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Pelleted manure compost improves mine spoil properties enhancing plant growth and phyto-stabilization of potentially toxic metals

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Abstract

Feedlot manure is rich in plant nutrients and can immobilize potentially toxic metals. However, pelleted manure compost as an amendment material in mine spoils (chat) is not well studied. This study was conducted to investigate the impact of pelleted cattle manure on improving chat properties facilitating phyto-stabilization and the establishment of grasses. A greenhouse pot experiment was conducted with unamended and amended chat (lime treated) with pelleted manure at three rates (60, 120, and 180 Mg ha⁻¹) with and without bentonite (B), using two native grasses, switchgrass (*Panicum virgatum* L.) and wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve). Leachates from pots were collected periodically until harvest. Nutrients and metal concentrations were measured in chat treatments, and metal concentrations were measured in plant tissues and leachates. Manure-amended chat reduced leachate Cd and Zn on average by >75% and >80%, respectively. Above-ground dry matter yield increased by >2.5-fold and >4-fold, respectively, in switchgrass and wheatgrass with the increase of 3-fold manure rate. The manure rate at 180 Mg ha⁻¹ reduced plant Cd and Zn by 50% and 20%, respectively, in wheatgrass, and 30% and no reduction, respectively, in switchgrass, compared to the 60 Mg ha⁻¹ manure rate. Overall, pelleted manure compost significantly increased available nutrients and decreased available metals in amended chat, with no significant effect of B. This study indicated that pelleted manure, preferably at 180 Mg ha⁻¹ rate with lime, can be used in acidic metal-contaminated chat to facilitate the establishment of perennial native grasses and reduce the potentially toxic metal availability.

Key words: mine spoils, native grasses, phyto-stabilization, pelleted manure compost, potentially toxic metals

Résumé

Riche en éléments nutritifs, le fumier de bovins peut immobiliser des métaux potentiellement toxiques. Cependant, on ne s'est pas beaucoup intéressé au compost d'agglomérés de fumier comme amendement pour les déblais miniers (conglomérats cherteux). Les auteurs voulaient établir dans quelle mesure de tels agglomérés peuvent améliorer les propriétés des conglomérats cherteux qui favorisent la stabilisation de la végétation et l'établissement des graminées. Dans cette optique, ils ont cultivé du panic raide (*Panicum virgatum* L.) et de l'agropyre de l'Ouest [*Pascopyrum smithii* (Rydb.) A. Löve], deux graminées indigènes, en serre, dans des pots de conglomérats cherteux (chaulés), amendés ou pas avec 60, 120 ou 180 Mg d'agglomérés de fumier de bovins par hectare, avec ou sans bentonite. Ensuite, ils ont recueilli le lixiviat des pots à intervalles réguliers jusqu'à la récolte. Les auteurs ont mesuré la concentration d'oligoéléments et de métaux dans les conglomérats cherteux traités et celle des métaux dans les tissus végétaux et le lixiviat. Le fumier servant d'amendement aux conglomérats cherteux réduit la lixiviation du Cd et du Zn respectivement de plus de 75 % et de 80 %, en moyenne. Le rendement en matière sèche des organes aériens de panic raide et d'agropyre de l'Ouest augmente respectivement de plus de 2,5 et de quatre fois, avec la triple ration d'agglomérés. Comparativement au taux de 60 Mg par hectare, l'application de 180 Mg de compost par hectare diminue respectivement la concentration de Cd et de Zn de 50 % et de 20 % chez l'agropyre, et de 30 % ou pas du tout chez le panic. En général, le compost d'agglomérés de fumier augmente de façon significative la quantité d'oligoéléments disponibles et diminue la concentration de métaux disponibles dans les conglomérats cherteux bonifiés, mais la bentonite n'a aucun effet significatif. Selon les résultats de l'étude, l'application de 180 Mg de compost d'agglomérés de fumier par hectare avec de la

chaux faciliterait l'établissement des graminées indigènes vivaces sur les conglomérats chertueux acides contaminés par des métaux et permettrait de réduire la quantité de métaux potentiellement toxiques disponibles. [Traduit par la Rédaction]

Mots-clés : déblais miniers, graminées indigènes, phytostabilisation, compost d'agglomérés de fumier, métaux potentiellement toxiques

Introduction

Sphalerite (zinc sulfide) and galena (lead sulfide) wastes after mining, smelting, or other various industrial processes in the tri-state mining district (TSMD) of southeastern Kansas City, USA, were left behind in some places as chat piles (USEPA 2020). High levels of lead (Pb), zinc (Zn), and cadmium (Cd) were reported in the chat collected from these sites (Pierzynski et al. 2002a; Baker et al. 2011; Indraratne et al. 2021). Wind and rain cause the chat to scatter and spread contaminated metals to surrounding water bodies and land. The harmful effects of these metal components are visible despite the remediation efforts made by the US Government (Juracek 2013; Park et al. 2020). Even after many efforts in the TSMD, remediation is unsuccessful to counteract groundwater, chat, and sediment contamination (Johnson et al. 2016; Juracek and Drake 2016). The elevated amounts of heavy metals in soil can increase human and animal exposure through the food chain transfers, ingestion by wind-blown dust or by direct ingestion of soil (Pierzynski et al. 2002a). Cost-effective remedial alternatives should be applied to highly contaminated sites to prevent further dispersion of contaminants (Johnson et al. 2016). Physical, chemical, and biological remediation methods have been applied to minimize the impact of mine tailings on environmental bodies (Ciarkowska et al. 2017; Song et al. 2017). Phyto-stabilization, a phytoremediation strategy where metals are stabilized by the plant root activity in the rhizosphere, is an eco-friendly way to control the mobility of heavy metals in mine tailings (Wang et al. 2020).

Revegetation of disturbed areas with tall, warm season, native grasses (i.e., switchgrass and wheatgrass) is one of the remedial options identified for chat piles in TSMD (USEPA 2020). The installation of plant cover over mine spoil piles stabilizes pollutants and reduces spread of contamination and leaching losses (Wang et al. 2020). Vegetation on chat piles is very scant due to the soil's poor physicochemical properties and high concentrations of Cd, Pb, and Zn (Baker et al. 2011; Indraratne et al. 2021). Previous studies on Pb and/or Zn mine wastes have shown the ability of soil amendments such as biosolids, diammonium phosphate, and tall fescue grass to restore a plant cover on tailings and reduce the erosive potential of these wastes (Pierzynski et al. 2002a, 2002b; Brown et al. 2007). Nevertheless, the success of revegetation and subsequent metal immobilization is closely related to the physical and chemical properties of mine spoils (Li and Huang 2015). Organic products, such as compost, biochar, and ash, have been successfully used to enhance the phyto-stabilization of contaminated soils (Radziemska et al. 2021). Studies showed that the addition of animal wastes such as cattle manure greatly increases plant growth and survival in mine spoils (Pierzynski et al. 2002b; Novak et al. 2019).

