

Understanding and Mitigating Variability in Field Uptake of Heavy Metals in Winter Wheat

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INTRODUCTION

The United States Food and Drug Administration (FDA) recently released its Closer to Zero (C2Z) Action Plan to reduce exposure to heavy metals from foods eaten by infants and young children to the lowest levels reasonably achievable (FDA, 2024). The motivation behind this action plan was the 2021 Congressional Staff Report on high levels of arsenic (As), cadmium (Cd), and lead (Pb) found in store-bought infant foods, (House of Representatives, 2021) and the potential neurocognitive impairment to infants and young children exposed to these toxic heavy metals (Roy & McDonald, 2015; Thomas, 2021; Wasserman et al., 2014). The FDA is anticipated to set action levels for heavy metals in “baby and young children’s foods” with significant economic implications for growers and food processors. To ensure a safe food supply, it is crucial to understand the factors and risks that drive crop uptake of heavy metals.

In Michigan, winter wheat (*Triticum aestivum* L.) is the third largest row crop in area (USDA-NASS, 2023). Wheat flour is used as a multi-grain cereal component in infant and young children's food (Gerber website, 2024). Among the heavy metals, Cd was the most prevalent and highest in translocation from soil to plant in grain crops (Ata-Ul-Karim et al., 2020; Bashir et al., 2020), but the understanding of As and Pb accumulation in cereals is limited. Ensuring low winter wheat heavy metal accumulation is critical not only for the substantial number of sensitive consumers and potential neurocognitive impairments but also to limit the rejection of winter wheat at time of sale by food processors and substantive economic losses to growers.

OBJECTIVES

Soil Amendment Trial - Investigate winter wheat uptake of heavy metals and determine whether specific nutrients or soil amendments may reduce heavy metal uptake or concentrations within winter wheat.

Slope Trial – Evaluate the spatiotemporal variability of heavy metal (As, Pb, and Cd) uptake from soil and winter wheat grain on a gradual slope from summit to mid-slope to toe-slope.

METHODOLOGY

Amendment Trial

A field experiment was established in Lansing, MI (42.6891765, -84.4850320). Plots were 12 rows wide (8.5 ft. width by 25 ft. length by 7.5 inch. row spacing) and planted using a Great Plains 3P600 drill (Great Plains Manufacturing, Salina, KS). The experimental site was established on a Conover loam soil (Fine-loamy, mixed, active, mesic *Aquic Hapludalfs*) with a surface layer of 41.6% sand, 39.2% silt and 19.2% clay (National Cooperative Soil Survey, 2018). Soft red winter wheat variety ‘Wharf’ was planted at 1.8 million A⁻¹. Pre-plant soil characteristics (0–8 inch.) included 7.1 pH (1:1 soil/water) (Peters et al., 2015), 33 mg kg⁻¹ P (Bray-P1) (Frank et al., 2015), 80 mg kg⁻¹ K (ammonium acetate method) (Warncke & Brown, 2015), 23 g kg⁻¹ soil organic matter (loss-on-ignition) (Combs & Nathan, 2015), and 3.4 mg kg⁻¹ Zn (0.1 M HCl) (Whitney, 2015). Soil nitrate concentration (0–12 inch.) was collected prior to planting with 3 mg NO₃-N kg⁻¹ soil (nitrate electrode method) (Gelderman & Beegle, 2015). Prior to the field experiment, the average soil cadmium (Cd), arsenic (As), and lead (Pb) concentrations were 0.46, 4.70, and 12.46 mg kg⁻¹ soil.

A randomized complete block design with four replications was established. Treatments investigated included: 1) control, 2) pre-plant agricultural lime (2 T A⁻¹), 3) pre-plant dairy compost (5 T A⁻¹), 4) pre-plant biochar (2 T A⁻¹), 5) pre-plant gypsum (1 T A⁻¹), 6) pre-plant granular ZnSO₄ (10 lbs. Zn A⁻¹) and foliar ZnSO₄ (1 pint A⁻¹) at Feekes (FK) 9, 7) low N (50 lbs. N A⁻¹) at FK 4, 8) moderate N (100 lbs. N A⁻¹) at FK 4, 9) high N (150 lbs. N A⁻¹) at FK 4, and 10) biodegradable chelating agent ethylenediaminedisuccinic acid (EDDS) sprays with a 2 mmol L⁻¹ concentration applied at FK 5, FK 5 + 1 week, and FK 5 + 2 weeks. Autumn starter fertilizer was top-dressed at a rate of 125 lbs. A⁻¹ during planting except check. All treatments received a base green-up N application rate of 100 lbs. A⁻¹ of urea (46-0-0) except check and N fertilizer treatments.

