
**Concentrations of selected elements, with special reference to Cd and Zn,
in the seeds of cultivated and wild plants from the DePue Wildlife
Management Area, DePue, Illinois**

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Final Report

28 February 2000

ABSTRACT

We examined concentrations of barium (Ba), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) in buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), giant ragweed (*Ambrosia trifida*), Pennsylvania smartweed (*Polygonum pennsylvanicum*), and water hemp (*Amaranthus rudis*) seeds collected at the "3-Is" waterfowl impoundment at DePue Wildlife Management Area (DWMA), DePue, Illinois, in the autumn of 1997. Concentrations in these species (except giant ragweed) were compared with samples collected at Anderson Lake Conservation Area (ALCA), a reference site located 146 km from DWMA.

Mean Cd, Cu, Zn concentrations were lowest, and Ba and Pb were not detectable, in corn seeds; Fe concentrations were lowest in smartweed seeds. Cd, Fe, and Zn concentrations were highest in seeds of water hemp, while Cu was highest in giant ragweed. Pb was detectable in some smartweed and water hemp samples. Mean and maximum Cd, Fe, and Zn concentrations were higher in buckwheat, corn, smartweed, and water hemp seeds from DWMA than in samples from ALCA. Seeds of the plant species examined exhibited little variation in Ba and Cu concentrations between sites. Pb was detected in a lower proportion of water hemp seeds, but a higher proportion of smartweed seeds, from DWMA, when compared to ALCA.

We detected a wide range of variation in Cd and Zn concentrations in seeds within and across the cell comprising the impoundment, and this was further complicated by variation among species. As a general trend, individual values tended to be higher at the north end of the easternmost cell of the impoundment, the east end of the northcentral cell, and the central portion of the west/southcentral cell. The Zn:Cd ratio in seeds was higher in corn and smartweed seeds, and lower in water hemp seeds, from DWMA as compared to seeds from ALCA. The relationship between Cd and Zn concentrations in seeds varied among species and between sites, suggesting an interaction between species and soil concentrations of these elements.

Seed Cd and Zn concentrations in our study were above lower effects levels for mammals and birds observed in some studies but not in others, suggesting some poorly-definable risk to granivorous species utilizing DWMA. Cd concentrations were within known effects levels for laboratory mice, however, this group may be more sensitive to Cd exposure than wild species. Based on the information available from prior studies, it seems unlikely that concentrations of Cd and Zn observed in seeds from DWMA are high enough to cause adverse effects in ducks or gallinaceous birds that might consume them, although there might be some risk to young of these avian groups. Studies of the effects of many contaminants to wild species at environmentally-realistic concentrations are lacking, making plausible inferences about risks to species consuming seeds at DWMA difficult.

The bioavailability of metals present in plants needs to be considered in determining exposure risk to granivorous mammals and birds from ingesting seeds at DWMA, given the presence of elevated concentrations of elements which antagonize Cd and Zn. Additionally, the relative importance of the plant species examined as food items in the diet of each species needs to be considered, as do other exposure routes for granivorous animals. We suggest that, given elevated concentrations of Cd and Zn in the seeds of plant species growing within the impoundment (that exceed or approach effects levels in some studies) further information is needed on exposure, tissue concentrations, population levels/demographics, and individual health

of representative granivorous species (e.g. white-footed mice) present at DWMA.

INTRODUCTION

Gibb and Cartwright (1982), and later Cahill and Steele (1986) reported elevated concentrations of Cd and Zn, along with other metals, in the sediments of DePue Lake, a backwater lake located along the Illinois River by the town of DePue, Illinois, as a result of past smelting operations (Fig 1). Cd, Zn, and to a lesser degree Ba and Pb concentrations in a soil core taken from within the waterfowl impoundment (DWMA) at DePue Wildlife Management Area, located adjacent to DePue Lake, were elevated compared to a core collected adjacent to it (Illinois Waste Management and Research Center, unpubl. data). Cahill et al. (1995) observed greatly elevated concentrations of Cd (26 to 173 ppm) and Zn (3,130 to 11,500 ppm) in the top soil horizon (0-15 cm) of the impoundment, which was originally constructed to contain sediments dredged from DePue Lake. Cu (91 to 535 ppm) and Pb (150 to 1,900 ppm) concentrations in DWMA were similar to the neighboring floodplain, but together were also higher than background levels for Illinois soils (Cahill et al. 1995).

Currently, the area is managed to provide sanctuary and predictable food resources for migrating waterfowl. Limited public waterfowl hunting opportunities are available on the area. When conditions allow, approximately 32 ha of the 37.4 ha impoundment are cultivated and planted to agricultural crops, primarily corn (*Zea mays*). Seed-bearing annual "moist soil" plants are generally abundant in non-cultivated portions of the area. During years when river levels are above flood stage into mid-summer the area can not be cultivated, and only wild plants are present. The impoundment is flooded in October using water pumped from the Illinois River, and water levels are maintained throughout the fall migrational period to emulate natural river cycles.

Since the growth of annual seed-bearing plants is encouraged to meet site objectives of providing food for migrating ducks, we collected seeds from the more abundant annual, seed-bearing plants present in 1997 to examine levels of contaminants of primary interest. Specifically, our objectives were to 1) compare concentrations of Ba, Cd, Cu, Fe, Pb, and Zn in seeds from abundant annual plant species found at DWMA with samples collected at a reference site having a similar management regime, and 2) compare concentrations of elements detected in seeds from DWMA, particularly Cd and Zn, with published information on effects levels in ducks and other granivorous wildlife.

METHODS

Seeds were collected from the five most abundant annual plant species which are used as food by dabbling ducks. These included corn, buckwheat (*Fagopyrum esculentum*), Pennsylvania smartweed (*Polygonum pennsylvanicum*), water hemp (*Amaranthus rudis*), and giant ragweed (*Ambrosia trifida*). Seeds were collected 30 September through 2 October, 1997. Anderson Lake Conservation Area (ALCA), located on the Illinois River 146 km southwest of DWMA, served as a reference area or control. Cd and Zn concentrations in surface soil from ALCA averaged 1.83 and 106.3 ppm, respectively (n= 3). Plants were selected by stratified random sampling; we selected at least 2 portions of each cell, located stands of target plant

species (if available) within those areas, and then randomly located sampling stations within that patch (Fig. 2). A marker was planted after a station was located and seeds were collected from three plants located closest to the station marker. Approximately 5 grams of seeds were stripped from seed heads by hand using clean, powder-free latex gloves. Seeds were stripped directly into sterile Whirl-pacs, transferred to wet ice, and later frozen until preparation for analysis.

