ENVI 102
Independent
Project
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Bioaccumulation of Chromium in a Food Chain of Bridges Pond

Introduction

We have all seen the warning signs around Bridges Pond which say that the fish in the pond have levels of chromium which surpass safe levels for food, and should not be eaten.

I want to see if this claim is true and if the chromium is bioaccumulating up the food chain. If it is, then it represents a greater threat to people eating the fish, because it becomes very concentrated in the tissues of organisms high on the food chain such as fish, especially game fish, which are predatory.

Bioaccumulation

Bioaccumulation of substances in the tissues of living organisms can occur when the substance is available to the biota for assimilation into organisms and is not biodegradable or able to be removed from the body ~~for~~ by excretion.

A substance in the sediment which is available to the biota may be taken up by organisms at the bottom of the food chain, the producers. In the pond these would be aquatic plants such as *Elodea* and filamentous green algae. These plants have a certain average concentration of chromium in their tissues, say X. This concentration tends to be low - the plants do not take up chromium by active transport because it is not an essential element. The organisms which eat the plants, such as insects, have higher chromium levels in their tissues because they eat many plants during its life time and all the chromium stays in their tissues. The other parts of the plant are digested and pass through the beetles. The chromium, unable to be removed by the body, remains. After each meal, there is a bit more chromium in the organism. When small fish eat the insects, the higher levels of chromium enter their bodies and remain, slowly building up. This is repeated at each step in the food chain. At the top of the food chain the organisms to be very long-lived (relatively speaking). The eat a vast number of meals over their lifetimes, so they get a huge number of small doses of chromium which accumulate. This can harm the organisms, because the concentrations in their tissues can easily reach toxic levels. Humans, at the top of the food chain, can find themselves in this position.

fix a sentence

Measurement of Chromium

The chromium levels in our samples were measured by atomic absorption spectrometry. A description of this method appears below under the heading 'Analysis'.

Procedure

Lab methods used frequently

The following procedures will not be explained in the method, but it is implicit that they are done as below:

Cleaning glassware - rinse thoroughly with dilute HCl then rinse 3 times with DDW.

Dilutions - pour solute into volumetric flask, spray down neck with DDW to make sure all solute is in bulb, top up to line with DDW using bottom of meniscus as boundary. Cap securely and invert repeatedly to dissolve solute and mix the solution so it has uniform concentration.

Precautions

Safety - all work with acid was done wearing gloves and goggles with all apparatus in the hood with the screen as low as conveniently possible.

Outline

I worked in conjunction with Rob Seidenwurm. I analysed vegetation from the pond, he analysed sediments. We had to:

- calibrate the Atomic absorption Spectrometer to chromium,
- collect samples of sediment and vegetation from various places around the pond and
- prepare the samples for chromium analysis in the Atomic absorption Spectrometer's (A.A. for short) graphite furnace.
- Analyse the samples with the A.A.

Details of Methods

Calibration of the A.A.

(see under 'Analysis' for description of A.A)

The A.A. had to have a frame of reference for its measurements: the absorption of radiation by chromium in the furnace is directly proportional to the amount of chromium present, but the A.A. has no way to know what amount chromium causes a given amount of

absorption. If we give it known standard solutions and tell it what their concentrations are, it can make a standard curve which shows exactly how much chromium causes a given amount of absorbance and it can use this curve to measure absolute chromium levels in the range of the curve.

Making up the standard - a stock solution of potassium dichromate ($K_2Cr_2O_7$)

Potassium dichromate is a good compound to use when making standard solutions because it is easy to work with and very soluble.

Knowing the chemical formula of potassium dichromate, we calculated (see calc.#1) how much of it we had to dissolve in how much water to make solutions in known concentrations. We made the concentrations between 10 ppb and 100 ppb, because the A.A. works well over this range.

CALCULATION # 1

To make Chromium Standard:

Weigh out 0.2829 g $K_2Cr_2O_7$

$$0.2829\,g\left(\frac{mole}{294.2g}\right)\left(\frac{2\,mole\,Cr}{1\,mole\,K_2Cr_2O_7}\right)\left(\frac{52.00g}{mole}\right) = 0.100\,g\,Cr$$

Dilute to 1 l

$$\left(\frac{0.100\,g}{l}\right)\left(\frac{1000\,mg}{g}\right) = \frac{100\,mg}{l} = 100\,ppm$$

Take 10 ml of this 100ppm solution and dilute to 1 L.

$$(10\,ml)(100\,ppm) = (1000\,ml)(1\,ppm)$$

Concentration = 1 ppm
= 1000 ppb.