Revegetation of severely phytotoxic, metal-contaminated mine spoils is a challenge since chat is a poor growing

medium (Wong 2003; Vega et al. 2004). In a previous experiment, we observed the growth of sorghum (*Sorghum bicolor* L. Moench) in chat amended with cattle manure at 134 Mg ha^{-1} and CaO at 5.5 Mg ha^{-1} relative to no growth in chat without cattle manure in a controlled greenhouse environment (Indraratne et al. 2021). Hence in this experiment, we determined the economical rate of sole use of pelleted cattle feedlot manure compost that would be effective in covering chat piles with native grasses. Besides pelleted manure, clay minerals can also improve chat properties as clay minerals have been predicted to promote plant growth in mine spoils by decreasing the available forms of potentially toxic metals (Vega et al. 2004; Ling et al. 2007; Wang et al. 2016). To this purpose, bentonite (B), a montmorillonite clay with strong retention ability for bioavailable forms of heavy metals, could be used.

Therefore, the objectives of the study were to (a) determine the effects of pelleted manure compost at different rates on the yield of native grasses, nutrient and heavy metal accumulation, and soil metal stabilization, and (b) evaluate the benefits of using bentonite blend with pelleted manure compost on the phyto-stabilization of heavy metals. We hypothesized that pelleted manure, lime, and bentonite amendments improve the physical, chemical, and biological properties of chat, which, in turn, enhances the phyto-stabilization process in mine spoils.

Materials and methods

Manure-amended (with or without bentonite) chat treatment combinations

Mine spoil or chat samples were collected from Galena, KS, USA ($37^{\circ}9'16''\text{N}$; $94^{\circ}50'2''\text{W}$) at 0–20 cm depth. The chat was sieved through a stainless steel 2 mm screen, air-dried, and stored in plastic containers at room temperature ($22\text{--}24^{\circ}\text{C}$). Composted beef manure obtained from a local feedlot farm and subjected to a commercial pelletization was used for the study. Pelleted manure compost was sieved through a 2 mm screen prior to application. Wyoming bentonite (Wyo-Ben, Inc., Billings, MT) was mixed with the pelleted manure during the pelletization process at a rate of 50 g of bentonite kg^{-1} of compost to improve the physical properties of the final product, as explained in Baker et al. (2011). The chat was mixed with pelleted compost at three different rates: 60, 120, and 180 Mg ha^{-1} . Each manure rate was mixed with (+B) or without B (–B). There was a total of seven treatment combinations: control (chat only), 60 Mg ha^{-1} (60), $60 \text{ Mg ha}^{-1} + \text{B}$ ($60 + \text{B}$), 120 Mg ha^{-1} (120), $120 \text{ Mg ha}^{-1} + \text{B}$ ($120 + \text{B}$), 180 Mg ha^{-1} (180), and $180 \text{ Mg ha}^{-1} + \text{B}$ ($180 + \text{B}$). The chat material was corrected for acidity by adding CaO at a rate of 5.5 Mg ha^{-1} in all treatments to improve the growth medium's qualities

(Pierzynski et al. 2002b; Baker et al. 2011; Indraratne et al. 2021).

Greenhouse experiment

A greenhouse pot experiment was carried out at the Kansas State university, Manhattan, KS with two native grass species, switchgrass and western wheatgrass. The seven treatments with four replicates were arranged as a repeated measure design to compare the changes in leachate toxic elements in the amended chat. Materials necessary for each treatment (chat, manure, CaO, and/or bentonite) were mixed well and 1 kg of amended or control chat was placed in a 4 L plastic pot with holes in the bottom covered with cheesecloth. Properties of media with respect to each treatment are listed in Table 1. A plastic container was kept under each pot to collect the leachate. Each pot was leached with 1 L of deionized water to remove excess salt and then allowed to equilibrate for 1 week before seeding.

After germination, thinning was conducted leaving 12 plants of switchgrass or wheatgrass in a pot containing chat treatments. Leachates were collected from the bottom containers twice per week after plant emergence till final harvest (approximately 62 days), while adding enough deionized water (100–150 mL) to collect around 50 mL of leachate. Deionized water was added as needed to each pot to keep the moisture levels at field capacity for the days that the leachate was not collected. The daytime and nighttime temperatures in the greenhouse were controlled to 26 °C and 18 °C, respectively, and the day length was 16 hours. After sufficient biomass was generated, all pots were harvested. After harvest, chat samples were air-dried and sieved through a 2 mm stainless-steel mesh and used for further analysis.

Chat, leachate, and plant analysis

Initial and after harvest, chat mixtures were analyzed for pH, Mehlich III-extractable P, electrical conductivity (EC), available Ca^{2+} , Mg^{2+} , K^+ , NH_4^+ , NO_3^- , and total and bioavailable Cd, Pb, and Zn. Soil pH was determined in a 1:1 soil-deionized water mixture with a Ross combination pH electrode (Thermo Orion, Beverly, MA). Exchangeable basic cations (Ca, Mg, and K) were determined after extracting with 1 mol/L NH_4OAc at 1:10 soil-to-solution ratio, and the concentrations were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; Accuris 141; Fisons Instruments, Beverly, MA). EC was measured in a saturated paste extract using an EC meter (USDA 1954). Inorganic N (NH_4^+ and NO_3^-) was extracted by shaking with 1 mol/L KCl solution (1:10) on an orbital shaker for 2 hours, and inorganic P was extracted with the Mehlich-III method (Mehlich 1984). After extractions, P and N were analyzed colorimetrically using a flow-injection analyzer (Lachat Quikchem 8500).

Nitric acid-digested total metals (Cd, Pb, and Zn) were determined by using 2 g of chat (≤ 2 mm) with 20 mL of 4 mol/L HNO_3 (trace metal grade) acid at 80–85 °C for 4 hours (Sposito et al. 1982). Filtered digests were then analyzed for Cd, Pb, and Zn with ICP-OES. Available soil metal concentrations were determined by extracting with 0.1 mol/L $\text{Ca}(\text{NO}_3)_2$, (soil-to-solution ratio 1:20, 4-hour shaking at 200 rpm) and mea-

sured with ICP-OES. Cadmium, Pb, and Zn bioavailability was also assessed with the diffusive gradients in thin films (DGT) method (Sonmez and Pierzynski 2005).

The leachates were collected periodically, and a 20 mL aliquot was sent to ICP-OES for total Cd, Pb, and Zn determinations, after adding a drop of concentrated HNO_3 acid (trace metal grade). Cumulative mass of Cd, Pb, and Zn was estimated by multiplying metal concentration with total volume in each leachate collection and then summing up totals.

