

This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-96SR18500 with the U. S. Department of Energy.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, nor any of their contractors, subcontractors or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or any third party's use or the results of such use of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof or its contractors or subcontractors. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Key Words:
Wetland, Sediment
Metal Retention
Contaminant Removal
Remobilization

Retention:
Permanent

**ASSESSMENT OF CONTAMINANT DISTRIBUTION AND STABILITY
IN SEDIMENT OF THE A-01 CONSTRUCTED WETLAND
2005 REPORT**

**Anna Knox, Mike Paller, Eric Nelson,
Winona Specht, and Nancy Halverson**

**Savannah River National Laboratory
Environmental Science and Biotechnology Department
Environmental Analysis Section**

DECEMBER 2005

Savannah River National Laboratory
Westinghouse Savannah River Company
Savannah River Site
Aiken, SC 29808

**Prepared for the U.S. Department of Energy Under
Contract Number DE-AC09-96SR18500**



DISCLAIMER

This report was prepared for the United States Department of Energy under Contract No. DE-AC09-96SR18500 and is an account of work performed under that contract. Neither the United States Department of Energy, nor WSRC, nor any of their employees makes any warranty, expressed or implied, or assumes any legal liability or responsibility for accuracy, completeness, or usefulness, of any information, apparatus, or product or process disclosed herein or represents that its use will not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, name, manufacturer or otherwise does not necessarily constitute or imply endorsement, recommendation, or favoring of same by Westinghouse Savannah River Company or by the United States Government or any agency thereof. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Printed in the United States of America

**Prepared For
U.S. Department of Energy**

Key Words:
Wetland, Sediment
Metal Retention
Contaminant Removal
Remobilization

Retention:
Permanent

**ASSESSMENT OF CONTAMINANT DISTRIBUTION AND STABILITY
IN SEDIMENT OF THE A-01 CONSTRUCTED WETLAND
2005 REPORT**

**Anna Knox, Mike Paller, Eric Nelson,
Winona Specht, and Nancy Halverson**

**Savannah River National Laboratory
Environmental Science and Biotechnology Department
Environmental Analysis Section**

DECEMBER 2005

Savannah River National Laboratory
Westinghouse Savannah River Company
Savannah River Site
Aiken, SC 29808

**Prepared for the U.S. Department of Energy Under
Contract Number DE-AC09-96SR18500**



TABLE OF CONTENTS

LIST OF FIGURES	iv
LIST OF TABLES	v
LIST OF ACRONYMS	vi
EXECUTIVE SUMMARY	1
1.0 INTRODUCTION.....	3
2.0 OBJECTIVE	5
3.0 MATERIALS AND METHODS	6
3.1 TASK 1, METAL DISTRIBUTION IN THE SEDIMENT PROFILE	6
3.2 TASK 2 & 3, METAL MOBILITY AND RETENTION.....	7
3.2.1 Sequential Extraction of Organic and Inorganic Layers	7
3.2.2 Collection of Floc Material	8
3.2.3 Analytical Methods on Floc Material	9
3.3 DATA ANALYSIS.....	10
4.0 RESULTS AND DISCUSSION	11
4.1 GENERAL SEDIMENT PROPERTIES	11
4.2 VERTICAL AND HORIZONTAL DISTRIBUTION OF CONTAMINANTS IN SEDIMENT	13
4.2.1 Total Concentration of Cu and Other Metals in the Sediment Profile	13
4.2.2 Total Hg and Methyl Hg in Sediment.....	19
4.3 POREWATER CHEMISTRY AND METAL CONTENT	21
4.3.1 Porewater Chemistry and Metal Content.....	21
4.4 METAL MOBILITY AND RETENTION IN SEDIMENT	30
4.4.1 Metal Speciation	30
4.4.1.1 Floc layer	30
4.4.1.2 Organic and Inorganic Layer	36
4.4.2 Potentially Mobile Fraction and Recalcitrant Factor	39
4.4.3 Distribution Coefficient (K_d) Values for Selected Metals in the Wetland Sediment.....	43
5.0 CONCLUSION	45
6.0 REFERENCES.....	47
APPENDIX A. SEQUENTIAL EXTRACTION PROCEDURE	51

LIST OF FIGURES

Figure 1. Schematic of the A-01 Wetland Treatment System and Sampling Locations 4

Figure 2. Sediment pH as a Function of Depth..... 12

Figure 3. Floc Sedimentation Rates ($g/m^2/day$) in 4A and 4B Cells 12

Figure 4. Organic Matter Distribution (%) in the Sediment Profile 13

Figure 5. Copper, Pb, and Zn Concentrations (in ppm) in the Sediment Profile..... 16

Figure 6. Iron and Mn Concentrations (in ppm) in the Sediment Profile 17

Figure 7. Principal Components Analysis of the Total Digestion Data from the A-01
Constructed Wetland 18

Figure 8. Mercury Concentration (ppm) in the Sediment Profile of the Core Samples
Collected in 2004. The samples were analyzed by ICP-MS. 20

Figure 9. Mercury Concentration (ppm) in Floc Samples Collected in 2005. The
samples were analyzed by CVAFS (FGS-069). 20

Figure 10. Methyl Mercury in Floc Samples Collected in 2005 21

Figure 11. Porewater pH Values as a Function of Depth; Samples Collected from the
Sediment of Wetland Cells 4A and 4B 22

Figure 12. Dissolved Organic Carbon (DOC) and Total Inorganic Carbon (TIC)
Concentrations (ppm) in Porewater..... 22

Figure 13. Average Sulfate Concentrations (ppm) in the Porewater Collected from Cells
4A and 4B..... 23

Figure 14. Average Fe and Mn Concentrations (ppb) in the Porewater Collected from
Cells 4A and 4B 24

Figure 15. Average Cd, Cu and Zn Concentrations (ppb) in the Porewater Collected
from Cells 4A and 4B..... 26

Figure 16. Average Hg, Ni and Pb Concentrations (ppb) in the Porewater Collected
from Cells 4A and 4B..... 28

Figure 17. Principal Components Analysis of Metal Concentrations in Porewater from
the A-01 Constructed Wetland 29

Figure 18. Sequential Extraction of Cu, Pb, and Zn from Sediment Collected from
Wetland Cells 4A and 4B 38

Figure 19. Average Potentially Mobile Fractions (PMF) and Recalcitrant Factors (RF)
for Elements in the Floc (0-2 cm), Organic (2-5 cm), and Inorganic
(5-10 cm) Layers 41

Figure 20. Principal Components Analysis of Exchangeable Metal Fractions in
Sediment from the A-01 Constructed Wetland 42

Figure 21. Average K_d Values for Cu, Pb, and Zn in the Wetland Sediment..... 44

LIST OF TABLES

Table 1. Sequential Extraction Procedure Employed for the Geochemical Fractionation of Elements in Wetland Sediment 8

Table 2. Concentrations of Selected Elements in floc Samples from the A-01 Sediments.. 14

Table 3. Concentrations of Selected Elements in the Organic Layer of the A-01 Sediments 14

Table 4. Concentrations of Selected Elements in the 5-10 cm Inorganic Layer of the A-01 Sediments 15

Table 5. Concentrations of Selected Elements in the 10-20 cm Inorganic Layer of the A-01 Sediments 15

Table 6. Sequential Extraction of Cu from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)..... 31

Table 7. Sequential Extraction of Pb from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B) 32

Table 8. Sequential Extraction of Zn from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B) 33

Table 9. Sequential Extraction of Fe from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B) 34

Table 10. Sequential Extraction of Mn from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)..... 35

Table 11. Sequential Extraction of Cu from Sediment Collected from the Inflow to the Outflow of Wetland Cells 4A and 4B 37

LIST OF ACRONYMS

ANOVA	analysis of variance
CW	constructed wetland
CVAFS	cold vapor atomic fluorescence detector
DOC	dissolved organic carbon
ICP-AES	inductively couple plasma - atomic emission spectroscopy
ICP-MS	inductively couple plasma – mass spectroscopy
K_d	distribution coefficient (partition coefficient)
MMHg	monomethyl mercury
NPDES	National Pollution Discharge Elimination System
OM	organic matter
PCA	principal component analysis
PMF	potentially mobile fraction
RF	recalcitrant factor
SRNL	Savannah River National Laboratory
SRS	Savannah River Site
SOM	soluble organic complex
SOP	standard operating procedures
TIC	total inorganic carbon
WTS	wetland treatment system

EXECUTIVE SUMMARY

Constructed wetlands have the ability to remove pollutants from water and retain them in sediment. However, there is considerable variation among metals and between wetlands in the degree to which metals are removed. The A-01 wetland treatment system (WTS) was designed to remove metals (primarily copper) from the A-01 effluent at the Savannah River Site, Aiken, SC. The main purpose of research conducted during 2004 and 2005 was to evaluate the distribution and retention of contaminants in the sediment profile.

Most of the metal removed by the wetland cells was accumulated in the two top layers; i.e., the floc and organic layer. Principal components analysis revealed a strong spatial gradient in the sediment metal data, with floc layer samples from the A cell at one extreme and inorganic layer samples from both cells at the other. This gradient was strongly correlated with percent organic matter, pH, and the concentration of all metals except mercury. These results showed that most metals in the A-01 wetland sediments behaved similarly: their concentrations decreased as sediment depth increased.

Copper, Cd, and Zn distribution in the sediment porewater profile was similar, and the concentrations of these elements generally decreased as a function of depth. The horizontal and vertical distributions of sulfate, Fe, and Mn in the porewater indicated the oxidation status of the sediments. The presence of sulfate in the porewater showed that cell 4A, especially the top organic layer, was in an oxidized state. The porewater sulfate concentration in all layers was higher in 4A cell than in 4B cell. The lower inorganic layers, especially closer to the effluent discharge, were more reduced as indicated by higher concentrations of Fe and Mn.

The stability of contaminants in the wetland sediment profile was evaluated by calculating the potentially mobile fraction (PMF), recalcitrant factor (RF), and distribution coefficient (K_d) values. The highest PMF values were in the floc layer, followed by the organic layer, with the lowest values in the inorganic layer. The PMF value for Cu in the inorganic layer was 20%, but increased to 40% and 60% in the organic and floc layers, respectively. Similar patterns of increases in PMF with decreasing sediment depth were shown by Zn and Pb. RF values for Cu, Pb, and Zn were high in the inorganic layers indicating that all three elements were strongly bound in the deeper sediments and not easily extractable. However, RF values were low in the floc layer indicating high potential desorption and solubility.

K_d values were highly variable, but were clearly much higher for Pb than for Cu and Zn. This result was consistent with the Cu, Zn, and Pb data from the sequential extraction, which showed that Pb concentrations in the exchangeable fraction were much lower than the Cu and Zn concentrations, especially in the organic layer.

Our conclusions agree with previous studies conducted on the A-01 WTS and other constructed wetlands, which indicate that substratum (sediment) is the primary sink for metals. Metal concentrations in the A-01 WTS tended to be greater in the surface layers of sediment, which were rich in organic matter. Association of metals with soluble organic complexes in the floc layer is substantial and should be further evaluated.

Although there have been a variety of engineering improvements for constructed wetlands, much less attention has been paid to optimizing the microbial processes responsible for metal removal. An understanding of biotic interactions may prove to be crucial for designing an effective wetland system incorporating microbial processes for achieving the highest rates of metal removal from effluents. An enhanced understanding of the complex biotic and abiotic interactions involved along with the technical possibilities for exploiting these interactions will enable wetland technologies to be used on a broader scale.

1.0 INTRODUCTION

The principal objective of wastewater treatment is to eliminate or reduce contaminant levels that cause adverse effects on humans or the receiving environment. However, traditional active treatment processes such as reverse osmosis, ion exchange, microfiltration, and the addition of chemicals are either not very efficient or quite costly. In some cases they are simply unfeasible. In addition to physicochemical techniques, biotechnology presents novel possibilities with many still under development.

Constructed or artificial wetlands (CWs) are an alternative to conventional water treatment of contaminated waters (Hammer, 1989; Odum et al., 2000). This cost-effective, ecologically friendly, “passive” technology is used extensively worldwide to treat a range of wastewater and effluents from a variety of sources, including mine drainage, landfill leachates, urban stormwater, and agricultural runoff. CWs are man-made ecosystems that mimic their natural counterparts. The complex ecosystem of plants, microorganisms, and substrate in a CW act together as a biogeochemical filter, efficiently removing low levels of contamination from very large volumes of water and providing protection for natural water resources such as rivers, lakes, estuaries and ground waters.

The way in which a wetland is constructed ultimately determines how wastewater treatment occurs and what mechanisms will be involved. CWs typically consist of four principal components participating in pollutant removal: substrates (soil/sediment), plants, water, and associated microbial populations (Kosolapov et al., 2004). The two main types of CWs are:

- 1) surface flow wetlands consisting of vegetation planted in shallow, relatively impermeable soil, clay or mine spoil
- 2) subsurface wetlands consisting of vegetation planted in a deep, permeable mixture of substrate such as soil, peat moss, spent mushroom compost, sawdust, straw/manure, hay bales and gravel, often underlaid with limestone

In surface CWs, the dominant treatment processes occur mainly in the shallow surface layer. In subsurface wetlands, the water primarily flows through the substrate and treatment involves major interactions within the substrate (Stottmeister et al., 2003).

The A-01 wetland treatment system is a surface flow wetland designed to remove metals (primarily copper) from the A-01 industrial effluent. The treatment system consists of a stormwater retention basin, a splitter box, and four sets of two sequential, treatment cells (Figure 1) with surface flow. Each treatment cell is a one-acre wetland that contains *Schoenoplectus californicus* (giant bulrush) and has about a 24-hour retention time, depending on flow. A-01 effluent flows from the retention basin to the splitter box, where it is distributed to the four A-cells. The effluent flows through the A-cells into the B-cells, through the B-cells to the wetland outfall, and into Tims Branch.

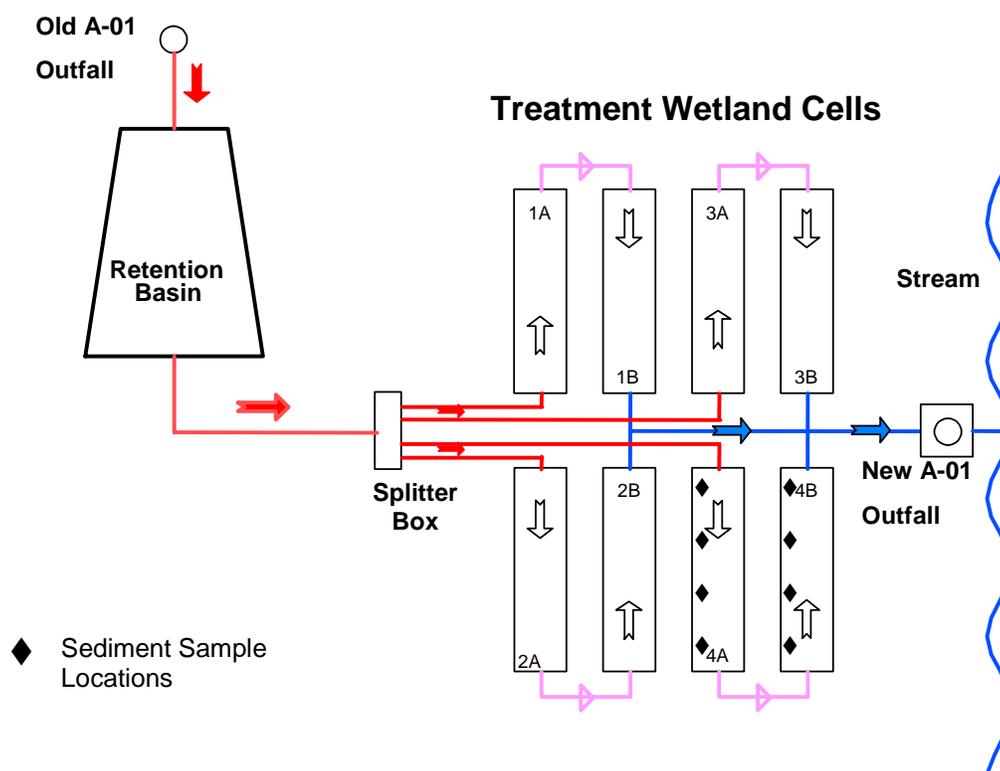


Figure 1. Schematic of the A-01 Wetland Treatment System and Sampling Locations

When the wetland treatment cells were constructed, the hydrosol was amended with organic matter (primarily coarse wood chips), fertilizer (Osma-coat, 14-13-14 formula) at the rate of 3920 kg/hectare (1.75 tons/acre), and gypsum at the rate of 2240 kg/hectare (1 ton/acre) (Specht and Nelson, 2002). A layer of hydrosol, followed by the amendments and an additional 6 inch layer of hydrosol were added to the cells and lightly disked to mix them together. The treatment system was constructed during the winter/spring of 2000 and began receiving A-01 effluent in July 2000. Since the beginning of the A-01 WTS operation, water samples have been routinely collected at the compliance point (i.e., New A-01 outfall; Figure 1) for monthly reporting. For research purposes, water samples were also collected at numerous locations from the inflow of the system through the discharge to the receiving stream in 2000 and 2001 (Nelson et al., 2002). Sediment samples were also collected and analyzed to provide baseline data on metal concentrations and general sediment chemistry early in the operational life of the treatment system in September 2001 (Specht and Nelson, 2002).

Another set of water and sediment samples were collected in 2003 (July-August). A detailed description of 2003 sampling, analytical methods and results is presented in Knox et al. (2004). In June 2004 and 2005 additional sediment samples from two wetland cells (4A and 4B) were collected. The data and findings from the 2004 and 2005 study are presented in this document.

2.0 OBJECTIVE

Wetland sediments play an important role in maintaining water quality by removing contaminants from the water column. However, the long term effectiveness of these systems requires that the metals remain bound by the sediments and not be remobilized easily by changed biogeochemical conditions. The manner in which sediment contamination is treated ultimately controls its stability. Choosing the most appropriate treatment requires an understanding of how contaminants bind to the sediment and the conditions under which they will be released back into the water column.

