–3.06)	0.197 (0.184–0.206)	0.0562 (0.0046–0.0732)	0.965 (0.696–1.13)	20.2 (13.9–151)	0.784 (0.2895–1.37)	1086 (341–1400)	9.72 (7.51–16.8)	1.06 (0.971–1.34)

Table 3
The pH, E_h, and $NH_2OH \cdot HCl$-extractable Mn and Fe content of sediments, and Mn, Fe, and SO_4^{2-} content of the interstitial water of the sediments, in Hamell Lake, West Nesootao Lake, and Schist Lake (medians of 3 or 5 replicate values, with ranges in parentheses). Number of replicate samples analysed: Hamell Lake, 5; West Nesootao Lake, 3; Schist Lake, 5. Abbreviations: conc. = concentration

Lake	Sediment					Interstitial water			
	pH	E_h	$NH_2OH \cdot HCl$-extractable metal conc. (μmol/g) and Mn/Fe ratio of extract (mol/mol)			Mn (μmol/L)	Fe (μmol/L)	Mn/Fe (mol/mol)	SO_4^{2-} (mmol/L)
			Mn	Fe	Mn/Fe				
Hamell	6.40 (6.20–6.50)	−130 (−170 to −130)	2.24 (1.41–3.91)	6.86 (3.545–10.95)	0.357 (0.2395–0.473)	1.46 (0.364–5.64)	1.97 (1.43–15.2)	0.382 (0.145–1.02)	0.579 (0.231–0.891)
West Nesootao	6.55 (6.45–6.55)	−115 (−135 to −75)	3.27 (3.21–3.66)	10.1 (8.42–11.2)	0.3265 (0.325–0.381)	5.10 (3.46–6.55)	95.8 (75.7–123)	0.415 (0.0361–0.0865)	0.115 (0.0604–0.181)
Schist	6.70 (6.65–6.70)	−290 (−305 to −270)	0.637 (0.197–1.505)	11.9 (6.43–13.85)	0.0581 (0.0306–0.109)	18.2 (7.46–25.1)	10.2 (2.86–13.3)	2.22 (1.78–2.61)	3.04 (2.42–3.21)

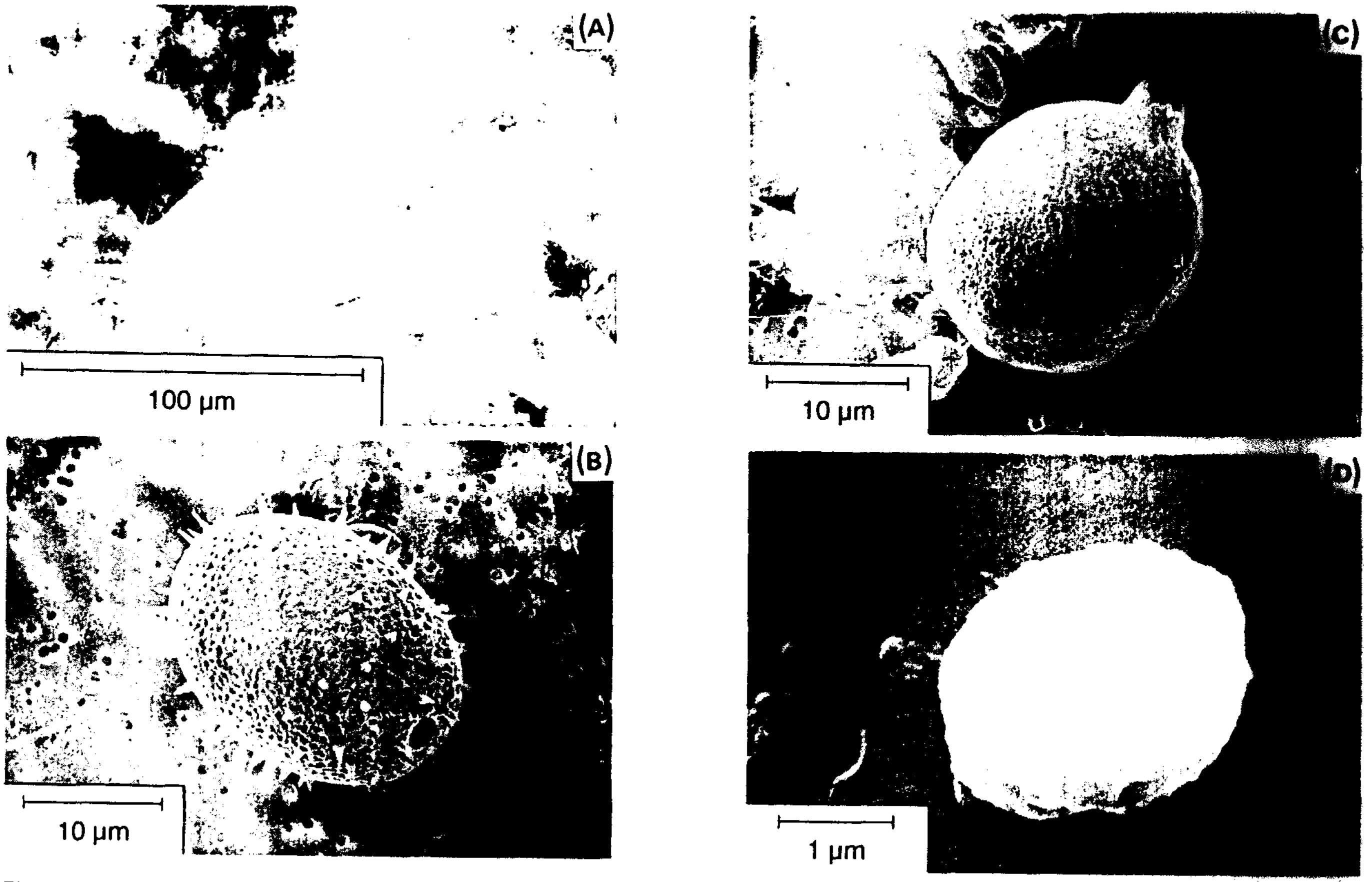

Fig. 2. Electron micrographs of a *Keratella* lorica from Schist Lake (A) and a *Trachelomonas* lorica (B), *Strombomonas* lorica (C), and chrysophyte cyst (D) from Hamell Lake. The microscopy was performed with the Philips-505 scanning electron microscope.

Table 4

Composition of plankton hard parts from Hamell Lake, West Nesootao Lake, and Schist Lake as determined by the AMR-1000/TN-11 system. Element concentrations are expressed as peak heights normalised to a lifetime of 240 sec. Except for data from a single *Strombomonas* lorica, median values are given for the various classes of specimen, with the ranges shown in parentheses. Number of specimens per lake: Crustacean exoskeleton fragments: 5 from Hamell Lake, 5 from West Nesootao Lake, 7 from Schist Lake. *Keratella* loricae: 2 from West Nesootao Lake, 4 from Schist Lake. Peak height of "0" means below limit of detection (< ~ 10 counts/sec). Abbreviations: n.d. = no data; L. = Lake

The nature and source of the specimens	Peak height (counts/sec)						Peak height ratio	
	Cu	Zn	Pb	Fe	Mn	S	Zn/Cu	Mn/Fe
Crustacean exoskeletons								
Hamell L.	130	250	0	470	190	1200	2.6	0.48
	(33–170)	(87–680)	(– [a])	(240–2720)	(80–1950)	(380–2400)	(1.5–4.5)	(0.070–0.833)
West Nesootao L.	130	890	0	1490	1750	1700	6.85	1.0
	(90–185)	(190–1320)	(– [a])	(120–2500)	(120–3290)	(990–3100)	(2.1–7.43)	(0.54–1.6)
Schist L.	59	50	0	70	0	1390	0.71	0
	(10–320)	(10–160)	(– [a])	(0–5610)	(0–300)	(320–2900)	(0.22–1.60)	(0–0.75)
Keratella loricae								
West Nesootao L.	107.5	280	0	460	775	1900	2.6	1.6
	(90–125)	(220–340)	(– [a])	(400–520)	(270–1280)	(1750–2050)	(2.4–2.7)	(0.675–2.5)
Schist L.	35	36	n.d.	142	0	1030	1.1	0
	(20–52)	(10–60)		(0–200)	(– [a])	(760–6440)	(0.33–1.3)	(– [a])
Strombomonas lorica								
Hamell L.	180	1290	90	5500	2750	1800	7.2	0.500

[a] All replicate values = 0.