Above-ground plant materials, harvested at 62 days after plant emergence, were thoroughly washed, first with deionized water, then with a 5 g kg^{-1} sodium lauryl sulfate solution ($\text{CH}_3-(\text{CH}_2)_{10}\text{CH}_2\text{OSO}_3\text{Na}$), and finally with deionized water to remove adhering soil particles. Washed plant samples were oven-dried at 55 °C to a constant weight and dry matter weights were recorded. Oven-dried plant samples were finely ground before analyzing for total metal (Cd, Pb, and Zn) concentrations. Subsamples of 0.5 g of plant materials were digested with trace metal grade, concentrated HNO_3 acid for 4 hours at 120 °C, and Cd, Pb, and Zn in the digests were analyzed by ICP-OES.

Statistical analysis

This study was a repeated measures design with four factors: manure rate, bentonite, grass species, and time. The analysis was conducted using the PROC MIXED procedure of SAS (version 9.4, SAS Institute 2014) for leachate metal concentrations collected from different treatment combinations. A separate statistical analysis was performed for initial and after harvest soil properties using the PROC MIXED procedure (SAS 9.4) to determine the effect of manure rate, bentonite, and grass species on properties of the growth media. Similarly, plant dry matter yield and metal concentrations were also analyzed using the PROC MIXED procedure.

The Kenward–Rogers denominator degrees of freedom method and Tukey–Kramer adjustment were used for multiple comparisons using pairwise differences comparison (PDIF) statement. Least square means statement (LSMEANS) was used to assess differences. A residual analysis was performed to test the normality and homogeneity of variances. If normality assumption and homogeneity of variances were not acceptable, variance stabilization transformation (lognormal distribution) was conducted. Back transformations of log-transformed means were done manually ($=\text{EXP}(X)$).

Correlation analysis was performed to determine the significance of various relationships between soil properties and plant parameters. For all statistical analyses, significance was set at $\alpha = 0.05$.

Results and discussion

Initial properties of manure-amended (with or without bentonite) and unamended chat

According to the basic analysis of materials (data not shown), chat prior liming had low pH (5.2), low nutrients (0.35 mg kg^{-1} of total N) and high concentrations of Cd (45 mg kg^{-1}), Pb (583 mg kg^{-1}), and Zn (6154 mg kg^{-1}). Pelleted manure compost had alkaline pH (8.1), high nutrients

Table 1. Initial soil characterization of lime-added chat mixed with different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B) before planting grass species.

Treatments	60-B	60+B	120-B	120+B	180-B	180+B	Control
pH (1:1, distilled water)	6.78d	6.97 cd	7.23bc	7.51b	7.42b	8.97a	6.85 cd
EC (dS m ⁻¹ , saturated paste)		4.1c		5.23b		6.17a	2.11d
Available P (mg kg ⁻¹)		148b		182a		194a	37c
Available K ⁺ (mg kg ⁻¹)	1053c	923c	1641b	1728b	2599a	2323a	133d
Available NH ₄ ⁺ (mg kg ⁻¹)	30a	29a	30a	31a	32a	33a	29a
Available NO ₃ ⁻ (mg kg ⁻¹)		28c		37b		47a	11d
Available Mg ²⁺ (mg kg ⁻¹)	427c	411c	622b	678b	915a	824a	136d
Available Ca ²⁺ (mg kg ⁻¹)	4037b	3653bc	3562bc	3758bc	3902bc	5403a	3372c
Total Pb (mg kg ⁻¹)		255a		224ab		199b	258a
Total Zn (mg kg ⁻¹)	6236a	4735a	4939a	5200a	4732a	5098a	5385a
Total Cd (mg kg ⁻¹)	35.1a	32.9a	32.4a	33a	30.4a	32.1a	37.5a
DGT-Cd (μg) ^Y	1.29a	0.63b	0.52b	0.51b	0.74ab	0.49b	0.91ab
DGT-Zn (μg)	84.3ab	114.9a	100.1ab	85.5ab	50.2bc	16.1c	119.5a
Ca(NO ₃) ₂ Cd (mg kg ⁻¹) ^X	9.1b	10.8b	4c	4.7c	4.2c	1.1d	18.8a
Ca(NO ₃) ₂ Zn (mg kg ⁻¹)	52.2bc	78.7b	22.5 cd	32.9 cd	36.9 cd	3.5d	127.7a

Note: pH, Ca²⁺, K⁺, Mg²⁺, DGT-Zn, DGT-Cd, Ca(NO₃)₂-Cd, and Ca(NO₃)₂-Zn had significant (Manure × Bentonite) interaction effect; EC, P, NO₃⁻, and total Pb had significant manure effect; NO₃⁻ had significant Bentonite effect (+B > -B). Different letters within a soil attribute represent a statistical significance at *p* = 0.05.

^YDiffusive gradient thin films extractable (bioavailable).

^XCalcium nitrate extractable.

(13.5 g kg⁻¹ of total N and 7.5 g kg⁻¹ of total P), low concentrations of Cd (1.3 mg kg⁻¹) and Pb (not detectable, detection limit 0.004 mg L⁻¹), and moderate concentration of Zn (496 mg kg⁻¹).

Initial properties of pH, available Ca²⁺, K⁺, Mg²⁺, and DGT-Zn, and -Cd, Ca(NO₃)₂-Zn, and -Cd had significant manure × bentonite interaction effects, while EC, P, NO₃⁻, and total Pb had significant manure effect (Table 1). Further soil NO₃⁻ had a significant bentonite effect (data not shown). Addition of CaO increased the pH of chat from 5.2 to 6.9 in the control. The pH of the pelleted manure amendments ranged from 6.8 at 60 Mg ha⁻¹ without B to 9.0 at 180 Mg ha⁻¹ + B. Bentonite significantly increased available NO₃⁻, +B having higher average available NO₃⁻ (38 mg kg⁻¹) than without B (30 mg kg⁻¹). EC significantly increased with pelleted manure additions, increasing from 2.1 dS m⁻¹ in the unamended control to 6.2 dS m⁻¹ at 180 Mg⁻¹ ha manure rate.

Unamended chat (control treatment) had the lowest NO₃⁻, P, K⁺, Ca²⁺, and Mg²⁺ as expected. Addition of pelleted manure significantly increased plant nutrients, increasing with the rate (Table 1). The nutrient increases were tremendous for NO₃⁻, P, K, and Mg but at a lesser extent for Ca. No significant manure or bentonite effect was detected for NH₄⁺. At the highest manure rate, +B had higher Ca²⁺ than -B.

