CORRELATION BETWEEN FROG MALFORMITIES AND HEAVY METALS IN WARD MARSH, WEST HAVEN, VT, AND MUD CREEK, ALBURG, VT.

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Abstract

Studies performed by the Vermont Agency of Natural Resources (VTANR) and Jim Andrews of Middlebury College have found frog malformations in a number sites across the Lake Champlain Basin. The surveys documented rates of morphological abnormalities in the Northern Leopard frog (*Rana pipiens*) ranging from 2% to 45% depending on the site (VTANR, 1998). Further laboratory research by VTANR, using the Frog Embryo Teratogenesis Assay: Xenopus (FETAX), showed that frog embryos grown in *water* from Ward Marsh WMA, in West Haven, VT, had an 89% malformity rate with a 6.6% mortality rate. In the assays using *sediment* from Ward Marsh, there was a 100% malformity rate and a 38.3% mortality rate (VTANR, 1997). This evidence indicates that the sediment is a potential source of teratogens. Mud Creek, in Alburg, VT, which was used as a control in this study, has very little evidence of malformities in the field and laboratory FETAX assays.

In other studies, frogs exposed to Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} in FETAX assays have shown these metals to be teratogens, causing malformities and mortality. The malformities of frogs exposed to these metals include retinal depigmentation and pelvic and hind limb malformities (Luo et al, 1993; Plowman et al., 1991,1994). These types of malformities have been found in Ward Marsh by VTANR, which leads us to suspect that the frog malformities may be related to high concentrations of metals in the sediment.

EPA method 200.2 for total recoverable analytes was used to prepare sediment samples from Ward Marsh and Mud Creek for ICAP analysis. Samples at Ward Marsh and Mud Creek were collected in transects ranging from subaerially exposed soils adjacent to the marsh to subaqueous sediment in the marsh, including all levels in between. Concentrations of Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cr^{2+} in the sediment at the two sites were determined using the ICP, with replicate runs.

Sediment samples from Ward Marsh contain statistically higher concentrations of Zn^{2+} (mean=103 $\pm$ 34 mg/kg), Co^{2+} (mean=18.3 $\pm$ 3 mg/kg), Ni^{2+} (mean=39.4 $\pm$ 7.7 mg/kg), and Cr^{2+} (mean=43.4 $\pm$ 11.8 mg/kg), than Mud Creek. Cu^{2+} concentrations were similar between the two sites. Furthermore, metal concentrations in Ward Marsh indicate a trend ranging from low concentrations in soils farther from East Bay to higher concentrations in subaqueous sediment closer to the bay. This relationship implies that East Bay is a potential source of heavy metals, which are accumulating in the sediment and that elevated metals concentrations could be responsible for frog malformities observed in the field at Ward Marsh.

Acknowledgments

I would like to thank Grant Meyer for introducing me to geology and getting me hooked (i.e. Mojave desert trip). Who knows, I may have still been psych major if it wasn't for Grant and his alluvial fans. I wish him the best at his new job.

Of course I can not forget the rest of the folks in the department. Ray, Kim, Pat, Tom, and Trish thank you for all the support over the years. I consider myself extremely lucky to work with and learn from such a talented group of people.

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I. Introduction

Field Data

In the fall of 1995 a Minnesota middle school science class was shocked to discover malformed frogs with extra hind legs or missing limbs in a local wetland (Science News Oct.11,1997). This incident soon gained national attention, resulting in reports in 32 states and 4 Canadian provinces of deformities in at least 20 various species of amphibians.

After receiving reports from the general public of frog malformations in 12 sites throughout the Lake Champlain Basin, the Vermont Agency of Natural Resources (VTANR) conducted focused surveys in 4 of the 12 sites in October of 1996 (Levey, 1999). Their survey targeted young of the year Northern Leopard frogs (*Rana pipiens)* . Adult frogs were not examined due to the lack of malformities present in the adult population, which may be a result of increased mortality of abnormal frogs (Andrews, 1999). Of the 290 frogs observed, 13.1% were malformed, with missing or partial hind legs and missing or abnormal eyes being the most common malformities. During this time, abnormal and normal Northern Leopard Frogs were examined for parasite burden by parasitologist Stan Sessions of Hartwick College, who found no abnormal amounts of parasites in the frog tissue (Levey, 1999). In July and September of 1997, the VTANR, in association with Middlebury College, U.S. Environmental Protection Agency (USEPA), and the U.S. Fish and Wildlife Service (USFWS) surveyed 19 sites within the Champlain Valley. They found that 8% of the 2538 Northern Leopard Frogs yearlings collected had malformities, again primarily consisting of partial hind limbs and shortened or missing digits (Levey, 1999).

In July of 1997 the National Wildlife Health Center (NWHC) examined normal and abnormal frogs from four sites in the Champlain Valley for internal and external deformities. The results from the study suggest that there is no correlation between viruses, bacteria, or parasites and the frog abnormalities (Levey, 1999). Furthermore, radiographic examinations by NHWC show that the abnormalities were not trauma or disease related. From these studies, it is thought that the malformities are caused by early developmental errors as opposed to deformities resulting from mechanical errors (Meteyer, 1997). "Malformations represent the end point in a developmental process that incorporated an error long before the external manifestation of pathology." (Meteyer, 1997)

Morphogenesis is the term given to the development of an organism. Cells divide, proliferate, migrate, differentiate and die as the organisms grow. The timing of these stages is governed by chemical signals (Meteyer, 1997). If something blocks the normal sequence of morphogensis, pathology will occur in the form of malformities, primary errors in development, or deformities, results from mechanical errors (Meteyer, 1997). "Teratogenesis is the term given to the abnormal development that results in malformations" (Meteyer, 1997). The teratogensis can occur anywhere along the stage of morphogenesis, "caused either by genetic or environmental factors" called teratogens. (Meteyer, 1997).

Malformities seen in NWHC analysis of Northern Leopard Frogs include missing or partial hind legs, rotated femurs, and abnormalities in webbing (Meteyer, 1997). In cases of amelia or missing limbs, the lack of limb initiation or proximal truncation suggests primary error in the formation of the limb (Meteyer, 1997). Hemimelia, or partial limbs, ectrodactyly , or abnormal feet, and brachydactyly, or missing digits, were seen in the abnormal frogs (Meteyer, 1997).

Analyses by NWHC determined that the malformities found in frogs from Vermont differed from malformities seen in other states (Meteyer, 1997). Malformities in Vermont frogs were relatively uniform, with missing or partial limbs, as compared to frogs examined from other states that had multiple limbs. In fact, no frogs that were examined from Vermont had extra legs. Furthermore, malformed frogs in Vermont were found to be extremely emaciated compared to frogs from other states (Meteyer, 1997).

Table 1 shows data collected by VTANR. In 1997, the highest number of malformed yearling Northern Leopard Frogs in the Champlain Valley was found in Ward Marsh, also known as the Poultney River site. No malformities were found in Mud Creek that year. In 1998 there were significantly less malformities in Ward Marsh (Levey, 1999). The average percentage of malformed Northern Leopard Frogs in the Champlain Valley in 1997 was approximately 8%, and in 1998 lowered to 5.6%.

Table 1. VTANR Northern Leopard Frog (*Rana pipiens*) Survey 1997 and 1998

Mud Creek	Sample Size	% Malformed
7/21/97	54	0
7/10/98	74	6.7
7/30/98	91	3.2
9/01/98	79	3.7
Ward Marsh		
7/22/97	121	45.4
9/23/97	67	17.9
7/13/98	242	2.4
7/29/98	226	3.9
9/17/98	86	4.6

(VTANR, 1999)

FETAX Studies

In November of 1997 the National Institute of Environmental Health and Science (NIEHS) used the FETAX assay (Frog Embryo Teratogenesis Assay- *Xenopus*) to test the toxicity of water and sediment from Ward Marsh (affected site), Mud Creek (reference site) and two other sites in Vermont (Levey, 1999). FETAX is an established toxicity test in which embryos of *Xenopus laevis*, the South African clawed frog are grown in a laboratory in solutions of what ever is being tested.

In this case the FETAX test used water and sediment from Ward Marsh, Mud Creek and two other sites. The study measured development of embryos over the first four days (4 d), tail resorption over 14 days (14 d), and hind limb development over 30 days (30 d) (Fort et al., 1998).

In the 4 d development toxicity study, blastula-stage embryos were placed in water, aqueous sediment extracts, and whole bulk sediment samples from various sites. The aqueous extracts were prepared by mixing 200g of sediment with 800g of FETAX solution and tumbled for 48 hours. After they were centrifuged, the supernatant was used in the assay (Fort et al., 1998). For the whole bulk sediment tests, 35g (wet weight) of sediment was placed in a specimen jar with FETAX solution to dilute and the embryos were placed on a mesh insert just over the top of the sediment at the sediment/water interface (Fort et al., 1998).

The results of the 4d FETAX assay clearly show the teratogenic potential of Ward Marsh sediment. Examples of malformations found in the 4 d embryo include, stunting, gut miscoiling, craniofacial and mouth abnormalities, mal-development of the lens of the eyes, and muscular based tail kinking (Fort et al., 1998). Aqueous sediment extract results are excluded from

Figures 2 and 3 because they were not done for all sample sites. However, it is important to note that the sediment extract from Ward Marsh produced 100% mortality (Fort et al., 1998).

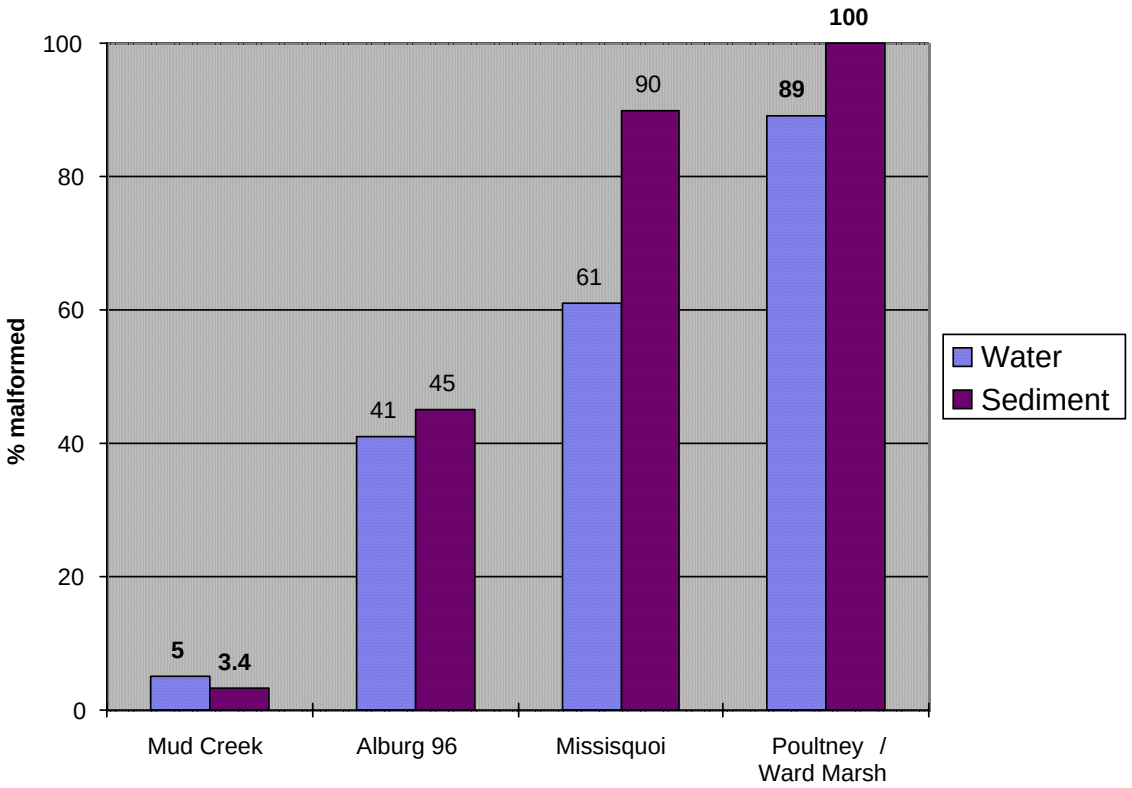


Figure 2. Percent of malformed frogs embryos using sediment and water from 3 affected sites and 1 reference site in FETAX assay. The sediment and water from Ward Marsh (affected site) has a significantly greater teratogenic potential than Mud Creek (reference site).

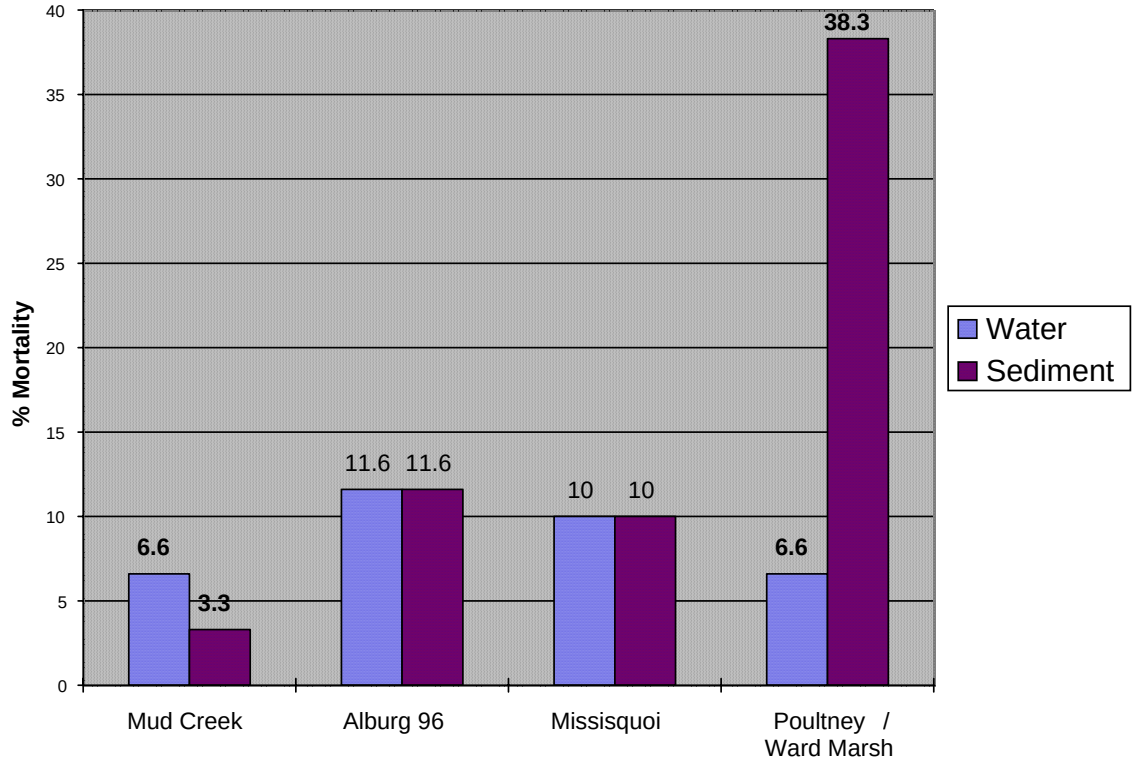


Figure 3. Percent mortality using sediment and water from 3 affected sites and 1 reference site in FETAX assay.

For limb development studies and tail resorption, frog embryos were placed in 10 gallon aquaria containing varying concentrations of the sample site water.

Results of the 30d limb development studies show that approximately 30% of the 30 day old frogs were malformed in water from Ward Marsh and 0% were malformed in water from Mud Creek (Fort et al., 1998). The abnormalities induced from the assay consisted of limb defects that show concordance with the malformities seen in the field (Levey, 1999). Furthermore, the tail resorption study showed that water from Ward Marsh "caused a significant reduction in the rate of tail resorption (Fort et al., 1998). Assessment of thyroid function of the frogs suggest that thyroid activity is being inhibited. NIEHS analysis of sediment form Ward Marsh shows natural estrogenic substances that may be inhibiting thyroid activity, UV sensitivity, and sensitivity to retinoids (Levey, 1999).

The laboratory FETAX assays show that sediment from Ward Marsh is more lethal than the water and that the aqueous extract is very toxic, resulting in 100% mortality. Furthermore, the sediment induces high malformity rates. Fort et al.(1998) suggest that "identical compound(s) induce both effects, and that the compound is soluble in aqueous extracts, but tends to accumulate in the pond sediment".

These data indicate that the sediment in Ward Marsh is a potential source of teratogens that could lead to malformities in Northern Leopard Frogs.

The Metal Link

FETAX toxicity assays have examined the teratogenicity of various heavy metals such as Co, Ni, Cd, Cu, and Zn (Luo et al., 1993; Plowman et al., 1991, 1994).

Results of Co^{2+} analysis show concentration-dependent malformities in frog embryos such as intestinal malformations, ocular anomalies, kinked tail, craniofacial abnormalities, cardiac deformities, and dermal blistering (Plowman et al., 1991).

Zn^{2+} and Cu^{2+} are also teratogens in FETAX tests producing malformities in frog embryos consisting of eye, gut, facial, notochord and cardiac anomalies (Luo et al., 1993a).

A FETAX study by Hopfer et al. (1990) showed that Ni^{2+} induced ocular, skeletal, and intestinal deformities with varying concentrations.

In 1994, Plowman et al. examined whether the malformities in *Xenopus* embryos caused by Ni^{2+}, Cu^{2+}, and Cd^{2+} persist after tadpoles metamorphose into young frogs. After 4 days of growing in metal rich water the tadpoles were put in a control solution and examined after 13 weeks. The malformities in the metal exposed frogs included retinal depigmentation, diastematomyelia, scoliosis, kyphosis, phocomelia, sacro-pelvic and hind limb deformities, dysplasias of the heart, kidney, ovary, and gut (Plowman et al. 1994).

Table 2. Median Teratogenic and Lethal concentrations from FETAX studies.

	Zn	Cu	Ni	Co	Cd
Median Teratogenic Concentration (mg/L)	**2.62**	**0.16**	**0.15**	**1.47**	**0.42**
Median Lethal Concentration (mg/L)	**55.58**	**1.40**	**21.42**	**612.91**	**3.60**

(Plowman et al., 1994)

Furthermore, in 1984, Abbasi and Soni grew *Rana tigrina* in solutions of Cr (VI) and found that all the tadpoles died within 72 hours at $\geq$ 2.0 mg/L with tail and fin abnormalities.

These metals were shown to cause malformities in frogs that mimic the malformities found in the field in VT and are similar to malformities produced in NIEHS FETAX assaying. Data suggest that the sediment, especially Ward Marsh, is a possible source of teratogens and that metals do lead to malformities in certain species of frogs.