Analytical prep and techniques

Seed samples were initially dried at room temperature, and were stored in desiccators until ready to be digested. The seed samples were dried at 140°C overnight and the concentrations were calculated as dry weight. Seeds were ground and digested with hydrogen peroxide and acid prior to measurements of metals concentration using inductively coupled argon plasma emission spectroscopy (ICP). Prior to digestion the seed samples were reduced to particle sizes that would pass through the fine bore tubing of the microwave digestion equipment by grinding for approximately three minutes using a cutting mill (Model M20-Universalmuhle, IKA®-Labortechnik, West Germany). It was necessary to re-grind the ragweed, buckwheat, and smartweed seeds for 20 minutes using a Spex 8000 Mixer/mill (Spex Certiprep, Inc., Metuchen, New Jersey, USA), as they clogged the tubing after the previous step.

Sample weights of 0.3 to 0.5 grams were used in the digestions. A mixed portion of the sample was weighed to 0.1 mg using an electronic top loading balance directly into a tared 50 mL conically tipped polypropylene centrifuge tube. The tubes were precleaned using a 24 hour 10% nitric acid (HNO₃) soak followed by a deionized water rinse. The samples and tubes were tared, 1.00 mL of 30% hydrogen peroxide (H₂O₂) added, and the weights recorded. Approximately 20 to 30 mL of an acid and internal standard solution were then added to the sample after taring and the weight recorded. The acid concentrations used were 2% HNO₃ and 5% hydrochloric acid (HCl). Beryllium (Be) was used as an internal standard, at a target concentration of 2.00 mg/L.

The samples were then homogenized into a slurry using a saw-toothed generator of titanium and TFE-fluorocarbon construction (Pro Scientific, Monroe, CT). The internal standard solution was used to rinse excess materials from the generator and the volume accounted for in the total weight. Sample preparations were completed using a SpectroPrep System automated microwave digestion system manufactured by CEM Corporation, Matthews, NC, using 10 mL sample loop. After heating, cooling, and filtering, about 9.0 mL of the sample were collected and deposited by the autosampler into 15 mL polypropylene test tubes. This digestate was then used for ICP analysis without any further treatment. High purity acids and hydrogen peroxide (Baker Ultrex and Fisher Optima brands, respectively) were used for all digestions and standard solutions.

Analysis of element concentrations in seeds was conducted using a Thermo Jarrell Ash (TJA) AtomComp Model 61 vacuum spectrometer. This instrument has a polychromator configured with fixed channels, including analytical lines for high and low concentrations of Ca, Mg, and Na. Blank subtraction and background correction were used to correct for matrix emissions. Standards used for the tissue analyses were prepared in a matrix of 2% HNO₃ and 5% HCl. Analytical methods followed USEPA Method 200.7, Revision 4.4, Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission

Spectroscopy, except that we used the digestion process described previously. Beryllium was chosen as an internal standard because it was not present in the samples, there are no spectral or background interferences, and it is very precisely detectable.

Statistical techniques

Due to low and disparate sample sizes, the Mann-Whitney U nonparametric test was employed to examine variation among means, however, most comparisons were descriptive. Comparisons of within-site variation focused primarily on Cd and Zn concentrations in corn and smartweed (and to a lesser extent water hemp), the most abundant seed-bearing plant species and those with samples from each of the cells within the impoundment.

RESULTS

Detection Limits and Measures of Accuracy and Precision

Detection limits (ppm dry weight) were as follows:

	<u>Ba</u>	<u>Cd</u>	<u>Cu</u>	<u>Fe</u>	<u>Pb</u>	<u>Zn</u>
buckwheat-	0.79	1.72	3.23	3.74	7.89	2.40
corn-	0.33	0.73	1.37	1.59	3.35	1.02
water hemp-	0.41	0.90	1.69	1.96	4.13	1.26
giant ragweed-	1.23	2.69	5.05	5.85	12.3	3.75
smartweed-	0.76	1.65	3.10	3.59	7.57	2.31

Recoveries from spiked blanks and (spiked samples) were (mean %): Ba 107 (109), Cd 109 (102), Cu 107 (106), Fe 116 (116), Pb 108 (101), Zn 107 (99). Recoveries from both matrix and analytical spikes were within $\pm 20\%$ and were therefore considered acceptable.

Between-site variation in concentrations of selected elements

Ba

Ba was not detectable in corn seeds from either site. Mean Ba concentrations were similar in composite buckwheat seed samples from both sites, as well as in giant ragweed seeds from DWMA (Table 1). Mean Ba concentrations were higher in water hemp seeds than in the other plants we examined. However, Ba levels in this species were nearly identical between sites. Smartweed seeds were intermediate among the plant species examined in mean Ba concentration. Although the maximum Ba concentration was higher in smartweed seeds from DWMA than in those collected at ALCA (Table 1), the average concentration was 20% higher in the sample

from ALCA ($U_{(2), 0.05, 5, 12} = 24.0, P = 0.57$).

Cd

The mean Cd concentration in the composite buckwheat samples from DWMA was 90% greater, and the minimum concentration was 41% greater, than in the sample from ALCA (Table 1). Mean and maximum concentrations in corn from both sites were lower than in the other species examined. The mean ($U_{(2), 0.05, 7, 9} = 22.0, P = 0.35$), minimum, and maximum values were higher in the corn samples from DWMA than in those from ALCA. The mean ($U_{(2), 6, 7} = 0.0, P = 0.001$), maximum, and minimum Cd concentrations in water hemp from DWMA were greater than in this species from ALCA, and were the highest of the five plant species collected. Giant ragweed seeds from DWMA ranked second in Cd concentration among the species examined (Table 1). The mean Cd concentration in smartweed seeds from DWMA was 53% higher than in those collected at ALCA ($U_{(2), 0.05, 5, 12} = 14.0, P = 0.10$).

Cu

The mean Cu concentration in the composite buckwheat samples from DWMA was 1.3 times (130%) greater, and the minimum concentration was 39% greater, than in the sample from ALCA (Table 1). Cu concentrations in corn seeds were similar between sites, and were lower than in the other species examined. Cu concentrations in water hemp seeds were also similar between DWMA and ALCA (Table 1). The mean Cu concentration was highest in giant ragweed seeds than in the other species examined. The mean and maximum Cu concentrations in smartweed seeds were highest in the samples from DWMA ($U_{(2), 0.05, 5, 12} = 21.0, P = 0.38$).

