Insights into sequential chemical extraction procedures from quantitative XRD: a study of trace metal partitioning in sediments related to frog malformities

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Abstract

Sequential chemical extraction (SCE) and quantitative X-ray diffraction (QXRD) were used in combination to assess trace metal speciation and availability in sediment from two separate marshes in western Vermont, USA, one with high (45%) and one with low (< 5%) northern leopard frog (*Rana pipiens*) malformity rates. The types of malformities observed in the field are very similar to malformities produced in laboratory studies where frogs are exposed to waters and sediments with elevated levels of trace metals such as Co, Cu, Ni, and Zn. Total recoverable metals, as determined by US EPA method 200.2 (HCl–HNO₃), are statistically higher in the malformity-affected marsh than in the marsh with low malformity rates, suggesting possible causality. Analysis of trace metal speciation by SCE and QXRD, however, implies that the most common reservoir of Co, Cr, Cu, Ni, and Zn in the sediment is trioctahedral clay minerals, including chlorite, high charge smectite and vermiculite. The only trace metal that is not associated with trioctahedral clay is Pb, which appears to be contained in poorly crystalline or amorphous oxyhydroxides. The proportions of trace metals in the exchangeable fraction are variable, with values of $\leq 3\%$ of total Cr and Pb, 2–17% of total Cu and Ni, and up to 24% of total Co and Zn. Total metals concentrations may be slightly elevated relative to background values, and the partitioning of Co, Cu, Ni, and Zn into the exchangeable fraction implies a mechanism for interaction with frogs. Furthermore, partitioning of Pb into poorly crystalline or amorphous oxyhydroxides suggests that it might be mobilized when water levels rise and cause reduction and dissolution of oxyhydroxides in submerged sediment. The difference in speciation between Pb on the one hand, and Co, Cr, Cu, Ni, and Zn on the other, implies that only Pb has an anthropogenic source. Mn speciation differs from all other metals in that it is subequally partitioned into exchange sites, hydroxides and trioctahedral clay. Fe is predominantly partitioned into trioctahedral clay, with lesser amounts in hydroxide form. QXRD indicates that expandable trioctahedral clay dissolves progressively throughout the SCE procedure, including during extractions intended to (1) oxidize sulfides and organic matter, and (2) reduce oxides and hydroxides, indicating that SCE procedures are not entirely phase selective. It also underscores the need for quantitative mineralogical analysis in conjunction with SCE, and has important implications for the interpretation of metal speciation by SCE alone and without corroborating QXRD analyses. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Sequential chemical extraction; XRD; Chlorite; Smectite; Trace metals

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1. Introduction

Analysis of trace metal content in soil and sediment is essential for determining anthropogenic environmental contamination, contributions from chemical weathering, and the relationships between bioavailability and sequestering of metals (Tessier et al., 1979; Szefer et al., 1995; Dabkowska-Naskret, 1997; Cabral and Lefebvre, 1998). While analyses of total metals concentration in soils and sediments are useful in some cases, it is often necessary to determine metal partitioning and availability under natural conditions. Given that heavy metal speciation is primarily a function of the mineralogy and chemistry of the solid fraction (Tessier et al., 1979; Calloway et al., 1998; Song et al., 1999), a number of sequential extraction procedures have been applied to the analysis of metal partitioning between such fractions as exchangeable sites, organic matter, and minerals of varying solubility (Tessier et al., 1979; Kersten and Förstner, 1986; Ure et al., 1993). The problem with such procedures, however, is the unequivocal, quantitative determination of phase presence, stability and solubility during the various chemical extractions (Tessier et al., 1979; McCarty et al., 1998; Song et al., 1999). Most studies make assumptions regarding phase stability based on thermodynamic models and semi-quantitative X-ray diffraction analyses and, as a result, phase dissolution has been commonly characterized as operationally defined (Tessier et al., 1979; Ure et al., 1993; Cabral and Lefebvre, 1998; Usero et al., 1998).

One of the pioneering studies of sequential chemical extraction is that of Tessier et al. (1979). In that study, the authors designed a series of sequential extractions intended to progressively release (1) adsorbed and exchangeable cations, (2) metals contained in carbonates, (3) metals bound in Fe and Mn oxides, (4) metals bound in organic matter and sulfides, and (5) residual metals contained primarily in silicate minerals. In order to verify which minerals dissolved during the extractions, XRD patterns were obtained prior to and after each extraction. These analyses allowed for qualitative interpretations of mineral proportions but only semi-quantitative evaluations due to doubts as to whether the reagents were perfectly selective and concerns regarding particle orientation in XRD mounts (Tessier et al., 1979; Cabral and Lefebvre, 1998).

X-ray diffraction (XRD) is commonly applied to the identification of crystalline phases, particularly in fine-grained specimens. However, without careful sample preparation, it is impossible to use XRD analyses for quantitative analysis. The main reason for this is the problem of preferred orientation of minerals with anisotropic forms such as micas, clays, and feldspars (Bish and Reynolds, 1989; Hillier, 1999). The degree of orientation controls the intensity of diffraction peaks, and without a means of eliminating preferred orientation, quantitative XRD analysis of sediments and soils is exceedingly difficult. Furthermore, differences in particle orientation in sample mounts prepared before and after a given chemical extraction could lead to inaccurate interpretations of phase dissolution and/or abundance if particle orientation differs between sample mounts. Quantitative XRD analyses are only possible if either (1) degree of particle orientation can be accurately measured, or (2) sample mounts can be prepared in which particles are randomly oriented. A spray dry method developed by Hillier (1999) produces sample mounts with no preferred orientation, and thus makes feasible quantitative mineralogical analyses of soils and sediments.

The purpose of this study is to combine sequential chemical extraction (SCE) with accurate, reproducible quantitative X-ray diffraction (QXRD) analyses to determine the availability and speciation of trace metals in marsh sediment from two wetlands in the Champlain Valley of the eastern USA. These particular sediments are of interest for two reasons. One is the documentation of abnormally high frog malformity rates (*Rana pipiens*; Northern Leopard Frog) in one of the wetlands studied (Meteyer, 1997; Fort et al., 1999). A subsequent study documented significantly higher total extractable metals in sediments from the pond with high malformity rates (Wall, 1999; Wall and Ryan, 1999). In order to assess quantitatively the phase dissolution associated with each extraction, QXRD patterns were run before and after each extraction procedure and quantified using an internal standard (corundum), which was added to the specimen after chemical extraction. All powders were spray dried to enable quantitative mineralogi-cal analysis (Hillier, 1999) before and after each chemical extraction.

1.1. Previous work

The impetus for this study results from previous field studies of northern leopard frog (*Rana pipiens*) malformities in marshes adjoining Lake Champlain in western Vermont (Meteyer, 1997) and related laboratory studies (Meteyer, 1997; Fort et al., 1999). Frog embryo teratogenesis assays on *Xenopus* frogs (FETAX) clearly demonstrate that trace metals such as Co, Cr, Cu, Ni, and Zn cause developmental malformities such as missing hind limbs and eyes and slow tail resorption (Harfenist et al., 1989; Luo et al., 1993; Plowman et al., 1991, 1994). In response to observations of similar malformities in numerous Vermont marshes, Meteyer (1997) and Fort et al. (1999) carried out in vitro developmental studies of *R. pipiens* in sediment obtained from marshes with high rates of frog malformities. These studies documented malformities similar to those produced in the FETAX analyses of Luo et al. (1993) and Plowman et al. (1991, 1994), suggesting trace metals as a potential cause of malformities. A study by Wall (1999) and Wall and Ryan (1999) revealed a positive correlation between rates of occurrence of physiological malformities in *R. pipiens* and the concentration of heavy metals in associated wetland sediment. Results indicated statistically higher concentrations of Zn, Ni, Co, and Cr in the "affected" wetland sediment as compared to sediment from the control site (Fig. 1). However, the actual availability of metals in the

sediment to the frogs was unknown. Metals were extracted from sediment according to EPA method 200.2, an aqua regia (HNO_3–HCl) extraction that does not reveal metal speciation, and provides no information on the bioavailability of metals.

Positive correlations among Al, Fe, Mg, Co, Cr, Cu, Ni, and Zn and preliminary, non-quantitative XRD analyses suggested that the trace metals might have been released by dissolution of chlorite during aqua regia extraction. An example of such correlation between Ni and Al is given in Fig. 2. These results are significant because (1) they implied differing availabilities of these metals, and (2) the primary metal source might not be anthropogenic activity but rather chemical weathering of minerals. Both of these points have important implications for metals bioavailability, source, and the relationship between metals and observed frog malformities. The current study was designed to address the issue of environmental availability and phase partitioning using a more sophisticated sequential extraction procedure and accurate QXRD.

