Relationships between the Partitioning of Trace Metals in Sediments and Their Accumulation in the Tissues of the Freshwater Mollusc *Elliptio complanata* in a Mining Area

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Surficial sediment samples and specimens of a benthic freshwater pelecypod, *Elliptio complanata*, were collected at eight sites located along a metal contamination gradient downstream from the mining and smelting complex in Rouyn–Noranda, Quebec. The molluscs were dissected, digested, and analysed for Cu, Pb, Zn, Fe, and Mn by flame atomic absorption spectrophotometry. Each sediment sample was wet sieved and the <70-μm granulometric fraction was subjected to a sequential extraction procedure designed to determine the partitioning of the trace metals among various geochemical phases. Regression analysis indicates that Cu, Pb, and Zn levels in various tissues of *E. complanata* (or in the whole organism, without the shell) are best related, not to total metal concentrations in the adjacent sediment, but rather to one or more of the relatively easily extracted fractions. Accumulation of Cu, Pb, and Zn was also influenced by the protective or competitive effect of other sediment constituents, notably amorphous iron oxyhydroxides and, to a lesser degree, organic matter.

Des échantillons de sédiments de surface et des spécimens du pélécypode d'eau douce *Elliptio complanata* ont été prélevés à huit sites situés dans un gradient de contamination en métaux, en aval du complexe d'opérations minières et de raffinage de Rouyn–Noranda, Québec. Les mollusques ont été disséqués, digérés et analysés pour Cu, Pb, Zn, Fe et Mn par spectrophotométrie d'absorption atomique. Chaque échantillon de sédiments était tamisé par voie humide et la fraction granulométrique <70 μm était soumise à une procédure d'extractions séquentielles conçue pour déterminer la répartition de métaux traces entre diverses phases géochimiques. L'analyse de régressions indique que les niveaux de Cu, Pb et Zn dans divers tissus de *E. complanata* (ou dans l'organisme entier, sans la coquille) sont mieux corrélés avec l'une ou plusieurs des fractions de métaux extraites relativement facilement des sédiments plutôt qu'avec les concentrations totales des métaux dans ces sédiments. L'accumulation de Cu, Pb et Zn était aussi influencée par l'effet protecteur ou compétitif d'autres constituants des sédiments, notamment des oxyhydroxydes de fer amorphe et, à un degré moindre, de la matière organique.

The tendency of many aquatic organisms to accumulate trace metals from their environment has led to the use of some of these organisms for the assessment of trace metal pollution (Phillips 1977; Wright 1978). Bivalve molluscs have been used extensively for quantitative assessment of trace metal contamination, especially in the marine environment (Huggett et al. 1973; Darracott and Watling 1975; Phillips 1976; Klumpp and Burdon-Jones 1982; Ritz et al. 1982). The use of freshwater pelecypods as biological indicators has also been suggested (Curry 1977; Foster and Bates 1978; Jones and Walker 1979; Forester 1980). Direct proportionality between environmental concentrations and tissue levels is frequently assumed in such studies. However, the processes and factors controlling trace metal availability to these organisms are not completely understood, and simple relationships are seldom found in natural systems between trace metal levels in the organisms and total metal concentrations in either the sediment or the water to which the organisms are exposed.

The objective of this study was to identify factors influencing trace metal bioavailability to freshwater benthic organisms; to this end, we have investigated the relationships between the partitioning of trace metals in sediments and their accumulation in various tissues of the benthic pelecypod *Elliptio complanata*. This filter feeder is widely distributed in the streams and lakes of northeastern North America (Clarke 1973; Curry 1977; Heit et al. 1980; Magnin and Stanczykowska 1970); it is a long-lived sedentary organism that comes into contact with both sediments and water when feeding and respiring, and can accumulate trace metals. These characteristics make *E. complanata* a useful organism for studying the factors influencing trace metal bioavailability in the aquatic environment.

Laboratory experiments have shown that the ingestion of sediments can be an important route of trace metal uptake for some marine bivalves and that the uptake should depend upon the physicochemical associations of the trace metals in the sediments (Jenne and Luoma 1977; Luoma and Jenne 1977). An alternative route of entry involves the direct absorption of trace metals from water (Jenne and Luoma 1977; Luoma and Jenne 1977; George and Coombs 1977; Simkiss et al. 1982; Zamuda and Sunda 1982). Recent studies (Oakley et al. 1981; Luoma

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and Davis 1983) suggest that adsorption onto specific geochemical phases might control the trace metal concentrations in the ambient water at the sediment–water interface (i.e. the interstitial water or the water in intimate contact with the surface of the sediments). The physicochemical forms of trace metals in the sediments could thus affect the bioavailability of particulate trace metals ingested by filter-feeding organisms, and also reflect the dissolved trace metal concentrations in the solution to which these organisms are exposed. If these observations may be generalized for natural systems, there should be relationships between the trace metal levels in specific tissues (or in the whole organisms) and the concentrations of certain metal forms in the sediments.

Study Area

Necessary conditions for the present study included the existence of a spatial gradient of trace metal concentrations in the sediments and the presence of an appreciable bivalve population along this gradient. Such conditions were met in the mining area of Rouyn–Noranda, located in northwestern Quebec some 500 km northwest of Montreal, for stations chosen in Lakes Beauchastel, La Bruère, and Montbeillard. Sources of metal contamination in the area include abandoned mines, current mining operations, accumulated residues from the smelting process, and atmospheric fallout from the smelter emissions. Lakes Beauchastel and La Bruère are influenced by point sources of metal contamination, while Lake Montbeillard is affected only by the diffuse smelter emissions.

