Crop uptake of heavy metals in response to the environment and agronomic practices on land near mine tailings in the Zambian Copperbelt Province.

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Abstract

A field experiment was undertaken on farmers' fields adjacent to a large mine tailings dam in the Zambian mining town of Kitwe. Experimental plots were located close to the tailings (≤200m) or further away (300-400m) within the demarcated land farmed by the same community. This study evaluated the uptake of Cd, Cu, Ni, Pb and Zn by pumpkin leaves and maize grown in soil amended with lime and manure applied at agronomic rates, and the subsequent risk of dietary exposure to the local community, typical of many similar situations across the Zambian Copperbelt. Treatments, combinations of lime and manure (present or absent), were applied to subplots selected independently and randomly within each main plot, which represented variable geochemistry across this study site as a result of windblown/rain-driven dust from the tailings. Total elemental concentrations in crops were determined by ICP-MS following microwave-assisted acid digestion. Concentrations of Cu and Pb in pumpkin leaves were above the prescribed FAO/WHO safe limits by 60 – 205%

and by 33 – 133% respectively, while all five metals were below the limit for maize grain. Concentration of metals in maize grain were not affected by the amendments. However, lime at typical agronomic application rates significantly reduced concentrations of Cd, Cu, Pb and Zn in the pumpkin leaves by 40%, 33%, 19% and 10%, respectively and for manure Cd reduced by 16%, whilst Zn increased by 35%. The uptake of metals by crops in locations further from the tailings was greater than closer to the tailings because of greater retention of metals in the soil at higher soil pH closer to the tailings. Crops in season 2 had greater concentrations of Cu, Ni, Pb and Zn than in season 1 due to diminished lime applied only in season 1, in line with common applications on a biannual basis. Maize as the staple crop is safe to grow in this area while pumpkin leaves as a readily available commonly consumed leafy vegetable may present a hazard due to accumulation of Cu and Pb above recommended safe limits.

Keywords: heavy metals, pumpkin leaves, maize, lime, manure, mine tailings, agronomic amendments

1. Introduction

Heavy metal pollution of soil is widespread in Africa (Yabe et al. 2010). Due to the health risks to humans associated with heavy metals, the extent of soil and water pollution has been of considerable interest in many environmental studies. For heavy metal contaminated soils, there is a risk of toxicity to plants and subsequent transfer of metals to the food chain (Fosu-Mensah et al. 2017; Latif et al. 2018; Njagi et al. 2017). Despite this evidence, land near potential soil contaminants is widely used for cultivation of food crops in most urban areas in Africa (Kneen et al. 2015; Nakaona et al. 2019; Kaninga et al 2020a) as a result of scarcity of arable land and proximity to water for irrigation from tailings dams. In Zambia's Copperbelt Province, land around mine wastes is cultivated with the staple crop, maize alongside vegetable crops such as pumpkin, ubiquitously grown for both its fruit and leaves. The occupants of such land are usually resource-poor smallholder farmers who in most cases are unaware of the potential danger posed by the mine wastes and are primarily concerned with the productivity of the land. This is the case in the study area, Mugala Village in Kitwe, Copperbelt Zambia (Nakaona et al. 2019).

Studies conducted on land contaminated with heavy metals have shown that soil pH and organic matter are key factors determining their bioavailability to crops (Gray et al. 2006; Angelova et al. 2013; Kubátová et al. 2016). As such, application of lime and organic resources, usually at high rates, have been recommended as remediation strategies for contaminated soils (Zaniewicz-Bajkowska et al. 2008; Yi et al. 2010). However, field-based studies, considering the uptake of heavy metals by crops grown on farmer fields close to pollution sources such as tailings are scarce, especially in Zambia. In typical tropical soils such as those found in Northern Zambia, low pH and organic matter content are known to limit crop production (Lungu et al. 1993; Shitumbanuma et al. 2015; Chapoto et al. 2016). Farmers are encouraged to amend their soils with lime and organic materials to optimise crop production. However, the inadvertent effect of these agronomic amendments on uptake of heavy metals by crops grown on contaminated agricultural soils has not been investigated.

Classical agronomic experiments are generally undertaken at uniform sites, selected to be representative of conditions where the experimental findings will be applied (Ligowe et al.

2020; Manzeke et al. 2020). However, in real field situations, site characteristics may be highly heterogeneous. For example, the environmental heterogeneity in areas affected by mining activities is of intrinsic interest, and it is necessary to understand how responses to agronomic practices might differ in contrasting environments. In our study setting, proximity to mine tailings is important because previous observation shows that wind-dispersal of tailings material and deposition on the site takes place, which has the potential to enrich the soil with material in the tailings, particularly in locations near to the dam itself (Lark et al. 2017). The soils in the area under study have inherently low pH (pH<5) (Soil Survey Unit 1991). However, preliminary observations showed that the pH of soils closer to the tailings is substantially higher because the tailings are limed prior to disposal (Lark et al. 2017; Hamilton et al. 2020). There is therefore a possibility that, along with spatial variations in the input of heavy metals via wind or rain driven transfer, different microenvironments in the study region, determined primarily by distance to the tailings, have different baseline soil chemical conditions that influence their effect on the uptake of metals by crops.

For this reason, we set-up an agronomic experiment in contrasting microenvironments at the site, with the aim of evaluating the uptake of metals by crops and subsequent hazard for humans consuming crops in lime- and manure-amended soils close to mine tailings. To achieve this aim we had the following objectives; 1) to design and implement a field experiment in which the main effects and interactions of proximity to tailings and a set of agronomic treatments on crop uptake could be measured; and 2) to maintain this experiment over two seasons, typical for assessment of agronomic applications to examine short-term residual effects of the agronomic treatments.

2. Materials and methods

2.1. Study site and field experimentation

The study was undertaken during the 2016–17 and 2017–18 cropping seasons (November to May), in the Mugala village area, on an agricultural field adjacent to a large mine tailings dam along the Kalulushi – Mufulira road, north of Kitwe town in the Copperbelt province of Zambia (12°47’20’’S and 28°06’10’’E). The site is in the high rainfall, climatic region III of Zambia. The area is characterised by highly weathered soils; according to the FAO-UNESCO system, the soils in this area are classified as Rhodic Ferrasols (Soil Survey Unit 1991).