1.2. Field sites

The two sites selected for this study are in the Champlain Valley of western Vermont, USA (Fig. 3). Both sites are wetlands that border Lake Champlain. Ward Marsh, a site with a very high (45%) malformity rate in 1997, is at the southern end of Lake Cham-
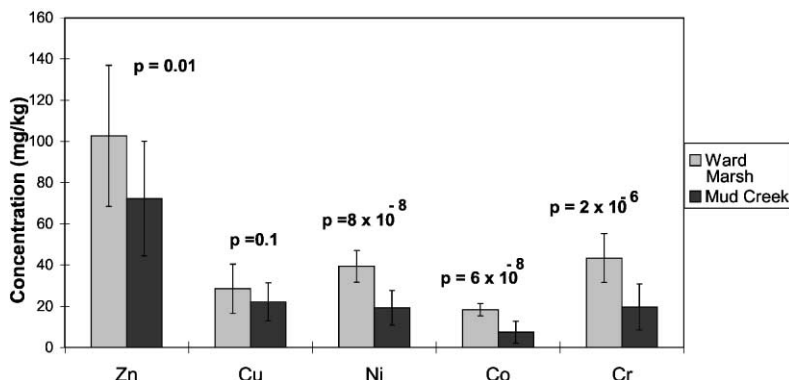

Fig. 1. Comparison of Zn, Cu, Ni, Co, and Cr concentrations (aqua regia-recoverable analytes) in sediment from Ward Marsh (45% malformed frogs in 1997) and Mud Creek (<5% malformity rate). Concentrations are in mg kg^{-1} (ppm). Error bars represent the standard deviation of the sample population. Values of $p \leq 0.05$ indicate statistically significant difference.
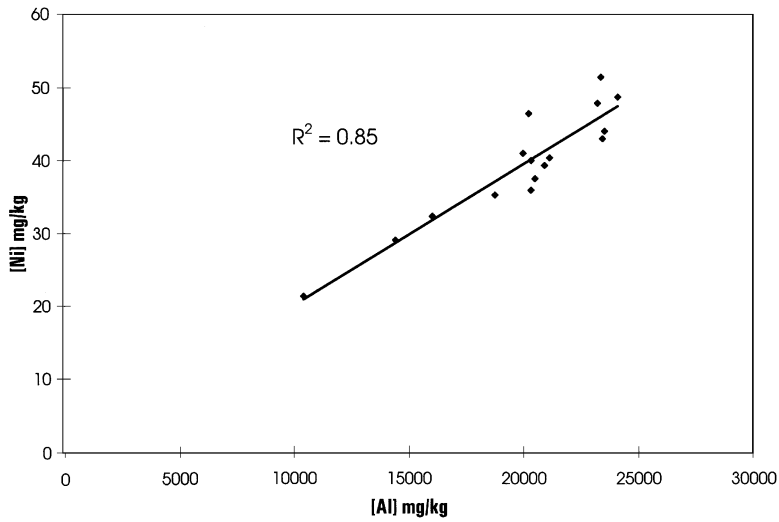
Fig. 2. Correlation of Al and Ni concentrations in sediment from Ward Marsh, shown to illustrate the positive correlation between trace metals and silicate clay components.
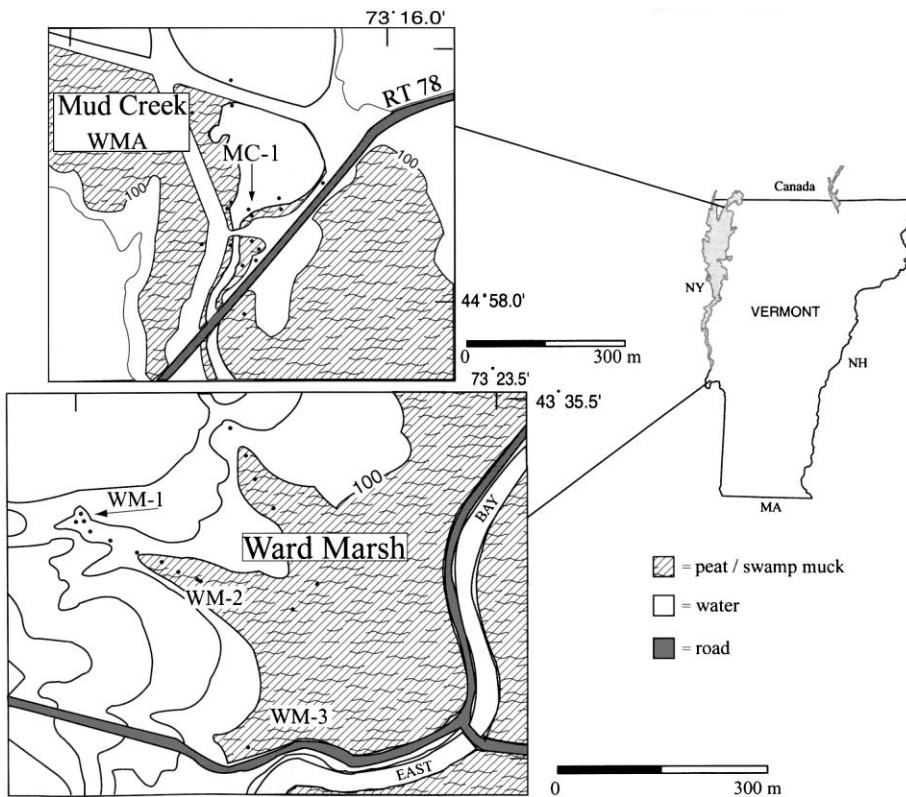

Fig. 3. Location maps. Dots represent sample localities. Those denoted with WM or MC indicate sites analyzed by sequential extraction. All other sites were analyzed for aqua regia-extractable metals and mineral content and quantity. Sources: Rouses Point and Whitehall 15′ US Geological Survey Quadrangle Maps.

plain, at the confluence of the East Bay of the lake and the Poultney River. The source areas of sediment in this marsh are the Taconic Slate Belt and the metamorphic rock-dominated Green Mountains (Doll et al., 1961). The Mud Creek site, characterized by background ($\leq 5\%$) malformity rates, is a wetland that borders Lake Champlain and is bisected by a channel through which a small, stagnant stream (Mud Creek) flows. Sediment source is the limestone-dominated Champlain lowlands of southeastern Quebec. In both marshes the sediment is rich in clay and organic material. Both marshes are affected by variations in Lake Champlain water level, although Ward Marsh is affected to a greater extent due to its low gradient and broad, expansive banks. The Mud Creek wetland is narrower and has steep, well-defined banks.

1.3. Materials and methods

In July of 1999, three 40 cm cores were collected from Ward Marsh: one at a site above the high-water mark that is isolated from the normal fluctuation of water level (WM-1), a second in the interior of the marsh above the typical water level but well below the high water mark (WM-2), and a third from a perennially subaqueous site near the confluence with the Poultney River (WM-3). A core sample of submerged clay-rich sediment was taken from Mud Creek (MC-1) at a site that remains subaqueous and, like WM-3, is not prone to changing redox conditions.

All core samples were obtained using a stainless steel corer that was rinsed in dilute nitric acid and deionized water (DI) and thoroughly immersed in and mixed with sediment surrounding the sample to prevent contamination from a previous location. Samples from the top 10 cm of the core are demarcated with an A, whereas the bottom 10 cm (30–40 cm depth) are indicated with a D (e.g. WM-1A, WM-1D). Specimens were stored at room temperature for 2 days in sealed polyethylene bottles, and then were oven dried at 60 °C for 24 h in acid rinsed beakers prior to sequential extraction. All analyses were performed within 3 weeks of sampling. Prior to drying, sediments were stored under conditions essentially the same as the field conditions. However, storage and transport at room temperature, and handling under ambient atmosphere, may have imparted minor changes to the redox composition of the sediments.

Sediment samples were subjected to the modified BCR three-step sequential extraction procedure developed by the Standards, Measurements and Testing Programme (formerly BCR) of the European Commission (Rauret et al., 1999). In addition, aqua regia extraction US EPA 200.2 comprised the fourth stage of the method. All reagents were trace metal grade. Certified reference material S33 (BCR standard) was used to gauge analytical accuracy and recovery, and to assess the effect of sequential extraction on mineral stability in a well-known reference material.

Oven dried sediment samples were sub-sampled into 2.4-g aliquots using a sample splitter. Each sample was oven dried at 105 °C to drive off adsorbed water. All results were calculated using oven-dried weights. Soil pH was determined in deionized water (DI) at a 1:1 ratio of soil solids: DI.

The reagents used in the BCR method, along with the operationally defined extracted sediment components, are described below (Usero et al., 1998).

• <u>Exchangeable ions and acid extractable (carbonates)</u>: 100 ml of 0.11 M acetic acid (HOAc) was applied to each 2.4-g sample. Sample was shaken overnight in an end-over-end shaker at room temperature. Supernatant was separated from the residue by centrifuging at $3000 \times g$ for 20 min. Supernatant was then filtered through EDTA washed No. 542 filter paper into polypropylene bottles for analysis. Residue was rinsed with Millipore water in an end-over-end shaker and separated by centrifugation.

• <u>Reducible (Fe-, Mn-oxides)</u>: 100 ml of 0.5 M hydroxylamine hydrochloride (HA–HCl) adjusted to pH 2 with 2 M HNO_3 was added to the rinsed residue, which was processed according to the procedure for HOAc.

• <u>Oxidizable (sulfides, organic matter)</u>: 25 ml of 8.8 M hydrogen peroxide (H_2O_2) was added to each residue and allowed to digest for 1 h with occasional manual mixing. Samples were transferred into a water bath to continue digestion at 85 °C (bottle tops loosened to allow release of pressure) for 1 h after which the bottle caps were removed and samples were allowed to evaporate down to near dryness. This step was repeated. 125 ml of 1 M ammonium acetate (NH_4OAc) adjusted to pH 2 with 2M HNO_3 was used to extract ions from the residue and procedures were repeated as described in the previous steps.

• Residual (some silicates, well-crystallized oxides): EPA method 200.2, an aqua regia extraction that is a precursor to the current method 3050b, was used to determine the concentration of total recoverable analytes by ICP-AES. This method is "designed to dissolve almost all elements that could become environmentally available" (http://www.epa.gov/epaoswer/hazwaste/test/3050b.pdf). The aqua regia solution, comprised of 10 ml (1:1) HNO_3 and 25 ml of (1:4) HCl, was added to each 2.4-g aliquot of residue and placed in a 95 °C water bath for 30 min. Samples were filtered and the supernatant was transferred to a 250-ml volumetric flask.

The exchangeable and carbonate fractions were not assessed separately due to time constraints, and XRD analyses indicated the occurrence of carbonate (dolomite) in only trace amounts ($\leq 1\%$ of the untreated powders).