Initial total Cd, Pb, and Zn concentrations in the lime added, unamended chat (control) were 38, 258, and 5385 mg kg⁻¹, respectively, with no significant differences following treatment additions (Table 1). This indicated that mixing pelleted manure compost and B in treatments did not significantly dilute the metals in the chat. Previous research also indicated nonsignificant effect of limestone and biosolid-compost application on total Zn and total Cd concentrations for highly contaminated soils (Li et al. 2000). Pelleted manure decreased Cd and Zn extracted with Ca(NO₃)₂ and DGT, con-

sidered as bioavailable or mobile fractions. Available Cd as extracted by Ca(NO₃)₂ was reduced from 19 to 1 mg kg⁻¹ and available Zn from 128 to 4 mg kg⁻¹ with manure rate from chat only to 180 Mg ha⁻¹ plus bentonite (Table 1). Available Pb was not detectable.

Properties of manure amended (with or without bentonite) and unamended chat after harvesting grass species

Pelleted manure rate × bentonite × grass species interaction effects were significant for pH, EC, P, K⁺, Mg²⁺, and NH₄⁺, while pelleted manure rate × bentonite interaction effect was significant for Ca²⁺ and manure rate and grass species main effects were significant for NO₃⁻ (Table 2). All treatments maintained a pH in the neutral range until time of harvest, switchgrass from 6.2 to 7.4 and wheatgrass from 6.6 to 7.6, indicating suitability for plant growth. Increase of pH using liming materials can be identified as the first step in phyto-stabilization process in acid mine spoils (Pierzynski et al. 2002a, 2002b; Alvarenga et al. 2008).

Though the EC in manure treatments were >4.0 dS m⁻¹ initially, all treatments were nonsaline (EC <2 dS m⁻¹) at the time of harvest in both switchgrass and wheatgrass, except at 180 Mg ha⁻¹ under wheatgrass. However, no deleterious effects on germination of switchgrass or wheatgrass were seen in any manure-amended chat.

Manure addition effects on plant nutrients were still present at harvest in both switchgrass and wheatgrass grown chat, but the soil NO₃⁻ was severely reduced in the manure-amended chats even at the highest rate (Table 2). Nitrate losses were mainly due to leaching and plant uptake, as found by an increase in dry matter yield with pelleted manure rate. Exchangeable K⁺, Mg²⁺, Ca²⁺, and available P in switchgrass

Table 2. Soil properties of chat mixed with different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B) after harvesting switchgrass (SG) and wheatgrass (WG).

	Available values													
	pH		EC		P		K ⁺		NH ₄ ⁺		Mg ²⁺		M × B	M
	SG	WG	SG	WG	SG	WG	SG	WG	SG	WG	SG	WG	Ca ²⁺	NO ₃ ⁻
60–B	6.16e	7.07abc	1.55bcd	1.57bcd	116e	160c	438e	499e	10b	16.9a	377de	284e	2857b	3.8a
60+B	6.37de	7.28ab	1.72bcd	0.62 cd	123e	156c	422e	497e	6.8b	18.7a	392de	294e	2921b	
120–B	6.95bc	7.32ab	1.82abcd	1.43bcd	129de	222b	849d	997bcd	8b	17.0a	629b	478cd	3129b	1.9b
120+B	6.76 cd	7.49a	1.86abcd	1.19 cd	129de	221b	838d	935 cd	10b	16.9a	649b	459d	3121b	
180–B	6.93bc	7.42ab	1.72bcd	2.85ab	125e	292a	1303abc	1344ab	9.3b	16.8a	868a	592bc	3239b	2.1b
180+B	7.43ab	7.56a	2.02abc	3.28a	148 cd	296a	1367ab	1501a	9.4b	16.8a	896a	613b	4395a	
Control	6.67cde	6.58cde	0.39d	0.42d	44f	45f	124f	83 g	8b	15.7a	111f	72f	2778b	1.7c

Note: All parameters except NO₃⁻ and Ca²⁺ had significant Grass (G) (Manure (M) × Bentonite (B)) interaction effect. Available Ca²⁺ had significant M × B interaction and G effect. Available NO₃⁻ has significant M and G effects; WG (4.8 mg kg⁻¹) had higher NO₃⁻ than SG (2.1 mg kg⁻¹). Different letters within a soil attribute represent a statistical significance at p = 0.05.

Table 3. Concentrations of total and available ($\text{Ca}(\text{NO}_3)_2$ extractable and DGT) Cd, Pb, and Zn in chat mixed with different rates of manure compost (60, 120, and 180 Mg ha^{-1}), with/without bentonite (B) after harvesting SG and WG.

