Water Air Soil Pollut (2009) 202:229–243
DOI 10.1007/s11270-008-9972-9

# Evaluation of Poultry Litter Amendment to Agricultural Soils: Leaching Losses and Partitioning of Trace Elements in Collard Greens

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Received: 20 March 2008 / Accepted: 22 December 2008 / Published online: 16 January 2009
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**Abstract** Leaching of trace metals and greenhouse plant growth (Collard greens; *Brassica oleracea var. acephala)* response studies were conducted in two types of soils with contrasting characteristics amended with varying rates (0 to 24.70 Mg ha$^{-1}$) of poultry litter (PL) or 1:1 mixture of PL and fly ash (FA). Leaching of Cr, Zn, Cd, Cu, and Pb from soils amended with PL or PL + FA (1:1) increased with increasing rates of amendment. Leaching losses were greater from coarse-textured soil compared to that from medium-textured soil. Crop performance study indicated that growth as well as trace elements concentrations increased with increasing rates of amendments only up to 12.35 Mg ha$^{-1}$. Trace element concentrations in plant parts were greater in plants grown in Candler fine sand (CFS) compared to that grown in Ogeechee loamy sand (OLS). Trace element concentrations were greater in the above ground plant parts (leaf and stem) than those in roots. This study demonstrated beneficial effects of PL or mixture of PL + FA amendments to soils at rates not exceeding 4.94 Mg ha$^{-1}$. Further field studies are recommended to evaluate the long-term impact of using poultry litter and fly ash on plant growth and tissue trace metal concentration as well as environmental impact.

**Keywords** Accumulation · Collard greens · Dry matter yield · Fly ash · Growth performance · Leaching · Partitioning of trace elements · Poultry litter · Tissue trace element concentrations

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## 1 Introduction

Animal manures (Cabrera and Sims 2000. Eneji et al. 2001; Pederson et al. 2002) and other domestic and industrial by-products (Basta 2000; Sajwan et al. 2003; Zvomuya et al. 2006) are used as soil amendments in the United States as organic sources of plant nutrients and for improving physical, chemical, and biological properties of soils. Animal manures can be soil applied as fresh material or after composting (Moss et al. 2002). However, one major concern associated with land application of the above by-products is the potential for contamination of environment by trace elements and/or pathogens (Gupta

and Charles 1999; Jackson et al. 2003; Basta et al. 2005; Pirani et al. 2006) and, in turn, entry of these contaminants into human food chain. With the increased interest of producers and consumers towards the use of organic amendments as substitute for conventional chemical fertilizers, the use of organic amendments in agriculture will increase significantly. This trend could contribute to potential for increased pollution of soil and water due to over-use of organic amendments.

In the United States, about 317 million metric tons of animal manure is produced annually from about 238,000 animal feeding operations (USDA-NRCS 2000) in addition to an unestimated amount of manure produced from grazing animals. Nearly 90% of about 11.4 million metric tons of poultry litter (PL) produced annually is land applied (Cabrera and Sims 2000). Poultry litter applied to agricultural lands serves as a source of macro nutrients such as N, and P for major crops, pasture and hayfields (Nyakatawa and Reddy 2002; Pederson et al. 2002). Poultry litter also contains high concentrations of some trace elements such as Cu, Zn, and As (van der Watt et al. 1994; Moore et al. 1998). These trace elements are apparently derived from growth promoters or biocides in poultry feed (Sims 1995).

Fly ash (FA) is a major coal combustion by-product. Coal-fired electric power utilities in the USA generate approximately 122 million metric tons combustion by-products annually and about 60% of this is FA (Am. Coal Ash Assoc. 2004). Results of several studies have indicated the benefits of FA application to agricultural lands since it contains K, Ca, S, B, Mo, and many other essential micronutrients that can be taken up by the plants (Martens 1971; Page et al. 1979; Elseewi et al. 1980; Ransome and Dowdy 1987). Alkaline property of FA renders it to be an effective material for neutralizing soil acidity (Adriano et al. 1980). However, FA lacks N and P content and may contain high concentrations of some toxic heavy metals, depending on the origin of coal. These factors, in turn, explain limited acceptance of FA as a sole amendment to agricultural soils. Adriano et al. (1980) reported that the use of FA as an agricultural amendment can be enhanced by blending it with organic by-products such as sewage sludge (SS), poultry litter (PL), or cattle manures (CM). The organic amendments, named above, provide a source of N and P and also can complex heavy metals in FA, rendering them less phyto-toxic. However, additional research is needed to evaluate the chemistry and plant availability of heavy metals in soils amended with a mixture of FA and different organic by-products.

Jackson et al. (1999) examined the solubility, leachability and availability of selected trace elements to corn plants from land application of extremely high rates of (100 and 200 Mg ha$^{-1}$) FA + PL and/or FA + SS under field conditions. Application of PL to agricultural land should not exceed 6 Mg ha$^{-1}$ per application or about 12.5 Mg ha$^{-1}$ per annum in order to minimize soil and water quality problems (Schroeder et al. 2004; Martinez and Harmel 2006). Land application rates of PL is determined by the soil test values of N and P, and the amount needed to replenish N and P removed by the crop harvested from that land. Beneficial effects of soil amendment of FA plus with PL can be evaluated based on the chemistry, transport, and plant availability of various elements.

The current study was designed to (1) quantify leaching and (2) evaluate the partitioning of selected trace elements in the above and below ground parts of Collard green *(Brassica oleracea var. acephala)* grown in a greenhouse in two soils amended with different rates (0 to 24.7 Mg ha$^{-1}$) of PL or 1:1 mixture of PL and FA.

## 2 Materials and Methods

### 2.1 Soils

Soils were collected from 0–30 cm depth of a Candler fine sand (sandy, hyperthermic, uncoated, Typic Quartzipsamments) Polk County, Florida and an Ogeechee loamy sand (Siliceous, thermic Typic Ochragult) from Chatham County, Georgia. Selected properties of the soil samples and the amendments used in this study are presented in Table 1.