There are various other metals that influence the development of frogs negatively and positively. Miller and Landesman (1978) showed that Mg^{2+} can reduce the toxic effects of Pb, Cd, and Mn ions. Along similar lines, a study by Luo et al. (1993a) showed that the lack of Mg^{2+} enhances the effects of Ni^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+}, while the presence of Mg^{2+} diminishes the toxic and teratogenic effects. They theorize that Mg^{2+} replaces other divalent metal ions during cellular uptake or other molecular binding (Luo, 1993b). Dale et al. (1985) have shown that the LC_{50} for Al in *Xenopus* is 0.90 mg/L in pH 6.0 water.

Some studies have determined the concentration and toxic effects that metals have specifically on *Rana pipiens*. A study by Birge et al. (1977) suggests that percent survival depends on the concentration of Cd and the stage of development of the frog. For embryos exposed to 2.5 mg/L of Cd during the cleavage stage, no embryos survived. At 0.5 mg/L, 30% survived and at 0.1 mg/L, 63% survived. For embryos at the neurula stage, 0.5 mg/L produced a 51% survival rate and 0.1mg/L a 70% survival rate (Birge et al. 1977).

Adult *Rana pipiens* seem to have a higher tolerance for Pb with the LC_{50} (the concentration at which 50% of the sample dies) being 105 mg/L (Kaplan et al., 1967). They found that Pb resulted in a loss of semi-erect posture, muscular twitches, decreased red and white blood cells and at 300 mg/L produced a 100% mortality rate (Kaplan et al., 1967). Other studies demonstrated that concentrations of 2.5×10^{-4} % cause extensive loss of muscular activity (Takeno et al., 1977).

Birge and Just (1975) also looked at the effect of mercury on various stages of development of *Rana pipiens*. In concentrations of 0.01 mg/L Hg, no embryo in the cleavage and blastula stage survived and at 0.1mg/L, none of the embryo in the gastrula and neurula stage survived. Concentrations above 1mg/L of Hg were lethal to all developmental stages.

Landè and Guttman (1973) examined the toxic effects of Cu in *Rana pipiens* and found that the eggs were not affected by Cu concentrations of 0.04-1.56 mg/L, but levels greater than 0.31 mg/L was fatal to tadpoles**.**

A study in 1980 by Pytasz et al. found that frogs *(Rana ridibunda)* collected near a metallurgic work in Poland had lower metabolic rates than those captured farther away.

Study Area

Ward Marsh

Ward Marsh in West Haven, VT marks the confluence of the East Bay of Lake Champlain and the Poultney River (Fig. 4). The marsh lies on Precambrian gneiss (Geologic Map of Vermont, 1981) and the sediment consists of clay rich sediment.

A total of 18 samples were collected from Ward Marsh (Fig. 5). Two water samples were taken: WM-1 and WM-9. The remainder were sediment samples and only these were analyzed. Samples were taken from two transects, one of which consisted of WM-1 through WM-11 and WM-17. The northern transect included WM-12 through WM-16. WM-18 was taken 6 m north of the dirt road, and was 15 m from East Bay. Samples sites ranged from aerially exposed, dry sediment to sub-aqueous muck.

It is important to note that WM-2 and WM-3 were located in a small man-made pond that provided drinking water for cattle. This pond was isolated from the main wetland and did not receive its water from East Bay. This provides a control within this site. The land adjacent to the marsh is open pasture land and a dirt road marks the southern edge of the marsh. East Bay itself had a slow velocity and its brown color indicated heavy sediment load.

Mud Creek

Mud Creek in Alburg, VT consists of sediment ranging from clay to sand size. It lies on calcareous shale that grades upward into limestone and dolomite beds (Geologic Map of Vermont, 1981). Eighteen sediment samples were collected for analysis (Fig. 6). MC-1 through MC-12 were sampled in pairs. One of each pair was obtained at the water's edge and the other was sampled from sediment below a meter of water. MC-1 through MC-6 were taken just north

of the foot bridge and MC-7 through MC-12 just south of the foot bridge. The remainder of the samples were obtained from throughout the marsh close to the edge of the abandoned railroad bed or Route 78.

Mud Creek has a well defined channel through which the fastest water flows. The middle of the marsh was open moving water.

The sample site is bordered by Route 78 on the south east, and two abandoned railroad beds on the North and West. The beds on the north side are made in part with slag, most likely from mining refuse. The slag appears to be not reactive and apparently is not a significant source of metals.

Figure 4. Map of Vermont showing the two sample sites. Ward Marsh (affect site) is located at the southern end of Lake Champlain and Mud Creek (reference site) is situated at the northern end.

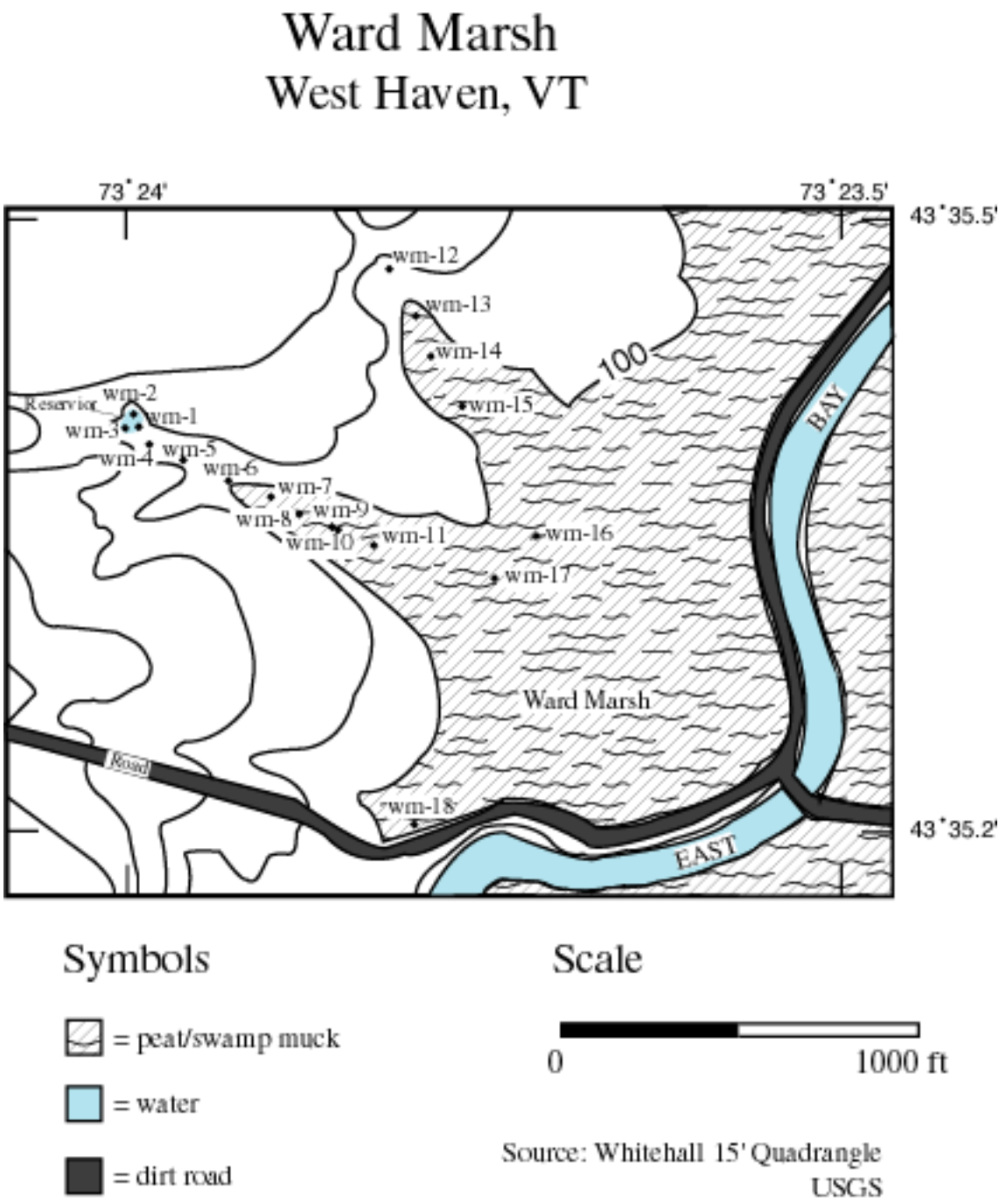


Figure 5. Site map of Ward Marsh in West Haven, VT.

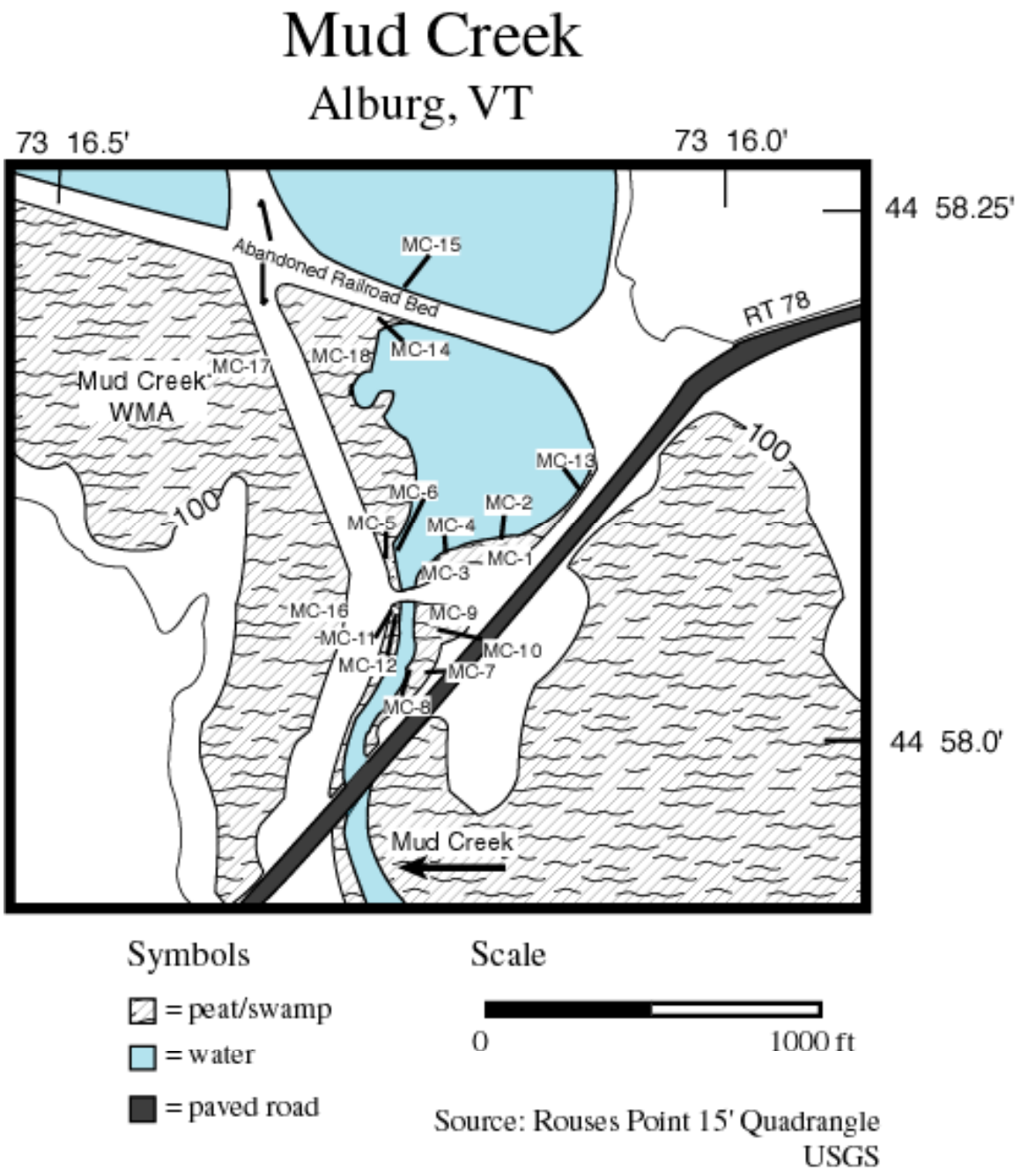


Figure 6. Site map of Mud Creek in Alburg, VT.

Figure 7. A picture of Ward Marsh from WM-12 looking east toward WM-15. Note the variation in sediment conditions from aerially exposed to sub-aqueous.

Figure 8. Looking north from MC-2 in Mud Creek across the open water toward the abandoned railroad bed.

Purpose and Goals

This study compares the concentrations of Zn, Ni, Co, Cr, and Cu in the sediments of Ward Marsh and Mud Creek. These metals were chosen because they have been known to cause frog malformities in laboratory settings that are similar to ones found in the field. Sediment rather than water was chosen to be analyzed because of its obvious teratogenicity in the FETAX assay (Fig. 2&3). Furthermore, water samples were not studied in detail because the sediment is a better indicator for metal accumulation due to the fluctuation and short residence time of analytes in the water column (Singh et al., 1997). There are many other components of the sediment that could have been and should be analyzed, such as organic complexes. However, this study focuses on metals partly due to the scope of this senior thesis research, but mostly due to the past research that has shown the teratogenic effects of metals in frogs. I hypothesize that metal concentrations will be higher in Ward Marsh (affected site) than at Mud Creek (reference site).

The issue of malformed frogs is very complex and much more work needs to be done in order to gain a better understanding of the problem. This study is one link in the chain that can help tease apart the cause of the malformities. We can not derive any causation from the results of this study; the bioavailability of the metals in uncertain. However, a correlation between metal concentrations in the sediment and a high rate of frog malformities can set the foundation for future research.

II. Background

Northern Leopard Frogs

The habitat and life style of the frogs must be understood in order to realize where and how the frogs are being exposed to metals in the environment.

The habitat of the Northern Leopard frog extends across the fields and meadows of the United States and southern Canada. During breeding season they are typically found at the waters edge of marshes, lakes and streams. Part of the Ranidae family, the frog usually is brown or green with dark spots, typically grows to about 5 inches, and has an average life span of 3 years. In late April the adults move from over-wintering sites under water to their "terrestrial home range". Adults soon migrate to breeding ponds. Exhibiting a distinctive behavior on rainy nights, they move from the meadows to the water by the thousands. Jim Andrews of the Middlebury Department of Biology takes advantage of this by placing drift fences parallel to the marsh's edge. The fences funnel the frogs into deep buckets into which the frogs fall. From this he is able to survey many frogs at once and examine them for deformities. In early May the male frogs arrive at the mating ponds and begin advertising their presence. Once the female selects the male, the couple swims to the egg laying site, which are typically laid in communal nesting sites. The female extrudes the eggs mass which is about 6 inches long, 2 inches wide and contains 5000-6000 tiny black eggs that the male then fertilizes. This process takes approximately five minutes. The eggs hatch in two to three weeks and transform from tadpole to frog in two to three months. The young frogs "rarely get far from where they hatch" (Tyning, 1990).

The Northern Leopard frog tadpoles are classified as a suspension feeder that primarily feeds on plankton and minute algae. The adult frog's diet consists of insects that are snatched

with its tongue. Another important characteristic pertinent to this study is the species' winter survival habits. In order to withstand the freezing temperatures, the frogs overwinter in oval mud pits at the bottom of a body of water (Tyning, 1990).

Heavy Metals and Wetland Sediment

Heavy metals in aquatic environments have been studied extensively in recent years due to the drastic impact they can have on the ecosystem. Metal pollution is problematic because it is not easily eliminated from aquatic environments like most organic pollutants (Forstner, 1981). Furthermore, they act as good indicators of water quality and of the extent of potential anthropogenic contamination (Singh et al., 1997). Pollutants such as heavy metals can be present in water, suspended sediment, and bottom sediment of aquatic systems.

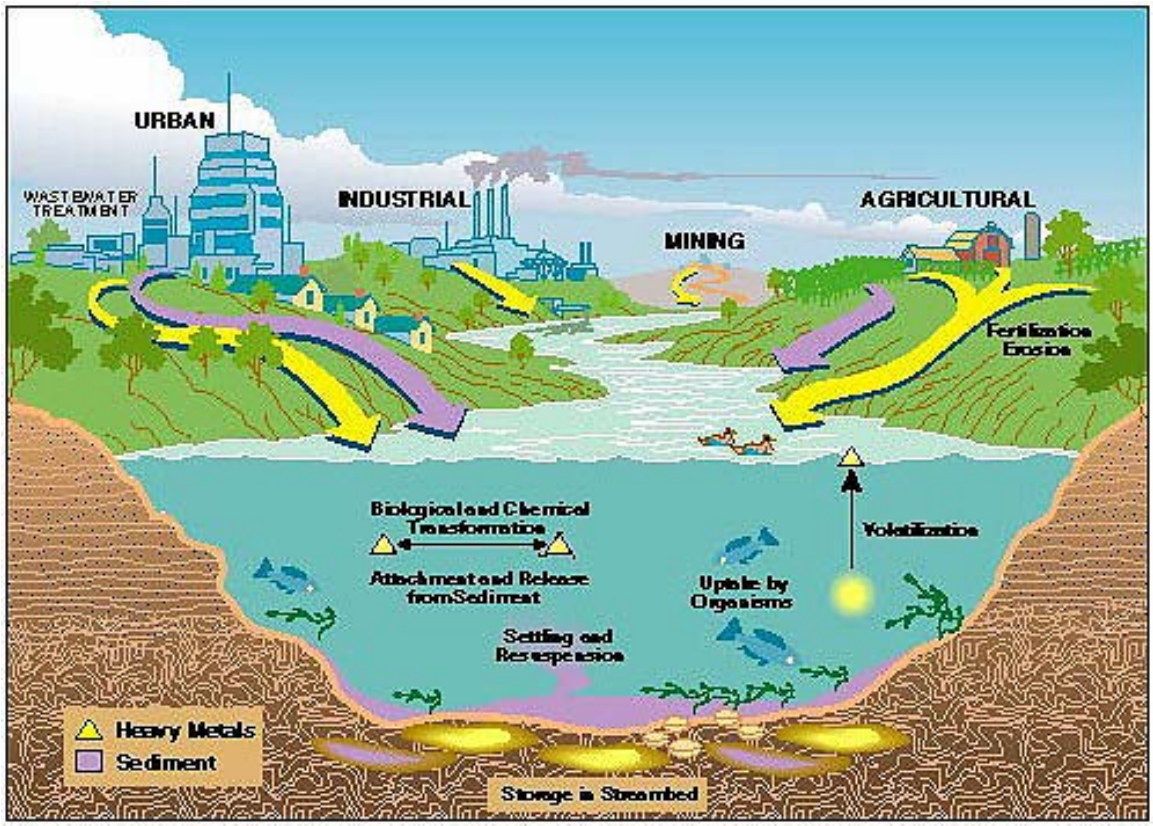
Sources

Heavy metals are those having densities five times greater than water. These include such elements are Fe, Pb, Ni , Zn, Co, and Cr. Metals from bedrock parent material exist naturally in sediment. However, it is usually human interaction that results in toxic concentrations of metals (Gambrell, 1993).