In order to facilitate QXRD analyses, five individual sub-samples were prepared from each sample site. One sub-sample was prepared for QXRD without any chemical treatment. The remaining four sub-samples were treated by one or more steps of the sequential chemical extraction before preparation for QXRD. Thus one of the sub-samples was exposed to HOAc, one to HOAc followed by HA−HCl, one to HOAc, HA−HCl and H_2O_2, and one to HOAc, HA−HCl, H_2O_2 and HNO_3−HCl·HNO_3−HCl. This procedure was necessary in order to insure that sufficient sample was available for XRD. ICP-AES analyses were performed on extracted supernatants at each step, and QXRD analyses were carried out on the residues. All powders were prepared for QXRD analysis according to the spray drying method of Hillier (1999). An accurately weighed 20% addition of corundum was added to the powder to serve as an internal standard for quantitative analysis. This mixture of powder and standard (≈ 3 g) was suspended in approximately 10 ml of a 0.5% (w/v) aqueous solution of polyvinyl alcohol (PVA), and milled for 12 min in a McCrone Micronizing Mill. The resulting slurry was spray dried directly from the mill, producing powders consisting of 50 μm diameter aggregates of particles showing no preferred orientation. These prepared powders were toploaded and analyzed in the air-dried state. The <2-μm fraction was separated from selected samples to enable detailed analysis of (1) the mineralogy of the fine, reactive clay-size fraction, and (2)

detailed mineralogic characteristics of clay minerals. Oriented mounts were prepared according to the filter peel-membrane filtration technique of Drever (1973). These samples were analyzed in air-dried, ethylene glycol-solvated, and heated (250 °C) states.

XRD data were collected on an automated Siemens D-5000 diffractometer with a θ/θ goniometer, Co source, a diffracted beam monochromator, 1° slits, and 0.02° steps with 2-s counts per step. Additional, detailed XRD analyses with 10-s counts were performed on selected powders to enable more precise mineral quantification (MC-1A and WM-2A).

Peak intensities for quantitative analysis were determined by measuring integrated areas using Jade® software(http://www.mdi.com). Reference intensity ratios (RIRs) for each mineral were calculated by (1) analyzing 50:50 (by wt.) spray dried mixtures of corundum (internal standard) and pure mineral, (2) determining integrated peak area intensities of diagnostic corundum (e.g. 2.09 Å) and mineral peaks (see below), and (3) determining ratios of standard: mineral, which were entered into a spreadsheet to facilitate mineral % determinations from sediment samples. Muscovite (dioctahedral mica) determinations were based on peak areas of the 02,11 peak at 4.48 Å, the 001 at 10 Å, and the 060 at 1.50 Å. Trioctahedral clay was quantified by measuring the peak area of the 4.67-Å 02,11 peak and verified by comparison to the 060 at 1.54 Å (quartz subtracted). The 4.67-Å peak occurs as a distinct peak on the low-angle shoulder of the 4.48-Å muscovite 020 peak. The percentage of chlorite was determined by measuring the peak area of the 7.1-Å peak and subtracting the area of the 7.1-Å kaolin 001 peak. Kaolin is defined as the 7.1-Å peak area remaining after aqua regia extraction — following aqua regia, there is no evidence of a trioctahedral 020 peak, indicating that the post-aqua regia 7-Å peak cannot be produced by chlorite (or serpentine). Other peaks used are as follows: for quartz, the 4.26-Å peak (100); for Na-feldspar, peaks at 3.19 Å (002, 040) and 4.04 Å (201); and for K-feldspar, the combined 020 and 004 peaks at 3.24 Å. Hornblende was quantified using the 110 peak at 8.4 Å. Other measurable peaks were used for verification of each phase. Serpentine, if present, is indistinguishable from chlorite in this study. First, the methods used would not be capable of detecting small amounts of serpentine, and second, serpentine is compositionally and structurally similar to chlorite, with

similar XRD characteristics (Bailey, 1988; Bailey et al., 1994; Ryan and Reynolds, 1996). Unless stated otherwise, expanded uncertainty using a coverage factor of 2, i.e. 95% confidence, is given by $\pm X^{0.35}$, where X = concentration in wt.%, e.g. 30 wt.% ± 3.3.

Carbon and nitrogen content in the sediments was determined using an automated catalytic oxidation technique on an NA1500 Carlo Erba Elemental Analyzer.

2. Results

Concentrations of aqua regia-extractable metals are significantly higher ($p < 0.05$) in Ward Marsh, the site with elevated malformities, than in Mud Creek, with the exception of Cu, which has a p-value of 0.1 (Fig. 1). Aqua regia extractable trace metal concentrations are similar to the composition of average shale (Turekian and Wedepohl, 1961; Taylor and McLennan, 1985), are slightly elevated relative to background values for some soils (McBride, 1997) and are similar to some soils with naturally elevated metals content (McLean and Bledsoe, 1992; Lottermoser, 1997).

2.1. Speciation by specific extractant

The distribution of metals extracted by the different reagents is presented in Fig. 4 and Table 1. In Table 1, steps 1–4 correspond to the first four stages of the SCE procedure, and step 5 is the result of the aqua regia extraction on fresh, unaltered powder. With the exception of Pb, all the metals showed similar speciation, with the greatest concentration of metal released during aqua regia extraction, followed by HA–HCl extraction. Relatively little metals are contained in exchangeable sites and oxidizable phases, with the notable exception of Mn in WM-1A and WM-3A. The overwhelming majority of Pb was released during HA–HCl extraction.

2.1.1. Exchangeable (HOAc)

A relatively small proportion of metals found in both Ward Marsh and Mud Creek were released during HOAc extraction, implying that relatively small amounts of metals are contained in exchangeable sites. The notable exception is Mn, for which 36% (range = 8–58%) of total Mn recovered was extracted

by HOAc. Of the heavy metals, Co, Ni, Cu, and Zn had the highest percentages associated with HOAc extraction, ranging from 4% to 24% (1–3 mg kg^{-1}). Site WM-3A generally had higher concentrations of exchangeable metals with 24% of Zn in this first stage. WM-3D, which was sampled from 30 to 40 cm below the surface, had only 6% of Zn in exchangeable sites. Even though the proportion of Zn in exchangeable sites (vs. other sites) is less than the percentage exchangeable for other metals, the amounts by mass of exchangeable Zn are higher, ranging from 1 to 18 mg kg^{-1}, reflecting higher total recoverable Zn concentrations. In contrast, Pb and Cr contained ≤ 1 mg kg^{-1} in exchangeable sites, or $\leq 2\%$ of total recoverable.

2.1.2. Oxides, hydroxides (HA–HCl)

Pb speciation is significantly different than all of the other metals. The majority of Pb (63–90% of total recoverable Pb) was liberated during the HA–HCl extraction. By contrast, only 14% to 27% of Ni and Cr, 21% to 38% of Cu and Zn, and 25% to 46% of Co (% of total recovered) were liberated during HA–HCl extraction. HA–HCl extractable Mn was similar to the majority of metals with one notable exception, WM-2D (30–40 cm depth), where 73% of Mn was extracted by HA–HCl. From 14% to 31% of Mn was extracted by HA–HCl in the other samples. High amounts of Pb released during HA–HCl imply that Pb is contained in easily-to-moderately reducible oxide or hydroxide phases. The lack of oxide and hydroxide peaks in XRD patterns, combined with the release of appreciable amounts of metals, particularly Fe, by HA–HCl, is consistent with the presence of amorphous hydroxides. However, we cannot rule out the possibility that HA–HCl extraction caused desorption of Pb (and other metals to a lesser extent) that were not extracted by the relatively weak HOAc extraction.