From calculation #1:

I put a watchglass on the electronic balance and zeroed it. I weighed out about 0.6g of $K_2Cr_2O_7$ onto the watchglass and put it in the dessication oven @ 105° C for 1 hour. This drove off all water (The $K_2Cr_2O_7$ granules were red when hot and dry, orange when cold and moist.) so that the mass was all $K_2Cr_2O_7$ and not part $K_2Cr_2O_7$ and part water. (If the $K_2Cr_2O_7$ was damp, then I would not get as much per gram as the calculation showed and all my concentrations would be too low.) I took the watchglass to another lab which had a top-loading electronic balance which could read to 0.0001g and weighed out 0.2832g of $K_2Cr_2O_7$ onto a piece of weighing paper, then poured it into a 1l volumetric flask. I diluted the $K_2Cr_2O_7$ to 1l. This solution's concentration was 100 ppm. I transferred 10ml of this solution to another clean 1l volumetric flask and diluted it to 1l. This was a 1000 ppb solution. I labelled both solutions accordingly.

Making the standard curve

The 1000 ? or 100? ppb solution was put into the A.A. which diluted it to varying amounts so that it could analyse solutions of 50 ppb, 25 ppb, 20 ppb, 10 ppb and 5 ppb. The A.A. constructed a standard curve from these results so that it could measure chromium on an absolute scale.

Collecting the Samples

In mid-April, Rob and I went out onto Bridges Pond in a small inflatable boat with a pair of hand-held dredger jaws. We paddled around the pond and took samples of the bed material from the same sites (roughly) that we tested during the winter. (see map, 'Sampling Locations in Bridges Pond') We put the samples, sediment and vegetation, into zip-lock bags, labelled with the site number. The bags were brought back and put in the cold room (@ 5°C) overnight.