Natural sources include bedrock, especially slate, which is enriched in heavy metals compared to sandstone, granite, and carbonate rocks (Faure, 1998).

Anthropogenic sources include domestic effluent, mine drainage and industrial effluent such as papermills, petroleum refineries, and metal works (Forstner, 1981). Industry such as laundries, soft drink and ice cream manufacturers discharge effluent enriched in Cu, textile dyeing and laundry waste may emit Cr, and Ni is associated with bakery wastes (Forstner, 1981).

Figure 9. An illustration taken from Garbarino (1995) that shows the sources and sinks of heavy metals and sediment.



Physical factors influencing metal concentration in sediment and availability

Gibbs (1977) describes 5 major mechanisms for metal accumulation in or on sediments as: 1) adsorption on fine grained material, 2) precipitation of metal compounds, 3) co-precipitation with hydrous Fe and Mn oxides and carbonates, 4) organometallic bonding with organic matter, and 5) incorporation within crystalline minerals.

Adsorption is the condensation of atoms, ions, or molecules on the surface of clays, Fe hydroxides, and humic acids. Organometallic bonding is attachment of any metal to carbon, i.e. diethylzinc, methyl and dimethyl mercury, and tetraethyl lead (Horowitz, 1985)

Grain Size

Grain size can affect the concentration of metals in soil and sediment. It is an important physical characteristic because it incorporates surface area and surface charge. As grain size decreases, surface area increases (Forstner,1981). Clay sized sediment, which is very fine grained, has surface areas of square meters per gram while sand sized sediment has surface areas of only tens of square centimeters per gram (Forstner,1981).

Table 3. Material with surface area less than 2 micrometer diameter.

Material	***Surface Area m^2/g***
Calcite	12.5
Clay minerals	
Kaolinite	10-50
Illite	30-80
Montmorillonite	50-150
Iron hydroxide	300
Organic mater (Humic acids)	1900

(Forstner, 1981)

As surface area increases, the substance is able to adsorb more metals (Horowitz, 1985). Furthermore, substances such as clays have negative surface charges because the anion is exposed at the edge of the octahedral layer (Drever, 1997). This negative surface charge is able to hold on to positively charged cations.

Cation exchange capacity

Cation Exchange Capacity is the ability of a soil component to sorb cations from solution and replace them with equivalent amounts of ions. CEC depends on the negative anionic site like $SiOH^-$, $Al(OH)_2^-$, and $AlOH^-$ (Forstner, 1981). The CEC sites also occur between layers of silicates in expandable clays (Forstner, 1981). Expandable clays such as smectite and vermiculite have high CEC (Drever, 1997).

<u>Clays</u>

The grain size, surface area, and CEC of clay minerals makes them good metal concentrators. Hirst (1962) determined the trace metal sorbing abilities in descending order:

Montmorillonite>vermiculite>illite+chlorite>kaolinite

The affinity of metals for clay minerals is as follows (Forstner, 1981):

Pb>Ni>Cu>Zn

Recent studies in some European rivers appear to indicate that organics and hydrous oxides (i.e. FeOH and MnO) actually carry out the concentration of metals and the clays acts as a mechanical substrate onto which these collectors can concentrate (Jenne, 1976; from Horowitz, 1985).

Hydrous Metal Oxides

Hydrous Fe and Mn oxides are very good at sorbing metals from solution because they are fine grained, amorphous, have large surface area, high CEC, and have negative surface charges (Forstner, 1981).

Organic Matter

Organic matter such as humins, humic acid, fulvic acid, and yellow organic acids also have characteristics similar to hydrous Fe and Mn oxides that make them good collectors of metals (Forstner, 1981). Dissolved organic substances are capable of complexing metals and increasing metal solubility, altering the distribution between oxidized and reduced forms of metals, alleviating the toxic affects and availability of metals to aquatic life, influencing the extent to which metals are adsorbed on suspended mater, and affecting the stability of metal-containing colloids (Forstner, 1981).

Shapiro (1963) found that organic acids are able to keep Fe and other metals in solution even with high pH and high Eh (Forstner, 1981).

The Irving-Williams Series describes the stability (high stability indicating low mobility) of organic-metal constituent complex in soils as follows (Forstner, 1981):

Pb>Cu>Ni>Co>Zn>Cd>Fe>Mn>Mg

Chemical factors influencing metal concentration in sediment

The bioavailability of contaminated sediment to organisms in the ecosystem depends on the chemical conditions of the environment. For instance, if the concentration of a particular contaminant is low in the aqueous phase, then the contaminants bound to sediment are likely to have a greater impact on organisms (et al., 1996).

Oxidation and Reduction

Oxidation and reduction status in wetland sediment is an important factor that influences metal mobility. There are situations, though, even in reducing environments, when the sediment can become more oxic, such as at the sediment water interface or the roots of some aquatic plants. The oxic conditions can remobilize the metals, thus increasing the bioavailability (et al., 1996). Dissolution of FeO and MnO and hydroxides at redox boundaries can lead to metal release.

pH

The pH is also an important factor. The pH can be reduced as a result of acid mine drainage, acid rain, and acidic industrial effluent. The acidic conditions can release metals in the system. In general, wetland sediment is anaerobic with a pH between 6.5 and 7.5, which indicates that metals in these conditions would be relatively immobile (Gambrell, 1993). However, metal mobility can alter with change in the pH as a result of oxidation. Gambrell et al. (1991) continuously stirred wetland sediment for 8 days under air atmosphere. The initial pH of 7 dropped to 3 by the end of the study. Another study by Prasana (1994) stirred sediment for 14 days in an argon atmosphere and the initial pH of 7.3 remained the same, but the identical sediment stirred in an air environment dropped to a pH of 3.5. These studies show that acidity

can develop under natural oxidizing situations (Gambrell, 1993). Other studies have shown that oxidized wetland sediment has a lower pH than reduced sediment (Gambrell, 1980).

The precipitation of metal species is dependent upon the concentration of the ions and the pH of the environment (Forstner, 1981). For hydroxides, the minimum solubility values lie within pH = 9 - 12 while at lower pH (~4) there is total dissolution (Forstner, 1981). Fe, Mn, and Cd sulfides are soluble in HCl while Ni and Co sulfides are more difficult to disassociate (Forstner, 1981). Copper, Pb, and Hg are only soluble in oxidizing agents such as HNO_3. Acid extraction works because H^+ replaces the metal at the exchange site.

Bioavailability

Metals can exist in various chemical forms in the environment and their toxicity to organisms depends on their availability.

Table 4. Classification of naturally occurring metals according to their toxicity and availability in the hydrologic environment (Wood, 1974) (taken from Garbarino, 1995)
[Metals that normally do not exist as dissolved species in natural waters or are very rare in crustal rocks are in italics]

Nontoxic		Low toxicity		Moderate to high toxicity			
Aluminum	Magnesium	Barium	*Praseodymium*	*Actinium*	Indium	Polonium	Uranium
Bismuth	Manganese	*Cerium*	*Promethium*	Antimony	*Iridium*	*Radium*	Vanadium
Calcium	Molybdenum	*Dysprosium*	*Rhenium*	Beryllium	Lead	*Ruthenium*	Zinc
Cesium	Potassium	*Erbium*	*Rhodium*	Boron	Mercury	Silver	*Zirconium*
Iron	Strontium	*Europium*	*Samarium*	Cadmium	Nickel	*Tantalum*	
Lithium	Rubidium	*Gadolinium*	Scandium	Chromium	*Niobium*	Thallium	
	Sodium	*Gallium*	*Terbium*	Cobalt	*Osmium*	Thorium	
		Germanium	Thulium	Copper	Palladium	*Titanium*	
		Gold	*Tin*	*Hafnium*	Platinum	*Tungsten*	
		Holmium	*Ytterbium*				
		Neodymium	Yttrium				

- Water-soluble metals such as free ions (e.g., Zn^{2+}), inorganic complexes, organic complexes and exchangeable metals are the most mobile forms (Gambrell, 1993).

Metals precipitated as inorganic compound are generally immobile except in low pH conditions perhaps resulting from the oxidation of reduced sediment (Gambrell, 1993). All heavy metal chlorides (except $PbCl_2$) and sulfates are soluble while carbonates, hydroxides and sulfides dissolve with difficulty (Forstner,1981). The general formula for the disassociation of inorganic metal compound is as follows:

$$MX_2(s) \text{<---->} M^{2+}(aq) + 2X^- (aq)$$

- Metals complexed with large molecular-weight humic materials are considered to be stable in reducing environments due to the strength of the bonds under these conditions (Gambrell, 1993).
- Hydrous oxides of Fe and perhaps Al and Mn have been known to adsorb metals but can be less stable in reducing conditions (Gambrell, 1993). In the fringes of wetland these oxides can form, holding on to metals, and during flood events become reduced and release the metals (Gambrell, 1993).
- Metals precipitated as sulfides are insoluble in reducing conditions. Metals in the pore water are removed by sulfide precipitation in anoxic environments but can become mobile again in oxic condition due to the mineralization of organic matter and oxidation of sulfide (Gambrell, 1993).
- Metals bound within the crystalline lattice structure of primary minerals are not available to organisms and metals only become releases after extensive weathering (Gambrell, 1993).

Sediment Quality Assessment

The U.S. Environmental Protection Agency has no specific health guidelines for heavy metals associated with suspended or bed sediments. This lack of national guidelines based on concise scientific criteria causes difficulty when evaluating the environmental effects of heavy metals in sediments. Table 5 lists criteria for open-water disposal of polluted sediments from Garbarino (1995).

Table 5. U.S. Environmental Protection Agency maximum contaminant levels for heavy-metal concentration in drinking water and water supporting aquatic life, and criteria for open water disposal of polluted sediments.

[μg/L, microgram per liter; μg/g, microgram per gram; >, greater than; <, less than; --, no guideline available]

	Cadmium	Chromium	Copper	Lead	Mercury	Uranium
Drinking water, in μg/L[1]	5	100	[2]1,000	15	2	[3]20
Water supporting aquatic life, in μg/L[4]	12	100	20	100	0.05	–
Natural sediments, nonpolluted, in μg/g[5]	--	<25	<25	<40	<1	–
Natural sediments, moderately polluted, in μg/g[5]	--	25 to 75	25 to 50	40 to 60	--	–
Natural sediments, heavily polluted, in μg/g[5]	>6	>75	>50	>60	>1	–

[1]U.S. Environmental Protection Agency, 1992.
[2]Secondary maximum contaminant level based on esthetic water quality.
[3]Proposed maximum contaminant level.
[4]U.S. Environmental Protection Agency, 1982.
[5]Great Lakes Water Quality Board, Dredging Subcommittee, 1982.

(Garbarino, 1995)

Calmano et al. argues that water quality standards should be applied to sediment because of its strong influence on the water quality. However, total metal concentration in sediment is not a good estimation of bioavailability. Different phases of sediments (such as sulfides) can vary in toxicity with the same concentration (Calmano et al., 1996).

Chapman (1986) developed a rubric, through which sediment quality can be assessed, called the Sediment Quality Triad. Three components are assessed including identification and quantification of contaminants in the sediment, sediment toxicity tests, and *in situ* biological effects. Chapman points out the advantage is that it can be used with any sediment type and it does not require "assumptions concerning the specific mechanism of interaction between organisms and contaminants" (Calmano et al. 1996). However, it does not provide causation nor does it account for an unmeasured contaminant that may be causing the toxic effects.

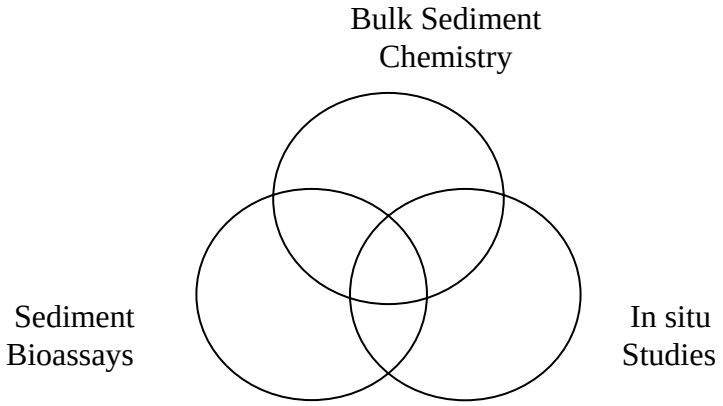


Figure 10. Conceptual model of the sediment quality triad, which combines data from chemistry, bioassay and in situ studies. Areas where the three facets of the triad show the greatest overlap (in terms of either positive or negative results) provide the strongest data for determining numerical sediment criteria.
(Taken from Calmano et al., 1996)

Van de Guchte (1991) based his evaluation of contaminated sediment from the Rhine on the triad. He analyzed 49 substances, both organic and inorganic, used *in situ* biological analyses, and toxicity assays. He established two parameters for Sediment Quality Criteria (SQC): safe level and warning value. The criteria are presented in the following table.

Table 6. Some indicative criteria in the triad assessment of contaminated Rhine sediments (Van de Guchte, 1993)

Parameter	Criterion 1	Criterion 2
Chemicals		
-Metals, PAH, PCB, OCP	SQC- safe level	SQC- warning value
Field Surveys		
-Chironomids	10% deformities	20% deformities
-Maximum density	1500 individuals/m^2	500 individuals/m^2
Bioassays		
-Daphnia reproduction	10% effect	50% effect

Taken from Calmano et al., 1996

Through the 1980's, studies found that sediment (top 3 cm) in Collingwood Harbour, Lake Heron, Ontario, had exceeded the limits of heavy metals for open-water disposal of dredged material. In 1992, sediment quality criteria were established based on both laboratory bioassays and field research (Krantzberg, 1992). Table 7 shows these limits as the Lowest Effect Level in μg/g.

Table 7. Sediment Quality Criteria for sediment in Collingwood Harbour, Lake Heron, Ontario, using the concentrations of metals (μg/g) that were associated with the least alteration of the local benthic population.

	Zn	**Cu**	**Ni**	**Cr**	**Cd**	**Hg**	**Pb**	**Fe**
Lowest Effect Level (μg/g)	120	16	16	26	0.6	0.2	31	20,000

Source: Collingwood Harbour RAP Team and Public Advisory Committee (1992)

III. Methods

This project followed EPA method 200.2 for spectrochemical determination of total recoverable analytes in sediment from Ward Marsh and Mud Creek.

Field Methods

All sample containers were soaked in 10% HNO_3 for > 24 hours.

Clean latex gloves were used during all sediment sampling. Bottles were pushed into the mud and the sediment was hand scooped in to the bottles. Caps were screwed on tightly to prevent air exchange between sample and atmosphere. No metallic sampling devices were used. Samples were collected from the upper 15 cm of marsh sediment.

Laboratory methods

Prior to lab analysis, samples were stored at 20° C in airtight bottles. Method 200.2 calls for storage at 4° C. However, the storage conditions used in this study are within the range of natural conditions for theses samples (e.g. T, pH, Eh, H_20 content), so the only alterations to the samples, if any, will be similar to those produced in the field.

The samples were mixed thoroughly and a 100g aliquot was weighed. Samples were dried at 60° C in beakers that were soaked in 10% HNO_3 for > 24 hours and rinsed with de-ionized water. Weight was recorded before and after drying. The dried sediment samples were ground in an acid rinsed (10% HNO_3) mortar and pestle. A 1.0 $\pm$ 0.01 g aliquot was placed into a 250 ml flask. The acid extraction used 4 ml of (1:1) HNO_3 and 10 ml of (1:4) HCl. (1:1) HNO_3 was made with equal amounts of trace metal grade 70% HNO_3 and deionized water. (1:4) HCl was prepared by adding 200ml of concentrated trace metal grade 37% HCl to 400 ml of deionized water and then diluting to 1L.

Flasks were covered with a watch glass and set on a calibrated hotplate at 95° C for extraction. The samples were heated for 30 minutes at a gentle boil. They were then cooled and quantitatively transferred to a 100 ml volumetric flask, diluted to 100 ml with distilled water, stoppered and mixed. Samples were filtered in acid soaked filters that were analyzed by SEM to ensure that soaking did not influence the pore size.

Cation concentrations (mg/L) were determined by inductively coupled argon plasma (ICAP) atomic emission spectrometry. Calculations expressed in mg/kg were derived from ICAP results. All reported results are expressed in mg of metal / kg of soil.

ICAP Analysis

A Thermo Jerrell Ash In-Board 2500 R.F. Generator Inductively Coupled Argon Plasma Spectrometer (ICAP) was used to analyze metal concentrations. The acid extract is introduced to the torch as a aqueous aerosol. The excitation of the atoms or ions emits light that is converted into electrical signals that is measured by the spectrometer. The intensity of the signal is converted to concentration using a measured standard (Thompson and Walsh, 1989). For this study, a high standard of 1 mg/L was used for Zn, Ni, Cu, Co, and Cr and the low standard was a blank. The high standard for Na, P, K, and Mn was 100 mg/L and for Mg, Al, Ca, and Fe the high standard was 500 mg/L. The low standard was 1 mg/L for Na, P, K, and Mn and 5 mg/L for Mg, Al, Ca, and Fe.

Methods for pH Analysis

The Soil Paste method (Palmer and Troeh, 1995) was used to assess the pH of the sediment samples. Ten grams of dried sediment was place in 20 ml of deionized water and stirred intermittently for 20 minutes. The pH was then obtained using an Orion Research Inc. Model 210 A pH meter standardized to pH 4 and 7 solutions.

Mercury Analysis

The concentration of mercury in sediment from Ward Marsh and Mud Creek was determined by using a cold vapor Atomic Absorption Spectrometer (AAS).

Lead Analysis

Analysis of lead concentration of sediment was attempted but due to equipment problems the results were unreliable.

IV. Results

Teratogenic Metals

ICAP analyses indicate that the concentrations of Zn, Ni, Co, and Cr are statistically higher in sediment from Ward Marsh (affected site) than Mud Creek (reference site). Tables 8a-b summarize the mean, and median concentrations of five metals from Ward Marsh and Mud Creek. Concentrations of individual sample sites can be found in Appendix A.

Table 8a: Concentrations of Zn, Cu, Ni, Co, and Cr in sediment from Ward Marsh using ICAP spectrometry. Concentrations are in mg kg^{-1} (ppm).

Ward Marsh	Zn	Cu	Ni	Co	Cr
mean	**103**	**28.5**	**39.4**	**18.3**	**43.4**
median	97.3	25.9	39.9	17.9	40.5
stdev	34	11.9	7.7	2.9	11.8

Table 8b: Concentrations of Zn, Cu, Ni, Co, and Cr in sediment from Mud Creek using ICAP spectrometry. Concentrations are in mg kg^{-1} (ppm).