The region is densely forested and has a low relief (<80 m); drainage is not well developed, and swampy areas, densely covered with vegetation, are abundant. The drainage network is part of the Ottawa River watershed. The bedrock is covered by locally derived till and, at the surface, by lacustrine clays deposited during the existence of the proglacial Lake Barlow–Ojibway; the mean depth of unconsolidated material is 15–20 m. The lacustrine clay fraction (<2 μm) contains the following minerals: illite > vermiculite > chlorite > feldspar > quartz > hornblende. Many metal anomalies are observed in the lacustrine clays (Lalonde and Lasalle 1983) and, in particular, high Cu, Pb, and Zn concentrations are reported for some soil samples. Metal concentrations found in the sediments may thus be considered to have been introduced both by anthropogenic activities and by natural processes of weathering and erosion.

Methods

During a preliminary sampling effort, in May 1981, surficial sediments were collected with an Ekman dredge at 46 sites in the lakes lying downstream from the major mining and smelting activities in Rouyn–Noranda. A diver equipped with SCUBA noted the presence or absence of an appreciable mollusc population at each site. To evaluate the spatial variability of metal levels in the sediments, total concentrations of Cu, Pb, and Zn were determined for each of these samples. A second sampling campaign was then carried out between 3 and 5 September 1981 at eight sites (Fig. 1) located along the spatial gradient identified earlier. At each site, bottom sediments were obtained with an Ekman dredge; the surficial, oxidized portion was collected with a plastic spoon, placed in a polyethylene bag, and kept at 4°C until analysed; analysis was started within 4 d following the sampling. Specimens of *E. complanata* were

obtained by a diver at each site, within a radius of 20 m from the sediment collection point. The specimens were cleaned by brushing, kept at 4°C during the field work and transport to the laboratory, and then frozen until needed for analysis.

All bivalves analysed were between 6.5 and 9.5 cm long, and all but one specimen were between 9 and 13 yr old. Specimens were dissected while still frozen (Smith et al. 1975) into foot, muscle, visceral mass, hepatopancreas, gills, and mantle. Each organ was dried (105°C), weighed, and individually digested and analysed for Cu, Pb, Zn, Fe, and Mn. The soft parts of the organisms remaining after dissection, comprising mainly the intestinal tract contaminated with sediments, were discarded; this material represented about 10% of the total soft parts (dry weight). For digestion of the organs, the following steps were employed (Borg et al. 1981): treatment with 10 mL of HNO_3 (70%) overnight at room temperature, evaporation to near dryness, and treatment with 10 mL of H_2O_2 (30%) heated to 85°C for 6 h. Nitric acid (final concentration 2%) was then added and the resulting solution analysed by flame atomic absorption spectrophotometry. Mean concentrations, C_R, for the whole organisms were calculated as follows for each trace metal:

$$(1) \quad C_R = \Sigma C_i W_i / \Sigma W_i$$

where C_i and W_i represent the trace metal level and the dry weight of the ith organ or tissue, respectively. The values of C_R could not be calculated for Pb, as Pb levels in some tissues were below the analytical detection limit. To estimate the size of particles ingested by *E. complanata*, the contents of the digestive system were obtained from five specimens from different sampling points and combined. The accompanying mucus was then destroyed with H_2O_2 (30%), and the distribution of particles was determined on a volumetric basis (Fig. 2) with an electronic particle counter (Coulter counter, model TA).

The sediment samples were wet sieved with a 70-μm Nylon screen, and the <70-μm granulometric fraction, assumed to be representative of that ingested by *E. complanata* (see Fig. 2), was retained. In the size separation, minimum amounts of water were used to avoid excessive solubilization of solid material; during the sieving of a given sample, the water was clarified by high-speed centrifugation and reused. The <70-μm granulometric fraction was subjected to a sequential extraction procedure designed to partition the particulate trace metals, M, into the following five fractions: (a) M(F1): *exchangeable metals*. The sediment sample was extracted for 10 min with 0.5 mol $MgCl_2$/L at pH 7.0; (b) M(F2): *metals bound to carbonates or specifically adsorbed*. The residue from (a) was leached for 5 h with 1 mol sodium acetate (NaOAc)/L adjusted to pH 5.0 with acetic acid (HOAc); (c) M(F3): *metals bound to Fe–Mn oxides*. The residue from (b) was extracted at 96°C for 6 h with 0.04 mol $NH_2OH \cdot HCl$/L in 25% (v/v) HOAc; (d) M(F4): *metals bound to organic matter and sulfides*. The residue from (c) was extracted at 85°C for 5 h with 30% H_2O_2 adjusted to pH 2 with HNO_3 and then at room temperature with 3.2 mol NH_4OAc/L in 20% (v/v) HNO_3; (e) M(F5): *residual metals*. The residue from (d) was digested with a 5:1 mixture of concentrated hydrofluoric and perchloric acids.