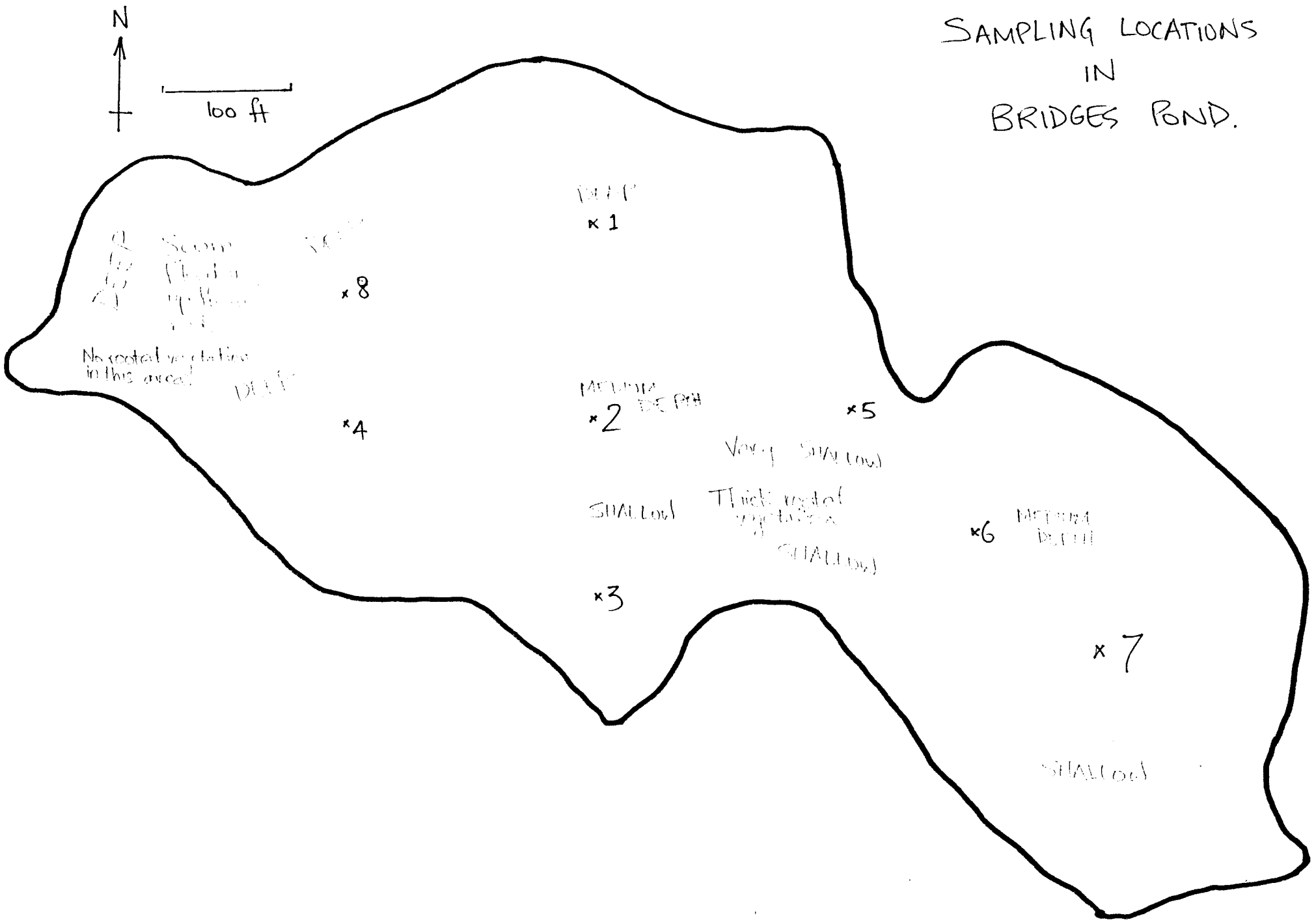
Preparing the samples for analysis

The preparation involves cleaning, drying, ashing, dissolving and diluting the sample to yield a solution which has the contents of the vegetation dissolved in it (the A.A. can only handle liquids), with chromium at a concentration the A.A. can handle.

Cleaning and Identifying the Samples

Sediment

The mud, debris and vegetation was dumped out of a bag into a brass (stainless steel has chromium in it) mesh seive. Any vegetation was taken out and put into a labelled beaker The beakers were labelled K[site number] e.g. K4 for site 4. (the 'K' was to distinguish my samples from Rob's which were labelled with an 'R'). The mud was worked through the mesh into the collecting pan below so that there would be no debris or large chunks in the sample. (I found that


N
100 ft
SAMPLING LOCATIONS
IN
BRIDGES POND.
×1
×8
No rooted vegetation in this area!
×4
×2
×5
Very shallow
Thick rooted vegetation
SHALLOW
×6
×3
×7
SHALLOW

it helped to force the mud through the mesh by placing a small watchglass face down on the mud and pressing it sharply into the mesh. Large watchglasses had too much volume in the concave side, so they allowed the mud to stay above the mesh.) When there was about half an inch depth of mud in the collecting pan, we spooned some onto a clean watchglass, trying to get as little water as possible (I found that tipping the pan to make all the water go to one side helped). When we had a watchglass of each sample, they were all put in the hood to air dry.

Vegetation

I washed the vegetation samples vigorously and repeatedly with distilled water (DW) to remove sediment and other debris. I identified the plants to genus. All but one could be identified with the unaided eye. I used a microscope to identify the algae sample.

To remove any dirt which did not come off with rinsing, I put Tween detergent in each beaker and swished it around. I put a watchglass over each beaker and after about eight hours I vigorously rinsed off the Tween with DW. I could not rinse off the Tween from the surface sample because it was all microscopic organisms. Thus, this sample was not used. I should have measured the exact volume of Tween I put in. Then I could have accounted for the mass of the Tween and run a Tween blank through the A.A. to see if there was any chromium contamination.

Drying the Samples

I left the watchglasses and beakers in the hood for a day to let the samples air dry (no watchglasses on beakers). We weighed each watchglass and beaker, then put them all in the dessicating oven (@ 105°C) to drive off all remaining moisture. After 8 hours we weighed each again to determine the percent water content of the samples after air drying. (isn't this pointless? shouldn't I have weighed them when they were still wet and alive ?) → This helps to standardize the procedure from lab to lab. Some people may have "wetter" wet + alive samples than others

Ashing the Samples

We had to dry ash our samples so that we would be able to cleanly dissolve them in the acid. Dry ashing combusts all organic material so that only the inorganic solids are left, including the metal being tested for. Getting rid of organics means that there is much less to dissolve and to interfere with the acid. The oven temperature must not exceed the melting point of the test metal or it will be evaporated. (from PERKIN-ELMER 'Analysis of Plant Tissue' Jan 1982)

I labelled the bottom of a crucible with a sample number, weighed it, then, if it was vegetation, stuffed as much of that sample into it as I could. I weighed the crucible again to get the mass of sample. Rob put pieces of dry sediment into labelled crucibles of known mass and weighed them.

When we had a crucible full of each sample, we put all the crucibles into an ashing oven @ about 600°C. After about 24 hours we removed them. The organic matter had been burned off and all that was left was ash. (Some of the vegetation samples still had normal shapes, but when dumped into flasks, I found that the pieces turned to dust.) We weighed each crucible-sample again and subtracted the mass of the each crucible to find the percent yield of ash from dry vegetation.

Dissolving the Samples

We were testing for 'Acid-extractable metals', defined as "the concentration of metals in solution after treatment of an unfiltered sample with hot dilute mineral acid." We made the assumption that this method dissolves all of the chromium.