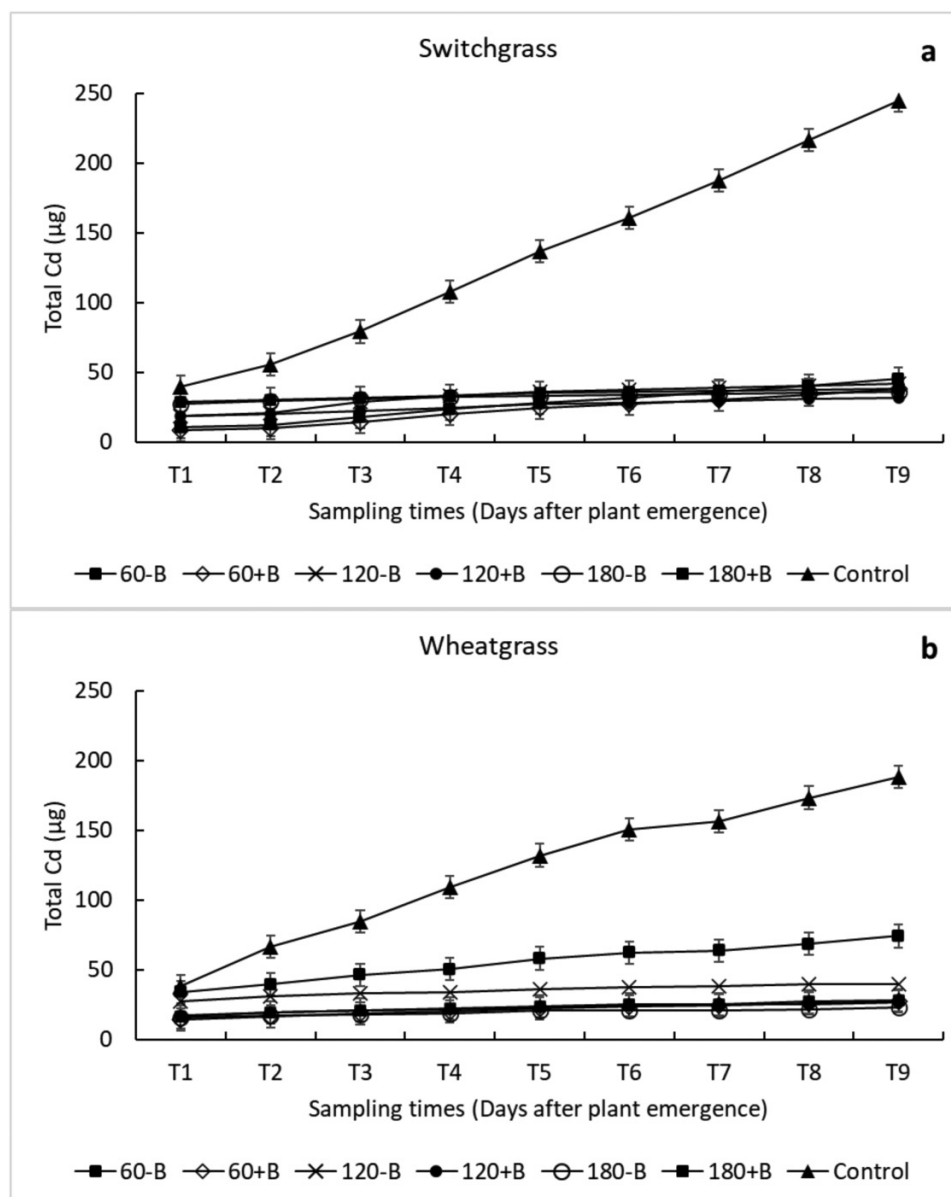
	Total Pb		Total Zn		DGT-Cd ^X		DGT-Zn		Total Cd	$\text{Ca}(\text{NO}_3)_2$ Cd ^Y	$\text{Ca}(\text{NO}_3)_2$ Zn
	SG	WG	SG	WG	SG	WG	SG	WG			
60–B	282cde	532a	6060bc	8023a	0.58de	0.89ab	59.6c	59.4c	N/A	5.3b	229b
60+B	278cde	425ab	5983bc	6953ab	0.64cd	0.81bc	80.3b	50.6cd	N/A		
120–B	260de	357bc	5662bc	6223bc	0.37efg	0.63cd	22.2ef	34.9de	N/A	2.6c	111c
120+B	249de	337bcd	5844bc	6243bc	0.47de	0.43def	28.5ef	23.4ef	N/A		
180–B	268cde	320bcd	6049bc	6063bc	0.23fg	0.48de	14.8f	22.9ef	N/A	1.6d	65d
180+B	228e	266cde	5604bc	5194c	0.19g	0.36efg	10.8f	15.0f	N/A		
Control	307cde	287cde	6862ab	6116bc	0.88ab	1.06a	88.8b	113.5a	N/A	10.6a	546a
Properties of soils after harvest											
Grass											
SG	N/A		N/A		N/A		N/A		42.6b	2.2b	103b
WG	N/A		N/A		N/A		N/A		51.1a	5.2a	211a

Note: All parameters except $\text{Ca}(\text{NO}_3)_2$ Cd, and Zn and total Cd had significant Grass (G) (Manure (M) \times Bentonite (B)) interaction effect. Total Cd had significant G effect. $\text{Ca}(\text{NO}_3)_2$ Cd and Zn had significant M and G effects; available Pb was not detectable. N/A, not applicable.

^YDiffusive gradient thin films extractable (bioavailable).

^XCalcium nitrate extractable.

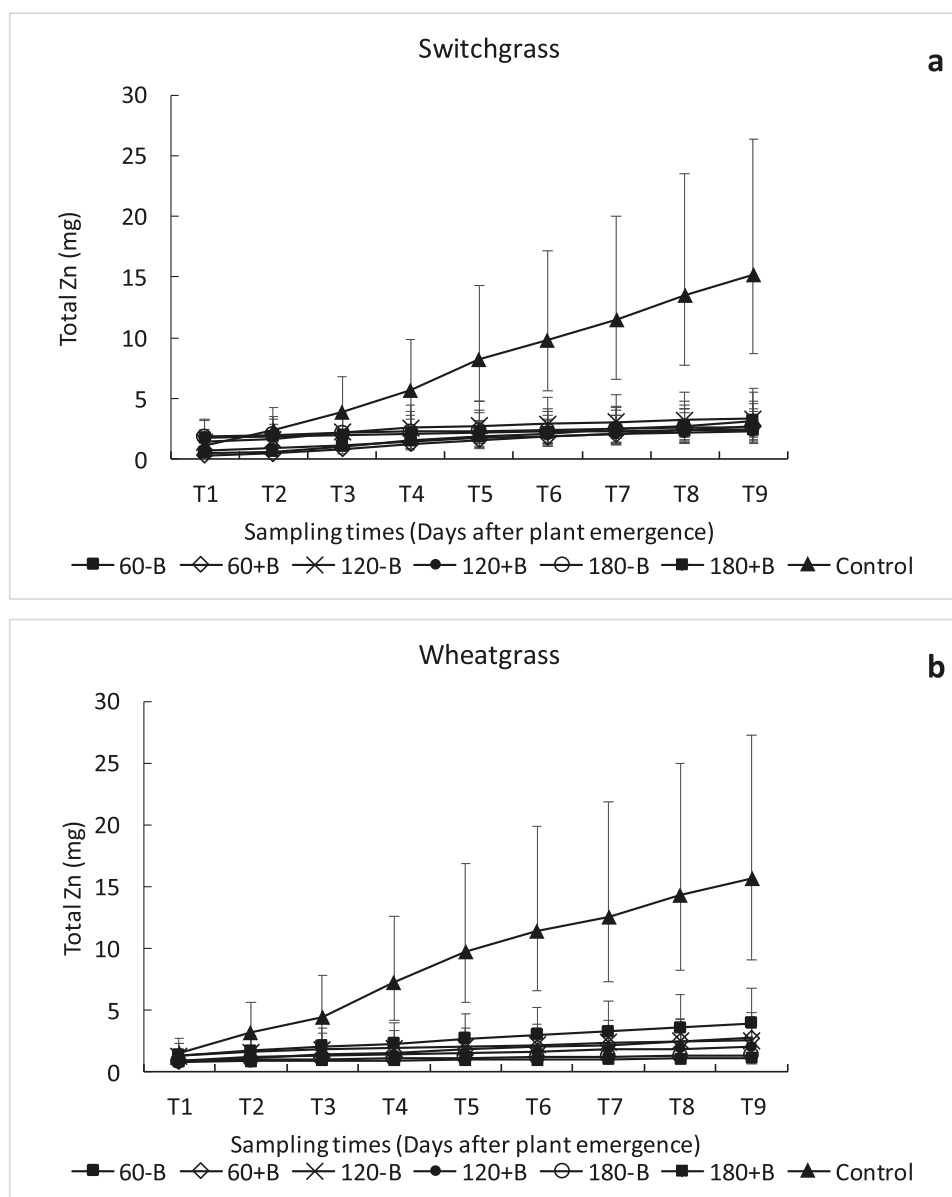
Fig. 1. Cadmium in leachates collected from switchgrass (SG) (a) and wheatgrass (WG) (b) pots, filled with chat and different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B). Nine sampling times (days after plant emergence): T1 = 0; T2 = 36; T3 = 40; T4 = 44; T5 = 47; T6 = 51; T7 = 55; T8 = 58; T9 = 62. Vertical bars represent the standard error of the mean. There were significant four-way interactions (time (Compost × Bentonite × Grass type)).



