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Fate and Impacts of Priority Pollutant Metals in Coal Fly Ash–Soil–Switchgrass Plant Mesocosms

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ABSTRACT

Coal fly ash (CFA) is a coal combustion by-product containing heavy metals (HMs) that can enter and accumulate in environmental matrices. The current study assessed the fate and effects of HMs contained in CFA on Armour silt loam (ASL) soil, switchgrass plant, and leachate. Soil impact indices compared levels of HMs in the CFA-soil admixtures to control soil (without CFA). These indices include contamination factor (C_f), modified degree of contamination (mC_d), and pollution load index (PLI). The ratio of the levels of HMs in switchgrass root and shoot tissues to their levels in the CFA-soil admixtures were used to determine bioaccumulation factor (BAF) and enrichment factor (EF). The levels of HMs in the leachate were compared with U.S. Environmental Protection Agency (US EPA) water quality limits. Switchgrass was grown in greenhouse pots containing 0%, 7.5%, and 15% (wt/wt) CFA-soil admixtures for 90 days. Separate portions of the admixtures were inoculated with arbuscular mycorrhizal fungi (AMF) or fortified with glutathione (GSH). Representative samples of CFA-soil admixtures and plant tissues were digested in a microwave accelerated reaction system (MARS) and digests were analyzed for selected US EPA priority pollutant HMs (Cu, Zn, Cr, Cd, Pb, Ni, and As) using inductively coupled plasma optical emission spectroscopy (ICP-OES). Results showed $C_f < 3$ for Cu, Zn, Cr, Cd, Pb, and Ni in all CFA-soil treatments, except for As, which had $C_f > 3$. The 7.5% and 15% CFA-soil admixtures had $mC_d < 4$ and $mC_d > 4$, respectively, on day 0, which were further reduced by day 90, whereas PLI values in all treatments were >1. Switchgrass showed a preferential uptake of Cu and Zn (BAF > 1, EF > 1) when grown in the CFA-soil admixtures, whereas the concentrations of priority metals in the leachate were within the US EPA water quality limits, except for As. The effect and fate of HMs in the CFA-soil-switchgrass mesocosms varied temporally with CFA concentrations, AMF, and GSH in the soil.

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1. Introduction

Coal fly ash (CFA) has once more come under consideration as a valuable resource for agricultural production (Yunusa et al., 2012; Tsadilas, 2014; Dzantor et al., 2015). The by-product has been investigated for improving soil nutrient statuses (Rautaray et al., 2003; Tsadilas, 2014), moisture relationships (Chang et al., 1977; Phung et al., 1978; Garg et al., 2003; Dixit et al., 2016), pH ameliorations (Matsi and Keramidas, 1999; Basu et al., 2009), and wasteland reclamation (American Coal Ash Association, 1998; Jala and Goyal, 2006). However, the presence of toxic heavy metals (HMs) such as As, Cd, Pb, and Se (Adriano et al., 1980; Rautaray et al., 2003) may contaminate soil, posing threats to human and ecosystem health and changing physicochemical property of soils, leading to land degradation including accelerated erosion rates (Gupta et al., 2012).

Depending on its composition and property, the application of certain types and levels of CFA to soils could result in significant alterations of physicochemical properties of those soils and their contamination with several metal species (Basu et al., 2009). Nearly

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5–30% of toxic elements present in CFA, especially Cd, Cu, and Pb, are leachable (Natusch and Wallace, 1974); accordingly, these metals can readily percolate down and contaminate groundwater or nearby waterbodies (Carlson and Adriano, 1993; Gupta et al., 2012). Amending agricultural soils with CFA has been reported to lead to increasing concentrations of extractable Ca, Ba, Mo, Se, S, B, Pb, and Cd (Adriano et al., 1980; Singh et al., 2010; Swamy et al., 2010; Dash et al., 2015). Furthermore, increased activity of certain HM species could hinder the soil microbial activities (Adriano et al., 1978).

Experiments have shown that accumulation and translocation of HMs depends on CFA-soil admixture ratios (Gupta and Sinha, 2009; Pandey et al., 2009; Gupta et al., 2012; Singh et al., 2016). Application rates of up to 100% (wt/wt) CFA-soil as soil amendment for cultivation of food crops have been reported (Pandey et al., 2009). According to Singh et al. (2010), metal uptake and mobilization, as well as tolerance to potentially toxic metals across plant membranes are done by root cells, driven by ATP-dependent proton pumps that catalyze H^+ extrusion across the membrane. Toxic metal–tolerant plants have developed protective mechanisms that sequestrate toxic HMs in their vacuolar compartment, thereby excluding them from cellular sites (Singh et al., 2010). Moreover, metal competition in soils and within plant tissues may complex with metal-binding peptides, metallothioneins, and phytochelatins, resulting in the alleviation of the toxicity of HMs in plants (Singh et al., 2010).

Over the decades, several indices have been developed to assist in assessing levels and degrees of pollution/contamination by metal intrusions into the environment from anthropogenic sources. They include (1) bioaccumulation factor (BAF), (2) enrichment factor (EF), (3) translocation factor (TF), (4) contamination factor (C_f), (5) modified degree of contamination (mC_d), (6) geoaccumulation index (I_{geo}), and (7) pollution load index (PLI). BAF is the ratio of the metal concentration in plant roots to the surrounding environment (e.g., soil or water) (Badr et al., 2012; Majid et al., 2014). EF is the ratio of the metal concentration in plant shoots to the surrounding environment (Sutherland et al., 2000; Mmolawa et al., 2011; Majid et al., 2014). TF is the ratio of metal concentrations in plant shoots to root tissues, or the ratio of EF to BAF (Srivastava et al., 2006; Yoon et al., 2006; Usman and Mohamed, 2009; Majid et al., 2014). BAF, EF, and TF have been used in environmental toxicology and risk assessment as essential indices to assess plant tolerance and to determine the phytoremediation potential of plants that are hyperaccumulators, accumulators, indicators, and excluders (Ghosh and Singh, 2005; Majid et al., 2014). The bioenergy crop, switchgrass (*Panicum virgatum* L.) is a good choice of test crop for cultivation on soil amended with fly ash because it is not a food crop for humans and because HMs in switchgrass are less likely to enter the food chain in other ways. Therefore, determining the phytoremediation efficiency of switchgrass in fly ash–amended or fly ash–contaminated soil could serve a dual purpose of both phyto–metal uptake and bioenergy production.