Slope Trial

A field experiment was established in Clarksville, MI (42.8736347, -85.2586937). Plots were 11.3 ft width by 50 ft. length. Soft red winter wheat variety ‘Wharf’ was planted at 1.8 million A⁻¹. Pre-plant soil characteristics (0–8 inch.) included 6.3 pH (1:1 soil/water) (Peters et al., 2015), 125 mg kg⁻¹ P (Bray-P1) (Frank et al., 2015), 184 mg kg⁻¹ K (ammonium acetate method) (Warncke & Brown, 2015), 1.6 g kg⁻¹ soil organic matter (loss-on-ignition) (Combs & Nathan, 2015), and 4.6 mg kg⁻¹ Zn (0.1 M HCl) (Whitney, 2015). Prior to the field experiment, the average soil cadmium (Cd), arsenic (As), and lead (Pb) concentrations were 0.47, 5.50, and 13.46 mg kg⁻¹ soil.

Transects (six replicates) were established across three slope positions (summit, midslope, and toeslope) resulting in 18 sampling locations per crop-year. For maintenance, 100 lbs. N A⁻¹ of 46-0-0 was applied at Feekes 5.

Data Gathering

Both trials: Four random soil cores (0-8 inch. depth) were sampled from each plot at Feekes 4, 9 (slope trial only), and post-harvest. Tillers at Feekes 4 and flag leaves at Feekes 9 were washed with tap water to remove soil particles followed by two washes with deionized water. At Feekes 4, the tillers were separated into shoots and roots using a Teflon knife after air drying. The shoots were retained while the roots were discarded. Wheat shoots and flag leaves were dried at 158 °F for 72 hours before being ground to 1 mm (UDY Cyclone sample mill). Grain samples were manually cleaned by removing excess husk. Winter wheat grain (50g) was ground into a coarse powder using an electric coffee grinder (Hamilton Beach®, Richmond, VA) for 1 minute.

Amendment Trial only: Plant tiller counts were taken from 1ft² plot⁻¹ at Feekes 4. At Feekes 11.1, plant height, head density ft⁻², and head length were measured. Plant height was determined from the soil surface to the top of each spike. Head density was calculated by counting the head-bearing tillers within 1ft². Head length was measured using a digital vernier caliper. On 01 July 2024, the outer 5ft. of plots were mowed prior to harvest. Grain yield was collected from the center 5ft. by 20ft. in each plot using a plot combine (Kincaid Equipment Manufacturing, Haven, KS).

Slope Trial only: Soil moisture (0-8 inch. depth) was measured in five random points each plot using Spectrum TDR 250 Economy Moisture Meter (Bridgend, UK).

AMENDMENT TRIAL RESULTS

Grain yield: Grain yield ranged from 18.2-109.2 bu. A⁻¹ with a mean of 83.6 bu. A⁻¹. Low N decreased grain yield ($P < 0.0001$) by 19.1-35.8 bu. A⁻¹ compared to the remaining soil amendments (data not shown) indicating few adverse effects from tested soil amendments on wheat grain yield.

Bulk soil heavy metal concentrations: Bulk soil samples at Feekes 4 had comparable soil Cd, As, and Pb levels with check (Table 1). Soil Cd levels ranged from 0.13-0.36 mg kg⁻¹ soil with a mean of 0.25 mg kg⁻¹ soil. Soil As levels ranged from 2.58-3.89 mg kg⁻¹ soil with a mean of 3.27 mg kg⁻¹ soil greater than the statewide average of 2.50 mg kg⁻¹ soil (State of Michigan, 2023). Soil Pb levels ranged from 8.52-25.37 mg kg⁻¹ soil with a mean of 12.06 mg kg⁻¹ soil.

Bulk soil samples at harvest had comparable soil As and Pb levels with check (Table 1). Among soil amendments, ZnSO₄ increased soil Cd level by 0.047 mg kg⁻¹ soil. Soil Cd levels ranged from 0.15-0.43 mg kg⁻¹ soil with a mean of 0.24 mg kg⁻¹ soil. Soil As levels range from 1.56-3.99 mg kg⁻¹ soil with a mean of 2.47 mg kg⁻¹ soil. Soil Pb levels ranged from 7.76-12.11 mg kg⁻¹ soil with a mean of 9.91 mg kg⁻¹ soil. Across both sampling periods, the order of soil heavy metal concentrations was Pb > As > Cd (Table 1).

Plant tissue heavy metal levels: For both Feekes 4 and 9, Pb was the most prevalent heavy metal followed by Cd and As. However, Cd was the most dominant heavy metal at harvest followed by Pb and As (Table 2).

The biomass of soil amendments at Feekes 4 and grains at harvest had comparable Cd concentrations with check (Table 2). Cadmium accumulation began in biomass at Feekes 4 ranging from 0.22-0.60 mg kg⁻¹ dw with a mean of 0.34 mg kg⁻¹ dw. At Feekes 9, all soil amendments decreased flag leaf Cd level by 0.26-0.28 mg kg⁻¹ with lime showing the greatest reduction compared to the control. Flag leaf total Cd levels ranged from 0.04-0.99 mg kg⁻¹ dw with a mean of 0.15 mg kg⁻¹ dw. Grain total Cd levels at harvest ranged from 0.019-0.053 mg kg⁻¹ fw with a mean of 0.033 mg kg⁻¹ fw. Across sampling, the order of winter wheat stages with plant tissue Cd levels was Feekes 4 > Feekes 9 > grains at harvest.