The detailed experimental procedure is given in Tessier et al. (1979, 1980). Trace metal concentrations in the leachates were determined by flame atomic absorption spectrophotometry. Quantification was achieved with appropriate calibration curves prepared with the components of each extraction solution (sediments) and the digestion solution (mollusc tissues).
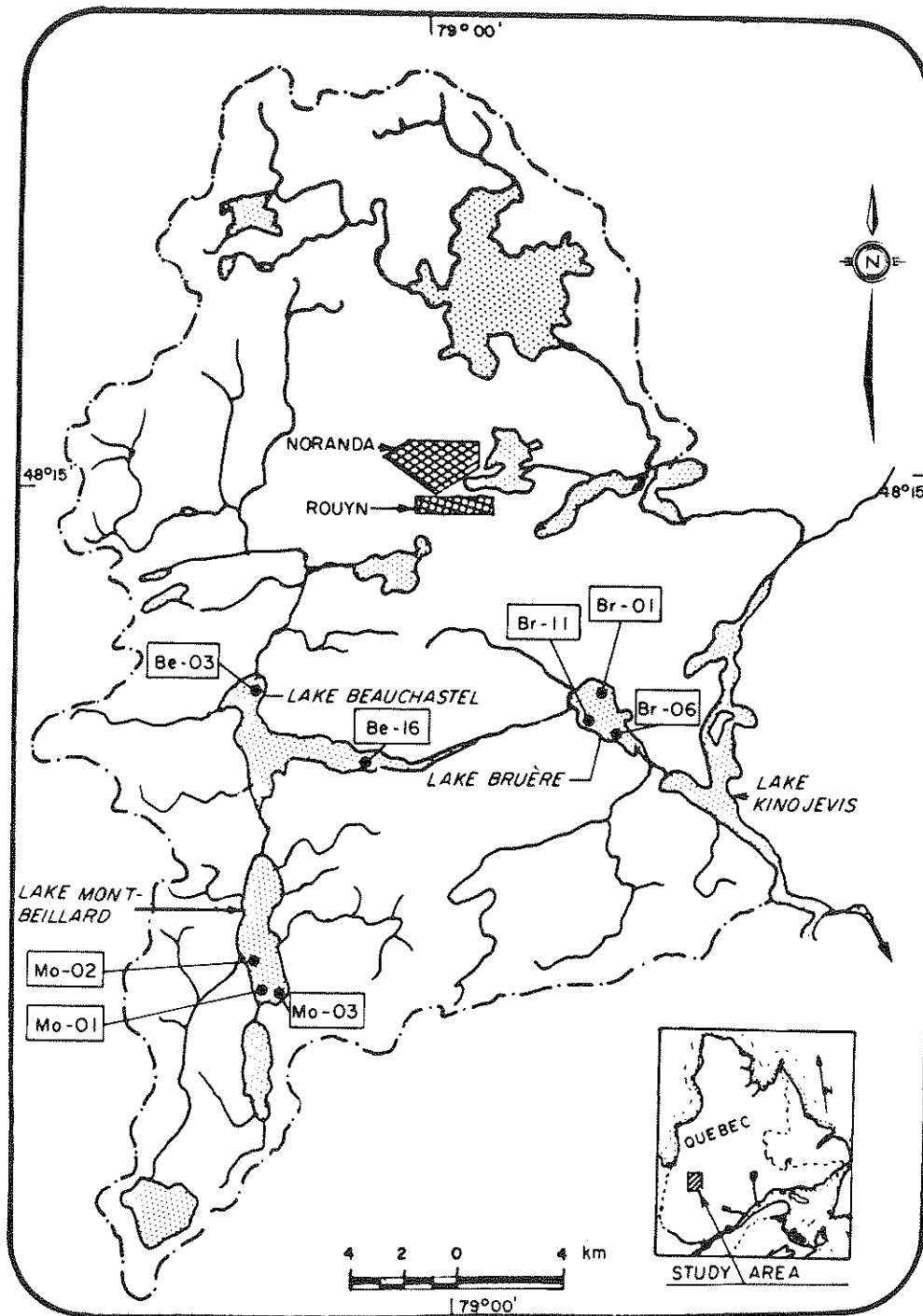
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FIG. 1.   Sampling stations in the Rouyn–Noranda area, Quebec.

Particulate sulfur and carbon were determined with Leco Total Sulfur (model IR-32) and Carbon (model IR-12) analyzers, respectively. Inorganic carbon was assumed to be equal to the difference between total and organic carbon concentrations, the latter being measured after acid treatment (6 mol HCl/L, 30 min) of the sediment to dissolve the carbonates present. Silicon and Al concentrations were determined by atomic absorption spectrophotometry (nitrous oxide–acetylene flame), after fusion with lithium metaborate followed by dissolution of the fused material in 1.2 mol HCl/L.

Results

Trace Metal Distribution in the Sediments
(<70 μm Granulometric Fraction)

Total trace metal concentrations, M(S5), were consistently higher in sediments from Lakes Beauchastel and La Bruère than in those from Lake Montbeillard; the same trend was generally observed for the concentrations in the individual fractions 1–5 (Table 1). For total trace metal values, the ratios of maximum to

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Metal concentrations in the tissues of *E. complanata* varied from station to station (Table 2); the lowest levels were generally found for the sites located in Lake Montbeillard, while the highest were observed for the sites located in either Lake La Bruère (Cu, Pb) or Lake Beauchastel (Zn). A hierarchical analysis of variance indicated that significant differences in the tissue levels ($P < 0.05$) existed between sites in Lake Montbeillard and sites BR-01 (Cu in the gills, muscle, and in the total organism; Pb in the gills and mantle) or BE-03 (Zn in the muscle, hepatopancreas, gills, and in the total organism). The mean bioconcentration ratios (concentration in the molluscs/total concentration in the sediment) were 0.38 and 1.11 for Cu and Zn, respectively; these values are much lower than the concentration ratios of 4 (Cu) and 7 (Zn) reported by Heit et al. (1980) for specimens of *E. complanata* collected from Lake George.

For all metals, the highest concentrations were generally found in the gills and mantle, whereas the lowest values occurred in the foot and adductor muscle; intermediate levels were associated with the hepatopancreas and visceral mass. However, the standard deviations in Table 2 are high, and at a given station statistically significant differences were not always observed between different tissues. A hierarchical analysis of variance indicated that the only significant differences between the tissue levels ($P < 0.05$) at most stations are foot and adductor muscle different from gills, mantle, and hepatopancreas (for Cu) and adductor muscle and visceral mass different from gills and mantle (for Zn, Fe, and Mn). The large standard deviations are too high to be attributed to analytical error only ($\pm 8\%$), suggesting that biological variability (age, sexual condition, etc.) is largely responsible for the observed variations at each station (Boyden 1974, 1977; Cossa et al. 1979; Julshamn 1981).

The highest contributions to the metal body burden were from the gills (Cu, Zn, Mn) and the mantle (Fe) whereas the lowest were from the foot and adductor muscle (Table 3). Similar tissue distributions of Fe and Mn have been reported for the same species (Hobden 1970; Seah and Hobden 1969); especially high contributions from the gills (20% for Fe; 78% for Mn) and mantle (27% for Fe; 16% for Mn) were also observed by these authors.