The objective of the 2004 and 2005 sediment studies was to investigate metal binding in the A-01 Constructed Wetlands. Specific tasks include assessment of:

1. Metal distribution in the sediment profile
2. Metal mobility and retention in the wetland sediment
3. Influence of organic floc on metal mobility and retention

3.0 MATERIALS AND METHODS

The study consists of three tasks. Materials and methods for each task are described in this report. Data produced by all three tasks were documented in a laboratory notebook (WSRC-NB-2004-00111).

3.1 TASK 1, METAL DISTRIBUTION IN THE SEDIMENT PROFILE

In June 2004, 16 sediment core samples were taken from cells 4A and 4B, with each cell spatially divided into 4 quarters along its long axis. Two replicate cores were taken near the center of each quarter. Each core was divided into four layers: floc (0-2 cm), organic (2-5 cm), inorganic (5-10 cm), and inorganic (10-20 cm) that were visually described. The sediment samples were dried at ambient temperature (about 21°C), ground in a porcelain mortar, and passed through a 2 mm sieve. A fraction under 2mm from the organic and inorganic layers was used for the determination of pH, organic matter content, total concentration of metals and metal fractionation. Floc samples collected in 2005 were used for fractionation and organic matter analysis of the floc layer.

Percent organic matter (OM) was estimated by loss-on-ignition at a temperature of 375°C. The pH was determined from a 1:1 mineral/water equilibration solution. Total concentration of metals was determined by a total microwave digestion of 0.6 g of sediment material with concentrated acids (10 mL of HNO₃, 4 mL of H₂SO₄, and 2 mL of HCl). The resulting extract solution was analyzed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Total concentration of Hg in sediment was analyzed by Inductively Coupled Plasma-Mass Spectroscopy (IC-MS).

Additionally, porewater samples were separated from three layers: organic (2-5 cm), inorganic (5-10 cm), and inorganic (10-20 cm). The porewater samples were taken from one replicate sediment core from each quarter 12 to 24 hours after collection of the core. The separation of sediment and porewater was carried out using 50 mL centrifuge filter tubes each fitted with a 20-mL capacity filter insert with a 10 µm polypropylene mesh (Vectaspin tubes). Typically, three of four tubes were filled to the insert capacity with sediment and centrifuged at 3000 rpm for 30 minutes. The collected porewater was subsequently filtered with a 0.20µm filter and analyzed for dissolved organic carbon (DOC) by an organic carbon analyzer (model 1020A). Porewater redox potential and pH for each sample were measured immediately following separation from the sediment.

Anion analyses on the porewater samples were performed using a Dionex DX-500 ion chromatograph. Metal concentrations in porewater samples were determined by ICP-MS.

Data from these analyses were used to address the horizontal and vertical distribution of metals in the wetland sediment.

3.2 TASK 2 & 3, METAL MOBILITY AND RETENTION

3.2.1 Sequential Extraction of Organic and Inorganic Layers

In Task 2, a sequential extraction was conducted on the floc layer, organic matter layer, and upper inorganic sediment layer (5-10 cm depth) with the objective of assessing metal mobility and retention in each sediment layer. Organic matter layer and inorganic sediment layer samples were taken from the eight cores collected in June 2004. Floc samples were collected in floc traps in May 2005 (described in greater detail later).

A sequential extraction scheme based on modified methods of Tessier et al. (1979) and Hall et al. (1996) was used for the organic matter and inorganic layers. This extraction scheme was chosen because of the potential mineralogical phases residing in the core samples. The following fractions were extracted from 1 g subsamples from each layer:

- Exchangeable
- Carbonate
- Amorphous Fe and Mn oxides
- Crystalline Fe and Mn oxides
- Organic
- Sulfides
- Residual

The metal fractionated scheme employed in this study is presented in Table 1. All extractions, except the final digestion, were conducted in 50-mL polypropylene centrifuge vials to minimize losses of solid material. Between each successive extraction, separation was achieved by centrifuging at 3000xg for 20 min. The supernatant was removed with a pipette, filtered through a 0.45- μ m filter, and stored for analysis. The residue was washed with 10 mL of nanopure water and centrifuged, and the supernatant was added to the sample extract. The extracts from each fraction were analyzed for metal concentrations by ICP-AES.

Table 1. Sequential Extraction Procedure Employed for the Geochemical Fractionation of Elements in Wetland Sediment

Step	Operationally defined fraction	Reagents	Extraction time
Organic matter and inorganic layer*			
1	exchangeable	1.0 M MgCl ₂	1 h
2	carbonates	1.0 M CH ₃ COOHNa	6 h
3	amorphous oxides	0.25 M NH ₂ OH·HCl in 0.25 M HCl	2 h
4	crystalline oxides	1.0 M NH ₂ OH·HCl in 25% CH ₃ COOH	3 h
5	organic	0.1 M Na ₄ P ₂ O ₇	24 h
6	sulfide	4.0N HNO ₃	30 min
7	residual	HNO ₃ /H ₂ SO ₄ /HCl/H ₂ O ₂	
Floc layer**			
1	soluble organic	0.1 M Na ₄ P ₂ O ₇	24 h
2	exch. and carbonates	1.0 M CH ₃ COOHNa	6 h
3	amorphous oxides	0.25 M NH ₂ OH·HCl in 0.25 M HCl	2 h
4	crystalline oxides	1.0 M NH ₂ OH·HCl in 25% CH ₃ COOH	3 h
5	insoluble organic	30% H ₂ O ₂ /HNO ₃ /CH ₃ COOCH ₄	5 h

*sequential extraction conducted on dry mass of samples

**sequential extraction conducted on wet mass of samples

3.2.2 Collection of Floc Material

Task 3 focused on the floc layer that collected on top of the sediments. Samples of floc were collected from cells 4A and 4B with each cell spatially divided into 4 quarters along its long axis. One floc sample was collected from each quarter using “floc traps,” each of which consisted of a 13 cm deep, 25 cm wide, and 64 cm long plastic container anchored to the bottom with bricks. Floc traps were left in place for two weeks to collect sufficient material for analysis, then sealed while still under water with a water-tight cap to keep the floc and pore-solution in place when lifting the trap from the water. The floc traps were subsequently stored in a dark cooler at less than 4 °C to preserve the physical and chemical integrity of the sample material and promptly transferred to the analysis labs.

3.2.3 Analytical Methods on Floc Material

Each floc sample was split into two sub-samples. One was sent to Frontier Geosciences Inc. for total and methylmercury analysis, and the other was processed for total metal content and sequential extraction at the Savannah River National Laboratory (SRNL). Total metal content was analyzed by total digestion as described in Task 2. Because floc samples are high in organic matter, the sequential extraction was modified to include extraction of a soluble organic fraction followed by exchangeable, amorphous oxides, oxides, and organic fractions (Table 1). The residual fraction was not run due to insufficient material. The extracts from the sequential extractions and total metal digestion were analyzed for metal by ICP-AES by the analytical laboratory at the University of Georgia. Additional analytical work on the floc samples included percent organic content by loss-on-ignition at a temperature of 375 °C.

The sediment sub-samples sent to Frontier Geosciences were prepared with an aqua regia (80% HCl/20% HNO₃) cold digest. Digested samples were analyzed for total Hg in accordance with the standard operating procedures (SOPs) described in the Frontier Geosciences Quality Assurance manual. Aliquots of each digest (0.5-5 mL) were reduced in pre-purged reagent water to Hg⁰ with SnCl₂, and then the Hg⁰ was purged onto gold traps as a preconcentration step. The Hg contained on the gold traps was analyzed by thermal desorption into a cold vapor atomic fluorescence detector (CVAFS) using the dual amalgamation technique. Peak heights were measured by a chart recorder and recorded on bench sheets in "chart units" to the nearest 0.2 units.

For methyl Hg analysis, because of the potential for significant positive monomethyl mercury (MMHg) artifact formation during the distillation of samples containing high levels of inorganic Hg (Bloom et al., 1997), sediment samples were cold extracted rather than distilled. Aliquots of these samples (approximately 0.5 grams) were accurately weighed into 40 mL Teflon® centrifuge tubes, and 5 mL of H₂SO₄/KBr solution plus 1 mL of 1 M CuSO₄ were added. After mixing, 10.0 mL of methylene chloride (CH₂Cl₂) was added to each tube, and the samples were shaken for 1 hour. The samples were then centrifuged at 3000 RPM for 30 minutes to separate the solvent from the aqueous layer. Exactly 2.0 mL of CH₂Cl₂ was removed from each sample and pipetted into a Teflon vial containing 57.6 mL of deionized water. The samples were heated to 45 °C and purged with N₂ to volatilize the CH₂Cl₂, thus releasing the MMHg to the pure aqueous phase.

Aliquots of extracted samples were analyzed using aqueous phase ethylation, purging onto carbotrap, isothermal GC separation, and CVAFS detection (Bloom, 1989; Liang et. al., 1994; Bloom and Fitzgerald, 1987). The reporting limit (calculated by annual 40 CFR study) is 0.020 ng/g MMHg, as Hg. Prior to ethylation, the pH of the sample was brought to 4.9 with the addition of acetate buffer. Samples were ethylated by the addition of sodium tetraethyl borate, and then the volatile ethyl analogs purged with N₂ onto carbotrap.

After a trap drying step, the mercury ethyl analogs were thermally desorbed into a 1 m isothermal GC column (15% OV-3 on Chromasorb WAW-DMSC) held at 100 °C for separation. The column resolves the following peaks: elemental Hg, dimethyl Hg, methyl ethyl Hg, and diethyl Hg. Because of the wet chemistry used, only methyl ethyl Hg, the MMHg analog is quantified for this assay. The organo-Hg compounds were pyrolytically broken down to Hg⁰ prior to entering the CVAFS detector for quantification. Peak heights were accessed by chart recorder, and recorded on bench sheets in "chart units" to the nearest 0.2 units.

3.3 DATA ANALYSIS

Comparisons involving multiple variables measured in numerous samples can be efficiently summarized with ordination techniques that "map" the samples in two or three dimensions such that the distances between samples are proportional to the dissimilarities among samples. We used Principal Component Analysis (PCA) (Dillon and Goldstein, 1984) to ordinate samples collected from different depths and cell quarters based on dissimilarities in their metal concentrations. Concentration data were log₁₀ transformed prior to analysis to better conform to the normal model. Pearson correlation coefficients were used to quantify the influence of each contaminant on the Principal component axes. Separate ordinations were constructed for the total digestion data, porewater data, and exchangeable metal fractions as explained in greater detail later.

Mantel tests (McCune and Mefford, 1999) were used to assess the correspondence between the distribution of total metals in the sediments and metals in the porewater and between the distribution of total metals in the sediment and exchangeable metals in the sediments. Bray-Curtis dissimilarities calculated from the log₁₀ transformed metal concentration data were used as the distance measures in this analysis. Results were expressed as the standardized Mantel statistic (*r*), which ranges from -1 to 1 and is a measure of the strength of the relationship between two distance matrices. A permutation method was used to test the significance of *r*.

Two-way factorial analysis of variance (ANOVA) with depth (i.e., sediment layer) and treatment cell (4A vs. 4B) as main effects was used to assess the significance of spatial differences in metal concentrations, pH, and organic matter concentrations. Concentration data were log₁₀ transformed and percent data were arcsine transformed prior to analysis to meet the assumptions of normality and homogeneity of variance. The critical level for all statistical tests was $P \leq 0.05$.

Statistical analyses were conducted with PC-ORD (McCune and Mefford, 1999) and SYSTAT (SYSTAT Software Inc., 2002).

4.0 RESULTS AND DISCUSSION

4.1 GENERAL SEDIMENT PROPERTIES

Hydrosoil (sediment) added to the A-01 WTS during construction consisted of local soils typical of the southeast United States, with a slightly acidic pH (5.7) and a cation exchange capacity of 1.4 meq/100g. The particle size distribution was approximately 90% sand and 10% silt and clay. Organic matter content ranged from 1 to 2 percent (Murray-Gulde, 2003). Based on the initial characterization and the design objectives for the constructed wetland, soils were amended during construction with pine mulch, agricultural lime to neutralize pH, gypsum as a source of sulfur, and Osmocote time release fertilizer. After 18 months of operation, organic matter content in the sediment remained at 2 to 3 %, CEC slightly increased to 5.6 meq/100g, and pH increased to 6.4 (Murray-Gulde, 2003). The sediment redox measured from the 8 wetland cells at that time ranged from -17 to -261mV (Murray-Gulde, 2003). Yu (1991) also showed that the pH of acidic sediments increased after they were submerged from 4.5 to near 5.5 after one week and a final stable pH of 6.4 after about 2 weeks. When submerged, sediment pH is influenced by a number of reactions (Yu, 1991). The impact of these reactions on sediment pH changes as the redox status changes. In general, reduction reactions in aqueous systems take up protons, or make the sediment more basic.

During this study (2004 and 2005), the pH of the sediment samples was lowest in the organic layer (5.0 to 5.4, respectively, for cell 4A and 4B) and increased with sediment depth (Figure 2). Two-way ANOVA of the pH data indicated a significant difference among depths ($P < 0.001$) and a significant interaction between depth and cell ($P < 0.001$). The latter finding reflected changes in the relationship between cells at different sediment depths.

The sedimentation rate ranged from 7.2 to 76.6 g/m²/day with a mean of 19.9 and 27.5 g/m²/day, respectively, for cells 4A and 4B (Figure 3). In comparing the spatial distribution of sedimentation rates among sediment traps located from the inflow to outflow of the wetland cells, no differences were observed, except location 4B-A where the sedimentation rate was unusually high. Murray-Gulde (2003) reported slightly lower mean sedimentation rates (2.2 to 28.6 g/m²/day) for all 8 wetland cells (samples collected in 2002).

Organic matter content (as indicated by loss-on-ignition) was very high (54.4 and 65.6%, respectively for 4A and 4B) for the floc layer and, to a lesser extent, the organic matter layer (depth of 2-5 cm; 16.7 and 11.8% respectively for 4A and 4B) and low (about 3%) for the inorganic layers at 5-10 cm and 10-20 cm (less than 3%) (Figure 4). Two-way ANOVA of arcsine transformed percent organic matter data indicated that organic matter content significantly differed among sediment layers ($P < 0.001$) and that organic matter content was significantly greater in cell A than in cell B ($P = 0.031$).

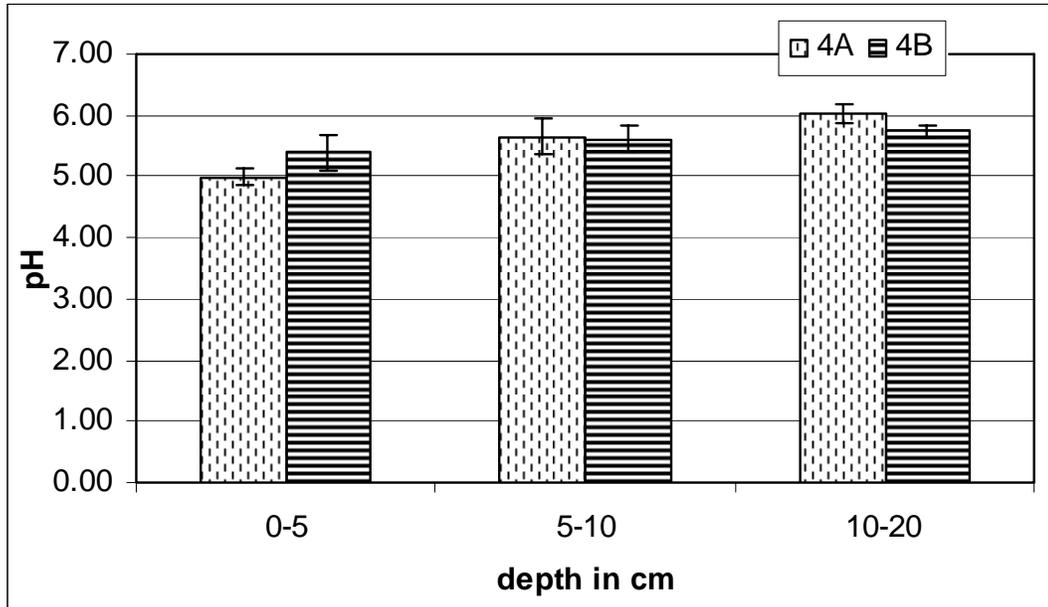


Figure 2. Sediment pH as a Function of Depth

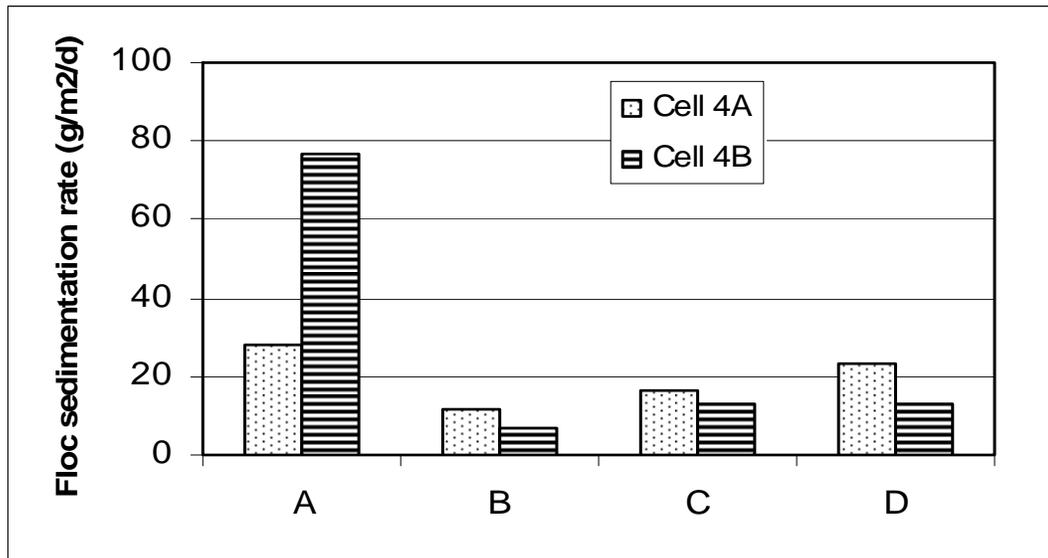


Figure 3. Floc Sedimentation Rates (g/m²/day) in 4A and 4B Cells

The role of organic matter in wetlands is to provide a carbon source for sulfate reducing bacteria as well as to provide organic ligands for binding Cu and other metals. Material collected in the sediment traps provides a conservative measure of the quantity and associated organic matter content of particulates settling from the water column. In general, the organic matter content measured in the A-01 wetland agreed with organic matter content observed in other wetlands (Fennessy et al., 1994).