Mud Creek	Zn	Cu	Ni	Co	Cr
mean	**72.3**	**22.2**	**19.2**	**7.41**	**19.6**
median	64.1	24.6	16.9	5.19	15.2
stdev	27.7	9.1	8.4	5.3	11.1

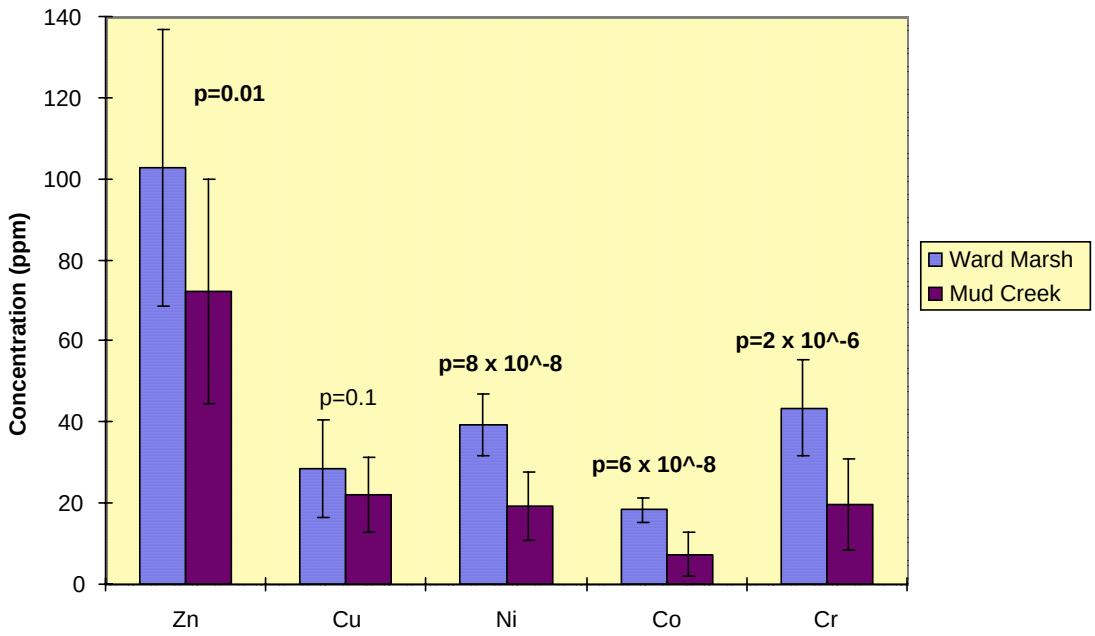


Figure 11. Analysis of Zn, Cu, Ni, Co, and Cr concentrations in sediment from Ward Marsh and Mud Creek. Concentrations are in mg kg^{-1} (ppm). Error bars represent the standard deviation of the sample population.

It is important to note that the results for Mud Creek (Table 1b) do not include two anomalously high sample sites (MC-11 and MC-12). These spurious peaks did not represent the characteristic Zn concentrations of the marsh. Table 2 shows the mean, median, and standard deviation of metal concentrations in Mud Creek *including* MC-11 and MC-12.

Table 9. Concentrations of Zn, Cu, Ni, Co, and Cr in sediment from Mud Creek including MC-11 and MC-12.

Mud Creek	**Zn**	**Cu**	**Ni**	**Co**	**Cr**
mean	**135.9**	**23.0**	**18.9**	**7.15**	**19.1**
median	69.1	24.9	16.9	5.19	15.2
stdev	197.5	8.9	8.2	5.1	10.7

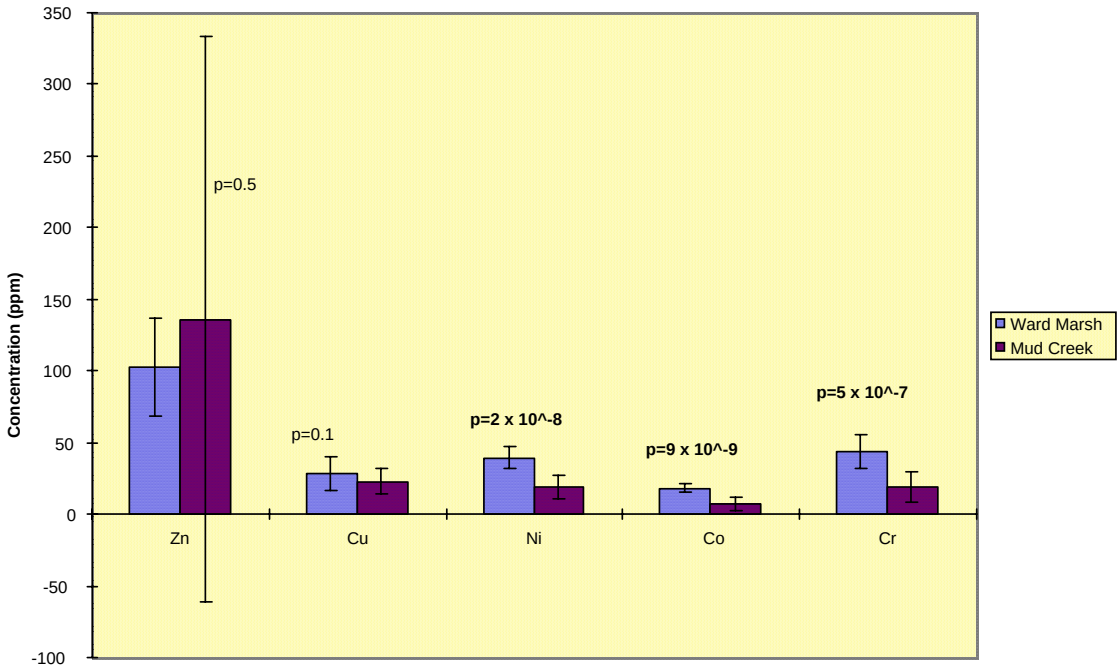


Figure 12. Summary of the concentrations of Zn, Cu, Ni, Co, and Cr in sediment from Ward Marsh and Mud Creek. Concentrations are in mg kg^{-1} (ppm). Error bars represent the standard deviation of the sample population. This figure includes MC-11 and MC-12.

Including the two anomalous samples, the standard deviation of the sample population is larger than the mean. This shows that MC-11 and MC-12 are skewing the mean and are not representative of Zn levels in the marsh. By comparing Table 2 and Table 1b it is clear that the removal of these spurious peaks only alters the mean and median concentrations for Zn. The four other metals are not significantly affected by these changes. For these reasons MC-11 and MC-12 have been eliminated from the analysis and concentrations from Table 1b are the reported values. MC-11 and MC-12 were located next to a foot bridge that is used as a fishing spot.

T-test analysis shows statistically higher values of Zn, Ni, Co, and Cr in Ward Marsh versus Mud Creek. The t-test show no difference between levels of Cu in sediment between the sites. The following table summarizes the statistical analysis. P values less than 0.05 represent a

statistically significant difference. That is, there is 95% certainty that the differences are not due to chance.

Table 10. T-test analysis of metal levels in sediment from Ward Marsh and Mud Creek.
$P \leq 0.05$ represents a statistically significant difference. Concentrations are in mg kg^{-1} (ppm).

Zn

	Ward Marsh	*Mud Creek*
Mean	**103**	72.3
P(T<=t) two-tail	0.01	

Cu

	Ward Marsh	*Mud Creek*
Mean	28.5	22.2
P(T<=t) two-tail	0.1	

Ni

	Ward Marsh	*Mud Creek*
Mean	**39.4**	19.2
P(T<=t) two-tail	8E-08	

Co

	Ward Marsh	*Mud Creek*
Mean	**18.3**	7.41
P(T<=t) two-tail	6E-08	

Cr

	Ward Marsh	*Mud Creek*
Mean	**43.4**	19.6
P(T<=t) two-tail	2E-06	

Other Elements

Concentrations of non teratogenic elements were analyzed using ICAP spectrometry in sediment from the two sites. Individual sample site concentrations appear in Appendix B. Concentrations of Mg, Al, K, Fe, and Mn are statistically higher in sediment from Ward Marsh versus sediment from Mud Creek. There is statistically more Ca in Mud Creek sediment that Ward Marsh and levels of Na and P are similar between the sites.

Table 11a. Mean and median concentrations of common elements in sediment from Ward Marsh in mg kg^{-1} (ppm).

Ward Marsh	Na	Mg	K	Ca	Al	P	Mn	Fe
mean	188	9280	2340	5330	20000	827	528	35200
median	184	9600	2390	4650	20400	848	420	36500
stdev	51	2090	612	3240	3700	141	203	5360

Table 11b. Mean and median concentrations of common elements in sediment from Mud Creek in mg kg^{-1} (ppm).

Mud Creek	Na	Mg	K	Ca	Al	P	Mn	Fe
mean	173	3670	1020	8650	9300	703	262	19200
median	154	3550	668	5530	8930	736	188	17200
stdev	98	2020	929	5740	5040	306	175	9860

The following figures summarize the concentrations of common elements in Ward Marsh vs. Mud Creek. Concentrations are in mg kg^{-1} (ppm) and $p \leq 0.05$ represent statistically significant differences.

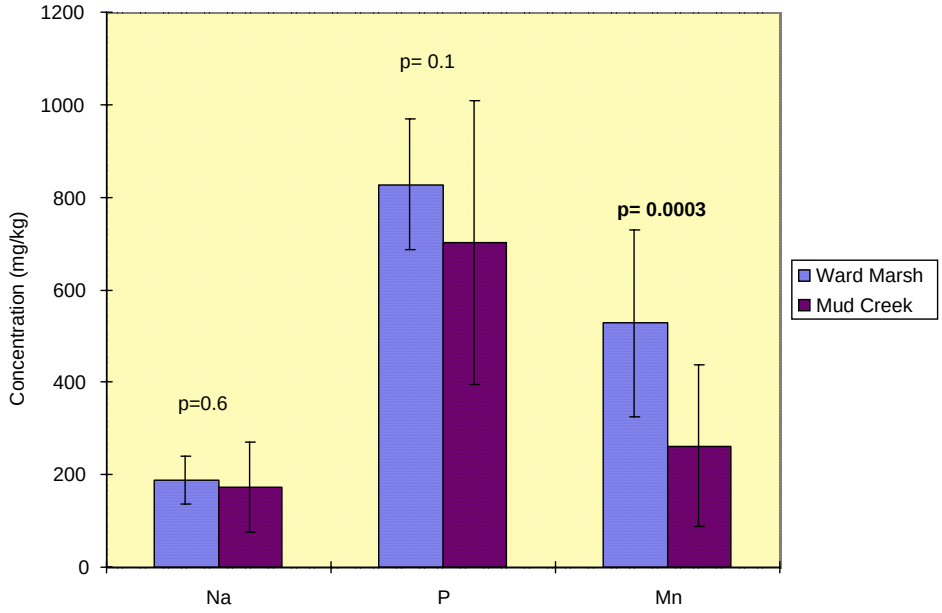


Figure 13. Concentrations of Na, P, and Mn in sediment of Ward Marsh and Mud Creek. Concentrations are in mg kg^{-1} (ppm). $p \leq 0.05$ represents statistically significant differences.

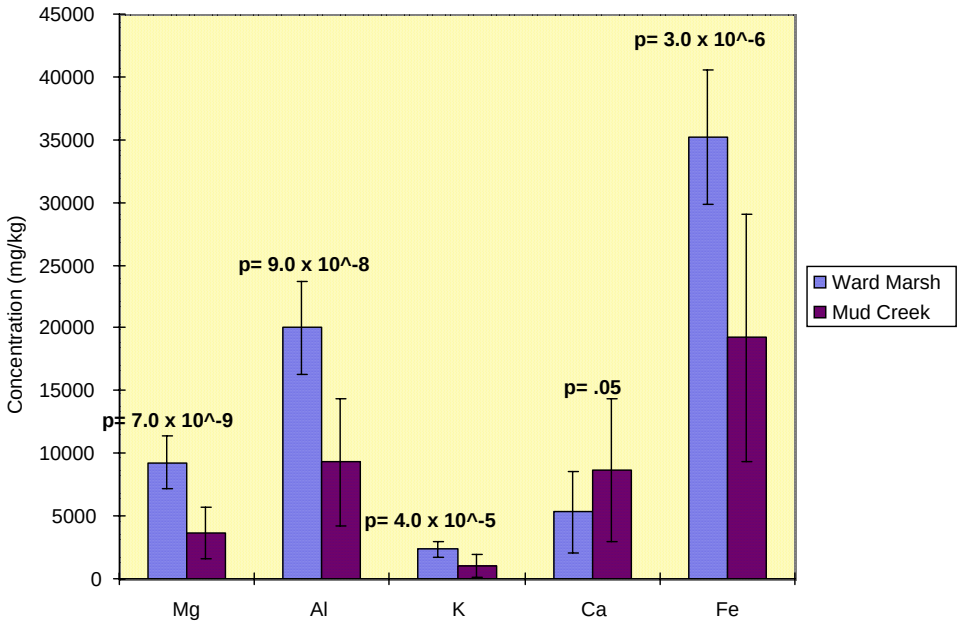


Figure 14. Concentrations Mg, Al, K, Ca, and Fe in sediment of Ward Marsh and Mud Creek. Concentrations are in mg kg^{-1} (ppm). $p \leq 0.05$ represents statistically significant differences.

Ward Marsh Transect - Teratogenic Metals

Transect A-A' in Ward Marsh lends itself to some interesting analysis. Sample sites WM-2 and WM-3 are located in a < 1 acre reservoir that supplies drinking water for the local livestock. The cattle pond is isolated from the main wetland. Due to its location above the high water mark it does not receive its water and sediment directly from East Bay over flows, but rather from the uplands. This provides a control within the site for analysis of potential sources of contaminants. The transects allow analysis of metal concentrations in sediment ranging from aerially exposed sediment distal from the bay to subaqueous samples proximal to East Bay. The concentrations in the following figures are all in

mg kg^{-1}.

Figure 5 shows a transect of Zn concentration in sediment from Ward Marsh in transect A-A'. Concentrations of Zn in sediment from WM-2 and WM-3 in the isolated cattle pond are lower than the remainder of the samples from within the wetland. The concentration increases closer to the bay with a small peak at WM-4 and WM-5.

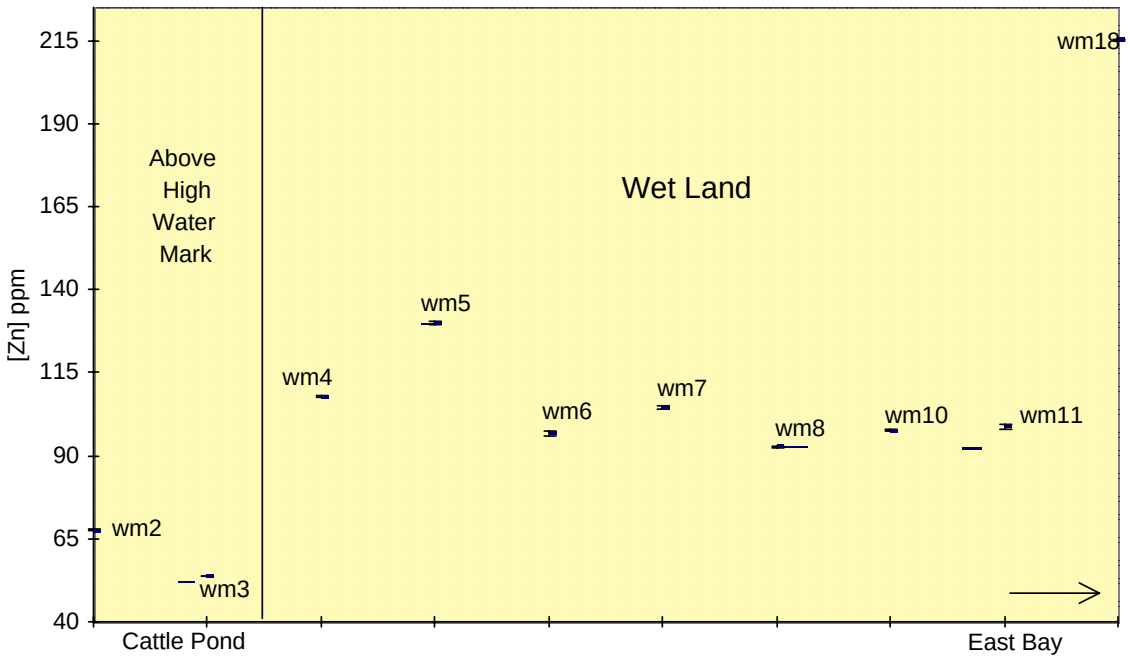


Figure 15a. Concentration (mg kg^{-1}) of Zn in Ward Marsh along transect A-A'. Cattle pond is hydrodynamically isolated from the main wetland.

Similar trends can be seen in the four other teratogenic metals. In all cases the concentrations in sediment from the isolated cattle pond are lower than the remainder of the marsh.

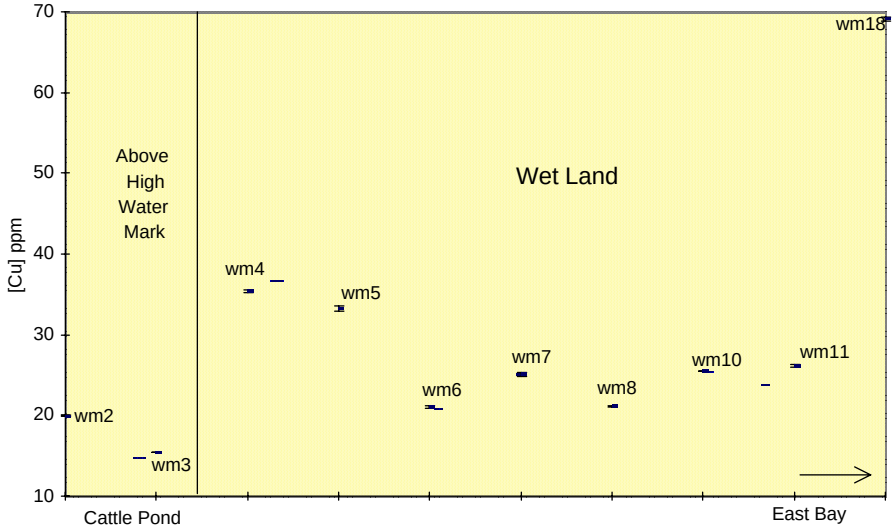


Figure 15b. Concentration (mg kg^{-1}) of Cu in Ward Marsh along transect A-A'. Cattle pond isolated hydrodynamically from the main wetland.