Fe

The mean, minimum, and maximum Fe concentrations in buckwheat seeds collected at DWMA were greater than the sample from ALCA (Table 1). The mean ($U_{(2), 0.05, 7, 9} = 9.0, P = 0.02$) and maximum values for Fe in corn seeds from DWMA were 261% and 805% greater, respectively, than in corn from ALCA. Mean ($U_{(2), 0.05, 6, 7} = 20.0, P = 0.95$) and maximum Fe concentrations were also greater in water hemp seeds from DWMA (Table 1). Fe concentrations in ragweed seeds were approximately intermediate in concentration, when compared to the other species sampled at DWMA. Fe concentrations in smartweed seeds were the lowest of the wild plant species collected, and mean ($U_{(2), 0.05, 5, 12} = 13.0, P = 0.08$) and maximum concentrations were higher in the samples from DWMA than in those collected at ALCA.

Pb

Pb concentrations in all corn, buckwheat, and ragweed samples were < MDL. Only 3 of 7 (42%) of the composite water hemp samples from DWMA (4.92 to 6.96 ppm) were > MDL, whereas 4 of 6 (67%) water hemp samples from ALCA (5.00 to 7.73 ppm) were > MDL. However, none of the Pb concentrations in water hemp from either site were at least 100% > MDL. Four of 12 (33%) composite smartweed samples from DWMA (9.68 to 19.3 ppm) were above the MDL, whereas only 1 of 5 samples from ALCA (10.3 ppm) was > MDL. The Pb concentration of one sample from DWMA approached (14.7 ppm), and another exceeded (19.3 ppm), 2 times the MDL.

Zn

The mean Zn concentration in buckwheat from DWMA was 255%, and the maximum concentration was 289%, higher than the composite sample from ALCA (Table 1). Mean ($U_{(2), 0.05, 7,9} = 0.0, P < 0.001$) and maximum Zn concentrations in corn seeds from DWMA were higher than in corn from ALCA, though were lower than in buckwheat. Zn concentrations in water hemp collected at DWMA were the highest of any species from either site; the mean concentration was 330% higher than in this species from ALCA ($U_{(2), 0.05, 6,7} = 0.0, P = 0.001$). The mean Zn concentration in giant ragweed was similar to that of buckwheat and smartweed from DWMA (Table 1), while the mean ($U_{(2), 0.05, 5,12} = 3.0, P = 0.002$) and maximum Zn concentrations in smartweed were greater in the samples from DWMA than in those collected at ALCA.

Within-site variation in concentrations of selected elements

Cd

The mean and maximum Cd concentrations in corn were slightly higher in the samples from cell A than cell C (Table 2). Cd concentrations were lowest and least variable in cell B. Individual values for composite samples within each cell were highest at stations A1, B4, and C7 (Fig. 2). The mean Cd concentration in the composite smartweed samples was highest in cell B, although the maximum value was highest in cell C. Cd levels in smartweed seeds were lowest in cell A. Individual values for composite samples within each cell were highest at stations A1, B3, and C4 (Fig. 2).

The mean Cd concentration in water hemp was highest in cell C, although the maximum values were similar in cells B and C (Table 2). The single composite sample from cell A fell within the range of values for B, but was lower than the minimum value for cell C. The highest Cd concentration for an individual composite sample from within cell B was observed at station 3 (Fig. 2).

Zn

The mean and maximum Zn concentrations in corn were similar across the 3 cells (Table 2); Zn concentrations were most and least variable in cells C and B, respectively. Values for individual composite samples within each cell were highest at stations A1, B5, and C6 (Fig. 2).

Among-cell Zn concentrations were more variable in smartweed seeds than in corn (Table 2). The mean Zn concentration was highest in cell B and lowest in cell C, although the maximum concentration was observed in the latter. Zn concentrations were least variable in cell A. Individual values for composite samples within each cell were highest at stations A2, B3, and C8 (Fig. 2).

Zn concentrations in water hemp seeds were highly variable across cells (Table 2). The mean, maximum, and minimum Zn concentrations were highest in cell C, while the composite sample from cell A had the lowest observed Zn concentration. The highest Zn concentration for an individual composite sample from within cell B was observed for station 4 (Fig. 2).

Cd-Zn ratios

The mean Zn:Cd ratio in seeds from DWMA was higher than in seeds from ALCA for corn (51.1 vs. 38.4) and smartweed (12.6 vs. 8.4), but lower for water hemp (13.3 vs. 15.5). A moderate negative correlation existed between Cd and Zn in corn from DWMA ($r = -0.389$, $P = 0.30$), whereas a stronger positive relationship was observed between Cd and Zn in corn from ALCA ($r = 0.60$, $P = 0.16$). The relationship between Cd and Zn concentrations in water hemp seeds from DWMA was weak ($r = 0.12$, $P = 0.85$); a stronger, negative correlation existed between Cd and Zn in this species from ALCA ($r = -0.51$, $P = 0.30$). Cd and Zn concentrations were highly correlated in smartweed seeds from DWMA ($r = 0.78$, $P = 0.003$), though only weakly so in samples from ALCA ($r = 0.12$, $P = 0.85$).

DISCUSSION

Plant - Metals - Soil Interrelationships

Peles et al. (1996) noted increased concentrations of Cd, Cu, and Zn in the seeds of soybean plants grown on sewage sludge-amended soil. The authors failed to detect a difference in the Cd, Pb, and Zn content of buckwheat seeds among treatments, as these metals primarily accumulated in the leaves of this species. Seeds of plants collected at DWMA had higher concentrations of certain elements examined (Cd, Fe, and Zn), than those from ALCA. Concentrations of these elements in a limited sample of seeds collected from DWMA in 1997 (IL Waste Management and Research Center, unpubl. data) fell below, within, or above the range of values examined in our study, suggesting that our sampling did not encompass the entire range of variation present within DWMA.

Seeds of agricultural crop plants had lower concentrations of the elements examined than did wild plant species. Concentrations in corn in particular were lower than in wild plants; in some cases buckwheat was higher than wild species for certain contaminants. In contrast, Peles et al. (1996) reported that Cd, Pb, and Zn uptake was greater in soybeans and buckwheat than in the old-field plant species examined from the same study site (Brewer et al. 1994). That species may differ widely in accumulation of certain elements is well documented (Brewer et al. 1994; Logan and Chaney 1983; Doyle et al. 1973; Turner 1973). Additionally, pH, cation exchange capacity, and organic-matter content of soil affect its metal binding capacity and therefore uptake by plants (Ross, 1994; Logan and Chaney 1983). Certain elements, Cd included, may escape the "Soil-Plant Barrier", which limits uptake of elements in soil by plants (see Logan and Chaney 1983).