The experiment consisted of 16 plots (four treatments and four replicates) which were set up in a Randomised Complete Block Design. The treatments included: lime applied with no manure (L1M0), chicken manure applied with no lime (L0M1), lime and manure both applied (L1M1), and a control treatment with no amendments (L0M0). As stated in the introduction, our interest was in the effect of agronomic practices in the setting of different environments in the landscape close to the tailings. Two sites (B1 and B2 in Fig. 1) were selected, each about 300 - 400 m from the tailings dam, and these were designated as ‘far’ (Dam1). Another two sites were selected which were about 100 – 200 m from the tailings (B3 and B4 in Fig.1), designated as ‘near’ (Dam2). These sites were the main-plots in our experiment. Within each of these main-plots, each of the four agronomic treatments were allocated independently and at random to the sub-plots.

Each sub-plot was 45 m^2, consisting of six 10 m long ridges, spaced 90 cm apart. Soil samples were collected from each plot and characterized for basic soil fertility, and heavy metal concentrations before amendments were applied as detailed in Kaninga et al. (2020b). Lime

($CaCO_3$) and pre-treated chicken manure (designated ‘manure’) were applied only within the ridge area, i.e. not including the inter-row spaces. Both amendments were applied to the soil only at the start of the study in 2016. Lime was applied on 23 November 2016, at a rate equivalent to 2.0 t ha^{-1} (9.0 kg $plot^{-1}$), while manure was applied four weeks later (21 December, 2016), at a dry weight equivalent to 5.0 t ha^{-1} (22.5 kg $plot^{-1}$). Maize and pumpkin plants were sown within the ridge by intercropping on 23rd December 2016 and 9th December 2017.

2.2. Plant sampling, and preparation

Six weeks after sowing, pumpkin leaf samples were collected within a ‘harvest plot’ within each sub-plot. The harvest plots were made by eliminating a meter from both ends of each ridge (row) and the two border rows in each plot. About 200 g of pumpkin leaves were picked with their stalks at about 5 cm from the ground. Plant samples were washed with tap water 3 times and rinsed once with distilled water. The authors acknowledge that not all dust will be possible to remove from the plants, based on previous experience (Joy et al. 2015; Watts et al. 2019). The leaf samples were then oven-dried at 55^{0}C, cooled in a desiccator and finely ground using stainless steel coffee grinders before being stored in paper bags, pending analysis.

Five months after sowing, maize cobs and stover were sampled from the same harvest plot marked for pumpkin leaves. A sample of stover was collected and prepared as described for pumpkin leaves. The cobs were then shelled and about 100 g of grain was oven-dried overnight at 55 ^{0}C, cooled, and finely ground in coffee grinders before being stored in paper bags pending analysis. All elemental analyses were conducted at the British Geological Survey, Inorganic Geochemistry Laboratory, in Keyworth, UK.

2.3. Plant sample analysis

Vegetation samples were analysed for elemental composition using an ICP-QQQ-MS (Agilent 8900 ICP-QQQ-MS) following acid-digestion in a microwave digestion system as described by Watts et al. (2019). For quality assurance, blanks, duplicates and certified reference materials (CRMs) including NIST 1573a (tomato leaves), NIST 1570a (spinach) and NIST 1567b (wheat flour) were analysed alongside the plant samples – the latter are presented in Supplementary Table 1 demonstrating good analytical performance. Soil chemistry data was reported in Kaninga et al. (2020b). For this paper, only crop data is considered for exposure related to direct human consumption of food produce.

2.4. Risk of exposure to consumers of crops

The risk of exposure to heavy metals among the population consuming crops grown on contaminated land is commonly assessed by comparison with maximum allowable concentration limits (Fosu-Mensah et al. 2017; Njagi et al. 2017). Additionally, calculation of the Hazard Quotient (HQ) takes into account the concentration of an element which is accumulated in the edible portion of the crop and an estimate of the daily oral exposure below which no adverse health effects are likely in one's lifetime (Hough et al. 2004). Of major concern is the HQ for maize, since it is consumed almost daily in the study area and generally in the central and southern African regions. On average, per capita consumption of maize in Zambia is about 371 g day^{-1}, equivalent to ~70-80% of calorific intake (FAOSTAT, 2017), although this can be as much as 500 g day^{-1} (De Groote et al 2015). The maize, which is mostly made into pap, is consumed alongside leafy vegetables especially. According to the national food balance sheet, per capita consumption of leafy vegetables by an average

Zambian is about 57g day^{-1} (FAOSTAT, 2017). Equation 1 (Mwesigye et al. 2016) was used to compute the HQ.

$$\boldsymbol{HQ} = \frac{C \times ADI \times FWC}{RfD \times BW} \qquad (1)$$

Where C is the concentration of a heavy metal in the grain, on a dry weight basis in mg kg^{-1}. ADI is the fresh weight average per capita daily intake of foods. RfD is the reference dose (mg kg^{-1} day^{-1}) which is an estimate of the daily oral exposure below which no adverse health effects are likely in one's lifetime (see Table 3 for values). The FWC is the Fresh weight conversion (moisture) factor; BW is the average adult body weight of the population in kg, which was taken as 60 kg (Nakaona et al. 2019).

2.5. Statistical analysis of data

Data analysis was computed on the R platform (R Core Team 2017). A linear mixed model in the nlme library for R platform (Pinheiro et al. 2017) was used to analyse the treatment effects on metal uptake by crops, which involved repeated measures within the sub-plots over two cropping seasons. The 'Field', and 'Plot within Field' were the random effects while the fixed effects were Dam, level 1 (far) or 2 (close), 'Season', Lime, Manure and their interactions. Only the two-way interactions were considered in our analysis. The response variable of interest were the metal concentrations in pumpkin leaves and, maize stover and grain.

After the model was fitted, a plot of the standardised residuals against the fitted values, and a plot of the quantiles of the residuals against the theoretical normal values (QQ plot) was examined to test the plausibility of the assumption that the random effects in the model are normally distributed and with a uniform variance (Webster and Lark 2019). The ANOVA results were then interpreted after satisfactorily meeting the assumptions for ANOVA.

3. Results

Initial soil characteristics

Table 1 and 2 shows the range of some initial chemical and physical characteristics of the soils each block. The organic carbon and total nitrogen content of the soil ranged from moderate to high, while phosphorus was generally low. The cation exchange capacity (CEC) ranged from low to moderate and a moderately acidic to neutral pH range (5.1 – 7.2). Thus, the soils in this study were of low to medium fertility, with a clay loam texture. The total heavy metals in the soil, except Cu were below FAO maximum permissible limits for agricultural soils and their distribution was fairly uniform.