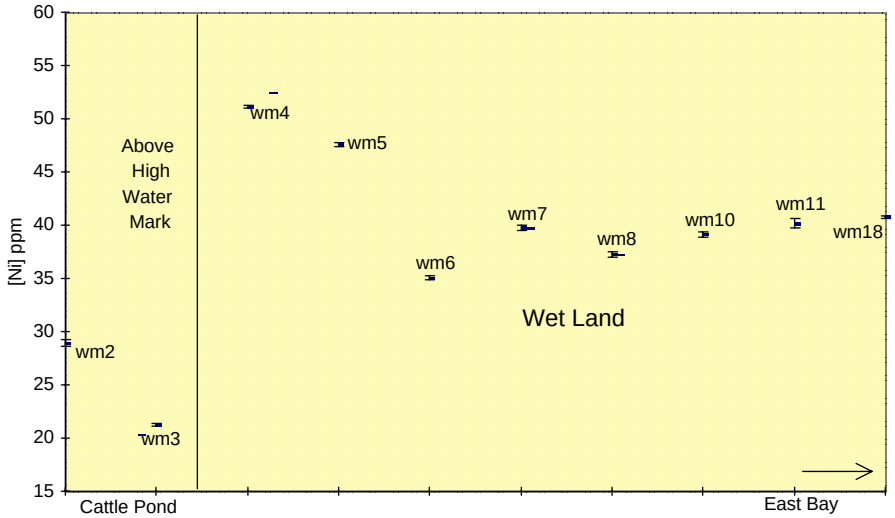


Figure 15c. Concentration (mg kg^{-1}) of Ni in Ward Marsh along transect A-A'. Cattle pond isolated hydrodynamically from the main wetland.

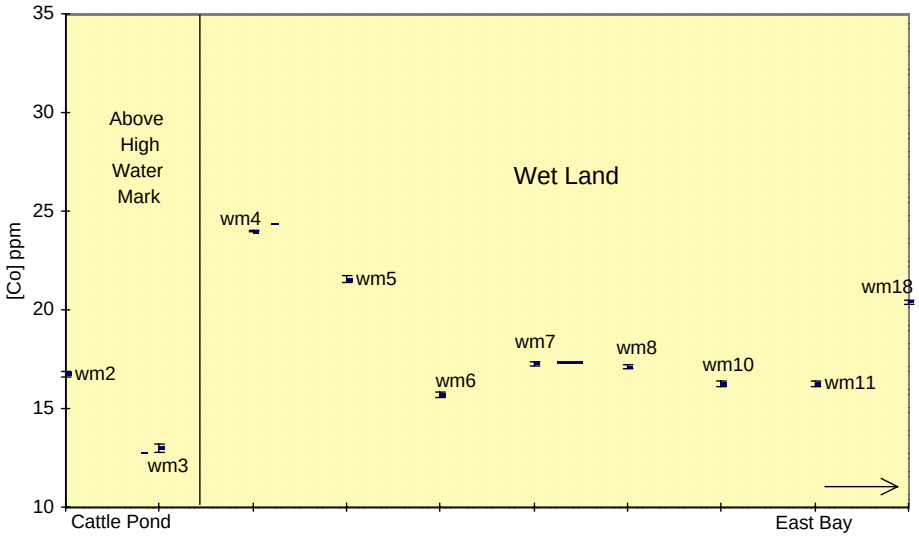


Figure 15d. Concentration (mg kg^{-1}) of Co in Ward Marsh along transect A-A'. Cattle pond isolated hydrodynamically from the main wetland.

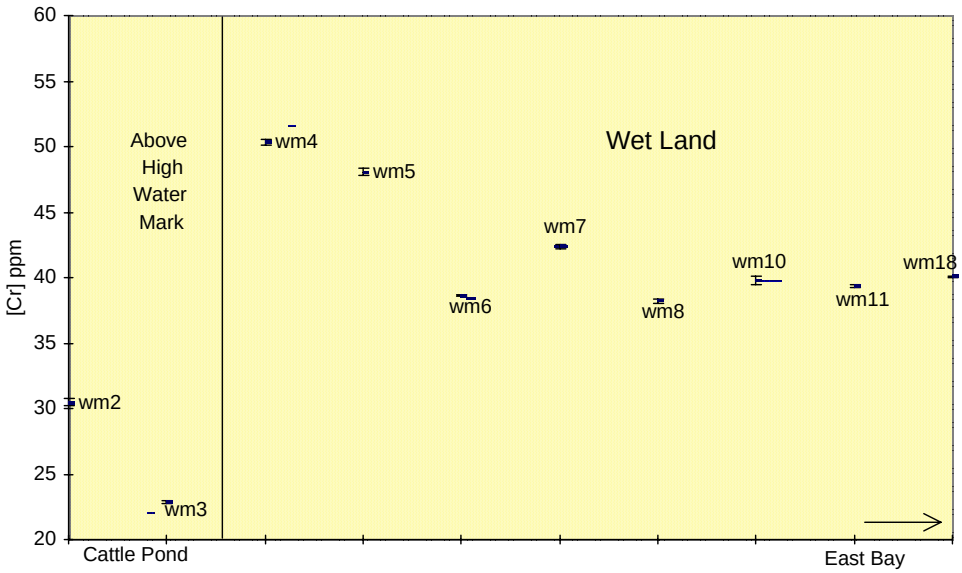


Figure 15e. Concentration (mg kg^{-1}) of Cr in Ward Marsh along transect A-A'. Cattle pond isolated hydrodynamically from the main wetland.

The second transect in Ward Marsh that includes WM-12 through WM-15 (Fig. 5) does not show a significant difference along the trend. The sample sites along this trend are analogous to WM-6 through WM-11 elevation wise, and thus are all below the high water mark.

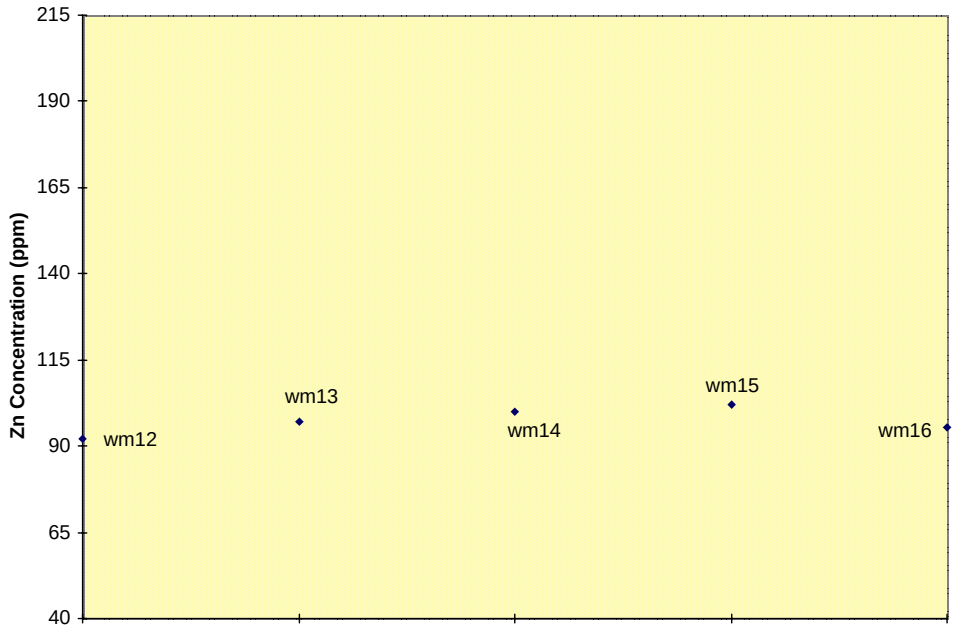


Figure 16. Concentration (mg kg^{-1}) of Zn in Ward Marsh along transect from WM12 - WM16. Concentrations remain relatively consistent along the trend. The transects of the four other teratogenic metals have similar shapes.

Ward Marsh Transect - Other Elements

Concentrations of 8 other common elements in sediment from Ward Marsh were plotted along transect A-A'. Na, Mg, K and Ca follow similar trends. Ca concentrations are relatively low throughout the marsh except for sample site WM-4. Similar to the teratogenic metals, the concentrations in the sediment for these four elements are lower in sediment from the cattle pond.

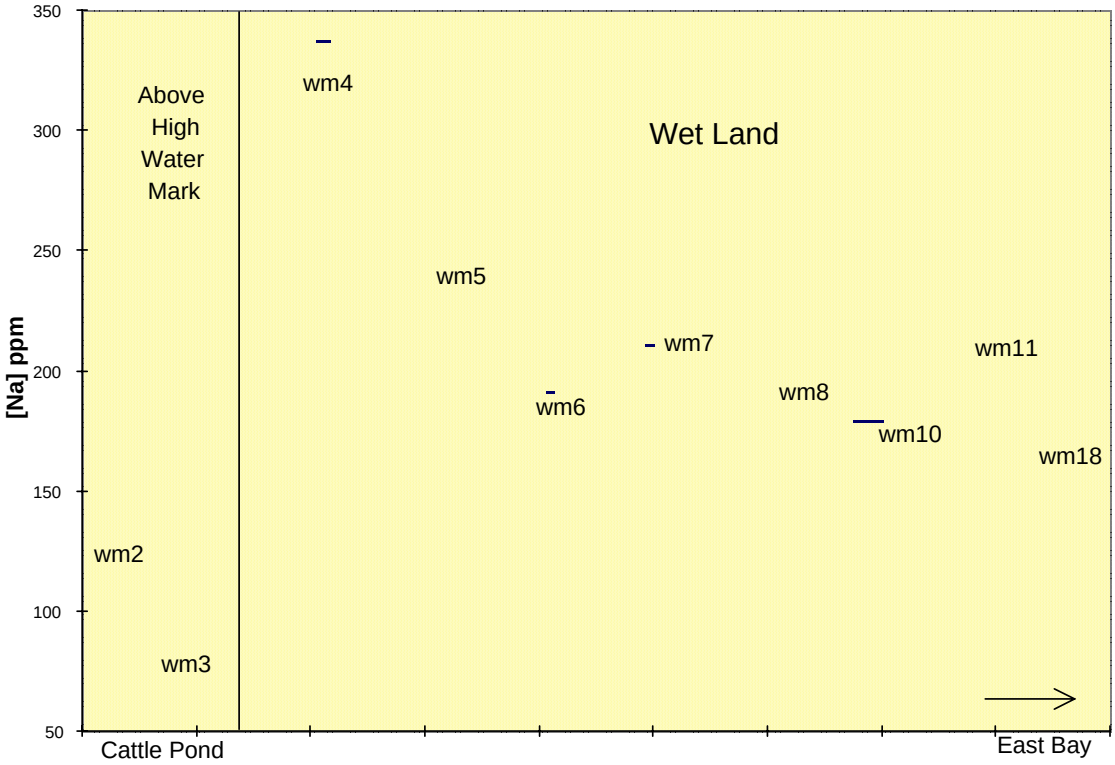


Figure 17a. Concentration of Na in sediment from Ward Marsh along transect A-A' in mg/kg.

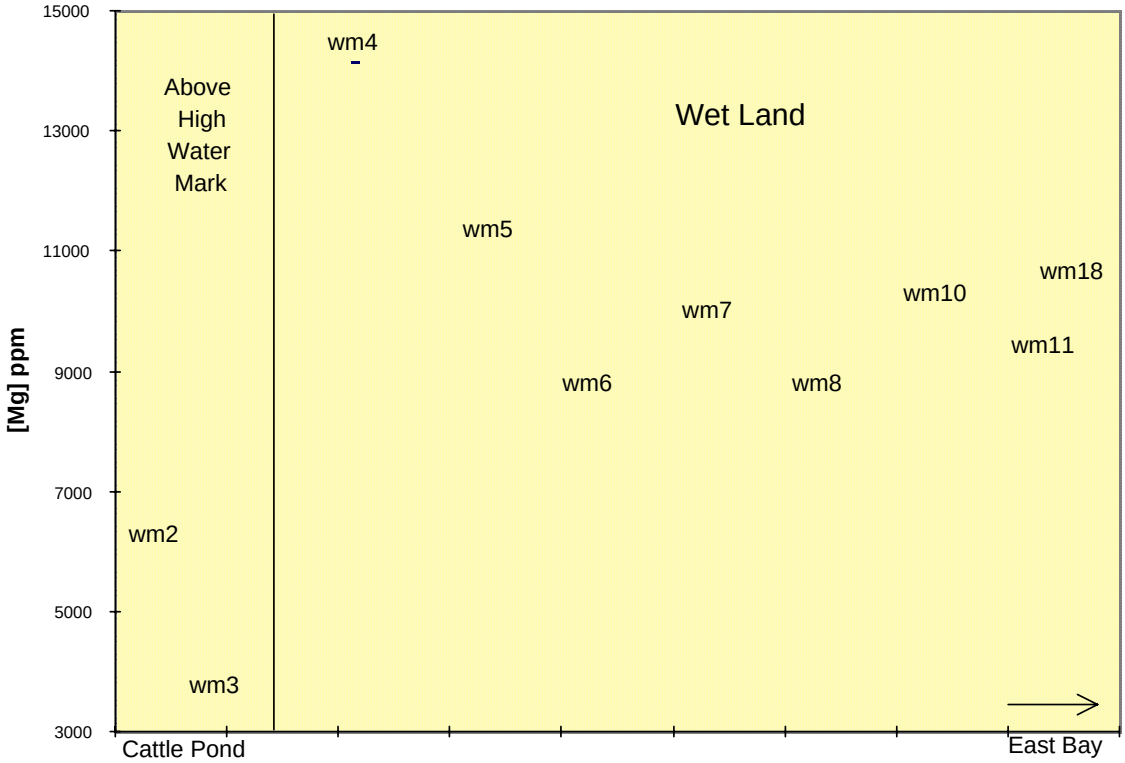


Figure 17b. Concentration of Mg in sediment from Ward Marsh along transect A-A' in mg/kg.

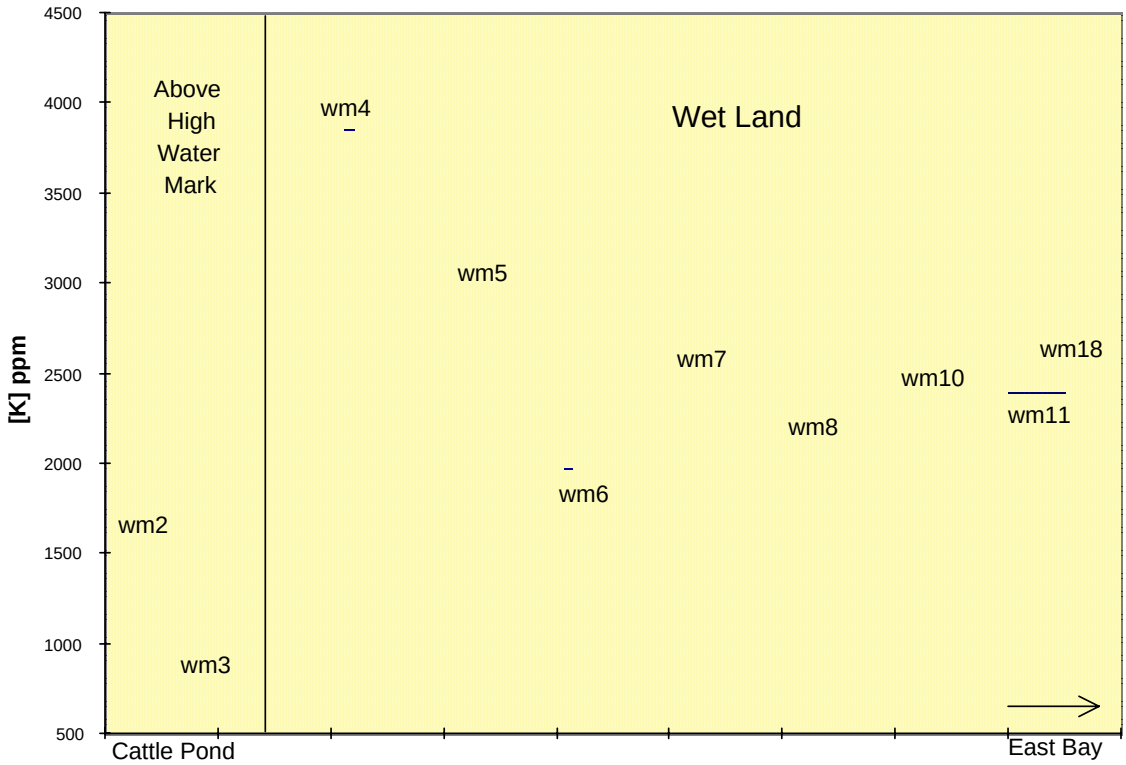


Figure 17c. Concentration of K in sediment from Ward Marsh along transect A-A' in mg/kg.

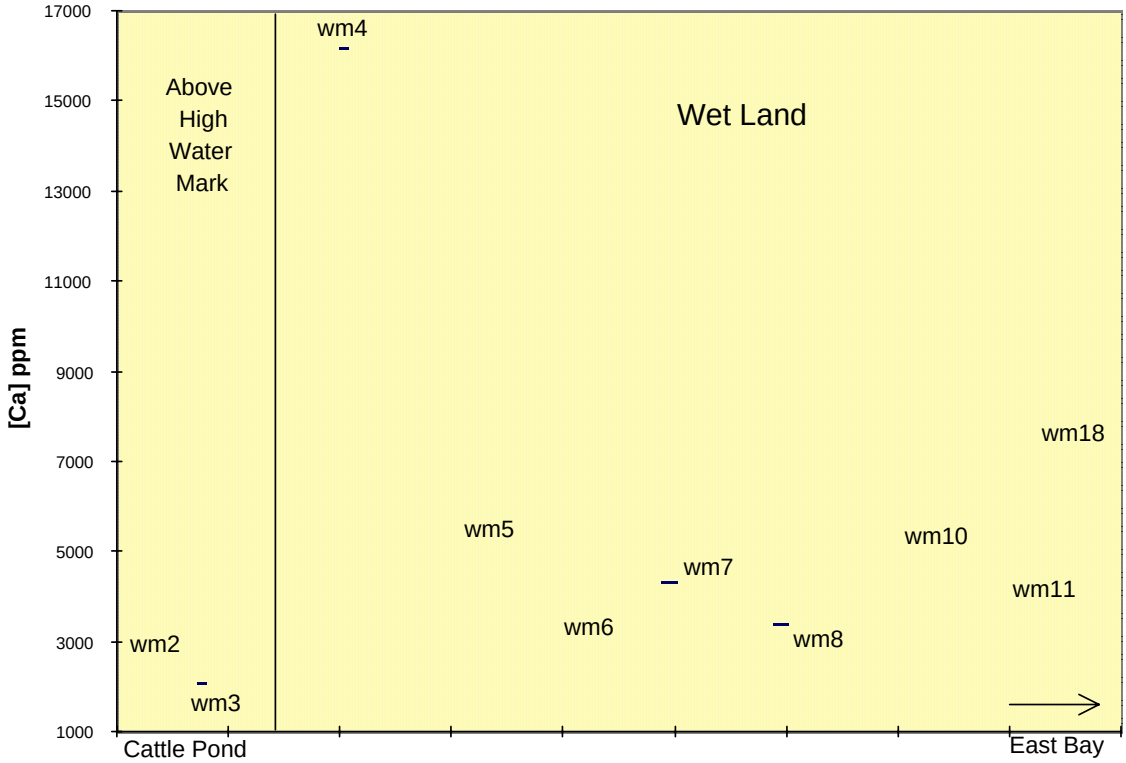


Figure 17d. Concentration of Ca in sediment from Ward Marsh along transect A-A' in mg/kg.