Table 5

Composition of plankton hard parts from Hamell Lake and Schist Lake as determined by the Philips-505/EDAX 9100/60 system. Each set of data represents analysis of a single specimen. "0" = not detected

The nature and source of the specimen	Element concentration (mmol/g)							Element ratio (mol/mol)	
	Cu	Zn	Pb	As	Fe	Mn	S	Zn/Cu	Mn/Fe
Hamell Lake									
Trachelomonas lorica	0.129	1.51	0	0.871	11.2	0	0.605	11.7	0
Strombomonas lorica	0.185	1.28	0.0265	0.2305	10.55	0.178	0.637	6.92	0.0169
Chrysophyte cyst	0.430	3.03	0.4335	0	0.311	0	0	7.04	0
Schist Lake									
Keratella	0.235	0	0	0	1.65	0	6.06	0	0

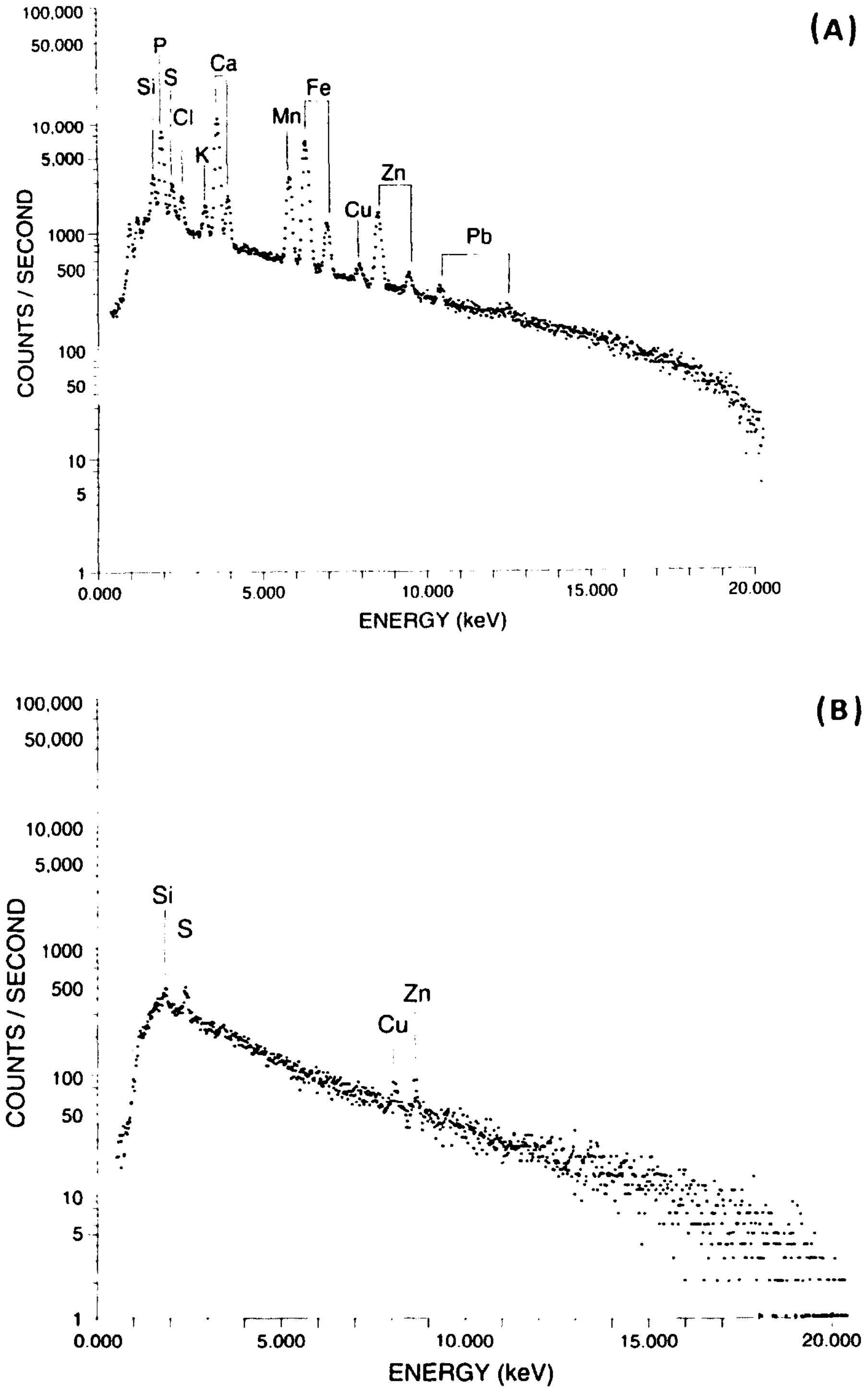

Fig. 3. Energy dispersive X-ray micro-analysis spectrum of a *Strombomonas* lorica from Hamell Lake (A) and associated background spectrum (B) (generated by the AMR-1000 scanning electron microscope and TN-11 analytical system). Lifetime = 240 sec.

(Fig. 1, A–E); (2) chitinous loricae of the rotifer *Keratella* (Fig. 2A), which were found only in NWSL and WNL; (3) Mn- and Fe-impregnated "pectic" or "mucilaginous" loricae of the euglenophyte genera *Trachelomonas* and *Strombomonas* (Bold and Wynne, 1985; Dunlap and Walne, 1985) (Fig. 2, B and C), which were found only in HL; and (4) a siliceous chrysophyte cyst (statospore) from HL (Fig. 2D). Concentrations of different metallic and nonmetallic elements in these specimens as estimated by micro-analysis are listed in Tables 4 and 5, and typical examples of sample and background spectra are shown in Fig. 3. All plankton samples contained measurable quantities of Cu and Zn, but few had detectable As or Pb, and none had detectable Cd.

3.2. *Relationships between different elements in the plankton hard parts*

Micro-analysis of the remains of crustacean exoskeletons revealed a significant positive correlation between Cu and Fe in each lake (Table 6), and the data for all three lakes conform to a single highly significant regression line (Fig. 4A); suspended algal cells and mineral grains in the Wabigoon River, Ontario showed a similar relationship (Jackson and Bistricki, unpublished data). In contrast, Zn increased with increasing Fe only up to a critical Fe concentration, then dropped abruptly almost to the detection limit and leveled off (Fig. 4B). On the other hand, Cu had a generally much weaker affinity for Mn than for Fe (Table 6, Fig. 4C; compare Fig. 4A) (although in WNL the difference was small), but Zn showed a strong positive correlation with Mn (Table 6; Fig. 4D; compare Fig. 4B). The Zn/Cu ratio varied in essentially the same way as Zn concentration with respect to the Mn and Fe concentrations (Fig. 5A, B). The close association of Zn with Mn suggests that the complex relationship between Zn and Fe was a secondary manifestation reflecting variation of Mn as a function of Fe (Fig. 6; compare Fig. 4B). In brief, the data demonstrate a specific affinity between Cu and Fe and between Zn and Mn, and this applies to all three lakes, both

Table 6
Relationships between Cu, Zn, Fe, Mn, and S in crustacean exoskeleton remains from Hamell Lake, West Nesootao Lake, and Schist Lake. Explanation of symbols: *r* = correlation coefficient; *P* = significance probability. Number of samples: Hamell Lake, 5; West Nesootao Lake, 5; Schist Lake, 7

Lake	Correlation matrix						
		Cu		Zn		S	
		r	*P*	*r*	*P*	*r*	*P*
Hamell	Fe	0.893	>0.02, <0.05	0.651	≫0.1	0.721	>0.1
	Mn	0.568	≫0.1	0.897	>0.02, <0.05	0.913	>0.02, <0.05
	S	0.884	>0.02, <0.05	0.989	>0.001, <0.01	–	–
West Nesootao	Fe	0.913	>0.02, <0.05	0.969	>0.001, <0.01	0.868	>0.05, <0.1
	Mn	0.905	>0.02, <0.05	0.980	>0.001, <0.01	0.562	≫0.1
	S	0.861	>0.05, <0.1	0.846	>0.05, <0.1	–	–
Schist	Fe	0.998	<0.001	0.811	>0.02, <0.05	0.282	≫0.1
	Mn	0.641	>0.1	0.989	<0.001	0.399	≫0.1
	S	0.399	≫0.1	0.443	≫0.1	–	–