The parameters C_f, mC_d, and PLI assess contamination by comparing the current levels of metal concentrations in the affected soils to those in unaffected ones (Rahman et al., 2012; Likuku et al., 2013; Ahmad et al., 2014; George et al., 2014). Various studies have involved application of contamination indices to assess the effects of metal entry into a wide range of environmental matrices. These include roadside soils in Botswana (Mmolawa et al., 2011) and Nigeria (Mafuyai et al., 2015), a copper-nickel mine in Botswana (Likuku et al., 2013), and agricultural soils around a coal-fired thermal power plant in India (Sengupta et al., 2010; George et al., 2014).

In spite of the increasing application of these indices to assess the effects of anthropogenic metal contamination, their use on CFA-soil admixtures is limited. This article evaluates (1) the contamination effects (C_f, mC_d, PLI) and fate of selected priority pollutant HMs in CFA-soil admixtures, (2) bioaccumulation (BAF and EF) of HMs in switchgrass plants grown in the CFA-soil admixtures, and (3) levels of HMs in leachates collected from CFA-soil admixtures.

Soil fortification with exogenous glutathione (GSH) or inoculation with arbuscular mycorrhizal fungi (AMF) has been suggested as ways to protect plants against the effects of HMs (Hossain et al., 2012; Emamverdian et al., 2015; Firmin et al., 2015). Huang et al. (2005) have shown that AMF (*Glomus mosseae*) decreased the availability of excessive Zn, Cu, and Pb for maize (*Zea mays* L.) grown on soil contaminated with HMs. Likewise, exogenous GSH has been demonstrated to protect plants against HM toxicity using hydroponic systems (Cai et al., 2010; Wei et al., 2010; Cao et al., 2013; Mostofa et al., 2014). Hence, this study also investigated the fate and effects of the HMs in the CFA-soil-switchgrass mesocosms with separate applications of AMF (*Rhizophagus clarum*) and GSH.

In general, this study addressed the questions on the fate and effects of selected priority pollutant HMs contained in CFA when used as a soil amendment. The fly ash used was alkaline; other fly ash materials will yield different results. The 15% CFA application rate used in this study is to represent a worst-case scenario. If the plants do not accumulate HMs to concerned levels when soil is treated at this rate, it could be concluded that the fly ash treatment is safe. It is important that the conclusions are not overextrapolated but are restricted to the type of fly ash, the type of soil, and the test plants used in this study.

2. Materials and Methods

2.1. Greenhouse pot study

Armor silt loam (ASL) soil collected from Tennessee State University Agricultural Research, Education Center, Nashville, was sieved through a 2-mm sieve. Appropriate amounts of the coal fly ash (CFA) provided by Tennessee Valley Authority, Kingston, were added to separate portions of the soil to provide CFA-soil admixtures (0%, 7.5%, and 15% [wt/wt] CFA-soil). The CFA used in this study is a class C type of fly ash mainly produced as a by-product of lignite coal combustion. Exactly 1.5 kg of each portion of CFA-soil admixture was introduced into plastic pots (14 cm deep, 15 cm top diameter, and 10.5 cm base diameter).

Seeds of the 'Alamo' variety of switchgrass obtained from Star Seeds Inc., Osborne, KS, were started in germination trays containing potting mix (Fafard #2 mix). Switchgrass seedlings at the two-leaf stage (4 weeks old) were transplanted into pots containing the CFA-soil admixtures.

Treatments consisted of (1) soil with CFA-soil admixtures only, (2) CFA-soil admixtures fortified with 0.4 mg/kg exogenous GSH (obtained from Acros Organic, Morris Plains, NJ), and (3) CFA-soil admixtures inoculated with 3% AMF substrate (*R. clarum*, WV234) obtained from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, Morgantown, WV.

Table 1
The pH, total dissolved solids, and levels of selected U.S. Environmental Protection Agency priority pollutant metals in the coal fly ash (CFA)–soil admixtures and at the start of the greenhouse pot study[1]

Physicochemical properties	Soil + 0% CFA (control)	Soil + 7.5% CFA	Soil + 15% CFA	CFA	Range in soil[2]	Range in CFA[2]
pH	5.39[a]	7.98[b]	8.59[c]	10.7[d]	–	–
Total dissolved solids (mg/kg)	101[a]	337[b]	488[c]	383[d]	–	–
Heavy metals (mg/kg)						
Cu	12.8[a]	18.6[b]	24.6[c]	42.4[d]	2–100	14–2800
Zn	160[a]	161[a]	193[ab]	221[b]	10–300	10–3500
Cr	28.3[a]	33.0[ab]	37.0[b]	58.4[c]	5–3000	10–1000
Ni	26.2[a]	28.3[a]	31.4[b]	34.3[b]	5–500	6.3–4300
Cd	2.06[a]	2.36[b]	3.06[c]	3.73[d]	0.01–0.7	0.7–130
As	0.20[a]	2.30[a]	7.70[b]	21.5[c]	1–50	2.3–6300
Pb	36.7[a]	35.4[a]	34.8[a]	12.4[b]	2–200	3.1–5000

[1] Data in a row followed by the same letter indicate no significant difference between CFA-soil admixtures ($p < 0.05$, Duncan multiple range test).
[2] Basu et al. (2009).

Pots were arranged in a completely randomized design on greenhouse benches with four replications. Plants were watered every other day with approximately 100 ml of deionized water, and leachate was collected into 500-mL Nalgene bottles (Thermo Scientific, Suwanee, GA) via Tygon tubing connected to the base of each pot. The leachate samples collected every other day were analyzed on day 30. A new batch of leachate was collected every other day from day 31 to day 60 and from day 61 to day 90 into new bottles to limit any external contamination (e.g., dust or microbial growth). Greenhouse set conditions were temperature (daytime, 75°F; night, 78°F), humidity (80%), and day-night schedule (sunrise, 8:53 am; sunset, 7:04 pm).

