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Response of *Amaranthus hybridus* to metal stress and manure amendment in contaminated soil

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Abstract

The effects of heavy metal contamination and manure amendment in soil on the growth of green *Amaranthus hybridus* were studied by pot experiments. A mineral soil was stressed with different doses (100 – 500 mg kg^{-1}) of Cu and Pb using a binary mixture of the metal nitrates and/or amended with poultry or swine manure (at 10:1 soil:manure ratio). *A. hybridus* was sown and monitored for changes in growth rate, above-ground dry biomass and tissue Cu and Pb concentration for 49 days. *A. hybridus* was seemingly intolerant to metal stress as the plants exhibited yellowish appearance, stunted growth and decreased biomass at elevated metal doses in soil, though with reduced severity upon manure (especially poultry) amendment. Growth profiles were sigmoid at lower metal doses (0 – 300 mg kg^{-1}) but plateau-like at higher doses (400 – 500 mg kg^{-1}). Tissue Cu and Pb increased linearly as metal doses in unamended soils and non-linearly in manure-amended soils. Soil-to-plant transfer factors, f (%), indicated that Cu ($11.2 \le f$ (%) ≤ 41.1) was more phytoavailable to *A. hybridus* than Pb ($8.4 \le f$ (%) ≤ 17.0). Step-wise modeling of tissue Cu and Pb concentrations from soil pH, organic matter, plant available and pseudototal metal content by multiple regression analysis suggested that the models were more reliable with plant available metal as a covariate than pseudototal metal content.

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Introduction

Amaranthus hybridus is one of the over 60 species of the genus *Amaranthus* extensively grown and consumed in many temperate and tropical regions of the world as a green, annual leafy vegetable (DAFF, 2010). As vegetable, *Amaranthus* species can produce up to 40 ton ha^{-1} of the edible fresh biomass with high content of carbohydrates, proteins, fats, minerals, vitamins and fibres (DAFF, 2010; Rusu *et al*., 2010). In Nigeria, *Amaranthus* species, including other common vegetables, though frequently grown by the urban populace in a bid to augment personal incomes and offset food insecurity occasioned by rural-urban drift, is often consumed by a greater part of the entire population (Shagal *et al*., 2012). Under this system, all forms of available lands including contaminated sites such as derelict waste dumps, banks of polluted rivers and streams, high way shoulders and industrial areas are indiscriminately cultivated owing to land tenure problems, inadequate regulation and enforcement system. The contaminated sites as well as the soil amendments (fertilizers, manures, sludge, compost), irrigation water and pesticides frequently applied to enhance soil fertility and boost the yield may, inadvertently, bear chemical stressors including the toxic heavy metals – lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (Wuana and Okieimen, 2011). Evidence keeps mounting that vegetables and other food crops grown under such unwholesome conditions may bioaccumulate the toxic heavy metals at levels exceeding statutory or advisory limits whether measured as metal concentrations in produce or expressed as projected daily intakes (Nabulo *et al*., 2010; 2011). Consequently, in Nigeria, the uptake of heavy metals by dietary vegetables has received much attention in recent times. The uptake of heavy metals including Cu and Pb by *Amaranthus* species, for instance, has been recently studied in soils at refuse dump sites, animal waste dumpsites and other forms of contaminated soils (Adekunle *et al*., 2009; Adefila *et al*., 2010; Adefemi *et al*., 2012; Akubugwo *et al*., 2012; Shagal *et al*., 2012). An index of metal phytoavailability frequently used in environmental monitoring is the degree to which an extractable solid-phase quantity is correlated with measured tissue concentration (Wuana *et al*., 2012). Using data obtained from chemical extractions, different parameters such as soil-to-plant transfer factor, the translocation index, mobility factor and regression models have been used to assess and/or predict metal uptake by *Amaranthus* species (Ahmad-Mahir *et al*., 2009; Ngole 2011; Adefemi *et al*., 2012, Akubugwo *et al*., 2012).

The present study was aimed at assessing the effects of different doses of Cu and Pb and/or manure application to soil on the growth potential of *A. hybridus* as well as predicting Cu and Pb phytoavailability to this vegetable from key soil parameters using step-wise multivariate regression models.

Materials and methods

Chemicals and apparatus

Chemicals and apparatus used for the study included lead nitrate, copper nitrate, calcium chloride, glassware, weighing balance (Gallenkamp 80), pH meter (Fisher Hydrus 300 model), mechanical shaker (Model TT 12F, Techmel and Techmel, Texas, US), electric heater, centrifuge (Model TGL-16G, Shanghai, China), atomic absorption spectrophotometer (Buck Scientific Model 200A, Norwalk, Connecticut, US).