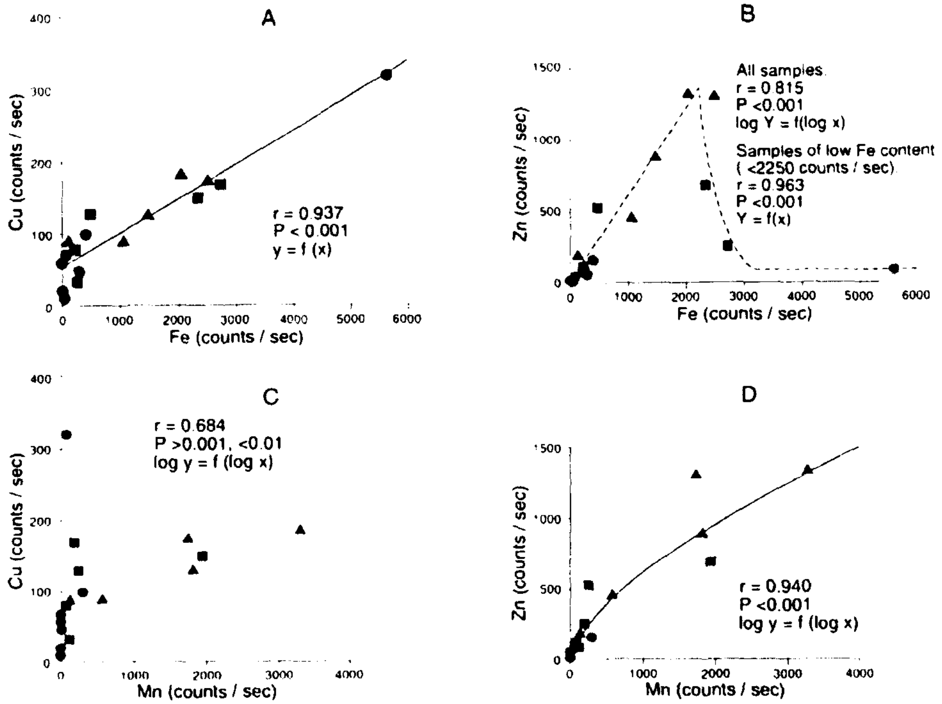

Fig. 4. Relationships between Cu and Fe (A), Zn and Fe (B), Cu and Mn (C), and Zn and Mn (D) in remains of crustacean exoskeletons from Schist Lake (●), Hamell Lake (■), and West Nesootao Lake (▲). Analyses were done with AMR-1000/TN-11 system.

individually and collectively. Cu and Zn in *Keratella* loricae gave similar patterns of variaton with respect to Fe and Mn, making allowances for the fact that their Fe content fell within the range of concentrations in which Zn increased steadily with increasing Fe (Figs. 7, 8).

The observed relationships are not limited to chitinous material, as the data for a *Strombomonas* lorica conformed to the same trends as data for crustacean remains (Fig. 9). The *Strombomonas* lorica, however, was richer in Fe and Mn, and therefore richer in Cu and Zn, than the crustacean remains; presumably for that reason, it was the only specimen in which Pb was detected (Table 4).

Another point of interest is that HL, whose sediments were poorest in free sulfide (Table 2), was the only lake in which the Zn bound to crustacean exoskeletons was associated to a significant degree with S (Table 6; Fig. 10). Among the crustacean specimens from the three lakes as a whole, the correlation coefficients representing the affinity of Cu and Zn for S increase progressively as the free sulfide content of the sediment decreases (Fig. 11). The percentages of the total sedimentary Cu and Zn that were extractable with the chelating agent DTPA also increase with decreasing free sulfide concentration (Fig. 12), demonstrating that sulfides depress the bio-availability of the metals. [Incidentally, the plots in Fig. 12 include data for Phantom Lake, which is situated near the other three lakes (Jackson, 1984).] These observations suggest that much of the Cu and Zn bound to hard parts of plankton is in the form of complexes of thiol compounds such as metallothioneins and phytochelatins (Gekeler et al., 1988; Robinson, 1989) released into the water by aquatic

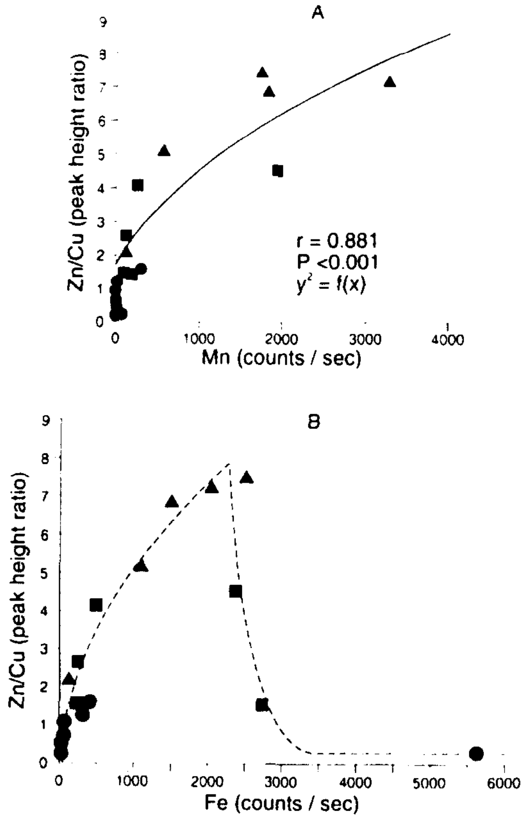

Fig. 5. Variation of Zn/Cu ratio with respect to Mn (A) and Fe (B) in crustacean exoskeletons in Schist Lake (●), Hamell Lake (■), and West Nesootao Lake (▲). Analyses were done with AMR-1000/TN-11 system.

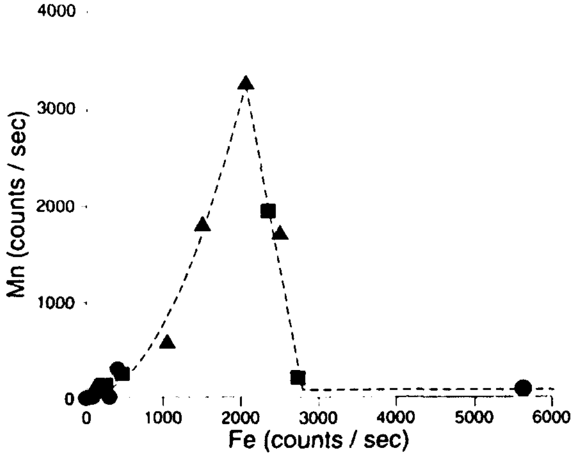

Fig. 6. Relationship between Mn and Fe in crustacean exoskeletons from Schist Lake (●), Hamell Lake (■), and West Nesootao Lake (▲). Analyses were done with AMR-1000/TN-11 system.

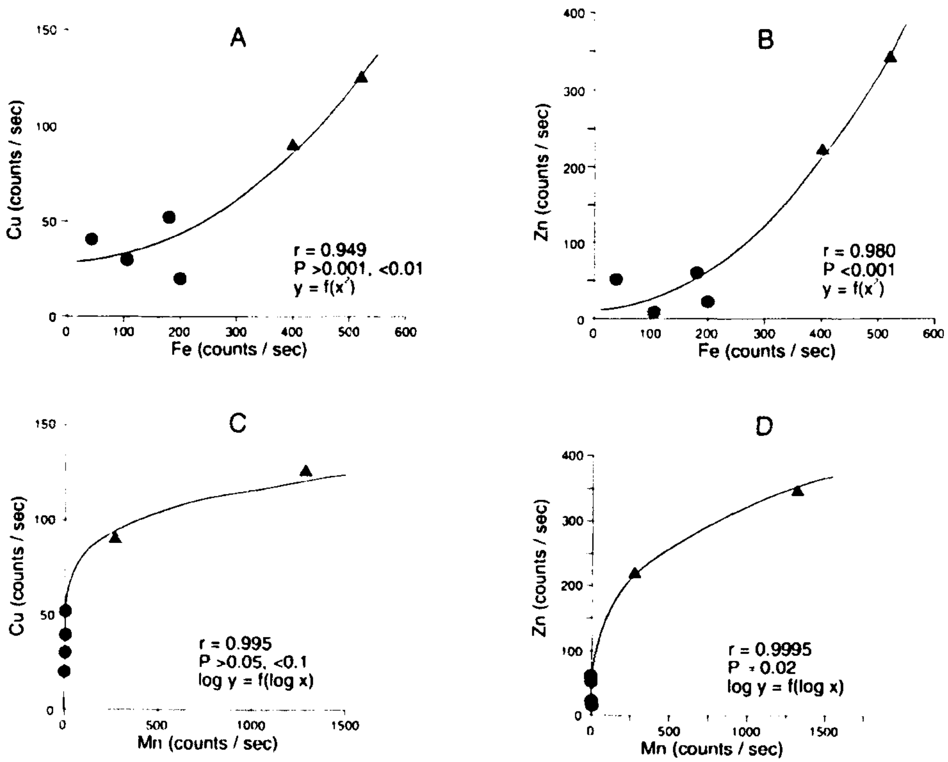

Fig. 7. Relationships between Cu and Fe (A), Zn and Fe (B), Cu and Mn (C), and Zn and Mn (D) in *Keratella* loricae from Schist Lake (●) and West Nesootao Lake (▲). Analyses were done with AMR-1000/TN-11 system.