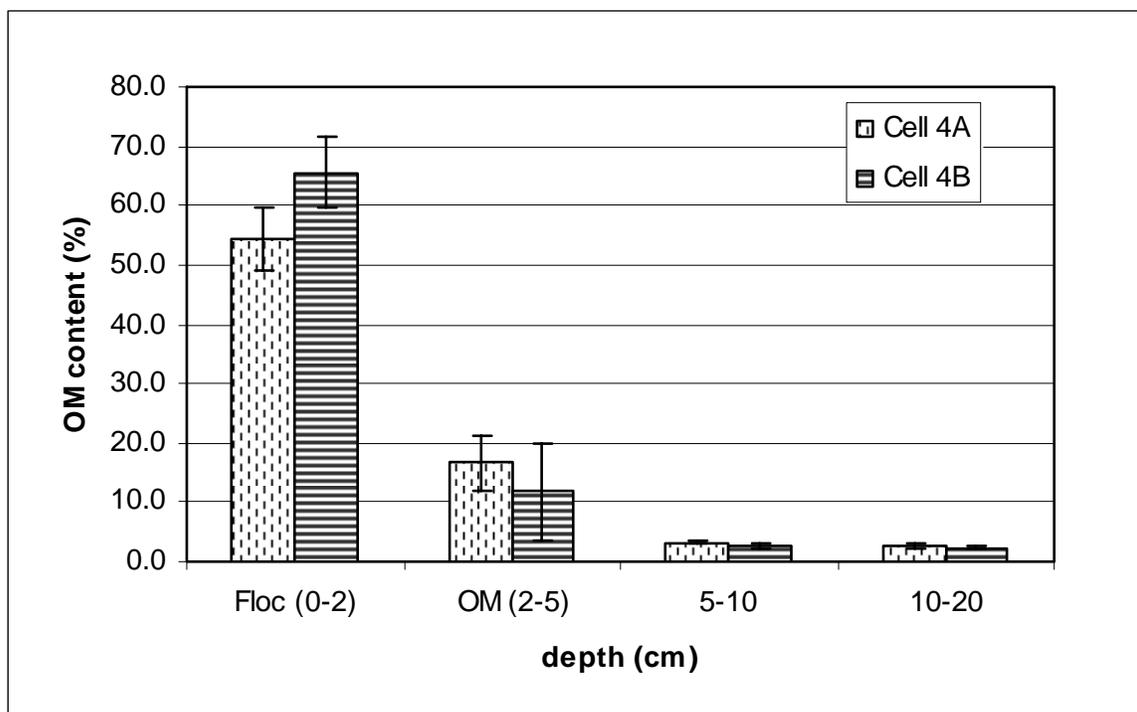


Figure 4. Organic Matter Distribution (%) in the Sediment Profile

4.2 VERTICAL AND HORIZONTAL DISTRIBUTION OF CONTAMINANTS IN SEDIMENT

4.2.1 Total Concentration of Cu and Other Metals in the Sediment Profile

Metal concentrations in the sediment samples from cells 4A and 4B are presented in Table 2 through Table 5. Concentrations of most metals were highest in the floc and decreased strongly with sediment depth and, to a lesser extent, with distance from influent (i.e., from cell 4A to cell 4B, Figure 5 and Figure 6). For example, Cu concentrations were highest in the floc layer from transects 4A-A1 and 4A-A2 (closest to the influent), 1276 ppm and 1139 ppm, respectively, and lowest in the last transect in cell 4B (Table 2). These relationships are summarized in the PCA of the total digestion data (Figure 7).

Table 2. Concentrations of Selected Elements in floc Samples from the A-01 Sediments

Sample Locations	Cd ppm	Cu ppm	Ni ppm	Hg Ppm	Zn ppm	Pb ppm	Fe ppm	Mn ppm
4A-A1	2.17	1276	33.2	0.90	909	54.8	20200	466
4A-A2	2.04	1139	29.7	1.04	767	57.8	19250	390
4A-B1	1.56	808	14.8	2.75	657	32.7	7082	338
4A-B2	1.63	896	20.5	1.52	726	36.2	9384	386
4A-C1	1.33	820	16.3	1.46	522	40.2	7154	264
4A-C2	0.88	501	13.5	1.00	398	27.4	6980	239
4A-D1	0.75	398	14.1	0.95	389	31.2	13561	182
4A-D2	1.02	534	23.8	1.35	451	37.0	9709	299
Avg	1.4	796	20.7	1.4	602	39.7	11665	320
Stdev	0.5	310	7.5	0.6	191	11.0	5429	93
4B-A2	0.57	269	8.2	1.95	214	22.8	7702	186
4B-B1	0.51	357	16.3	3.17	225	32.2	11421	265
4B-B2	0.39	274	15.0	0.51	210	23.8	10570	135
4B-C1	0.45	293	9.0	2.55	220	21.8	4309	158
4B-D1	0.63	274	22.0	3.62	318	24.0	5163	231
4B-D2	0.34	200	23.0	2.16	175	22.0	7021	202
Avg	0.5	278	15.6	2.3	227	24.4	7698	196
Stdev	0.1	50	6.3	1.1	48	3.9	2845	47

Table 3. Concentrations of Selected Elements in the Organic Layer of the A-01 Sediments

Sample Locations	Cd ppm	Cu ppm	Ni ppm	Hg Ppm	Pb ppm	Zn ppm	Fe ppm	Mn ppm
4A-A1	0.44	205.3	7.2	0.62	16.8	155.8	18839	87.3
4A-A2	0.59	313.9	5.9	3.97	17.7	218.8	7410	146.3
4A-B1	0.66	344.0	8.4	1.07	19.9	269.0	13690	146.1
4A-B2	0.31	187.1	4.7	5.05	11.1	141.8	4891	157.2
4A-C1	0.28	192.4	4.7	1.83	15.0	125.6	5475	76.8
4A-C2	0.14	101.6	3.5	0.96	9.3	81.1	4837	73.5
4A-D1	0.45	188.7	9.2	0.75	18.7	211.9	12901	140.8
4A-D2	0.12	103.6	5.2	1.68	12.3	97.0	6442	83.8
Avg	0.37	204.6	6.1	1.99	15.1	162.6	9311	114.0
Stdev	0.20	86.9	2.0	1.64	3.8	65.1	5196	36.5
4B-A1	0.11	33.3	3.0	0.31	7.2	28.0	3516	41.1
4B-A2	0.12	181.3	4.4	0.33	12.1	169.6	6363	167.1
4B-B1	0.24	91.5	4.0	0.86	11.6	82.6	5875	40.6
4B-B2	0.08	46.0	2.1	0.25	6.7	47.5	2621	36.7
4B-C1	0.08	39.9	3.5	0.99	8.5	35.3	5388	33.8
4B-C2	0.13	58.3	3.3	0.36	7.3	60.8	4895	31.6
4B-D1	0.07	30.4	1.1	0.30	4.3	41.9	1709	35.9
4B-D2	0.10	39.3	3.8	0.72	9.1	47.2	3869	39.2
Avg	0.12	65.0	3.2	0.52	8.4	64.1	4280	53.3
Stdev	0.06	50.9	1.1	0.29	2.6	45.8	1630	46.1

Table 4. Concentrations of Selected Elements in the 5-10 cm Inorganic Layer of the A-01 Sediments

Sample Locations	Cd ppm	Cu ppm	Ni ppm	Hg Ppm	Zn ppm	Pb ppm	Mn ppm	Fe ppm
4A-A1	0.04	22.8	1.9	1.62	19.6	6.2	46.1	4685
4A-A2	0.04	20.4	1.3	0.47	15.9	4.5	27.3	3229
4A-B1	0.04	15.0	1.7	0.74	15.5	5.1	26.2	4597
4A-B2	0.03	9.8	1.4	2.25	13.9	3.9	30.2	3341
4A-C1	0.02	11.3	2.3	0.58	12.9	5.8	26.7	6101
4A-C2	0.06	15.9	2.9	0.63	13.1	6.7	49.4	4030
4A-D1	0.30	8.2	1.7	4.09	8.9	4.9	59.2	4562
4A-D2	0.11	11.8	1.7	1.08	10.9	5.6	43.5	3377
Avg	0.08	14.4	1.8	1.4	13.8	5.3	38.6	4240
Stdev	0.09	5.1	0.5	1.2	3.3	0.9	12.6	964
4B-A1	0.11	10.3	2.7	0.25	9.3	5.0	86.9	2621
4B-A2	0.00	6.2	0.7	4.81	5.4	3.5	45.0	1713
4B-B1	0.00	6.3	0.9	0.35	5.6	4.0	12.4	2955
4B-B2	0.01	9.5	1.5	0.27	8.3	4.7	20.3	2313
4B-C1	0.03	12.2	3.3	0.50	9.1	7.4	33.3	5955
4B-C2	0.00	8.2	2.0	0.25	7.0	4.6	21.2	5403
4B-D1	0.02	7.5	1.7	3.69	9.7	5.0	48.4	2118
4B-D2	0.02	6.9	2.0	0.37	7.5	5.4	53.4	4016
Avg	0.02	8.4	1.84	1.3	7.7	4.9	40.1	3387
Stdev	0.04	2.1	0.85	1.8	1.7	1.2	24.0	1576

Table 5. Concentrations of Selected Elements in the 10-20 cm Inorganic Layer of the A-01 Sediments

Sample Locations	Cd ppm	Cu ppm	Ni ppm	Hg ppm	Zn ppm	Pb ppm	Mn ppm	Fe ppm
4A-A1	0.02	6.5	0.8	2.81	5.6	3.6	73.4	1662
4A-A2	0.02	4.8	0.8	1.04	4.6	3.3	46.7	1849
4A-B1	0.01	6.2	0.8	0.69	5.2	3.8	63.3	2750
4A-B2	0.01	5.7	2.1	1.33	5.9	4.1	84.9	4548
4A-C1	0.07	10.6	2.1	1.01	10.1	6.2	70.6	3689
4A-C2	0.01	11.3	2.6	0.20	10.2	4.5	71.1	5238
4A-D1	0.12	7.7	1.2	1.59	6.5	4.3	93.3	4180
4A-D2	0.12	10.7	2.9	0.69	10.7	7.3	124.9	5000
Avg	0.05	7.9	1.66	1.17	7.3	4.6	78.5	3614
Stdev	0.05	2.6	0.84	0.79	2.5	1.4	23.3	1385
4B-A1	0.12	8.3	1.5	0.15	8.2	3.6	149.3	2305
4B-A2	0.00	5.7	1.1	2.24	4.7	3.7	88.2	2377
4B-B1	0.00	7.2	1.9	0.08	7.2	4.6	41.4	3503
4B-B2	0.01	6.4	0.7	3.54	6.0	3.7	63.4	2452
4B-C1	0.00	12.5	2.2	0.66	9.4	6.4	35.8	2438
4B-C2	0.01	6.1	1.5	0.31	5.7	4.7	48.4	3077
4B-D1	0.04	16.0	5.9	2.24	13.5	9.5	157.1	6152
4B-D2	0.01	10.1	2.3	0.54	7.8	6.0	107.2	4853
Avg	0.02	9.0	2.1	1.2	7.8	5.3	86.3	3394
Stdev	0.04	3.6	1.6	1.3	2.8	2.0	47.7	1408