Bivalve–Sediment Relationships

Trace metal levels in the soft tissues of *E. complanata* were first compared with the trace metal concentrations in each of the five extraction fractions ([M(F1)] to [M(F5)]) and with the partial sums of these concentrations ([M(S2)] to [M(S5)]). Multiple regressions were then performed with the trace metal concentration in the tissues as the dependent variable and the concentrations of metal in the various sediment fractions as the independent variables. Due to the high multicolinearity existing between the sediment fractions, the selection of the first and largest partial regression coefficient explained a large proportion of the variance, with little gained (2–3%) upon inclusion of further additive combinations of the fractions. Such inclusion also resulted in partial regression coefficients that were highly variable. Finally, the concentrations in each sediment fraction or combination were normalized with respect to the iron oxide or organic carbon content of the sediments (i.e. $[M(F1)]/[Fe(F3)]$ to $[M(S5)]/C_{org}$), and bivariate analyses between the normalized parameters and tissue concentrations were
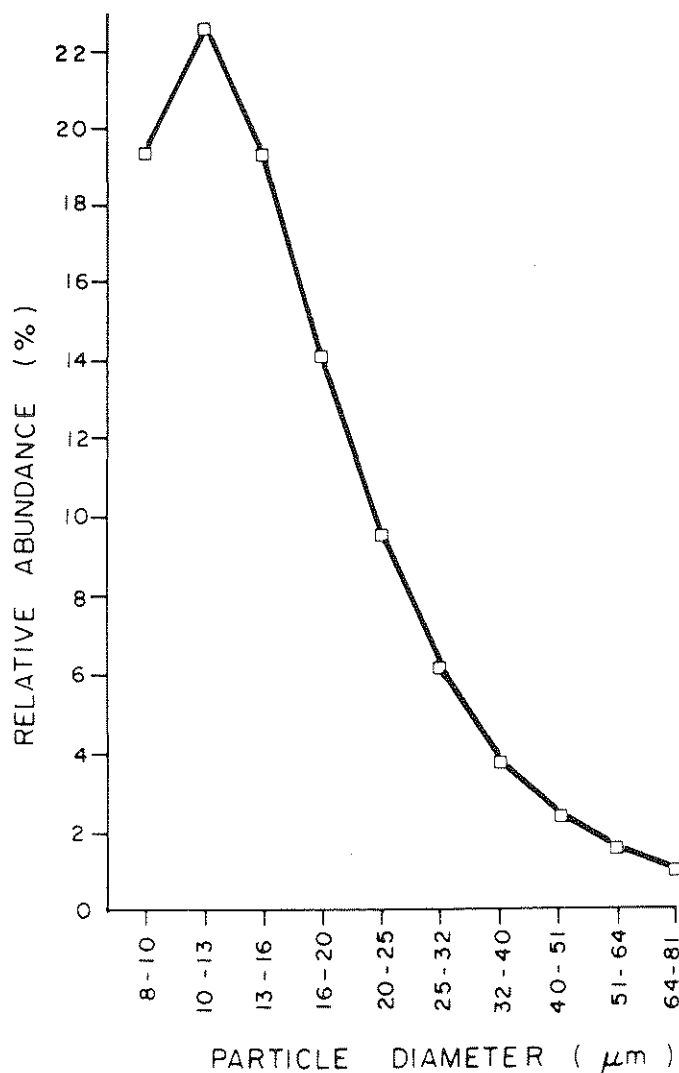

FIG. 2. Size distribution of particles ingested by *E. complanata*.

minimum concentration lay between 5 (Pb, Zn) and 8 (Cu); total Fe concentrations, on the other hand, were less variable (ratio < 2). A somewhat greater variation was observed when the individual nondetrital fractions were considered (F1–F4), for which the corresponding ratios ranged from 5 (Pb(F3)) to 34 (Zn(F1)).

The distribution of a given metal among the various fractions obtained by the sequential extraction procedure (Table 1) does not necessarily reflect the scavenging action of discrete sediment phases, but rather should be considered as operationally defined by the methods of extraction (Tessier et al. 1979). As has been observed in previous studies (Forstner and Wittmann 1981; Tessier et al. 1980, 1982), each metal exhibited a distinctive partitioning pattern among the nondetrital fractions 1–4. For example, the distribution of Cu showed the characteristic predominance of fraction 4, approximately equal contributions from fractions 2 and 3, and virtually undetectable levels in fraction 1 (i.e. F4 > F3 ~ F2 ≫ F1). In the case of Pb, fraction 3 dominated the distribution, the contributions from fractions 2 and 4 being approximately equal and that from fraction 1 again being quantitatively negligible (i.e. F3 > F2 ≥ F4 ≫ F1). A similar distribution pattern was observed for Zn, except that its exchangeable fraction contributed appreciably to the total metal load (i.e. F3 > F2 ≥ F4 ≥ F1).

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TABLE 1. Physicochemical characteristics of the <70-μm granulometric fraction of the sediment samples: trace metal concentrations as determined with the sequential extraction procedure; S, Al, Si, inorganic carbon, and organic carbon concentrations. All the results are based on sediment dry weight; [M(F1)]–[M(F5)] represent metal concentrations in fractions 1–5 following the sequence given in the text; [M(S5)] represents the sum of metal concentrations for the five fractions; C_O and C_I represent organic and inorganic carbon concentrations, respectively.