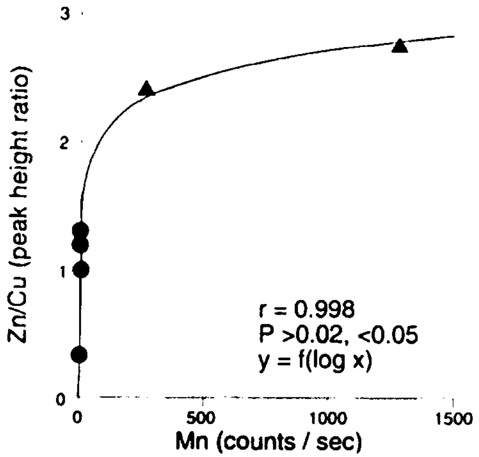

Fig. 8. Variation of Zn/Cu ratio with respect to Mn in *Keratella* loricae from Schist Lake (●) and West Nesootao Lake (▲). Analyses were done with AMR-1000/TN-11 system.

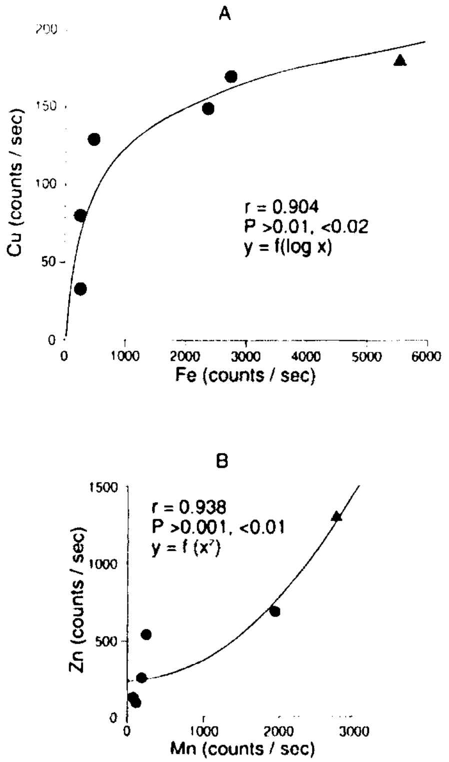

Fig. 9. Relationships between Cu and Fe (A) and Zn and Mn (B) in crustacean exoskeletons (●) and a *Strombomonas* lorica (▲) from Hamell Lake. Analyses were done with AMR-1000/TN-11 system.

organisms as a defense against metal toxicity wherever environmental sulfide levels are too low to provide adequate protection by immobilising the metals in the sediments.

Analysis of plankton hard parts yielded only scant information about As, which was detected solely in certain specimens analysed with the Philips/EDAX system; but the

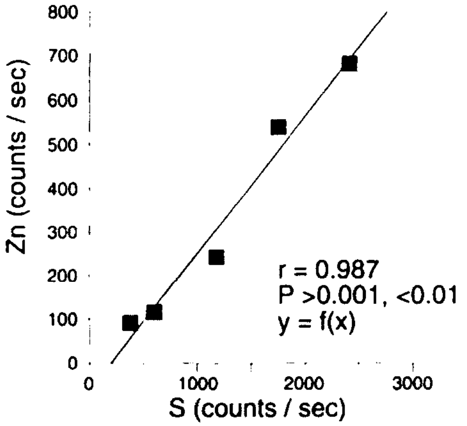

Fig. 10. Relationship between Zn and S in crustacean exoskeletons from Hamell Lake. Analyses were done with AMR-1000/TN-11 system.

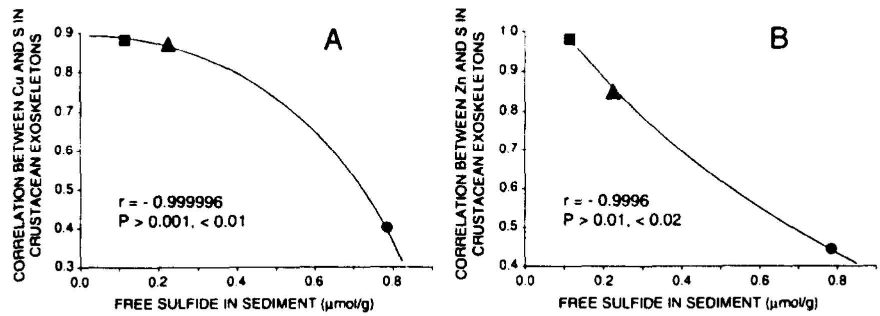

Fig. 11. Correlation coefficients relating Cu to S (A) and Zn to S (B) in crustacean exoskeletons plotted against the free sulfide content of the bottom sediments. The data represent Schist Lake (●), West Nesootao Lake (▲), and Hamell Lake (■). Analyses of exoskeletons were done with AMR-1000/TN-11 system.

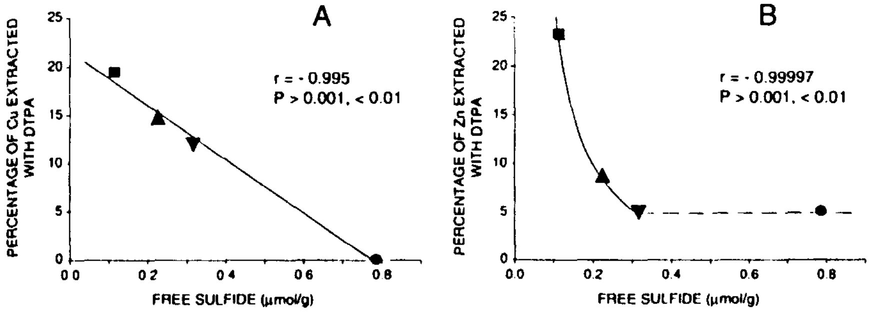

Fig. 12. Relationships between the percentages of sediment-bound Cu (A) and Zn (B) extracted with DTPA and the free sulfide content of the sediments. The data represent Schist Lake (●), Phantom Lake (▼), West Nesootao Lake (▲), and Hamell Lake (■).

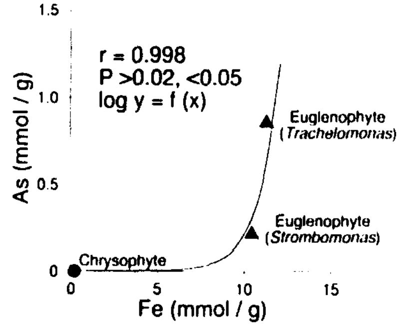

Fig. 13. Relationship between As and Fe in euglenophyte loricae (▲) and a chrysophyte cyst (●) from Hamell Lake. The analyses were done with the Philips-505/EDAX 9100/60 system.

available data indicate a strong affinity between As and Fe (Fig. 13). Detectable As occurred in the Fe-rich *Trachelomonas* and *Strombomonas* loricae in HL plankton but not in the associated Fe-poor chrysophyte cyst (Table 5; Fig. 13). In contrast, As showed no relationship with Mn, which was only detected in one of the specimens (*Strombomonas*) in any case (Table 5). Surprisingly, Cu, Zn, and Pb correlated *inversely* with Fe and varied independently of Mn (Table 5), but this probably reflects chemical peculiarities of the chrysophyte cyst rather than a contradiction of the previously discussed relationships of Cu and Zn with Fe and Mn (Tables 4, 6; Fig. 9): The concentrations of Cu, Zn, Pb, and Fe in the two euglenophyte loricae were very similar, as would be expected in the case of two such closely related species inhabiting the same environment, whereas the chrysophyte cyst was much richer in Cu, Zn, Pb but poorer in Fe (Table 5).

The accumulation of Cu, Zn, Pb, and As by hard parts of plankton can be attributed to adsorption by, and coprecipitation with, Fe and Mn oxyhydroxide coatings precipitated on the armoured surfaces of the organisms as spatially separated phases. Oxyhydroxide deposits are known to occur on loricae of *Trachelomonas* (Dunlap and Walne, 1985; Dunlap et al., 1983) and exoskeletons of aquatic insects (Cain et al., 1992; Hare, 1992); the ability of oxyhydroxides to adsorb trace elements is well established (Jenne, 1968; Lee, 1975); and there is evidence that oxyhydroxide coatings on aquatic insects scavenge trace elements from water (Cain et al., 1992; Hare, 1992). Our data suggest, moreover, that the adsorbed Cu and Zn species include thiol complexes as well as inorganic ions.