2.1.3. Sulfides, organic material (H_2O_2)

Based on relatively low metals concentrations recovered during H_2O_2 extraction, oxidizable phases are not a significant source of metals in these sediments. Proportions of Co, Ni and Cu liberated by H_2O_2–NH_4OAc were similar to values for HOAc extraction. It is worth noting that virtually no Zn was liberated during this stage, whereas up to 27% of Cr is speciated into oxidizable phases.
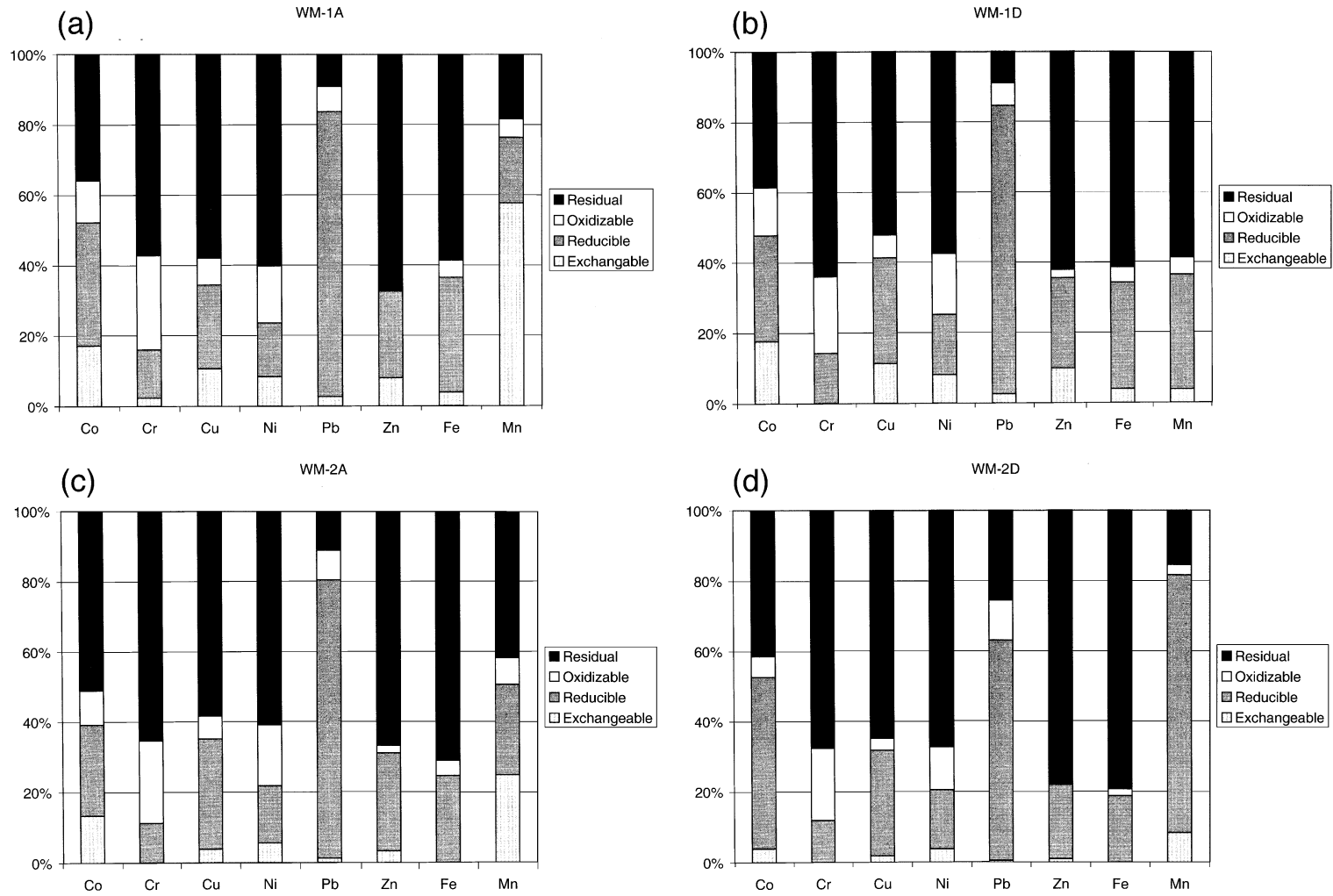
Fig. 4. Partitioning of trace metals as determined by sequential chemical extraction. Note significant difference between Pb and other metals.
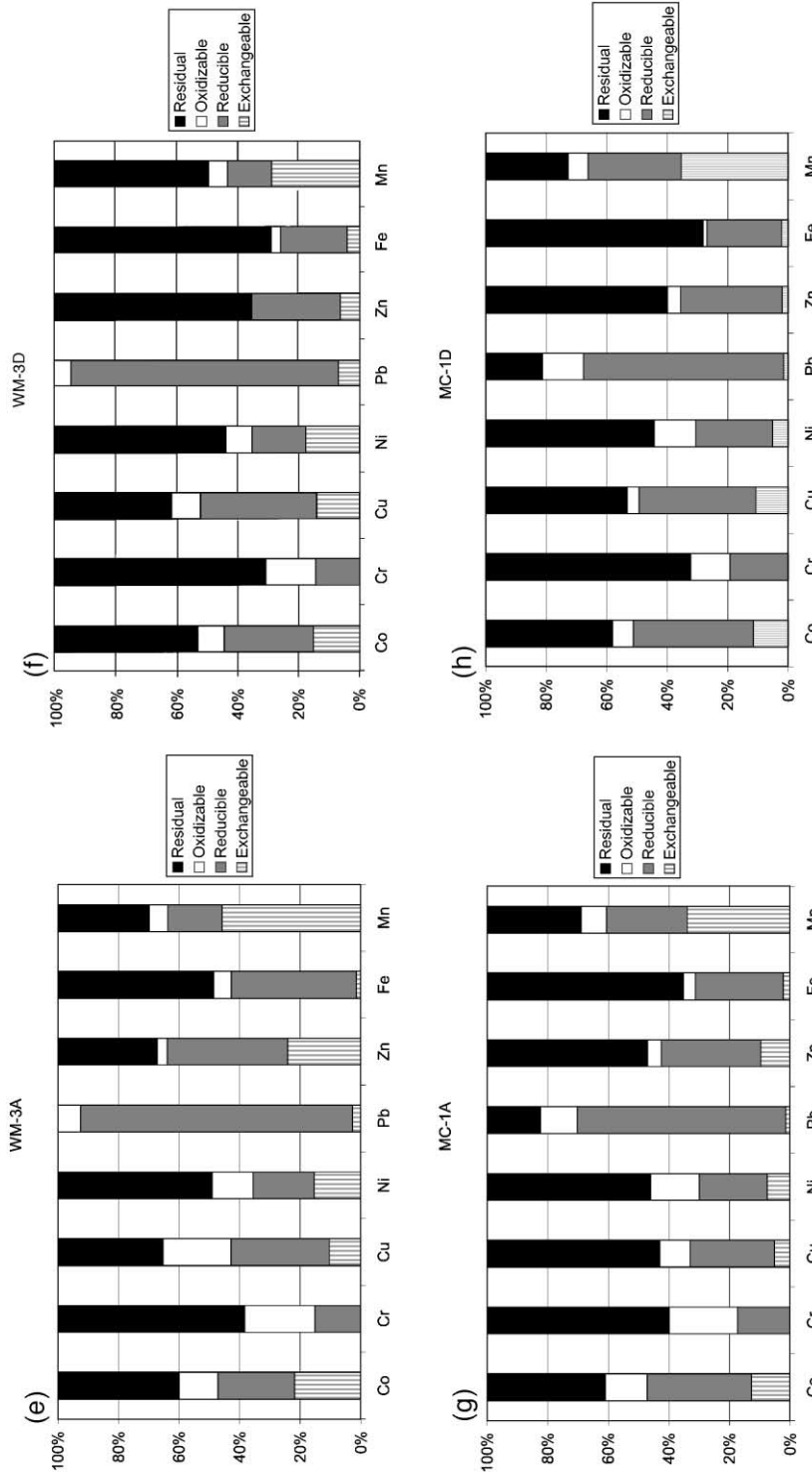
Fig. 4 (*continued*).

Table 1
Results of sequential extraction, expressed in mg/kg. Fractions 1–5 represent successive chemical extraction stages

Fraction	WM-1A	WM-1D	WM-2A	WM-2D	WM-3A	WM-3D	MC-1A	MC-1D	SRM 33
Cd 1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	BD	BD	BD	8.7±1.9
2	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.9±0.1
3	BD	BD	BD	BD	BD	BD	BD	BD	BD±0.0
4	<0.032	<0.032	<0.032	<0.032	<0.032	<0.032	<0.032	<0.032	0.1
5	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	10.9
Co 1	2.4±0.0	3.2±0.1	2.0±0.0	0.8±0.1	1.5±0.0	1.1±0.1	1.6±0.1	1.9±0.1	2.5±0.1
2	4.9±0.1	5.4±0.3	3.9±0.0	10.3±1.4	1.8±0.0	2.2±0.1	4.3±0.1	6.6±0.2	3.9±0.1
3	1.7±0.2	2.5±0.0	1.5±0.1	1.3±0.0	0.9±0.2	0.7±0.1	1.7±0.1	1.1±0.1	1.4±0.1
4	5.0	6.9	7.7	8.8	2.8	3.5	4.8	7.0	6.0 0.1
5	13.4	17.6	14.8	19.3	6.8	16.9	11.1	15.9	13.2
Cu 1	2.0±0.1	2.8±0.1	1.1±0.1	0.4±0.0	1.9±0.2	2.4±0.1	1.0±0.0	3.3±0.1	55.4±1.4
2	4.5±0.1	7.5±0.1	8.7±0.0	6.1±0.1	5.9±0.4	6.4±0.1	5.4±0.2	11.9±0.1	133.2±1.2
3	1.5±0.0	1.6±0.0	1.8±0.1	0.7±0.0	4.1±1.9	1.6±0.1	2.0±0.1	1.2±0.1	59.5±2.3
4	11.1	13.0	16.3	13.2	6.3	6.5	11.1	14.4	38.8 1.4
5	20.1	25.6	29.0	20.5	19.3	40.5	19.4	30.2	281.9
Ni 1	1.9±0.1	2.7±0.1	2.0±0.1	1.3±0.1	2.3±0.2	2.8±0.1	1.7±0.1	1.7±0.1	17.1±0.2
2	3.5±0.1	5.8±0.0	6.0±0.1	6.0±0.6	3.0±0.1	2.8±0.2	5.1±0.1	8.3±0.0	32.8±0.3
3	3.7±0.2	5.9±0.0	6.5±0.0	4.4±0.2	2.0±0.8	1.3±0.1	3.7±0.1	4.5±0.2	13.4±0.3
4	13.9	19.5	22.7	24.2	7.6	9.0	12.3	18.1	24.4 0.4
5	23.6	34.8	38.0	35.0	15.5	40.9	22.9	31.9	87.4
Pb 1	0.5±0.1	0.5±0.1	0.3±0.0	0.1±0.0	0.3±0.0	0.7±0.0	0.2±0.0	0.2±0.0	3.5±0.2
2	13.9±0.6	16.4±0.5	17.2±0.1	10.3±0.5	11.0±0.3	8.5±0.3	8.5±0.6	6.9±0.4	136.4±2.1
3	1.2±0.0	1.3±0.0	1.8±0.0	1.9±0.0	0.9±0.1	0.5±0.0	1.5±0.0	1.4±0.0	8.2±0.7
4	1.6	1.8	2.4	4.2	0.0	0.0	2.2	2.0	8.6 1.7
5	16.3	19.1	20.7	15.0	12.6	24.5	11.9	9.4	142.2
Zn 1	4.4±0.1	7.5±0.1	3.0±0.0	0.7±0.0	17.5±6.2	2.5±0.1	6.6±0.2	1.5±0.0	213.9±1.8
2	13.6±0.2	19.6±0.2	25.7±0.1	15.6±0.2	28.9±17.1	11.5±0.2	22.6±0.3	25.1±0.1	126.0±2.8
3	BD	1.8±0.1	2.0±0.1	BD	2.4±3.1	BD	3.1±0.2	3.3±0.1	35.0±2.4
4	37.5	47.8	61.9	58.2	23.8	26.1	36.3	45.0	76.8 3.1
5	60.0	81.7	97.1	77.1	68.7	106.1	70.6	77.3	444.4
Fe 1	891.2±24.9	1146.4±12.5	12.0±0.5	3.2±0.7	292.2±26.0	582.2±9.4	522.5±11.3	807.7±23.6	105.1±4.4
2	7576.2±101.6	8777.7±59.4	8031.3±69.9	6715.7±161.6	8197.9±245.6	3257.2±35.6	6594.3±66.8	8914.4±60.5	9327.8±68.8
3	1140.2±31.8	1265.6±16.5	1415.2±16.3	694.5±19.6	1150.2±389.6	425.3±19.6	895.5±24.0	436.9±4.1	1024.4±30.4
4	13,638.6	17,883.7	23,257.1	28,415.7	10,183.9	10,646.7	14,750.6	25,920.6	18,109.4 229.5
5	23,269.1	29,417.7	31,921.9	35,822.7	19,154.9	35,706.2	25,428.0	36,464.8	28,034.3
Mn 1	320.0±5.1	290.9±1.9	100.8±1.6	163.8±9.1	128.7±6.9	55.3±0.5	82.5±1.3	172.5±1.2	200.0±0.9
2	103.8±2.1	126.1±1.0	104.7±0.8	1449.1±286.9	50.4±1.6	28.3±0.4	64.8±1.5	149.8±2.6	159.0±4.4
3	29.2±0.5	35.7±0.2	30.7±0.2	56.9±7.4	17.1±5.0	12.1±0.2	20.0±0.1	32.1±0.1	22.7±0.6
4	102.0	139.3	170.1	305.4	84.6	98.2	75.5	132.9	178.8 1.2
5	541.6	584.9	407.3	1734.9	275.8	473.5	230.5	464.5	545.1
Cr 1	0.6±0.1	BD	BD	BD	BD	BD	BD	BD	2.6±0.1
2	3.5±0.2	4.8±0.1	4.3±0.0	4.4±0.1	1.7±0.1	1.6±0.1	5.0±0.1	7.5±0.2	54.7±0.6
3	6.9±0.1	7.3±0.0	9.1±0.1	7.7±0.1	2.6±0.5	1.8±0.0	6.5±0.2	5.1±0.0	160.7±1.3
4	14.8	21.5	25.4	25.3	6.9	7.6	17.4	26.3	31.1 0.2
5	25.3	34.1	39.0	36.5	11.4	27.7	28.5	38.5	244.9