### 2.2 Amendments

A PL (obtained from a commercial farm in Glenville, Georgia, USA) and a FA (Port Wentworth Electric Power plant of Georgia Power, formerly known as Savannah Electric, Chatham County, Georgia) were used in this study. Treatments included application of PL as sole amendment or as PL + FA (1:1 ratio) at

**Table 1** Selected properties of poultry litter (PL) and fly ash (FA) and the soils used in this study

| Properties[a] | PL[b] | FA[c] | CFS[d] | OLS[e] |
|---|---|---|---|---|
| pH (1:1 water–soil) | 8.90 | 11.40 | 6.80 | 5.50 |
| | | mS $cm^{-1}$ | | |
| EC | 3.40 | 1.60 | 0.10 | 0.10 |
| | | g $kg^{-1}$ | | |
| C | 395.00 | 147.00 | 5.00 | 12.00 |
| N | 45.70 | 23.00 | 0.40 | 0.60 |
| S | 39.00 | 28.00 | 0.10 | 0.20 |
| P | 34.30 | 00.23 | 0.11 | 0.21 |
| K | 11.80 | 00.58 | 1.30 | 1.20 |
| Ca | 39.00 | 8.44 | 0.17 | 0.28 |
| Mg | 10.00 | 4.66 | 0.06 | 0.06 |
| Fe | 0.73 | 17.52 | 0.59 | 0.66 |
| | | mg $kg^{-1}$ | | |
| Mn | 305.20 | 137.50 | 14.50 | 14.10 |
| Cu | 248.20 | <1.00 | 38.40 | 1.20 |
| Zn | 250.20 | 34.70 | 72.60 | 4.40 |
| Pb | <10.00 | <5.00 | 20.90 | 4.90 |
| Cd | <2.00 | 3.00 | 0.72 | 0.40 |
| Ni | 40.00 | 13.80 | nd | nd |
| Cr | 7.90 | 28.50 | 7.40 | 4.30 |
| | | g $kg^{-1}$ | | |
| Sand | | | 967.00 | 860.00 |
| Silt | | | 8.00 | 100.00 |
| Clay | | | 25.00 | 40.00 |
| Texture | | | Fine sand | Sandy loam |

*CFS* Candler fine sand, *OLS* Ogeechee loamy sand

[a] Elemental compositions were determined by ICP-OES on acid-digest

[b] Collected from a poultry farm in Glenville, Georgia, USA

[c] Collected from Port Wentworth Power Plant of Savannah Electric Company, Georgia, USA

[d] Collected from Polk County, Lake Alfred, Florida, USA

[e] Collected from Chatham County, Savannah, Georgia, USA

either 2.47, 4.94, 12.35, or 24.70 Mg (dry weight basis) $ha^{-1}$. An unamended control treatment was also included. Triplicate leaching columns were set for all the treatments

## 2.3 Leaching Column Study

Plexiglass columns, 32-cm long and 7-cm inner diameter, were used to study the leaching of various trace elements from soils amended with various rates of either PL or 1:1 mixture of PL + FA. Soils were dried and sieved to pass a 2-mm sieve. A Whatman No. 42 filter paper was placed at the bottom of the leaching column and the soil was packed to a height of 30 cm to attain ~1.5 g $cm^{-3}$ bulk density. Pre-weighed quantity of the amendment, as per treatment, was mixed with top 2.5 cm soil that was removed from each soil column and placed back to the same column and packed to the original bulk density. Then soil columns were saturated with de-ionized water in an acid-washed bucket. Excess water was allowed to drain overnight. A filter paper was placed on top of the soil surface and deionized water was applied at 1.5 mL $min^{-1}$ flow rate using a peristaltic pump. Leachate was collected at one half-pore volume fractions (220 mL which was equivalent to 5 cm rainfall) at each leaching event. Upon completion of each leaching event, soil columns were allowed to dry until the next leaching event (6 days) at room temperature (~25–30°C). Leaching and drying con-

ditions in this study were adapted to mimic soil conditions in Florida and southeastern Georgia during the spring and summer months characterized by rainfall and dry periods. The leaching/dry cycle was repeated 28 times (about 7 months) for a total amount of leachate equivalent to 140 cm rainfall, which is very close to the mean annual rainfall (125 cm) in southeastern coast of Georgia. The electrical conductivity and pH of each leachate fraction were measured using Hanna conductivity meter (HI 8733 conductivity meter, Hanna Instruments, Singapore) and Accumet Model 15 pH meter) Fisher Scientific, Pittsburg, PA, USA) respectively. The ionic strength (*I*; in mM) was calculated using EC (μS $cm^{-1}$) values i.e. $I$= 0.013 EC (Griffin and Jurinak 1973; Alva et al. 1991).

#### *2.3.1 Analysis of Trace Elements*

Concentrations of Cr, Zn, Cd, Cu, Ni, Fe, and Mn in leachate were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES; Plasma RL 3300, Perkin Elmer Inc., Norwalk, CT, USA). The amount of trace element leached in each fraction was calculated using the concentration of each element and the volume of leachate fraction. Cumulative leaching of each element was then calculated for the total leachate quantity.

### 2.4 Partitioning of Trace Elements in Collard Greens

This experiment was conducted using the treatments described above for the leaching column experiment with three replicates. Polyethylene pots lined with acid-washed gravel (1-cm thickness) and then filled with 3 kg of amended soil as per the application rates were used. The amended soils were incubated in the greenhouse for 4 weeks at field capacity soil water content (7% and 14% on gravimetric basis for Candler fine sand and Ogeechee loamy sand, respectively). Ten seeds of *Collard greens (Brassica oleracea var. acephala)* were planted per pot. After 1 week, the pots were thinned to seven plants per pot and grown for 6 weeks. Pots were watered to maintain field capacity water content throughout the growing period. The greenhouse temperature was maintained at 30 (day) and 24 (night) ± 3°C.

Six weeks after planting, above ground portion of the plant was clipped at the first node and leaves and shoots were separated. The root system was collected from the soil with care without loosing any portion of roots. Harvested plant parts were rinsed thoroughly in 10% HCl acid bath and then in four successive deionized water baths. The washed plant parts were air-dried at room temperature and then placed in paper bags and oven dried at 70°C for 72 h, and dry weights were recorded. The plant part dry matter was ground in a Wiley mill to pass a 22 mesh (841 μm) sieve and dry-ashed in muffle furnace set at 550°C for 6 h and dissolved in 20 mL of 1 M $HNO_3$ (Council of Soil testing and Plant Analysis 1980). The concentrations of trace elements were analyzed by ICP-OES. Standard reference plant samples (from NIST) were used to check and confirm the analytical quality of ICP-OES.

### 2.5 Statistical Analysis

Experimental data were analyzed using SAS (version. 8.1; SAS 2000) as completely randomized design (CRD) with factorial treatment arrangement (2 Soil×2 Sources of amendment×5 rates of application) with three replicates per treatment combination. Means of cumulative trace elements (individual elements) leached were compared separately for soils by using Duncan's Multiple Range Test (DMRT) at 0.05 probability level. Similarly, means of dry matter yield and concentration of various trace elements in above and below ground parts of Collard greens were compared by using Duncan's Multiple Range Test (DMRT) at 0.05 probability level.