An especially interesting result of our research is the evidence that the FeOOH and MnOOH phases discriminate between different trace elements, FeOOH accumulating Cu and As in preference to Zn whereas MnOOH preferentially binds Zn. Selective adsorption of elements has also been observed in Fe- and Mn-enriched phases of Mn nodules (Burns and Fuerstenau, 1966; Calvert and Price, 1970 and Calvert and Price, 1977), and there is indirect evidence that FeOOH and MnOOH discriminate between different Hg species (Jackson, 1988a), suggesting that such effects are common in aquatic environments, although more information is required to explain them. It remains to be determined whether precipitation of oxyhydroxides on plankton is a specific epitaxial phenomenon — that is, whether the molecular structure of the biopolymer on which the MnOOH and FeOOH coatings are deposited determines which polymorphs of the minerals are formed. Nonetheless, the data shown in Figs. 9 and 13, though preliminary, suggest that the mucilagenous, or pectic, matrices of euglenophyte loricae provide more favourable surface environments for the precipitation of FeOOH and MnOOH than do the chitinous products of zooplankton and the siliceous cysts of chrysophytes. Accordingly, euglenophyte loricae appear to be particularly effective scavengers of Cu, Zn, Pb, and As.

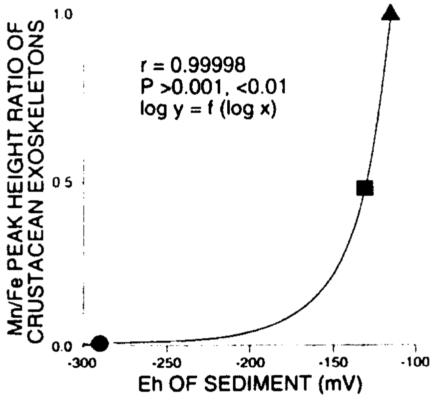

Fig. 14. Relationship between median Mn/Fe ratios of crustacean exoskeletons and E_h values of sediments in Schist Lake (●), Hamell Lake (■), and West Nesootao Lake (▲). Analyses of exoskeletons were done with AMR-1000/TN-11 system.

3.3. Effects of sedimentary environment on accumulation of metals by plankton

Relationships between the composition of the plankton specimens and physicochemical properties of the associated bottom sediments and interstitial water indicate that the Mn, Fe, Zn, and Cu concentrations and Mn/Fe and Zn/Cu ratios of plankton hard parts depend on the oxidation–reduction conditions in the sediments and overlying water. Thus, the Mn/Fe ratios of crustacean exoskeletons increase with the E_h of the sediment (Table 7; Fig. 14). Individually, Mn and Fe show the same tendency, although it is much weaker (Table 7). These relationships undoubtedly reflect the fact that at a given pH FeOOH precipitates at lower E_h and dissolved O_2 levels than MnOOH does, with the result that dissolved Fe diffusing into the water column from anoxic sediments is apt to precipitate as FeOOH even if the water is poor in O_2, whereas Mn remains in solution and precipitates as MnOOH only at relatively high dissolved O_2 levels. Probably an important contributing factor is that in low-E_h sediments generating copious free sulfide, as in NWSL, relatively little Fe is released into the water because much of the Fe is precipitated as FeS. The solubility of Fe and Mn depends on pH as well as oxidation–reduction conditions, but pH need not be taken into account in the comparison of biogeochemical pathways of metals in NWSL, WNL, and HL, as the pH values of the sediments and water were practically the same in all three lakes (Tables 1 and 3) — except, perhaps, in NWSL, where photosynthesis and SO_4^{2-} reduction may create localised alkaline environments in the water and sediments, respectively (Jackson, 1978; see below).

Table 7
Results of regression analyses relating sediment E_h to (1) the Mn and Fe content and Mn/Fe ratios of crustacean exoskeleton remains and (2) the concentrations of extractable Mn and Fe fractions, and the corresponding Mn/Fe ratios, of sediments in Hamell Lake, West Nesootao Lake, and Schist Lake. The regression analyses for crustacean specimens were performed on the median values for the three lakes. Explanation of symbols: r = correlation coefficient; P = significance probability. Sample sizes used for regression analyses: exoskeleton data, 3; sediment and interstitial water data, 13

Description of samples and sample fractions analysed for Mn and Fe	Correlation matrix		
		E_h of sediment	
		r	P
Crustacean exoskeletons	Mn/Fe	0.99998	>0.001, <0.01
	Mn	0.966	>0.1
	Fe	0.966	>0.1
$NH_2OH \cdot HCl$ extract of sediment	Mn/Fe	0.919	<0.001
	Mn	0.847	<0.001
	Fe	−0.524	>0.05, <0.1
DTPA extract of sediment	Mn/Fe	−0.953	<0.001
	Mn	−0.938	<0.001
	Fe	0.957	<0.001
Interstitial water of sediment	Mn/Fe	−0.925	<0.001
	Mn	−0.862	<0.001
	Fe	0.540	>0.05, <0.1

The variations in the concentrations of extractable Mn and Fe fractions in the sediments with respect to sediment E_h (Tables 3 and 7) are consistent with the relationships between crustacean exoskeleton composition and E_h, and they can be explained in terms of the same processes. Thus, the Mn/Fe ratio of the $NH_2OH \cdot HCl$-extractable fraction of the sediment correlates positively with E_h, but the Mn/Fe ratios of the DTPA-extractable fraction of the sediment and the interstitial water are inversely related to E_h (Table 7). The extractable Mn fractions by themselves show comparable but slightly weaker correlations with E_h (Table 7). Unlike the equivalent Mn fractions, $NH_2OH \cdot HCl$-extractable Fe is inversely correlated, and DTPA-extractable Fe and interstitial Fe are positively correlated, with E_h (Table 7). These relationships indicate that Mn is relatively soluble at low E_h whereas Fe is only slightly soluble, implying that most of the $NH_2OH \cdot HCl$-extractable Fe in the sulfide-rich sediments of NWSL consists of FeS rather than FeOOH.

These observations suggest that exposure of zooplankton to different dissolved O_2 and ambient E_h levels during their vertical movements through the water column accounts for the complex relationship between Mn and Fe (Fig. 6), and hence between Zn and Fe (Fig. 4B), in crustacean exoskeletons. Vertical movements of zooplankton through regions of water with different oxidation–reduction regimes could result from diurnal migration, transport by turbulent water currents, and sinking. The variation in the Mn content of exoskeletons with increasing Fe (going from left to right in Fig. 6) is interpreted as follows: (1) The low levels of both Mn and Fe in most NWSL specimens reflect the extremely reducing conditions and abundant H_2S in the sediments combined with thermal stratification of the water and a lack of dissolved O_2 in the hypolimnion; dissolved Mn diffusing into the hypolimnion from the sediments remained in solution, whilst Fe was immobilised as FeS in the sediments. (2) Specimens showing an increase in Mn with increasing Fe had been exposed to anoxic but H_2S-poor sediments overlain by O_2-rich water; both Fe and Mn were released from the sediment and then precipitated as oxyhydroxides on plankton surfaces in the water. (3) The abrupt drop in Mn content with further increase in Fe demonstrates the combined effect of anoxic but H_2S-poor sediments and O_2-deficient water; on release of Fe and Mn from the sediment, much of the Fe precipitated as FeOOH on plankton surfaces, whereas Mn mostly remained in solution.

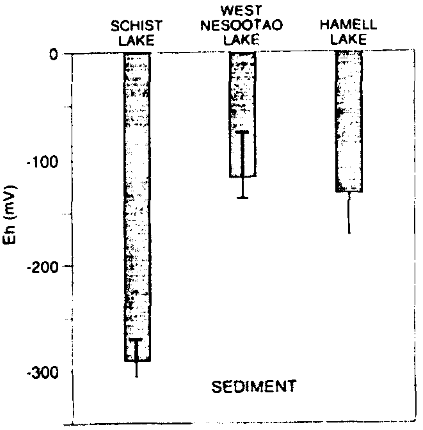

Fig. 15. The E_h values of sediments from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges).