2.2. Determination of selected priority pollutant HM concentrations in samples (CFA-soil admixtures, plant, and leachate)

Air-dried soil samples (0.25 g) at days 0 and 90 were digested with 10 mL of concentrated HNO_3 (U.S. Environmental Protection Agency [US EPA], 2007). Oven-dried (70°C) plant tissues (root and shoot, 0.25 g) were digested with 1:3 concentrated H_2O_2:HNO_3 (US EPA, 1996) in a microwave-assisted accelerated reaction system (MARS5 v194A08; CEM Corporation) at 170°C for 10 minutes. Digests were diluted to 100 mL with deionized H_2O and filtered through Whatman 42 ashless filter paper (GE Healthcare, Buckinghamshire, U.K.). To 250 mL of leachate samples collected, 3 mL of nitric acid was added before leachate samples were filtered. The concentrations of Co, Cu, Fe, Pb, Mn, Ni, and Zn in the filtrates were determined using an inductively coupled plasma spectrometer (iCAP 7000 series, Thermo Scientific). The inductively coupled plasma–optical emission spectroscopy (ICP-OES) detection limits were 5 μg/L for Cu, Zn, Cr, Ni, and Cd and 50 μg/L for As and Pb.

2.3. Measurement of pH and total dissolved solids in CFA-soil admixtures and leachate samples

To determine the pH of soil, CFA, and CFA-soil admixtures, 10 g of respective samples were weighed and added to 50-ml diluent (10 mM $CaCl_2$) in 125-mL Nalgene bottles (Thermo Scientific). The lids on the bottles were tightened and the mixture agitated at 15 rpm for 1 hour on a platform shaker, after which pH was measured using a portable instrument (ORION 4 Star, Thermo Scientific). The pH of leachate samples was measured directly.

To determine the total dissolved solids (TDS) in soil, CFA, and CFA-soil admixtures, 10-g soil samples were weighed and added to 50 mL of deionized H_2O in 125-ml Nalgene bottles. The lids on the bottles were tightened and the mixture agitated at 15 rpm for 1 hour on a platform shaker, after which TDS was measured using the portable instrument (ORION 4 Star). Total dissolved solids in leachate samples were measured directly. The pH, TDS, and concentrations of selected HMs in the CFA-soil admixtures and CFA used in this study are presented in Table 1, along with the typical ranges of the HMs found in soils and CFA (Basu et al., 2009).

2.4. Impact analysis

To estimate the effects of HM-containing CFA on the soil and switchgrass, metal concentration data were subjected to modified analytical methods of contamination and pollution indices (Rahman et al., 2012; Likuku et al., 2013; Liu et al., 2016) and accumulation (Majid et al., 2014) as expressed in Eqs. 1–5.

$$C_f^i = (C_m \text{ in CFA-soil admixture}/C_m \text{ in control soil}) \tag{1}$$

$$mC_d = \sum_{i=1}^{i=n} C_f^i/n \tag{2}$$

$$PLI = \left(C_f^1 \times C_f^2 \times C_f^3 \times \ldots \times C_f^n\right)^{1/n} \tag{3}$$

$$BAF = C_m \text{ in plant root}/C_m \text{ in soil} \tag{4}$$

$$EF = C_m \text{ in plant shoot}/C_m \text{ in soil} \tag{5}$$

C_m is the concentration of metals, and n is the number of metals analyzed.

Table 2 describes the classifications and descriptions of effects on the basis of C_f, mC_d, PLI, and BAF, respectively.

2.5. Statistical analysis

The results obtained for the HM concentrations were expressed as mean values, and the impact factors were expressed in bar graphs. The final data from the HM concentrations were subjected to one-way analysis of variance (ANOVA) to compare means. Based on the outcome of ANOVAs on all data, post hoc analysis was performed

Table 2
Classes and description of contamination factors (C_f), modified degree of contamination (mC_d), pollution load indices (PLI), and bioaccumulation factor (BAF)

Classification	Description
C_f class	Described by Rahman et al. (2012); Likuku et al. (2013)
$C_f < 1$	Low contamination
$1 \leq C_f < 3$	Moderate contamination
$3 \leq C_f < 6$	Considerable contamination
$C_f \geq 6$	Very high contamination
mC_d class	Described by Rahman et al. (2012); Likuku et al. (2013)
$mC_d < 1.5$	Nil to very low degree of contamination
$1.5 \leq mC_d < 2$	Moderately contaminated
$2 \leq mC_d < 4$	Moderate degree of contamination
$4 \leq mC_d < 8$	High degree of contamination
$8 \leq mC_d < 16$	Very high degree of contamination
$16 \leq mC_d < 32$	Extremely high degree of contamination
$mC_d \geq 32$	Ultra-high degree of contamination
PLI class	Described by Ahmad et al. (2014); George et al. (2014)
PLI < 0.5	No pollution, no drastic rectification is needed
0.5 < PLI < 1	Low pollution, more detailed study is needed
PLI = 1	Baseline pollution, immediate intervention is needed
PLI > 1	Moderate to high pollution, immediate intervention is needed
BAF class	Described by Ma et al. (2001)
BAF < 1	Excluder
BAF > 1	Accumulator
BAF > 10	Hyperaccumulator

with Duncan's multiple range test to separate the means pairwise. The statistical tools used were SPSS IBM v20.0 and the Microsoft 2010 Excel package.

3. Results

3.1. Effect of CFA amendment, AMF inoculation, and GSH fortification on soil pH, TDS, and HM concentrations

The initial (day 0) pH, TDS (milligrams per kilogram), and HM concentrations (milligrams per kilogram) in the CFA-soil admixtures increased significantly ($p < 0.05$) with increased levels of CFA in the soil, except for Pb. The Pb concentrations in the CFA-soil admixtures decreased with increased levels of CFA in the soil (Table 1). The pH, TDS, and concentrations of nitric acid–extractable HMs in different treatments of CFA-soil admixtures after 90 days of the greenhouse pot experiment are presented in Table 3.