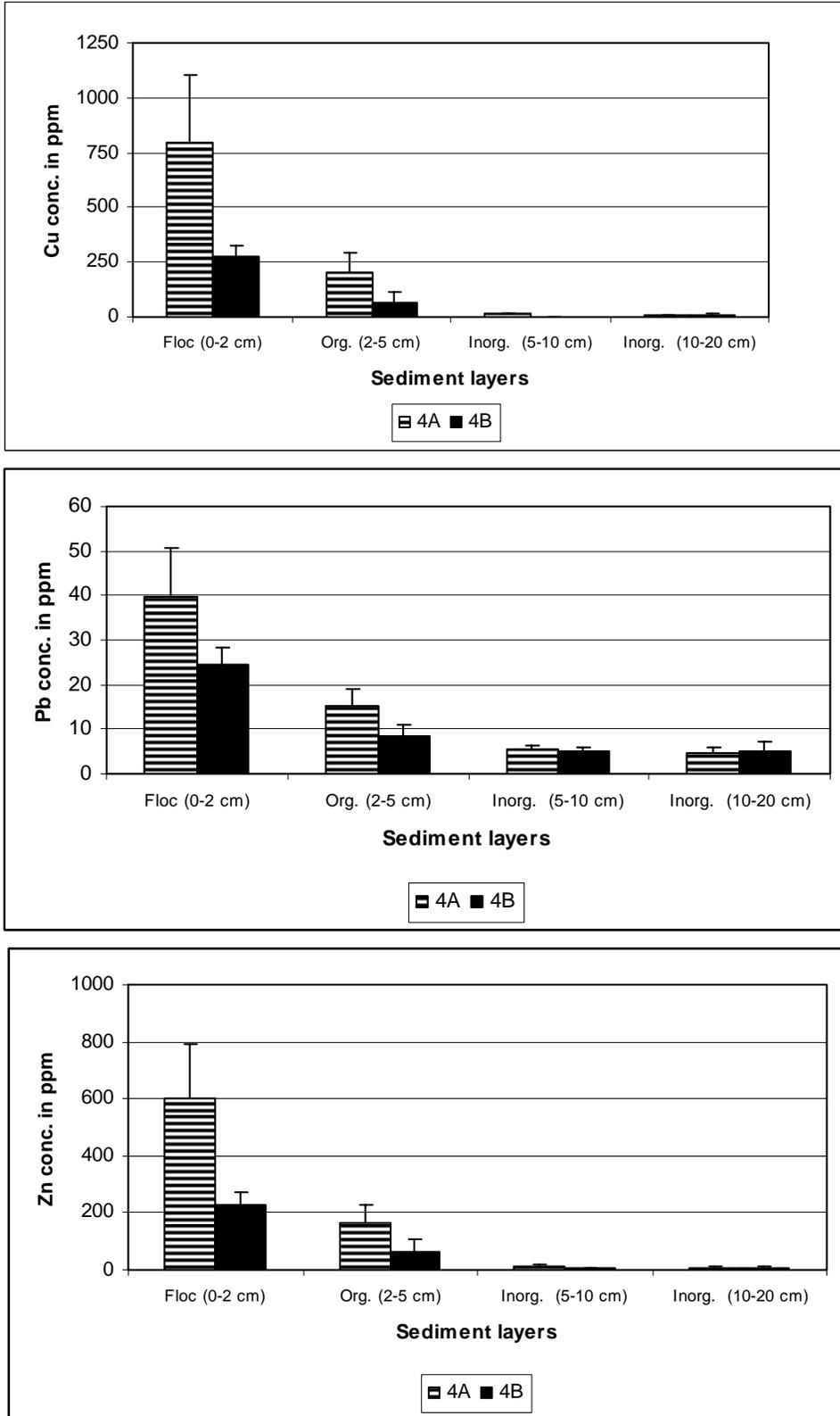


Figure 5. Copper, Pb, and Zn Concentrations (in ppm) in the Sediment Profile

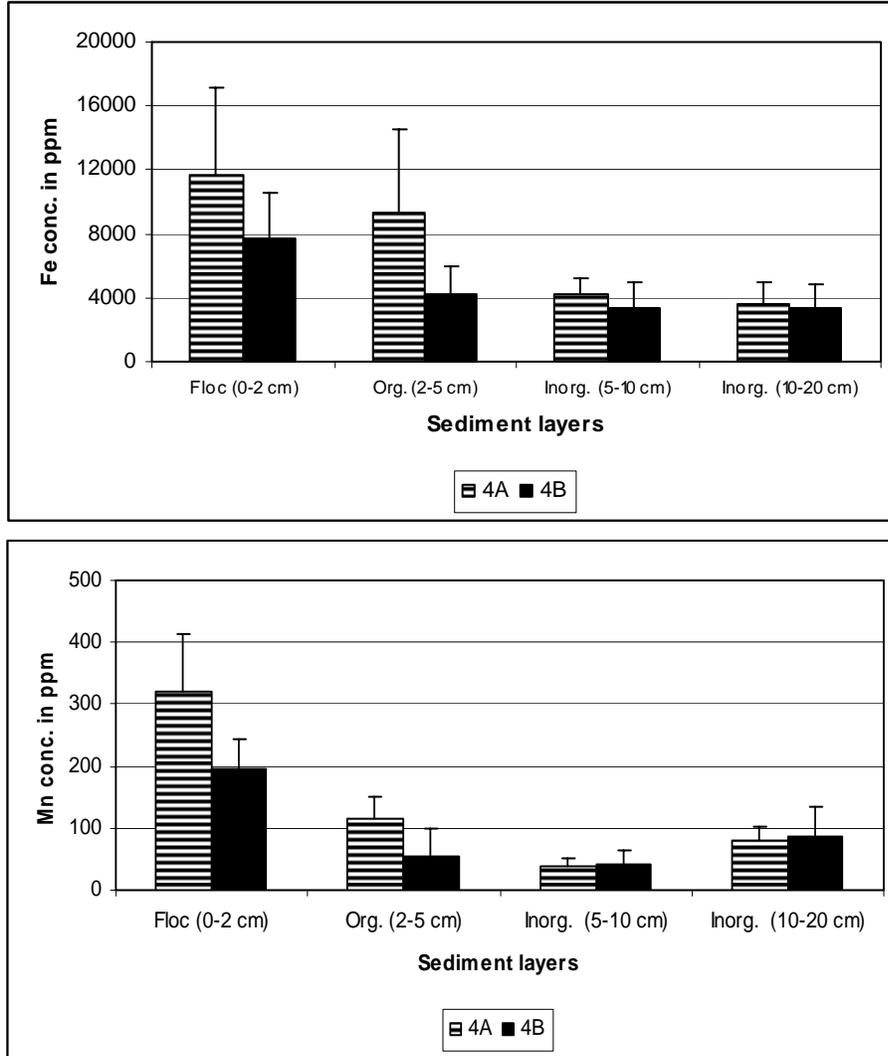


Figure 6. Iron and Mn Concentrations (in ppm) in the Sediment Profile

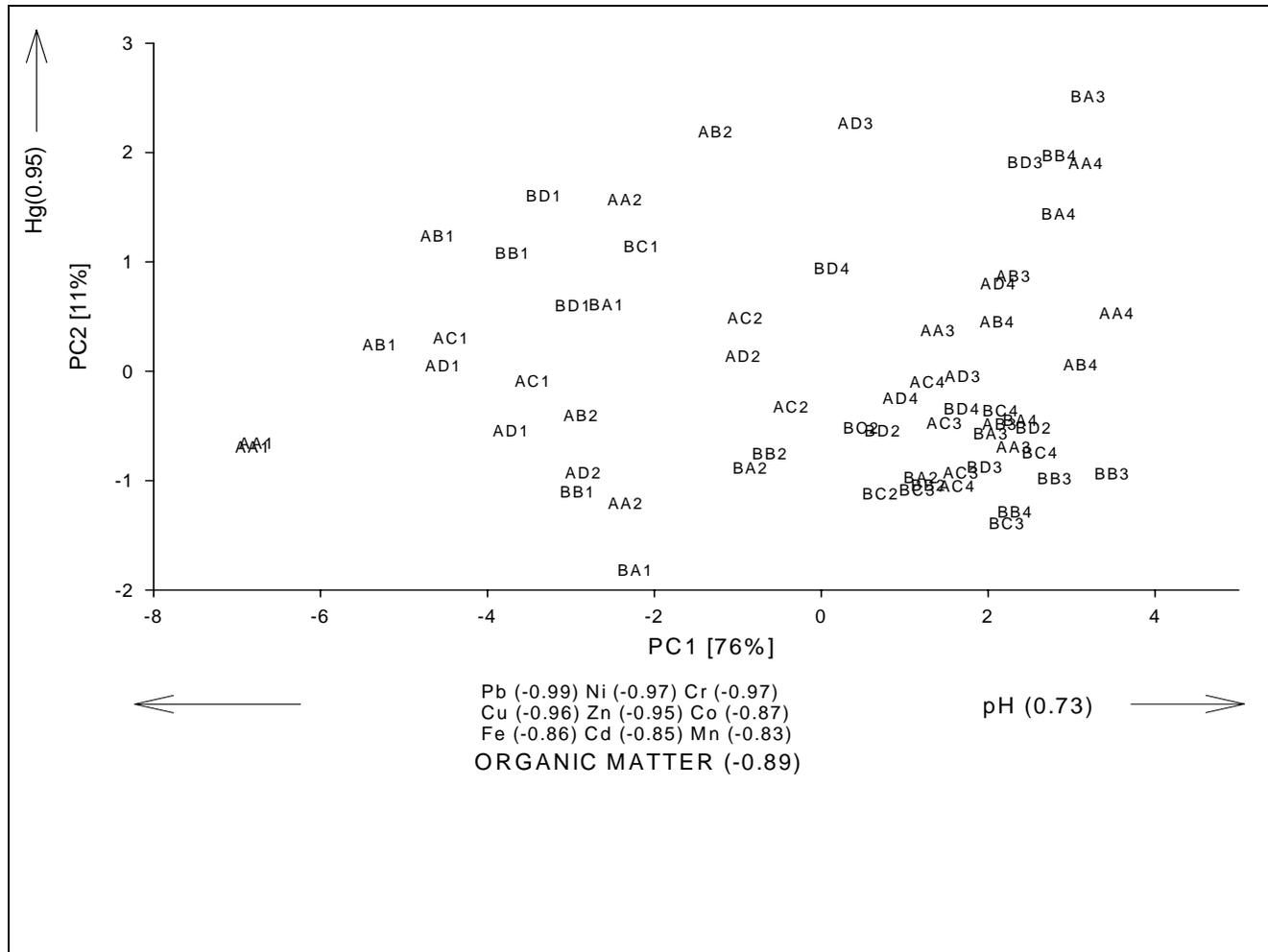


Figure 7. Principal Components Analysis of the Total Digestion Data from the A-01 Constructed Wetland

The first letter of each symbol represents the cell (A or B), the second letter represents the cell quarter (A, B, C, or D), and the number represents the depth (1=floc layer, 2=organic matter layer, 3=5-10 cm inorganic layer, 4=10-20 cm inorganic layer). Also shown are the variables correlated with each axis, the Pearson correlation (in parentheses) and the direction of increase (shown by the arrows). The proportion of the variance accounted for by each principal component score is shown in brackets.

Principal component 1 of the PCA accounted for 76% of the variance in the sediment metal data and represented a gradient with floc layer samples from the A cell at one extreme and inorganic layer samples from both cells at the other. This gradient was strongly correlated ($r=-0.83-0.99$) with the concentration of all metals except mercury, percent organic matter ($r=-0.89$) and pH ($r=0.73$). These results show that most metals in the A-01 wetland sediments behaved similarly: their concentrations decreased as sediment depth increased.

Principal component 2 accounted for 11% of the variance in the sediment metal data and was strongly weighted by only Hg, indicating that Hg behaved differently than the others metals in the A-01 wetland. Cu (\log_{10} transformed) was selected as a representative metal for two-way ANOVA to assess the significance of depth and cell related concentration differences. Depth, cell, and the interaction between depth and cell were all significant at $P<0.001$. The interaction resulted from the fact that 4A cell Cu concentrations were higher than 4B cell Cu concentrations in the floc layer, but not in the inorganic layers as clearly shown in Figure 5.

The distribution of metals in the sediment profile of the A-01 WTS agrees with the findings of other researchers. For example, Ye et al. (2001) reported that the concentration of metals such as Co and Ni tended to be higher in the top 5 cm of sediment than in the 5 to 10-cm and 10 to 15-cm sediment layers in a Pennsylvania constructed wetland treating coal combustion by-product leachate.

4.2.2 Total Hg and Methyl Hg in Sediment

Mercury concentrations in the A-01 sediments are presented in Table 2 - Table 5 and Figure 8 - Figure 10. Total Hg concentrations in the core samples collected in 2004 did not decrease with depth (Figure 8) or distance from the influent (Table 2 - Table 5) as did concentrations of the other metals. Nor did total mercury concentrations in the floc samples collected from 2005 decrease with distance from the influent (Figure 9). However, methyl mercury concentrations in the floc samples increased with distance from the influent (i.e., transect A of both cells had the lowest methyl Hg) and concentrations in the B cell were higher than in the A cell (Figure 10).

Total Hg in the floc layer from the 2004 core samples ranged from about 1.4 to 2.3 ppm compared with about 0.3 to 0.8 ppm in the floc samples collected with the sediment traps in 2005. This difference could be due to the use of different total digestion methods. Samples collected in 2005 were digested with aqua regia, but samples collected in 2004 were digested with a mixture of concentrated acids ($\text{HNO}_3/\text{H}_2\text{SO}_4/\text{HCl}$) and 30% hydroxyl peroxide (H_2O_2). The higher Hg concentration in the 2004 samples could have produced more realistic results, because addition of hydroxyl peroxide enhances organic matter digestion. Both floc and organic matter layers were very rich in organic compounds.

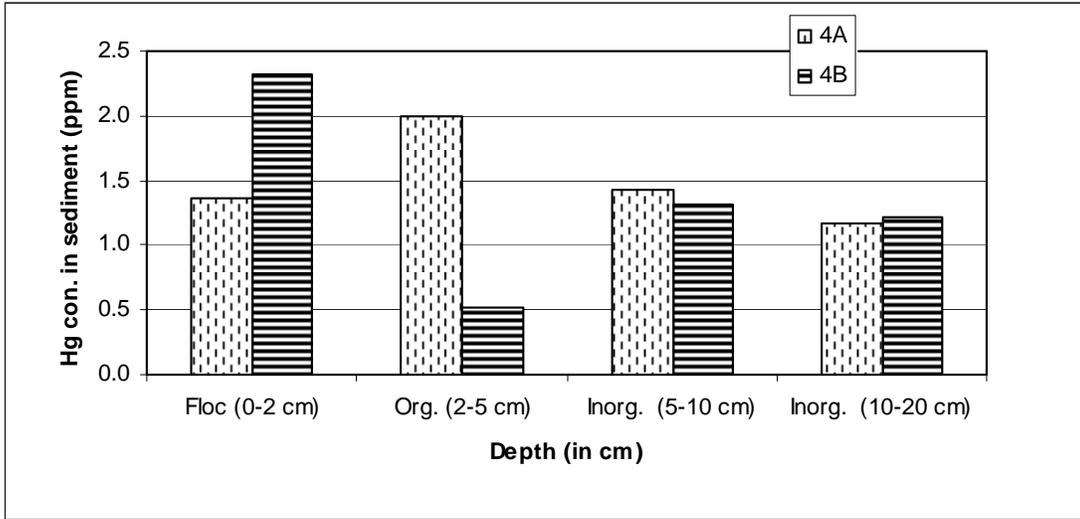


Figure 8. Mercury Concentration (ppm) in the Sediment Profile of the Core Samples Collected in 2004. The samples were analyzed by ICP-MS.

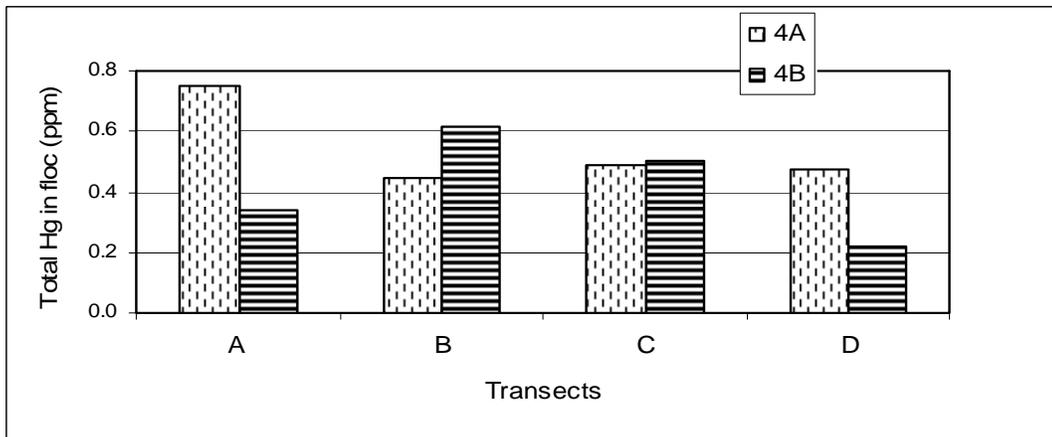


Figure 9. Mercury Concentration (ppm) in Floc Samples Collected in 2005. The samples were analyzed by CVAFS (FGS-069).

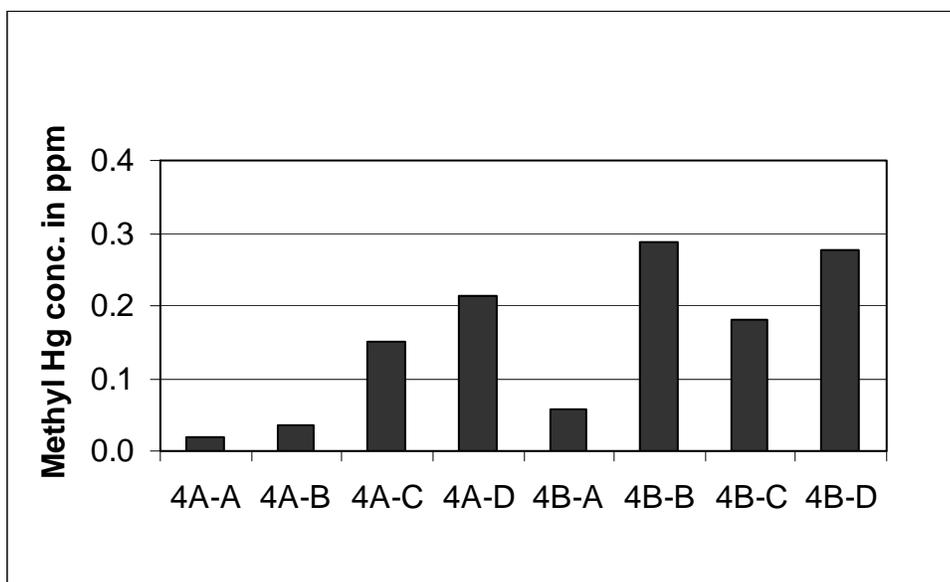


Figure 10. Methyl Mercury in Floc Samples Collected in 2005

4.3 POREWATER CHEMISTRY AND METAL CONTENT

4.3.1 Porewater Chemistry and Metal Content

Porewater from wetlands is characterized by sulfidic and low oxygen conditions, and anoxic and sulfidic processes play major roles in wetland environments. The porewater pH ranged from about 6.5 to 6.6 and varied little throughout the sediment profile (Figure 11). DOC and total inorganic carbon (TIC) were analyzed in only the organic matter layer and the 5-10 cm inorganic layer in cells 4A and 4B, and the 10-20 cm organic layer from cell 4A. There was insufficient sample in the 10-20 cm inorganic layer from cell 4B for this analysis. The concentration of DOC in both the organic and inorganic layers was higher in cell 4B than in cell 4A (Figure 12). A similar pattern was observed for TIC concentrations (Figure 12), which also differed between cells but not layers. DOC concentrations were about ten times greater than TIC concentrations in both cells (Figure 12).

The presence of sulfate, Fe and Mn concentrations in porewater indicate the oxidation status of the sediment. Unlike DOC and TIC, the porewater sulfate concentrations in all layers were higher in cell 4A than in cell 4B (Figure 13). The presence of sulfate, plus low concentrations of Fe, and Mn in the porewater (Figure 14) show that cell 4A, especially the top organic layer, was in an oxidizing state. The lower inorganic layers, especially closer to the effluent showed more reduced conditions as indicated by the presence of relatively high concentrations of Fe and Mn (Figure 14). Porewater sulfate is of particular importance because of its relationship with sulfate-reducing bacteria and the ability of the sediment to sequester metals. Gilmour and Henry (1991) speculated that the optimal sulfate concentration for mercury methylation by sulfate-reducing bacteria in sediments was in the range of 200-500 μM . Above this concentration, sulfide would inhibit methylation, while at lower sulfate levels, sulfate reduction and hence methylation would be limited by available sulfate. Higher sulfate concentrations could be responsible for the lower concentrations of methyl Hg in cell 4A (Figure 10).