## 3 Results and Discussion

### 3.1 Ionic Strength of Leachate

Ionic strength of leachate provides better indication about the amount of various salts present in soil solution or in leachate following the application of water to soil amended with varying rates of soil amendments such as PL alone or as 1:1 mixture of PL + FA. Greater ionic strength of leachate means greater the salt content in leachate/soil solution which leads to substantial adverse effect on plant growth by affecting water absorption by plant roots. Ionic strength (*I*) of the leachate across all leaching events followed similar pattern regardless of treatment types

whether it is PL or PL + FA amendments (Figs. 1 and 2) used in this study. Ionic strength increased with increasing rates of amendment across both soils and sources of amendments. Peak *I* was attained at the third leaching event and decreased rapidly for the subsequent leachates and attained the background *I* values (~1.0 mM) similar to the unamended soils by about 26 to 28 leaching event. However, this continuous rapid decrease in *I* values were not observed in Ogeechee loamy sand amended with higher rates of either PL or PL + FA At a given application rate, *I* values were two- to threefold greater for the Candler fine sand as compared to that for the Ogeechee loamy sand. The peak I for the CFS soil was about 140 mM while it was about 49 mM for the OLS soil. These values are 14- to 15-fold greater than that expected for irrigation water, i.e., EC of <0.7 mS $cm^{-1}$ (FAO 1985). With the lowest rate (4.94 Mg $ha^{-1}$) of PL amendment, peak I was 26 and 13 mM respectively, for the CFS and OLS soils. The corresponding *I* values increased to 32 and 14.7 mM for the respective soils which received 4.94 Mg $ha^{-1}$ 1:1 mixture of PL + FA.

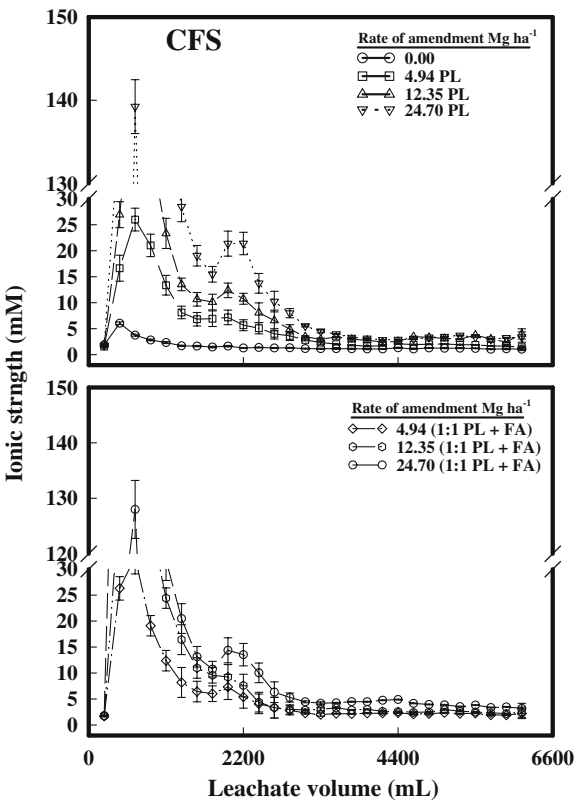


**Fig. 1** Ionic strength of leachate collected at various leaching events from Candler fine sand (CFS) amended with varying rates of PL or 1:1 mixture of PL + FA and in unamended CFS soil. *Error bar* on each point represents standard error of the means

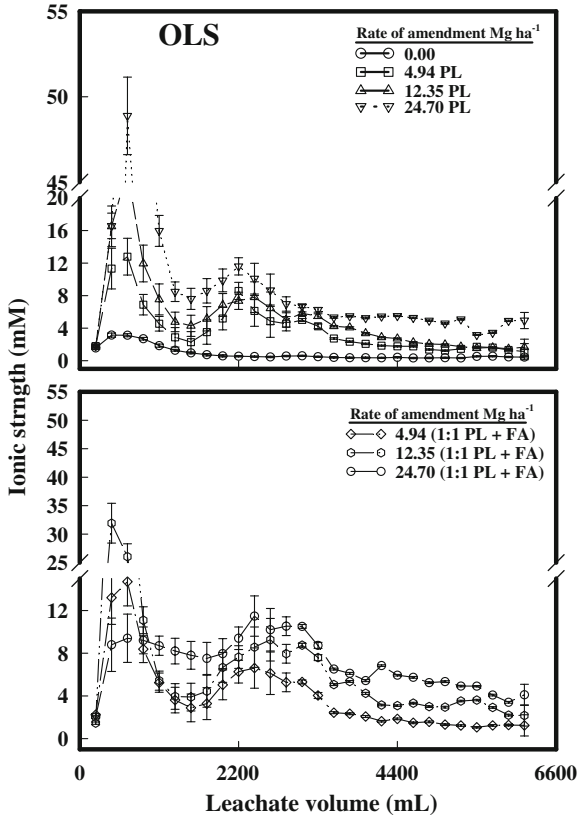


**Fig. 2** Ionic strength of leachate collected at various leaching events from Ogeechee loamy sand (OLS) amended with varying rates of PL or 1:1 mixture of PL + FA and in unamended OLS soil. *Error bar* on each point represents standard error of the means

The ionic strength values estimated from electrical conductivity measurements of leachate samples were extremely high and that would pose serious salinity problem. This high salinity problem could cause poor germination and poor growth performance for crops which are salt intolerant. Periodic and repeated electrical conductivity measurements made (date not presented) in both soils amended with either PL or 1:1 mixture of PL + FA through out the 1 month incubation period prior to seeding the pots with collard greens supported the salinity problem. The effect of high salinity on germination and growth performance of collard green was observed clearly in both CFS and OLS soils amended with the highest application rate (24.7 Mg $ha^{-1}$) of either PL or 1:1

mixture of PL + FA. Substantially poor growth of collard green was observed in CFS amended with the highest application rate of either PL or 1:1 mixture of PL + FA which resulted in the lowest dry matter yield (Figs. 5 and 6). This also resulted in not enough plant tissue samples for the analysis of trace elements in collard green plant parts and was reported as 'nda—no data available' in Tables 3, 4, and 5. Similar observations due to undesirable level of high salt contents were reported by other researchers in soils amended with high application rates of unprocessed (raw) sewage sludge materials (Sajwan et al. 1995; Alva et al. 1999; Basta 2000).

### 3.2 Leachate pH

In the CFS soil, leachate pH was not influenced by either the rates or the sources (PL vs. PL + FA) of amendments (Figs. 3 and 4). Leachate pH was about 6

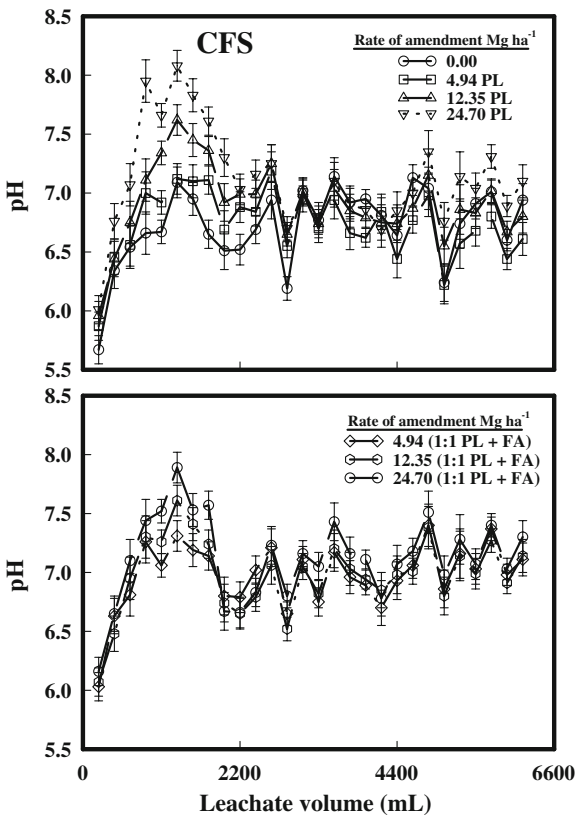


**Fig. 3** pH of leachate collected at various leaching events from Candler fine sand (CFS) amended with varying rates of PL or 1:1 mixture of PL + FA and in unamended CFS soil. *Error bar* on each point represents standard error of the means