The soil pH ranged from a minimum of 5.34 in control soil to a maximum of 8.11 in soil mixed with 15% CFA (Table 3). Total dissolved solids ranged from 173 mg/kg in control soil to 458 mg/kg in soil mixed with 15% CFA (Table 3). The pH and TDS increased significantly ($p < 0.05$) in the CFA-amended soils compared with the control (unamended) soil. In the control soil, the HM with the highest concentration (milligrams per kilogram) was Zn (96.9), and As had the lowest concentration (0.18). With 7.5% CFA added to the soil, the highest metal concentration was Zn (71.2) and the lowest was As (0.63). With 15% CFA in the soil, the highest metal concentration was Zn (78.9) and the lowest was Cd (1.58). The concentrations of extractable Cu, Zn, and Pb were reduced in the CFA-amended soils compared with the control, whereas Cr, Ni, Cd, and As were higher in the CFA-amended soils after 90 days. These variations in metal concentrations were significant ($p < 0.05$) for Cu, Zn, Cr, Ni, Cd, and Pb with 7.5% CFA in the soil and for Cu, Cr, Ni, Cd, As, and Pb for 15% CFA in the soil compared with the control (Table 3).

In CFA-soil admixtures (0%, 7.5%, and 15% [wt/wt] CFA-soil) inoculated with AMF alone (Table 3), the pH and TDS were reduced compared with the CFA-soil admixtures that were not inoculated with AMF. The variations in pH between the AMF-inoculated and the uninoculated CFA-soil admixtures were significant ($p < 0.05$), whereas the variation in TDS was not significant. In the 0% CFA-soil admixture inoculated with AMF alone compared with the control soil, Cu, Zn, Cr, As, and Pb were reduced, Ni increased, and Cd remained unchanged. In the 7.5% CFA-soil admixture, Cu, Zn, Cr, and As were increased, whereas Ni, Cd, and Pb were reduced with AMF inoculation compared with the uninoculated. In the 15% CFA-soil admixture, Cu and As were increased, whereas Zn, Cr, Ni, Cd, and Pb were reduced with AMF inoculation compared with the uninoculated. These variations in the concentrations of HMs were significant ($p < 0.05$) for Cu and Pb in the 0% CFA-soil admixture and significant ($p < 0.05$) for Cr in the 15% CFA-soil admixture inoculated with AMF compared with the uninoculated (Table 3).

In the 0% CFA-soil admixture fortified with GSH alone compared with the control soil (Table 3), the pH and TDS were reduced, with the reduction in TDS being significant ($p < 0.05$). Meanwhile, in the 7.5% and 15% CFA-soil admixtures that were fortified with GSH, the pH and TDS were increased compared with the nonfortified CFA-soil admixtures, with the increase in pH being significant ($p < 0.05$). The concentrations of metals varied with GSH alone in the CFA-soil admixtures. In the 0% CFA-soil admixture fortified with GSH, all the metals were reduced compared with the control soil, with the variations being significant ($p < 0.05$) for Cu and

Table 3
Average values of pH, total dissolved solids (TDS), and selected U.S. Environmental Protection Agency priority pollutant metals in different soil treatments at the end of the 90-day greenhouse pot study[1]

Soil treatment	pH	TDS (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	As (mg/kg)	Pb (mg/kg)
0% CFA (control)	5.34^{a}	173^{a}	10.5^{a}	96.9^{a}	15.0^{ab}	12.6^{ab}	0.98^{a}	0.18^{a}	23.4^{a}
7.5% CFA	7.74^{c}	316^{c}	6.73^{e}	71.2^{bc}	17.9^{cd}	15.2^{cde}	1.28^{b}	0.63^{a}	21.3^{b}
15% CFA	8.11^{f}	459^{d}	8.88^{bc}	78.9^{abc}	21.4^{e}	17.6^{f}	1.58^{c}	3.06^{c}	20.4^{bc}
0% CFA + AMF	5.26^{b}	142^{ab}	7.28^{de}	85.5^{ab}	14.5^{ab}	13.4^{abc}	0.98^{a}	ND	21.0^{b}
7.5% CFA + AMF	7.68^{d}	305^{c}	7.43^{de}	71.7^{bc}	18.1^{cd}	15.2^{cde}	1.23^{b}	1.04^{ab}	20.3^{bc}
15% CFA + AMF	8.02^{g}	457^{d}	9.83^{ab}	76.3^{bc}	18.8^{cd}	16.0^{def}	1.53^{c}	4.01^{c}	19.1^{cd}
0% CFA + GSH	5.30^{a}	124^{b}	7.38^{de}	65.2^{c}	12.9^{a}	11.7^{a}	0.93^{a}	0.02^{a}	21.9^{ab}
7.5% CFA + GSH	7.78^{e}	319^{c}	8.43^{cd}	122^{d}	16.7^{bc}	14.3^{bcd}	1.24^{b}	2.81^{b}	20.5^{bc}
15% CFA + GSH	8.12^{f}	468^{d}	10.4^{a}	84.8^{ab}	19.4^{de}	17.2^{ef}	1.63^{c}	3.31^{c}	18.4^{d}

Note: AMF = arbuscular mycorrhizal fungi; CFA = coal fly ash; GSH = glutathione; ND = not detected.
[1] Data in a column followed by the same letter indicate no significant difference between CFA-soil admixtures ($p < 0.05$, Duncan multiple range test).