and wheatgrass grown chat were greater in higher pelleted manure rates. Ammonium concentrations were not affected by manure rate but showed higher concentrations in wheatgrass than in the switchgrass. Grass species effect was significant for NO₃⁻, wheatgrass (4.8 mg kg⁻¹) having higher NO₃⁻ than switchgrass (2.1 mg kg⁻¹; data not shown). Available P and pH were also higher in wheatgrass as compared with that in switchgrass. Decreased EC, suitable pH range, and availability in nutrients by the end of harvest indicated improvements of soil fertility by addition of pelleted feedlot manure. Though the interaction effect of three factors was significant (grass species, manure, and bentonite), bentonite did not show any significant effect (at each manure rate) on chat fertility status.

All metal parameters except Ca(NO₃)₂ extractable Cd, and Zn and total Cd had significant grass species × manure × bentonite interaction effect. Wheatgrass grown chat had higher total Cd and Ca(NO₃)₂ extractable Cd and Zn than switchgrass (Table 3). Furthermore, Ca(NO₃)₂ extractable Cd and Zn had significant manure rate effect, higher the manure rate lower the available Cd and Zn. Novak et al. (2019) reported significant reduction of bioavailable Cd and Zn concentrations, respectively from 20.2 to 1.4 mg kg⁻¹ and 346–14 mg kg⁻¹ with addition of 5% cattle litter biochar and compost in TSMD. Decreased bioavailability of potentially toxic metals was probably achieved by the combined effect of immobilization of metals by humified organic matter in the manure-treated soils and the formation of insoluble carbon-

Fig. 2. Zinc in leachates collected from SG (a) and WG (b) pots, filled with chat and different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B). Nine sampling times (days after plant emergence): T1 = 0; T2 = 36; T3 = 40; T4 = 44; T5 = 47; T6 = 51; T7 = 55; T8 = 58; T9 = 62. Vertical bars represent the standard error of the mean. There were significant four-way interactions (time (Compost × Bentonite × Grass type)).



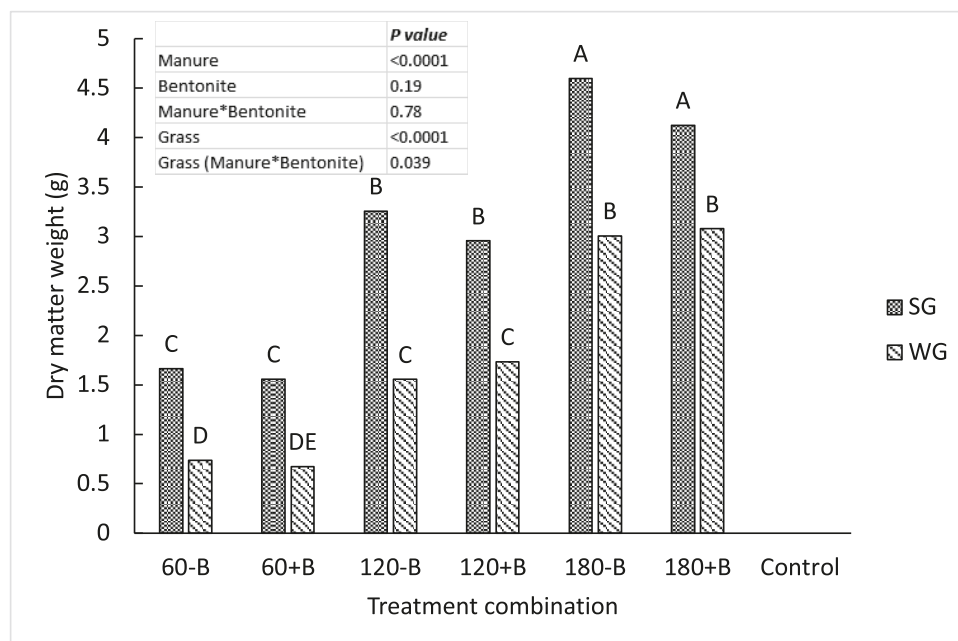
ates and/or phosphates with increased pH, a consequence of the application of composts and liming materials (Walker et al. 2004; Al-Wabel et al. 2015; Jiang et al. 2019). Addition of composts improved physicochemical properties of soil and reduced the bioavailability of heavy metals through sorption processes onto the organic materials (Clemente et al. 2006). Indirect measurements, such as soil pore water concentrations or DGT, are acceptable as viable methods to estimate soil metal concentrations that are available for biological uptake (Martin and Ruby 2004). Therefore, the best treatment for phyto-stabilization and establishment of grasses on chat piles based on DGT extractable Cd and Zn was chat amended with 180 Mg ha⁻¹ manure rate (Table 3). The addition of B did not result in significant reduction in DGT or Ca(NO₃)₂-

extractable Cd or Zn (except in 60 manure rate for DGT-Zn in switchgrass) when compared with respective manure treatment. Organic substances provided with manure could be the main adsorptive phase to sequester cationic metals in manure by-products (Basta et al. 2005; Zhou and Haynes 2010). A previous study suggested that dairy cattle and poultry manure were capable of complexing >60% of available Zn in growth medium (Bolan et al. 2004).

Heavy metal contents in leachates

The four-way interaction (manure rate × bentonite × time × grass species) effect was significant for total Cd in leachates. Total mass of Cd leached from una-

Fig. 3. Dry matter weights of above-ground plant parts of SG and WG grown in chat mixed with different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B). Different letters represent a statistical significance at $p = 0.05$.



mended chat was approximately 200 μg and significantly lower quantities of Cd were found in leachates from all manure-amended treatments (<50 μg) during the growing period (Fig. 1) in both grass species. Leachates of the manure-amended chat treatments had 38–42 μg (on average 83% reduction than the control) and 28–74 μg Cd (on average 73% reduction), respectively, from switchgrass and wheatgrass (Figs. 1a and 1b). Total mass of Cd in switchgrass leachates was not significantly different among pelleted manure-amended treatments while wheatgrass leachates had significantly higher Cd mass in 60 – B treatment than the other pelleted manure-amended treatments. At the lower pelleted manure rate where adsorption sites in the amended chat were limited, B-treated amendment (60 + B) played a role by retention of more Cd than manure-amended chat without B (60 – B). At 120 and 180 Mg ha⁻¹ manure rates, B had no significant effect on Cd retention.