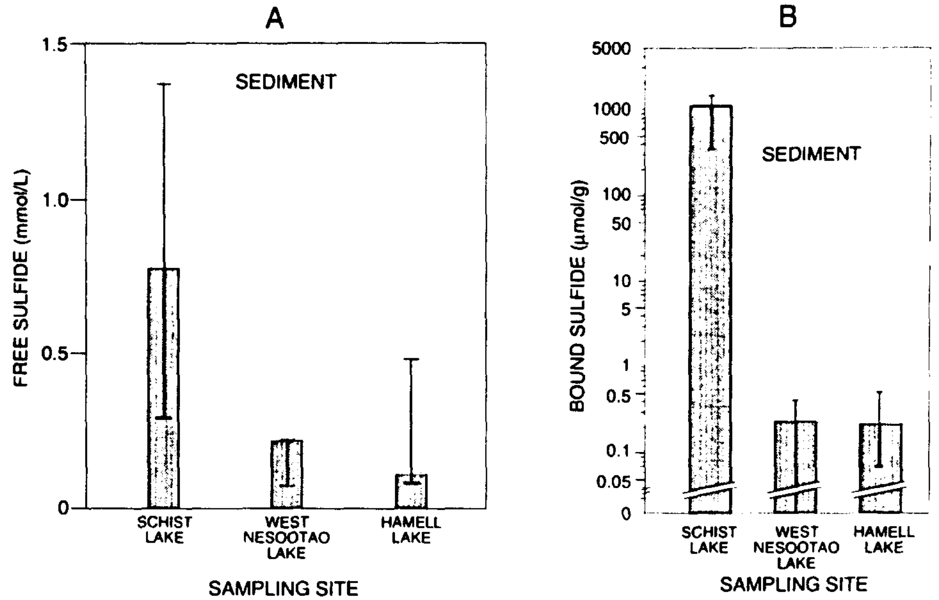

Fig. 16. The free sulfide (A) and bound sulfide (B) concentrations in sediments from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges).

The effects of sedimentary environment on the accumulation of FeOOH and MnOOH deposits and adsorbed Cu and Zn on surfaces of chitinous plankton hard parts are illustrated in a series of bar diagrams comparing characteristics of samples from different sampling sites (Figs. 15–20). NWSL sediment is sharply distinguished from WNL and HL sediments by its low E_h and high sulfide concentrations (Fig. 15 and 16). Accordingly, NWSL sediments were poor in $NH_2OH \cdot HCl$-extractable Mn, though rich in $NH_2OH \cdot HCl$-extractable Fe, and their $NH_2OH \cdot HCl$ extracts had low Mn/Fe ratios (Fig. 17); in contrast, the NWSL sediments were rich in DTPA-extractable Mn and interstitial dissolved or dispersed Mn but had a low DTPA-extractable Fe content (Fig. 17). These results demonstrate weak bonding, preferential solubilisation, and high bio-availability of Mn accompanied by immobilisation of Fe in an unavailable form — presumably FeS, not FeOOH. The NWSL sediments had the highest total Cu and Zn levels (Fig. 18) owing to continual loading of Cu and Zn by tailings pond effluent (in addition to atmospheric fallout) and entrapment of these metals in the sediments through the combined effects of (1) reaction with H_2S (assisted by alkaline conditions created by SO_4^{2-} reduction in sedimentary micro-environments), resulting in precipitation of CuS, ZnS, and FeS containing coprecipitated Cu and Zn (Fig. 16); (2) the scavenging of Cu and Zn from the water column by algal blooms and other suspended matter; and (3) precipitation, as well as enhanced adsorption by particulate matter, in the epilimnion due to alkaline conditions created locally by photosynthesis (Jackson, 1978). However, the concentrations of DTPA-extractable Cu and Zn were lowest in NWSL (Fig. 18) owing to immobilisation of the metals by sulfides. The NWSL sediment had the lowest overall Zn/Cu ratio, but its DTPA extract had the highest Zn/Cu ratio (Fig. 18), showing that Zn was more readily solubilised than Cu. The preferential solubilisation of Zn may, to some extent, reflect the dearth of MnOOH in the sediments (Fig. 17), but it

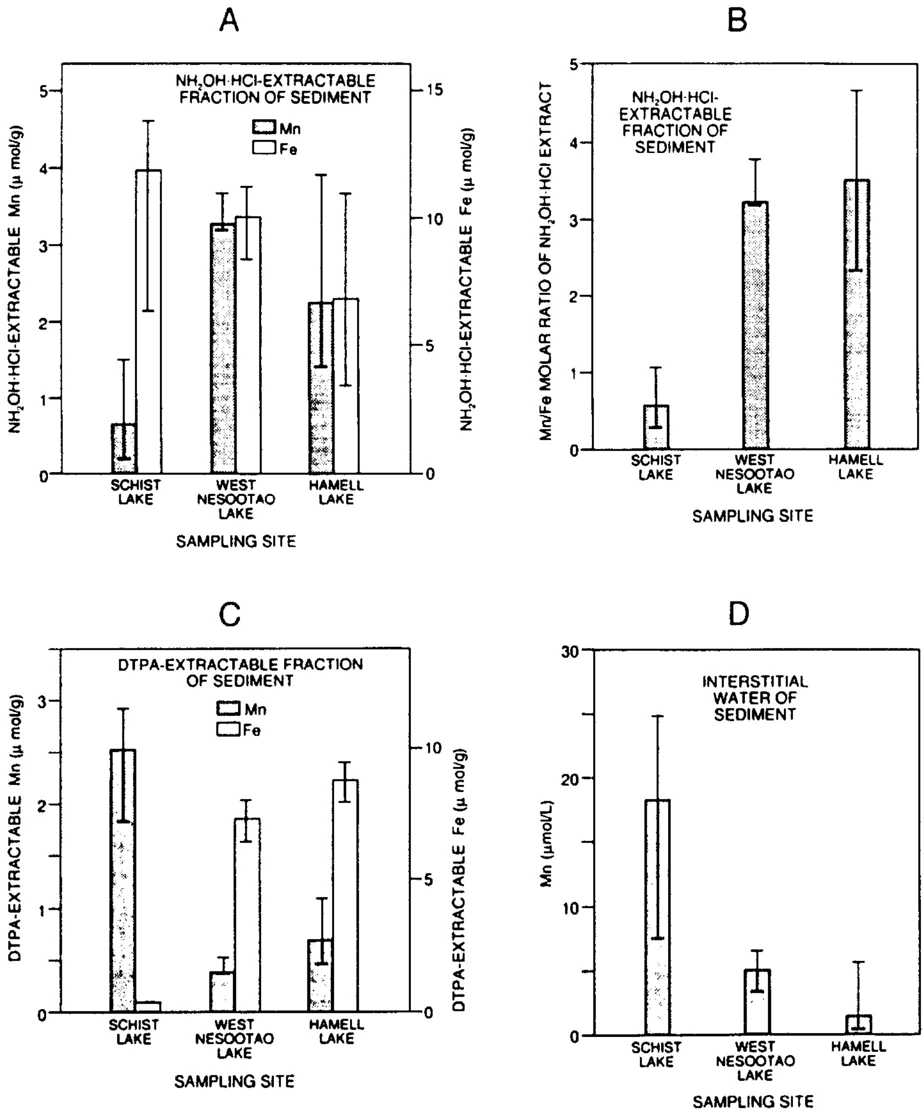

Fig. 17. Mn and Fe data for sediments from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges): $NH_2OH \cdot HCl$-extractable Mn and Fe content (A), Mn/Fe ratios of $NH_2OH \cdot HCl$ extracts (B), DTPA-extractable Mn and Fe content (C), and Mn concentrations in interstitial water (D).

is probably due primarily to preferential binding of Cu by sulfides and differential partitioning of Cu and Zn between sulfide and organic matter: In NWSL, Cu forms more stable bonds with sulfide than Zn does because the standard entropy of CuS exceeds that of ZnS, but Zn has a greater affinity for organic matter (Jackson, 1978, 1979). In brief, NWSL sediment, because of its highly reducing, sulfide-rich environment, is distinguished by high Mn solubility, low Fe solubility, an $NH_2OH \cdot HCl$-extractable fraction with a low Mn/Fe ratio, low bio-availability of both Cu and Zn, and a high proportion of Zn to Cu in its reservoir of bio-available metals.