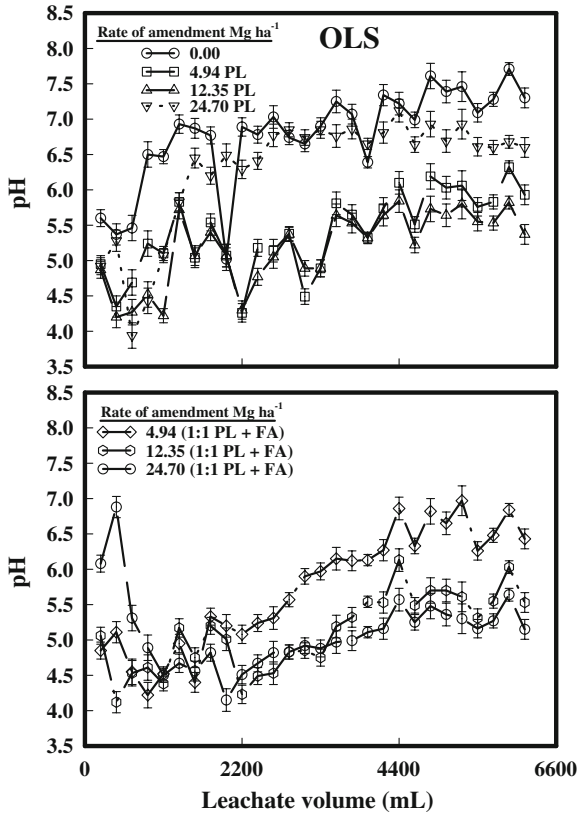


**Fig. 4** pH of leachate collected at various leaching events from Ogeechee loamy sand (OLS) amended with varying rates of PL or 1:1 mixture of PL + FA and in unamended OLS soil. *Error bar* on each point represents standard error of the means

at the beginning of leaching, increased 7–8 by fifth to sixth leaching events and then declined and remained relatively steady towards the end of the experiment. Leachate pH in the OLS soil varied from 4.0 to 8.0 across leaching events, but did not follow any clear cut trend with respect to application rates of either PL or PL + FA (Fig. 2). Wide variation in the leachate pH for the OLS soil amended with different rates of either PL or mixture of PL + FA could be due to the presence of various cations and anions in this soil and are not reported in this manuscript and will be presented elsewhere.

### 3.3 Cumulative Leaching of Trace Elements

Results showed that the leaching of trace elements was greater from the CFS as compared to that from the OLS (Table 2). These results support greater leaching of trace elements in a coarse textured soil

**Table 2** Cumulative quantities and mean concentrations of trace elements in leachate collected from two soils amended with various rates of poultry litter (PL) or 1:1 mixture of poultry litter + fly ash (PL + FA) and from unamended soils

| Treatments (Mg ha$^{-1}$) | µg metals in 6,160 mL leachate/mean concentrations µg L$^{-1}$ (values in parentheses) | | | | |
|---|---|---|---|---|---|
| | Cr | Zn | Cd | Cu | Pb |
| Candler fine sand | | | | | |
| 4.94 (PL) | 204 de (33) | 730 c (119) | 80 b (13) | 7,780 d (1263) | 1,116 c (181) |
| 12.35 (PL) | 212 d (34) | 770 c (125) | 77 b (13) | 11,250 c (1826) | 1,195 c (194) |
| 24.70 (PL) | 268 a (43) | 1070 b (174) | 78 b (13) | 14,770 b (2398) | 1,242 c (202) |
| 4.94 (PL + FA) | 228 cd (37) | 620 d (101) | 86 a (14) | 7,390 d (1200) | 1,250 c (203) |
| 12.35 (PL + FA) | 233 bc (38) | 930 b (151) | 85 a (14) | 12,870 c (2089) | 1,617 b (263) |
| 24.70 (PL + FA) | 248 b (40) | 1310 a (213) | 84 a (14) | 18,540 a (3010) | 2,440 a (396) |
| Unamended | 198 e (32) | 310 e (50) | 73 b (12) | 1,640 e (266) | 365 d (59) |
| Ogeechee sandy loam | | | | | |
| 4.94 (PL) | 141 b (23) | 327 c (53) | 15 b (2) | 80 cd (13) | 146 c (24) |
| 12.35 (PL) | 143 b (23) | 332 c (54) | 24 a (4) | 110 b (18) | 208 b (34) |
| 24.70 (PL) | 264 a (43) | 438 b (71) | 25 a (4) | 250 a (41) | 384 a (62) |
| 4.94 (PL + FA) | 129 c (21) | 296 d (48) | 14 b (2) | 87 c (14) | 105 d (17) |
| 12.35 (PL + FA) | 129 c (21) | 326 c (53) | 14 b (2) | 94 c (15) | 130 c (21) |
| 24.70 (PL + FA) | 131 c (21) | 501 a (81) | 16 b (3) | 113 b (18) | 142 c (23) |
| Unamended | 116 d (19) | 117 e (19) | 11 b (2) | 77 d (13) | 96 d (16) |

Elemental compositions in leachate were determined by ICP-OES. Similar letters after a mean indicate no significant difference ($p<0.05$) among treatment means within a column for each soil according to Duncan's Multiple Range Test

compared to that from a medium textured soil. As expected, leaching of trace element increased with increasing rates of amendments. Substantially greater cumulative amounts of Cu, Zn, and Pb leaching were observed in both soils amended with varying rates and sources of amendments compared to that of Cr and Cd. This substantial difference of cumulative leaching among elements might be due to the presence of inherently greater amounts of Cu, Zn, and Pb in both source of amendments (poultry litter (PL), fly ash (FA)) and in soils (Tables 1 and 2). Also, elemental (Cu, Zn, Pb, Cd, and Cr) concentrations were greater in the CFS than those in the OLS soil (Table 1). Adriano (2001) noted that availability of trace elements for leaching and plant uptake will be affected by the total amounts of trace elements present in soils and amendments, other soil factors such as organic matter content, cation exchange capacity, pH and chelating agents present in the medium.