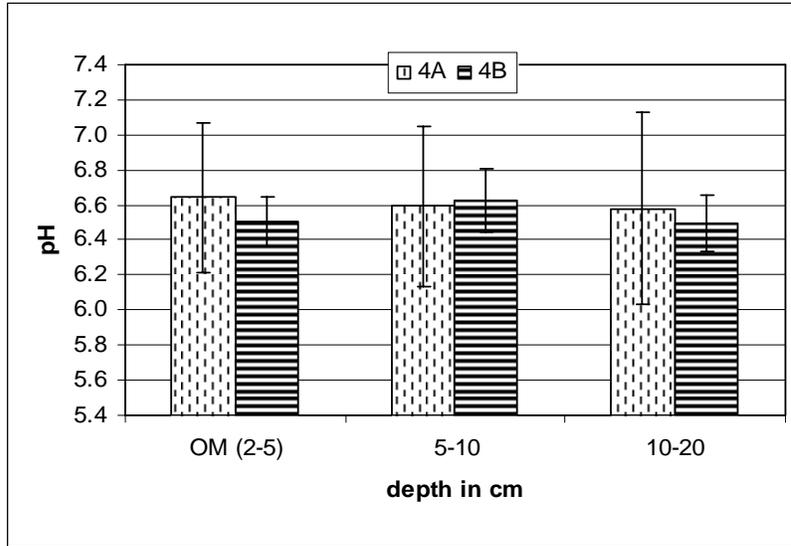


Figure 11. Porewater pH Values as a Function of Depth; Samples Collected from the Sediment of Wetland Cells 4A and 4B

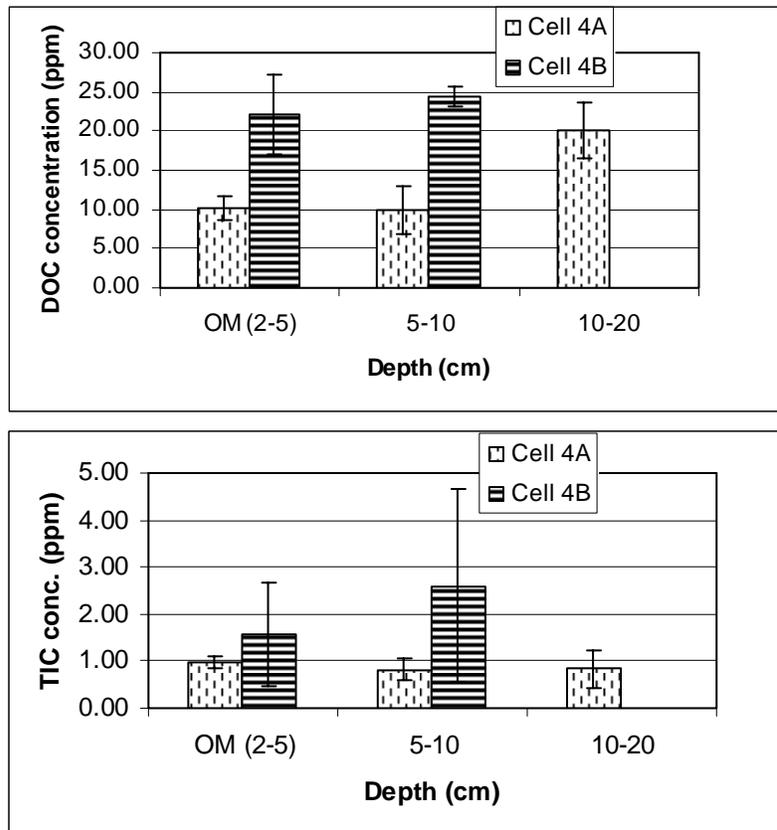


Figure 12. Dissolved Organic Carbon (DOC) and Total Inorganic Carbon (TIC) Concentrations (ppm) in Porewater

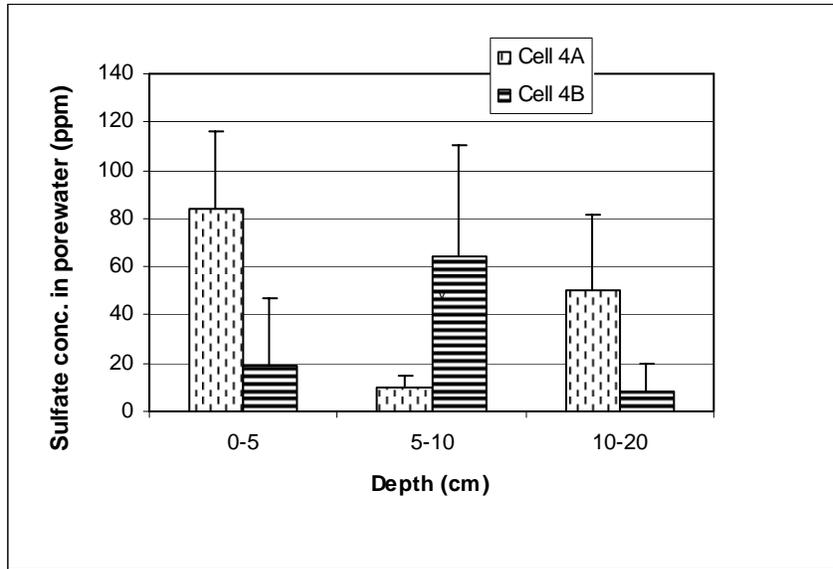


Figure 13. Average Sulfate Concentrations (ppm) in the Porewater Collected from Cells 4A and 4B

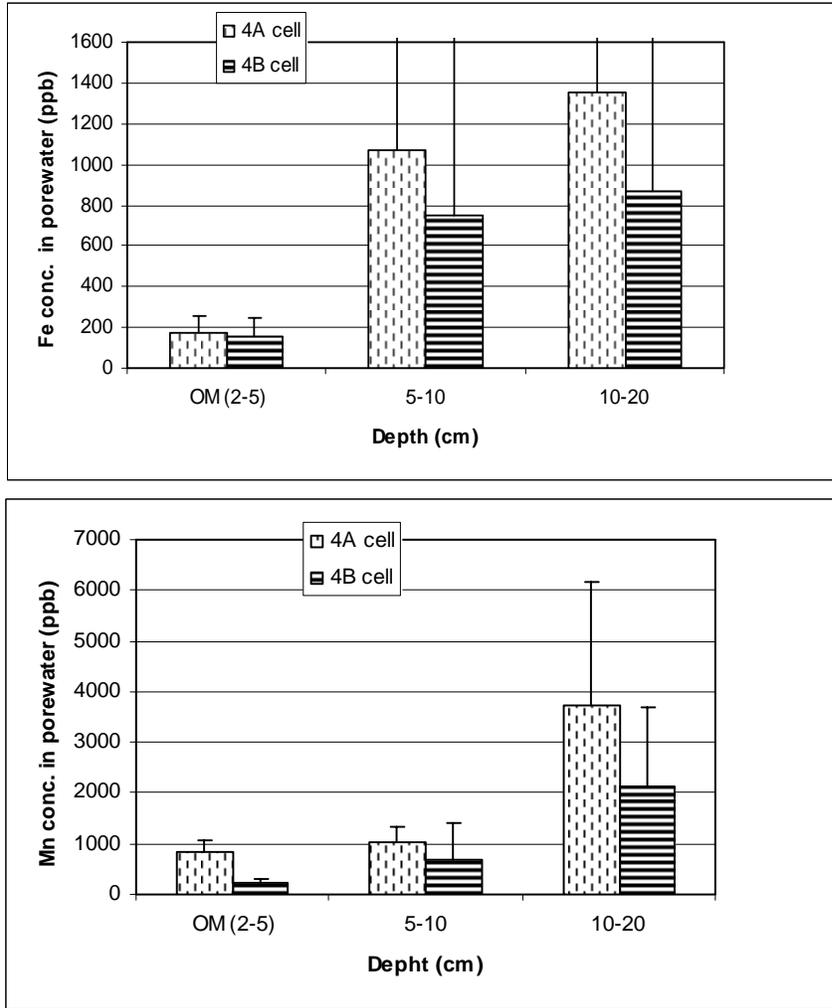


Figure 14. Average Fe and Mn Concentrations (ppb) in the Porewater Collected from Cells 4A and 4B

The pattern of Fe and Mn distribution in the porewater from the wetland sediment was different not only from sulfate but also from the other elements. Iron and Mn concentrations increased from the top layer (organic matter) to the deepest inorganic layer (10-20 cm) (Figure 14). The release of Mn and/or Fe to water depends on the redox chemistry of these two elements as well as the solubility of the solids formed under the redox potential present in the wetland sediment. Mineral $\text{Fe}(\text{OH})_3$ controls the release of Fe^{3+} , while the other two minerals FeCO_3 and $\text{Fe}(\text{OH})_2$ control the release of Fe^{2+} . Under reducing conditions, FeCO_3 and $\text{Fe}(\text{OH})_2$ are stable in the pH range of 7 to approximately 10, as a carbonate solid rather than a hydroxide solid (Evangelou, 1998). The pH of the porewater was about 6.5 (Figure 11) indicating that FeCO_3 and $\text{Fe}(\text{OH})_2$ were dissolved. The stability of Mn species is also Eh and pH dependent (Evangelou, 1998). Under reducing conditions, the solids controlling the release of Mn^{2+} are MnCO_3 and $\text{Mn}(\text{OH})_2$, which are stable in the pH range of 7.5 to approximately 11.2. High Mn concentrations could also be related to the high concentration of Fe^{2+} . Manganese precipitates will return to solution in the presence of ferrous iron (Fe^{2+}) (Stumm and Morgan, 1981). Also iron- and manganese-reducing microorganisms can dissolve insoluble Fe(III) and Mn(IV) oxides, resulting in the release of soluble Fe(II) and Mn(II), as well as the trace metals bound by the Fe(III) or Mn(IV) minerals (Lovley, 2000). Iron (III) and Mn(IV)-reducing microorganisms can thus affect the fate of other contaminant metals through both direct enzymatic reduction and indirect reduction catalyzed by biogenic Fe(II) and Mn(II) (Lovley, 2000). These microorganisms have a detrimental effect on wetland efficiency as they remobilize iron and other metals which may be exported from the system.

The predominant factors governing dissolved metal speciation and reactivity in porewaters are metal oxidation state and metal complexes including inorganic and organic ligands. Relatively little is known about metal speciation in wetlands, primarily because of the small sample volumes that are usually available for experimental research and because of the dramatic changes in metal chemistry that can occur on a millimeter vertical scale (Luther, 1995).

Although variable, Cu, Cd, and Zn concentrations in the sediment porewater profile generally decreased with depth. This pattern was clearer in the sediment profile from cell 4A (Figure 15).

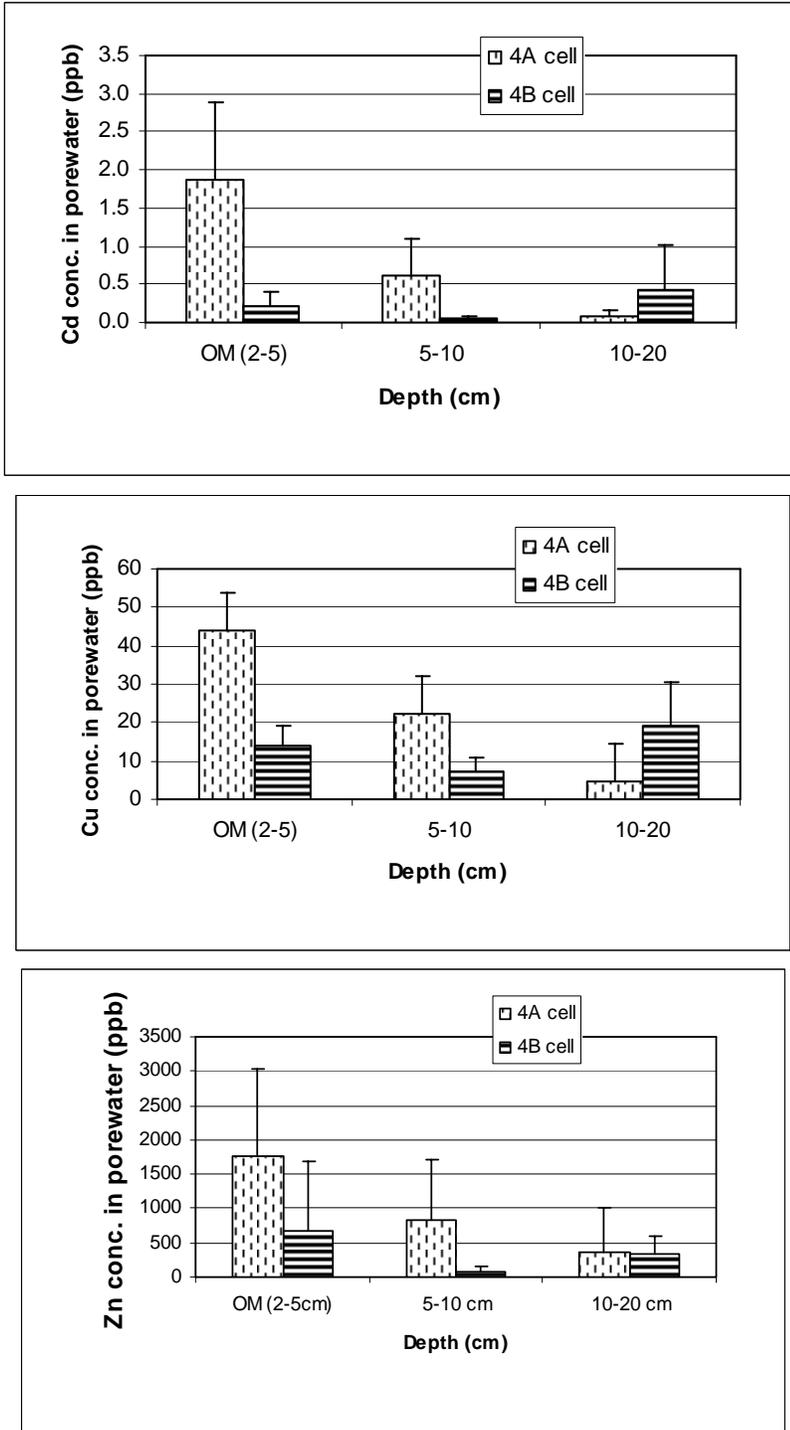


Figure 15. Average Cd, Cu and Zn Concentrations (ppb) in the Porewater Collected from Cells 4A and 4B

Concentrations of these elements in the organic and 5-10 cm inorganic layers were higher in cell 4A than 4B. However, concentrations in the 10-20 cm inorganic layer from cell 4B were higher or almost the same as in cell 4A.. McBride et al. (1997) also showed that DOC significantly influenced the mobility of heavy and transition metals. It should be noted that the average dissolved Cu porewater concentrations in cells 4A and 4B at all depths in this study were below the National Pollution Discharge Elimination System (NPDES) permit limit (60 ppb on monthly average), and well below the limit set for drinking water in the United States (1.0 ppm). Unlike Cu, Cd, and Zn concentrations, the Hg, Ni, and Pb concentrations in the porewater did not exhibit consistent patterns among depths or cells (Figure 16).

PCA was used to summarize spatial trends in metal concentrations in porewater from the A-01 wetland (Figure 17). The first principal component (PC1) accounted for 45% of the variance in the porewater data and was strongly related to the concentration of several metals ($r=-0.77$ to -0.94); however, the ordination of sites on PC1 did not correspond to depth or horizontal position (i.e., distance from the influent). These results parallel Figure 15 and Figure 16 which show that horizontal and vertical concentration gradients differed among metals and were poorly defined for some. The second principal component (PC2) accounted for 17% of the variance in the porewater data and was strongly weighted by only Fe and Mn ($r=0.63-0.73$). PC2 represented a gradient of increase from shallow to deeper sediments and paralleled the results shown in Figure 14 for Fe and Mn.

A Mantel test was used to assess the correspondence between trends in metal concentrations in the sediments and metal concentrations in the porewater. Although significant ($P=0.005$), the standardized Mantel statistic (r) was low ($r=0.22$) indicating weak correspondence between the spatial distribution of metals in the sediment and porewater and suggesting that other factors in addition to total metal levels determine porewater metal concentrations.

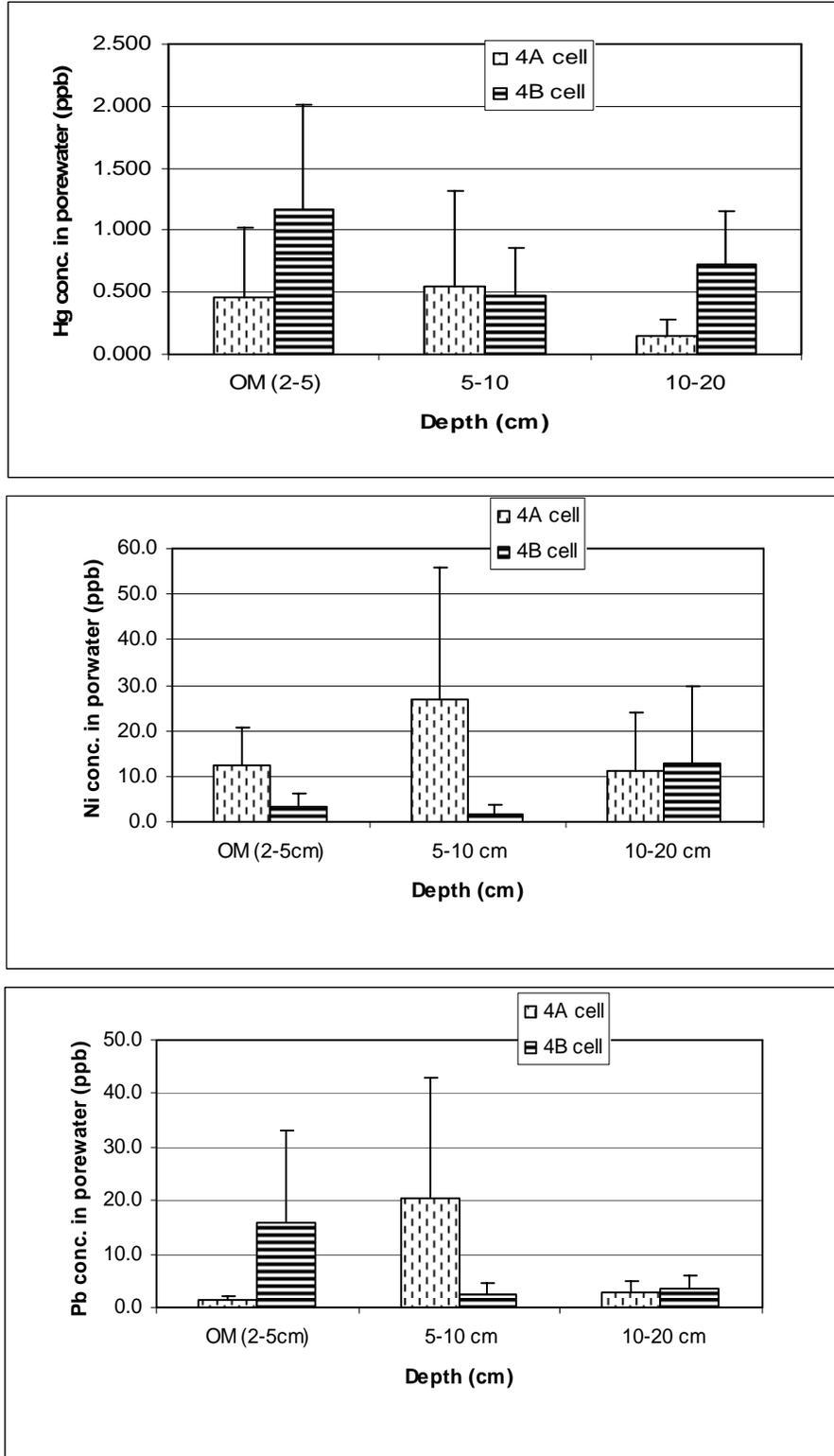


Figure 16. Average Hg, Ni and Pb Concentrations (ppb) in the Porewater Collected from Cells 4A and 4B