Al and Fe concentrations in the main marsh are higher than the cattle pond with high concentrations at WM-4 and WM-5. The concentrations of elements along transect A-A' are relatively similar to the other elements that were analyzed.

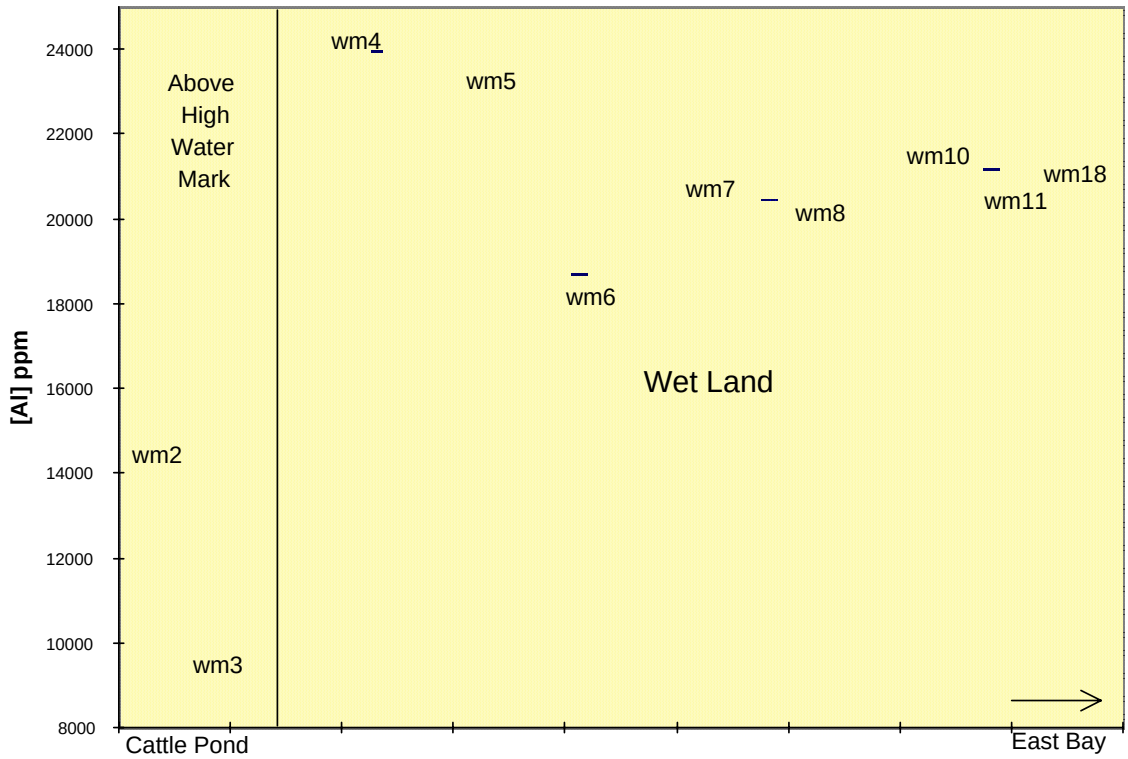


Figure 17e. Concentration of Al in sediment from Ward Marsh along transect A-A' in mg/kg.

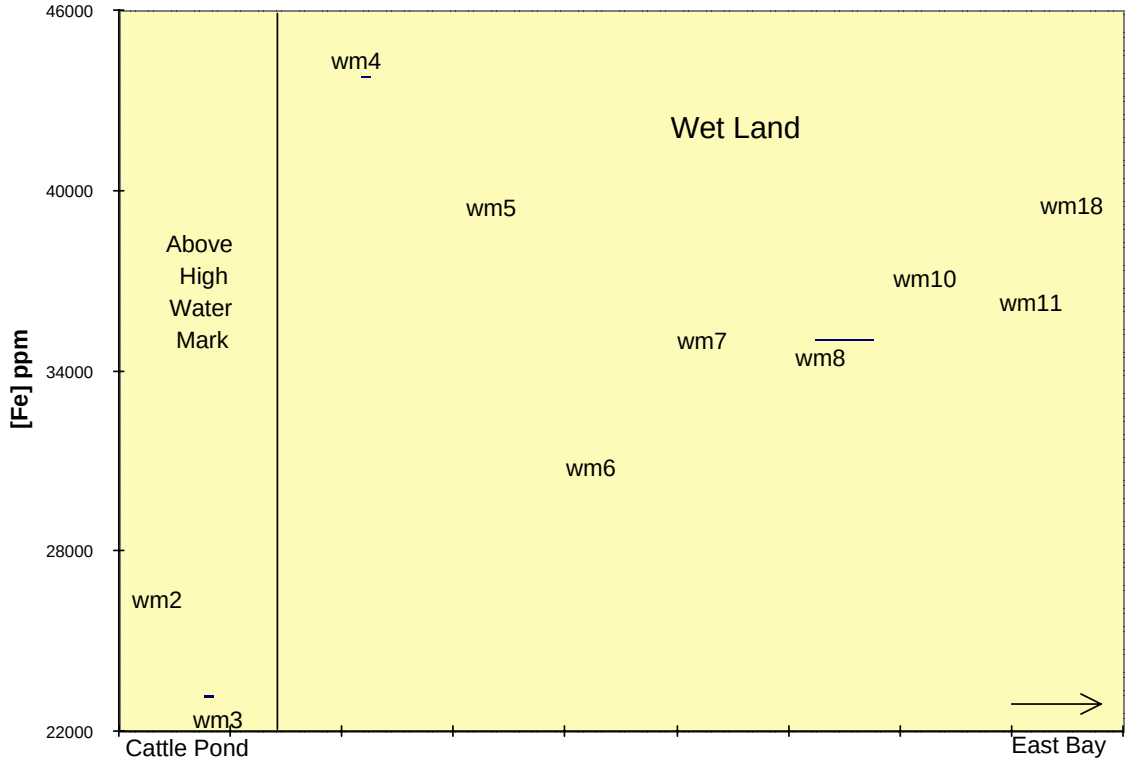


Figure 17f. Concentration of Fe in sediment from Ward Marsh along transect A-A' in mg/kg.

Concentrations of Mn differ from the other metals along transect A-A'. Concentrations in sediment in the main section of the marsh have lower concentrations than the cattle pond. Levels of Mn also peak at sample site WM-4 and WM-5 with concentrations approximately two times greater that the rest of the marsh.

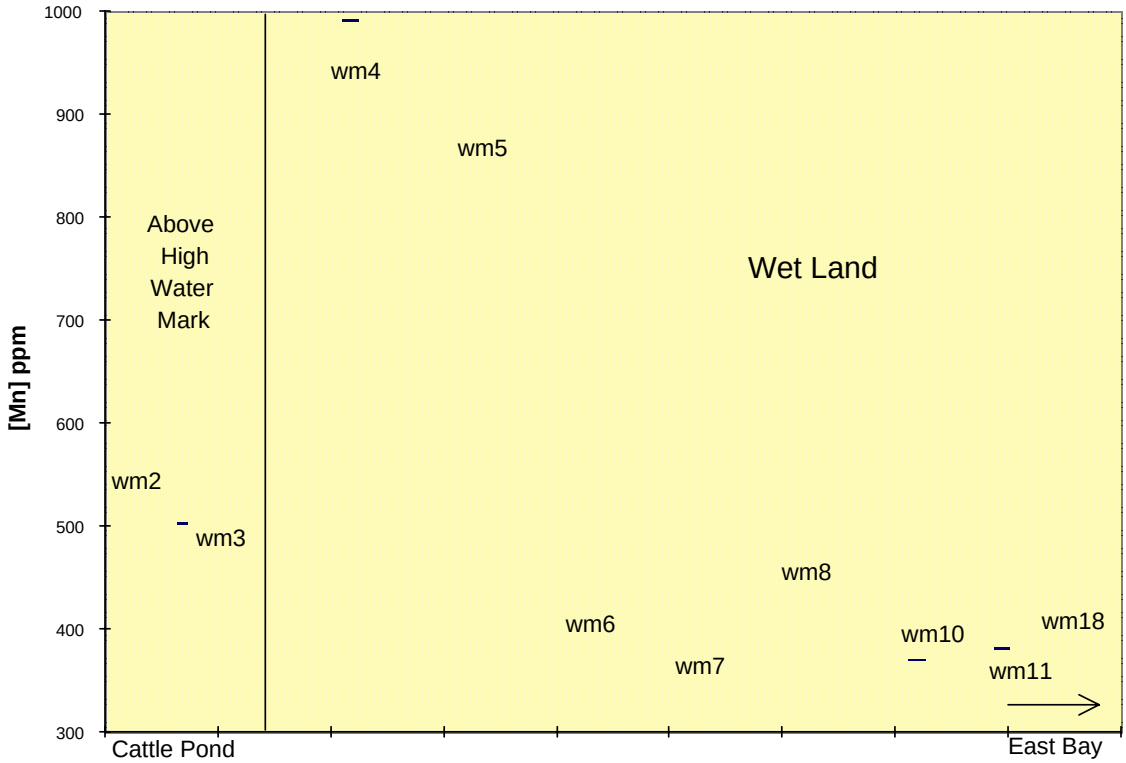


Figure 17g. Concentration of Mn in sediment from Ward Marsh along transect A-A' in mg/kg.

The concentration of P in the sediment is more variable in the main wetland than the other elements, however the concentrations are still higher than the cattle pond.

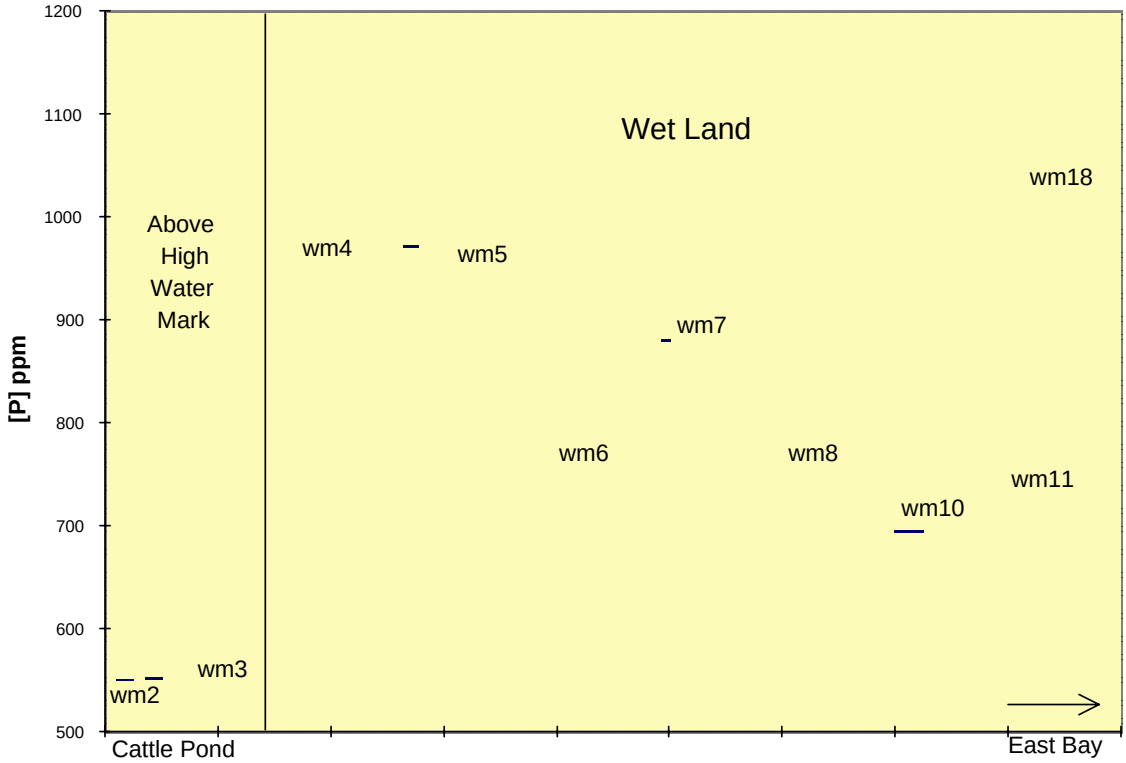


Figure 17h. Concentration of P in sediment from Ward Marsh along transect A-A' in mg/kg.

Ward Marsh Element Correlation

In order to determine the potential source of the teratogenic metals and other elements it is necessary to plot the concentrations of elements at each sample site against each other. In other words, a regression analysis of concentrations of two elements plotted against each other can show how well the distributions of metals across the marsh correlate with each other. Under this assumption, particular elements that correlate well may have been introduced into the sediment contemporaneously, or by similar sources.

The concentration of Zn and Cu in the sediment show a strong correlation. The closer the R^2 value is to 1.0 the better the two elements correlate.

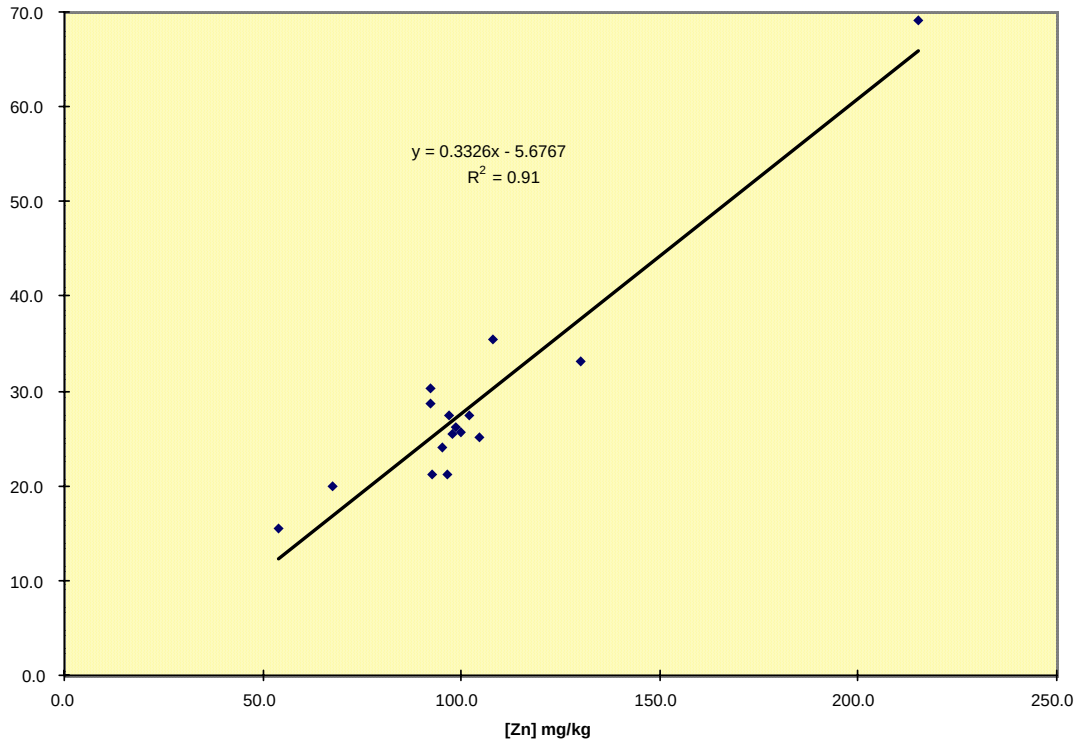


Figure 18. Concentration of Cu and Zn in sediment from Ward Marsh.

The concentrations of Cu and Zn correlate well with each other, however, the distribution of the other metals do not correlate well with Cu or Zn.

Zn correlates moderately well with Fe (R^2=0.60), and poorly with Ni (R^2=0.18), Co (R^2=0.21), Cr (R^2=0.03), Mn (R^2=0.00), Al (R^2=0.16), Mg (R^2=0.22), or P (R^2=0.43).

Similarly, the distribution of Cu throughout Ward Marsh sediment only correlates well with Zn and not with Fe (R^2=0.21), Ni (R^2=0.16), Co (R^2=0.24), Cr (R^2=0.03), Mn (R^2=0.00), Al (R^2=0.08), Mg (R^2=0.17), and P (R^2=0.40).

The concentrations of Fe and Ni in the sediment from Ward Marsh correlate well, as seen in Figure 19.

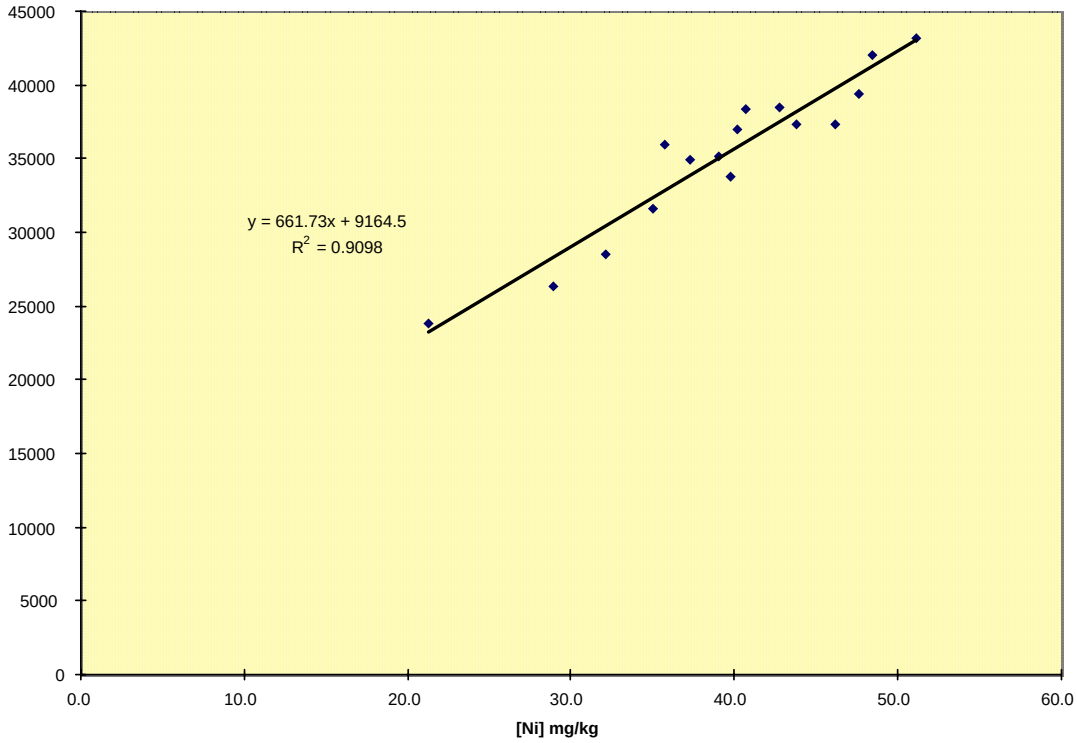


Figure 19. Concentration of Fe and Ni in sediment from Ward Marsh.