Biomass total As levels were below the detection limit ($< 1.736 \times 10^{-6}$ mg kg⁻¹) at Feekes 4. Arsenic accumulation began in the flag leaf at Feekes 9 ranging from 0.06-0.64 mg kg⁻¹ dw with a mean of 0.16 mg kg⁻¹ dw (Table 2). Soil amendments reduced flag leaf As concentrations by 0.18-0.24 mg kg⁻¹ at Feekes 9 compared to the check plot. Grains at harvest had comparable As levels with check ranging from 0.002-0.006 mg kg⁻¹ fw with an average of 0.003 mg kg⁻¹ fw. Across sampling, the order of winter wheat stages with plant tissue As levels was Feekes 9 > grains at harvest >> Feekes 4.

Biomass at Feekes 4 and grains at harvest had comparable Pb levels with check (Table 2). Lead accumulation began in biomass at Feekes 4 ranging from 0.34-3.54 mg kg⁻¹ dw with a mean of 1.33 mg kg⁻¹ dw. At Feekes 9, the addition of ZnSO₄ decreased flag leaf Pb level by 2.90 mg kg⁻¹ dw. Flag leaf total Pb levels ranged from 0.06-7.71 mg kg⁻¹ dw with a mean of 1.57 mg kg⁻¹ dw. Grain total Pb levels at harvest ranged from 0.003-0.073 mg kg⁻¹ fw with a mean of 0.016 mg kg⁻¹ fw. Among the mean grain Pb concentrations of soil amendments, lime (0.01 mg kg⁻¹) and gypsum (0.007 mg kg⁻¹) were within the allowable limit set by FDA (0.01 mg kg⁻¹). Across sampling, the order of winter wheat stages with plant tissue Pb levels was Feekes 4 = Feekes 9 > grains at harvest.

Correlations between grain As, Cd and Pb concentrations with in-season measurements:

Grain Cd levels were weakly negatively correlated with head length (R = -0.37) and moderately negatively correlated with soil pH (R = -0.42). Grain Pb levels were weakly negatively correlated with plant height (R = -0.34) and grain yield (-0.32) (Fig. 1).

DISCUSSION

Pre-plant broadcast lime at 4.5 MT ha⁻¹ reduced flag leaf Cd and grain Cd concentrations by 0.28 mg kg⁻¹ dry weight (dw) and 0.012 mg kg⁻¹ fresh weight (fw), respectively, compared to the control. In soil solution, Cd occurs predominantly as Cd²⁺. As alkalinity increases, Cd adsorption decreases due to the competitive adsorption of Ca²⁺ and Mg²⁺ ions.

Pre-plant broadcast lime at 4.5 MT ha⁻¹ and pre-plant broadcast biochar at 1.8 MT ha⁻¹ reduced flag leaf As concentrations, by 0.24 and 0.23 mg kg⁻¹, respectively, compared to the

control, while all soil amendments maintained grain As levels between 0.003–0.006 mg kg⁻¹. The FDA action level for inorganic As in cereals is 0.1 mg kg⁻¹. The current study does not quantify inorganic As forms such as arsenate [As(V)] or arsenic trioxide [As(III)]. Nonetheless, using total As levels, we attempted to demonstrate winter wheat As accumulation. Hypothetically, with only 10–20% of total As being inorganic, the current samples are approximately 300–400 times lower than the FDA's computed total As action level (1–2 mg kg⁻¹). Lime provides CaCO₃ which arsenate ions may substitute for CO₃²⁻ in calcite, suggesting that As may be immobilized. On the other hand, carbon-based organic amendments, such as wood-based biochar, may act as electron donors and provide energy sources for soil microorganisms, promoting the reduction of As(V) to the more mobile inorganic form As(III). Arsenic accumulation began only at Feekes 9 and was lowest in grains suggesting that As is less mobile than Cd and Pb and may not pose a significant threat.

Pre-plant broadcast lime at 4.5 MT ha⁻¹ and gypsum (CaSO₄·2H₂O) were within the allowable Pb level set by the FDA (0.01 mg kg⁻¹). Lead exists in oxidized conditions as Pb²⁺ and becomes less soluble with increasing pH (> 6) because phosphates, hydroxides, and carbonates are the most dominant insoluble forms of Pb. Calcium ions from gypsum may have displaced Pb in the soil exchangeable complex, while SO₄²⁻ ions may have reduced Pb phytoavailability through the formation of metal-sulfate complexes.