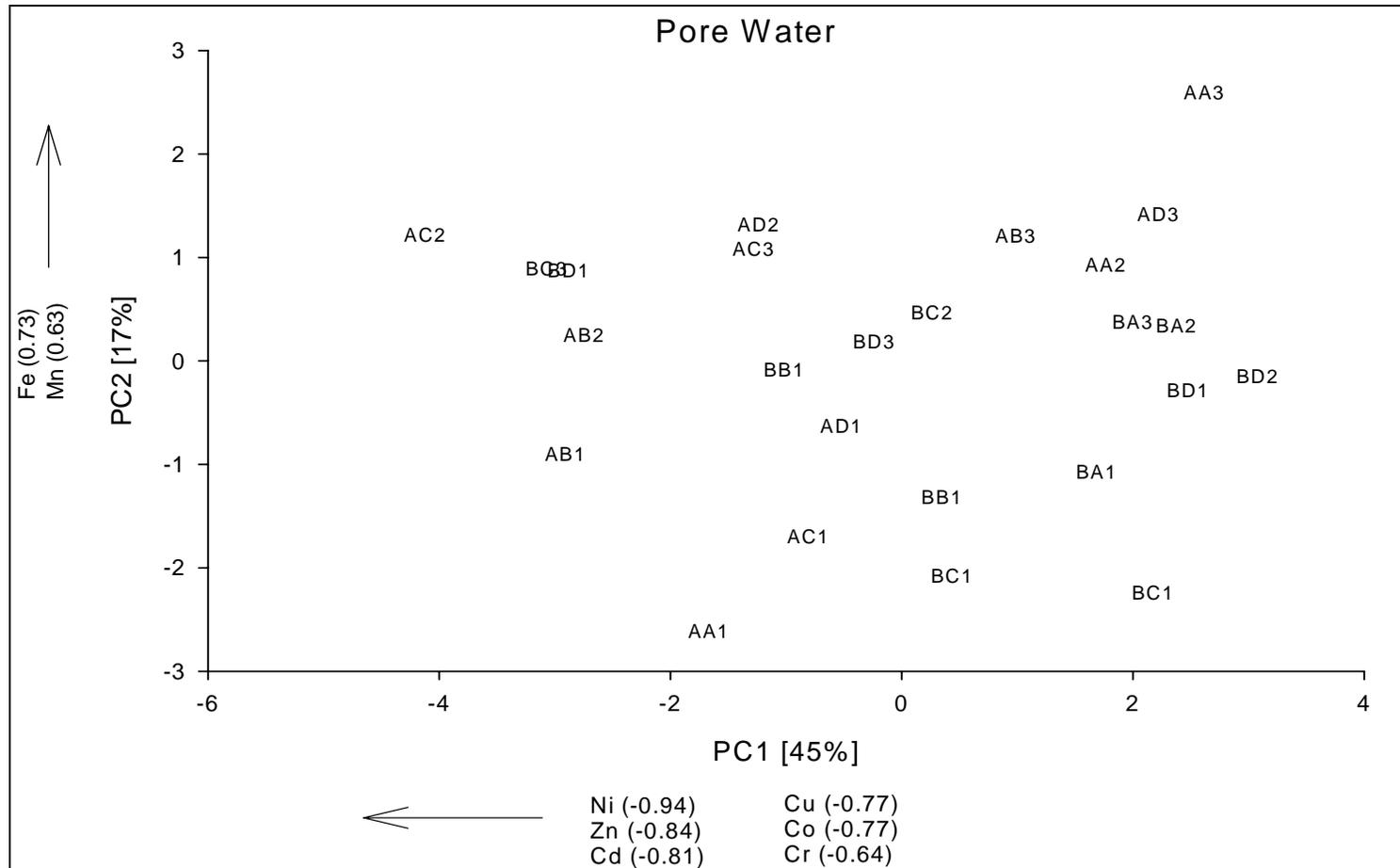


Figure 17. Principal Components Analysis of Metal Concentrations in Porewater from the A-01 Constructed Wetland

The first letter of each symbol represents the cell (A or B), the second letter represents the cell quarter (A, B, C, or D), and the number represents the depth (1=organic matter layer, 2=5-10 cm inorganic layer, 3=10-20 cm inorganic layer). Also shown are the metal concentrations correlated with each axis, the Pearson correlation (in parentheses) and the direction of increase (shown by the arrows). The proportion of the variance accounted for by each principal component score is shown in brackets.

4.4 METAL MOBILITY AND RETENTION IN SEDIMENT

The mobility and retention of trace elements in sediments strongly depends on their specific chemical and mineralogical forms and their binding characteristics. This implies that these forms and characteristics should be studied in addition to total metal concentrations. Since the early 1980s, single and sequential extraction schemes have been designed for the speciation of metals in soils and sediments (Tessier et al., 1979; Quevauviller et al., 1997). In this study, stability of removed contaminants in the wetland sediment was evaluated by potentially mobile fraction, recalcitrant factor and K_d values.

Sequential chemical extraction results are operational in nature (e.g., extraction times and choice of reagents), which may confound interpretation (Nirel and Morel, 1990). Nevertheless, despite the dangers of uncritical acceptance of results from sequential extractions analysis, the procedure is still widely used for assessing the likelihood of mobilization and retention of metals in soils or sediments. Unlike methods often used to determine K_d values, sequential extraction methods explicitly account for both adsorption and precipitation processes that might affect metal mobility and retention.

4.4.1 Metal Speciation

4.4.1.1 Floc layer

Due to the high content of organic matter in floc, a soluble organic matter fraction (SOM) was the first fraction extracted from floc samples. This fraction was extracted with 0.1 M sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$). The results indicated that the SOM fraction was important for Cu retention, with an average of 64 ppm found in this fraction (Table 6). Other divalent elements such as Pb and Zn also occurred largely in this fraction. Similarly, Sobolewski (1996) observed that Cu was predominantly in organically-bound and oxide-bound phases in wetland sediments rich in organic matter. The exchangeable and carbonate fractions in floc samples were extracted with 1 M NaOAc adjusted to pH 5. This fraction, which includes metals weakly sorbed via specific adsorption and precipitation reactions as well as those sorbed via outer-sphere reactions (Tessier et al., 1979), is termed exchangeable because it is readily exchanged with other cations. It also contains metals associated with carbonate minerals. Copper, Pb, Zn, Fe, and Mn concentrations were low in this fraction (Table 6 - Table 10). Similarly, Fujiyoshi et al. (1996) found that Zn sorbed on marine sediment was not extractable with 1 M NaOAc, implying that sorption did not occur via a nonspecific, outer sphere, electrostatic mechanism. However, the results and data presented by Fujiyoshi et al. (1996) contrast with results for metal fractionation in freshwater sediments, where exchangeable metals are often substantial (Jain, 2004). This reflects the importance of the ionic strength of the aqueous matrix, which in some freshwater systems allows aqueous metals to occupy cation exchange sites. Emmerson et al. (2001) have shown that metals sorbed to sediments are released from exchangeable sites via displacement by cations such as Na and K.

Table 6. Sequential Extraction of Cu from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)

Locations	SOM	Exch.	Amorphous Oxides	Cryst. Oxides	OM	Sum	Total D.	Recovery
	ppm	ppm	ppm	Ppm	ppm	ppm	ppm	%
4A-A	173	6.8	333	121	53	686	669	103
4A-B	63	4.7	177	62	32	339	331	102
4A-C	110	4.4	266	132	88	601	350	172
4A-D	66	2.6	170	97	71	407	300	136
Avg of 4A	103	5	237	103	61	508	412	128
Stdev of 4A	51	2	78	31	24	163	172	33
4B-A	21	1.0	67	28	23	140	135	103
4B-B	34	0.2	54	41	54	183	212	86
4B-C	38	0.3	76	36	38	189	185	102
4B-D	9	0.3	23	22	33	88	58	151
Avg of 4B	26	0	55	32	37	150	148	111
Stdev of 4B	13	0	23	9	13	47	68	28
Avg of 4A & 4B	64	3	146	67	49	329	280	119
Stdev of 4A & 4B	54	2	111	44	22	221	186	30

Table 7. Sequential Extraction of Pb from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)

Locations	SOM	Exch.	Amorphous Oxides	Cryst. Oxides	OM	Sum	Total D.	Recovery
	ppm	ppm	ppm	Ppm	Ppm	ppm	ppm	%
4A-A	33	1	24	11	17	86	81	106
4A-B	7	1	16	9	19	51	51	100
4A-C	17	1	18	6	14	55	40	121
4A-D	11	0	15	5	13	44	37	114
Avg of 4A	17	1	18	8	16	59	52	110
Stdev of 4A	11	0	4	3	3	18	20	9
4B-A	30	1	11	4	15	62	23	139
4B-B	15	1	9	1	7	33	37	97
4B-C	15	1	13	1	11	41	50	82
4B-D	9	1	8	0	6	24	19	130
Avg of 4B	17	1	10	2	10	40	32	112
Stdev of 4B	9	0	2	2	4	16	14	27
Avg of 4A & 4B	17	1	14	5	13	45	42	111
Stdev of 4A & 4B	10	0	5	4	4	19	19	19

Table 8. Sequential Extraction of Zn from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)

Locations	SOM	Exch.	Amorphous Oxides	Cryst. Oxides	OM	Sum	Total D.	Recovery
	ppm	ppm	ppm	Ppm	ppm	ppm	ppm	%
4A-A	441	26	107	20	11	605	570	106
4A-B	256	19	60	11	6	353	311	114
4A-C	510	42	140	25	9	726	440	165
4A-D	495	43	150	26	9	724	518	140
Avg of 4A	426	32	114	21	9	602	460	131
Stdev of 4A	117	12	40	7	2	175	113	27
4B-A	147	18	61	9	6	240	197	122
4B-B	113	14	97	20	7	251	256	98
4B-C	152	19	131	18	7	328	297	111
4B-D	106	13	111	21	6	257	195	132
Avg of 4B	130	16	100	17	6	269	236	116
Stdev of 4B	24	3	30	6	1	40	49	15
Avg of 4A & 4B	278	24	107	19	8	436	348	123
Stdev of 4A & 4B	176	12	34	6	2	213	144	22

Table 9. Sequential Extraction of Fe from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)

Locations	SOM	Exch.	Amorphous Oxides	Cryst. Oxides	OM	Sum	Total D.	Recovery
	ppm	ppm	ppm	Ppm	ppm	ppm	ppm	%
4A-A	9425	7	4451	5638	2460	21981	22116	99
4A-B	8813	9	4039	6210	2468	21540	19761	109
4A-C	7388	6	3389	3556	1456	15794	10186	155
4A-D	6730	5	3067	3203	1365	14371	12797	112
Avg of 4A	8089	7	3737	4652	1937	18421	16215	119
Stdev of 4A	1245	2	625	1494	609	3903	5640	25
4B-A	5780	6	1738	2611	1295	11429	11469	100
4B-B	3714	5	1674	1172	698	7264	8804	83
4B-C	3828	51	1532	1399	976	7785	7175	109
4B-D	5563	51	2400	1466	777	10257	8186	125
Avg of 4B	4721	28	1836	1662	937	9184	8908	104
Stdev of 4B	1102	26	386	645	266	1986	1835	18
Avg of 4A & 4B	6405	17	2786	3157	1437	13803	12562	111
Stdev of 4A & 4B	2104	21	1124	1921	690	5710	5507	21

Table 10. Sequential Extraction of Mn from Floc Layer Samples Collected from the Inflow to the Outflow of Two Wetland Cells (4A and 4B)

Locations	SOM	Exch.	Amorphous Oxides	Cryst. Oxides	OM	Sum	Total D.	Recovery
	ppm	ppm	ppm	Ppm	ppm	ppm	ppm	%
4A-A	311	29	44	17	17	417	402	104
4A-B	379	34	53	14	11	491	449	109
4A-C	426	29	36	13	10	514	324	159
4A-D	346	23	26	10	8	412	358	115
Avg of 4A	365	29	40	13	11	458	383	122
Stdev of 4A	49	5	11	3	4	52	54	25
4B-A	102	7	11	6	7	133	115	116
4B-B	303	16	20	7	4	349	374	93
4B-C	316	19	23	6	6	372	348	107
4B-D	723	37	35	9	4	809	590	137
Avg of 4B	361	20	22	7	5	416	357	113
Stdev of 4B	260	13	10	2	1	283	194	18
Avg of 4A & 4B	363	24	31	10	8	437	370	118
Stdev of 4A & 4B	174	10	13	4	4	190	133	21

Oxyhydroxide (oxide) minerals, along with organic matter, have been recognized as the predominant metal sorbents in aquatic systems. In comparison with carbonate minerals, amorphous oxide minerals have a surface site density that is three to four orders of magnitude greater and a larger surface area (oxides up to 300m²/g, organic matter up to 1900m²/g, and carbonates usually <1m²/g) (Forstner and Wittmann, 1979; Benjamin and Leckie, 1981; Bilinski et al., 1991). The geochemical fractionation results from the floc layer are consistent with the high affinity of metals for amorphous oxide minerals. Association with amorphous oxide minerals (defined by extraction with 0.25 M NH₂OH·HCl in 0.25 M HCl) was very important for Cu, Pb, and Zn retention (Table 6 - Table 8). In contrast to amorphous minerals, lower concentrations of Cu, Pb, and Zn were observed in the operationally defined crystalline oxide fraction. This probably reflects the much greater surface area of amorphous minerals in comparison with more crystalline material (Kampf et al., 2000). The H₂O₂-extractable fraction is assumed to reflect metals strongly bound to sediment organic material. The results indicate that this fraction was important for Cu retention. In contrast, relatively lower proportions of Pb and Zn were associated with organic matter (Table 7 and Table 8).

4.4.1.2 Organic and Inorganic Layer

The modified sequential extraction technique developed by Tessier et al., (1979) and Hall et al. (1996) was used for the organic and inorganic layers. The idea behind this sequential extraction method is that the successive application of selective reagents (singly or in progressively stronger mixtures), sequentially liberates the metals associated with specific fractions, including very labile (exchangeable-adsorbed), carbonates, oxyhydroxides, organic matter, sulfides, and non-labile (crystalline) forms. The distribution of Cu fractions differed among cells and layers (Table 11). In the organic layer from cell 4A, Cu was predominantly in the exchangeable, sulfide, and residual fractions (23.8%, 21.4%, and 20.3%, respectively) (Figure 18). However, in the inorganic layer (5 – 10 cm), only 4% of the Cu was in the exchangeable, carbonate and crystalline oxide fractions (Figure 18). The high exchangeable Cu concentrations in the organic layer could be related to Fe(III) and Mn(IV) reducing microorganisms. These microorganisms can dissolve insoluble Fe(III) and Mn(IV) oxides, resulting in the release of soluble (Fe(II) and Mn(II)), as well as the trace metals bound by the Fe(III) or Mn(IV) minerals (Kosolapov et al., 2004). Additionally, the high proportion of organic matter in the organic layer (more than 10% compared with 3% in the inorganic layer) may have included a large pool of Cu that desorbed from soluble organic complexes and contributed to the exchangeable fraction. The soluble organic fraction was one of the largest fractions in the floc layer (Table 6). The majority of Cu in the inorganic layer was associated with the residual and amorphous oxide fractions (Table 11 and Figure 18). In cell 4B the Cu associated with exchangeable fraction was higher in the organic layer than in inorganic (8.9% and 0.6%, respectively) as in cell 4A (Figure 18). However, unlike cell 4A the largest Cu fractions in both layers from cell 4B were the residual, amorphous oxide and organic fractions (Figure 18). The Cu distribution in the inorganic layer from both cells paralleled the results of Murray-Gulde (2003), which were based on samples collected from the A-01 wetland following 569 days of Cu loading. They showed that the lowest copper concentrations were associated with the exchangeable and carbonate fractions, and the highest with the residual, organic, and oxide fractions. Differences in the amount of organic matter present undoubtedly contributed to differences in metal speciation between the organic and inorganic layers. The former was about 12-16% organic matter and the latter about 3% (Figure 4).

Table 11. Sequential Extraction of Cu from Sediment Collected from the Inflow to the Outflow of Wetland Cells 4A and 4B

Locations	Exch.	Carb.	Am. Ox.	Cryst. Ox.	Organic	Sulfide	Res.	Sum
	Ppm	ppm	ppm	ppm	ppm	ppm	Ppm	ppm
4A-A OM	22.4	4.4	15.7	23.5	2.0	9.8	12.5	90.3
4A-A5-10	1.2	0.5	4.0	0.0	1.7	1.0	11.7	20.1
4A-B OM	30.3	5.2	28.3	15.4	2.5	24.6	16.8	123.2
4A-B 5-10	0.3	0.2	4.5	0.0	2.1	1.1	8.2	16.4
4A-C OM	13.8	2.2	13.0	0.0	2.2	16.2	11.3	58.6
4A-C 5-10	0.0	0.1	4.6	0.0	1.8	0.8	8.2	15.4
4A-D OM	8.6	1.2	3.3	0.0	1.9	10.6	13.5	39.1
4A-D 5-10	0.0	0.6	2.5	0.0	1.9	0.3	10.6	15.9
4A-OM avg	18.8	3.2	15.1	9.7	2.2	15.3	13.5	77.8
4A-OM stdev	9.6	1.9	10.3	11.7	0.3	6.8	2.4	36.9
4A 5-10 avg	0.4	0.3	3.9	0.0	1.9	0.8	9.7	17.0
4A 5-10 stdev	0.6	0.2	1.0	0.0	0.2	0.4	1.8	2.1
4B-A OM	1.6	3.0	4.8	0.0	1.8	0.7	12.3	24.1
4B-A 5-10	0.0	0.0	2.5	0.0	2.1	0.2	8.8	13.6
4B-B OM	4.9	0.7	3.8	0.0	1.8	6.8	20.8	38.7
4B-B 5-10	0.1	0.0	1.9	0.0	2.2	0.0	10.3	14.6
4B-C OM	1.9	2.1	4.8	0.0	1.8	1.3	9.8	21.6
4B-C 5-10	0.0	0.0	1.9	0.0	4.3	0.3	12.5	19.0
4B-D OM	1.7	1.9	4.5	0.0	2.0	1.9	11.5	23.6
4B-D 5-10	0.2	0.0	1.2	0.0	2.5	0.5	11.1	15.5
4B-OM avg	2.5	1.9	4.5	0.0	1.8	2.7	13.6	27.0
4B-OM stdev	1.6	1.0	0.5	0.0	0.1	2.8	4.9	7.9
4B 5-10 avg	0.1	0.0	1.9	0.0	2.8	0.2	10.7	15.7
4B 5-10 stdev	0.1	0.0	0.5	0.0	1.0	0.2	1.5	2.4

The data represent an organic layer (OM, 2 – 5 cm depth) and an inorganic layer (5-10 cm depth). The second upper case letter stands for transect (transect A was nearest to the influent and transect D nearest to the outflow).