Site	[M(F1)] (μg/g)				[M(F2)] (μg/g)				[M(F3)] (μg/g)				[M(F4)] (μg/g)			
	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe
Mo-01	0.1	<0.9	1.5	0.9	2.1	4.3	5.1	67.0	4.3	14.5	31.8	2830	5.2	<2.0	4.0	110
Mo-02	0.1	<0.9	1.1	2.6	1.9	2.6	7.2	90.7	6.0	20.8	51.5	4980	7.7	2.0	6.9	205
Mo-03	<0.1	<0.9	0.4	4.3	2.8	2.6	7.8	57.0	6.4	14.4	44.8	3880	4.8	<2.0	5.9	115
Be-03	<0.1	<0.9	19.1	7.0	17.5	8.6	64.5	670	13.8	26.8	177	7220	40.3	6.3	15.2	240
Be-16	<0.1	<0.9	16.0	2.7	13.7	14.9	65.5	280	7.9	27.0	206	5610	69.1	10.5	40.2	140
Br-01	0.2	<0.9	7.8	0.9	18.1	13.0	17.5	144	20.2	49.5	123	4950	28.7	8.3	13.3	920
Br-06	0.3	0.9	13.8	2.6	23.7	12.2	41.4	190	17.8	51.6	163	5160	54.9	8.4	18.6	195
Br-11	0.1	<0.9	13.4	2.7	27.8	16.7	49.1	185	31.4	77.0	215	5820	61.5	21.0	33.0	290

Site	[M(F5)] (μg/g)				[M(S5)] (μg/g)				C_O (%)	C_I (%)	S (%)	Al (%)	Si (%)
	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe					
Mo-01	12.0	10.0	36.0	21 300	23.7	28.8	78.4	24 300	0.43	0.48	0.03	12.5	68.1
Mo-02	25.0	20.0	68.0	29 300	40.7	45.5	135	34 600	0.90	0.86	0.05	13.3	61.3
Mo-03	21.0	13.3	60.0	28 000	35.0	30.3	119	32 100	0.46	0.61	0.04	13.5	65.3
Be-03	35.7	23.3	65.7	27 700	107	65.0	342	35 800	0.74	0.89	0.07	12.9	63.7
Be-16	54.7	33.3	105	37 300	145	85.7	433	43 300	1.54	1.30	0.12	14.3	57.5
Br-01	38.3	26.7	80.7	27 700	106	97.5	242	33 700	0.56	0.76	0.05	13.7	63.1
Br-06	45.3	26.7	81.3	28 000	142	100	318	33 500	0.75	0.91	0.06	13.5	62.0
Br-11	59.3	36.7	112	32 700	180	151	423	39 000	0.84	1.26	0.06	14.0	61.6

conducted. This generally resulted in greater coefficients of determination (r^2). Since our goal was to obtain the best overall single predictor among the fractions and normalized parameters, all subsequent statistical analyses were bivariate. The correlation coefficient values (r) were calculated in each case and the data were examined graphically to verify spread and linearity. For all the calculations, mean values for the tissues (or organism) at each station were used. Rather than present all the statistical parameters, we shall limit the following discussion to the general trends observed for each metal (Tables 4–6).

For Cu (Table 4), significant correlations generally occurred between the metal concentration in the soft tissues of *E. complanata* and [Cu(F2)] or [Cu(F3)] ; correlation coefficients were not calculated with [Cu(F1)], as the latter values were lower than or close to the analytical detection limit (Table 1). The r values increased when the various Cu concentrations in the sediments were normalized with respect to the organic content in the sediments or, more importantly, with respect to [Fe(F3)]; when normalization of the trace metal concentrations in the sediments was effected, higher correlation coefficients were obtained if [Cu(S3)] was considered in the calculation instead of [Cu(F2)] or [Cu(F3)], where [Cu(S3)] is the sum of [Cu(F1)], [Cu(F2)], and [Cu(F3)]. The ratio [Cu(S3)]/[Fe(F3)] in the sediments (Fig. 3) was the best predictor of the Cu concentration in the whole mollusc.

A slightly different trend was observed for Zn (Table 5). Few significant correlations were observed between Zn concentration in the soft tissues of the bivalves and [Zn(F1)], [Zn(F2)], or [Zn(F3)]; the correlations with [Zn(F4)] were the most numerous and statistically significant (Table 5). In contrast with Cu, normalizing the various Zn concentrations with respect to the organic carbon content of the sediments systematically

decreased the r value; however, normalizing [Zn(F4)] or [Zn(S3)] with respect to [Fe(F3)] did increase the correlation coefficients. The ratio [Zn(F4)]/[Zn(F3)] in the sediments (Fig. 4) was the best predictor of the Zn concentration in the whole mollusc.

For Pb, a statistically significant correlation coefficient was found between the metal level in the gills and [Pb(F3)] (Table 6); correlation coefficients involving either [Pb(F1)] or the foot and adductor muscles were not calculated, as the Pb concentrations were lower than the analytical detection limits. The r values increased when the various Pb concentrations in the sediments were normalized with respect to the organic content in the sediment or, more importantly, with respect to [Fe(F3)]. When normalization was effected, higher correlation coefficients were obtained when [Pb(S3)] was considered in the calculation instead of [Pb(F3)].

Discussion

Copper, Pb, and Zn levels in various tissues of *E. complanata* (or in the whole organism) can be predicted, in the study area, from trace metal concentrations extracted from the sediments. As expected, correlations are better for relatively easily extractible metals (e.g. F2, F3, F4) than for more tightly bound metals (residual; F5), or for the total trace metal concentrations (S5). This suggests that the availability of a particular metal is inversely related to its binding strength to the various substrates in the sediments. Extracting the sediments and normalizing with respect to sediment components (e.g. [Fe(F3)], organic carbon) increased the explained variance in accumulated metals in the tissues of the organisms over that explained by the total metal concentrations in the sediments. For example, using [M(S3)]/[Fe(F3)] instead of [M(S5)] as the predictor increased r^2 by 33%

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TABLE 2. Metal concentrations in the tissues of *E. complanata* from various sampling sites. Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1); ND, not detectable.

