HEAVY METAL BASELINES FOR WILD RICE FROM NORTH CENTRAL WISCONSIN

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ABSTRACT

Wild rice grain samples from North America have been found to have elevated concentrations of heavy metals, raising concern for potential effects on human health. Wild rice plants growing either in polluted waters or near heavy metal ore bodies in north central Wisconsin could contain elevated heavy metals because of release of metals in area waters and soils. Determining the baselines of heavy metals in various parts of wild rice plants would be useful for biomonitoring these elements in the future. Wild rice plants were collected from four areas in Wisconsin in September 1997 and 1998 and divided into four plant parts for elemental analyses: roots, stems, leaves, and seeds. A total of 194 samples from 51 plants were analyzed across the localities, with an average of 49 samples per plant part depending on the element. Samples were cleaned of soil, wet digested, and analyzed by inductively coupled argon plasma spectrophotometry (ICP) for Ag, As, Cd, Cr, Cu, Hg, Mg, Pb, Se, and Zn. Roots contained the highest concentrations of Ag, As, Cd, Cr, Hg, Pb, and Se. Copper was highest in both roots and seeds, while Zn was highest just in seeds. Magnesium was highest in leaves. Baseline ranges for the 10 elements were established for all plant parts using the 95% confidence intervals of the medians. Using the criteria of highest concentration and lowest confidence interval relative to the median determined that most of the heavy metals are best monitored in the roots. It is also recommended to sample As and Pb in seeds because levels from the localities studied may be elevated. To measure the health of wild rice, it is recommended to sample Zn in seeds and Mg in leaves.

INTRODUCTION

Wild rice, *Zizania aquatica* L., is a staple in the diet of native peoples in the north central United States and has become a central component of Native American identity and culture in the Great Lakes region. Wild rice, or *manoomin* in Ojibwe language, has been endowed with spiritual attributes and is a central theme in legends told by the Ojibwa people. Wild rice has also been recognized as an important factor in European settlement in the Great Lakes region as settlements and fur trading posts tended to be established near the plants' natural stands (Vennum 1988).

Wild rice is recognized as an important factor in the ecology of lakes and streams in the Great Lakes region. It is an important source of food for many species of waterfowl and provides roosting areas for mature birds and brood cover for young birds. Wild rice stands maintain water quality by binding loose soils, retaining nutrients, and reducing wind erosion in shallow lakes.

Today, many historic rice beds have been lost. Wild rice is vulnerable to pollution, boat wakes, exotic species, and changes in water levels. Many water bodies that supported wild rice in the past have been dammed, resulting in the destruction of rice habitat. To address these issues, the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) has developed a wild rice management and enhancement program. Wild rice abundance is monitored in northern Wisconsin waters. Cooperating with state and federal agencies, GLIFWC is attempting to protect existing wild rice stands, restore historic rice beds, and introduce wild rice in appropriate habitats.

North central Wisconsin is underlain by massive sulfide ore deposits that are rich in technogenic heavy metals (DeMatties 1990). Wild rice is threatened by possible mineral development of these deposits because these developments have the potential of altering water levels in streams and lakes near the mine site. In addition, massive sulfide mining can produce runoff that contains heavy metals and acids and that may be toxic to wild rice.

Because of the importance of preserving wild rice stands and the health of people who consume its seeds, several studies have explored the levels of contamination in wild rice (Bennett et al. 1999; Nriagu and Lin 1995; Pip 1993). These studies suggest that wild rice accumulates heavy metals within its tissues. It is therefore reasonable to hypothesize that wild rice may be a valuable species to use in biomonitoring efforts aimed at assessing environmental change in lakes and streams located near point sources of pollution. This study presents preliminary baseline contaminant levels for wild rice roots, stems, leaves, and seeds as well as an initial assessment of wild rice as a biomonitor.