Nutrient dynamics and available elemental composition for leaching and plant uptake in soils are affected by combination of various factors. Cumulative amount of Cd leached was three- to sevenfold greater from the CFS soil as compared to that from the OLS soil, despite slightly greater total Cd content in the latter as compared to that in the former soil (Tables 1 and 2). It is important to note that the units for cumulative amount of Cd reported in leachate (µg) was different than that for the concentration of Cd reported for soils and amendments mg kg$^{-1}$). Naidu and Harter (1998) reported pH values ≥7.0 significantly enhance Cd availability. In addition to soil pH, low soil organic matter content and associated low CEC in the CFS soil could have contributed to greater leaching of Cd compared to that of from OLS. Leaching of Cu is expected to be greater in low pH soils (Locascio 1978). However, in this study leaching of Cu was greater in the CFS than that from the OLS soil. Affinity to form organic complexation is greater for Cu and Pb as compared to Zn and Cd (Martinez and McBride 1999). It is important to note that most of the trace elements monitored in leachate or collard green tissue samples were substantially greater in CFS than that of in OLS and their quantities in original soils are reported in mg kg$^{-1}$ units (Table 1). Furthermore, the soil texture and other soil factors also could play significant role in determining the amount of leaching of elements from soils (Adriano 2001). About 70% to 80% of cumulative quantities of most metals leached from the CFS soil were accounted for in the first three to six pore volumes while seven to ten pore volumes of leachate

required to account for 60% to 80% of cumulative leaching of most of the trace metals from the OLS soil. The differences in leaching of trace elements from CFS and OLS were possibly mainly due to textural differences of these two soils.

### 3.4 Average Concentration of Trace Elements in Leachate

The average concentrations of Cr in the leachate from the CFS soil amended with PL and PL + FA varied from 33 to 43 and 37 to 40 μg $L^{-1}$, respectively. The respective ranges for the OLS soils were 23 to 43 and 21 μg $L^{-1}$. Variation in average concentrations of Cd in the leachate from the CFS soil amended with PL and PL + FA were 13 and 14 μg $L^{-1}$, respectively. The corresponding values for the OLS were 2 to 4 and 2 to 3 μg $L^{-1}$, respectively. The maximum contaminant levels of Cr and Cd for fresh water are 6 and 3 μg $L^{-1}$ (Bowen 1979). The average concentrations of Cr and Cd in the leachate from unamended CFS soil were 32 and 12 μg $L^{-1}$ respectively and 19 and 2 μg $L^{-1}$ respectively for the unamended OLS soil. The observations of reduced leaching of trace elements in soils amended with FA + PL (organic amendments) are in close agreement with the observation of Adriano et al. (1980) who attributed the formation of complex of trace elements with organic amendments or organic matter in soils as the possible reason for rendering them less available for leaching or plant uptake.

The average concentrations of Zn and Cu in the leachate from the CFS soil amended with 4.94 Mg $ha^{-1}$ PL were 119 and 1,263 μg $L^{-1}$ respectively, whereas these values were 101 and 1,200 μg $L^{-1}$ respectively for the CFS soil amended at the same rate with PL + FA. The corresponding concentrations Zn and Cu for OLS soil amended with PL and PL + FA were 53 and 13 and 48 and 14 μg $L^{-1}$ respectively. The average concentration of Zn in fresh water is 15 μg $L^{-1}$ (range 1 to 100) while that of Cu is 3 μg $L^{-1}$ (range 0.2 to 30) respectively (Bowen 1979). The average concentrations of Zn and Cu from unamended CFS soil were 50 and 266 where as the respective values for the unamended OLS soil were 44.3 and 29.6 μg $L^{-1}$. The average concentrations of Zn and Cu in the leachate from both soils in unamended treatment were greater than those in fresh water. The poultry litter used in this study had close to 250 mg $kg^{-1}$ each of Zn and Cu while FA had about 35 mg $kg^{-1}$ Zn but negligible concentration of Cu (Table 1). Furthermore, concentrations of Zn and Cu were much greater in the CFS soil as compared to those in the OLS soil. The CFS soil was collected from a citrus grove, which received repeated Cu loading at low quantities as an ingredient in commonly used fungicide for citrus production. Indeed, studies have shown high Cu concentrations in citrus producing soils (Alva and Graham 1991; Zhu and Alva 1993; Alva 1993; Alva et al. 1995).

The average concentration of Pb in the leachate from CFS soil amended with PL and PL + FA varied from 181 to 202 and 203 to 396 μg $L^{-1}$, respectively. The corresponding Pb concentrations in the leachate from OLS soil were 24 to 62 and 17 to 23 μg $L^{-1}$, respectively. The average concentration of Pb in fresh water is 3.0 μg $L^{-1}$ and allowable range is 0.06 to 120 μg $L^{-1}$ (Bowen 1979). The average concentration of Pb in leachate collected from unamended CFS and OLS soils were 59 and 16 μg $L^{-1}$, respectively. The average concentrations of Pb in the leachate from both unamended soils were greater than average concentrations found in fresh water.

### 3.5 Plant Dry matter Production

Dry matter production of above ground parts (leaves and stems) increased with increasing rates of amendments up to12.35 Mg $ha^{-1}$ across both soils and sources of amendments. In general, coefficients of determination of the regression equations to show the quadratic relationships ($r^2$ values in the range of 0.92 to 0.99) between dry matter yield of collard green and rates of application were substantially greater for all plant parts grown in both CFS and OLS (Figs. 5 and 6).

However, the coefficients of determination of regression equations for the quadratic relationships between the dry matter yield of collard green roots grown in OLS and rates and sources of amendments were substantially low. Importantly, the lowest dry matter yield was noticed at the highest application rate of both PL and 1:1 mixture of PL + FA and that was due to high salinity level of soil which resulted in poor growth collard green plant. This observation was particularly true for leafy portion of collard green biomass yield at the highest rate (24.7 Mg $ha^{-1}$) of amendment of PL or PL + FA. This trend was more prominent in the CFS soil as compared to that in the OLS soil (Figs. 3 and 4). The results of this study

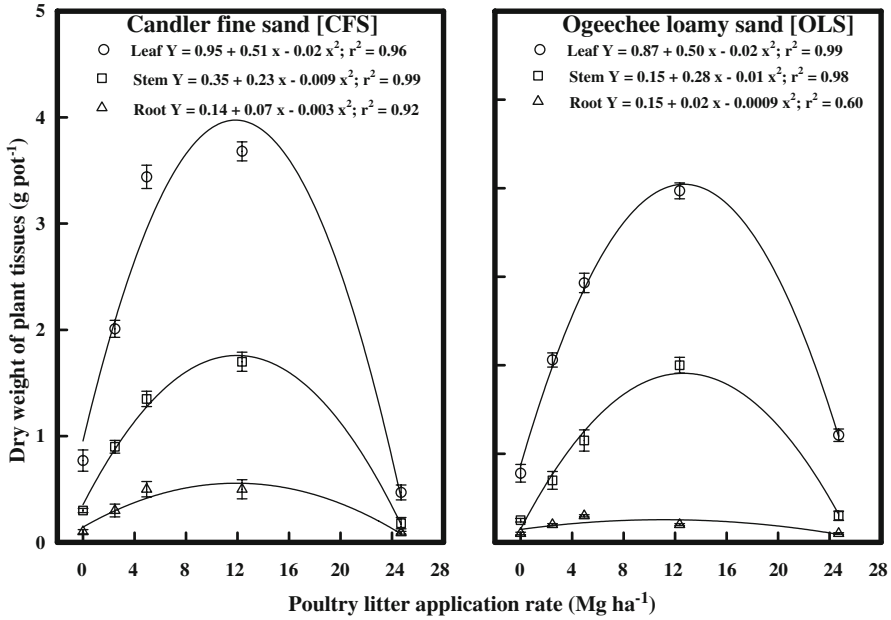


**Fig. 5** Relationship of application rates of poultry litter (PL) and dry weight of leaf, shoot, and root of collard green crop grown in light-textured Candler fine sand (CFS) from Lake Alfred, FL and in medium-textured Ogeechee loamy sand (OLS) from Savannah, GA. *Error bar* on each point represents standard error of the means