I labelled and weighed a clean 100ml volumetric flask on the top-loading electronic balance. Sediment samples were still in chunks, so I ground them to dust in the crucibles with a silver metal spatula. This was I could pour small masses at a time.? I tapped about 0.3g of ash from that sample's crucible onto a piece of weighing paper on the top-loading electronic balance. I poured the ash into the flask and weighed it again to find the mass of ash that had been transferred from the weighing paper to the flask. (It is possible that some ash would spill or be blown away during pouring, so the mass

of ash on the weighing paper might not be equal to the mass of ash in the flask.)

I pipetted 5ml of DDW into the flask and swirled it to make the ash into a paste and then put 5ml of concentrated hydrochloric acid (c.HCl) into the flask with a 5ml volumetric pipette. The c.HCl was put in all at once because if it is put in slowly, a lot of it will tend to boil off when it touches the water. By putting it in rapidly, the solution is made acid quite quickly so very little boils off. A little more DDW is used to wash any ash on the neck of the flask into the bulb and a stirring flea is dropped in. To make sure the ash was completely dissolved, the flask was then swirled and heated for 1 hour on the magnetic stirring hot plate. (This was done in the hood so that any vapors from the c.HCl would be removed.)

Diluting the Samples

The flasks were each topped up to the 100ml line (judging by the bottom of the meniscus) with DDW, first by pouring from a clean beaker, then by squirting from a plastic bottle. They were inverted repeatedly and shaken when upside down to thoroughly mix the solution.

A rough calculation was done to determine how much more the solutions should be diluted so that the chromium concentration would be on the range measurable by the A.A. (10 ppb to 100 ppb). 20ml of solution from one of the samples was transferred to another

clean 100ml volumetric flask with a 10ml pipette and the flask filled up to the 100ml mark. This solution was run through the A.A. to see if the chromium level was in the measurable range. It was about 6 ppb, below the low end of the range within which the A.A. can measure accurately. Thus, I decided to take 30ml from each solution instead of 20ml.

I used a 25ml and a 5ml volumetric pipette to transfer 30ml of solution from each flask into another clean 100ml volumetric flask and diluted each to the 100ml mark. These flasks were labelled dil.K[site number] e.g. dil.K4 for site 4. (the 'K' was to distinguish my samples from Rob's which were labelled with an 'R') They were ready for analysis in the A.A.

Controls

To give an indication of the levels of chromium typically found in plant tissues, we made up solutions from pine and citrus leaves. They were made in exactly the same way that the other samples were.

To determine any constant errors in the method I made up a method blank: It was made as the others but only DDW and c.HCl were put into the first 100ml flask. This control was to measure the amount of chromium contamination occurred in the method.

Analysis of the Samples and Controls

The samples and controls were analysed in the A.A. The basic principle of the A.A. is that metals emit and absorb radiation of a certain frequency which is specific to the metal in question.

In atomic absorption spectrometry the sample is aspirated into a flame or furnace (the latter in our case) and a light beam is directed through the flame, into a monochrometer and on to a detector that measures the amount of light absorbed by the atomized element in the flame. Each metal has its characteristic absorption/emission wavelength, so a lamp of the metal being tested for is used. The amount of radiation absorbed is proportional to the amount of the metal in the sample.

An A.A. consists of a light source emitting the line spectrum (light of only one color - a single line in the electromagnetic spectrum which is of a characteristic wavelength for that metal) of an element (hollow cathode lamp or electrodeless discharge lamp), a device for vaporizing the sample (in our case a graphite furnace heated electrically), a means of isolating an absorption line (monochrometer or filter and adjustable slit) and a photoelectric detector with its associated electronic amplifying and measuring equipment.

The A.A. has to be programmed for the metal being tested for. At Williams College it had not been used for chromium before (May 1990), so Tim Sulliavan (class of '90, chemistry major and TA for ES 102) and Sandy Brown (lab scientist, helped ES 102 students), the only people who were farmiliar with the A.A. based the chromium

new word!

programming on that which they had used for aluminium and modified it after a bit of trial and error. The program consisted of temperature changes over precise times (in seconds) which prepared the sample for testing, atomized it, then burned it off. The temperatures ranged from room temperature to 2500°C.

What do you mean by this?

Matrix modifier - potassium nitrate.

Data

The vegetation samples were identified as follows:

K2 - *Elodea*

K3 - *Elodea*

K4 - watermilfoil (*Myriophyllum*)

K5 - *Elodea*

K6 - watermilfoil (*Myriophyllum*)

K7 - watermilfoil (*Myriophyllum*)

K-grab - *Elodea*

K-surface - filamentous green algae

(from the Fresh Water Algae, by G.W. Prescott.)