Table 1: Soil fertility parameters before application of the amendments

Proximity to tailings dam	Block	Organic carbon (%)	pH	Total N (%)	Available P (mg/kg)	CEC (cmol(+)/kg)	Texture (%clay)
Dam 1 (≤100m)	B1	1.42-1.83	5.2-5.6	0.29-0.45	6.60-24.05	8.2-13.5	Clay loam (29.8)
	B2	1.55-1.92	5.1-5.3	0.13-0.34	4.49-20.66	10.1-13.3	Clay Loam (27.2)
Dam 2 (300 - 400m)	B3	1.1-2.11	6.4-6.7	0.20-0.34	12.38-19.46	9.0-13.6	Clay loam (28.5)
	B4	1.55-1.96	6.3-7.2	0.20-0.31	14.06-30.31	12.1-21.0	Loam (23.9)

Table 2: Soil heavy metal concentration before application of amendments

Block	Total metal concentrations (mg/kg)				
	Cd	Cu	Ni	Pb	Zn
B1	0.12±0.01	874±84	36±1.6	9.4±0.5	32±2
B2	0.11±0.01	935±31	44±1.6	10.2±0.2	32±0.6
B3	0.11±0.01	947±66	41±0.9	9.6±0.5	26±1.2
B4	0.09±0.01	979±85	40±1.5	8.1±0.3	24±1.5
Mean	0.11	934	40.9	9.3	28.5
FAO/WHO max allowable	3	100	50	100	300

Maize grain yield

According to the analysis of variance (Table 3), maize grain yield was significantly increased by application of lime (p=0.024) and manure (p=0.031), while there was no evidence to reject the null hypothesis of no interaction between these factors (p=0.084). The grain yield was larger in season S1 than S2 (p=0.025) as shown in Table 2, but there was no evidence for an interaction between season and either the effects of lime or of manure. In season S1, maize grain yield increased from 1280 kg ha^{-1} to 1950 kg ha^{-1} under lime, to 1980 kg ha^{-1} under manure and to 2240 kg ha^{-1} with both amendments. In season S2, it increased from 1030 kg ha^{-1} to 1710 kg ha^{-1}, 1630 kg ha^{-1} and 1620 kg ha^{-1}under lime, manure and their interaction respectively. There was no difference in maize grain yield between Dam 1 and Dam 2 (p=0.437), nor for any interactions between the Dam effect and any other fixed effect in the model.

Table 3: ANOVA for the effect of the fixed effects on maize grain yield

Comparison	Denominator degrees of freedom	F-value	P-value
Season	8	7.64	0.025
Dam	2	0.93	0.437

Lime	6	9.05	0.024
Manure	6	7.91	0.031
Season·Dam	8	4.27	0.073
Season·Lime	8	0.27	0.617
Dam·Lime	6	0.086	0.779
Season·Manure	8	0.84	0.385
Dam·Manure	6	0.29	0.605
Lime·Manure	6	4.3	0.084

Table 4: Maize grain yield (Mean ± standard deviation) under each treatment

Season	Treatment	Grain yield [a] (kg ha^{-1})
S1	L0M0	1279 ± 385
	L1M0	1953 ± 635
	L0M1	1980 ± 496
	L1M1	2243 ± 396
S2	L0M0	1033 ± 214
	L1M0	1708 ± 476
	L0M1	1629 ± 171
	L1M1	1615 ± 122

[a]average of four replications

3.1. Metal concentration in crops

The concentrations of Cd, Cu, Ni and Pb found in pumpkin leaves and maize grain are shown in Sup. Table 4 and in relation to FAO/WHO limits in Fig. 2. In pumpkin leaves, Cd ranged from 0.02 – 0.06 mg kg^{-1}, which was a similar range to that found for maize stover, while 0.001 – 0.005 mg kg^{-1} was found in maize grain. Concentrations of Cu ranged from 22 – 71 mg kg^{-1} in pumpkin leaves, 1.5 – 3.0 mg kg^{-1} in maize grain and 5 – 35 mg kg^{-1} in maize stover. Nickel ranged from 1.2 – 4.5 mg kg^{-1} in pumpkin leaves, 0.04 – 0.20 mg kg^{-1} in maize grain and 1.2 – 13 mg kg^{-1} in maize stover, while Pb was 0.30 – 4.2 mg kg^{-1} in pumpkin leaves, trace – 0.04 mg kg^{-1} in maize grain and 0.10 – 0.70 mg kg^{-1} in maize stover. Zinc concentrations in pumpkin leaves, maize grain and stover were 17 – 60 mg kg^{-1}, 11 – 22 mg kg^{-1} and 2.5 – 12 mg kg^{-1}, respectively.

3.2. Risk of exposure

Values of HQ for Cd, Cu, Ni, Pb, Zn were calculated according to Equation 1 for an average person weighing 60 kg are shown in Table 5. For both pumpkin leaves and maize grain, Cd presented the lowest HQ; Copper the largest HQ for pumpkin leaves, while both Cu and Zn were highest for maize grain.

Table 5: Hazard quotients associated with consumption of pumpkin leaves and maize grain at Mugala village, Copperbelt Zambia

Heavy metal	Median conc. in pumpkin leaves (mg kg^{-1})	Median conc. maize grain (mg kg^{-1})	FAO/WHO max. allowable limit (mg kg^{-1})	RfD[a] (mg kg^{-1} day^{-1})	HQ for pumpkin leaves FWC=0.078	HQ for maize grain FWC=0.896
Cd	0.033	0.002	0.2	0.001	0.002	0.004
Cu	49.0	1.8	20	0.04	0.091	0.093
Ni	2.34	0.073	67	0.02	0.009	0.008
Pb	0.478	0.011	0.3	0.0035	0.01	0.007
Zn	39.8	14.0	99	0.3	0.01	0.096

[a] US EPA Iris database (2015)
FWC is the moisture factor

3.3. Uptake of heavy metals by Pumpkin leaves

Table 6 shows the results for the analysis of variance of the fixed effects on uptake of Cd, Cu, Ni, Pb, Zn by pumpkin leaves. The results show a significant relationship between lime and the uptake of Cd (p=0.0004), Cu(p=0.0001), Pb (p=0.048) and Zn(p=004). Compared to the control treatment, application of lime led to a reduction of 40% in concentration for Cd in pumpkin leaves and 33% for Cu, 19% for Pb and 10% for Zn. There was a significant relationship between manure application and Cd (p=0.038) and Zn (p=0.004) uptake. Compared to the control treatment, application of manure reduced Cd uptake by an average of 16%, while Zn uptake was increased by 35%. No evidence for an interaction between lime and manure for Cd (p=0.711), Ni (p=0.733) and Pb (p=0.281) was found, while Cu was significantly reduced (p=0.008) and Zn increased (p=0.048).