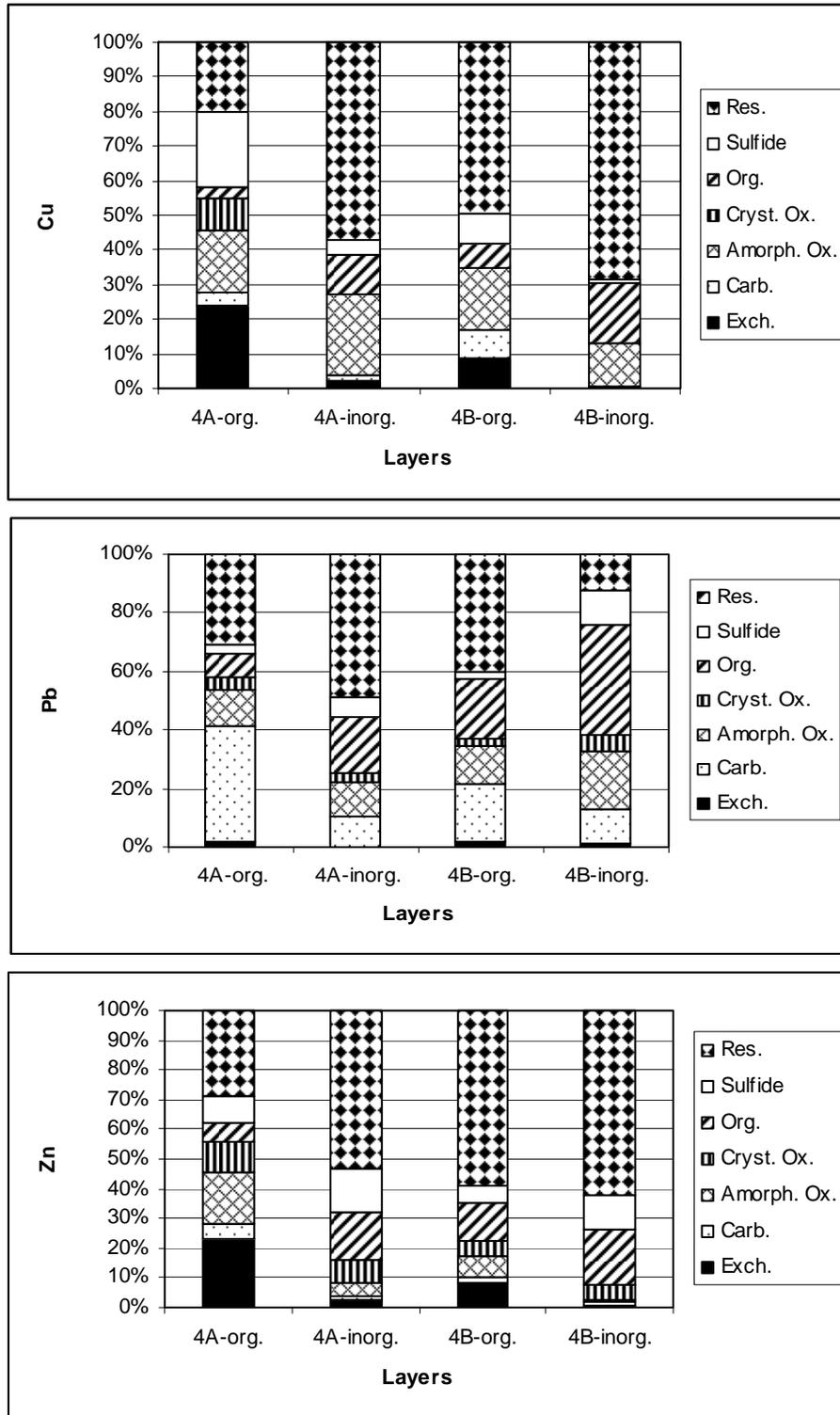


Figure 18. Sequential Extraction of Cu, Pb, and Zn from Sediment Collected from Wetland Cells 4A and 4B

4.4.2 Potentially Mobile Fraction and Recalcitrant Factor

Sequential extraction results can be summarized using the concepts of PMF and RF (Knox et al., 2005). Early extraction steps (water soluble, exchangeable, carbonate, amorphous Mn and Fe oxides, and organic fractions) tend to recover metal fractions that are less strongly bound than the fractions collected in the later extraction steps (crystalline oxides, sulfides, and residue fractions). These less strongly bound fractions can be termed the PMF because they constitute the contaminant fraction that has the potential to enter into the mobile aqueous phase in response to changing environmental conditions, such as pH, Eh, temperature, etc.

The PMF for the organic and inorganic layers was calculated using Equation 1:

Equation 1 **Potentially Mobile Fraction = 100 – (F_{Cry. oxides} + F_{Sulfide} + F_{Residual})**

F _{Cry. oxides}	=	crystalline Fe oxide fraction (wt-%)
F _{Sulfide}	=	sulfide fraction (wt-%)
F _{Residual}	=	residual fraction (wt-%)

The PMF for the floc layer was calculated using Equation 2:

Equation 2 **Potentially Mobile Fraction = 100 – (F_{Cry. oxides} + F_{INOM})**

F _{Cry. oxides}	=	crystalline Fe oxide fraction (wt-%)
F _{INOM}	=	insoluble organic matter fraction (wt-%)

The sulfide and residual fractions were omitted from this equation, because these steps were not included in the sequential extraction scheme for floc.

The idea behind these constructs is that the entire contaminant pool may not be accessible to the mobile aqueous phase. The strongly bound fractions collected in the later extraction steps are likely to include metals that will be retained by the wetland sediments. Fractions of the contaminant pool that are very strongly bound by the sediment include crystalline oxides, sulfides or silicates, and aluminosilicates. These strongly bound fractions were used to calculate the RF. The RF is the ratio of strongly bound fractions to the total concentration of the element (i.e., sum of all fractions) in the sediment. The meaning of the RF is opposite to the PMF; i.e., the RF indicates the virtually irreversible retention of metals by the solid phase. For this study the RF for organic and inorganic layer was calculated using Equation 3:

Equation 3 **Recalcitrant Factor = $\left[\frac{C_{cry.oxides} + C_s + C_{residual}}{C_{exch} + C_c + C_{org} + C_{oxides} + C_s + C_{residual}} \right] \times 100$**

where C represents concentration, and the subscripts *crystalline oxides (cry. oxides)*, *sulfides (S)* and *residual*, represent the three final fractions of the sequential extractions. The subscripts *exch*, *C*, *org*, and *oxides* stand for water soluble and exchangeable metals, carbonates, organically bound metals, and metals bound to amorphous Fe oxides and occluded Mn oxides, respectively. For the floc layer, in Equation 3 the organic fraction was

subsidized by soluble organic complex and insoluble organic fraction, and the sulfide and residual fractions were not present. This construct provides an estimate of the percentage of a contaminant in the sediment that was resistant to remobilization; i.e., retention of contaminants in wetland sediments.

PMF and RF in the floc, organic matter, and inorganic layers were calculated for Cu, Pb, and Zn (Figure 19). The highest observed values for PMF were in the floc layer, followed by organic layer, with the lowest values in the inorganic layer. Concentrations of all three metals also showed a trend of decrease from the A cell influent (transect 4A-A) to the B cell effluent. The PMF and RF values for the inorganic layer from cells 4A and 4B cells agree with results presented by Knox et al. (2004), where PMF and RF averaged 23% and 77%, respectively, for Cu indicating that most Cu was strongly retained by the sediments and not easily extractable.

In the current study, the PMF and RF values for Cu in the inorganic layer were 20 and 80%, respectively. However, PMF values for Cu increased to 40% and 60% in organic and floc layers (Figure 19), respectively. Similar patterns of increasing PMF with decreasing sediment depth were observed for Pb and Zn (Figure 19). RF values for Zn, Cu Pb were high in the inorganic layers, indicating that most of the Zn and Pb were strongly bound in the deeper sediments and not easily extractable. Like the other metals, RF values for Fe and Mn were relatively low in the floc layer indicating high potential desorption from the sediment and solubility (Figure 19).

As a final step in the analysis, PCA was used to summarize spatial distribution patterns of exchangeable metals in the wetland sediments (Figure 20). Principal component 1 accounted for most (75%) of the variance in the exchangeable metal data and represented a gradient with organic layer samples from the A cell at one extreme and inorganic layer samples from both cells at the other. This gradient was strongly correlated with the concentration of all exchangeable metals included in the PCA except mercury ($r=0.70-0.97$). These results were in accord with (Figure 19) and clearly showed that exchangeable metal concentrations were highest in the organic layer from the transects nearest the influent (i.e., A cell transects). The PCA shows that metals are concentrated in potentially mobile forms in the upper (0-5 cm) organic layers of the wetland sediment and suggest that this layer plays a critical role in the incorporation of metals into the sediment.

A Mantel test was used to assess the correspondence between total metal concentrations in the sediments and exchangeable metal concentrations in the sediments. The standardized Mantel statistic (r) was 0.38 ($P=0.007$), which was greater than for total metal concentrations and the porewater metal concentrations (0.22), but still only moderate. As with porewater metal levels, these results suggest that other factors besides total metals levels influence exchangeable metal concentrations in the sediments.

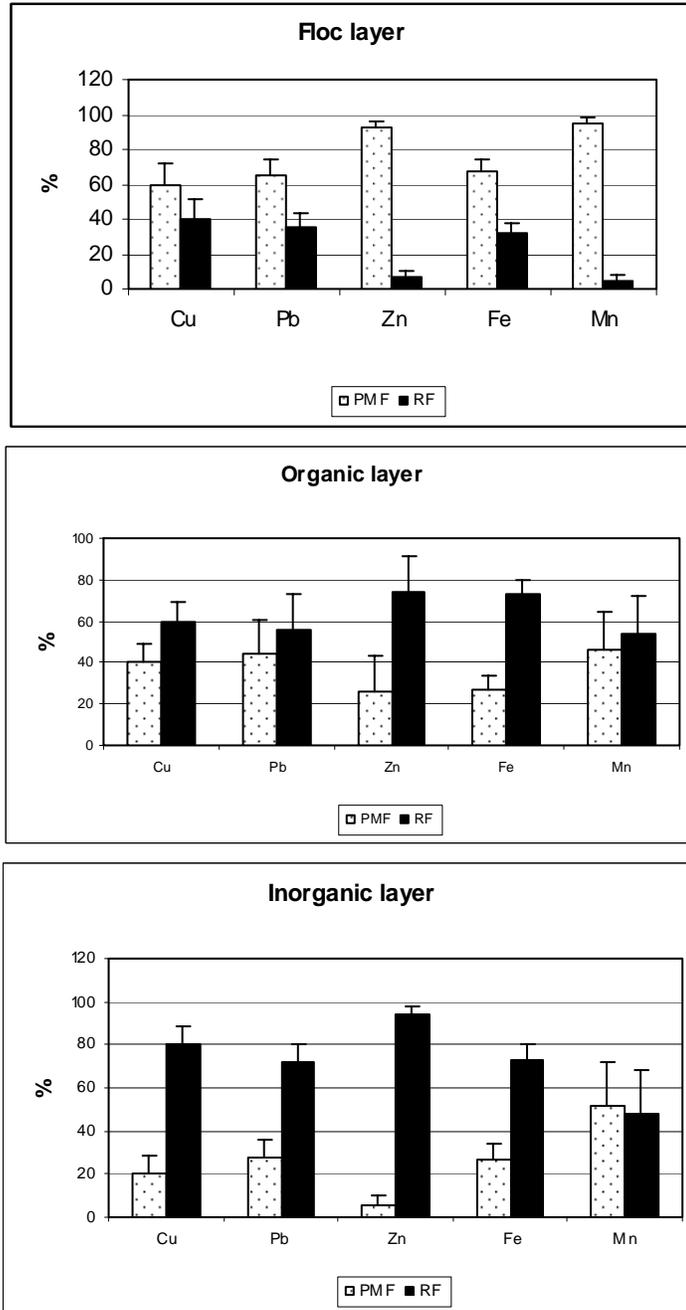


Figure 19. Average Potentially Mobile Fractions (PMF) and Recalcitrant Factors (RF) for Elements in the Floc (0-2 cm), Organic (2-5 cm), and Inorganic (5-10 cm) Layers

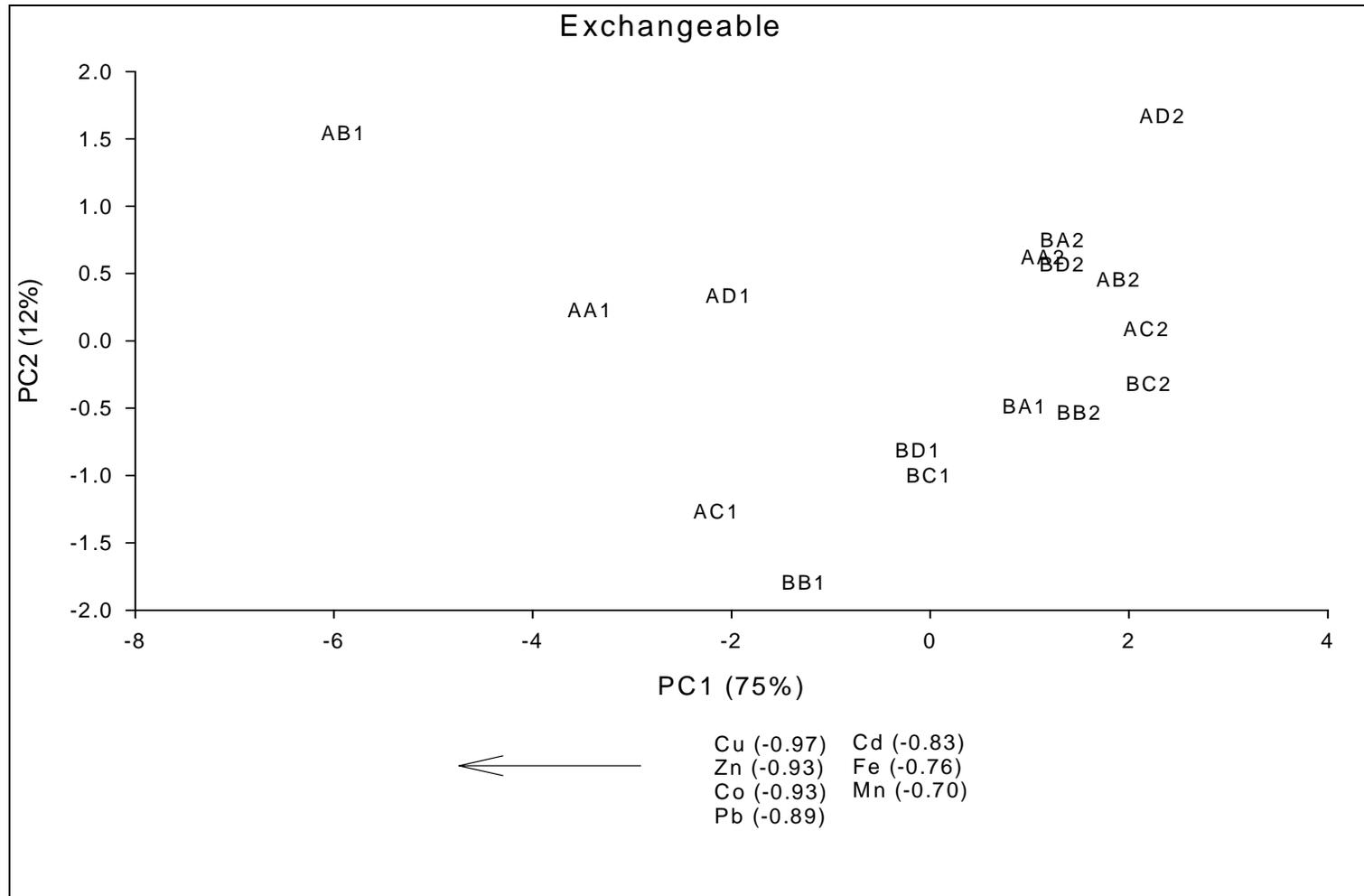


Figure 20. Principal Components Analysis of Exchangeable Metal Fractions in Sediment from the A-01 Constructed Wetland

The first letter of each symbol represents the cell (A or B), the second letter represents the cell quarter (A, B, C, or D), and the number represents the depth (1=organic matter layer, 2=5-10 cm inorganic layer, 3=10-20 cm inorganic layer). Also shown are the metal concentrations correlated with each axis, the Pearson correlation (in parentheses) and the direction of increase (shown by the arrows). The proportion of the variance accounted for by each principal component score is shown in brackets.

4.4.3 Distribution Coefficient (K_d) Values for Selected Metals in the Wetland Sediment

The K_d is very important in estimating the potential for the adsorption of dissolved contaminants in contact with sediment or soil. As typically used in fate and contaminant transport calculations, the K_d is defined as the ratio of the contaminant concentration associated with the solid to the contaminant concentration in the surrounding aqueous solution when the system is at equilibrium, Equation 4:

$$\text{Equation 4} \quad K_d = \frac{\text{Contaminant concentration in sediment (mg/kg)}}{\text{Contaminant conc. in the solution contacting the sediment (mg/L)}}$$

Ideally, for site-specific calculations, the transport modeler should use sorption values determined for site-specific materials at site-specific conditions. In the absence of such data, the modeler often selects a K_d value taken from the literature that was measured under conditions similar to those at the study site. However, very subtle properties of the solid and aqueous phases can have a profound effect on a contaminant's K_d . Thus it is essential for the modeler selecting K_d values to recognize these obstacles and use the right method to determine a K_d value.