Shown below is the data about the samples as they are being prepared for dissolving in acid.

ASHING THE SAMPLES

MASS OF CRUCIBLE	SAMPLE	MASS BEFORE(g)	MASS AFTER(g)	YIELD (%)	
9.80	Kgrab	1.55	0.25	16.1	
10.59	K2	0.47	-0.39	—	
11.88	K3	0.31	-0.47	—	
12.44	K4	1.85	0.29	15.7	
11.39	K5	1.16	0.33	28.4	
11.64	K6	1.64	0.24	14.6	
12.03	K7	1.56	0.23	14.7	
	R3			32.1	calculations done by Rob.
	R4			19.1	
	R5			45.9	
	Pine	5.00	0.11	2.2	Mass before provided by D. Dethier.
	Citrus	5.00	0.61	12.2	

Mass before and Mass after refers to the mass of ash alone. These values were determined by weight crucible and ash, then subtracting the mass of the crucible (weighed previously)

DIGESTING THEASHED SAMPLES

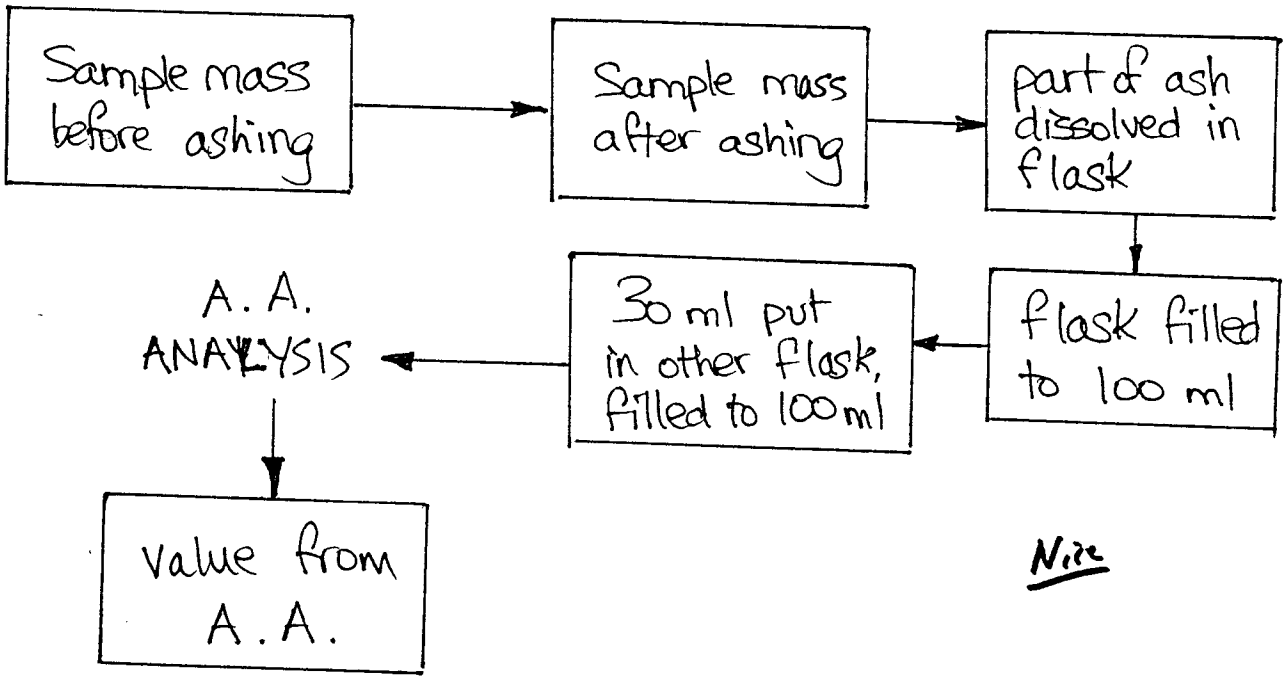
SAMPLE	MASS OF ASH USED (g)
K-grab	0.31
K4	0.32
K5	0.17
K6	0.30
K7	0.28
R3	0.35
R4	0.31
R5	0.37
Pine	0.11
Citrus	0.32

CALCULATION #2

What is the concentration of chromium in the dry sample (before ashing)?

The concentration measured by the A.A. is of an altered sample. Calculation must account for dilution and ashing procedures.

Procedural Flow Chart



N.B.