Table 1. Winter wheat cadmium, arsenic, and lead bulk soil concentrations (mg kg⁻¹) at Feekes 4, harvest, and post-harvest soil pH, Lansing, MI. 2024. Mean Cd, As, and Pb concentrations of check plots displayed. All other treatments display change in Cd, As, and Pb levels using Dunnett's test.

Treatment	Bulk soil at Feekes 4 ^a			Bulk soil at harvest ^b			Post-harvest
	Cd	As	Pb	Cd	As	Pb	Soil pH ^c
	mg kg ⁻¹ soil						
Check ^d	0.25	3.29	12.48	0.22	2.56	9.77	6.70
+ Lime	-0.016 ^{ns§}	+0.18 ^{ns}	-1.01 ^{ns}	+0.035 ^{ns}	-0.20 ^{ns}	-0.19 ^{ns}	-0.06 ^{ns}
+ Dairy compost	+0.019 ^{ns}	+0.10 ^{ns}	-1.39 ^{ns}	+0.031 ^{ns}	+0.42 ^{ns}	+0.61 ^{ns}	-0.05 ^{ns}
+ Biochar	+0.001 ^{ns}	+0.02 ^{ns}	-1.26 ^{ns}	+0.021 ^{ns}	-0.24 ^{ns}	+0.44 ^{ns}	-0.02 ^{ns}
+ Gypsum	-0.016 ^{ns}	-0.16 ^{ns}	-0.55 ^{ns}	+0.014 ^{ns}	-0.36 ^{ns}	-0.03 ^{ns}	-0.16 ^{**}
+ ZnSO ⁴	-0.040 ^{ns}	-0.26 ^{ns}	-0.96 ^{ns}	+0.047 [*]	-0.25 ^{ns}	+0.54 ^{ns}	-0.08 ^{ns}
+ Low N	NA [¶]	NA	NA	+0.028 ^{ns}	+0.37 ^{ns}	+0.35 ^{ns}	-0.04 ^{ns}
+ Mod. N	NA	NA	NA	+0.017 ^{ns}	-0.22 ^{ns}	+0.26 ^{ns}	-0.04 ^{ns}
+ High N	NA	NA	NA	-0.016 ^{ns}	-0.02 ^{ns}	-0.15 ^{ns}	-0.13 [*]
+ EDDS	NA	NA	NA	-0.011 ^{ns}	-0.35 ^{ns}	-0.25 ^{ns}	+0.03 ^{ns}
Range	0.13-0.36	2.58-3.89	8.52-25.37	0.15-0.43	1.56-3.99	7.76-12.11	6.39-6.85
Overall mean	0.25	3.27	12.06	0.24	2.47	9.91	6.64

^aDunnett's test degrees of freedom = 18; ^bDunnett's test degrees of freedom = 27; ^cDunnett's test degrees of freedom = 26; ^d Autumn starter fertilizer was top-dressed at a rate of 125 lbs. A⁻¹ during planting except check. All treatments received a base green-up N application rate of 100 lbs. A⁻¹ of urea (46-0-0) except check and N fertilizer treatments. §Asterisks indicate thresholds of significance (NS, P > 0.10; *, P < 0.10; **, P < 0.05; ***, P < 0.001); ¶NA - not applicable, treatments were excluded since they had not been applied before the Feekes 4 sampling.

Table 2. Winter wheat cadmium, arsenic, and lead tissue concentrations (mg kg⁻¹) at Feekes 4, flag leaf at Feekes 9, and grains at harvest, Lansing, MI. 2024. Mean As, and Pb levels of check plots displayed. All other treatments display change in Cd, As, and Pb levels using Dunnett's test.