clearly demonstrated that the growth performance of leafy portion of collard green can be significantly enhanced by application of either PL or 1:1 mixture PL + FA at a rate not exceeding 12.35 Mg ha$^{-1}$. Results of this study also indicated that addition of FA with PL had minimal effect on the collard green growth response as compared to that of PL alone. This observation is expectable since FA helps to neutralize soil acidity while FA generally lacks major plant nutrient elements such as N and P which mainly support plant growth.

## 3.6 Elemental Concentrations in Plant Tissues

### *3.6.1 Above-Ground Plant Part (Leaf)*

In general, concentrations of trace elements (metals) evaluated in this study increased with increasing rates

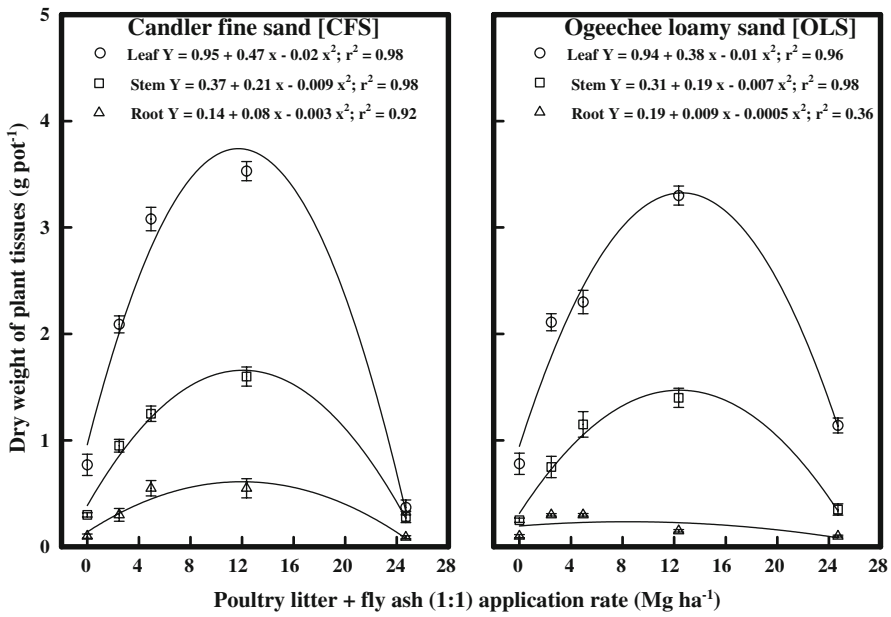


**Fig. 6** Relationship of application rates of 1:1 mixture of poultry litter and fly ash (PL + FA) and dry weight of leaf, shoot, and root of collard green crop grown in light-textured Candler fine sand (CFS) from Lake Alfred, FL and in medium-textured Ogeechee loamy sand (OLS) from Savannah, GA. *Error bar* on each point represents standard error of the means

of amendments (with exception at the highest rate of amendment application due to poor growth collard green crop and unavailability plant tissue for elemental analysis) irrespective of amendment sources and soils types (Table 3). Elemental concentrations in leafy part of collard green followed the following order Zn > Cu > Cr > Pb > Cd. Results also indicated that blending FA with PL at 1:1 ratio reduced the concentration of Zn, Cd, and Cu to certain extent in both soil types (Table 3).

However, the similar trend was not noticed for Cr and Pb. It is also important to note that concentrations of Cr in leafy part of collard green grown in both unamended light-textured Candler fine sand and medium-textured Ogeechee loamy sand were 4.80 μg $g^{-1}$ and 4.67 μg $g^{-1}$ respectively. These values were significantly different than the concentrations of Cr in leafy part of collard green crop grown in both soils amended at all the application rates irrespective of type of amendment with the exception of concentration of Cr in leafy part collard green grown in CFS amended with PL at the rate of 2.47 Mg $ha^{-1}$. High level of Cr in leafy part of collard green possibly coming from both amendment types and soils types used to grow collard green crop (Tables 1 and 3). Apparently, FA used in this study with greater Cr content compared to PL or soil types might have had some influence on the leaf Cr concentration (Table 1). However, it is difficult to support this claim since we did not have FA treatment alone in this study.

### *3.6.2 Above-Ground Plant Part (Stems)*

As observed in leaf, concentrations of trace elements evaluated in this study in stems also increased with increasing rates of amendments (with exception at the highest rate of amendment application due to poor growth collard green crop and unavailability plant tissue for elemental analysis) irrespective of sources of amendments and types of soils (Table 4). Elemental concentrations in stems of collard green followed the same order Zn > Cu > Pb > Cr > Cd like what was observed in leaf and the concentration values were

**Table 3** Concentration of trace elements in the collard green leaves grown in two soils with different rates of poultry litter (PL) or poultry litter + fly ash (PL + FA) amendments

| Treatments (Mg $ha^{-1}$) | Trace element concentration in collard green leaves (μg $g^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Cr | Zn | Cd | Cu | Pb |
| Candler fine sand | | | | | |
| 2.47 (PL) | 4.83 c | 196 e | 2.13 b | 12.50 c | 4.07 c |
| 4.94 (PL) | 4.99 b | 284 b | 2.13 b | 15.97 b | 4.23 c |
| 12.35 (PL) | 5.27 b | 526 a | 2.27 a | 20.20 a | 6.73 a |
| 24.70 (PL) | nda | nda | nda | nda | nda |
| 2.47 (PL + FA) | 4.87 c | 135 f | 2.03 c | 9.20 d | 3.60 c |
| 4.94 (PL + FA) | 5.07 b | 211 d | 2.13 b | 12.70 bc | 4.40 c |
| 12.35 (PL + FA) | 6.77 a | 233 c | 2.20 a | 13.57 b | 5.17 b |
| 24.70 (PL + FA) | nda | nda | nda | nda | nda |
| Unamended | 4.80 c | 190 e | 2.07 c | 12.00 c | 2.37 d |
| Ogeechee sandy loam | | | | | |
| 2.47 (PL) | 4.80 b | 61 c | 2.17 c | 5.73 b | 3.27 d |
| 4.94 (PL) | 4.87 b | 62 c | 2.33 ab | 5.87 b | 4.53 b |
| 12.35 (PL) | 5.00 a | 156 a | 2.40 a | 6.60 a | 4.77 b |
| 24.70 (PL) | 4.99 a | 80 b | 2.27 b | 6.23 a | 3.27 d |
| 2.47 (PL + FA) | 4.93 a | 24 d | 2.17 c | 4.43 d | 3.67 c |
| 4.94 (PL + FA) | 4.93 a | 26 d | 2.20 bc | 4.77 cd | 3.73 c |
| 12.35 (PL + FA) | 4.97 a | 89 b | 2.40 a | 5.03 c | 6.63 a |
| 24.70 (PL + FA) | 4.98 a | 29 d | 2.23 b | 5.00 c | 4.77 b |
| Unamended | 4.67 c | 60 c | 2.13 c | 4.73 cd | 3.17 d |