Unlike Cu and Zn, the distribution of Fe and Ni correlate well with other elements.

- Al correlates well with Fe (R^2=0.88) and Ni (R^2=0.85).
- Mg also correlates moderately well with Fe (R^2=0.81) and Ni (R^2=0.82).

Furthermore Mg correlates relatively well with Al (R^2=0.76).

- The distribution of Co correlates well with Fe (R^2=0.77), and Ni (R^2=0.80), moderately well with Mg (R^2=0.64), but not with Al (R^2=0.58).
- Cr concentrations correlated moderately well with Ni (R^2=0.73), Fe (R^2=0.62), and Al (R^2=0.60), and poorly and Mg (R^2=0.36).

Regression analysis was performed on other elements and demonstrated no significant correlations. However, Na correlated moderately with K (R^2=0.71).

In summary, regression analysis shows that the distribution of Zn and Cu are related to each other, but they are not related to the distribution of the other teratogenic metals, Ni, Co, and Cr. Furthermore, Ni distribution appears to be related to concentrations of Co and Cr, but the distributions of Co and Cr are not related to each other.

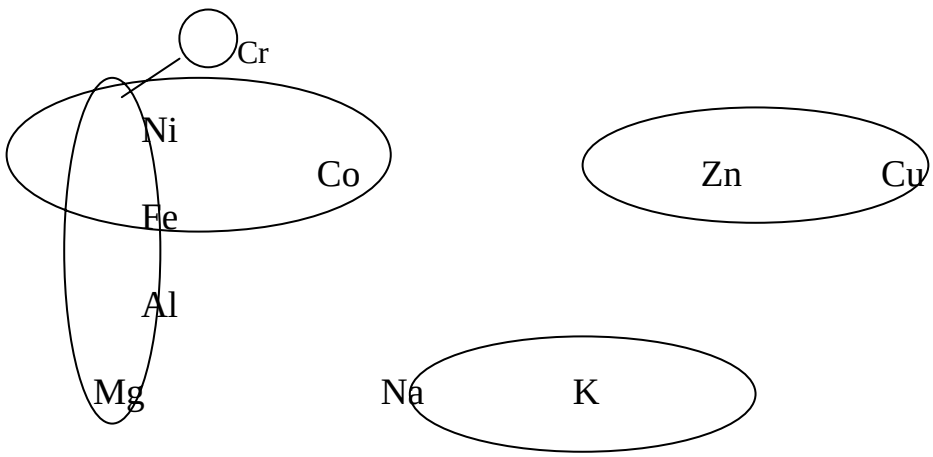


Figure 20. Schematic diagram of distribution relationships of elements in sediment from Ward Marsh.

Mud Creek Element Correlation

The distribution of metals in Mud Creek differs from that of Ward Marsh. Unlike Ward Marsh the distributions of Zn and Cu were dissimilar.

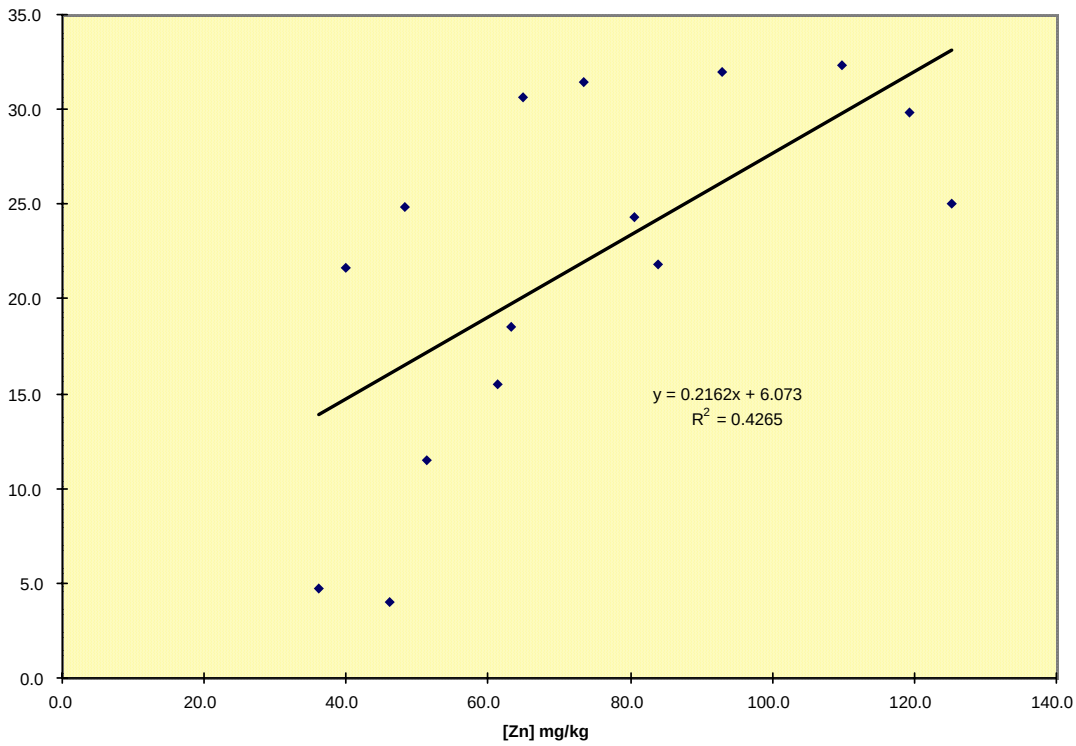


Figure 21. Concentration of Cu and Zn from sediment in Mud Creek.

- The distribution of Zn did not correlate well with Ni (R^2=0.43), Co (R^2=0.30), or Cr (R^2=0.28).
- Cu distribution correlated even more poorly with Ni (R^2=0.31), Co (R^2=0.19), and Cr (R^2=0.16).

Of the five teratogenic metals, only the distribution of Co, Cr and Ni were related to each other. The following figures show the plots of Cr vs. Co, Co vs. Ni, and Cr vs. Ni.

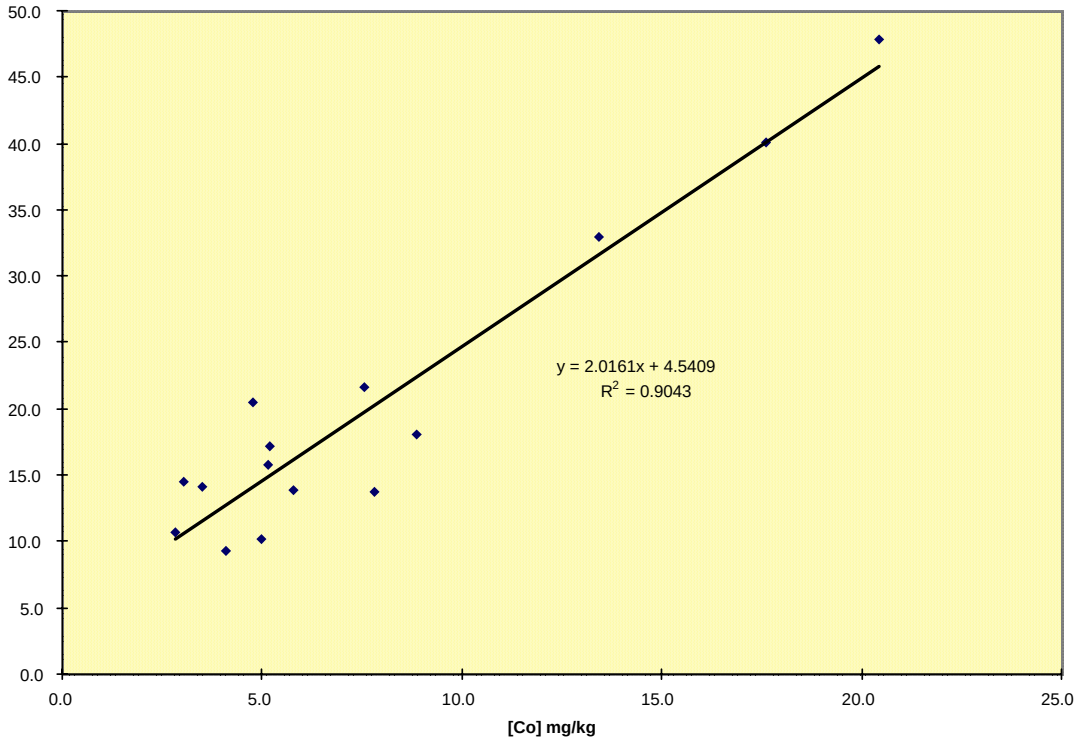


Figure 22. Concentration of Co and Cr from sediment in Mud Creek.

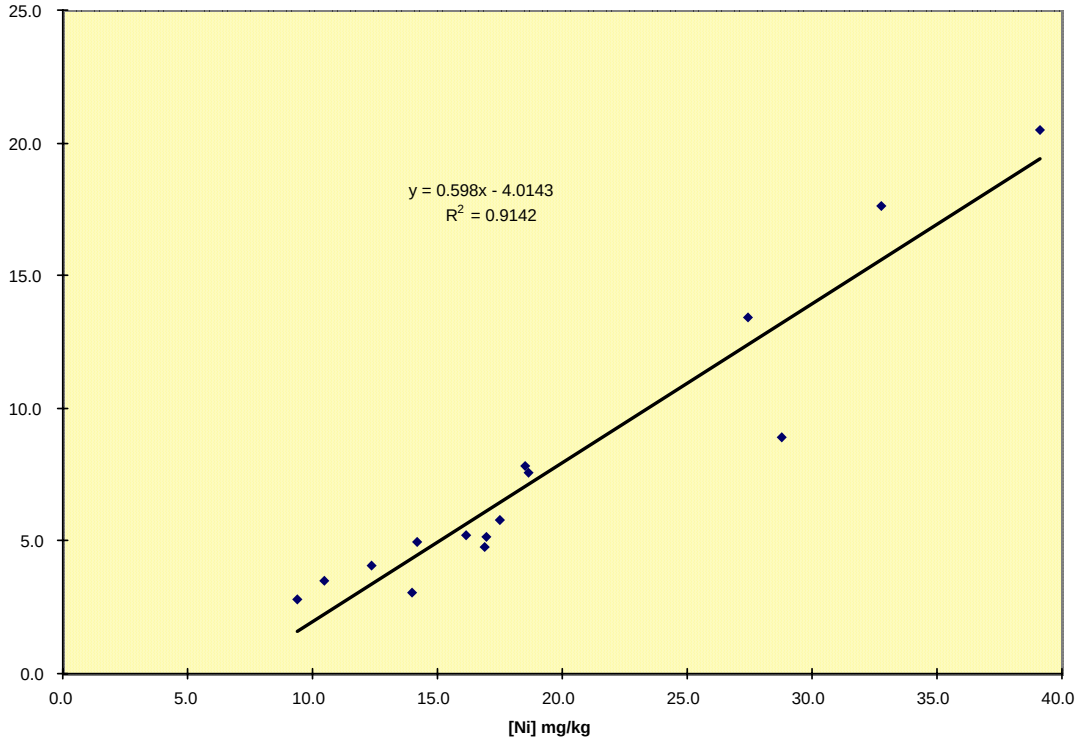


Figure 23. Concentration of Co and Ni from sediment in Mud Creek.

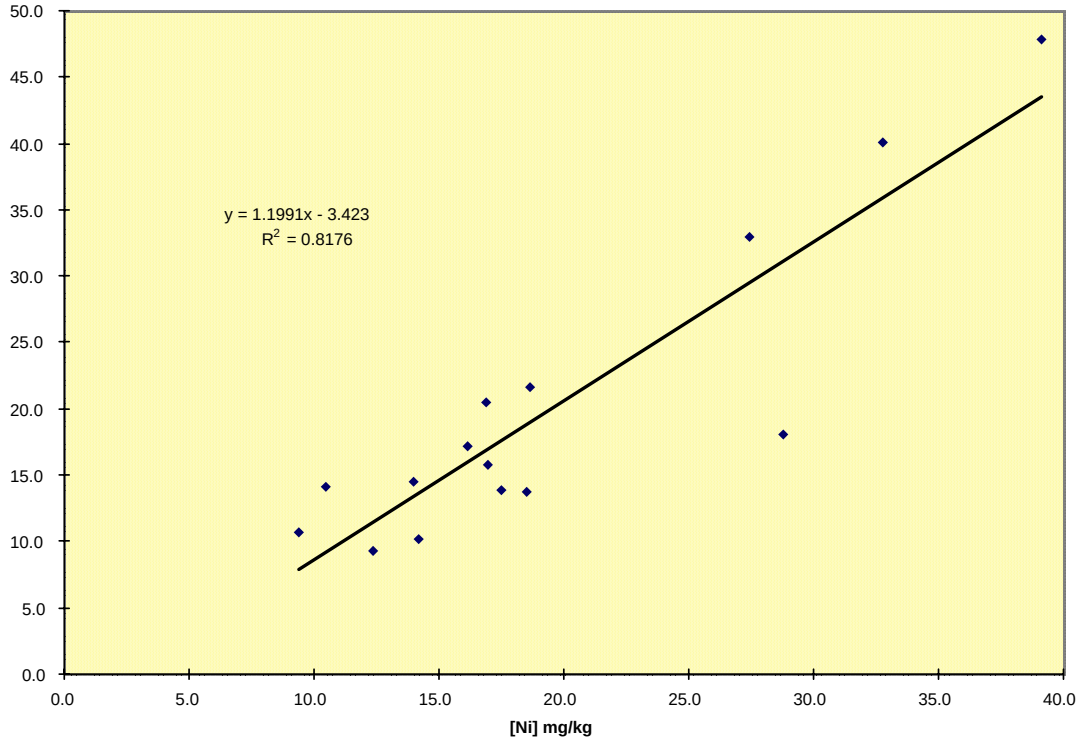


Figure 24. Concentration of Ni and Cr from sediment in Mud Creek.

The distribution of Co, Cr and Ni correlate well with each other in the sediment of Mud Creek.

As for the other elements that were analyzed, the distribution of Al and Mg correlated well.

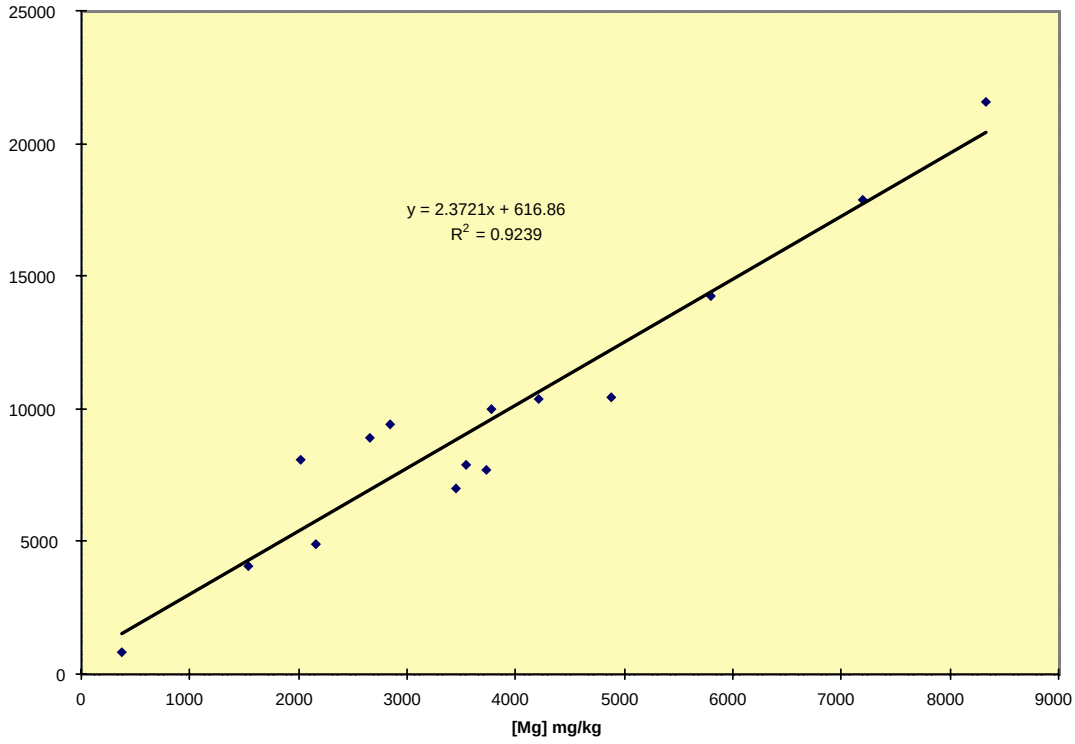


Figure 25. Concentration of Al and Mg from sediment in Mud Creek.

As seen above Al correlates well with Mg, Fe (R^2=0.81) and Cr (R^2=0.83), fairly well with Co (R^2=0.66), and Ni (R^2=0.62), but not with Zn (R^2=0.26), or Cu (R^2=0.08).

Similarly Mg correlates well with Fe (R^2=0.84), and Cr (R^2=0.85), but not with Zn (R^2=0.29), or Cu (R^2=0.16). However, unlike Al, Mg correlates moderately well with Ni (R^2=0.75) and Co (R^2=0.72).

The distribution of Fe correlates well with Mg and Al as seen above. Furthermore there is a good correlation with Cr (R^2=0.80) and Co (R^2=0.74) and a moderate correlation with Ni (R^2=0.69). Further analysis demonstrated no correlation between Na and K (R^2=0.08), Ca and Fe (R^2=0.21), and Ca and Mn (R^2=0.21).

In summary, this analysis shows that the distribution of Ni, Co, and Cr in the sediment from Mud Creek relate to each other but not with Cu and Zn. Furthermore, the distribution of

Cu and Zn are not related to each other, unlike at Ward Marsh. The following diagram shows the relationships of elements in the sediment of Mud Creek.