Treatment	Biomass Feekes 4 ^a		Flag leaf Feekes 9 ^b			Grains at harvest ^c		
	Cd	Pb	Cd	As	Pb	Cd	As	Pb
	mg kg ⁻¹							
Check ^d	0.32	1.06	0.39	0.36	3.16	0.041	0.003	0.010
+ Lime	-0.03 ^{ns} §	+0.66 ^{ns}	-0.28 [*]	-0.24 ^{**}	-1.06 ^{ns}	-0.012 [*]	-0.0006 ^{ns}	+0.000 ^{ns}
+ Dairy compost	-0.03 ^{ns}	+0.01 ^{ns}	-0.25 [*]	-0.21 ^{**}	-2.34 ^{ns}	-0.006 ^{ns}	-0.0006 ^{ns}	+0.005 ^{ns}
+ Biochar	-0.02 ^{ns}	+0.73 ^{ns}	-0.26 [*]	-0.23 ^{**}	-2.26 ^{ns}	-0.014 [*]	-0.0003 ^{ns}	+0.017 ^{ns}
+ Gypsum	+0.11 ^{ns}	+0.41 ^{ns}	-0.27 [*]	-0.21 ^{**}	-2.39 ^{ns}	-0.005 ^{ns}	-0.0004 ^{ns}	-0.003 ^{ns}
+ ZnSO ₄	+0.05 ^{ns}	-0.29 ^{ns}	-0.26 [*]	-0.21 ^{**}	-2.90 [*]	-0.011 [*]	-0.0004 ^{ns}	+0.001 ^{ns}
+ Low N	NA ¶	NA	-0.27 [*]	-0.18 ^{**}	-2.38 ^{ns}	-0.011 [*]	-0.0001 ^{ns}	+0.017 ^{ns}
+ Mod. N	NA	NA	-0.24 [*]	-0.21 ^{**}	-1.04 ^{ns}	-0.008 ^{ns}	+0.0000 ^{ns}	+0.011 ^{ns}
+ High N	NA	NA	-0.26 [*]	-0.20 ^{**}	-0.86 ^{ns}	-0.004 ^{ns}	+0.0004 ^{ns}	+0.008 ^{ns}
+ EDDS	NA	NA	-0.26 [*]	-0.19 ^{**}	-0.68 ^{ns}	-0.012 [*]	-0.0002 ^{ns}	+0.007 ^{ns}
Range	0.22- 0.60	0.34- 3.54	0.04- 0.99	.06- 0.64	0.06- 7.71	0.019- 0.053	0.002- 0.006	0.003- 0.073
Overall mean	0.34	1.33	0.15	0.16	1.57	0.033	0.003	0.016

^aDunnett's test degrees of freedom = 18; ^bDunnett's test degrees of freedom = 27; ^cDunnett's test degrees of freedom = 26; ^d Autumn starter fertilizer was top-dressed at a rate of 125 lbs. A⁻¹ during planting except check. All treatments received a base green-up N application rate of 100 lbs. A⁻¹ of urea (46-0-0) except check and N fertilizer treatments. § Asterisks indicate thresholds of significance (NS, P > 0.10; *, P < 0.10; **, P < 0.05; ***, P < 0.001); ¶ NA - not applicable, treatments were excluded since they had not been applied before the Feekes 4 sampling.

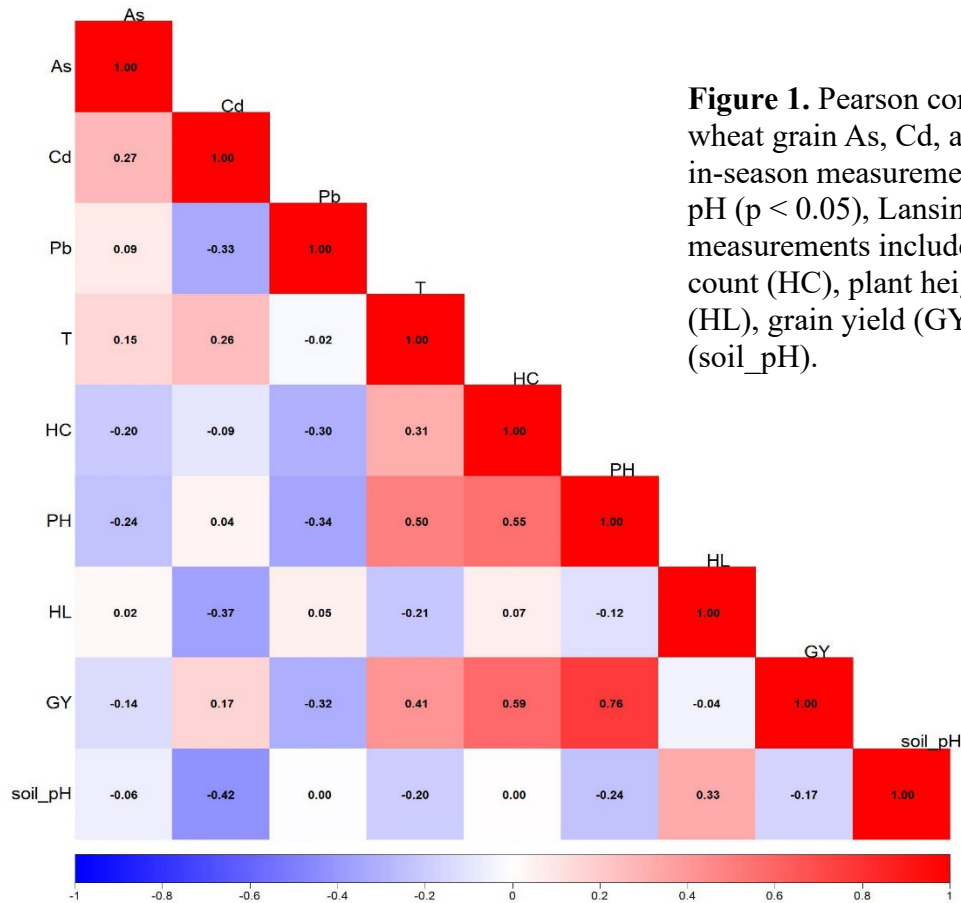


Figure 1. Pearson correlation between winter wheat grain As, Cd, and Pb at harvest with in-season measurements and post-harvest soil pH ($p < 0.05$), Lansing, MI. 2024. In-season measurements include tiller count (T), head count (HC), plant height (PH), head length (HL), grain yield (GY), post-harvest soil pH (soil_pH).