There was no evidence of a relationship between the season and uptake of Cd, Cu, Ni and Pb in pumpkin leaves, but a highly significant (p=<0.0001) relationship with Zn was seen: pumpkin leaves in S2 had more Zn than in S1 (Sup. Table 1). A significant relationship was found between the Dam and the uptake of Cd (p=0.0298), Ni (p=0.043) and Pb (p=0.0463), with pumpkin leaves in Dam1 having a larger concentration than in Dam2. There was a significant interactive effect of the season with the Dam for the uptake of Cd (p=0.0216), Cu (p=0038), Ni (p=0054) and Zn (p=0.004). The response to the interaction between Season and Dam was similar to that of the Dam effect, where combinations involving Dam1 had elevated heavy metal concentrations compared to Dam2 (Fig. 3). Furthermore, there were significant interactions of lime, with season for uptake of Cu (p=0.0305), and with Dam for uptake of Cd (p=0333), where pumpkin leaves under treatments with lime had less Cu than those without lime. Pumpkin leaves in Dam1 had more Cd than those in Dam2, but within each Dam, those under lime treatment had less Cd than those without lime (Fig. 4).

3.4. Uptake of heavy metals by Maize grain and stover

There was no evidence of an effect of both lime and manure, and their interaction, on maize grain Cd, Cu, Ni, Pb and Zn concentrations (**Error! Reference source not found.**7). There was however evidence of a significant seasonal effect (p=0.048) on maize grain for Pb, and with its interaction with lime (p=0.0375), where the content in S1 was greater than in S2 (Fig. 5a). Additionally, no evidence was found for an effect of the other fixed effects and their interactions on Cd, Cu, Ni, while a significant interaction of the season and Dam was seen for Zn grain content (p=0.0127), with interactions involving Dam1 having a greater Zn concentration than those with Dam2 (Fig. 5b).

Both lime (p=0.016), manure (p=0.0012) and their interaction (p=0.001) had significant effects on accumulation of Cu into maize stover (**Error! Reference source not found.**8). In comparison to the zero treatment, lime reduced the Cu concentration in maize stover by an average of 46%. Manure also reduced the Cu concentration in stover by 58% and the interaction of lime and manure reduced it by 45%. Concentrations of Cu in stover were significantly greater (p=0.0002) in season S2 than S1 and this was observed even with its interaction with lime (p=0.0195) and manure (p=0.0111). Similar trends between the seasons were also observed for Ni (p=0.0117), Pb (p=0.0287) and Zn (p=0.0004). There was a significant effect of lime (p=0.0069) and manure (p=0.0072) on stover Zn concentration. Lime reduced maize stover Zn by 48%, while manure reduced it by 46%. Similarly, to Cu, a significant interaction of season and lime (p=0.0419) was realised for maize stover Zn, where interaction with season S2 had greater maize stover Zn concentrations than those involving S1.

Table 6: ANOVA for the effect of the fixed effects on heavy metal uptake by pumpkin leaves

Comparison	Denominator degrees of freedom	Cd		Cu		Ni		Pb		Zn	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	2.15	0.18	1.11	0.322	1.8	0.216	4.69	0.062	265.1	< 0.000
Dam	2	32.03	0.029	5.44	0.145	21.9	0.043	20.09	0.046	12.04	0.074
Lime	6	51.097	0.000	83.4	0.000	0.000	0.994	4.51	0.048	20.7	0.004
Manure	6	7.066	0.038	5.15	0.064	1.196	0.316	1.66	0.244	21.1	0.004
Season·Dam	8	8.095	0.022	16.3	0.004	14.3	0.005	15.88	0.004	11.8	0.009
Season·Lime	8	3.557	0.096	6.88	0.031	0.11	0.753	0.004	0.949	0.014	0.909
Dam·Lime	6	7.567	0.033	1.3	0.299	1.68	0.243	0.079	0.787	0.12	0.743
Season·Manure	8	0.755	0.41	2.01	0.194	0.51	0.496	0.059	0.813	2.31	0.167
Dam·Manure	6	0.017	0.900	0.028	0.87	0.01	0.935	0.00	0.999	1.71	0.238
Lime·Manure	6	0.151	0.711	14.9	0.008	0.13	0.733	2.67	0.154	6.11	0.048

Table 7: ANOVA for the effect of the fixed effects on heavy metal uptake by maize grain

Comparison	Denominator degrees of freedom	Cd		Cu		Ni		Pb		Zn	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	4.79	0.06	0.229	0.637	1.31	0.285	14.9	0.005	1.15	0.314
Dam	2	0.53	0.542	0.0005	0.984	0.016	0.908	1.15	0.396	7.92	0.107
Lime	6	1.04	0.347	0.141	0.711	0.62	0.462	1.55	0.26	0.14	0.719
Manure	6	0.02	0.889	0.363	0.576	0.04	0.854	0.99	0.358	0.46	0.524
Season·:Dam	8	2.57	0.147	3.992	0.085	0.38	0.557	4.48	0.067	10.2	0.013
Season·Lime	8	2.57	0.147	0.016	0.894	0.064	0.806	6.2	0.038	1.26	0.293
Dam·:Lime	6	0.19	0.677	1.654	0.242	3.57	0.108	1.87	0.22	0.0006	0.981
Season·Manure	8	1.04	0.337	0.177	0.692	0.047	0.834	0.000	1.	0.323	0.585
Dam·Manure	6	0.02	0.889	1.219	0.312	0.49	0.510	0.25	0.636	0.012	0.917
Lime·Manure	6	0.53	0.493	2.326	0.179	1.43	0.277	0.062	0.812	1.09	0.337

Table 8: ANOVA for the effect of the fixed effects on heavy metal uptake by maize stover

Comparison	**Denominator degrees of freedom**	**Cd**		**Cu**		**Ni**		**Pb**		**Zn**	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	2.2	0.176	41.9	0.000	10.5	0.012	7.09	0.029	33.4	0.0004
Dam	2	0.04	0.858	2.43	0.259	0.36	0.61	0.005	0.951	11.48	0.077
Lime	6	1.29	0.299	10.9	0.016	0.11	0.749	0.120	0.741	16.25	0.007
Manure	6	0.51	0.500	32.9	0.001	2.27	0.183	1.885	0.219	15.95	0.007
Season·Dam	8	3.29	0.107	0.84	0.387	0.68	0.433	0.059	0.815	4.9	0.058
Season·Lime	8	0.47	0.513	8.46	0.0196	1.67	0.233	1.169	0.311	5.85	0.042
Dam·Lime	6	1.76	0.233	1.27	0.303	0.32	0.590	0.323	0.590	2.97	0.136
Season·Manure	8	3.1	0.116	10.7	0.011	0.79	0.399	1.240	0.298	1.73	0.224
Dam·Manure	6	0.15	0.713	2.63	0.156	1.91	0.216	3.40	0.115	0.013	0.910
Lime·Manure	6	0.48	0.514	35.2	0.001	0.099	0.764	0.323	0.590	3.03	0.132