The calculation follows this flow chart in reverse to find the concentration at step 1.

$$\left(\text{conc. of analysed solution}\right)\left(\text{Volume of analysed Solution}\right)\left(\frac{1}{\text{dilution factor}}\right)\left(\frac{1}{\text{mass of ash used}}\right)\left(\text{yield of ash from dry sample}\right) = \text{conc. of Cr in dry sample}$$

- conc. of analysed solution: show on A.A. data sheet, corrected from method blank.*
- Volume of analysed Solution: 0.1 l because the ash was dissolved in a 100 ml flask.
- $\frac{1}{\text{dilution factor}}$: $\frac{100\text{ ml}}{30\text{ ml}}$ because 30 ml of solution from the first flask was diluted to 100 ml.
- $\frac{1}{\text{mass of ash used}}$: dividing by this gives the concentration of Cr in ash.
- yield of ash from dry sample: multiplying by this scale factor adjusts the concentration to that in the dry vegetation before ashing.

UNITS

$$\left(\frac{\mu g}{l}\right)\left[\ L\ \right]\left[\frac{ml}{ml}\right]\left[\frac{1}{g}\right]\left(\frac{g}{g}\right) = \mu g.g^{-1}$$

* Correction of A.A. readings:

The method blank had a Cr concentration of $0.23\ \mu g.l^{-1}$ in the analysis solution. I am assuming that this represents the Cr contamination in my method, so I subtracted this from each $\{$conc. of analysed solution$\}$ value before calculating as above.

Calculation # 2 for samples.

For Pine

$$\left[\frac{32.63\,\mu g.l^{-1} - 0.23\,\mu g.l^{-1}}{1}\right]\left[0.11\right]\left(\frac{10ml}{3ml}\right)\left(\frac{1}{0.11g}\right)\left(\frac{0.11g}{5.00g}\right) = 2.16\,\mu g.g^{-1}$$

Table of Results - Chromium Levels in Dry Samples

Sample	Cr. Conc. $\mu g.g^{-1}$
Pine	2.16
Citrus	0.65
K-grab	0.33
K 4	0.19
K 5	0.68
K6	0.09
R 3	7.52
R 4	4.40
R 5	9.32

Conc. given to 3 significant figures because balance weighed to this accuracy (0.01g)

The accepted values of Cr. concentration for pine & citrus

Pine: $2.6 \pm 0.2\ \mu g.g^{-1}$

Citrus: $0.8 \pm 0.2\ \mu g.g^{-1}$

(from NBS Special Publication 260-88 (1984))

Mean Chromium Levels

Sample Type	Cr. Conc. $\mu g.g^{-1}$
Vegetation	0.32
Fish *	5.26
Sediment	7.08

* From ALLIANCE Techologies Corporation assessment of Phase I Landfill, Williamstown, MA Table 6.1 "Summary of Contaminants detected at Phase II site".

Only K4, K5, K6, K7 and K-grab were analysed, because there was limited time to use the A.A.