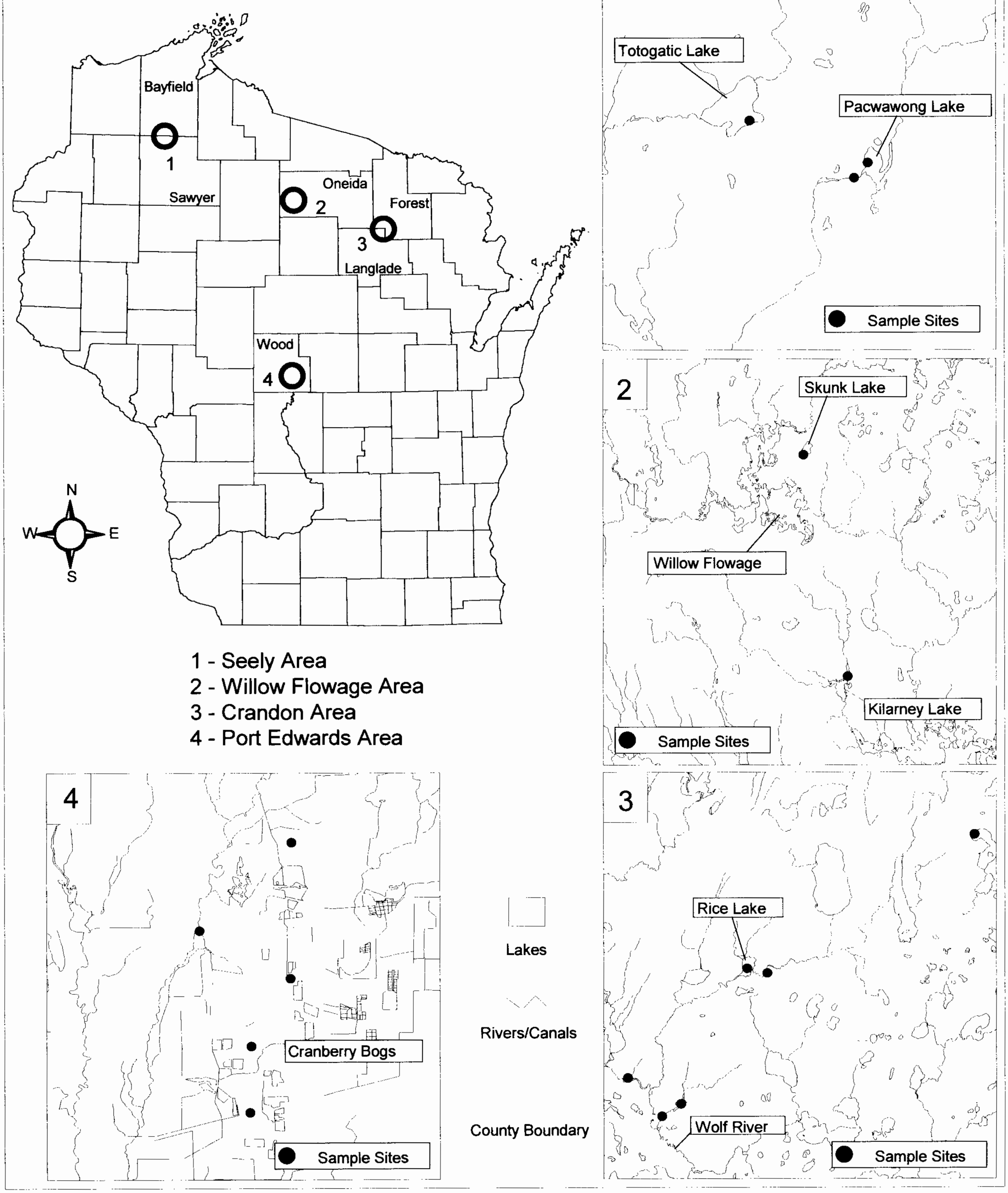
MATERIALS AND METHODS

Entire wild rice plants were collected at seed set time (September) in 1997 and 1998 from four localities in northern Wisconsin (see Figure 1): Seeley (Bayfield and Sawyer Counties), Willow Flowage (Oneida County), Crandon (Forest and Langlade Counties), and Port Edwards (Wood County). The first three localities were remote and not close to any point sources, while the last one is 15 km upwind (west) of a chlor-alkali plant, which is the largest atmospheric emitter of Hg in the state of Wisconsin. Nine plants were collected at Seely, 6 at Willow Flowage, 15 at Port Edwards, and 21 at Crandon, for a total of 51 plants. Plants were selected randomly and harvested by digging. The roots were washed in place and again back at the laboratory to remove soil.