2.1.4. Resistant minerals (HNO_3–HCl)

The final step aqua regia procedure is designed to extract metals remaining in the sediment after stages 1–3. It is not a total dissolution, and likely does not extract all remaining metals, but most likely does extract those remaining in well-crystalline oxides and some silicates. Phases that dissolved during this extraction are the dominant source of metals (excluding Pb) in these sediments, with percentages of total extracted ranging from 36% to 69%.

Aqua regia extractions were also performed on fresh powders. These results are reported in line 5 of Table 1. Sums of stages 1–4 are within 5% of aqua regia extraction on fresh powder.

2.2. Speciation by metal

Lead. The majority of Pb was released during HA–HCl extraction (63–90% of total recoverable metals), suggesting that Pb is primarily contained in easily or moderately reducible hydroxides or oxides. Very little Pb was liberated by HOAc extraction (1–3%), implying little exchangeable Pb. With one exception (WM-1), the concentrations of Pb are greater at the surface (<10 cm) than at depth (30–40 cm). There is no significant difference between total Pb concentrations between WM and MC. However, higher concentrations of Pb occur in the sites above low water mark (WM-1 and WM-2; 16–22 mg kg^{-1}) as compared to the perennially subaqueous sites WM-3 and MC-1 (10–12 mg kg^{-1}).

Nickel. The majority of Ni was released by aqua regia (51–67%), although it should be noted that a relatively high proportion (2–3 mg kg^{-1}; 17%) of exchangeable Ni occurs in WM-3.

Copper. Most of the Cu was released during aqua regia extraction (47–65%), except for site WM-3 where a relatively high proportion was released during HA–HCl extraction (~35%). HOAc and H_2O_2 liberated relatively small amounts of Cu (<3 mg kg^{-1}), implying little Cu in exchange sites, sulfides and organic matter.

Chromium. The majority of Cr in the sediment was released during aqua regia (57–69%) and essentially no Cr was liberated by HOAc (<0.7 mg kg^{-1}). This is consistent with numerous studies indicating that Cr is insoluble in these types of sediments (e.g. McGrath and Smith, 1990; McLean and Bledsoe, 1992).

Zinc. The highest proportion of Zn was released during aqua regia extraction (33–78%), suggesting that it is primarily contained in resistant minerals. The proportion of Zn in exchangeable sites is greater in surface samples (e.g. WM-3A, 24%) than at depth (e.g. WM-3D, 6%). HA–HCl treatment also resulted in a moderate release of Zn (21–40%), indicating a significant amount of Zn in reducible forms.

Cobalt. Co was released primarily by HA–HCl and aqua regia. Other than Pb and Mn, Co contained the lowest proportion of metal extracted by aqua regia (≤47%). There are higher concentrations of Co in the upper stretches of WM and in MC than in WM-3A.

Iron. The majority of Fe in the sediment is associated with aqua regia-extractable phases (60–80%), followed by HA–HCl-extractable phases (18–38%). This implies that Fe occurs in silicates and poorly crystalline hydroxides such as $Fe(OH)_3$, with very little Fe in sulfides or organic matter.

Manganese. Mn speciation is distinguished from the majority of metals in this study in that (1) Mn was preferentially partitioned into exchange sites (5–58% HOAc-extractable) as compared to the other metals (<24% HOAc-extractable), and (2) WM-2D contained 73% HA–HCl-extractable Mn, an anomalously high amount. It is similar to the majority of metals in that <10% of Mn was extracted by H_2O_2, implying little Mn is in sulfides or organic matter. For the two predominantly subaerial samples (WM-1 and WM-2), exchangeable Mn was much higher in the surface samples than those at depth. In WM-1A, a site that is virtually always subaerial (Fig. 3), HOAc-extractable Mn is 58% of total Mn, but at 30–40 cm depth, HOAc-extractable Mn is only 3% of total Mn. In WM-2A, which is more frequently subaqueous than WM-1A, HOAc-extractable Mn is 25% of total Mn, and HOAc-extractable Mn is 8% of total Mn. In WM-3, a perennially subaqueous site near the confluence of Ward Marsh and the Poultney River, HOAc-extractable Mn is 46% (<10 cm depth) and 29% (30–40 cm) of total Mn. HA–HCl-extractable Mn is generally greater at depth (30–40 cm) than in the surface samples.

2.3. QXRD and phase identification

Quartz, muscovite, albite, microcline and chlorite are the dominant minerals in sediment from Ward Marsh and Mud Creek (Figs. 5 and 6), comprising

(a)

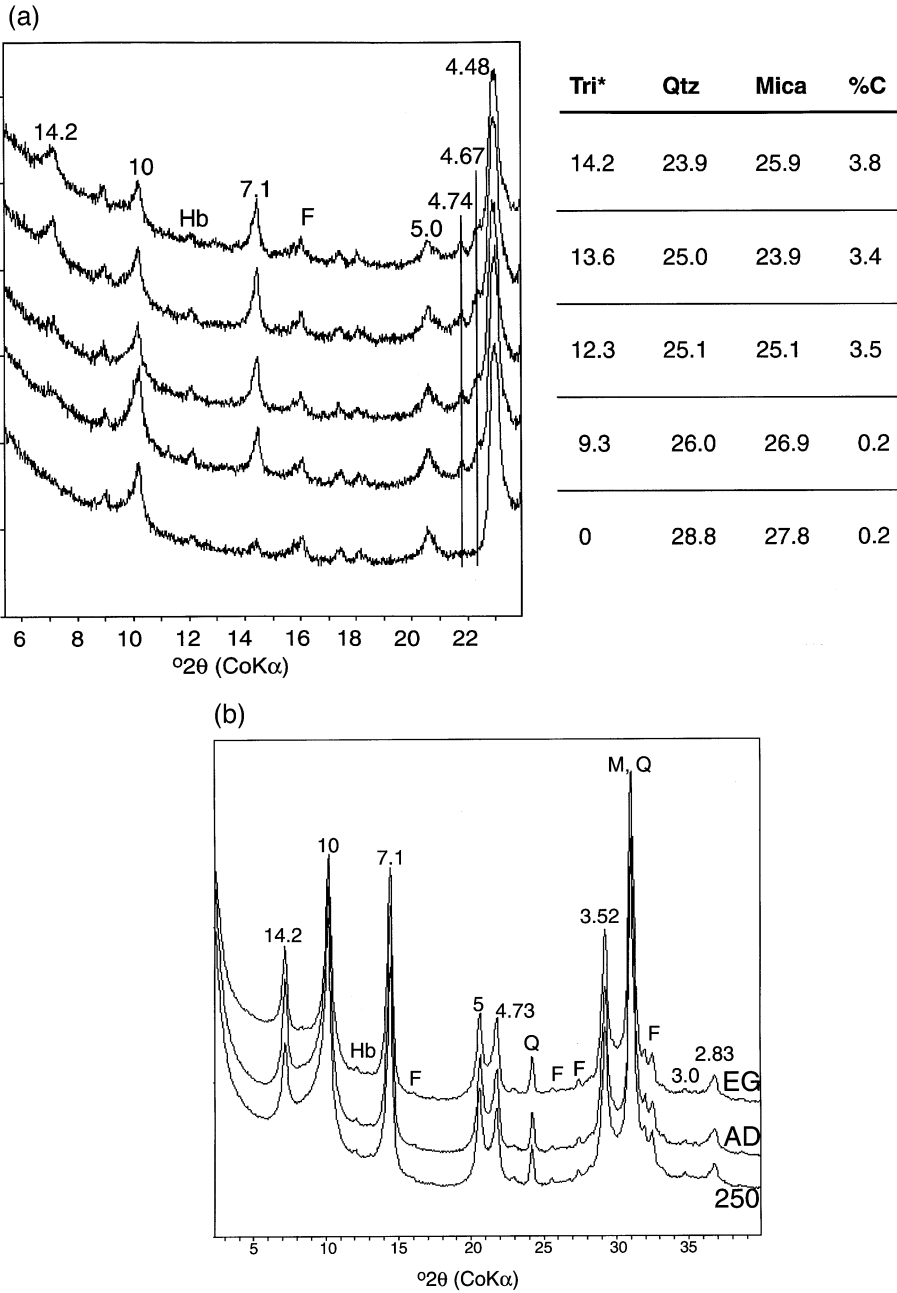| Tri*	Qtz	Mica	%C
14.2	23.9	25.9	3.8
13.6	25.0	23.9	3.4
12.3	25.1	25.1	3.5
9.3	26.0	26.9	0.2
0	28.8	27.8	0.2