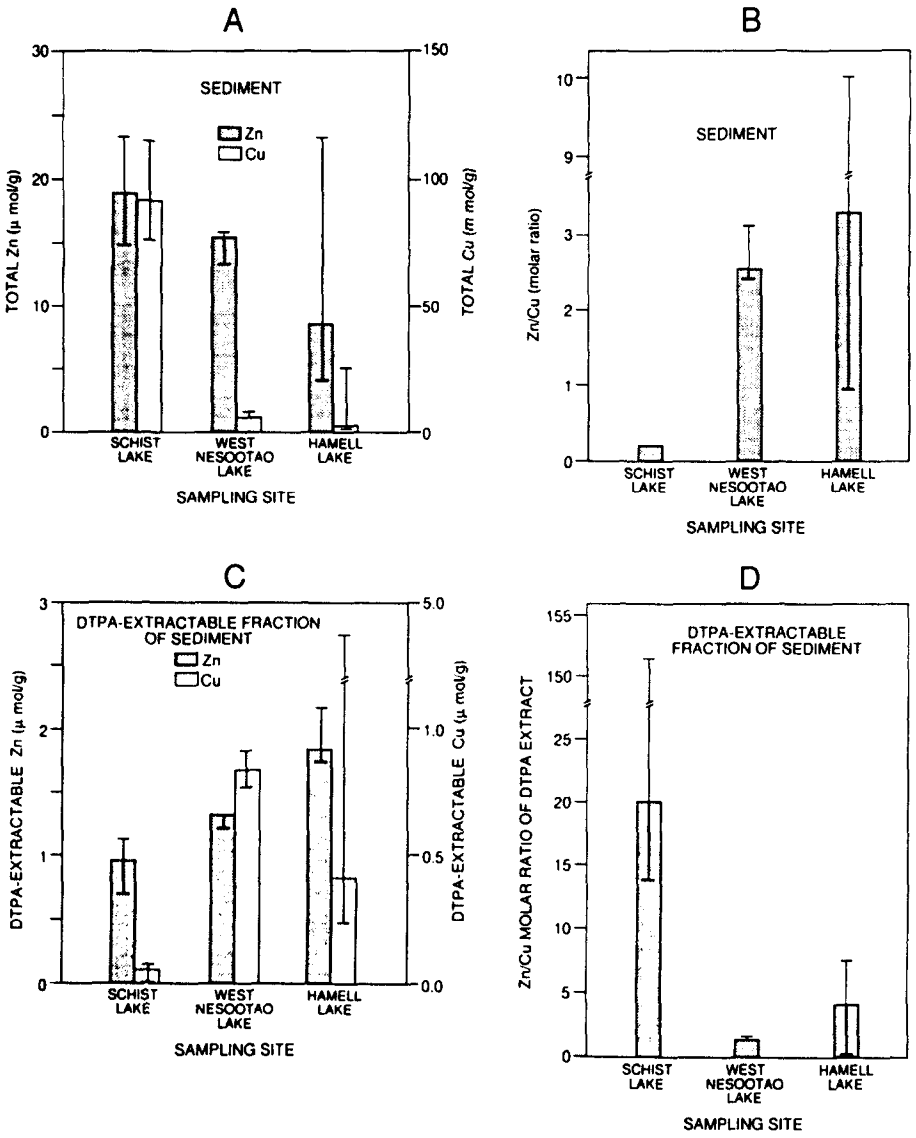

Fig. 18. Zn and Cu data for sediments from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges): total Zn and Cu content (A), Zn/Cu ratios (B), DTPA-extractable Zn and Cu content (C), and Zn/Cu ratios of DTPA extracts (D).

The situation in the sediments and hypolimnion is reflected in the composition of NWSL crustacean exoskeletons and *Keratella* loricae, which are poor in Fe, Mn, Zn, and Cu and have low Mn/Fe and Zn/Cu ratios (Figs. 19 and 20). Moreover, the analytical results yielded by the AMR/TN and the Philips/EDAX instruments were in agreement to the extent that comparison was possible, as both systems revealed that *Keratella* loricae from NWSL had low Zn/Cu and Mn/Fe ratios (Table 5). From inspection, it is obvious that the median Mn, Fe, Zn, and Cu concentrations and Mn/Fe and Zn/Cu ratios of the plankton

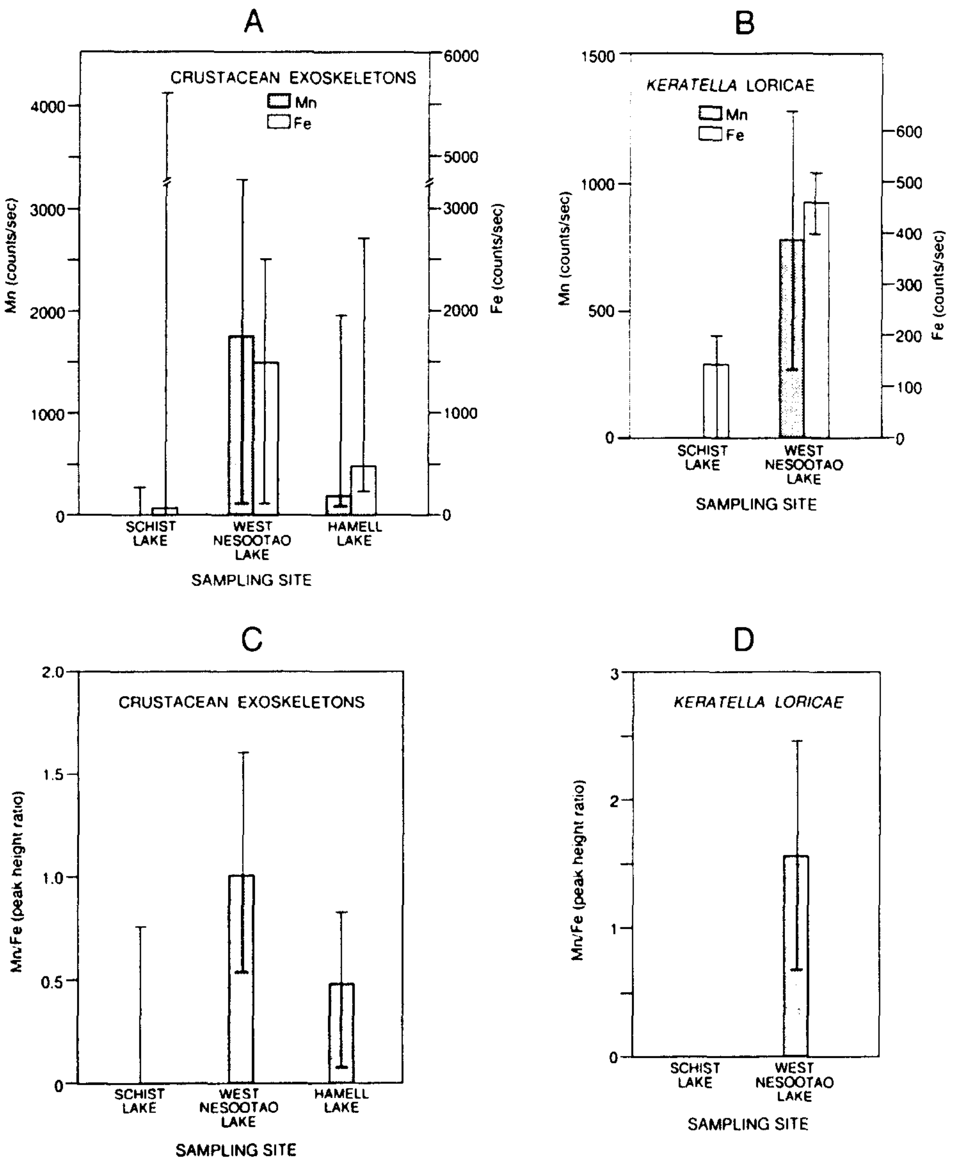

Fig. 19. Mn and Fe content of crustacean exoskeletons (A) and *Keratella* loricae (B), and Mn/Fe ratios of crustacean exoskeletons (C) and *Keratella* loricae (D), from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges). Analyses were done with AMR-1000/TN-11 system.

specimens from the three lakes (Figs. 19, 20) decrease progressively with sediment E_h (Fig. 15). We conclude that the reducing conditions and abundance of sulfides in the sediments of NWSL (together with O_2 deficiency in the hypolimnion) interfered with the accumulation of Cu and Zn by the plankton and lowered its Zn/Cu ratio by (1) preventing release of dissolved Fe from the sediments and subsequent reprecipitation as FeOOH on plankton surfaces, (2) preventing precipitation of dissolved Mn as MnOOH on the plankton