Table 4
Average concentrations of selected U.S. Environmental Protection Agency priority metals in the switchgrass tissues (root and shoot) grown on different soil treatments[1]

Soil treatment	Cu (mg/kg DW)	Zn (mg/kg DW)	Cr (mg/kg DW)	Ni (mg/kg DW)	Cd (mg/kg DW)	As (mg/kg DW)	Pb (mg/kg DW)
Root							
0% CFA (control)	14.8[a]	133[a]	1.80[ab]	0.30[a]	0.76[a]	ND	1.80[ab]
7.5% CFA	11.0[b]	221[c]	1.85[ab]	0.30[a]	0.76[a]	0.20[a]	1.10[ad]
15% CFA	12.0[c]	89.1[f]	2.40[b]	ND	1.52[c]	ND	ND
0% CFA + AMF	9.62[e]	207[b]	1.90[ab]	0.90[b]	0.76[a]	0.20[a]	3.30[b]
7.5% CFA + AMF	8.52[f]	83.0[d]	1.50[a]	0.30[a]	0.76[a]	0.30[a]	5.90[e]
15% CFA + AMF	9.72[e]	42.8[g]	1.90[ab]	ND	1.16[b]	0.70[a]	0.30[d]
0% CFA + GSH	13.0[d]	132[a]	1.90[ab]	4.30[c]	0.76[a]	0.60[a]	2.40[bc]
7.5% CFA + GSH	9.12[ef]	77.3[e]	1.50[a]	ND	0.76[a]	ND	0.50[d]
15% CFA + GSH	9.32[ef]	34.5[h]	1.90[ab]	ND	0.76[a]	3.50[b]	ND
Shoot							
0% CFA (control)	11.5[a]	52.2[a]	1.20[a]	3.20[a]	0.36[a]	3.80[a]	1.50[ab]
7.5% CFA	13.0[ac]	93.0[d]	1.00[ac]	7.60[b]	0.36[a]	ND	1.10[abc]
15% CFA	14.5[c]	163[g]	2.10[b]	2.60[c]	0.72[b]	1.80[b]	ND
0% CFA + AMF	9.62[b]	126[b]	0.70[ac]	7.50[b]	0.36[a]	0.70[b]	2.00[abc]
7.5% CFA + AMF	8.92[b]	54.8[e]	0.80[ac]	6.30[d]	0.36[a]	0.50[b]	1.30[abc]
15% CFA + AMF	9.22[b]	72.6[h]	0.50[c]	4.70[e]	0.36[a]	0.10[b]	0.50[acd]
0% CFA + GSH	12.9[a]	56.0[c]	0.90[ac]	4.90[e]	0.36[a]	0.40[b]	1.40[b]
7.5% CFA + GSH	11.6[a]	104[f]	0.80[ac]	4.70[e]	0.36[a]	0.40[b]	0.40[cd]
15% CFA + GSH	9.02[b]	63.7[i]	0.60[c]	3.60[f]	0.36[a]	0.10[b]	ND

Note: AMF = arbuscular mycorrhizal fungi; CFA = coal fly ash; DW = dry weight; GSH = glutathione; ND = not detected.
[1] Data in a column followed by the same letter indicate no significant difference between CFA-soil admixtures ($p < 0.05$, Duncan multiple range test).

Zn. In the 7.5% CFA-soil admixture fortified with GSH, Cu, Zn, and As were significantly ($p < 0.05$) increased, whereas Cr, Ni, Cd, and Pb were reduced compared with the corresponding admixture without GSH. In the 15% CFA-soil admixture, Cu, Zn, Cd, and As were increased, whereas Cr, Ni, and Pb were reduced with GSH in the admixture, with the variations being significant ($p < 0.05$) for Cu and Pb (Table 3).

All treatments of CFA-soil admixtures had $C_f < 3$ for Cu, Zn, Cr, Ni, and Cd and $C_f < 1$ for Pb. On day 0, CFA-soil admixtures (7.5% and 15%) had $C_f > 6$ for As and on day 90, 7.5% CFA-soil admixture had $C_f < 6$ for As. CFA-soil admixtures (7.5% and 15%) had $mC_d > 1.5$ on day 0; on day 90, 7.5% CFA-soil admixture had $mC_d < 1.5$. CFA-soil admixtures (7.5% and 15%) containing AMF or GSH had $mC_d < 1.5$ on day 90. All treatments of CFA-soil admixtures had PLI > 1.

3.2. *Effect of CFA amendment, AMF inoculation, and GSH fortification on the fate of HMs in plant tissues*

The metal concentrations in switchgrass tissues varied significantly ($p < 0.05$) between different treatments of CFA-soil admixtures. With CFA-soil admixtures alone, the total concentrations of HMs were highest in root tissues from the 7.5% CFA-soil admixture and lowest in shoot tissues from the control soil (Table 4). With CFA-soil admixtures inoculated with AMF, the total concentration of HMs was highest and lowest in root tissues from 0% and 15% CFA-soil admixtures, respectively (Table 4). With CFA-soil admixtures fortified with GSH, the total concentration of HMs was highest and lowest in root tissues from 0% and 15% CFA-soil admixtures, respectively (Table 4).

In CFA-soil admixtures only, switchgrass bioaccumulation showed BAF values were >1 for Cu and Zn (Table 5). In 0% and 7.5% CFA-soil admixtures inoculated with AMF, BAF values were >1 for Cu and Zn. The BAF values were >1 for Cu and Zn in 0% CFA-soil admixture fortified with GSH, for Cu in 7.5% CFA-soil admixture fortified with GSH, and for As in 15% CFA-soil admixture fortified with GSH (Table 5).

The enrichment (uptake of metals into the shoots) of switchgrass showed EF > 1 for Cu in 0%, 7.5%, and 15% CFA-soil admixtures only, with EF > 1 for Zn in 7.5% and 15% CFA-soil admixtures only (Table 5). The EF values were >1 for Cu in 0% and 7.5% CFA-soil admixtures inoculated with AMF and for Zn in 0% CFA-soil admixture inoculated with AMF. Similarly, the EF values were >1 for Cu in 0% and 7.5% CFA-soil admixtures fortified with GSH (Table 5).