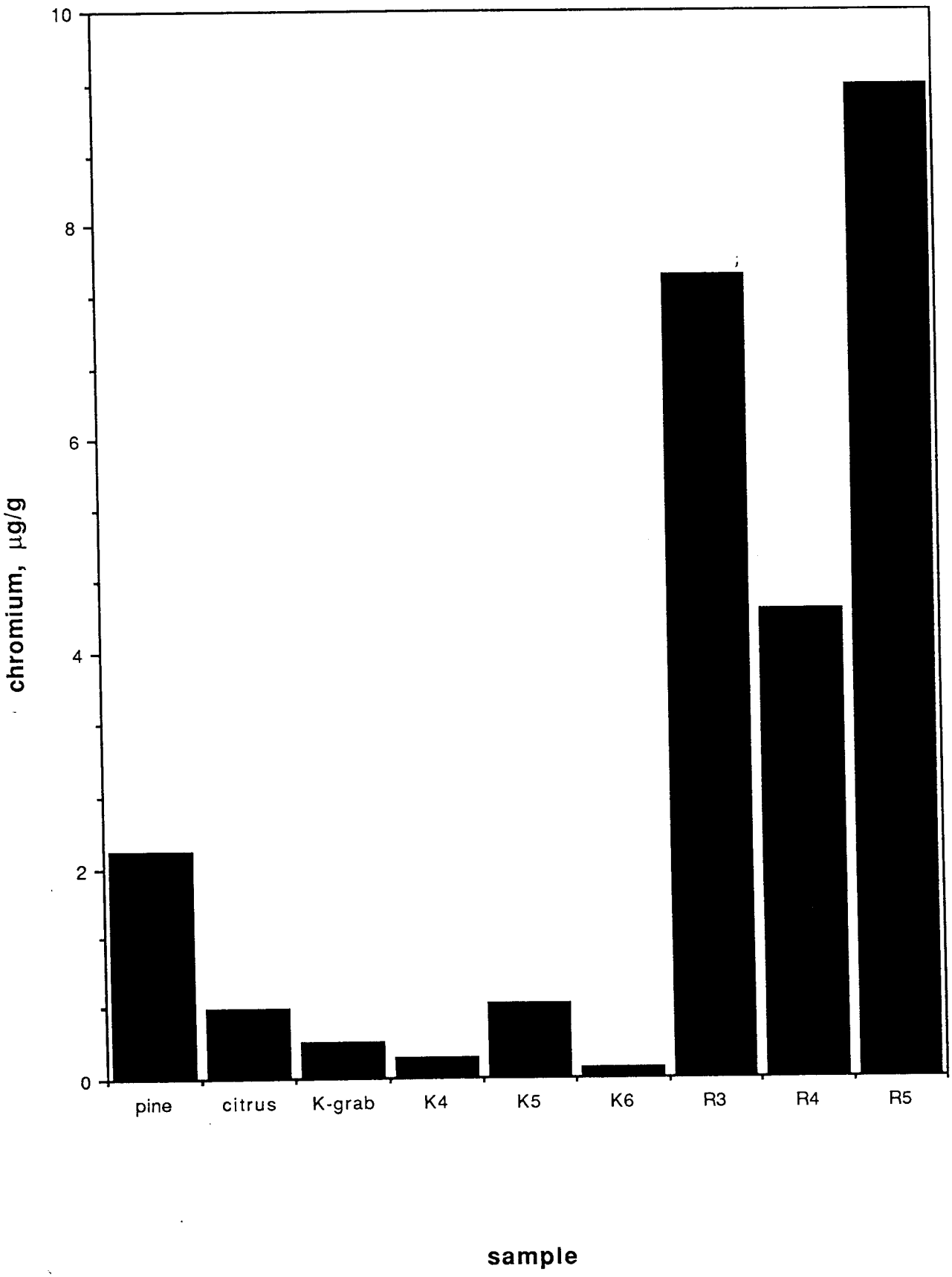
Of the sediment samples, R3, R4 and R5 were analysed.

The A.A. measured the concentration of chromium in the prepared solutions. Calculation #2 shows how the concentration of chromium in the original plant tissue or sediment was determined.

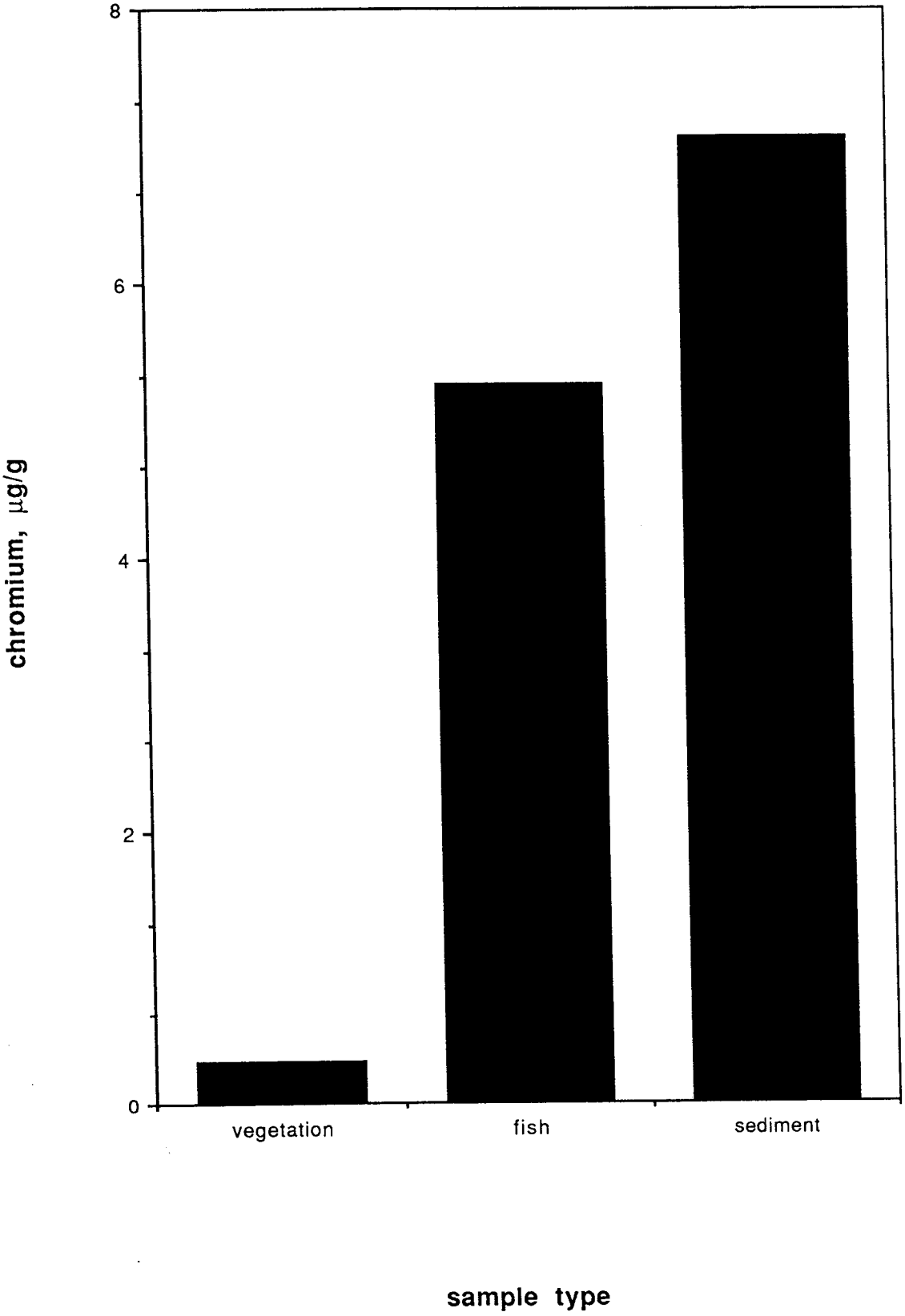
See the table 'Ashing the Samples'. In the vegetation section of the 'mass after' collumn (which show the masses of ash yielded), there are two negative coefficients. The other masses are lower that the masses which I later put into flasks for dissolving. (This is impossible, and I only used all of the ash from some of the samples.) The 'mass after' measurements were done by Rob and the others by me. One of us did something wrong (it is impossible to tell which one of us), and these data cannot be used. This means I cannot calculate the concentration of chromium in dry vegetation. I can only calculate the concentration in ash.