SLOPE TRIAL RESULTS

Soil moisture: The experimental site was established on a Lapeer sandy loam (Coarse-loamy, mixed, active, mesic *Mollic Hapludalfs*) with a surface layer of 64.6% sand, 25.6% silt and 9.8% clay (National Cooperative Soil Survey, 2018). Using surveying equipment, there was about 6 ft. elevation difference from summit to toeslope. Across sampling periods, both toeslope and summit had comparable soil moisture content except during Feekes 9 when there was 0.19 and 0.48 inch. total precipitation within 4 and 7-day before sampling (Table 3).

Bulk soil heavy metal concentrations: Mean soil Cd ($0.16\text{--}0.20\text{ mg kg}^{-1}$) and Pb ($8.00\text{--}8.50\text{ mg kg}^{-1}$) concentrations in toeslope and summit were comparable followed by midslope (0.10 mg kg^{-1} Cd, 6.01 mg kg^{-1} Pb) at Feekes 4 (soil Cd $P=0.0290$; soil Pb $P=0.0054$). Soil As levels were greatest in summit (2.11 mg kg^{-1}) followed by toeslope (1.66 mg kg^{-1}) and mid-slope (1.58 mg kg^{-1}) (Table 4) ($P=0.0480$).

Mean soil Cd ($0.09\text{--}0.13\text{ mg kg}^{-1}$) and Pb levels ($6.86\text{--}9.53\text{ mg kg}^{-1}$) across slope positions were comparable at Feekes 9 (soil Cd $P=0.3479$; soil Pb $P=0.1757$). Soil As levels were greatest at summit (2.47 mg kg^{-1}), followed by mid-slope (1.8 mg kg^{-1}) and toeslope (1.19 mg kg^{-1}) (Table 4) ($P=0.0042$).

Toeslope had greatest soil Cd (0.13 mg kg^{-1}) and Pb (7.64 mg kg^{-1}) concentrations at harvest followed by mid-slope (0.08 mg kg^{-1} Cd, 4.97 mg kg^{-1} Pb) and summit (0.06 mg kg^{-1} Cd, 4.50 mg kg^{-1} Pb) (soil Cd $P=0.0001$; soil Pb $P=0.0006$). Soil As levels were comparable at summit (2.11 mg kg^{-1}) and toeslope (1.90 mg kg^{-1}) followed by mid-slope (1.44 mg kg^{-1}) (Table 4) ($P=0.0616$).

Post-harvest soil pH was significantly different across slope positions with summit at the greatest soil pH (6.18), followed by toeslope (5.97) and mid-slope (5.89) ($P=0.0021$).

Plant tissue heavy metal concentrations: Average heavy metal Feekes 4 biomass concentrations across slope positions were comparable (Cd = $0.41\text{-}0.69 \text{ mg kg}^{-1}$; As = $0.48\text{-}0.52 \text{ mg kg}^{-1}$; Pb $1.44\text{-}2.66 \text{ mg kg}^{-1}$) (Table 5) (biomass As $P=0.9704$; Cd $P=0.3463$; Pb $P=0.4306$).

Mean flag leaf As and Pb concentrations at Feekes 9 were not statistically different across slope positions (As = $0.12\text{-}0.16 \text{ mg kg}^{-1}$; Pb = $0.94\text{-}1.92 \text{ mg kg}^{-1}$) (flag leaf As $P=0.3588$; Pb $P=0.6933$). Toeslope had greatest flag leaf Cd level (0.10 mg kg^{-1}) followed by summit (0.06 mg kg^{-1}) and mid-slope (0.04 mg kg^{-1}) (Table 5) (flag leaf Cd $P=0.0650$).

Grain heavy metal concentrations at harvest were comparable across slope positions with Cd ($0.03\text{-}0.04 \text{ mg kg}^{-1}$) > Pb ($0.009\text{-}0.02 \text{ mg kg}^{-1}$) > As (0.003 mg kg^{-1}) (grain As $P=0.2488$; Cd $P=0.1942$; Pb $P=0.6975$). Among the mean grain Pb concentrations of slope positions, summit (0.01 mg kg^{-1}) and mid-slope (0.009 mg kg^{-1}) were within the allowable limit set by FDA (0.01 mg kg^{-1}).