Total mass of Zn in leachates of manure-amended chat was similar to Cd with significant four-way interaction effect (Fig. 2). Untreated chat had around 15 mg of cumulative total Zn in the leachate. Manure-amended chat treatments had significantly lower Zn in the leachate compared to unamended chat, i.e., on average both grass species reduced by 83% of the total Zn in manure-amended treatments. There was no significant difference between the two grass species in relation to Zn loading in leachates. Lead in leachates was very low in all treatments and there were no significant differences among treatments (data not shown).

Potential for metals to be leached from contaminated sites by infiltration water, subsequently spreading the contamination and potentially polluting groundwater is a major environmental concern. The success of phyto-stabilization is also determined by the mobility of toxic elements with the

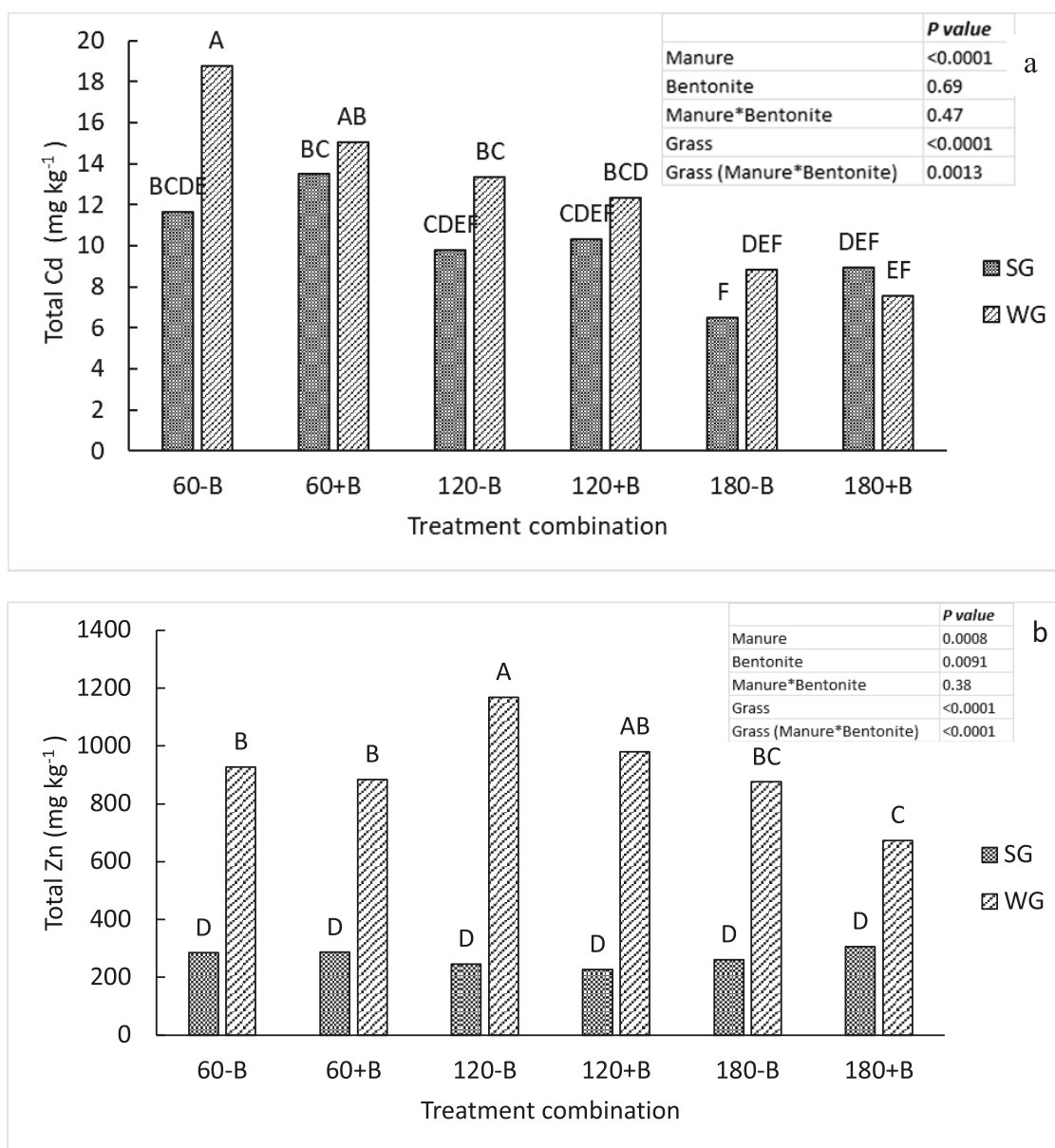
leachate (Martin and Ruby 2004). A combination of compost and other materials, such as ashes and steel shot-mixed amendments, reduced the leaching losses of Cd and Zn by >97% in a metal-contaminated soil (Ruttens et al. 2006). Therefore, manure-amended chat has a high potential to reduce mobility of potentially toxic metals, minimizing the contamination of groundwater from chat. Reduction of metal leaching could be an important advantage of in situ immobilization treatments and may play a role in groundwater protection and reduction of metal dispersion.

Plant growth in amended chat

Plants of neither species survived in unamended chat (limed with CaO only). Though soil acidity is the main constraint for the establishment of vegetation in acid mine spoil environments (Alvarenga et al. 2008), addition of lime only did not improve chat for plant establishment. Chat is a material with a low water-holding capacity, aggregation stability, and nutrient contents, and high toxic concentrations of metals (Pierzynski et al. 2002a, 2002b; Baker et al. 2011; Indraratne et al. 2021). Therefore, cattle manure is a good amendment to improve physical and chemical properties of chat and facilitate plant growth and survival (Novak et al. 2019).

Dry matter yields of grass species grown in pelleted manure-amended chat had a significant grass species \times pelleted manure \times bentonite interaction effect (Fig. 3). In this experiment, chat amended with pelleted manure increased the above-ground dry matter yields of both switchgrass and wheatgrass, the highest manure rate having the highest response in both grass types. Out of the two grass species, switchgrass performed significantly better (25%–56% higher dry matter yield) than the wheatgrass in manure-amended

Fig. 4. Total Cd (a) and Zn (b) concentrations of above-ground plant parts of SG and WG grown in chat mixed with different rates of manure compost (60, 120, and 180 Mg ha⁻¹), with/without bentonite (B). Different letters represent a statistical significance at $p = 0.05$



chat. However, the increase in yield following manure addition rate was more for wheatgrass (4-fold) than for switchgrass (2.5-fold). Addition of B with manure did not show any significant effect on both switchgrass and wheatgrass dry matter yields. Therefore, according to the dry matter yield, chat amended with 180 Mg ha⁻¹ manure with switchgrass seemed more beneficial to cover the contaminated lands than that of other combinations. Novak et al. (2019) noted improved switchgrass growth and lower Cd and Zn availability after amending mine soils with beef cattle manure compost + biochar mix. The choice of the plant is a very important aspect to consider in a phyto-stabilization (Alvarenga et al. 2008). Both grass species showed the suitability, however, with a better crop performance with switchgrass (for each

treatment). The higher capacity to supply essential macronutrients (N, P, K) and the stabilization of potentially toxic metals could be the main explanation for the higher relative growth of grasses at the 180 Mg ha⁻¹ treatment than at other manure rates.