Site	Organ	Mean ± SD (µg/g dry weight)				
		Cu	Pb	Zn	Fe	Mn
Mo-01	Fo	5.4±4.8	ND	127±36	85±21	144
	Mu	2.4±0.6	ND	83±13	67±27	60±5
	VM	8.5±2.0	5.8±3.6	78±11	390±270	320±280
	He	29.0±13.2	13.0	123±23	400±390	89±77
	Gi	29.5±19.4	18.8±6.9	265±45	840±360	5000±300
	Ma	17.6±9.5	13.9±4.2	173±19	3 700±1300	3000±3400
	RO	13.8±5.0	—	132±15	925±460	1330±450
Mo-02	Fo	5.4±1.8	ND	140±8	109±71	103
	Mu	2.7±1.1	ND	76±9	82±23	36
	VM	10.5±2.9	ND	84±9	770±290	147
	He	28.8±7.8	14.7±3.5	121±7	670±90	103
	Gi	27.8±11.9	12.9±1.9	280±9	1 450±740	4950
	Ma	11.8±0.8	14.4±4.1	160±14	5 600±1200	1560
	RO	13.6±2.3	—	136±4	1 470±500	1270
Mo-03	Fo	7.7±3.2	ND	130±31	119±32	52±15
	Mu	4.6±1.0	ND	95±10	300±130	113±32
	VM	11.8±3.6	4.8±1.8	98±10	1 080±500	650±60
	He	32.0±3.2	31	170±24	1 130±190	178±11
	Gi	28.2±16.4	12.2±2.8	320±90	2 590±1000	8600±3380
	Ma	18.4±2.0	21.1±9.7	280±56	10 400±400	5510±1240
	RO	16.0±2.5	—	180±50	3 000±330	2800±1250
Be-03	Fo	6.9±1.3	ND	144±10	114±66	47±17
	Mu	3.4±0.9	ND	88±10	115±71	74±31
	VM	11.1±3.3	1.4	90±12	650±150	400±140
	He	32.0±5.3	ND	119±10	880±340	230±95
	Gi	58.8±11.2	6.3	790±270	1 610±310	8800±1380
	Ma	43.9±6.9	7.7±1.7	275±87	4 070±1440	2580±610
	RO	23.3±3.4	—	202±38	1 220±260	1570±170
Be-16	Fo	7.9±1.9	ND	148±28	390±310	166±141
	Mu	7.7±5.8	ND	119±22	280±142	184±116
	VM	11.7±3.4	6.3	160±65	1 120±780	540±270
	He	37.5±8.2	13.1	208±23	1 490±810	260±84
	Gi	30.3±6.0	14.4±1.3	1360±890	3 440±720	8720±1940
	Ma	69.8±44.8	21.1±5.2	1190±980	11 100±2100	8590±2120
	RO	28.2±9.7	—	535±320	3 340±460	3230±510
Br-01	Fo	12.7±7.9	5.5±1.4	141±15	108±27	43±11
	Mu	11.7±7.7	3.8	100±11	135±56	59±19
	VM	16.5±4.4	6.9±0.8	114±6	585±212	286±136
	He	44.5±1.3	14.3±7.2	172±8	755±200	153±63
	Gi	214±110	36.0±2.7	1060±370	2 560±640	8660±1000
	Ma	94±90	33.3±8.0	332±117	3 420±1000	2260±350
	RO	59.1±22.3	—	295±43	1 160±180	1830±170
Br-06	Fo	13.1±6.9	ND	154±18	203±174	105±130
	Mu	10.7±3.9	ND	103±12	262±173	134±101
	VM	16.1±5.9	6.0±2.0	129±34	570±245	240±200
	He	72.9±39.2	15.3	187±9	800±560	140±80
	Gi	132±73	35.4±9.8	1030±140	2 480±1250	7120±640
	Ma	81.7±33.0	35.6±10.7	425±103	5 370±1240	2900±1400
	RO	46.8±8.3	—	320±46	1 520±180	1660±210
Br-11	Fo	12.8±2.9	ND	141±18	164±59	61±30
	Mu	5.6±2.1	ND	98±9	136±128	67±45
	VM	23.5	5.5	103	438	264
	He	56.6	20.2	187	880	199
	Gi	203	39.8	832	2 700	9870
	Ma	134	25.2	382	3 570	4290
	RO	68.2	—	272	1 260	2380

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TABLE 3. Relative contributions (%) of various tissues to the total metal burden in *E. complanata*.

Tissue	Cu	Zn	Fe	Mn	Relative dry weight
Gills	36±13	40±11	21±9	61±14	15±4
Mantle	28±13	25±11	58±11	31±14	17±3
Hepatopancreas	12±5	5±2	4±1	0.7±0.3	8±2
Visceral mass	18±7	16±4	15±4	7±3	36±5
Adductor muscle	3±1	7±2	1.4±0.4	0.6±0.1	15±2
Foot	3±1	7±2	0.8±0.2	0.3±0.2	10±2

for both Pb in the gills and Cu in the reconstituted organisms; using [Zn(F4)]/[Fe(F3)] increased r^2 by 21% over [Zn(S5)]. Based on the statistical analysis, however, it is difficult to associate bioavailability of sediment-bound trace metals to well-identified phases of sediment. Due to the restricted geographical area examined in this study, intercorrelations between the various metal fractions in the sediments are frequently observed, and for a given trace metal, tissue levels are often correlated with several metal fractions in the sediments. The difficulty in identifying key geochemical phases is compounded by the fact that the chemical extractions are not completely selective. To fully evaluate the predictive power of the various metal fractions in the sediments, studies should be conducted in watersheds of diverse geological characteristics.

The consistent increase in the correlation coefficient values that is observed when the trace metal concentrations extracted

from the sediments are normalized with respect to [Fe(F3)] suggests a "protective" or "competitive" role for Fe in the processes leading to the accumulation of trace metals by *E. complanata*. This Fe, extracted with hydroxylamine hydrochloride, presumably originates mainly from amorphous iron oxyhydroxides; well-crystallized iron oxides (e.g. goethite, hematite) are not easily attacked under these conditions (Rapin and Forstner 1983). The accumulation of Pb (Luoma and Bryan 1978) and As (Langston 1980) in *Scrobicularia plana* in various English estuaries was also well predicted by the ratios [Pb]/[Fe] and [As]/[Fe], respectively, in hydrochloric acid extracts of surface sediments; the Fe extracted with 1 mol HCl/L was considered by these researchers to be mainly amorphous iron oxides.