There are five methods of determining K_d values:

- laboratory batch method
- in-situ batch method
- laboratory flow-through (or column) method
- field modeling method
- K_{oc} method

Each method provides an estimate of the propensity of a contaminant to sorb to the solid phase. However, the techniques used and the assumptions underlying each method are quite different. Consequently, K_d values for a given system that were measured by different methods commonly have values ranging over an order of magnitude (Gee and Campbell, 1980; Relyea, 1982).

In this study, in situ K_d values were calculated using sets of “matched” porewater and soil samples where $K_d = \text{contaminant concentration in soil} / \text{contaminant concentration in porewater}$. The K_d values were highly variable, differing by an order of magnitude at even the same location (Figure 21). However, K_d values were clearly lower for Cu and Zn than for Pb. This finding is consistent with Cu, Zn and Pb data from the sequential extraction, which showed that the exchangeable fraction was much lower for Pb than for Cu and Zn, especially in the organic layer. A clear advantage of the in-situ batch method compared, for example, to the laboratory K_d method is that the precise solution chemistry and solid phase mineralogy is used for modeling.

Disadvantages of the in-situ batch method are that sediment samples are transported to a lab, and the aqueous phase is separated from the solid phase by centrifugation or filtration and then analyzed for the solute concentration. The time and operations needed to obtain porewater from the sediment samples can change aqueous phase parameters such as pH and redox. This obviously has the potential to change the K_d of elements.

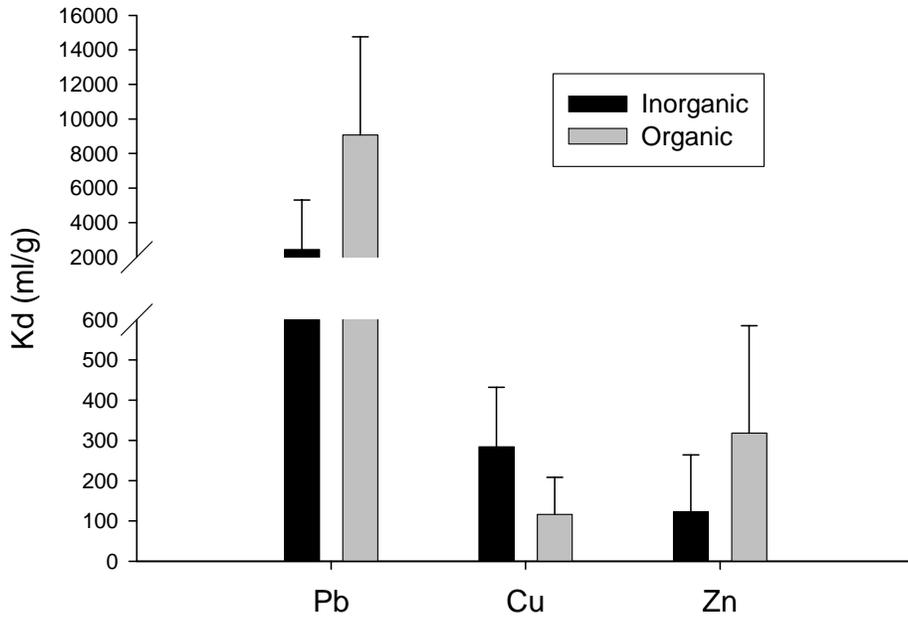


Figure 21. Average K_d Values for Cu, Pb, and Zn in the Wetland Sediment

5.0 CONCLUSION

Constructed wetlands have the ability to remove pollutants from water and retain them in sediment. However, there is considerable variation among metals and between wetlands in the degree to which metals are removed. The A-01 wetland treatment system (WTS) was designed to remove metals (primarily copper) from the A-01 effluent at the Savannah River Site, Aiken, SC. The main purpose of research conducted during 2004 and 2005 was to evaluate the distribution and retention of contaminants in the sediment profile.

Most of the metal removed by the wetland cells was accumulated in the two top layers; i.e., the floc and organic layer. Principal components analysis revealed a strong spatial gradient in the sediment metal data, with floc layer samples from the A cell at one extreme, and inorganic layer samples from both cells at the other. This gradient was strongly correlated with percent organic matter, pH, and the concentration of all metals except mercury. These results showed that most metals in the A-01 wetland sediments behaved similarly: their concentrations decreased as sediment depth increased.

Copper, Cd, and Zn distribution in the sediment porewater profile was similar, and the concentrations of these elements generally decreased as a function of depth. The horizontal and vertical distributions of sulfate, Fe, and Mn in the porewater indicated the oxidation status of the sediments. The presence of sulfate in the porewater showed that cell 4A, especially the top organic layer, was in an oxidized state. The porewater sulfate concentration in all layers was higher in 4A cell than in 4B cell. The lower inorganic layers, especially closer to the effluent discharge, were more reduced as indicated by higher concentrations of Fe and Mn.

The stability of contaminants in the wetland sediment profile was evaluated by calculating the potentially mobile fraction (PMF), recalcitrant factor (RF), and distribution coefficient (K_d) values. The highest PMF values were in the floc layer, followed by the organic layer, with the lowest values in the inorganic layer. The PMF value for Cu in the inorganic layer was 20%, but increased to 40% and 60% in the organic and floc layers, respectively. Similar patterns of increases in PMF with decreasing sediment depth were shown by Zn and Pb. RF values for Cu, Pb, and Zn were high in the inorganic layers indicating that all three elements were strongly bound in the deeper sediments and not easily extractable. However, RF values were low in the floc layer indicating high potential desorption and solubility.

K_d values were highly variable, but were clearly much higher for Pb than for Cu and Zn. This result was consistent with the Cu, Zn, and Pb data from the sequential extraction, which showed that Pb concentrations in the exchangeable fraction were much lower than the Cu and Zn concentrations, especially in the organic layer.

Our conclusions agree with previous studies conducted on the A-01 WTS and other constructed wetlands, which indicate that substratum (sediment) is the primary sink for metals. Metal concentrations in the A-01 WTS tended to be greater in the surface layers of sediment, which were rich in organic matter. Association of metals with soluble organic complexes in the floc layer is substantial and should be further evaluated.

Although there have been a variety of engineering improvements for constructed wetlands, much less attention has been paid to optimizing the microbial processes responsible for metal removal. An understanding of biotic interactions may prove to be crucial for designing an effective wetland system incorporating microbial processes for achieving the highest rates of metal removal from effluents. An enhanced understanding of the complex biotic and abiotic interactions involved along with the technical possibilities for exploiting these interactions will enable wetland technologies to be used on a broader scale.

6.0 REFERENCES

- Benjamin, M.M., and J.O. Leckie. 1981. Multiple-site adsorption of Cd, Cu, Zn, and Pb on amorphous iron oxyhydroxide. *J. Colloid Interface Sci.* 78:209-221.
- Bilinski, H., S. Kozar, M. Plavsic, Z. Kwokal, and M. Branica. 1991. Trace metal adsorption on inorganic solid phases under estuarine conditions. *Mar. Chem.* 32:225-233.
- Bloom, N. 1989. Determination of pictogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Can. J. Fish. Aquat. Sci.* 46:1131-1140.
- Bloom, N., J. A. Colman, and L. Barber. 1997. Artifact formation of methyl mercury during aqueous distillation and alternative techniques for the extraction of methyl mercury from environmental samples. *Fresenius J. Anal. Chem.* 358:371-377.
- Bloom, N., and W.F. Fitzgerald. 1988. Determination of volatile mercury species at the pictogram level by low-temperature gas chromatography with cold-vapour atomic fluorescence detection. *Anal. Chimica Acta.* 208:151-161.
- Dillon, W.R., and M. Goldstein. 1984. *Multivariate Analysis Methods and Applications.* John Wiley & Sons, Inc., New York.
- Emmerson, R.H.C., M.D. Scrimshaw, J.W. Birkett, and J.N. Lester. 2001. Solid phase partitioning of metals in managed realignment soils: Laboratory studies in timed soil sea-water batch mixtures. *Appl. Geochem.* 16:1621-1630.
- Evangelou, V.P. 1998. *Environmental Soil and Water Chemistry,* John Wiley & Sons, Inc. New York,
- Fennessy, M.S., C.C. Brueske, and W.J. Mitsch. 1994. Sediment deposition patterns in restored freshwater wetlands using sediment traps. *Ecol. Eng.* 3:409-428.
- Forstner, U., and G.T.W. Wittmann. 1979. *Metal pollution in the aquatic environment.* Springer-Verlag, Berlin.
- Fujiyoshi, R., T. Gomei, and M. Katayama. 1996. Sorption of Pb(II) on marine sediments by a sequential extraction-radiotracer technique. *Appl. Radiat. Isot.* 47:165-169.

Gee, G. W., and A.C. Campbell. 1980. Monitoring and Physical Characterization of Unsaturated Zone Transport-Laboratory Analysis. PNL-3304, Pacific Northwest Laboratory, Richland, Washington.

Gilmour, C.C., and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. *Environmental Pollution*. 71:131-169.

Hall, G. E. M., J. E. Vaive, R. Beer, M. Hoashi. 1996. Selective Leaches Revisited, with Emphasis on the Amorphous Fe Oxyhydroxide Phase Extraction. *J. Geochem. Exploration*, 56: 59-78.

Hammer, D.A. 1989. *Constructed Wetlands for Wastewater Treatment*, Lewis Publisher, Ann Arbor (MI) USA.

Jain, C.K., 2004. Metal fractionation study on bed sediments of River Yamuna, India. *Water Res.* 38:569-578.

Kampf, N., A.L. Scheinost, and D.G. Schulze. 2000. Oxide minerals. P. 125-168. In M.E. (ed.) *Handbook of soil science*. CRC Press, Boca Raton, FL.

Knox, A.S., D. Dunn, E. Nelson, W. Specht, J. Seaman. 2004. *Wastewater Treatment and Heavy Metals Removal in the A-01 Constructed Wetland*, Tech. Report WSRC-TR-2004-00228, Westinghouse Savannah River Company, Aiken, SC.

Knox, A.S., D. Dunn, M. Paller, E. Nelson, W. Specht, and J. Seaman. 2005. *Assessment of Contaminant Retention in Constructed Wetland Sediments*. Engineering Life Sciences (in press).

Kosolapov, D.B., P. Kusch, M.B. Vainshtein, A.V. Vatsourina, A. Wiebner, M. Kastner, and R.A. Muller. 2004. Microbial processes of heavy metal removal from carbon-deficient effluents in constructed wetlands. *Eng. Life Sci.* 4(5):403-411.

Liang, L., N.S. Bloom, and M. Horvat. 1994. Simultaneous determination of mercury speciation in biological materials by GC/CVAFS after ethylation and room-temperature percollection. *Clin. Chem.* 40(4):602-607.

Lovley, D.R. 2000. *Environmental microbe-metal interactions*. ASM Press, Washington, DC.

Luther, G. W., III. 1995. Trace metal chemistry in pore waters. In: H. E. Allen (ed.) *Metal Contaminated Aquatic Sediments*, Ann Arbor Press, Inc., Chelsea, Michigan, pp 65-75.

McBride, M.B., B.K. Richards, T.S. Steenhuis, J.J. Russo, and S. Sauve. 1997. Mobility and solubility of toxic metals and nutrients in soil fifteen years after sludge application. *Soil Sci.* 162:487-500.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data, Version 4. MjM Software Design, Gleneden Beach, Oregon.

Murray-Gulde, C.L. 2003. Assessment of a constructed wetland system for decreasing the concentrations and bioavailability of copper in a wastestream. Doctoral Dissertation, Clemson University, NC.

Nelson, E. A., W.L. Specht, J.A. Bowers and John B. Gladden. 2002. Constructed wetlands for removal of heavy metals from NPDES outfall effluent. Westinghouse Savannah River Company, Savannah River Technology Center, WSRC-MS-2002-00600.

Nirel, P. M., and F.M. M. Morel. 1990. Pitfalls of sequential extractions. *Wat. Res.*, 24: 1055-1056.

Odum, H.T., W. Wojcik, L. Pritchard Jr., S.Ton, J.J. Delfino, M. Wojcik, S. Leszczynski, J.D. Patel, S.J. Doherty, J. Stacik. 2000. *Heavy Metals in the Environment: Using Wetland for their Removal*, Lewis Publishers, Boca Raton, (FL), p. 893.

Relyea, J.F. 1982. Theoretical and experimental considerations for the use of the column method for the use of the column method for determining retardation factors. *Radioactive Waste Management and the Nuclear Fuel Cycle*, 3(2):151-166.

Quevauviller, P., G. Rauret, J.-F. Lopez-Sanchez, R. Rubio, A. Ure, and H. Muntau. 1997. Certification of trace metal extractable contents in a sediment reference material (CRM 601) following a three-step sequential extraction procedure. *Sci. Tot. Environ.* 205:223-234.

Sobolewski, A. 1996. Metal species indicate the potential of constructed wetlands for long-term treatment of metal mine drainage. *Ecological Engineering* 6:259-271.

Specht, W. L. and E. A. Nelson. 2002. Baseline hydrosol chemistry of the A-01 wetland treatment system, September 2001. Westinghouse Savannah River Company, Savannah River Technology Center WSRC-MS-2002-00174.

Stottmeister, U., A. Wiebner, P. Kusch, U. Kappelmeyer, M. Kastner, O. Bederski, R.A. Muller, H. Moormann. 2003. Effect of plants and micro-organisms in contracted wetlands for wastewater treatment, *Biotechnol. Adv.* 22:93-117.

Stumm, W. and J. Morgan. 1981. *Aquatic Chemistry*, 2nd edition. New York, Wiley.

SYSTAT Software Inc. 2002. SYSTAT 10.2. Richmond, CA.

Tessier, A., Campbell, P.G. C., and M. Bisson. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51: 844-850.

Ye, Z.H., S.N. Whiting, Z.-O. Lin, C.M. Lytle, J.H. Qian, and N. Terry. 2001. Removal and distribution of iron, manganese, cobalt, and nickel within a Pennsylvania constructed wetland treating coal combustion by-products leachate. *J. Environ. Qual.* 30:1464-1473.

Yu, T. R. 1991. Characteristics of Soil Acidity of Paddy Soils in Relation to Rice Growth. pp. 107-112. In: *Plant-soil Interactions at Low pH*. R. J. Wright et al. (eds.), Kluwer Academic Publishers, Netherlands.

APPENDIX A.
SEQUENTIAL EXTRACTION PROCEDURE

(based on Tessier et al., 1979 and Hall et al., 1996)

Fraction 1: Exchangeable (Tessier et al, 1979)

To 1 g of a dry sample in a 50-ml screw-cap centrifuge tube, add 8 ml of pH 7.0 1.0 M 1M MgCl₂ and vortex contents for 5-10 s and place in a horizontal shaker for 1 h. Centrifuge for 20 min at 4800 rpm (or at higher rpm) and decant supernatant liquid into a labeled test tube. Rinse residue with 10 ml of DI water, vortex and centrifuge again.

Fraction 2: Carbonates (Tessier et al, 1979)

The residue from fraction 1 extract with 8 ml of pH 5 1.0 M CH₃COONa (sodium acetate) and vortex contents for 5-10 s and place in a horizontal shaker for 6 h. Centrifuge for 20 min at 4800 rpm (or at higher rpm) and decant supernatant liquid into a labeled test tube. Rinse residue with 10 ml of DI water, vortex and centrifuge again

Fraction 3: Amorphous oxide (Hall et al, 1996)

The residue from fraction 2 extract with 20 ml of 0.25 M NH₂OH HCl in 0.25M HCl. Vortex contents for 5-10 s and place in a water bath at 60oC for 2h with cap loosened. Every 30 minutes, cap tightly and vortex the contents.

Centrifuge for 20 min at 4800 rpm (or at higher rpm) and decant supernatant liquid into a labeled test tube. Rinse residue with 10 ml of DI water, vortex, centrifuge again; and add supernatant rinses to the test tube. Dilute to 30 ml with DI water and analyze.

Fraction 4: Crystalline oxide (Hall et al, 1996)

The residue from fraction 3 extract with 30 ml of 1.0 M NH₂OH HCl in 25% CH₃COOH (acetic acid)

Vortex contents for 5-10 s and place in a water bath at 90oC for 3h with cap loosened. Every 20 minutes, cap tightly and vortex the contents

Centrifuge for 20 min at 4800 rpm (or at higher rpm) and decant supernatant liquid into a labeled test tube. Rinse residue with 10 ml of DI water, vortex, centrifuge again; and add supernatant rinses to the test tube. Dilute to 40 ml with DI water and analyze.

Fraction 5: Organic (modified Hall et al, 1996)

To the residue from fraction 4, extract at room temperature with 40 ml of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ by continuous agitation for 24 hours. Centrifuge, rinse with 10 ml DI water and add supernatant rinses to the test tube. Dilute to 50 ml and analyze.

Fraction 6: Sulfides (modified Hall et al, 1996)

To the residue from fraction 5, add 10 ml 4.0 N HNO_3 , cap and vortex. Place in a water bath at 90°C for 20 min. Centrifuge, rinse with 10 ml DI water and add supernatant rinses to the test tube. Dilute to 20 ml and analyze.

Fraction 7: Residual

To the dried residue from fraction 6, weigh out a 0.6-g sub-sample for digestion in the Star 6 system with HNO_3 (10ml), H_2SO_4 (4.0 ml), HNO_3 (4.5 ml), and HCl (2ml). Filter samples and dilute to 100 ml with DI water. Digestates should be slightly yellow in color.