Table 5
Bioaccumulation factor and enrichment factor of metal concentrations in switchgrass tissues

CFA/soil treatment	Cu	Zn	Cr	Ni	Cd	As	Pb
Bioaccumulation factor							
0% CFA/soil (control)	1.41	1.37	0.12	0.02	0.78	ND	0.08
7.5% CFA/soil	1.67	3.11	0.10	0.02	0.59	0.32	0.05
15% CFA/soil	1.36	1.13	0.11	ND	0.96	ND	ND
0% CFA/soil/AMF	1.32	2.43	0.13	0.07	0.78	ND	0.16
7.5% CFA/soil/AMF	1.15	1.16	0.08	0.02	0.62	0.29	0.29
15% CFA/soil/AMF	0.99	0.56	0.10	ND	0.76	0.17	0.02
0% CFA/soil/GSH	1.76	2.03	0.15	0.37	0.82	ND	0.11
7.5% CFA/soil/GSH	1.08	0.64	0.09	ND	0.62	ND	0.02
15% CFA/soil/GSH	0.90	0.41	0.10	ND	0.47	1.06	ND
Enrichment factor							
0% CFA/soil (control)	1.10	0.54	0.08	0.25	0.37	ND	0.06
7.5% CFA/soil	1.94	1.31	0.06	0.50	0.46	ND	0.05
15% CFA/soil	1.63	2.07	0.10	0.15	0.96	0.59	ND
0% CFA/soil/AMF	1.32	1.48	0.05	0.56	0.28	ND	0.10
7.5% CFA/soil/AMF	1.20	0.76	0.04	0.42	0.29	0.48	0.06
15% CFA/soil/AMF	0.94	0.95	0.03	0.29	0.24	0.02	0.03
0% CFA/soil/GSH	1.75	0.86	0.07	0.42	0.39	ND	0.05
7.5% CFA/soil/GSH	1.38	0.86	0.05	0.33	0.29	0.14	0.02
15% CFA/soil/GSH	0.87	0.75	0.03	0.21	0.22	0.03	ND

Note: AMF = arbuscular mycorrhizal fungi; CFA = coal fly ash; GSH = glutathione; ND = not defined because metal was not detected.

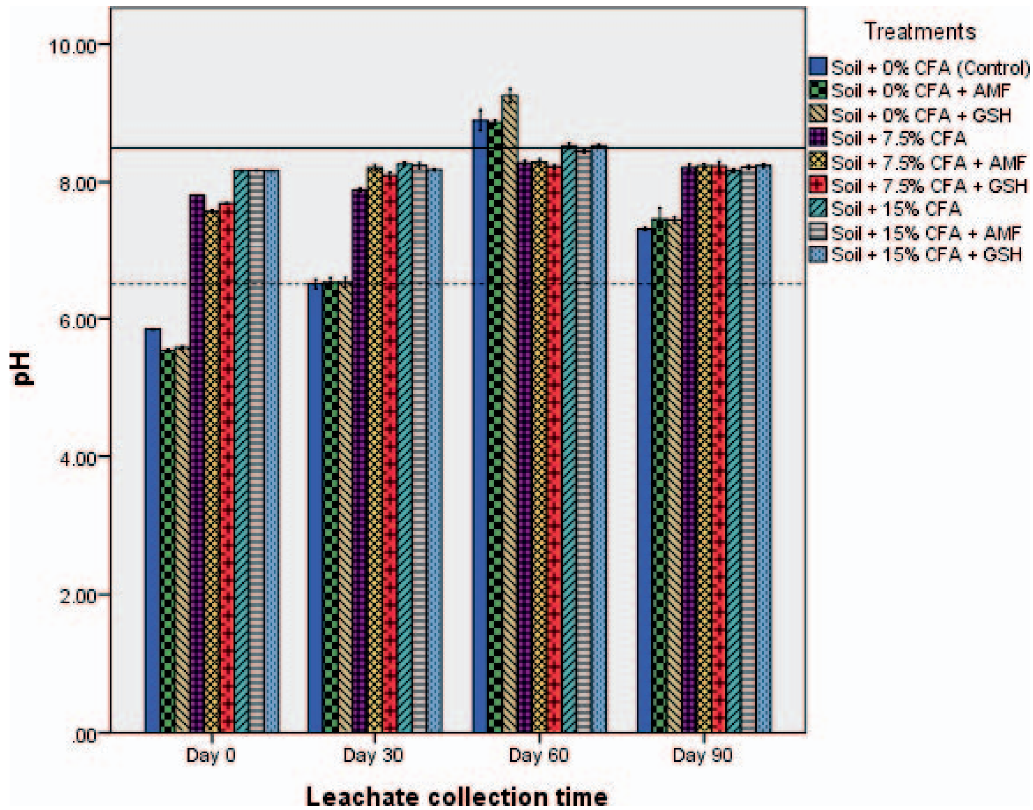

Fig. 1. The pH of leachate samples collected over a 90-day period. Reference horizontal lines indicate the upper (8.5) and lower (6.5) pH limits of U.S. Environmental Protection Agency (US EPA, 2001) regulatory levels.

3.3. Effect of CFA amendment, AMF inoculation, and GSH on the physicochemical properties and distribution of HMs in the leachate

The pH of the leachate collected from the 7.5% and 15% CFA-amended soils were significantly ($p < 0.05$) higher than the control soil on days 0, 30, and 90 but lower on day 60 (Figure 1). The temporal variations in leachate pH were within 1 unit with 7.5% and 15% CFA-soil admixtures compared with the leachate pH from control soil, which varied up to 3 units. The pH was within US EPA water quality limits for leachate collected from CFA-amended soils. TDS (milligrams per liter) in leachate samples varied significantly ($p < 0.05$) with time, and the highest and lowest values were on days 30 and 90, respectively (Figure 2). TDS was significantly ($p < 0.05$) higher with increased CFA concentrations and was beyond US EPA water quality limits in leachate collected from the CFA-amended soils.

The metal concentrations in the leachate collected across the duration of the greenhouse study were less than that of the CFA-soil admixtures and were below the US EPA water quality limits, except for As (Figure 3) in leachate collected mainly from 15% CFA-soil admixture on days 30, 60, and 90. The variations in pH and TDS of the leachate samples collected from CFA-soil admixtures containing AMF and GSH were similar to those of the CFA-soil admixtures only.