The plants were separated into their four parts, stored in brown paper bags, and oven dried at 70° C until a constant weight was obtained. All samples were then ground in a stainless steel Wiley mill and acid digested for analysis by ICP at the University of Wisconsin Soil and Plant Analysis Laboratory for 10 elements: Ag, As, Cd, Cr, Cu, Hg, Mg, Pb, Se, and Zn. Silver, As, Cr, Cu, and Se were not measured in the plants from Port Edwards. All data are expressed on a dry weight basis.

The total potential number of data points was 4 plant parts x 4 localities x 12.2 (average) plants/locality x 10 elements = 1950. However, because of missing plant parts (typically insufficient seed for analysis), one outlier value which was omitted, below detection limit values, and part of the design being incomplete, 1315 data points were available for analysis. Values below detection limits varied considerably between elements (see Table 1). Copper, Mg, and Zn had none; Ag, Pb, and Cd ranged from 5 to 14%; As and Cr averaged 27%; and Hg and Se were both over 50%. A decision had to be made on how to handle the below detection limit (BDL) values for statistical analyses. Miesch (1976) recommends not analyzing variables with BDLs greater than 20%, and replacing BDLs with 0.7BDL for variables that have less than 20% BDLs. Gough and others (1987) dropped variables with more than 33% BDLs, and used the same 0.7BDL replacement value for variables with less than 33% BDLs. Sparling and Lowe (1998) used 0.5BDL for replacement values and a 50% BDL threshold for inclusion. Another study dropped no variables at all no matter how many BDLs, and made the detection limit the replacement value (Mudrey and Bradbury 1993). Newman's theoretical study of BDLs (1989) found that at 50% BDLs the mean can be 40% higher than the true mean for normally distributed data, and less for lognormally distributed data. The mean was only 10% off at 25% BDLs. He recommended a maximum likelihood estimator for replacement values, but 0.7BDL was very close to the mean at low BDLs percentages, which agreed with Miesch (1976). Consequently, for statistical analyses, we did not analyze BDLs for Hg and Se, and substituted 0.7BDL for BDL values for As and Cr. All means and medians reported in the results are based on censored data for all elements. Means and medians for As and Cr based on uncensored data

Figure 1: Wild Rice Sample Sites



(with replacement BDLs) were 10% less than the censored means and medians on average.

Table 1. Below detection limit (BDL) sample numbers for 10 elements in wild rice from northern Wisconsin. Sample numbers are combinations of four plant parts and four localities.

Element	N	BDLs	Total	BDL%
Ag	127	7	134	5
As	97	37	134	28
Cd	166	28	194	14
Cr	99	35	134	26
Cu	134	0	134	0
Hg	64	130	194	67
Mg	194	0	194	0
Pb	180	14	194	7
Se	60	74	134	55
Zn	194	0	194	0
Total	**1315**	**325**	**1640**	**20**

Baselines are presented as 95% confidence intervals of the medians because they are better measures of central tendencies than means for data that are not normally distributed. Confidence intervals of medians and Mood's median significant tests were calculated using MINITAB.

RESULTS

Baseline values are presented for seeds, leaves, stems, and roots in Tables 2 through 5. Baselines are presented as medians +/- the 95% confidence interval of the median, and the interval as a percent of the median as a measure of how large the interval is. The nutritional elements, including Cu, Mg, and Zn, had the lowest variability with intervals between 10 and 30% of the medians. Chromium and Hg had the highest variability with intervals exceeding 100% of the medians depending on plant part. Seeds tended to have the greatest variability, with intervals averaging 57% of the medians. Stems were 40% on average, followed by leaves at 32% and roots were the lowest at 28%. Medians between the four plant parts were all significantly different ($P < 0.001$) for all 10 elements using Mood's median test.

Nine of the ten elements were significantly different between roots, stems, leaves and seeds (Bennett et al. 1999). Six elements were highest in roots: Ag, As, Cd, Cr, Pb, and Se. Copper and Zn were both highest in seeds, followed by roots, and then low in leaves and stems. Lead was almost three times higher in leaves and seeds than in stems, although still much lower than in roots. Magnesium was highest in leaves, followed by roots. Mercury was highest in roots and seeds compared to stems and leaves, although this was not significant at the 0.05 probability level.