4. Discussion

4.1. Maize grain yield

Lime and manure increased the yield of maize grain in both seasons by an average of 37% and 34% respectively but S1 had more yield than S2. This difference in yield between the two seasons can be largely attributed to differences in rainfall distribution as S2 was characterised by dry spells within the cropping season. In cropping seasons 2016/17 (S1) and 2017/18 (S2) the national average yields of maize grain by small scale farmers in Zambia were 2,120 kg ha^{-1} and 1,680 kg ha^{-1}, respectively (Central Statistics Office 2018; Ministry of Agriculture 2019). Thus, in the study area, yields achieved without lime and manure (1280 and 1030 kg ha^{-1}) were about 40% less than the national averages, while they were within close range (2060 kg ha^{-1} and 1,660 kg ha^{-1}) in amended plots. These results show that lime and manure application favour crop growth and increase yield as expected (Lungu et al. 1993). Soil pH is an important factor which affects the performance of crops. Soil acidity may cause fixation of some plant nutrients, e.g. phosphorus, rendering them unavailable for crops, and acidity will also increase the solubility of potentially toxic metals such as aluminium (Al), Pb and Cr. The et al. (2006) reported that the maize grain yield increase after application of lime in acid soils was largely due to reduction in exchangeable aluminium rather than to an increase in pH. This could explain the lack of differences in yield between Dam1 and Dam2 or the lack of an interaction between the Dam and lime as the soils in Dam1, though characterised as acidic (pH 5.1 – 5.6), had only trace levels of exchangeable Al (Kaninga et al. 2020b).

4.2. Concentration of heavy metals in crops

All pumpkin leave samples had concentrations of Cu and Pb above the FAO/WHO (2011, 2001) maximum permissible limit for vegetables while Cd, Ni and Zn were below the limit (Fig.2). Concentrations found in maize grain were below the FAO/WHO (2011, 2001) for all five heavy metals. The concentrations of Cd, Cu, Pb and Zn obtained in this study for maize grain are close to values (0.01, 2.72, 0.06 and 8.25 mg kg^{-1} respectively), reported by Mirecki and Agi (2015) for maize grown on soils which were contaminated by Pb and Zn smelting in Kosovo, although these values did not vary significantly with values obtained in uncontaminated control soils. Conversely, leafy vegetables which were grown in the same contaminated soil accumulated greater concentrations of heavy metals compared to those grown in the control soil indicating that plant physiological factors were the controlling factor (Baker 1981; Mirecki and Agi 2015). According to Puschenreiter et al. (2011), maize is classified as a low accumulator while leafy vegetables like lettuce and spinach are high accumulators. The results obtained in this study imply that consumption of pumpkin leaves grown in this area increases the risk of exposure to Cu and Pb, whereas, the risk is very minimal for maize, despite the considerable dietary contribution to daily food consumption.

Although, maize stover is not consumed by humans, its elemental content was of interest in order to determine the accumulation pattern of the heavy metals by the maize plant and as an indicator of plant uptake. In Zambia, maize stover is either fed to livestock on mixed farming systems or left as surface cover in the field, especially in conservation farming systems for organic reincorporation. In both cases, their heavy metal content is significant as they may find their way into the food chain, through ingestion by livestock or through their decomposition, potentially adding to available soil metal concentrations.

4.3. Risk of exposure to pumpkin leaves and maize grain

Values of HQ for Cd, Cu, Ni, Pb, Zn for both crops were all less than 1, implying that there is no potential adverse health effects expected from consumption of pumpkin leaves and maize grain grown in this area. This result confirms the interpretation made according to the concentrations found in the grain which were below the FAO/WHO (2011, 2001) maximum permissible limits. However, there is a discrepancy between the interpretation of the associated risk for Cu and Pb in pumpkin leaves between FAO/WHO limits and the HQs. According to the FAO/WHO (2011, 2001) limits, the median values of Cu and Pb found in pumpkin leaves were above the allowable thresholds, and so could pose harm to consumers. On the other hand, their associated HQs are at least an order of magnitude less than one, implying that they pose a minimal threat to consumers. This discrepancy suggests the need for standardising risk assessment tools in order to ensure consistency in findings. Although pumpkin leaves had larger concentrations of the metals than maize grain, their HQs were within the same order of magnitude with grain because of their smaller per capita consumption compared to maize grain.

4.4. Effect of the fixed-effects on heavy metal uptake by pumpkin leaves

The reduction in Cd, Cu, Pb and Zn concentrations of pumpkin leaves implies that lime applied in acidic soils for the purpose of ameliorating acidity, can inadvertently also reduce uptake of metals in these contaminated soils, even at agronomic application rates. Several studies have shown that amending soils with lime at much higher application rates for contaminated land scenarios reduced extractable concentrations of heavy metals, which are taken as surrogates for the plant-available fractions. For example, Vondráčková et al. (2013) reported a decrease in $CaCl_2$-extractable Cd and Zn following soil amendment with quicklime and dolomite. Similar results were reported for Cd and Pb by Abd El-Azeem et al.

(2013) after amending soils with calcite. Manure reduced the uptake of Cd but led to an increase for Zn. The soil metal immobilising ability of organic amendments has also been reported (Conder et al. 2001; Angelova et al. 2010; Angelova et al. 2013). Angelova et al. (2010) found that organic amendments were especially effective for reducing Cd uptake by potatoes. However, Zn uptake by pumpkin leaves was increased in this case because the manure used had a large concentration of Zn (434mg/kg total Zn). This result agrees with findings by Manzeke et al. (2020) that manure can improve the supply of Zn from soil for crop uptake. The form of the manure is however key as recent evidence showed that organic materials may limit Zn uptake into maize grain due to limitation in N availability (Manzeke et al. 2020). There was no significant interactive effect of lime and manure for Cd, Ni and Pb, while a reduction and an increase were observed for Cu and Zn respectively, suggesting that manure's effect on Cu and Zn uptake by pumpkin leaves depended on the soil pH. Although the amendments of lime and manure led to a significant reduction in uptake of some heavy metals, the reduced concentrations were not below the maximum permissible limit for Cu and Pb for leafy vegetables.

Zinc concentration in pumpkin leaves in season S2 was greater than in S1 probably due to mineralisation of organic matter in the soil. Pumpkin leaves in Dam1 had significantly more Cd, Cu, Ni, Zn than those in Dam2. This observation indicated the resultant effect of immobilisation of heavy metals in soil at higher pH (Gadepalle et al. 2007), because plots which were closer to the tailings had a higher pH than those which were further away. Similar trends were observed in all Dam interactions i.e. with season for Cd, Cu, Ni and Zn, and with lime for Cd. These results show that the initial soil chemical characteristics influence the effectiveness of the amendments in reducing heavy metal uptake by crops and therefore, should be taken into consideration when devising in-situ immobilisation strategies. Specifically for this area, this implies that liming of the tailings prior to disposal ensures the fixation/immobilisation of the heavy metals as they are deposited via wind-blown dust onto surrounding soils.