4. Discussion

Because of the alkaline nature of the CFA used in this study, its addition to ASL soil increased the soil pH. The relatively higher levels of TDS, Cu, Zn, Cr, Ni, Cd, and As in the CFA compared with the ASL soil also increased their corresponding levels in the CFA-soil admixtures. However, the concentrations of HMs in the CFA and in the CFA-amended or unamended soils used in this study were within their typical ranges in CFA and soil, respectively, except for Cd, which was beyond the typical soil range (Basu et al., 2009). Swamy et al. (2010), Dash et al. (2015), and Singh et al. (2016) have reported increases in pH, concentrations of HMs, or both with increased levels of alkaline CFA in the soil. It is important to note that soil contamination or pollution by CFA may vary temporally with the type, property, composition, and concentration of the CFA.

The impact factors or indices with reference to contamination, pollution, or both by HMs have been explored with the aim of categorizing the effect of anthropogenic activities on the environment (Rahman et al., 2012; Likuku et al., 2013). The contamination factor (C_f) assessed the levels of each HM in CFA-soil admixtures relative to their levels in control soil (Rahman et al., 2012; Likuku et al., 2013). In this study, all treatments of CFA-amended soils had low to moderate metal contamination levels, except for As. The contamination factor increased with increased concentrations of CFA in the soil and decreased with time. The modified degree of contamination (mC_d) averages the degree of contamination (C_d), which is the sum of C_f for all selected HMs; mC_d allowed the estimation of the overall mean value for a range of pollutants (Rahman et al., 2012; Likuku et al., 2013). In this study, soils amended with 7.5% and 15% CFA were moderately contaminated, and the degrees of contamination were decreased by day 90. Also on day 90, the CFA-amended soils containing AMF or GSH had a very low degree of

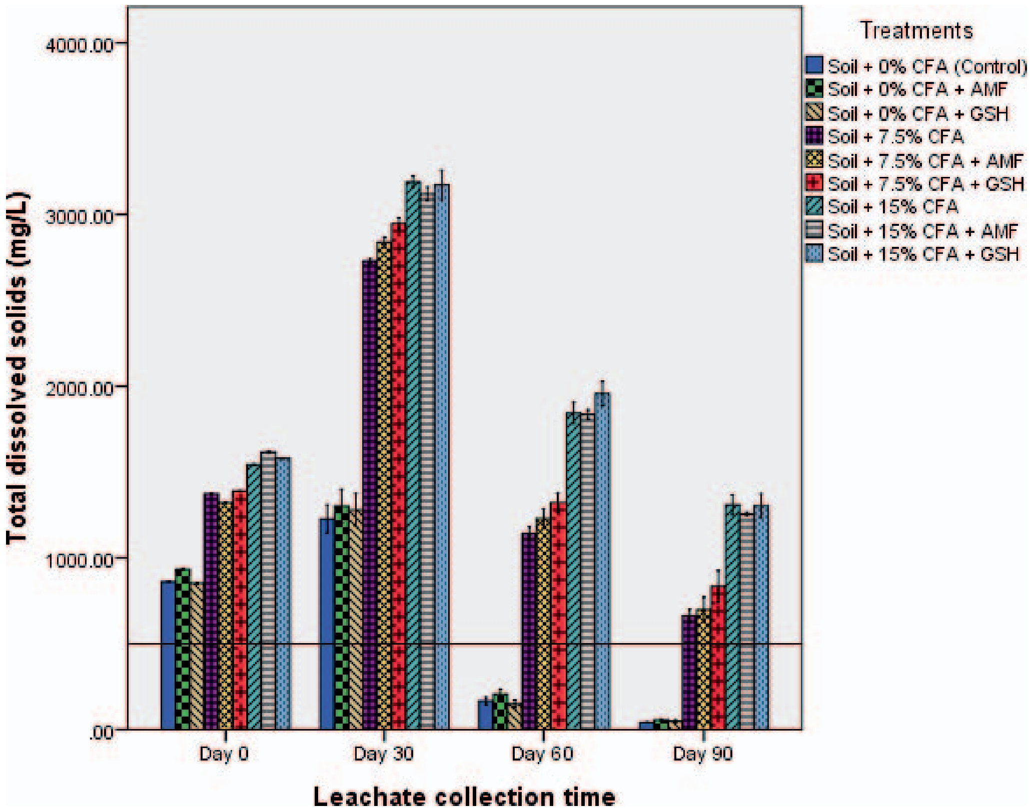

Fig. 2. Total dissolved solids in leachate samples collected over a 90-day period. Reference horizontal line indicates the U.S. Environmental Protection Agency water quality limit of 500 mg/L (US EPA, 2001).