Correlating grain As, Cd and Pb concentrations with soil moisture and bulk soil analysis at harvest: Grain As was weakly positively correlated ($R=0.27$) and grain Cd was weakly negatively correlated ($R=-0.23$) with soil moisture at harvest (Figure 2). Grain As was strongly negatively correlated with soil P ($R=-0.70$) but moderately negatively correlated with soil Ca, Mn, Zn, Cd, and Pb ($R=-0.44$ to -0.56). Grain Cd was moderately negatively correlated with soil Mg, V, Cr, Fe, Mn, Co, Ni, and As ($R=-0.44$ to -0.49). Grain Cd was also negatively moderately correlated with soil pH ($R=-0.40$). Grain Pb was moderately positively correlated with soil Mn and Pb ($R=0.41$ to 0.42). Grain Pb was weakly positively correlated with soil pH ($R=0.27$).

DISCUSSION

Toeslope and mid-slope consistently had the greatest and lowest soil moisture, respectively, while summit followed toeslope when rainfall occurred within a 4-day window prior to sampling. Among the heavy metals, Cd was the most predominant in grains. Cadmium retention in soil is governed by sorption reactions, with soil pH being the primary controlling factor. Soil Cd availability generally increases by 1.5 times with a one-unit decrease in soil pH. At harvest, toeslope and mid-slope had lower soil pH compared to the summit. However, toeslope had higher soil Cd concentrations than mid-slope resulting in relatively greater bioavailability. Cadmium uptake is not regulated by plant demand, and Cd absorption increases with higher soil Cd concentrations.

Toeslope had the greatest grain Pb concentrations exceeding the FDA action level. Soil Pb behavior depends on organic matter (OM) content as Pb has a significant affinity for partially degraded humic fractions. Although OM analysis has not yet been conducted to quantify total OM content across slope positions, it is reasonable to assume that OM deposition occurred from higher slope positions (i.e., summit) to the toeslope. This likely increased the opportunity for Pb to persist as organic ligands which are soluble and more readily available for plant uptake.

Table 3. Mean soil moisture (% VMC)^a in bulk soil across slope positions and total precipitation data (inch.)^b at Feekes 5, 9, and harvest, Clarksville, MI. 2024.

Feekes 5	Soil moisture (%)	13-16 April 2022 (4-day)	09-16 April (7-day)
Toeslope	10.2 a	0.00	0.55
Midslope	5.5 b		
Summit	9.0 a		
P > F ^c	**		
Feekes 9		11-14 May 2024 (4-day)	07-14 May 2024 (7-day)
Toeslope	15.1 a	0.19	0.48
Midslope	10.2 b		
Summit	10.7 b		
P > F	**		
Harvest		09-12 July (4-day)	05-12 July (7-day)
Toeslope	10.3 a	0.87	1.16
Midslope	6.9 b		
Summit	10.6 a		
P > F	***		

^aAverage soil moisture (0-8 inch. depth) measured using Spectrum TDR 250 Economy Moisture Meter (Bridgend, UK). ^bMSU Enviro-weather (<https://enviroweather.msu.edu/>, MSU, East Lansing, MI). ^cAsterisks indicate thresholds of significance (NS, P > 0.10; *, P < 0.10; **, P < 0.05; ***, P < 0.001). Values followed by the same lowercase letter are not statistically different at 0.10 probability level, Fisher's least significant difference (LSD).

Table 4. Mean cadmium, arsenic, and lead concentrations (mg kg⁻¹) in bulk soils at Feekes 4, 9, and harvest, Clarksville, MI. 2024.

Slope	Bulk soil at Feekes 4			Bulk soil at Feekes 9			Bulk soil at harvest			Post-harvest
	Cd	As	Pb	Cd	As	Pb	Cd	As	Pb	Soil pH
	mg kg ⁻¹									
Toeslope	0.16 a	1.66 b	8.50 a	0.12	1.19 c	7.03	0.13 a	1.90 a	7.64 a	5.97 b
Midslope	0.10 b	1.58 b	6.01 b	0.13	1.80 b	9.53	0.08 b	1.44 b	4.97 b	5.89 b
Summit	0.20 a	2.11 a	8.00 a	0.09	2.47 a	6.86	0.06 b	2.11 a	4.50 b	6.18 a
P > F ^a	**	**	**	NS	**	NS	*	**	**	**

^aAsterisks indicate thresholds of significance (NS, P > 0.10; *, P < 0.10; **, P < 0.05; ***, P < 0.001). Values followed by the same lowercase letter are not statistically different at 0.10 probability level, Fisher's least significant difference (LSD).

Table 5. Mean cadmium, arsenic, and lead concentrations (mg kg⁻¹) in biomass at Feekes 4, flag leaf, at Feekes 9 and grains at harvest, Clarksville, MI. 2024.