4.5. Effect of the fixed-effects on heavy metal uptake by maize

None of the treatments of lime and manure had a significant effect on maize grain heavy metal concentrations (**Error! Reference source not found.**7). This could be because very little of the heavy metals studied were assimilated into the grain, except for Zn which is an essential nutrient and particularly important for grain filling (Xue et al. 2019). However, analysis of the stover revealed that appreciable concentrations of the heavy metals were taken up by the maize plant but remained in the vegetative tissue. Interestingly for Zn, more was assimilated in the grain than in the stover. These results support the idea that plant physiological factors are an important consideration for the uptake of elements from soil and their assimilation into plant tissue. A significant interaction of season with Dam resulted in maize grain in Dam1 having a greater Zn concentration than in Dam2 (Fig. 5b), likely due to differences in soil pH.

Lime and manure both led to a reduction in stover Cu and Zn uptake due to their immobilisation in soil. Despite having a large Zn concentration, manure did not result in increased Zn content in maize. Manzeke et al. (2020) reported reduced Zn concentration in maize grown in soils treated with manure which they attributed to limited N availability. The significantly greater stover- Cu, Ni, Pb and Zn in season S2 than S1 could be due to a dilution effect in S1 (Cakmak 2008), as the yield was larger in S1 than S2.

5. Conclusion and recommendation

The findings obtained in this study suggest that maize grown in this area poses minimal risk of heavy metal ingestion by consumers because only small concentrations were accumulated - an important consideration for the affected community and many similar settings across Zambia and regionally, particularly given the dominance of maize in the diet. However, while the grain accumulated minimal concentrations of Cd, Cu, Ni, Pb and Zn, the stover had substantial amounts, which could require further investigation given the importance of maize stovers as an inexpensive and accessible livestock feed in Zambia and across Africa. Livestock may be at risk of ingesting detrimental amounts of heavy metals, therefore, this study area is not suited for livestock grazing. On the other hand, pumpkin leaves accumulated large concentrations of the heavy metals, with Cu and Pb above the FAO/WHO prescribed safety limit which could pose a threat to consumers, although when per capita consumption is considered, there is a minimal risk of exposure to all of the five heavy metals. Amendment by lime and manure at agronomic rates reduced the uptake of Cd, Cu and Zn by both maize (stover) and pumpkin leaves. This is important because using these amendments improves the soil quality for agricultural production, but also has a secondary benefit in reducing the potential for uptake of heavy metals by crops in contaminated soils. However, for the conditions under this study, the reduction in uptake of Cu and Pb, though noteworthy, did not reduce them below the FAO/WHO prescribed safety limits. Thus, at lime and manure rates equal or less than 2 t ha^{-1} and 5 t ha^{-1} respectively, pumpkin leaves may still accumulate more than safe concentrations of Cu and Pb, and therefore, it should not be grown in this area. Liming of the tailings prior to disposal effectively fixed the heavy metals in the soil, therefore unavailable for plant uptake, with greater immobilisation observed closer to the tailings. Interventions for reducing uptake of heavy metals in areas with contrasting micro-environments should consider the spatial environmental variation for effective planning of the application of agronomic amendments.

6. Acknowledgement

Declarations

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for non-life science journals

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Conflicts of interest/Competing interests

The authors declare that there were no competing or conflicting interests.

Availability of data and material (data transparency)

Additional data is provided in supplementary information to support the presented data tables and figures.

Code availability (software application or custom code)

Not applicable

Authors' contributions

All authors were involved in the preparation of the manuscript. Kaninga, Watts, Lark, Sakala, Chishala, Maseka and Young designed the study; Kaninga led the experimental work; Tye and Hamilton analyses.

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Crop uptake of heavy metals in response to the environment and agronomic practices on land near mine tailings in the Zambian Copperbelt Province.

Belinda Kaninga[1,2], R. Murray Lark[5], Benson H. Chishala[2], Kakoma K. Maseka[3], Godfrey M. Sakala[1], Scott D. Young[5], Andrew Tye[4], Elliott M. Hamilton[4] and Michael J. Watts[4]

Figure 1: Image of the study area showing location of the fields and their proximity to the tailings dam. Locations B1 and B2 were considered as far from the dam, while B3 and B4 were considered as close to the dam