Cd concentrations were highest in the leaves, followed by the roots, stems, and seeds, and Zn concentrations were highest in the leaves followed by the stems, roots, and seeds of buckwheat plants grown on sludge-amended soil without lime treatment (Peles et al. 1996). Based on the relationships among contaminant levels in various plant parts reported by Peles et al. (1996), and the levels of Cd and Zn in the seeds we collected, Cd and Zn concentrations in buckwheat leaves from DWMA would have averaged 121 and 241 ppm, respectively. This estimated average cadmium concentration is considerably higher than Peles et al. (1996) observed for buckwheat grown on sewage-amended soils ($\bar{x} = 19.8$ ppm). Although concentrations of Cd and Zn in seeds were considerably less than in surface soils (0-15 cm interval, $\bar{x} = 83$ ppm, min-max = 26-173

ppm, Cahill et al. 1995), the estimated buckwheat leaf Cd concentration was higher than the average soil concentration. As average and maximum Cd concentrations were higher in the seeds of wild plants than buckwheat seeds, they might be expected to have accumulated even higher leaf Cd concentrations.

Corn had lower concentrations of the elements examined than other plant species collected at DWMA. Similar results were obtained for a limited number of seed samples collected previously at DWMA (IL Waste Management and Research Center, unpubl. data). Munshower (1977) found that grasses (crested wheatgrass *Agropyron cristatum* and needle-and-thread grass *Stipa comata*) did not accumulate Cd above soil levels, and suggested that grasses may be poor accumulators of Cd. In contrast, Levine et al. (1989) reported that brome and foxtail had higher Cd, Cu, Pb, and Zn, and bluegrass had higher Cu, Pb, and Zn, concentrations than high-bush blackberry (*Rubus pennsylvanicus* {= *Rubus frondosus*}) when all four were grown on sewage-sludge treated fields. Brewer et al. (1994) found that the common (*Ambrosia artemisiifolia*) and giant ragweeds, both annuals, grown on sewage-sludge treated plots accumulated higher Cd, Cu, Pb, and Zn concentrations than an annual monocot, *Setaria faberii* or perennial dicot, *Solidago canadensis*. Mean Cd and Cu concentrations in giant ragweed in our study were higher, and Zn concentrations were lower, than Brewer et al. (1994) reported for this species.

Eisler (1985) concluded that background Cd levels in plants were usually < 1.0 ppm. However, concentrations in 48 of our 54 (89%) samples, including those from our reference site, exceeded 1.0 ppm. Bowen (1996) reported an average of 160 ppm Zn for woody angiosperms, and Doyle et al. (1973) found similar Zn concentrations in deciduous woody species, which had higher concentrations than in grasses and forbs. We did not examine woody species, of which willows (*Salix sp.*) were by far the predominant species present within the impoundment. Average and maximum Zn concentrations in Pennsylvania smartweed (*Polygonum pennsylvanicum*) from DWMA were higher, and from ALCA were lower, than in another smartweed species (*P. alaskanum*) from an area of molybdeniferous soils in northwestern Canada (63 and 67 ppm, Doyle et al. 1973). The mean Zn concentration in buckwheat seeds from DWMA was higher than in seeds of this species (62.8 ppm) grown on sludge-amended soil (Peles et al. 1996).

Ba and Cu exhibited little variation between sites. Doyle et al. (1973) found little variation in Cu concentration among plant species and habitats; concentrations were generally between 4 and 7 ppm although ranged as high as 19 ppm. Maximum concentrations in our study were as much as 50% greater than the highest values observed in that previous study. Mean and maximum concentrations in smartweed from both sites were greater than they reported (7.5 and 9.1 ppm, Doyle et al. 1973). Bowen (1966) cited an average of 14 ppm for woody angiosperms, with relatively little variation among groups of land plants.

Perhaps not surprisingly, Pb was detectable in few seed samples. Our detection limits for Pb were relatively high, and Pb is poorly translocated between soil and plants unless soil concentrations are very high (Logan and Chaney 1983).

Cahill et al. (1995) examined metal concentrations in the soils within the DWMA waterfowl impoundment, and reported the following relationships among cells with regard to Cd and Zn concentrations in upper soil horizons: Zn B>C>A; Cd B>A>C. Concentrations in seeds did not

reflect this pattern. This is not surprising, however, given differences in accumulation among species, the wide range of variation in concentrations within cells, and the fact that we did not sample in the exact locations as Cahill et al. (1995).

Smelting operations at DePue were terminated in 1971, thus deposition of contaminants via airborne emissions is no longer a concern at this site. However, dust-borne contamination at DWMA could result from wind-blown contaminated soils, particularly during cultivation. We rinsed 4 randomly-selected seed samples with deionized water, and did not detect Ba (MDL 0.004 mg/L), Cd (0.016 mg/L), Cu (0.005 mg/L), Fe (0.009 mg/L), or Pb (0.075 mg/L) in any of the samples. Zn was detectable (0.008 mg/L) in 3 of 4 rinse samples (0.01 to 0.06 mg/L). A combination of rain and wind prior to collection might be expected to have washed away any large accumulations of dust-borne contaminants present.

McKenna et al. (1993) noted that Zn:Cd ratios in leaves of spinach and lettuce plants increased along with Zn concentration of the growth medium. The relationship between Cd and Zn concentrations in plant seeds in the current study varied between sites and among species. Cd (1.83 ppm dry weight, n= 3) and Zn (106.3 ppm dry weight, n= 3) concentrations were considerably lower in surface soils at ALCA than at DWMA (Cd 83.3 ppm, Zn 5841 ppm, n= 26, Cahill et al. 1995), thus the Zn:Cd ratio in soils was higher at DWMA (70) than ALCA (58). An antagonism between Cd and Zn in plants has been documented (see Logan and Chaney 1983). Zn and Cd concentrations were significantly correlated in the leaves of elm and hawthorne trees growing in the vicinity of a smelter (Little and Martin, 1972). However, McKenna et al. (1993) found an antagonistic effect of Zn on Cd accumulation in spinach and lettuce leaves at low but not high concentrations, and suggested that Zn may not decrease Cd concentrations at highly contaminated sites. McKenna et al. (1993) found that Cd bioavailability in lettuce and spinach grown on Zn-Cd soils was lower than when grown on Cd-only soils, and that plant species differed in Cd bioavailability, regardless of identical Zn - Cd concentrations. Our results indicated that an interaction existed between plant species and soil Zn-Cd concentrations in determining bioavailability of these metals to plants.