Similar letters after a mean indicate no significant difference ($p<0.05$) among treatment means within a column for each soil according to Duncan's Multiple Range Test

*nda* no data available due to insufficient dry matter for digestion and elemental analysis

**Table 4** Concentration of trace elements in the collard green stems grown in two soils with different rates of poultry litter (PL) or poultry litter + fly ash (PL + FA) amendments

| Treatments (Mg ha$^{-1}$) | Trace element concentration in collard green stems (μg g$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Cr | Zn | Cd | Cu | Pb |
| Candler fine sand | | | | | |
| 2.47 (PL) | 1.33 c | 196 b | 0 | 0.90 d | 1.40 c |
| 4.94 (PL) | 1.43 b | 156 c | 0 | 15.97 b | 1.47 c |
| 12.35 (PL) | 1.57 a | 291 a | 0 | 19.57 a | 1.97 b |
| 24.70 (PL) | nda | nda | nda | nda | nda |
| 2.47 (PL + FA) | 1.20 d | 86 e | 0 | 0.27 f | 1.03 d |
| 4.94 (PL + FA) | 1.30 c | 115 d | 0 | 10.60 c | 1.27 cd |
| 12.35 (PL + FA) | 1.50 a | 147 c | 0 | 11.33 c | 2.43 a |
| 24.70 (PL + FA) | nda | nda | nda | nda | nda |
| Unamended | 1.30 c | 116 d | 0 | 0.53 e | 0.77 e |
| Ogeechee sandy loam | | | | | |
| 2.47 (PL) | 0.63 b | 78 c | 0 | 1.20 d | 2.30 c |
| 4.94 (PL) | 0.63 b | 100 b | 0 | 1.57 c | 3.87 a |
| 12.35 (PL) | 0.90 a | 133 a | 0 | 2.47 a | 4.13 a |
| 24.70 (PL) | 0.60 b | 58 cd | 0 | 1.53 c | 2.23 c |
| 2.47 (PL + FA) | 0.23 f | 29 e | 0 | 0.80 e | 2.17 c |
| 4.94 (PL+ FA) | 0.53 c | 30 e | 0 | 1.90 b | 3.40 b |
| 12.35 (PL + FA) | 0.63 b | 102 b | 0 | 2.33 a | 3.47 b |
| 24.70 (PL + FA) | 0.33 e | 29 e | 0 | 0.87 e | 3.07 b |
| Unamended | 0.43 d | 67 c | 0 | 0.47 f | 2.10 c |

Similar letters after a mean indicate no significant difference ($p<0.05$) among treatment means within a column for each soil according to Duncan's Multiple Range Test

*nda* no data available due to insufficient dry matter for digestion and elemental analysis

substantially lower than what was observed with leaf portion of the collard green. Interestingly, non-detectable amount of Cd was noted in shoots across all treatments and rates of amendment application. Results also indicated that blending FA with PL at 1:1 ratio reduced the concentrations of all the trace elements studied in both soil types (Table 4).

### *3.6.3 Below-Ground Plant Part (Roots)*

As observed in other plant parts of collard green, concentrations of most of the elements (metals) in roots increased with increasing rates of amendments (with exception at the highest rate of amendment application) irrespective of sources of amendments and types of soils (Table 5). However, no detectable uptake of Cd by roots in both soils amended with both type of amendments at all rates (Table 5). Greater concentrations of Cu were observed in roots compared to that of in stems/leaves in all treatments at all rates for both soils. In general, trace elements such as Zn, Mn, Ni, and B are distributed uniformly throughout the plants. However, in this study, we did not measure Mn, Ni, and B. Copper and Cd generally accumulate in roots but some times in shoots. But Pb and Cr mostly accumulate in roots and very little in shoots (Wallace and Romney 1977). However, in our investigation with collard green crop, most of the elements (metals) evaluated mainly accumulated in leaves compared to that in stems or roots with the exception of Cu. Greater Cu accumulation was noted only in roots of collard green crop grown in light-textured Candler fine sand amended with both types of amendments at all the application rates (Table 5) compared to leaf and shoots of the same crop (Tables 3 and 4).

### *3.6.4 General Discussion on Trace Element Accumulation in Collard Green*

*Concentration of Chromium in Plants* Concentrations of Cr in the foliage showed little relationship with the

**Table 5** Concentration of trace elements in the collard green roots grown in two soils with different rates of poultry litter (PL) or poultry litter + fly ash (PL + FA) amendments

| Treatments Mg ha$^{-1}$ | Trace element concentration in collard green roots (μg g$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Cr | Zn | Cd | Cu | Pb |
| Candler fine sand | | | | | |
| 2.47 (PL) | 1.27 d | 101 c | 0 | 11.80 c | 2.17 c |
| 4.94 (PL) | 1.40 c | 137 b | 0 | 47.60 ab | 2.27 c |
| 12.35 (PL) | 1.67 b | 216 a | 0 | 51.33 a | 4.47 a |
| 24.70 (PL) | nda | nda | nda | nda | nda |
| 2.47 (PL + FA) | 1.43 c | 89 d | 0 | 10.20 c | 1.60 d |
| 4.94 (PL + FA) | 1.63 b | 115 bc | 0 | 12.77 c | 2.57 c |
| 12.35 (PL + FA) | 1.87 a | 123 b | 0 | 42.73 b | 3.27 b |
| 24.70 (PL + FA) | nda | nda | nda | nda | nda |
| Unamended | 1.20 d | 65 e | 0 | 6.60 d | 1.30 d |
| Ogeechee sandy loam | | | | | |
| 2.47 (PL) | 1.67 c | 26 d | 0 | 2.50 d | 1.70 e |
| 4.94 (PL) | 2.80 a | 75 a | 0 | 2.93 c | 3.67 b |
| 12.35 (PL) | 3.13 a | 83 a | 0 | 5.60 a | 4.87 a |
| 24.70 (PL) | 2.07 b | 71 b | 0 | 4.07 b | 3.27 c |
| 2.47 (PL + FA) | 1.67 c | 29 d | 0 | 2.67 cd | 1.40 e |
| 4.94 (PL + FA) | 2.00 b | 29 d | 0 | 2.93 c | 2.30 d |
| 12.35 (PL + FA) | 2.07 b | 77 a | 0 | 4.53 b | 3.87 b |
| 24.70 (PL + FA) | 1.77 c | 37 c | 0 | 4.13 b | 2.27 d |
| Unamended | 1.03 d | 25 d | 0 | 2.40 d | 1.33 e |

Similar letters after a mean indicate no significant difference ($p<0.05$) among treatment means within a column for each soil according to Duncan's Multiple Range Test

*nda* no data available due to insufficient dry matter for digestion and elemental analysis

total Cr in the soil. In general, concentrations of Cr in plant tissues are below 1 ppm across a wide range of soil Cr levels and seldom exceed 5 ppm (Adriano 2001). Our study demonstrated grater accumulation Cr in collard green leaves as compared to that in shoots or roots (Tables 3, 4, and 5). Similar greater accumulation of Cr was observed by Shankar et al. (2005) in alfalfa shoots than root when grown in a growth medium containing 12 and 24 mg L$^{-1}$ Cr.