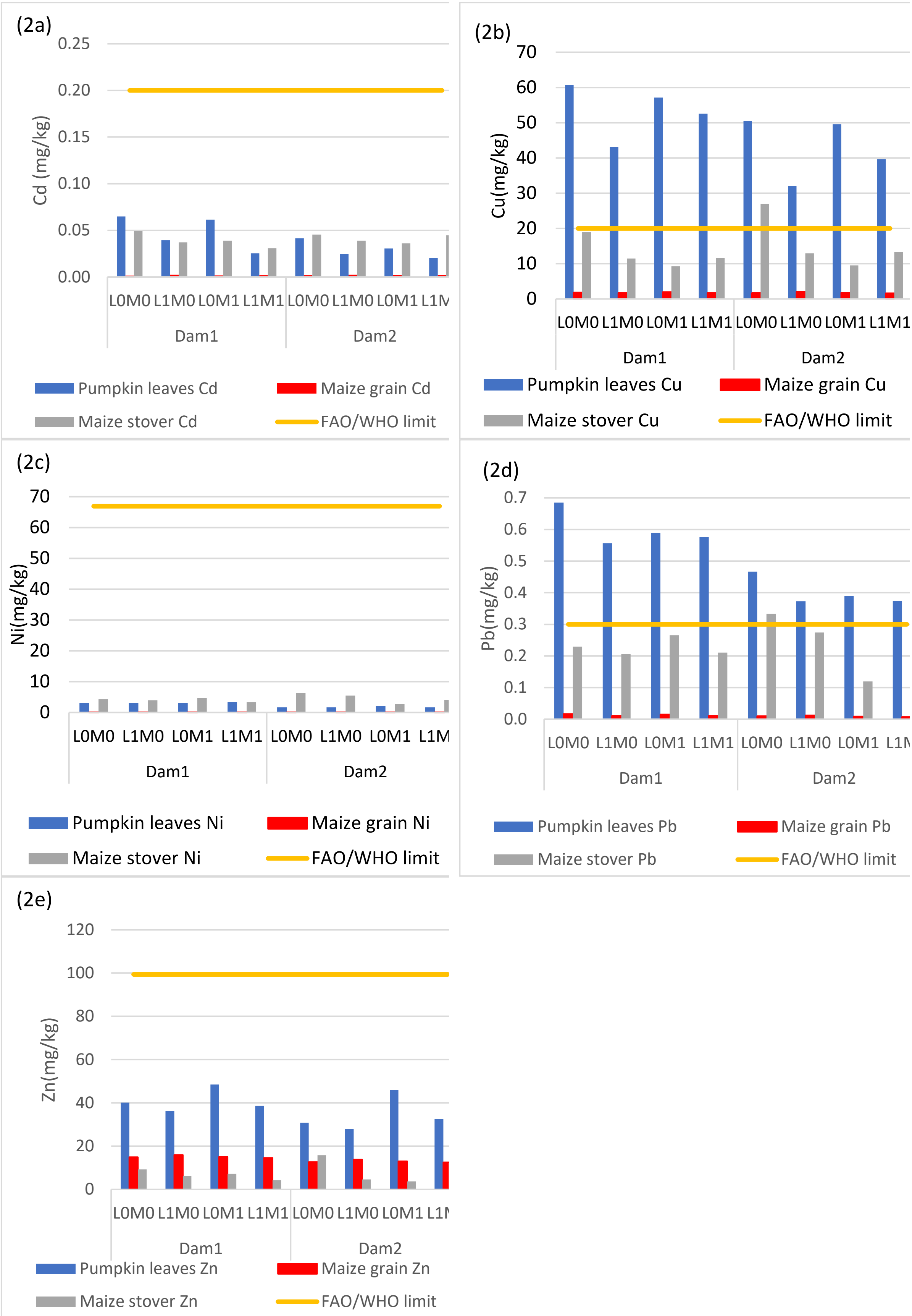
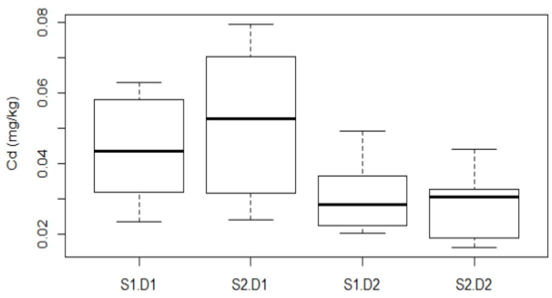


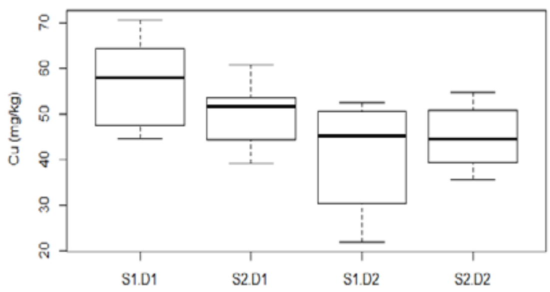
Figure 2: Concentrations of Cd, Cu, Ni, Pb, Zn in pumpkin leaves, maize grain, maize stover under each treatment per distance from the tailings (Dam), against the FAO/WHO maximum permissible

limits. L0M0, L1M0, L0M1 and L1M1 refer to zero, lime, manure and, lime and manure treatments respectively.

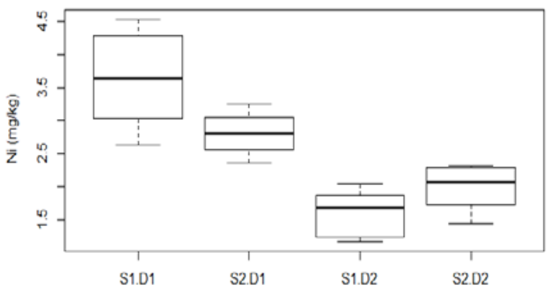
(3a)



(3b)



(3c)



(3d)

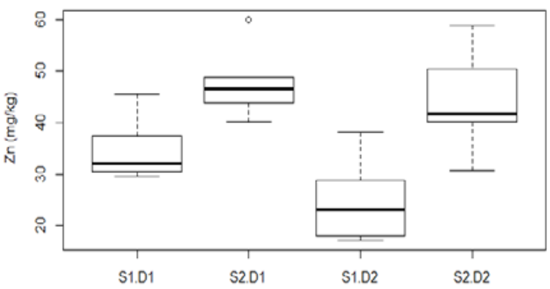


Figure 3: Boxplots showing the metal concentration in pumpkin leaves according to season and proximity to the tailings. S1 and S2 refer to seasons 1 and 2, D1 and D2 represent plots which were further and closer to the tailings dam, respectively.

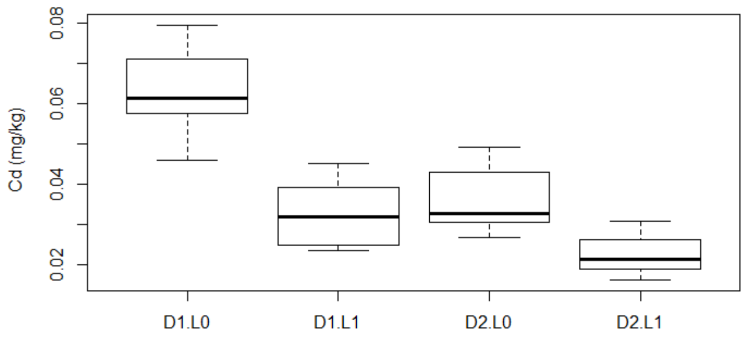
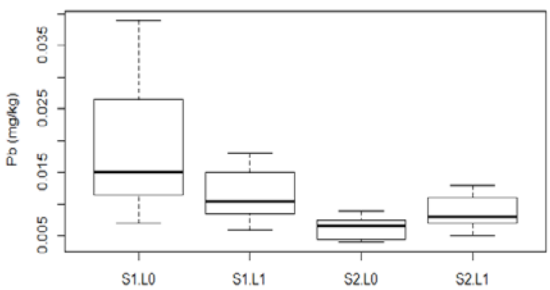


Figure 4: Boxplots showing the metal concentration in pumpkin leaves according to interaction of lime and proximity to the tailings. D1 and D2 represent plots denoted as 'near' and 'far' from the tailings dam.