Six elements were significantly different between the four localities: Ag, As, Cr, Mg, Pb, and Zn (Bennett et al. 1999). Plants at Crandon and Willow Flowage were highest for As, Pb, and Zn. Chromium and Mg were highest in plants at Crandon, and Ag was highest at Willow Flowage. Port Edwards or Seely generally tended to have plants with the lowest concentrations.

Concentrations of As, Cd, Cr, Mg, and Pb depended on the interaction between plant part and locality. Cadmium in roots was highest at all localities except at Seely, where leaves were higher. Cadmium in seeds and stems did not differ much between localities. Magnesium was highest in leaves at all localities except Crandon, where roots, leaves, and stems all had the same concentration. The highest root concentrations of Pb were found at Crandon and Willow Flowage, but the concentrations at the other two localities still exceeded the other plant parts, which hardly varied across localities at all. The high concentrations in the roots of Ag, As, Cr, Hg, and Se were all at Crandon. The highest concentrations of Cu and Zn in seeds were found at Crandon and Port Edwards.

DISCUSSION

A discussion of the distributions of the 10 elements in the plant parts is available elsewhere (Bennett et al. 1999). Here we will discuss the baseline values.

Table 2. Baselines (ppm) for 10 elements in wild rice seed from northern Wisconsin. Baseline is the 95% confidence interval of the median. The interval as a percent of the median is given in the last column.

Element	N	Lower	Median	Upper	Interval as % of the median
Ag	29	0.003	0.006	0.010	58
As	3	0.018	0.136	0.161	53
Cd	44	0.012	0.016	0.020	25
Cr	29	0.138	0.285	0.590	79
Cu	**29**	**3.18**	**4.15**	**5.80**	**32**
Hg	7	0.010	0.022	0.082	164
Mg	45	1012	1152	1255	11
Pb	44	0.151	0.250	0.371	44
Se	3	0.125	0.146	0.364	82
Zn	**45**	**36.8**	**43.4**	**47.0**	**12**

Table 3. Baselines (ppm) for 10 elements in wild rice leaves from northern Wisconsin. Baseline is the 95% confidence interval of the median. The interval as a percent of the median is given in the last column..

Element	N	Lower	Median	Upper	Interval as % of the median
Ag	33	0.00957	0.0139	0.0162	24
As	33	0.3046	0.51225	0.86021	54
Cd	48	0.02998	0.042	0.0701	48
Cr	33	0.37682	0.6711	1.14862	58
Cu	33	2.11721	2.45418	2.80048	14
Hg	18	0.012	0.016	0.026	44
Mg	**48**	**1855.69**	**2210.28**	**2662.63**	**18**
Pb	48	0.50537	0.743	0.809	20
Se	11	0.2291	0.33297	0.38168	23
Zn	48	8.3	10.6634	12.8311	21

Table 4. Baselines (ppm) for 10 elements in wild rice stems from northern Wisconsin. Baseline is the 95% confidence interval of the median. The interval as a percent of the median is given in the last column.

Element	N	Lower	Median	Upper	Interval as % of the median
Ag	36	0.00667	0.00883	0.0118	29
As	36	0.10252	0.25737	0.38564	55
Cd	51	0.015465	0.021913	0.0308	35
Cr	36	0.007	0.16912	0.39596	115
Cu	36	1.02952	1.30995	1.65584	24
Hg	13	0.011	0.016	0.026	47
Mg	51	997.95	1083	1293.98	14
Pb	51	0.16526	0.221	0.287	28
Se	10	0.19793	0.2829	0.388993	34
Zn	51	11.3951	14.0241	17.407	21

Table 5. Baselines (ppm) for 10 elements in wild rice roots from northern Wisconsin. Baseline is the 95% confidence interval of the median. The interval as a percent of the median is given in the last column.

Element	N	Lower	Median	Upper	Interval as % of the median
Ag	**36**	**0.015**	**0.0189**	**0.0265**	**30**
As	**36**	**4.6637**	**7.1423**	**13.2051**	**60**
Cd	**51**	**0.085**	**0.112499**	**0.153**	**30**
Cr	**36**	**3.1746**	**4.9441**	**6.4698**	**33**
Cu	**36**	**4.06436**	**4.53017**	**5.34216**	**14**
Hg	**26**	**0.0236**	**0.0305**	**0.03772**	**23**
Mg	51	1326.48	1746.5	1945.52	18
Pb	**51**	**3.4587**	**4.0174**	**6.4853**	**38**
Se	**36**	**0.62861**	**0.76265**	**0.91326**	**19**
Zn	51	21.8357	24.1	30.4988	18

Two methods for determining which plant parts to use for monitoring each element include basing it on the plant part that contains the highest concentrations of each element, or basing it on the parts that have the smallest confidence intervals relative to the medians.