Depending on the main route for accumulation of trace metals in *E. complanata*, at least three mechanisms could explain an inverse dependence of trace metal accumulation upon the iron oxyhydroxide concentration in the sediment.

Mechanism 1

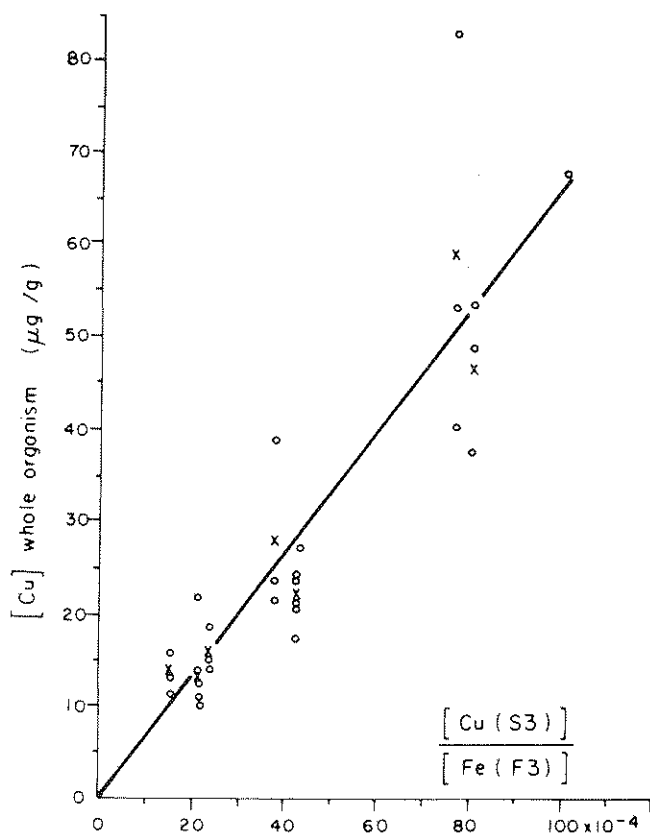If it is assumed that the major route for the accumulation of

FIG. 3. Relationship between the concentration of Cu in *E. complanata* and the ratio [Cu(S3)]/[Fe(F3)] in the sediments ($Y = 6854X - 0.02$; $r^2 = 0.95$). ○, individual specimens; ×, mean value at each station.

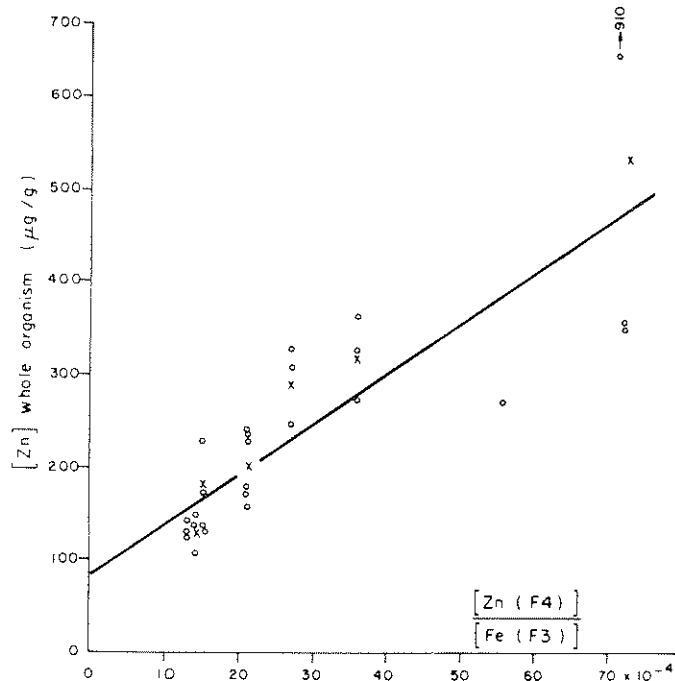FIG. 4. Relationship between the concentration of Zn in *E. complanata* and the ratio [Zn(F4)]/[Fe(F3)] in the sediments ($Y = 54256X + 86$; $r^2 = 0.79$). ○, individual specimens; ×, mean value at each station.

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TABLE 4. Correlation coefficients between Cu in sediments and Cu in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% levels (italicized) are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1).

Tissue	Cu(F2)	Cu(F3)	Cu(S3)	Cu(S5)	Cu(S3)/Fe(F3)	Cu(S3)/organic C
Fo	*0.846*	*0.852*	*0.872*	0.734	*0.945*	*0.898*
Mu	(0.595)	(0.463)	(0.548)	(0.556)	(0.652)	(0.617)
VM	0.826	*0.961*	*0.913*	0.757	*0.930*	*0.853*
He	0.805	(0.704)	0.778	0.718	*0.841*	0.732
Gi	0.790	*0.915*	*0.873*	(0.633)	*0.931*	*0.954*
Ma	*0.921*	*0.927*	*0.947*	*0.905*	*0.956*	*0.850*
RO	*0.873*	*0.943*	*0.930*	0.788	*0.974*	*0.921*

TABLE 5. Correlation coefficients between Zn in sediments and Zn in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% (italicized) levels are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1).