(5a)



(5b)

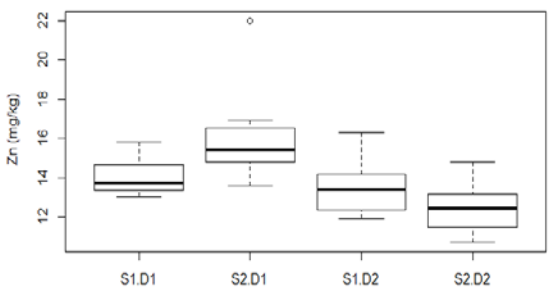


Figure 5: Boxplots showing the Pb and Zn concentrations in maize grain according to the interaction of season with lime (a) and with Dam (b) respectively. S1 and S2 represent season 1 and 2 respectively, and D1 and D2 represent dam1 and dam2 respectively.

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SUPPLEMENTARY MATERIAL

Sup. Table 1: Analytical performance data, based on 3 replicate measurements for certified reference materials and the limit of detection as 3 x standard deviation of the blank (with dilution factor).

Reference material	Concentration (mg kg^{-1})				
	Cd	Cu	Ni	Pb	Zn
Measured values					
NIST SRM 1567b	0.020 ± 0.002	1.9 ± 0.03	0.092 ± 0.006	0.015 ± 0.006	11.07 ± 0.04
NIST SRM 1570a	2.746 ± 0.009	11.36 ± 0.22	1.982 ± 0.048	0.162 ± 0.001	79.7 ± 0.9
NIST SRM 1573a	1.504 ± 0.066	4.46 ± 0.28	1.550 ± 0.084	0.563 ± 0.02	30.65 ± 1.38
Reference values					
NIST SRM 1567b Wheat Flour	0.026 ± 0.002	2.1 ± 0.2	-	0.02	11.6 ± 0.4
NIST SRM 1570a Spinach leaves	2.876 ± 0.058	12.22 ± 0.86	2.142 ± 0.058	-	82.3 ± 3.9
NIST SRM 1573a Tomato leaves	1.517 ± 0.027	4.70 ± 0.14	1.582 ± 0.041	-	30.94 ± 0.55
Instrument's limit of detection	**<0.002**	**<0.4**	**<0.004**	**<0.007**	**<0.2**

Sup. Table 2: Heavy metal concentrations in crops (Mean ± standard deviation) under each treatment, season

Variable	Treatment	Concentration[a]/ mg kg^{-1}	
		Season 1	Season 2
Pumpkin leaves Cd	L1M0	0.032 ± 0.01	0.036 ± 0.00
	L0M1	0.041 ± 0.00	0.034 ± 0.01
	L1M1	0.024 ± 0.01	0.026 ± 0.02
	L0M0	0.052 ± 0.02	0.053 ± 0.01
Pumpkin leaves Cu	L1M0	35.9 ± 9	39.5 ± 5
	L0M1	57.1 ± 12	49.6 ± 8
	L1M1	45.7 ± 13	46.5 ± 1
	L0M0	56.9 ± 10	54.3 ± 6
Pumpkin leaves Ni	L1M0	2.58 ± 1.1	2.32 ± 0.8
	L0M1	2.81 ± 1.4	2.44 ± 0.8
	L1M1	2.71 ± 1.5	2.42 ± 0.4
	L0M0	2.37 ± 1.2	2.42 ± 0.3

Pumpkin leaves Pb	L1M0	0.50 ± 0.2	0.43 ± 0.0
	L0M1	0.53 ± 0.2	0.45 ± 0.1
	L1M1	0.43 ± 0.1	0.48 ± 0.1
	L0M0	0.57 ± 0.1	0.58 ± 0.1
Pumpkin leaves Zn	L1M0	25.4 ± 7.7	38.7 ± 3.8
	L0M1	39.1 ± 6.5	55.3 ± 2.9
	L1M1	25.7 ± 6.4	45.4 ± 5.6
	L0M0	27.3 ± 5.0	43.7 ± 5.1
Maize grain Cd	L1M0	0.002 ± 0.001	0.003 ± 0.001
	L0M1	0.002 ± 0.002	0.002 ± 0.001
	L1M1	0.001 ± 0.001	0.004 ± 0.001
	L0M0	0.002 ± 0.001	0.002 ± 0.001
Maize grain Cu	L1M0	1.91 ± 0.73	1.84 ± 0.23
	L0M1	1.91 ± 0.28	1.79 ± 0.28
	L1M1	1.68 ± 0.08	1.60 ± 0.14
	L0M0	1.73 ± 0.09	1.77 ± 0.19
Maize grain Ni	L1M0	0.085 ± 0.04	0.064 ± 0.03
	L0M1	0.094 ± 0.05	0.074 ± 0.01
	L1M1	0.068 ± 0.02	0.061 ± 0.02
	L0M0	0.069 ± 0.01	0.071 ± 0.02
Maize grain Pb	L1M0	0.013 ± 0.00	0.010 ± 0.00
	L0M1	0.018 ± 0.01	0.006 ± 0.00
	L1M1	0.010 ± 0.01	0.008 ± 0.00
	L0M0	0.020 ± 0.01	0.007 ± 0.00
Maize grain Zn	L1M0	13.7 ± 1.8	15.9 ± 4.4
	L0M1	13.7 ± 1.1	14.3 ± 2.0
	L1M1	13.6 ± 1.2	13.5 ± 2.2
	L0M0	14.1 ± 1.2	13.5 ± 1.9
Maize stover Cd	L1M0	0.031 ± 0.01	0.045 ± 0.01
	L0M1	0.027 ± 0.01	0.053 ± 0.01
	L1M1	0.034 ± 0.01	0.042 ± 0.01
	L0M0	0.056 ± 0.02	0.039 ± 0.03
Maize stover Cu	L1M0	10.3 ± 1.5	14.1 ± 2.7
	L0M1	7.7 ± 2.6	11.0 ± 2.7
	L1M1	12.1 ± 2.0	12.8 ± 5.1
	L0M0	18.7 ± 1.7	27.2 ± 7.8
Maize stover Ni	L1M0	3.6 ± 2.4	5.9 ± 3.4
	L0M1	2.2 ± 0.4	5.2 ± 2.3
	L1M1	3.3 ± 1.0	4.1 ± 1.3
	L0M0	3.2 ± 1.4	7.4 ± 3.9
Maize stover Pb	L1M0	0.17 ± 0.03	0.31 ± 0.09
	L0M1	0.13 ± 0.04	0.26 ± 0.14
	L1M1	0.21 ± 0.09	0.20 ± 0.09
	L0M0	0.20 ± 0.02	0.36 ± 0.27
Maize stover Zn	L1M0	4.2 ± 1.3	6.7 ± 1.2
	L0M1	3.8 ± 1.2	7.1 ± 3.0
	L1M1	4.4 ± 0.8	4.1 ± 1.8
	L0M0	7.2 ± 1.1	9.5 ± 2.0

[a]average of four replications