Based on using the highest concentrations, seed would be best for sampling and monitoring Cu and Zn. Leaves would be best for Mg, while roots would be best for the other elements tested. Stems did not have the highest concentrations of any elements.

Based on using the smallest confidence intervals, seed would be best for Cr, Mg, and Zn. Leaves would be best for Ag, Cu, and Pb, while roots would be best for As, Cd, Hg, and Se. Stems did not have the lowest confidence intervals for any elements.

These two decision rules agree on Zn in seeds, and As, Cd, Hg, and Se in roots. It doesn't make a lot of sense to measure Mg in seeds when the highest concentration is naturally in the leaves, and the difference in the intervals as percentages is only 7%. Chromium is almost ten times higher in roots than seeds, which makes a compelling case of using roots even though the variability is more than twice that in seeds. Copper has the same level of variability in both roots and leaves, but the highest concentration is in the roots, suggesting that roots be used for Cu. Lead is so much higher in roots than leaves that it is compelling to monitor it in roots even though the variability is higher. Silver is about the same on both criteria in both roots and leaves with roots having a slightly higher concentration. These arguments point to a consensus that using the highest concentrations is the preferred decision rule for deciding on which plant part to use for biomonitoring. For 8 of the elements, roots would be the preferred part, and leaves for Mg and seeds for Zn (shown in bold in Tables 2 through 5).

Two exceptions to this recommendation concern arsenic and lead. Both were found to be elevated in the seed (Bennett et al. 1999). Although the seed concentrations were an order of magnitude lower than the roots, the concentrations were elevated compared to other literature values. Future sampling for arsenic and lead may want to include seed with roots because of this concern.

In conclusion, it appears that biomonitoring certain heavy metals using wild rice plants is possible in north central Wisconsin, and that roots are the best plant tissues to use for measurements. It is also recommended to sample As and Pb in seeds because of possibly elevated levels from the localities studied. To measure the health of wild rice, it is recommended to sample Zn in seeds and Mg in leaves.

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REFERENCES

Bennett JP, Chiriboga E, Coleman J, Waller D. 1999. Heavy metals in wild rice from northern Wisconsin. Science of the Total Environment (in review).

DeMatties TA. 1990. The Ritchie Creek main zone: a lower proterozoic copper-gold volcanogenic massive sulfide deposit in northern Wisconsin. Econ Geol 85:1908-16.

Gough LP, Jackson LL, Peard JL, Engleman EE, Briggs PH, Sacklin JA. 1987. Element baselines for Redwood National Park, California—Composition of the Epiphytic Lichens *Hypogymnia enteromorpha* and *Usnea* spp. Open-File Report 87-169. Denver: U. S. Geological Survey.

Miesch AT. 1976. Geochemical survey of Missouri—Methods of sampling, laboratory analysis, and statistical reduction of data. Geological Survey Professional Paper 954-A. Washington: U. S. Geological Survey.

Mudrey MG, Bradbury KR. 1993. Evaluation of NURE hydrogeochemical groundwater data for use in Wisconsin groundwater studies. Madison: Wis Dept of Natural Resources.

Newman MC, Dixon PM, Looney BB, Pinder JE. 1989. Estimating mean and variance for environmental samples with below detection limit observations. Water Res Bull 25:905-16.

Nriagu JO, Lin TS. 1995. Trace metals in wild rice sold in the United States. Sci Tot Env 172:223-8.

Pip E. 1993. Cadmium, copper and lead in wild rice from central Canada. Arch Environ Contam Toxicol 24:179-81.

Sparling DW, Lowe TP. 1998. Metal concentrations in aquatic macrophytes as influenced by soil and acidification. Water, Air and Soil Pollut 108:203-21.

Vennum T Jr. 1988. Wild rice and the Ojibway people. St Paul: Minnesota Historical Society Press.

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