(b)

Fig. 5. (a) Results of QXRD analyses on sample WM-2A, prepared by spray drying. Peak positions are shown in Å. Note the progressive disappearance of 14.2-Å peak, reflecting dissolution of expandable trioctahedral clay. The 7.1-Å peak is the chlorite 002 and kaolin 001. The 4.48-Å peak is the mica 020. Note the decrease in intensity of the 4.67-Å trioctahedral 02,11 peak and corresponding loss of 14.2-Å peak with increasing extraction stage (top to bottom). The data to the right of the XRD patterns are results of quantitative analysis (wt.%) of total sediment. Tri* refers to total trioctahedral clay, including chlorite and expandable phase. Hb = hornblende, F = feldspar, Qtz = quartz, %C = total organic carbon (wt.%), 10- and 5-Å peaks are K-mica. (b) XRD analysis of < 2-μm fraction of WM-2A, oriented mount. AD = air-dried, EG = ethylene glycol-solvated, 250 = heated to 250 °C for 1 h. Peak positions are shown in Å. Q = quartz, M = mica, other letter symbols are the same as in (a).
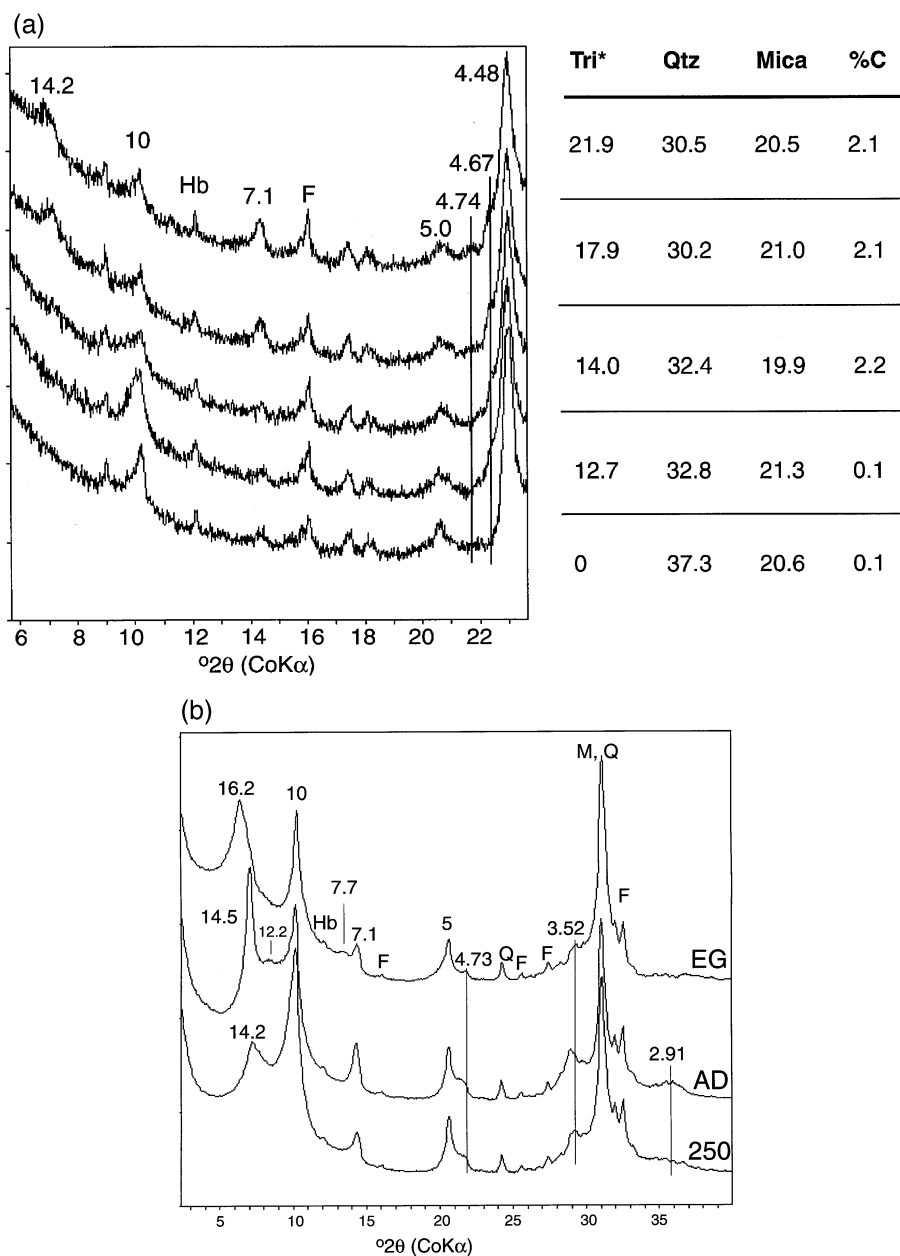
Fig. 6. (a) Results of QXRD analyses on sample MC-1A, prepared by spray drying. For annotations and additional information, see caption for Fig. 5A. (b) XRD analysis of <2-μm fraction of MC-1A, oriented mount. For symbols see Fig. 5A caption.

>90% (by wt.) of the total sediment. Kaolin and hornblende occur as minor phases, with estimated abundance of 1–3% and dolomite occurs in trace amounts of ≤1%. Organic matter comprises 1–8% (by wt.) as determined by C–N analyses. QXRD analysis did not reveal the presence of any crystalline oxides, hydroxides or sulfides in any samples. It should be noted that poorly crystalline iron hydroxides such as ferrihydrite produce weak and broad XRD peaks, and thus are difficult to identify in bulk XRD analysis.

Undisturbed sediment from both marshes contains 14–22% trioctahedral clay. Examination of the <2-μm fraction indicates coexistence of trioctahedral chlorite and an expandable trioctahedral component (Figs. 5B and 6B). Trioctahedral clay was observed to progressively dissolve throughout the extraction process (Figs. 5A and 6A), but with significant differences between sediment from the two marshes. The MC site has more trioctahedral clay than WM, and the trioctahedral clay that exists in MC appears to be more soluble than WM trioctahedral clay. In the case of WM-2A (Fig. 5A), the original sediment contained 14% trioctahedral clay. Following HOAc extraction, trioctahedral clay content had decreased to 12% and 9%, respectively. No trioctahedral clay was detected in post-aqua regia powders. For MC-1A (Fig. 6A), the amount of trioctahedral clay in the sediment progressively decreased through the extraction process, from 22% (original powder) to 18% (post-HOAc) to 14% (post-HA−HCl) to 13% (post-H_2O_2) and ultimately 0% (post-aqua regia).

Analysis of <2-μm fractions indicates difference in the type of expandable clay in WM and MC (Figs. 5B and 6B). The samples from WM contain a trioctahedral 14-Å phase that exhibits little change upon glycol and heat (250 °C) treatments (Fig. 5B). Modeling XRD patterns with NEWMOD (Reynolds, 1985) indicates that the subtle changes in peak and background intensity can be attributed to small amounts (e.g. <2%) of expandable layers in chlorite (cf. Reynolds, 1988), although a physical mixture of chlorite with <10% vermiculite can produce similar XRD effects. While the greatest amount of trioctahedral clay dissolution in WM samples occurred during aqua regia extraction, it is important to note that 15% dissolved during HA−HCl and an additional 20% dissolved during H_2O_2 extraction. Most of the HA−HCl and H_2O_2 dissolution was of a phase containing a strong 14-Å peak and weak 7-Å peak, consistent with vermiculite (Moore and Reynolds, 1997). Samples from MC, in contrast, contain a physical mixture of chlorite and a more highly expandable phase that expands from 12.3 to 16.1 Å upon ethylene glycol solvation and collapses to 11–14 Å after heating for 1 h at 250 °C (Fig. 6B). This behavior is consistent with high-charge smectite (Moore and Reynolds, 1997) that we interpret as trioctahedral based on positive correlation between intensities of the 14-Å 001 and trioctahedral 02,11 (4.67 Å) and 060 (1.54 Å) peaks (Figs. 5B and 6B).

Also, dissolution of the expandable phase and chlorite produces no decrease in dioctahedral peaks at 4.48 and 1.50 Å (020 and 060 peaks), consistent with dissolution of trioctahedral and not dioctahedral clay. For the MC samples, the 14-Å peak in spray dried, randomly oriented mounts represents the chlorite 001 and the 001 peak from smectite containing variable interlayer water and partially complete hydroxide interlayers. Analysis of <2-μm fraction oriented XRD mounts clearly indicates the presence of these two clay phases (Fig. 6B), with chlorite delineated by a 14-Å peak that is not altered by any of the three treatments. Comparison of relative intensities of 14-Å and 7-Å peaks of air-dried MC powders indicates that the high-charge smectite partially dissolves during HOAc and HA−HCl extractions. Loss of 14-Å peak intensity with no loss of 7-Å peak intensity represents dissolution of a phase with a strong 14-Å 001 peak and weak 7-Å 002 peak, consistent with air-dried smectite. The H_2O_2 extraction stage, which makes use of NH_4OAc as an extractant, causes complete removal of remaining post-HA−HCl 14-Å phase and simultaneous formation of trioctahedral 10-Å clay (presumably by dissolution of the interlayer hydroxide and/or loss of interlayer water) (Table 2). Based on the almost complete loss of the 7-Å peak following H_2O_2−NH_4OAc extraction, it appears that chlorite at least partially dissolved during H_2O_2

Table 2

Representative integrated peak areas used in QXRD. In particular, note (a) the elevated 10-Å peak area in post-H_2O_2 powders, and (b) decreases in 14-Å and 7-Å peak areas with increasing extraction stage. See text for abbreviations

	14 Å	10 Å	7 Å	4.67 Å	4.48 Å	4.26 Å	4.04 Å	2.09 Å
WM-2A								
Untreated	189	324	295	164	1008	1115	314	1343
HOAc	200	304	280	158	975	1163	283	1303
HA-HCl	134	341	233	142	958	1133	284	1277
H_2O_2− NH_4OAc	65	467	218	108	1032	1152	301	1388
Aqua regia	0	337	63	0	995	1264	316	1379
MC-1A								
Untreated	164	178	140	135	527	898	338	887
HOAc	174	157	102	110	528	862	333	879
HA−HCl	39	170	54	86	510	951	342	932
H_2O_2− NH_4OAc	0	291	55	78	535	958	340	900
Aqua regia	0	148	40	0	531	1171	281	931

extraction (Fig. 6A), although this particular point is difficult to quantify given low clay proportions.