Slope	Biomass at Feekes 4			Flag leaf at Feekes 9			Grains at harvest		
	Cd	As	Pb	Cd	As	Pb	Cd	As	Pb ^b
	mg kg ⁻¹								
Toeslope	0.41	0.48	2.66	0.10 a	0.16	1.28	0.03	0.003	0.02
Midslope	0.65	0.50	1.75	0.04 b	0.12	0.94	0.04	0.003	0.009
Topslope	0.69	0.52	1.44	0.06 ab	0.14	1.92	0.03	0.003	0.01
P > F ^a	NS	NS	NS	*	NS	NS	NS	NS	NS

^aAsterisks indicate thresholds of significance (NS, P > 0.10; *, P < 0.10; **, P < 0.05; ***, P < 0.001). Values followed by the same lowercase letter are not statistically different at 0.10 probability level, Fisher's least significant difference (LSD).

^b FDA action level for cereals (0.01 mg kg⁻¹).

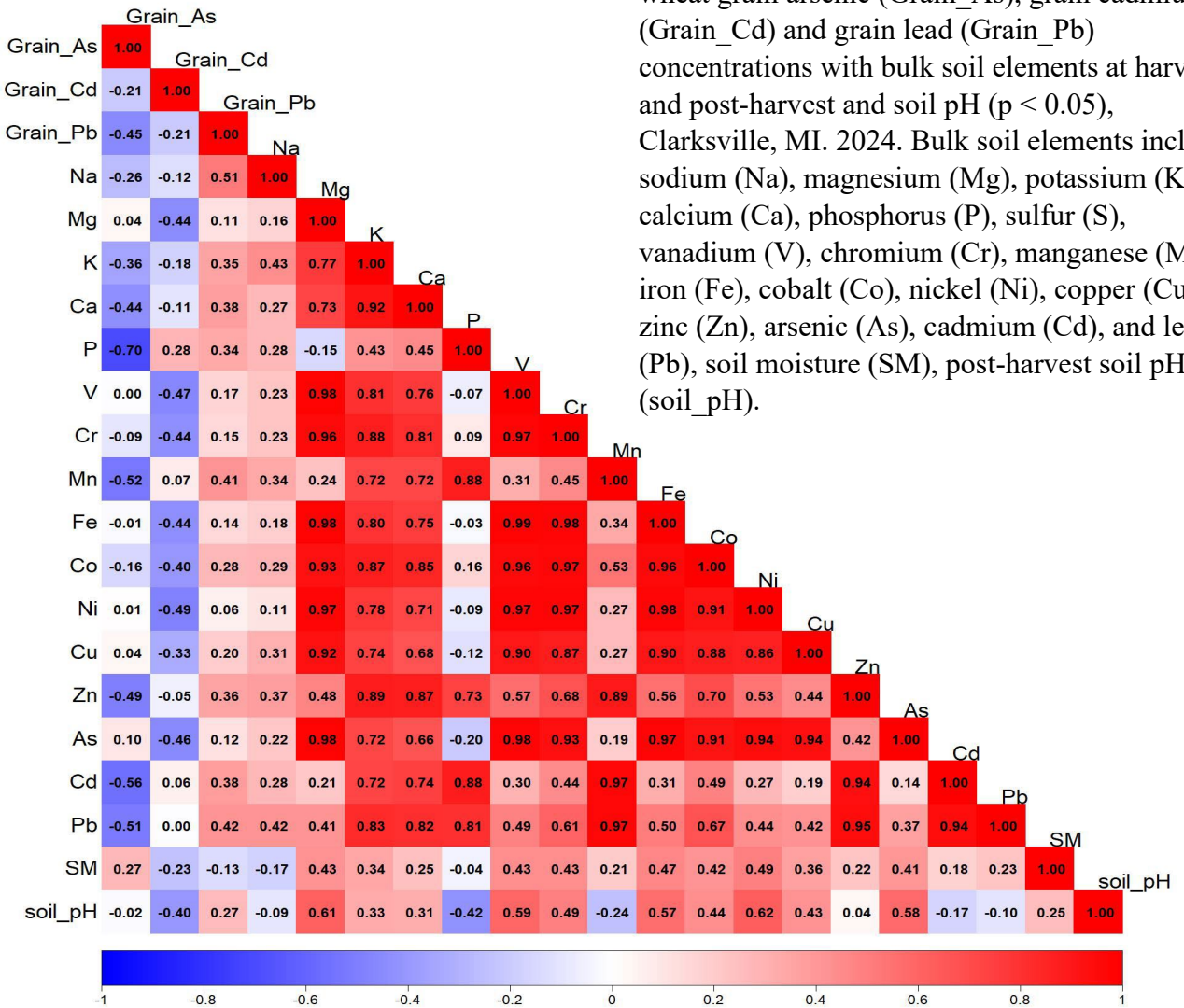


Figure 2. Pearson correlation between winter wheat grain arsenic (Grain_As), grain cadmium (Grain_Cd) and grain lead (Grain_Pb) concentrations with bulk soil elements at harvest and post-harvest and soil pH ($p < 0.05$), Clarksville, MI. 2024. Bulk soil elements include sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), sulfur (S), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb), soil moisture (SM), post-harvest soil pH (soil_pH).