Tissue	Zn(F1)	Zn(F2)	Zn(F3)	Zn(F4)	Zn(S3)	Zn(S5)	Zn(F4)/organic C	Zn(S3)/organic C	Zn(F4)/Fe(F3)	Zn(S3)/Fe(F3)
Fo	0.753	(0.690)	0.746	(0.599)	0.745	0.742	(0.541)	(0.603)	(0.556)	0.759
Mu	(0.548)	(0.586)	(0.664)	0.784	(0.648)	(0.692)	(0.636)	(0.367)	*0.829*	0.770
VM	(0.541)	(0.579)	(0.639)	0.771	(0.628)	(0.677)	(0.528)	(0.277)	0.814	0.748
He	(0.355)	(0.396)	(0.578)	0.737	(0.528)	(0.610)	(0.689)	(0.295)	0.803	0.688
Gi	0.793	0.753	*0.845*	0.802	*0.833*	*0.847*	0.727	(0.640)	0.793	*0.894*
Ma	(0.523)	(0.638)	(0.606)	0.829	(0.618)	(0.665)	(0.434)	(0.127)	*0.860*	(0.698)
RO	(0.621)	(0.671)	0.722	*0.860*	0.712	0.760	(0.600)	(0.331)	*0.889*	0.814

TABLE 6. Correlation coefficients between Pb in sediments and Pb in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% (italicized) levels are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle.

Tissue	Pb(F2)	Pb(F3)	Pb(S3)	Pb(S5)	Pb(S3)/Fe(F3)	Pb(S3)/organic C
VM	(0.334)	(0.291)	(0.300)	(0.264)	(0.524)	(0.341)
He	(−0.304)	(−0.086)	(−0.136)	(−0.160)	(−0.187)	(−0.053)
Gi	(0.648)	*0.873*	*0.856*	0.774	*0.964*	*0.916*
Ma	(0.545)	(0.631)	(0.638)	(0.573)	0.751	0.706

trace metals in *E. complanata* involves the digestive system, and that acidic and/or reducing conditions prevail in the intestinal tract, one can envisage the simultaneous solubilization of both Fe and trace metals (Cu, Pb, Zn). The Fe could then compete with the trace metals for uptake sites in the digestive system and reduce their uptake (cf. Luoma and Bryan 1978).

Mechanism 2

If it is again assumed that accumulation of trace metals occurs predominantly via the digestive system, but that conditions prevailing within the gut (pH, pε, residence time) are such that the iron oxyhydroxides are not solubilized but remain in particulate form, then the unreacted iron oxyhydroxides could compete with the uptake sites in the intestinal tract for the

solubilized trace metals. Higher levels of iron oxyhydroxides in the ingested sediment would reduce the concentrations of dissolved Cu, Pb, and Zn in the gut during digestion and thus result in lower uptake of these metals.

Mechanism 3

Alternatively, and perhaps more likely, the main route for accumulation of Cu, Pb, and Zn by *E. complanata* may involve not the ingestion of particulate material but rather the uptake of dissolved trace metals via the gills and mantle. Then the protective role of the iron oxyhydroxides could be explained by invoking adsorption in the *external* medium as the principal factor controlling the dissolved trace metal concentrations to which the organism is exposed. This adsorption can be

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characterized in a simplified manner by

(2)   M + S(k) = MS(k)

$$(3) \quad D(k) = \frac{[MS(k)]}{[M][S(k)]}$$

where D(k) is a distribution coefficient and [MS(k)], [M], and [S(k)] represent, respectively, the concentration of adsorbed trace metal M, the concentration of dissolved trace metal, and the number of sites available for adsorption on the substrate k (cf. Oakley et al. 1981). Clearly, as the concentration of adsorbing substrate ([S(k)] ≡ [Fe(F3)]) increases, the concentration of dissolved trace metal will decrease.

The high levels of trace metals associated with the gills and mantle (Table 2) and the high contribution of these two organs to the body burden (Table 3) suggest that uptake (or extracellular binding) of dissolved trace metals is an important route of accumulation of these elements in *E. complanata*. These two organs are physiologically remote from the digestive system of the organism, are in contact with large amounts of water necessary for feeding and respiration (approximately tens of litres per day; Mellanby 1938; Mohlenberg and Riisgard 1979), and have large surface areas. The above reasoning, coupled with the fact that most filter feeders selectively feed on phytoplankton and minimize their digestion of inorganic sediments, and with the observed inverse relationship between trace metal levels in the gills or mantle and [Fe(F3)], lends support to mechanism 3. The possibility of a direct uptake of particulate trace metals by the gills and mantle by endocytosis cannot be rejected (Brown 1982); Coombs (1980) has described such a process, i.e. engulfment of the particulate metal by the epithelial cell membrane, which then pinches off to form membrane-limited vesicles within the cell.

In addition to the iron oxyhydroxides discussed above, other sediment constituents might be expected to influence the availability of trace metals to benthic organisms; for example, manganese oxides and organic matter often constitute important sinks for trace metals in the oxidized layer of freshwater sediments (Tessier et al. 1980, 1982; Forstner and Wittman 1981; Lion et al. 1982). Normalizing the Cu and Pb levels in the sediments with respect to the organic carbon content of the sediments does indeed increase the correlation coefficients (Tables 4–6). Similar results were reported by Langston (1982) who found that Hg accumulation in *Scrobicularia plana* and *Macoma balthica* was best predicted by the ratio of the Hg concentration extracted from the sediment with concentrated HNO_3 to the organic content (ashing at 400°C) of the sediments. It should be noted that the apparent controlling effect of organic matter is observed for trace metals for which the stability constants with organic ligands are high (Hg, Cu, Pb) and not for other trace metals with lower stability constants (e.g. Zn; Table 5).

In conclusion, the present study demonstrated that (1) trace metal accumulation in *E. complanata* in the Rouyn–Noranda area is best related to one or more fractions of the total metal load (rather than to the latter) and (2) metal accumulation is also influenced by the protective or competitive effect of sediment constituents, notably amorphous iron oxyhydroxides. These observations confirm those made previously for benthic organisms in the estuarine environment (Luoma and Bryan 1978; Langston 1980).

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