Critical Concentrations

Cd

Cd concentrations in seeds from DWMA ranged from 0.75 to 15.7 ppm. Chronic intake of 0.5 ppm (10 μ g/day) Cd in drinking water produced maximal hypertension in rats; higher concentrations decreased blood pressure (Kopp et al. 1982). Changes in heart function and heart and kidney tissue metabolism were detected in rats given 1 ppm Cd. However, in another study, chronic administration of 5, 12, and 32 ppm Cd did not affect blood pressure and failed to produce lesions of cardiac or aortic tissue (Fingerle et al. 1982). Gatta et al. (1989) detected structural and functional nephropathy after 40 and 60 days in rats given 16 ppm CdCl₂ in drinking water. Sugawara and Sugawara (1974) found that chronic oral exposure of rats to 10 ppm Cd (dry weight) resulted in mild renal damage, while more severe tubular lesions and mortality, occurred at 50 ppm. Kostial et al. (1978) determined an LD₅₀ of 47 mg/kg CdCl₂ (administered orally by gavage) in 2 week-old rats.

A 25 g white-footed mouse consuming 7.8 gram of food/day containing 15.7 mg Cd/kg food,

would receive a dose of 4.8 mg Cd/kg body weight/day, or a total dose of 0.12 mg Cd/day. Dosing of pregnant rats with 2 mg CdCl₂/kg/day did not affect fecundity or development of offspring (Baranski 1985), although higher doses (12 and 40 mg/kg) resulted in reduced fetal weight, fetal resorption, and placental weight. Kotsonis and Klaassen (1977) determined an oral 14-day LD₅₀ of 225 mg Cd/kg (194-261 mg/kg) in rats. No differences in packed cell volume or blood hemoglobin or glucose concentrations, aniline hydroxylase activity, cytochrome P-450 concentrations, urinary protein concentrations, or blood pressure or heart rate were noted among rats given 0, 25, 50, 100, or 150 mg Cd/kg. However, the higher doses (100 and 150 mg/kg) caused reductions in body weight, hexobarbital oxidase activity, spermatogenesis (and subsequent lowered fecundity) and daily motor activity, and produced testicular lesions.

Borzelleca et al. (1989) determined a no-observed-adverse-effects-level of 25 mg/kg/day in rats administered CdCl₂ by gavage, and 18-23 mg/kg/day in rats given CdCl₂ in drinking water. Ten mg CdCl₂/kg body weight, when daily administered to rats by intubation, reduced liver and kidney lipid levels and increased lipid peroxidation in these organs after 15 days (Gill et al. 1989). Groten et al. (1990) noted reduced growth, anemia, increased plasma enzyme activities, and reduction or cessation of splenic extramedullary hematopoiesis in young rats fed 30 mg Cd/kg diet. No microscopic changes were observed in the other organs examined.

Most studies of Cd toxicity in mammals have been conducted using rats, however, mice are apparently more sensitive to Cd exposure. Siewicki et al. (1983) reported that diets containing as little as 3.6 ppm CdCl₂, and 1.8 ppm as Cd in oyster tissue, affected Fe metabolism and resulted in anemia in young lab mice. Ten ppm (mg/L) Cd in drinking water was immunosuppressive in laboratory mice, leading to a higher death rate due to lymphocytic leukemia (Blakley 1986), and exposure to as little as 3 ppm CdCl₂ for 70 days resulted in a reduced immune response which persisted well after exposure (Koller et al. 1975). The offspring of female bank voles (*Clethrionomys glareolus*) fed 10, 50, and 100 mg/kg Cd had increased body Cd concentrations, reduced weight gain, and increased mortality (Sawicka-Kapusta and Zakrzewska 1994); young laboratory mice were more sensitive to Cd exposure in their mothers than the voles.

Concentrations and hypothetical dosages in our study were within effects levels observed in the studies of Cd toxicity in mice that we reviewed. Threshold effects concentrations/doses in studies using rats have varied; maximum Cd concentrations in seeds from DWMA fell within effects levels from some studies, and well below in others. Differences in duration of exposure, age/sex of animals, form of Cd administered and method of introduction, and especially dietary factors can influence Cd absorption and retention. In mammals, only about 5% or less of ingested Cd is absorbed from the diet (Cooke and Johnson 1996). However, Ca and Fe deficiencies can increase this proportion. Conversely, addition of dietary Ca/phosphorus, protein, and fiber significantly reduced renal Cd concentrations in rats fed diets containing 192 ppm Cd (Omori and Muto 1977), and nutritionally-deficient diets can increase Cd accumulation (Muto and Omori 1977). In another study, oral administration of Fe alleviated Cd-induced Fe-deficiency anemia in rats (Sakata et al. 1988). Seeds from DWMA contained more Fe than those from ALCA. Mean Ca concentrations were higher in corn and water hemp, and maximum concentrations were higher in buckwheat, water hemp, and smartweed, seeds from DWMA than in samples from ALCA. Levengood et al. (1999) reported that Ca concentrations were considerably higher in the digested gizzard contents of mallards (*Anas platyrhynchos*) using

DWMA. Additionally, Zn, also an antagonist of Cd, was present in higher concentrations in seeds from DWMA. Thus, it is plausible that the elevated concentrations of Ca, Fe, and Zn present in seeds from DWMA may reduce the bioavailability of Cd.

After reviewing the literature on Cd in birds, Furness (1996) suggested that intake of < 1 mg/kg food was unlikely to produce toxic effects. Inclusion of 0.2, 2, and 20 mg/kg Cd into the diet of ringed turtle doves (*Streptopelia risoria*) resulted in metallothionein induction, however, no overt signs of toxicity were observed (Schuehammer and Templeton 1990). A variety of toxic effects were observed in hatchling Japanese quail (*Coturnix coturnix*) fed 75 mg Cd/kg diet for 4 or 6 weeks (Richardson et al. 1974).

Heinz and Haseltine (1983) found that one-week old ducklings from paired black ducks (*Anas rubripes*) fed 4 ppm Cd were hyperresponsive to fright stimuli, and the authors suggested that such behavior would be detrimental under natural conditions. However, Mayak et al. (1981) detected no differences in body weight to 13 weeks and no microscopic lesions in the kidneys of wood duck ducklings fed 0, 1, and 10 ppm Cd. White and Finley (1978) detected no differences in mortality, body and organ weights, hematocrit and hemoglobin concentration, or egg production in adult mallards provided food containing 0, 2, or 20 ppm Cd. White et al. (1978) noted that "first-year" mallards fed a diet containing 2 ppm Cd for up to 90 days did not sustain significant kidney or testicular lesions, while 20 ppm Cd induced slight kidney damage in one duck, and cessation of spermatogenesis in "some" ducks. Changes in food consumption, body and organ weights, and carbohydrate metabolism produced through food restriction were enhanced by addition of 10 or 50 ppm Cd to diets of drake mallards (Di Giulio and Scanlon 1985). Changes in blood parameters and kidney lesions were detected in ducklings fed 20 ppm Cd, though not in those fed diets containing 5 or 10 ppm Cd (Cain et al. 1983). Scheuhammer (1987) suggested that long-term exposure to relatively low concentrations (5-10 ppm) may prove toxic to birds. Mean or higher Cd concentrations in some species of plant seeds we examined exceeded or approached critical concentrations for effects in birds observed in some studies, but below these levels in others.