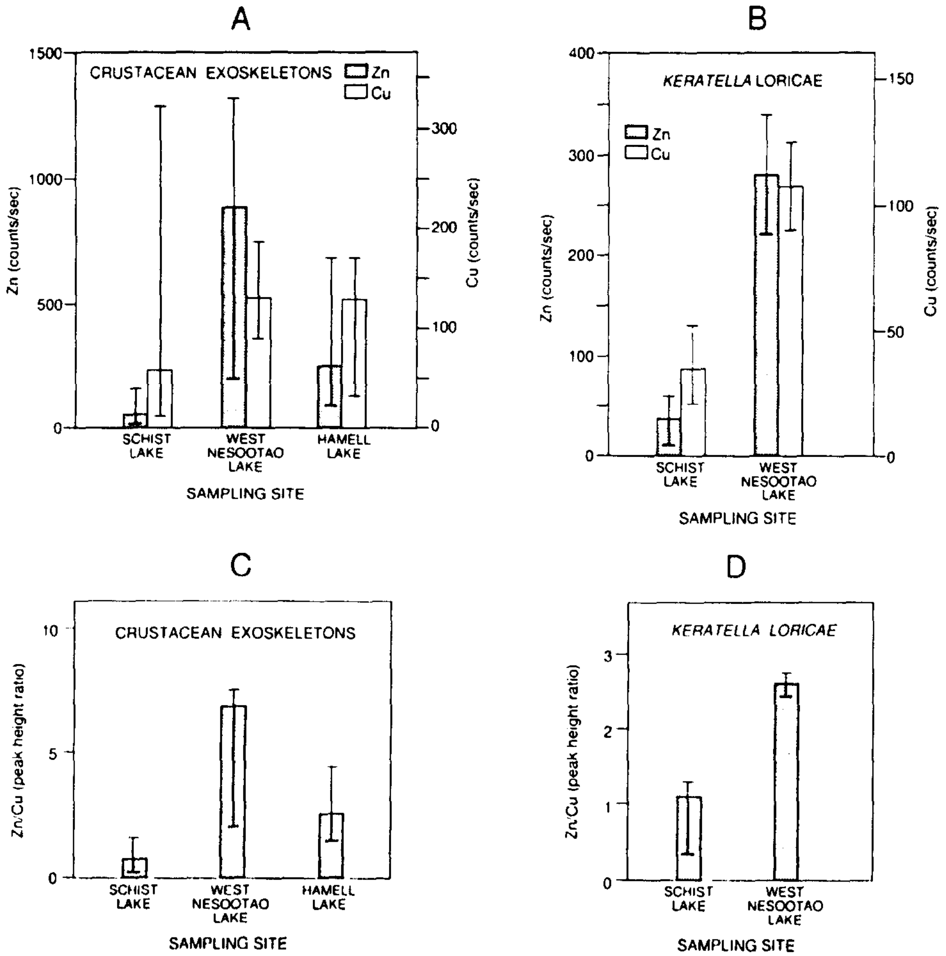

Fig. 20. Zn and Cu content of crustacean exoskeletons (A) and *Keratella loricae* (B), and Zn/Cu ratios of crustacean exoskeletons (C) and *Keratella loricae* (D), from Schist Lake, West Nesootao Lake, and Hamell Lake (medians and ranges). Analyses were done with AMR-1000/TN-11 system.

surfaces, and (3) depressing the solubility and bio-availability of sediment-bound Cu and Zn. Biodilution of Cu and Zn by the algal blooms of NWSL may also help to explain the low levels of these metals in NWSL plankton specimens. Furthermore, sulfides in the sediments appear to inhibit the uptake of Cu by crustacean exoskeletons much more effectively than they inhibit the uptake of Zn (Table 8). This confirms the previously discussed evidence that Cu is more stongly immobilised than Zn by the sulfides in NWSL sediments (Fig. 18) (Jackson, 1978, 1979). Organic substances as well as sulfides tend to interfere with the bio-accumulation of Zn, but their effect is much weaker (Table 8); in contrast, organic matter showed no tendency whatsoever to inhibit the bio-accumulation of Cu, and it even gave a weak *positive* correlation with Cu in exoskeletons (Table 8) (compare Cd data reported by Jackson et al., 1993). These relationships confirm the inferences drawn from sediment data regarding the relative affinities of Cu and Zn for sulfide and organic matter in NWSL (Jackson, 1978, 1979; see above).

Table 8

Correlation matrix showing variation of the Cu and Zn content of crustacean exoskeletons with respect to the concentrations of bound sulfide and organic C in the bottom sediments. The regression analyses were performed on median values of data for Hamell Lake, West Nesootao Lake, and Schist Lake. Explanation of symbols: r = correlation coefficient; P = significance probability. Sample size used for regression analysis: 3

	Crustacean exoskeletons			
	Cu		Zn	
	r	P	r	P
Bound sulfide in sediment	−1.000	<0.001	−0.898	>0.1
Organic C in sediment	0.439	≫0.1	−0.517	≫0.1

In view of the relationships shown in Figs. 4–9 and the fact that the Zn/Cu ratios of NWSL plankton are low (Fig. 20) despite the high Zn/Cu ratio of the DTPA-extractable fraction of NWSL sediment (Fig. 18), it would seem that the accumulation of Zn and Cu by MnOOH and FeOOH coatings on plankton is mainly controlled by the amounts of MnOOH and FeOOH precipitated on the plankton, although effects of sulfides, organic matter, and other metal-binding agents on the availability of Cu and Zn undoubtedly play a role as well. The quantities and proportions of MnOOH and FeOOH deposited on the plankton depend, in turn, on the E_h and sulfide content of the sediments and, presumably, on the O_2 and sulfide content of the overlying water; and the Mn/Fe ratio of the oxyhydroxide deposit largely determines the Zn/Cu ratio of the plankton.

4. Conclusions and implications

The results of the study are consistent with the following general conclusions: (1) FeOOH and MnOOH coatings commonly precipitate on the armoured surfaces of various planktonic freshwater organisms. (2) The oxyhydroxide coatings scavenge heavy metals and metalloids from the surrounding water, and this process is largely responsible for the passive accumulation of trace elements by plankton. (3) The FeOOH and MnOOH phases differ in their relative affinities for particular trace elements; therefore, the proportions of the trace elements bound by oxyhydroxide films vary with the MnOOH/FeOOH ratio of the film. (4) Environmental factors, including the E_h and sulfide levels in the bottom sediments and the O_2 content of the overlying water, control the adsorption of heavy metals by plankton, not only by affecting the speciation, solubility, and availability of the metals themselves but also — and perhaps primarily — by determining the absolute and relative quantities of FeOOH and MnOOH precipitated on the surfaces of planktonic organisms; but concentrations of metals in plankton may be unrelated to the total concentrations of these metals in the sediments. (5) Heavy metal bio-availability is limited primarily by sulfides produced in sediments or, if these are lacking, by special thiol compounds produced by the plankton itself as a defense against metal toxicity.

Finally, let us consider possible wider implications of our results and conclusions. The scavenging of toxic and nutrient heavy metals and metalloids by oxyhydroxide coatings on armoured surfaces of planktonic organisms may have an important bearing on the cycling and biological effects of these elements — for instance, by removing the elements from solution and hastening their transfer to the sediments through the settling out of biogenic carrier particles; by accelerating their uptake by animals that feed on plankton; by rendering them *less* available for uptake by living organisms, thereby making them less toxic or preventing them from performing beneficial biochemical functions such as the activation of enzymes; and by altering the proportions of bio-available elements in natural waters and aquatic food chains, thereby giving certain organisms an advantage over their competitors. Furthermore, the process could conceivably be applied to problems of pollution control, as in the design of settling ponds for efficient removal of metals from industrial effluents, such as tailings pond outflows (Jackson, 1978). For instance, a pond that is fertilised with sewage effluent and SO_4^{2-}, has dense plankton blooms which include numerous euglenophytes, is deep enough to be stratified, has anoxic bottom water, and has bottom sediments characterised by low E_h values and copious production of free sulfide should be particularly efficient in trapping and retaining heavy metals. In addition, the results of the study illustrate the advantages of energy dispersive X-ray micro-analysis over conventional bulk analysis in research on biogeochemical pathways involving complex, heterogeneous mixtures of microscopic entities such as plankton; but they also demonstrate the importance of integrating micro-analytical data with a wide spectrum of relevant information on the ecosystem and the conditions and processes that control its biogeochemical pathways. Without this body of related information, the study or monitoring of pollutant levels in plankton is likely to be of limited value owing to lack of sufficient basis for interpretation of the data. For example, without a detailed examination of sediment chemistry it would not have been possible to explain the seemingly paradoxical fact that the plankton in NWSL had the lowest metal concentrations even though the sediments in that lake were richest in metals. This leads to one other point: Our work shows the value of carrying out quantitative comparisons of data representing different ecosystems.

Acknowledgements

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