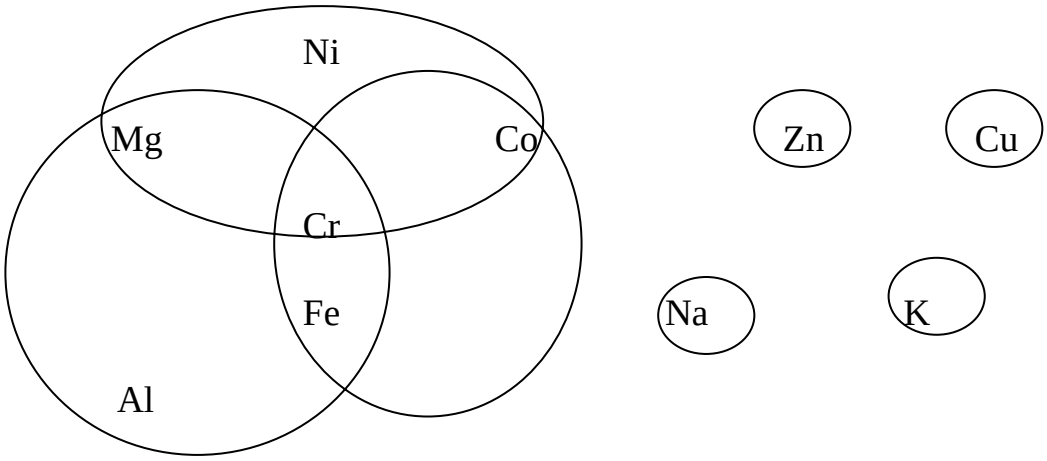


Figure 26. Schematic diagram of distribution relationships of elements in sediment from Ward Marsh.

Mercury Analysis

The average concentrations of Mercury in sediment from Ward Marsh and Mud Creek are shown in Table 11. They were obtained using an Atomic Absorption Spectrometer (AAS). A statistical test demonstrates no significant difference between the two sites.

Table 12. Average concentrations of Mercury in Sediment from Ward Marsh and Mud Creek, with statistical test showing no statistical difference ($p \geq 0.05$)

Ward Marsh		Mud Creek	
Mean	**84.21**	*Mean*	**107.75**
Median	**81.51**	*Median*	**101.40**
stdev	**20.33**	*stdev*	**57.53**

p= 0.13

pH Analysis

Table 13 shows the pH of sediment of Ward Marsh and Mud Creek.

Table 13. pH of sediment.

Sample	***pH***
Ward Marsh	
WM-2	6.11
WM-15	5.74
WM-18	6.64
Mud Creek	
MC-1	5.72
MC-5	6.29
MC-5	5.93

Error Analysis

Table 14. Error analysis using Standard Reference Material 2711 from the National Institute of Science and Technology.

	Zn	Cu	Ni	Co	Cr
measured	297	104	18	9	20
standard dev.	*2*	*0.3*	*0.2*	*0.2*	*0.2*
actual	290-340	91-110	14-20	7-12	15-25

Error analysis shows the accuracy of the ICAP and that there was no contamination due to laboratory procedures.

V. Discussion

Teratogenic Metals

The results from this study, along with field observations and FETAX laboratory tests, indicate that heavy metals are potentially responsible for the malformities seen in Northern Leopard Frogs (*Rana pipiens*) in the Ward Marsh. Concentrations of the teratogenic metals Zn, Ni, Co, and Cr are statistically higher in the sediment of Ward Marsh than in Mud Creek, which correlates with the field and laboratory observations. It does not appear that copper is influencing the system.

However, caution must be taken in comparing the concentrations determined in the sediment (Table 8a-b) to those concentrations found in laboratory studies (Table 2) due to the complicating factor of the bioavailability of the metals.

It is possible that the egg masses and tadpoles are being exposed to metals at the sediment water interface or the mother frog is accumulating metals in her tissue from over-wintering deep in the sediment and passing on the developmental abnormalities genetically. The metals may be entering the frog's system by permeating through the skin or through food inputs.

Considering the thermodynamics of an anoxic marshland environment, it is possible that the metals mostly exist in insoluble sulfidic phases, rendering the teratogenic metals unavailable to the developing frogs. However, this does not mean that all the metals exist in this form all the time. Metal sulfides can disassociate in oxic conditions (Gambrell, 1993), for example, due to the seasonal variability of water level. Furthermore, hydrous oxides on the edges of the wetland could dissolve during high water levels releasing metals into the system (Gambrell, 1993). Another explanation is that the sulfidic soil is becoming oxidized during dry conditions, which will result in the release of metals. A drop in pH from snow melt known as "acid surge" could be responsible for the remobilization of metals in the system. Considering these factors that

influence remobilization, it is possible that there is significant bioavailability of metals at certain times and variation in the oxic and anoxic conditions due to water fluctuation may explain the variability with the percent malformed seen in the field.

Even though the bioavailability of metals to the frogs is uncertain, it is certain that there are more teratogenic metals in the sediment from Ward Marsh than in Mud Creek, and it is entirely possible that these metals are available to the frog populations. Furthermore, concentrations of Cu, Ni, Cr and Fe in sediment from Ward Marsh significantly exceeds (with in standard deviation) sediment quality criteria established by research done in Collingwood Harbour, Lake Heron, Ontario, whereas Mud Creek only exceeds the quality criteria for Cu and Ni (Table 7).

I must not, however, ignore the possibility of other factors leading to malformities. Recently, researchers have discovered a correlation between frog malformities and tremotodes. This possibility seems unlikely in Vermont because of the difference in malformities observed in the field. The studies in Vermont have only found amelia (missing limbs), and not extra limbs as they have in other research. Stan Sessions, who worked on the most recently published project on frog parasites, examined the Vermont frogs in 1996 and found no evidence of parasite burden (Levey, 1999). Furthermore, the National Wildlife Health Center found no correlation between frog malformities and parasites (Levey, 1999).

It is possible that there is a synergistic effect between metals and UV radiation, pesticides, or biological agents, which have been identified by the Vermont Agency of Natural Resources as potential teratogens (Levey, 1999), and that a combination of teratogens are disrupting the development of the frogs.

Other elements

Previous research has shown that Mg^{2+} can reduce that toxic effects of certain heavy metals (Luo et al., 1993b). This does not seem to have an effect in Ward Marsh. The concentration of Mg^{2+} is higher in Ward Marsh than in Mud Creek, but it does not appear to be tempering the effects of the teratogenic metals. The statistically higher levels of Ca in the sediment from Mud Creek may be due to the local calcareous bedrock. This increase in Ca may behave like Mg in reducing the affects of metals in frogs.

Transects

Transect A-A' in Ward Marsh provides a good analysis of potential sources of metals found in the Marsh. The cattle pond is isolated from the main body of the wetland and is not likely to be influenced by suspended load and sediment deposition from flooding in East Bay or Poultney River.

In all cases the concentrations of the teratogenic metals were higher in the wetland than in the isolated cattle pond. This suggests that the source of the metals is East Bay. The concentrations also increase closer to the bay. This general trend can also be seen with the other non teratogenic metals, except for Mn.

There is a striking similarity with all the profiles. There is a peak at WM-4 and WM-5 with all of the metals that were analyzed. This peculiar feature is confounding and needs further research. Since these two sites were in oxidized soil above the water level, perhaps oxides formed and co-precipitated with metals. The oxides, having a strong affinity for metals, concentrate the metals, giving the spikes seen on the profile. This may also explain the variability of malformities seen in the field between 1997 and 1998. If the water is high and

floods over the oxides, in the reducing environment the oxides would dissolve, releasing the metals and making them available for uptake by developing tadpoles. However, this idea is not based on field data analyzing for the presence of oxide in the soil so future work will be needed to better determine this. Furthermore, lake level data does not corroborate the theory. At Rouses Point, NY the level of Lake Champlain was measured to be 99.25 ft on May 6, 1997 and on April 7, 1998, crested just below the record of 101.89 feet (USGS, 1999). Perhaps the sulfidic soil is becoming oxidized during periods of lower water, which results in the release of metals into the system.

Perhaps the difference in metal concentrations can be explained by the sediment type in Mud Creek compared to Ward Marsh. The metals are not remaining in the sediment in Mud Creek because it tends to be coarser grained. The sediment would not be able to adsorb metals as well as the clays in Ward Marsh.

Sources

The transects suggest that a possible source of these contaminants is East Bay, which marks the confluence of Lake Champlain and the Poultney River. Adult frogs may be exposed to contaminated sediment in the bay and accumulate metal in their tissue. Another possible explanation is that the tadpoles are exposed to metals in the sediment or the water column in Ward Marsh.

Anthropogenic sources include industry and waste disposal in the Poultney River drainage basin, and natural sources include bedrock, especially slate, which is enriched in heavy metals compared to sandstone, granite, and carbonate rocks (Faure, 1998).

More locally, some metals may be coming from metal waste that is present around the margins of the marsh. Large pieces of scrap metal are present along the road that forms the edge

of wetland. At one site in particular near WM-18, where East Bay flows into the marsh through a culvert, large sheets of rusting metal lie immersed in the channel. This may provide increased concentrations of Fe and perhaps Zn if the sheets were galvanized. Although, it is unclear how scrap metal in this location influences the metal concentrations in the remainder of the marsh, the presence of such wastes indicates the potential for anthropogenic sources. More research should be done to determine the type of metals being released from the wastes.

To determine potential sources of the metals, concentrations of the elements were plotted against each other to show how well the distribution of metals across the marsh correlate with each other. Under this assumption, particular elements that correlate well may have been introduce contemporaneously or by similar source(s).

Data suggest that Zn and Cu in the sediment of Ward Marsh were deposited at the same time or came from the same source. The same can be said about Na and K. The correlation of Ni, Fe, Al, and Mg in Ward Marsh sediment implies a similar source, which could be chlorite, an aluminosilicate clay mineral (Bailey, 1988). This suggests that Zn and Cu may be from anthropogenic sources, while Ni, Fe, Al, and Mg may have originated from natural sources.

Brass is an alloy that consists of Zn and Cu. It is possible that the correlation of Cu and Zn in Ward Marsh is due to the weathering of brass, that may be present in trash that is littering the perimeter of the wetland. It is also known that there are ship wrecks in East Bay that may be contributing to concentrations of Zn and Cu or other metals (Manley,1999).

Data suggest that Zn and Cu in sediment from Mud Creek may be from anthropogenic sources, while Ni, Fe, Al, and Mg, may have originated from natural sources. Zn and Cu have no correlation with the other metals, but also no correlation with each other. Due to the poor correlation the brass theory does not work for Mud Creek.

It is difficult to ascertain sources using this type of analysis; it can only offer possibilities. There needs to be further research, such as chemical partitioning studies, that pertains to metal availability. This method determines what metals are in crystal structures (biologically unavailable), Fe/Mn oxide coatings (available if environment becomes reducing or an increase in biological activity occurs), metals associated with organics (available if ingested by organism), and adsorbed metals (readily available but in small percentages).

VI. Conclusion

The issue of deformed frogs being observed throughout North America and Europe is an extensive problem that has no simple answer. It is difficult to tease apart the exact cause of the abnormalities, but every piece of research directed toward this problem helps us gain a better picture of possible sources. This study suggests that metals may be leading to the malformities observed in Vermont. Perhaps other agents are causing other types of irregularities seen elsewhere. Fortunately, much research is addressing the problem across the country and with time we will have a better idea about what is causing the malformities. I hope that this study serves as a launching point for future research with heavy metals and malformed frogs.

VII. Future Work

It would be interesting to examine frogs and tadpoles from Ward Marsh and Mud Creek in particular, to see if there is accumulation of metals in the tissue. Also, it would be beneficial to analyze the sediment from other sites across the Champlain Valley where there have been malformed frogs.

Chemical partitioning studies would also be important in determining the availability of metals in the sediment. It would be helpful to ascertain in which phases the metals exist in the marsh sediment.

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Appendix A:

Concentrations of Zn, Cu, Ni, Co, and Cr in Ward Marsh and Mud Creek (mg/kg).

	ICP 3/9/99									
	Zn	***stdev***	**Cu**		**Ni**		**Co**		**Cr**	
wm2	67.57	*0.37*	19.92	*0.11*	28.90	*0.29*	16.73	*0.16*	30.49	*0.24*
wm2a	66.37	*0.34*	30.37	*0.14*	37.96	*0.13*	16.50	*0.04*	32.05	*0.54*
wm3	53.81	*0.14*	15.45	*0.07*	21.24	*0.17*	13.00	*0.22*	22.86	*0.09*
wm4	107.80	*0.20*	35.41	*0.14*	51.14	*0.11*	23.98	*0.05*	50.33	*0.18*
wm5	129.90	*0.40*	33.16	*0.33*	47.57	*0.18*	21.55	*0.16*	48.07	*0.27*
wm6	96.67	*0.56*	21.11	*0.18*	35.04	*0.22*	15.71	*0.13*	38.61	*0.09*
wm7	104.40	*0.50*	25.11	*0.20*	39.76	*0.20*	17.27	*0.12*	42.37	*0.15*
wm8	92.69	*0.29*	21.13	*0.11*	37.26	*0.25*	17.11	*0.10*	38.21	*0.14*
wm10	97.63	*0.42*	25.47	*0.00*	39.08	*0.25*	16.26	*0.14*	39.82	*0.34*
wm11	98.77	*0.83*	26.18	*0.20*	40.13	*0.44*	16.24	*0.15*	39.33	*0.11*
wm12	92.11	*0.09*	30.27	*0.08*	46.16	*0.17*	21.25	*0.19*	62.93	*0.26*
wm13	96.93	*0.50*	27.50	*0.00*	48.42	*0.24*	21.72	*0.24*	67.95	*0.40*
wm14	99.92	*0.83*	25.57	*0.26*	43.76	*0.31*	19.21	*0.16*	57.68	*0.35*
wm15	102.10	*0.40*	27.50	*0.00*	42.73	*0.15*	19.91	*0.21*	43.90	*0.22*
wm16	95.42	*0.32*	24.06	*0.14*	35.70	*0.18*	18.44	*0.24*	40.99	*0.25*
wm17	92.23	*0.36*	28.73	*0.14*	32.15	*0.11*	14.34	*0.08*	31.12	*0.12*
wm18	215.20	*0.30*	69.13	*0.24*	40.73	*0.13*	20.40	*0.10*	40.06	*0.05*
wm18a	202.80	*0.60*	65.68	*0.27*	36.53	*0.19*	18.52	*0.00*	35.44	*0.23*
	Zn	***stdev***	**Cu**		**Ni**		**Co**		**Cr**	
mc1	73.37	*0.10*	31.47	*0.29*	27.41	*0.26*	13.44	*0.18*	32.89	*0.41*
mc2	125.20	*0.60*	25.01	*0.18*	18.63	*0.03*	7.54	*0.17*	21.61	*0.21*
mc3	119.30	*0.60*	29.86	*0.70*	39.11	*0.12*	20.46	*0.21*	47.80	*0.33*
mc4	80.57	*0.23*	24.34	*0.08*	32.78	*0.18*	17.63	*0.08*	40.11	*0.19*
mc5	51.27	*0.26*	11.52	*0.07*	16.18	*0.10*	5.21	*0.10*	17.13	*0.19*
mc6	83.96	*0.30*	21.78	*0.14*	16.96	*0.11*	5.17	*0.04*	15.78	*0.09*
mc7	48.30	*0.27*	24.83	*0.11*	16.90	*0.18*	4.79	*0.08*	20.51	*0.20*
mc8	59.83	*0.32*	26.34	*0.18*	14.57	*0.16*	3.35	*0.07*	13.27	*0.21*
mc9	63.20	*0.18*	18.51	*0.00*	17.51	*0.16*	5.79	*0.04*	13.91	*0.18*
mc10	92.84	*1.00*	32.00	*0.36*	14.00	*0.26*	3.06	*0.11*	14.52	*0.22*
mc11	829.60	*5.10*	30.28	*0.45*	10.41	*0.40*	3.10	*0.07*	10.05	*0.20*
mc12	460.90	*2.10*	28.44	*0.11*	21.51	*0.08*	7.00	*0.14*	20.13	*0.21*
mc13	61.38	*0.17*	15.51	*0.08*	18.53	*0.19*	7.80	*0.06*	13.73	*0.11*
mc14	64.90	*0.42*	30.65	*0.13*	14.20	*0.09*	4.98	*0.08*	10.17	*0.03*
mc15	40.02	*0.21*	21.61	*0.00*	12.38	*0.20*	4.10	*0.10*	9.29	*0.25*
mc16	46.09	*0.21*	3.99	*0.08*	10.44	*0.13*	3.50	*0.00*	14.10	*0.17*
mc17	36.03	*0.24*	4.69	*0.13*	9.42	*0.12*	2.81	*0.10*	10.66	*0.05*
mc18	109.80	*0.30*	32.36	*0.07*	28.80	*0.09*	8.88	*0.10*	18.08	*0.09*

Appendix B:

Concentrations of other elements in Ward Marsh and Mud Creek (mg/kg).

2/25/99	Na	Mg	Al	P	K	Ca	Mn	Fe
wm2	123	6298	14430	548	1664	2953	525	26370
wm3	93.9	4465	10430	589	1108	2531	526	23760
wm4	334	13900	23380	922	3780	16160	980	43150
wm5	239	11370	23230	965	3054	5485	867	39430
wm6	192	9267	18770	797	1980	3930	405	31640
wm7	211	9766	20350	881	2498	4318	384	33830
wm8	191	9079	20520	785	2285	3398	434	34950
wm10	179	9927	20930	694	2348	4826	372	35200
wm11	203	9643	21150	758	2391	4430	382	37020
wm12	185	9531	20250	930	2541	8444	567	37330
wm13	162	9673	24120	997	2431	5192	825	42050
wm14	168	9572	23530	930	2546	4538	674	37310
wm15	172	10180	23440	816	2775	4754	397	38510
wm16	203	8964	20340	708	1924	2863	388	35970
wm17	176	6992	16050	888	1672	4870	350	28490
wm18	183	9906	20000	1024	2384	6612	365	38360
2/25/99	Na	Mg	Al	P	K	Ca	Mn	Fe
mc1	133.9	5805	14270	796.5	1830	4700	158.5	27850
mc2	292.1	3777	9992	927.1	1242	16860	185.6	18600
mc3	255.2	8336	21560	881.6	3542	5488	351.2	41740
mc4	194.1	7190	17900	779.5	2979	4423	271.7	33220
mc5	63.56	4210	10360	343.9	632.5	3312	104.3	14700
mc6	153.9	3545	7911	641.3	667.6	14930	249	17220
mc7	343.7	3729	7676	930.6	853.6	14400	181.3	14530
mc8	3193	2688	6840	1061	526.9	26070	318.7	11300
mc9	84.72	3444	7021	650.5	556.3	5525	187.9	18790
MC8 *	3205	2799	7191	1130	549.2	27680	343.3	11700
mc10	391.7	2655	8929	972.2	401.6	19750	462.9	14360
mc11	175.2	2097	4383	1447	992	16160	235.1	12870
mc12	181.1	3675	10350	736.4	759.2	9752	602	35160
mc13	73.01	373.6	853.1	123.8	270.3	848.1	53.66	2749
mc14	146.9	1538	4044	290.6	362.5	9142	633.6	19400
mc15	74.43	2165	4889	455.6	451.5	3751	94.92	13060
mc16	89.11	2851	9427	654.1	583.9	3644	138.7	12950
mc17	102.1	2028	8095	565.5	389.7	4854	121	11180
mc18	181.1	4887	10430	747.4	883.5	9582	423.2	18410