Zn

The maximum Zn concentration observed in seeds for the different plant species from DWMA ranged from 30 to 201 mg Zn/kg seed. Maita et al. (1981) were unable to detect detrimental effects of feeding 300 and 3,000 ppm ZnSO₄ to mice and rats for 13 weeks. The higher concentration corresponded to dosages of 458 (males) and 479 mg/kg/day in mice, and 234 (males) and 243 mg/kg/day in rats. A 25 g white-footed mouse consuming 7.8 gram of food/day containing 201 mg Zn/kg food, would receive a dose of 62.7 mg Zn/kg body weight/day, or a total dose of 1.57 mg Zn/day. Saxena et al. (1989) found that 500 ppm Zn (as ZnSO₄) caused structural and functional pathologies of the testes and male accessory organs of rats after 3-6 weeks. Mice provided 0.5% (5,000 ppm) Zn as ZnCl₂ in a low Ca diet lost weight, and had reduced serum Ca concentrations, brittle femurs, and developed chromosomal aberrations (Deknudt and Gerber 1979). These effects were absent or less pronounced in animals fed a diet containing normal Ca levels. Two month-old rats given 0.12 mg Zn/cm³ (~ 12 mg Zn/kg body wt.) ZnCl₂ in drinking water experienced decreased RBC counts and hemoglobin concentrations (Zaporowski and Wasilewski 1992), although an erythropoietic response was observed in anemic

rats. In another study (Evenson et al. 1993), 12 mg Zn/kg diet did not affect spermiogenesis or chromatin structure (in the absence of high levels of silicon). Aughey et al. (1977) noted histopathologic changes in adrenals, pancreas, and pituitary gland of mice given 0.5 g/L Zn (as ZnSO₄) in drinking water for up to 14 months. However, treated animals did not lose weight, appeared healthy, and tissue Zn concentrations and plasma insulin and glucose concentrations did not differ from controls. Serum and liver cholesterol levels were not affected in rats fed 0.58 mg Zn/kg diet for 15 weeks (Fischer et al. 1980). Smith and Larson (1945) found that a diet containing 1% (10,000 ppm) Zn caused up to 75% mortality in rats. Reducing the concentration to 0.7% Zn (7,000 ppm) produced a microcytic, hypochromic anemia after 4 weeks, though "permitted the rats to live for relatively long periods of time".

Chicks fed 630 mg/kg Zn (ZnO) in a purified diet greatly increased tissue Zn levels and affected pancreatic function; however, feeding of 2,000 mg/kg Zn in a nonpurified diet produced only small increases in tissue Zn levels and did not affect pancreatic function (Lu and Combs 1988). Addition of 100 mg Zn/kg to diet of poultry chicks did not affect food intake, growth, organ weights, or Cu or Fe utilization, though the chicks accumulated more zinc and experienced nonsignificant impairment of immune function (Stahl et al. 1989a). Intake of 178 mg/kg Zn (dry weight in ration) fed to poultry hens induced slight immunosuppression in their progeny (Stahl et al. 1989b). However, in another study, supplementation of hen diets with up to 2,000 ppm ZnSO₄ did not affect food intake, hen performance, reproductive performance, or growth of progeny (Stahl et al. 1990).

Although diets containing 3,000 ppm to 12,000 ppm (dry weight) Zn resulted in anorexia, weight loss, changes in relative organ weights, paralysis, and anemia in young mallard ducks (Gasaway and Buss 1972), diets containing as high as 2,000 ppm Zn produced little or no effects on performance or hematology in poultry (*Gallus sp.*) chicks (Southern and Baker 1983), or physical and reproductive performance in hens (Stahl et al. 1990). Toxic effects were observed in chicks fed 800 ppm Zn in a diet lacking plant protein, however, these effects were not observed at 2,000 ppm when corn was added to the diet (Berg and Martinson 1972).

CONCLUSIONS

Cd, Fe, and Zn concentrations were higher in seeds of annual plants collected at DWMA than at a reference site. Mean and maximum concentrations tended to be lower in agricultural (i.e. buckwheat and corn) crops than in wild plant species. Metal concentrations in seeds were highly variable within and across the cells comprising the waterfowl impoundment.

Seed Cd and Zn concentrations in our study were within lower effects levels for mammals and birds observed in some studies. Mice are more sensitive to Cd administration than rats, and Cd concentrations were within known effects levels for the former. Although this suggests that wild, granivorous mice present on this site may be at risk, at least one previous study determined that laboratory mice are more sensitive to Cd and Pb exposure than wild rodent species. Based on the information available from prior studies, it seems unlikely that concentrations of Cd and Zn observed in seeds from DWMA are high enough to cause adverse effects in mature ducks or gallinaceous birds that might consume them, although there may be some risk to the very young of these avian groups.

Determination of critical levels has varied among studies, presumably due to differences in species/strains used, age, diet, form of contaminant administered, length of exposure, etc.. Based on critical concentrations observed in other studies, at least some exposure risk to granivorous animals utilizing DWMA exists. In determining exposure risk to granivorous mammals and birds from ingesting seeds at DWMA it is important to consider the bioavailability of metals present in plants. The presence of other elements which antagonize Cd and Zn, i.e. Ca, Cu, and Fe, along with Cd and Zn themselves, particularly when available in elevated concentrations could reduce bioavailability of Cd. Additionally, the relative importance of food items in the diet of each species needs to be considered. Although the seeds of wild plants we examined had the highest concentrations of Cd and Zn, buckwheat and especially corn were more important foods for mallards along the upper Illinois River during the fall migration in terms of percent volume in the gizzard than Pennsylvania smartweed, giant ragweed, or water hemp (Havera 1999). Although corn was by far the single most important food for wood ducks in terms of volume, water hemp figured prominently in diet of wood ducks in Illinois.

Our objective in this study was to examine the potential risk to wildlife consuming seeds at DWMA, since the production of annual plants is encouraged at this site. We acknowledge that other routes of exposure, e.g. ingestion of soils, plant parts other than seeds, and invertebrates may also be important exposure routes for granivorous animals, depending on the species, season, etc.. Studies of the effects of many contaminants in wild species at environmentally-realistic concentrations are lacking, making plausible inferences about risk difficult. Further information is needed on exposure, tissue concentrations, population levels/demographics, individual health of representative granivorous species (e.g. mice) present at DWMA.