In general, the above-ground biomass of switchgrass and wheatgrass did not give any indication of metal toxicity in manure-amended chat treatments. The three-way interaction effect of grass species \times manure rate \times bentonite was significant for plant tissue Cd and Zn. Significantly higher Cd and Zn concentrations were found in wheatgrass than in switchgrass (Fig. 4). Cadmium concentrations significantly decreased with increasing manure rate, having 52% reduction in wheatgrass and 40% reduction in switchgrass at

Table 4. Pearson’s correlation coefficients (*r*) between properties of chat (*n* = 56) mixed with different rates of compost, with/without bentonite (after harvesting SG and WG) and harvested plant (*n* = 48) parameters of dry matter (DM), total Cd, and Zn.

	Chat parameters after harvest										Plant parameters	
	pH	EC	Available values									
			P	K ⁺	NH ₄ ⁺	NO ₃ [−]	Cd-A ^Z	Zn-A	Cd(DGT) ^Y	Zn(DGT)	DM	Cd
EC	0.28*											
P	0.69***	0.64***										
K ⁺	0.64***	0.68***	0.74***									
NH ₄ ⁺	0.53***	ns	0.53***	ns								
NO ₃ [−]	ns	ns	ns	ns	0.45***							
Cd-A	ns	−0.54***	−0.46***	−0.63***	0.29*	ns						
Zn-A	−0.26*	−0.52***	−0.48***	−0.62***	ns	ns	0.98***					
Cd(DGT)	−0.38**	−0.48***	−0.42**	−0.81***	ns	ns	0.75***	0.72***				
Zn(DGT)	−0.62***	−0.57***	−0.65***	−0.89***	ns	ns	0.72***	0.72***	0.86***			
DM	ns	0.42**	ns	0.73***	−0.44**	−0.36*	−0.73***	−0.75***	−0.84***	−0.73***		
Cd	ns	−0.42**	ns	−0.65***	0.35*	0.42**	0.73***	0.74***	0.78***	0.67***	−0.78***	
Zn	0.57***	ns	0.64***	ns	0.84***	ns	0.53***	0.48***	0.42**	ns	−0.53***	0.43**

Note: ns, nonsignificant. *, **, and *** denote significance at *p* value of 0.05, 0.01, and < 0.001, respectively. ^ZCalcium nitrate extractable.
^YDiffusive gradient thin films extractable (bioavailable).

180 Mg ha⁻¹ than at 60 Mg ha⁻¹ treatments (Fig. 4a). The Zn concentration also decreased with increasing manure rates and was significant in wheatgrass with 20% reduction between 60 Mg ha⁻¹ and 180 Mg ha⁻¹ treatments (Fig. 4b). In general, Zn concentrations in crop tissues ranged from 25 to 150 mg kg⁻¹, while Cd levels are usually less than 1 mg kg⁻¹ (Page et al. 1981). Here, the Cd concentrations in the shoots of switchgrass and wheatgrass were eight- to 14-fold higher, whereas the Zn concentrations in wheatgrass had four- to seven-fold higher values than the respective normal Cd and Zn values in plants. The low Cd and Zn concentrations in switchgrass may have promoted the growth having higher dry matter yield than the wheatgrass. On the other hand, switchgrass with 25%–56% higher dry matter yield than wheatgrass may have caused dilution effect on metal concentrations. Producing high dry matter yields and accumulating moderate to high levels of metals in its biomass qualified these two native grass types in establishing plant cover in chat. In the long run, switchgrass would have better survival capacity than the wheatgrass, based on crop yield and metal accumulation in plant tissues. Previous research has indicated that compost additions decreased plant Zn concentration and allowed more plant survival in Zn contaminated soils (Shuman et al. 2001). Addition of biosolids to soils has been found to favor the formation of nonexchangeable Zn and reduction in water-soluble and exchangeable Zn at pH > 5.8 (Yoo and James 2002). Moreover, increasing pH of chat with CaO and application of manure allowed the formation of insoluble metal precipitates, which may have, in turn, reduced the bioavailability of metals and thus plant uptake of potentially toxic metals (Basta et al. 2001).

Correlation analysis conducted on dry matter yields, nutrients, and potentially toxic metals in chat and plant materials for parameters measured after harvest showed significant positive relationships (Table 4). Results showed significant positive correlations between DGT-available Cd ($r = 0.73$, $p = 0.001$) and Zn ($r = 0.48$, $p = 0.001$) in chat with plant tissue metals. Furthermore, available Cd and Zn in chat and plant tissue Cd and Zn negatively correlated with dry matter yield, indicating negative impact of Cd and Zn on plant growth. Nutrient availability (P, K, and NH₄⁺) in chat correlated positively ($p < 0.001$) with pH, indicating that increase of pH due to addition of pelleted manure increased available nutrients in chat. All forms of tested bioavailable Cd and Zn in chat (Ca(NO₃)₂ extractable and DGT) had negative significant correlation with pH, indicating a decrease of metal availability with increase in chat pH.

Conclusions

The potential use of two perennial native grasses, switchgrass and wheatgrass, for phyto-stabilization of potentially toxic metals in chat was assessed by evaluating biomass production and nutrient/metal composition in response to the addition of pelleted manure compost with or without bentonite. Results of this study revealed that increasing manure rate up to 180 Mg ha⁻¹ improved the chemical properties of growth media, notably their major nutrient contents, which was translated in a higher dry matter yield of both grasses.

Manure addition to contaminated chat also reduced the mobility of potentially toxic metals, subsequently reducing the potential contamination of groundwater. Switchgrass had the ability to produce high dry matter yields and accumulate moderate potentially toxic metals in its biomass better qualifying as a native grass suitable for phyto-stabilization and revegetation of mine spoils. There were no significant benefits of mixing bentonite with pelleted manure compost to remediate mine spoils. In situ immobilization techniques need long-term monitoring of the remediated material due to potential of becoming available from the immobilized pool through organic matter decomposition and soil acidification with time.

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Author information

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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