Within the margin of error, the content of all other minerals (e.g. muscovite, feldspars, hornblende, kaolin) remains constant throughout the extraction procedure, with the possible exception of albite, which exhibited a $\leq 18\%$ decrease following aqua regia. Quartz peak areas increase by $8-15\%$ between the $H_2O_2-NH_4OAc$ and aqua regia extractions for all samples, presumably caused by increased abundance of resistant quartz in extracted powders due to dissolution of soluble materials.

Although measurable amounts of metals were released during oxidation and reduction stages, no oxides or sulfides were detected by XRD analyses, either in bulk XRD assays or in scans of the <2-μm fraction of selected samples.

2.4. C–N analyses

95% to 99% of organic carbon was removed from sediment during the $H_2O_2-NH_4OAc$ stage (Figs. 5A and 6A), indicating dissolution of organic matter by H_2O_2.

2.5. Standard reference material

The mineral content of S33 is much like that of the WM and MC samples. S33 is characterized by 32.8% quartz, 21.4% muscovite, 15.6% albite, 8.7% microcline, 8.6% trioctahedral chlorite clay (primarily chlorite), approximately 7% organic matter, and $2-3\%$ each of kaolin and hornblende, and 0.5% calcite. As with the Ward Marsh and Mud Creek sediment, trioctahedral clay was observed to dissolve progressively throughout the extraction procedure. Loss of the 14-Å peak intensity without the loss of the 7-Å peak intensity implies dissolution of a vermiculitic or smectitic phase (or changes in the interlayer) during HA–HCl and H_2O_2 extractions. Elemental recoveries of trace metals during the SCE procedure ranged from 72% to 126% of accepted values, with a mean value of 104%. Trace metal speciation of S33 is shown in Table 1.

3. Discussion

Perhaps the most significant result of this study is the unequivocal documentation of progressive clay mineral dissolution during sequential chemical extraction. Previous studies have suggested that sequential extraction procedures are not perfectly selective with respect to mineral dissolution (e.g. Horowitz, 1991; McCarty et al., 1998; Song et al., 1999), and without QXRD, the minerals that dissolve with each successive extraction are operationally defined (Tessier et al., 1979). The results of this study show that in some cases such operational definitions can lead to errors with respect to assessment of metal speciation. For the sediment analyzed in this study, without QXRD, the assumption would be that oxides and/or sulfides and organic matter are significant sources of Cu, Co, Cr, Ni, and Zn, and that clay dissolution does not contribute trace metals except in the aqua regia stage. However, QXRD analyses provide no evidence of dissolution of oxides, hydroxides or sulfides, but rather progressive dissolution of expandable trioctahedral clay. The drastic drop in the percent of carbon after $H_2O_2-NH_4OAc$ suggests that the selectivity of this stage for extracting metals from organics is accurate.

3.1. Speciation of Fe and Mn

The lack of metals extracted by H_2O_2 is somewhat surprising given that the sediments are dark brown-black, organic-rich and predominantly subaqueous. Seasonal low water levels periodically expose some of the sample sites to air, although some sites (e.g. MC-1, WM-3) are perennially subaqueous. The lack of Fe extracted by H_2O_2, combined with $18-38\%$ HA–HCl-extractable Fe, implies a significant amount of Fe in poorly crystalline hydroxides, and little or no Fe in sulfides. When comparing QXRD and SCE results, it becomes apparent that, while the majority of Fe is released by trioctahedral clay dissolution, lesser amounts of Fe are also partitioned into poorly crystalline hydroxides. Insufficient trioctahedral clay dissolved during HA–HCl to produce all of the Fe released during this stage (Fig. 7), and the lack of Fe-hydroxide phases in XRD analyses is interpreted here to indicate the presence of poorly crystalline hydrous iron oxides. The lack of distinctive XRD peaks indicates that iron oxides in these sediments are either (a) well crystalline but present in only trace amounts ($<1\%$), and/or (b) poorly crystalline or amorphous, in which case they would have dissolved during HA–HCl and would be difficult to identify by XRD due to
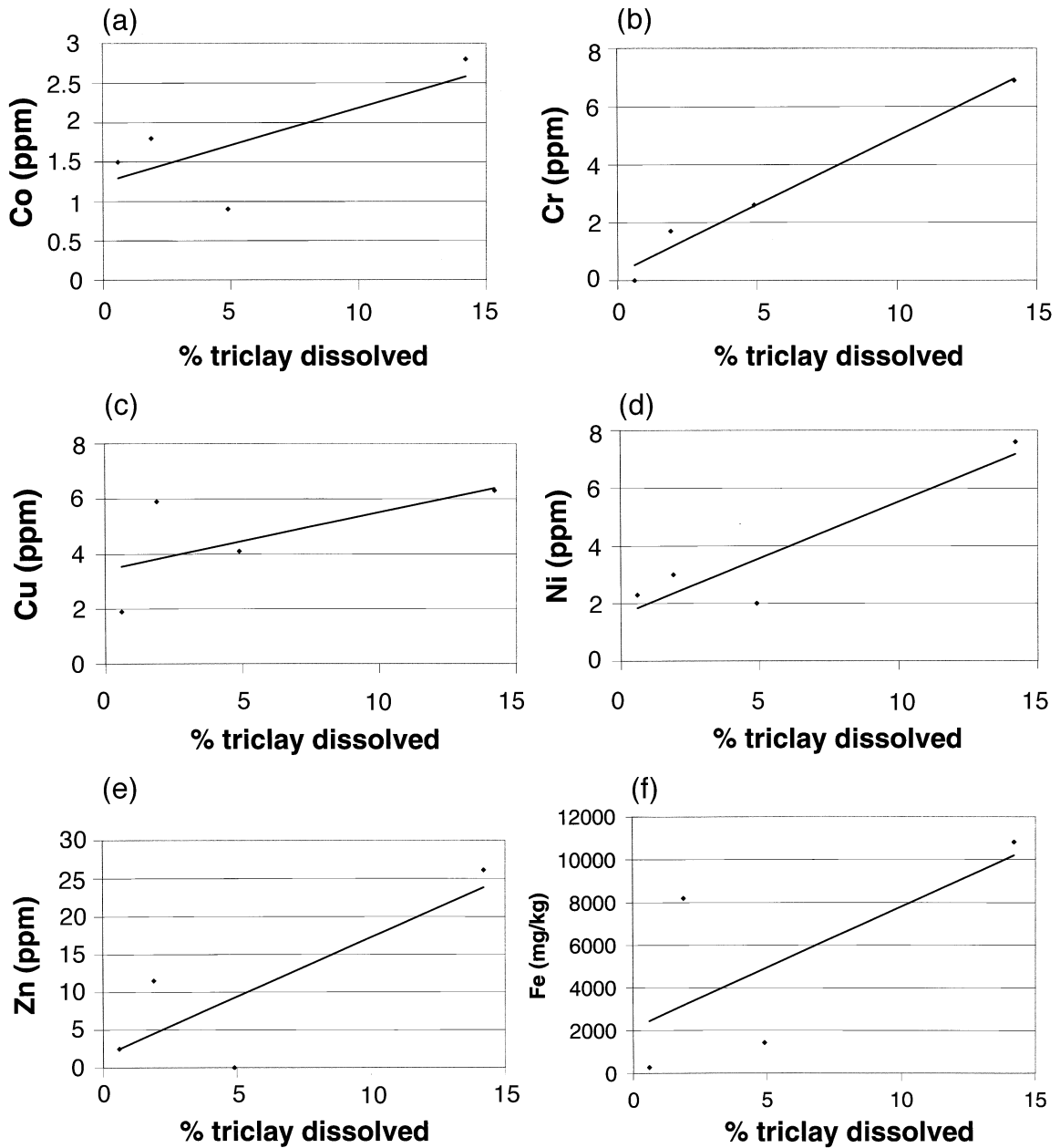
Fig. 7. Correlation between trioctahedral clay content and concentrations of specific metals. The *x* axis represents trioctahedral clay dissolution as % of sediment by weight; the *y* axis is the amount of trace metal released during SCE. For this particular specimen, 9.3% of sediment dissolved during aqua regia, and the greatest amounts of Co, Cr, Cu, Fe, Ni, and Zn were released during this stage. Less clay dissolved during other stages, and correspondingly less metal was released. Note the very strong positive correlations of clay with Cr and Ni, and poorer correlations of clay with Co, Cu, Fe, and Zn.

weak or unresolved XRD peaks. Taillefert et al. (2000) have shown that hydrous iron oxides form at the oxic-anoxic transition in stratified lakes and are preserved as complex aggregates with organic matter below the oxic-anoxic transition. This type of process may explain the apparent presence of Fe hydroxides in these predominantly anoxic sediments. Trace amounts of siderite are also possible, but they tend to occur where pH > 7 (Brookins, 1988; Drever, 1997) and thus are unlikely to occur given the slightly to moderately acidic pH of these sediments, which ranges from 5 to 6.5.