Acknowledgments - The assistance and expertise of the following is gratefully acknowledged: K. Caldwell, R. Cox, S. Davis, R. Farrell, C. Hine, M. Joselyn, S. Lasovsky, M. Piwoni, M. Resetich, A. Yetter, and B. Zercher. Funding was provided by the Illinois Waste Management and Research Center of the Illinois Department of Natural Resources.

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Fig. 1. Map of DePue, Illinois, area depicting the location of the DePue Wildlife Management Area waterfowl impoundment relative to DePue Lake, Illinois River, and the former industrial complex.

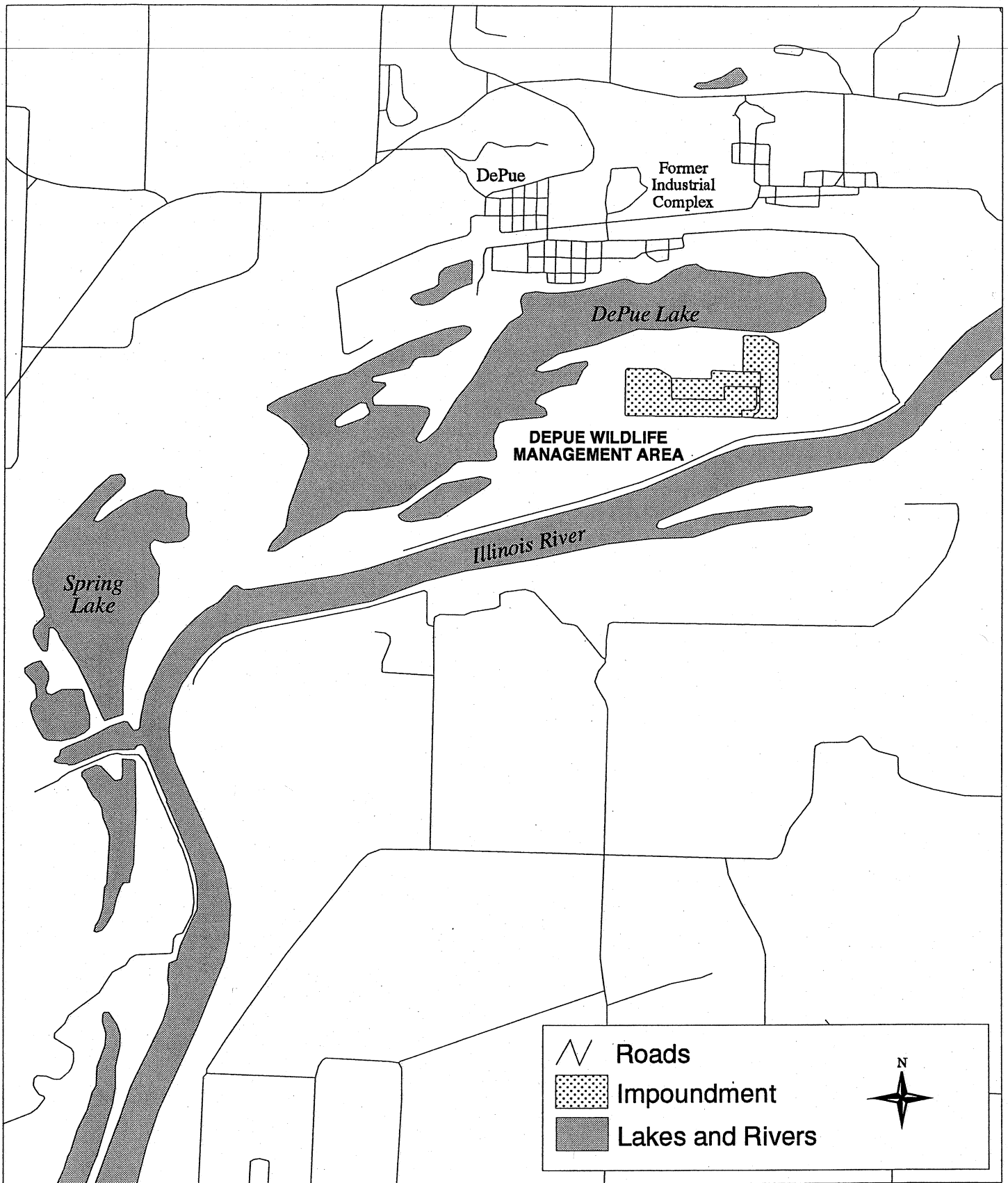


Fig. 2. Map depicting seed sampling locations within the three cells comprising the waterfowl impoundment at DePue Wildlife Management Area, DePue, Illinois.

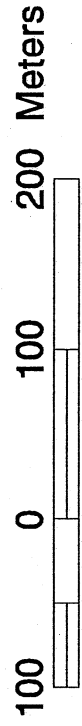
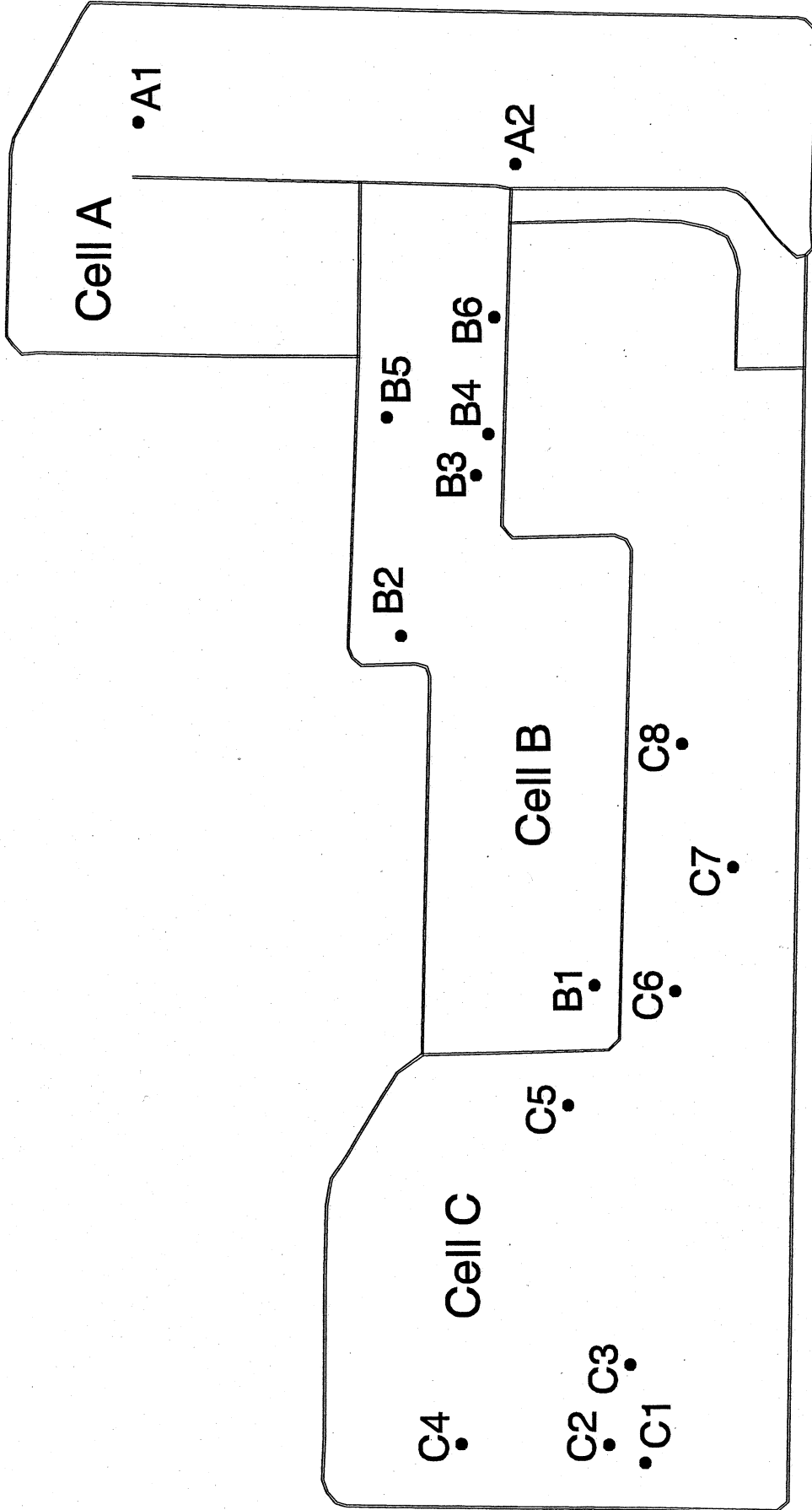