To get around this I took the mean yield of ash in pine and citrus and assumed (obviously with error) that this was the yield of the vegetation samples. Although the yields will not be the same, they

Chromium Levels in Dry Samples



Mean Chromium Levels



The chromium levels in the sediments are 35% higher than in the fish and 95% higher than in the vegetation.

Conclusions

There is distinct bioaccumulation of chromium in Bridges Pond. Producers - *Elodea* and *Myriophyllum* - have much lower chromium levels than high level consumers - sunfish.

Chromium is not apparently a trace element to plants. This partly explains why levels are naturally low in plants. Rocks and soils, on the other hand (including aquatic soils) contain varying amounts of chromium. Shacklette et al. (1971) reported a soil mean of 37 ppm (note: ug/g are equal to ppm) on the basis of 863 samples, but Wild reported values up to 125000 ppm in the Noro chromite (the commercially valuable chromium ore) mine in Zimbabwe (1974). Bridges Pond sediments have a mean chromium level of 7.08 ppm which is very low in comparison to the findings of Shacklette et al. Malyuga (1964) found the level of chromium in plants to be in the region of 1000 ppm (from work in Russia), much greater than the 0.32 ppm we found in Bridges Pond.

We found that the sediment had 16 times more chromium than the vegetation. Is seems likely that the sediments are acting as a trap for chromium. Lounamaa (1956) found that soils had 50 times more

for what locale?

How can you compare?

The Cr in soils is so region-specific that these #'s don't really tell you much. Did they assess them as "high" or "low"? What is a toxic level? To plants? To humans?

chromium that leaves in his work in Finland. Malyuga (1964) showed with work in Russia that chromium concentrations in plants followed fairly well the concentration in soils. There do not seem to be any marked trends in the ratio of chromium in plants and the soil they grow from.

The levels of chromium in the fish are similar to the levels in the sediment. However, I think it is unlikely that there is a significant direct chromium pathway between the sediments and the fish. The fish ingest limited amounts of sediment (male sunfish make nest in the spring by digging hollows with their mouths), but this is probably not significant.

Bugs eat sediment
↓
Fish eat bugs
↓
people eat fish

Sources of Error

The samples were uncovered while they were air drying, and chromium in the air could have settled into them.

which ones?

Stainless steel instruments were used, and because they contain chromium, they could have contaminated samples.

The problem of weighing errors certainly caused errors. The vegetation values were probably not good in relation to each other on an absolute scale, because the plan had been to have a separate yield calculation for each, and, as it is, there is a general yield factor

which does not have any necessary connection to the yield for aquatic plants.

Kevin,

Lots of work here! A little more discussion of toxic levels + the effects of Cr on plants + fish would have added depth to the paper.

An explanation of how the graphite furnace works would also have been a good thing to include.

Overall, nice job!

Sue