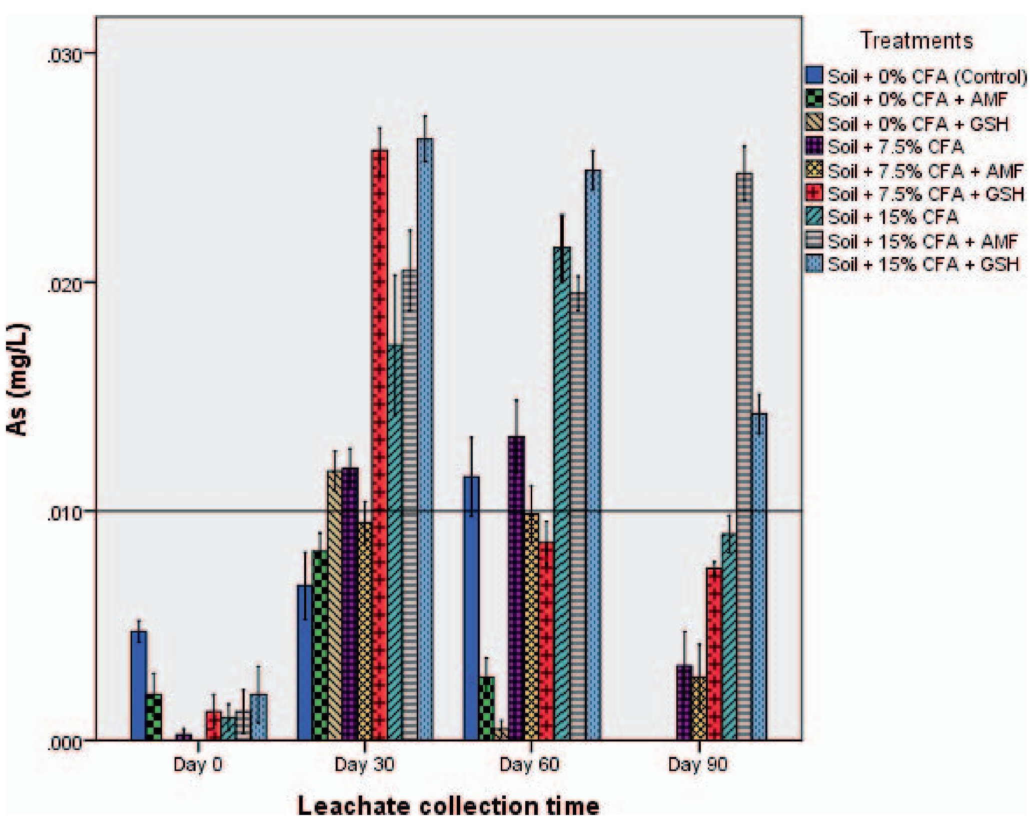

Fig. 3. Concentrations of As in leachate samples collected over a 90-day period. Reference horizontal line indicates the U.S. Environmental Protection Agency water quality limit of 0.01 mg/L (US EPA, 2001). Concentrations of Cu, Zn, Cr, and Pb in leachate samples were below the US EPA water quality limit, whereas Ni and Cd were not detected in the leachate.

contamination, which may be caused by assimilation of the metals into the cell walls of AMF hyphae (Emamverdian et al., 2015; Firmin et al., 2015), whereas exogenous GSH may allow plant assimilation of the metals (Cai et al., 2010; Wei et al., 2010; Cao et al., 2013). According to Rahman et al. (2012) and Likuku et al. (2013), the PLI proposed by Tomlinson et al. (1980) allows the detection of pollution and the necessary actions that need to be taken. The concentration of one HM may have a great influence on the values of mC_d and PLI. The high PLI reported in this study may be due to the high C_f for As, even though the concentration of As in the CFA, ASL soil, and CFA-soil admixtures were within the normal levels in uncontaminated soil.

Concentrations of HMs in the switchgrass tissues (root and shoot) varied with respect to individual metal levels in the CFA-soil admixtures, with highest and lowest metal uptake being Zn and Pb, respectively. Root uptake and translocation of HMs into the shoot were affected by the CFA treatments, AMF inoculation, and GSH fortification. For example, root uptake of Cu in CFA-amended soils was decreased compared with the unamended soils, whereas shoot uptake was increased. However, AMF or GSH added with CFA to soils reduced the uptake of Cu into the root and shoot. The reduced uptake of Cu into the plant tissues in the presence AMF or GSH may be due to efficient chelation or restriction of the metal within the plant rhizosphere (Huang et al., 2005; Mostofa et al., 2014). The availability, mobility, uptake, and translocation of HMs may be dependent on the interactions between a combination of HMs, soil, water, and plants (Singh et al., 2010; Chopra and Pathak, 2012). Singh et al. (2010) reported a significantly higher concentration of HMs in plant tissues (root and shoot) collected from CFA-contaminated sites than in uncontaminated sites.

The higher BAF for Cu and Zn in switchgrass may be due to a higher binding affinity of metallothioneins and phytochelatins for these elements (Singh et al., 2003, 2010). Plants have developed strategies to avoid toxic metal accumulation in roots and subsequent translocation into the shoots (Hossain et al., 2012; Emamverdian et al., 2015). Plants association with AMF (Firmin et al., 2015) or utilization of GSH (Hossain et al., 2012) may be also be an important strategy. AMF can effectively immobilize HMs and reduce their uptake by host plants during HM contamination and pollution by binding metal ions to hyphal cell walls and excreting several extracellular biomolecules (Emamverdian et al., 2015; Firmin et al., 2015). GSH serves a similar purpose by enhancing the plant's oxidative defense system (Hossain et al., 2012). The study carried out by Singh et al. (2010) on the accumulation and translocation of HMs in soil and plants from fly ash–contaminated sites showed variation in translocation specific to different metals in the contaminated soil than in the control soil, in which some were more, some were less. Singh et al. (2016) also reported increased metal accumulation in rice plant (*Oryza sativa* L.) because of CFA in the soil. Singh et al. (2010) attributed the variations in enrichment to the cytogenetic makeup of the plants, as well as a number of other factors (anatomical, biochemical, and physiological). Therefore, establishing a pattern of translocation of metals can be very useful in biological monitoring of HM contamination (Singh et al., 2010).

Leachate pH and TDS increased with increased CFA levels in the admixtures, but only temporally. TDS is directly related to the amounts of dissolved solutes leached out from the admixtures, whereas the increase in leachate pH ($\leq$3 units in control soil and $<$1 unit in CFA-soil admixtures) may be due to a number of factors, including the leachate collection strategy used in this study, watering volume/frequency (Costello, 2011; Singer, 2012), ion exchanges (e.g., NO_3^- uptake/OH^-, CO_3^{2-}, HCO_3^- release) during different growth stages of the plant (Nye, 1981; McClure et al., 1990; Muofhe and Dakora, 2000; Stoltz and Greger, 2002), root exudation (Jones and Darrah, 1994; Jones, 1998), or both. The concentrations of HMs in the leachate samples collected throughout the study were below the US EPA permissible limits for potable water, except for As, in the leachates collected on days 30 and 60 from CFA-soil admixtures. To reduce the As contamination, it is advisable to use lower concentrations of this alkaline fly ash in amending the soil.

5. Conclusions

Amending a silt loam soil with the alkaline CFA used in this study at rates of 7.5% and 15% elevated the levels of HMs in the soil, although with a minimal effect on contamination. Likewise, the concentrations of HMs in the leachates collected from the CFA-soil admixtures were within the US EPA water quality limits (except for As). The distribution of HMs within the plant tissues was affected by CFA-soil amendment rates, AMF inoculation, and exogenous GSH. Coapplication of CFA with AMF would be more effective than with GSH for amending agricultural or nutrient-deficient marginal soils to allow the cultivation of a nonfood bioenergy crop such as switchgrass for bioenergy production.

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