Table 1. Concentrations (ppm dry wt.) of selected elements in seeds collected at DePue Wildlife Management Area and Anderson Lake Conservation Area, Illinois, 1997.

Plant Species	Site ^a	n	Element				
			Ba	Cd	Cu	Fe	Zn
Buckwheat (<i>Fagopyrum esculentum</i>)	DWMA	2	1.68 ± 0.85 ^b 0.83 - 2.53	5.51 ± 0.57 4.94 - 6.07	18.3 ± 7.3 11.0 - 25.6	67.3 ± 6.4 60.9 - 73.6	77.8 ± 10.2 77.8 - 88.0
	ALCA	1	1.65 --- ---	2.90 --- ---	7.93 --- ---	59.8 --- ---	30.5 --- ---
Corn (<i>Zea mays</i>)	DWMA	9	--- ^c ---	1.13 ± 0.13 0.75 - 1.70	3.11 ± 0.14 2.50 - 3.77	57.5 ± 18.8 21.5 - 202.0	51.5 ± 1.9 39.5 - 57.2
	ALCA	7	--- ---	0.81 ± 0.16 0.37 - 1.19	3.02 ± 0.33 1.57 - 4.11	22.1 ± 1.4 16.3 - 25.1	24.7 ± 2.2 14.3 - 30.6
Water Hemp (<i>Amaranthus rudis</i>)	DWMA	7	16.0 ± 2.9 4.9 - 24.5	11.8 ± 1.3 7.0 - 15.7	8.40 ± 0.48 7.29 - 10.30	119.6 ± 16.1 79.0 - 203.0	144.3 ± 18.1 93.2 - 201.0
	ALCA	6	16.5 ± 2.6 8.87 - 24.6	2.91 ± 0.19 2.22 - 3.62	8.39 ± 0.33 7.21 - 9.10	106.1 ± 4.5 97.0 - 127.0	43.7 ± 2.5 35.7 - 51.6
Giant Ragweed (<i>Ambrosia trifida</i>)	DWMA ^d	4	1.66 ± 0.62 0.62 - 3.01	7.77 ± 1.05 5.55 - 10.40	22.3 ± 0.89 20.9 - 24.8	92.1 ± 32.7 41.3 - 188.0	76.5 ± 6.1 65.4 - 88.0
Penna. Smartweed (<i>Polygonum pennsylvanicum</i>)	DWMA	12	3.16 ± 0.43 1.45 - 5.81	6.69 ± 0.81 3.19 - 12.9	10.6 ± 2.0 5.58 - 30.50	71.2 ± 5.8 44.6 - 119.0	79.1 ± 7.5 29.9 - 126.0
	ALCA	5	3.80 ± 0.27 2.88 - 4.52	4.38 ± 0.71 2.32 - 6.60	7.91 ± 1.16 6.29 - 12.5	54.0 ± 6.5 39.0 - 76.3	33.3 ± 3.16 26.6 - 45.1

^aDWMA = DePue Wildlife Management Area; ALCA = Anderson Lake Conservation Area

^bData are mean ± S.E, minimum - maximum

^cAll corn samples were below MDL

^dGiant ragweed was not collected at ALCA

Table 2. Concentrations (ppm dry wt.) of cadmium (Cd) and zinc (Zn) in corn (*Zea mays*), smartweed (*Polygonum pennsylvanicum*), and water hemp (*Amaranthus rudis*) seeds collected from the "3-Is" impoundment at DePue Wildlife Management Area, Illinois, 1997.

Plant Species	Cell ^a	n	Element	
			Cd	Zn
Corn (<i>Zea mays</i>)	A	2	1.34 ± 0.36 ^b 0.98 - 1.70	49.2 ± 3.8 45.4 - 53.0
	B	3	0.81 ± 0.03 0.77 - 0.87	53.7 ± 1.2 51.4 - 55.4
	C	4	1.26 ± 0.20 0.75 - 1.64	50.9 ± 4.1 36.5 - 57.2
Penna. Smartweed (<i>Polygonum pennsylvanicum</i>)	A	3	6.31 ± 0.72 4.96 - 7.42	83.5 ± 3.8 76.4 - 89.2
	B	3	7.31 ± 1.16 5.33 - 9.35	93.7 ± 10.9 75.2 - 113.0
	C	6	6.57 ± 1.56 3.19 - 12.9	69.6 ± 13.5 29.9 - 126.0
Water Hemp (<i>Amaranthus rudis</i>)	A	1	10.6 --- ---	93.2 --- ---
	B	4	10.8 ± 2.08 7.00 - 15.7	129.8 ± 18.8 106.0 - 185.0
	C	2	14.5 ± 0.08 13.7 - 15.3	199.0 ± 2.0 197.0 - 201.0

^aCell A = east, Cell B = northcentral, Cell C = west/southcentral

^bData are mean ± S.E., minimum - maximum, and (sample size)