Mn speciation is unique among the metals analyzed in this study. In some samples (WM-2A, WM-3D), the highest proportion of Mn was extracted by aqua regia, implying partitioning into trioctahedral clay (Newman, 1987) and/or well-crystalline oxides. Mn^{+3} and Mn^{+4} are insoluble ions and tend to form oxides such as MnO_2, $MnOOH$, and Mn_3O_4, or become incorporated into silicates (McBride, 1997). The high proportions of Mn extracted by HOAc imply that Mn is (1) available on exchange sites, and (2) possibly also present in soluble carbonates such as dolomite, which occurs in trace amounts and dissolved during HOAc extraction. The Mn^{+2} ion is very soluble in water at pH < 7 (Brookins, 1988; McBride, 1997), and given the pH range of these sediments (5–6.5), soluble, exchangeable Mn^{+2} is likely. Significant amounts of Mn were also liberated by HA–HCl, particularly in WM-2D, where an anomalously high amount by mass (1449 mg kg^{-1}) and % (73% of Mn) was HA–HCl extractable. The presence of greater amounts of exchangeable Mn at the surface in subaerial sites (WM-1 and 2) suggests that oxidized sediment contains more exchangeable Mn than reduced sediment. However, equally high amounts of exchangeable Mn (similar to WM-1A and 2A) were also observed in perennially subaqueous samples WM-3 and MC-1, suggesting that redox potential does not control % exchangeable Mn in this case. Accordingly, it appears that Mn is widely speciated into exchangeable sites, oxides and silicate clays, but little in sulfides or organic matter.

3.2. Speciation, source and availability of CO, Cr, Cu, Ni, Zn

The greatest amounts of Co, Cr, Cu, Ni, and Zn were released by aqua regia extraction. Minerals that most commonly dissolve during this stage include silicate clays and well-crystalline iron oxides (Tessier et al., 1979; Ure et al., 1993). QXRD shows that trioctahedral clay was the main mineral dissolved during aqua regia extraction (Figs. 5A and 6A), with the occasional exception of small amounts of feldspar, implying that the largest reservoir for trace metals in these sediments is trioctahedral clays. Iron oxides and hydroxides are likely sources of trace metals and Mn as well (see below).

The occurrence of trace metals in silicate clay indicates that the majority of metals in the sediments are of only limited availability for uptake by organisms. Given that the proportions of Co, Mn and Ni extracted by HOAc are greater than other metals, it appears that they are the most environmentally available metals followed by Zn at some sample sites. Co, Ni, and Zn have been correlated in laboratory studies with the types of malformities observed at Ward Marsh (Luo et al., 1993; Plowman et al., 1991, 1994; Meteyer, 1997).

Mass calculations corroborate QXRD analyses in indicating that silicate clays are the dominant source of trace metals, with the exception of Pb. These calculations were made using a chlorite composition of $(MgFe_{2.5}Al_{2.0})(Si_3Al)O_{10}(OH)_8$, as determined by ICP-AES analyses of aqua regia-extractable fractions, and the assumption that all Al liberated during the chemical extractions is from trioctahedral clay. In this average unit cell, Al comprises 14–15% of the clay by weight. Partitioning all Al released by SCE into chlorite yields Al concentrations in chlorite of 11–17%. Assuming clays are the sole source of trace metals, concentrations in the trioctahedral clays would range from 600 (Co) to 7200 (Zn) mg kg^{-1}, consistent with values reported for chlorites in Newman (1987).

Speciation of trace metals into clays is further supported by positive correlation between individual trace metals extracted (in mg kg^{-1}) and extent of dissolution of trioctahedral clay during different SCE stages (Fig. 7). Cr and Ni correlate very strongly with clay dissolution, whereas Zn exhibits moderately strong correlation. Co and Cu also exhibit weaker correlations. By contrast, Pb does not correlate with clay dissolution, consistent with speciation of Pb into another phase, most likely amorphous or poorly crystalline oxyhydroxides, especially given that most Pb was released by HA–HCl. Relatively

poor correlations of Co, Cu, and Zn with trioctahedral clay dissolution, as compared to Cr and Ni, indicate partitioning of Co, Cu, and Zn into other phases in addition to clay, most likely poorly crystalline iron hydroxides. This interpretation is supported by (a) greater proportions of Co, Cu, and Zn (and Pb) extracted by HA–HCl than Cr and Ni, and (b) numerous studies that have shown that trace metals commonly coprecipitate with iron oxyhydroxide (McBride, 1997; Taillefert et al., 2000).

The cause of the difference in speciation between Cr and Ni, which both are almost exclusively partitioned into trioctahedral clay, and Co, Cu, and Zn, which are divided between trioctahedral clay and amorphous/poorly crystalline Fe-oxyhydroxide, is unclear. However, given that Cr and Ni both occur in highly elevated concentrations in serpentinite (McGrath and Smith, 1990; Lottermoser, 1997), perhaps their strong association with trioctahedral clay reflects the presence of Ni–Cr-bearing serpentine and chlorite minerals transported into these sediments as detritus from the Green Mountains and foothills.

3.3. Sources of the metal-bearing clays

The source of the metal-bearing chlorite and expandable trioctahedral clay appears to be chemical and physical weathering of serpentinite, carbonate and shale in the Green Mountains and Taconic ranges, which is upstream from the WM site, and carbonate lowlands upstream of the MC site. Shale and carbonate rock are exposed in the source areas of both marshes; serpentinite outcrops in the source area of Ward Marsh but not Mud Creek, which appears to be the best explanation for higher metals concentrations at Ward Marsh. Elevated concentrations of Co (>15 mg kg^{-1}), Cr (>150 mg kg^{-1}), and Ni (>100 mg kg^{-1}) have been observed in Taconic slates dominated by chlorite and illite (Garver et al., 1996), and highly elevated values of these same elements occur in Green Mountain serpentinites (>100 mg kg^{-1} Co and >1000 mg kg^{-1} Cr and Ni) dominated by chlorite and serpentine (Deregibus, 1982; Laundon, 2000). Typical limestone values for these metals are 0.1 mg kg^{-1} (Co), 11 mg kg^{-1} (Cr), 20 mg kg^{-1} (Ni) (Drever, 1997).

3.4. Trioctahedral clay dissolution—causes and implications

The progressive dissolution of trioctahedral clay throughout the extraction procedure has important implications for any study using sequential extractions to infer metal speciation. By tracking mineral dissolution with QXRD, it is clear that operationally defined phase stabilities are, at least in some cases, can lead to misinterpretation of speciation. It is significant to note that more clay dissolved during reduction and oxidation stages for the MC sediment than WM sediment. This is attributed to the presence of a greater proportion of expandable clay, and a more highly expandable clay, in the MC samples (Fig. 6B).

Poorly crystalline expandable clay, which possibly contains both Fe(II) and Fe(III), is unstable in the presence of the HA–HCl and H$_2$O$_2$ solutions used in the reduction and oxidation stages. Redox reactions involving Fe have been documented in various types of expandable clay (Stucki et al., 1996; Drits and Manceau, 2000), and may explain the expandable clay dissolution observed here. Alternatively, the presence of HNO$_3$ in HA–HCl and H$_2$O$_2$ solutions to adjust pH to 2 may be responsible as well. In either case, QXRD or some other quantitative means of tracking mineral dissolution during SCE studies is essential.

Formation of 10-Å layers following H$_2$O$_2$–NH$_4$OAc extraction (Table 2) is interpreted here to reflect stripping of hydroxide layers and/or loss of interlayer water from interlayers of the high-charge smectite in MC samples, and chlorite and/or vermiculite in WM samples. This process may be initiated by Fe oxidation, which destabilizes the interlayers. Once the interlayer material has been lost, the 2:1 layers collapse around NH$_4^+$ to create a trioctahedral mica-like mineral. This 10-Å phase is not present following aqua regia extraction.

3.5. Silica

The apparent increase in quartz content following aqua regia extraction in each of the powders analyzed is attributed to increases in the relative proportion of quartz in post-aqua regia residue relative to other components that dissolved during

extraction (mainly trioctahedral clay). The apparent lack of Si in post-aqua regia supernatants presumably reflects the low solubility of amorphous silica at low pH.

3.6. Pb speciation, source and mobility

The high proportion of Pb associated with HA–HCl extraction suggests that Pb is contained within hydroxides, although XRD analyses did not reveal the presence of any such phases. According to Tessier et al. (1979) and Kersten and Förstner (1986), the reducible forms extracted by HA–HCl include easily-to-moderately soluble oxides and hydroxides that are either amorphous or poorly crystalline. Such phases are difficult to detect by XRD, but the lack of oxide peaks can be used to infer that the oxides are in fact amorphous. Pb and Fe from the reducible fraction do not exhibit a strong correlation ($R^2 = 0.151$), but this low value is probably due to the fact that the majority of Fe (but not Pb) is released by dissolution of expandable trioctahedral clay during HA–HCl extraction (Figs. 5 and 6). Pb is known to bind strongly to poorly crystalline, hydrous Fe oxides in surface water (e.g. Balistrieri et al., 1992; Taillefert et al., 2000), and this is a common means of transferring Fe and Pb from the water column to sediment. Pb also exists in sulfides in anoxic sediment, but the virtual lack of Pb in the oxidizable fraction implies there is little or no Pb in sulfides. The drastic difference in partitioning of Pb and the other trace metals indicates different sources, and suggests that the Pb is anthropogenic, likely derived from automobile exhaust and/ or lead shot from waterfowl hunting.

3.7. Potential relationship between metals and frog malformities

In vitro FETAX studies have shown that the type of malformity observed at Ward Marsh (missing hind limbs and eyes) is produced by elevated concentrations of trace metals such as Co, Cr, Cu, Ni, and Zn (Luo et al., 1993; Plowman et al., 1991, 1994; Meteyer, 1997). By comparison to values published in McBride (1997), >80% of sediment samples from Ward Marsh contained slightly elevated Zn (>90 mg kg^{-1}), and slightly elevated concentrations of Co (>15 mg kg^{-1}) and Cu (>30 mg kg^{-1}) were found in 60%

and 40% of Ward Marsh samples, respectively. These values are more than double elevated metals rates at Mud Creek. While it is impossible to assess causality without actual analyses of frog tissue, the data do indicate a correlation between malformities and metals and also suggest the need for analysis of metals in frogs exhibiting these types of malformities. It is important to note that, while concentrations of individual metals at Ward Marsh are either similar to background values or only slightly elevated, the presence of a wide range of metals can lead to synergistic effects that produce toxicity at lower concentrations than commonly reported toxicity values determined for individual metals (Lefcort et al., 1998).

Although Pb does not produce the types of malformities observed in Vermont, the partitioning of Pb into amorphous oxyhydroxides implies that changing redox conditions could cause the release of Pb into pore water.

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