*Concentration of Zinc in Plants* Concentration of Zn was greater in collard green leaves than that in the shoot or root regardless of type or rates of amendment or soil type (Table 3, 4, and 5). Pederson et al. (2002) also reported greater Zn concentrations in leaves than that in stems, flowers or roots of 12 leguminous forage crops grown in soils amended with poultry litter. Tewolde et al. (2005) reported greater Zn partitioning into leaves as compared to the other parts of cotton plants grown in 11-L plastic pots containing 11 kg of 2:1 (*v*/*v*) sand to vermiculite treated with four levels (30, 60, 90, or 120 g pot$^{-1}$) of broiler litter.

*Concentration of Cadmium in Plants* Concentrations of Cd in crops vary significantly among species and even cultivars of the same species (Page et al. 1987; Alexander et al. 2006). Leaves usually contain greater concentration of Cd than the flowering or fruiting part of the plants (Page et al. 1987). In our study with collard green Cd accumulation was noted only in leaves but not in stems or roots (Table 3, 4, and 5). Concentrations of Cd in collard green leaves varied from 2.03 to 2.40 μg g$^{-1}$ irrespective of type of soil or amendments.

*Concentration of Copper in Plants* Copper is required only in very small amounts; 5 to 20 ppm of Cu in crop tissue is adequate for normal growth, while <4 ppm is considered to be deficient, and 20+ ppm is considered to be toxic (Jones 1972). As per this

classification, collard green grown in PL and in 1:1 mixture of PL + FA at various application rates indicated the presence of Cu in collard green leaves at sufficient level in both soil types and at deficient level in roots and stems grown in Ogeechee loamy sand at application rates (Table 3, 4, and 5). Concentrations of Cu in collard green stems grown in the light-textured CFS were within the normal range, except for the collard green stems sampled from unamended soil and at the lowest amendment application rate (2.47 Mg $ha^{-1}$). Pederson et al. (2002) reported peak Cu concentrations of 28.8, 18.1, 15.5, and 8.4 mg $kg^{-1}$ for the roots, flowers, leaves, and stem respectively among 16 forage crops (annual rye grass, three cereal crops and 12 leguminous crops) grown in poultry litter amended soil. Other researchers also indicated that roots have a greater Cu concentration than shoots in red clover (Vasseur et al. 1998), willow (*Salix* spp.; Punshon and Dickinson 1997), *Brassica* spp. (Ebbs and Kochian 1997), and wetland plants (Qian et al. 1999), Chinese cabbage and celery (Islam et al. 2007). Tewolde et al. (2005) reported greater Cu accumulation or partitioning in roots compared to other parts of cotton grown in 11-L plastic pots filled with approximately 11 kg of a 2:1 (*v*/*v*) sand to vermiculite growing mix amended with 0, 30, 60, 90, or 120 g $pot^{-1}$ rates of broiler litter. Greater Cu concentrations in leaves than that in stems were reported in grasses and legumes (Minson 1990) and in celery (Islam et al. 2007). Results of our study with collard greens indicated greater Cu concentration in roots than in leaves and stems irrespective of types and rates of amendments (Tables 3, 4, and 5).

*Concentrations of Lead in Plants* In many plants, Pb accumulates mostly in roots since it is poorly translocated to other plant parts. John and Van Laerhoven (1972) reported higher concentrations of Pb in roots rather than in leaves or tops of selected vegetable crops (lettuce, spinach, broccoli, cauliflower, radish, and carrot) grown in contaminated agricultural soils with 0 to 1,000 ppm Pb levels in western Canada. Chatterjee and Dube (2005) confirmed the above trend in vegetable crops such as potato, cauliflower, and cabbage grown in sewage-sludge-amended soils. Göthberg et al. (2004) also confirmed the above trend in water spinach grown in "hogland nutrient solution" amended with different amounts of Pb. In our study, however, concentration of Pb was higher in collard green leaves than that in stems or roots (Tables 3, 4, and 5).

## 4 Conclusions

Leaching of the trace metals examined in this study was lower in a medium-textured Ogeechee loamy sand than in a light-textured Candler fine sand with application of PL or 1:1 mixture of PL + FA within the range application range of 0 to 24.7 Mg $ha^{-1}$. Cumulative leaching of trace metals increased with increasing rates of amendments in both soils. Cumulative amount of trace metals leached with 14 pore volume (6160 mL) of leachate decreased in the order: (1) Cu > Pb > Zn > Cr > Cd in the CFS soil and (2) Zn > Pb > Cr > Cu > Cd in the OLS soil. The mean concentrations of the trace metals in the leachate in this study were greater than the recommended high concentration of the respective trace metals in the fresh water or drinking water.

The biomass production of collard green in both CFS and OLS soils increased with application of PL up to 12.35 Mg $ha^{-1}$ followed by a sharp decline at 24.7 Mg $ha^{-1}$ application rate. The sharp decline in biomass reduction at the highest application rate of either PL or 1:1 mixture of PL + FA was mainly due to poor growth performance attributed to high salinity observed with amended soils even after 4 weeks of incubation at field capacity moisture content. Supplementing PL with FA had no effect on biomass production of collard green in either soil. Concentrations of Cr, Zn, and Pb in collard green leaves were greater than in the stems or roots, while the concentrations of Cu were greater in the roots than in the leaves and the stems.

In summary, application of PL in moderate rates (≤12.35 Mg $ha^{-1}$) to a sandy or sandy loam soil can enhance the production of collard green. However, long-term field studies in multi locations are needed to confirm these findings and also to isolate any compounding effect of FA on PL under field conditions by having additional FA treatments alone at the similar application rates. Future studies also should accommodate residual elemental analysis on soils at the end of crop growth to develop recommendations on the maximum rate of application of PL, FA, and/or PL + FA that can benefit crop growth

and production without any potential long-term negative impacts on the soil and/or water quality.

**Acknowledgement** This research was funded, in part, by (DOE and EPA) contract number DE-FG09-96SR-18558, US Department of Energy/and Environmental Protection Agency and Savannah State University. Assistance offered by Dr. Ashim Heanacho to review statistical procedures and revised version of the manuscript is highly appreciated.

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