Soil sampling, characterization, artificial contamination and manure amendment

Five different samples of top soil were collected from an arable land in the vicinity of the Benue Industrial Layout, Makurdi (7.44°N, 8.33°E), north-central Nigeria in April 2011, pretreated, composited as the parent soil and characterized physicochemically in terms of pH (Maxted *et al*., 2007), bulk density (CDPRHAP, 1998), texture (Bouyoucos, 1962), organic matter (Schumacher, 2002), plant available Cu and Pb (Oliver *et al*., 1999) and pseudototal Cu and Pb (Khoudadoust *et al*., 2005). The parent soil was next, concurrently contaminated with various doses of Cu and Pb (100 – 500 mg kg^{-1}) by conjointly dissolving 0.38 and 0.16 g, 0.76 and 0.32 g, 1.14 and

0.48 g, 1.52 and 0.64 g and then, 1.90 and 0.80 g, respectively of $Cu(NO_3)_2.3H_2O$ and $Pb(NO_3)_2$ in deionized water in separate beakers and adding to separate 1-kg portions of the soil, followed by one-month equilibration. The metal-stressed soils were further treated with 111-g portions of sufficiently aged poultry or swine manures (previously characterized in terms of pH, organic matter and total Cu and Pb) to achieve 10 % *w/w* amendment and incubated for 1 month at ambient temperature.

Pot experiments

Pot experiments adopted the procedures of Nolan *et al.*, (2005) and Battaglia *et al.*, (2007) with modifications. Five seeds of *A. hybridus* were sown in perforated cylindrical plastic pots (volume = 1500 cm^3) each containing 1 kg of soil. The seedlings were thinned to three after emergence. Surface irrigation with deionized water was adopted to water the plants during growth. The plants were monitored for 7 weeks for changes in height, leaf breadth, above-ground dry biomass and tissue Cu and Pb concentrations. The above-ground plant biomass was harvested from the soils after 49 days, rinsed with deionized water and dried at 110°C for 72 h. The concentrations of Cu and Pb (mg kg^{-1} dw of plant biomass) was determined by $HNO_3 - H_2O_2$ digestion (Nolan *et al.*, 2005) followed by atomic absorption spectrophotometric measurements.

Results and discussion

Physicochemical properties of soil and manures

Table 1 presents some physicochemical attributes of the parent soil; manures used for the study and the metal-stressed and/or manure-amended soil sub-samples.

The parent soil, poultry and swine manure had pH 6.8, 7.2 and 7.5, respectively. These values are in the range reported for typical agricultural soils and the manures (Eriksson *et al.*, 1997; Awodun, 2007). In the metal-stressed soils, the pH decreased with increasing metal doses possibly due to the hydrolysis of the metal nitrates, furnishing the corresponding acids, thus lowering the soil pH. Manure amendments, however raised the soil pH. The growth of vegetable *Amaranthus* species is adversely affected by a soil pH 4.7 – 5.3; however, a soil pH of 6.4 could produce high yields (DAFF, 2010). Texturally, the percent of sand, silt and clay in the soil were 45.8, 23.0 and 31.2 %, respectively qualifying it as clay loam. The bulk density, porosity and particle size distribution of the soil suggest a high water retention capacity. The organic matter content of the soil, poultry and swine manures were 5.9, 31.5 and 23.2 %, respectively. The organic matter content of this soil qualifies it as a mineral soil (a characteristic of most agricultural soils). Manure amendments enhanced the soil organic matter justifying its widespread use to raise seedlings in tropical areas (Awodun, 2007; Ramadan *et al.*, 2007). Plant available Cu and Pb, Q_e, in the soil were 5.1 and 7.2 mg kg^{-1}; while the pseudototal Cu and Pb contents, Q_T were 17.5 and 51.0 mg kg^{-1}. These values were lower than their corresponding upper critical levels, defined as the range of values above which toxicity is considered to be possible (Maiz *et al.*, 2000) indicating that the parent soil was relatively uncontaminated in terms of Cu and Pb. Pseudototal Cu in poultry and swine manure were 120 and 250 mg kg^{-1}, respectively; and 5.0 and 9.5 mg/kg, respectively in the case of Pb. Manure amendments decreased the plant available Cu and Pb in the soil indicating the ability of the manures to bind the metals in the soil. In the poultry manure-amended soil (PMA) the binding efficiencies for Cu and Pb ranged from 28 – 51% and 27 – 45%, respectively. The ranges of metal binding efficiencies in swine manure-amended soils (SMA) were 15 – 41% and 8 – 32% for Cu and Pb, respectively. Poultry manure suppressed metal availability in soil to a greater extent than swine manure. The ability of the manures to bind metals, attenuate metal solubility, hence phytoavailability in soil is due to sorption or complexation onto the organic matter fraction of the manure.

Table 1. Some properties* of the parent soil (PS), poultry manure (PM), swine manure (SM) and experimental soils loaded with various metal doses before and after manure amendment.

Soil	Dose (mg kg^{-1})	pH	OM (%)	$Q_{e(Cu)}$ (mg kg^{-1})	$Q_{T(Cu)}$ (mg kg^{-1})	$Q_{e (Pb)}$ (mg kg^{-1})	$Q_{T (Pb)}$ (mg kg^{-1})
PS	–	6.5±0.1	5.9±0.2	12.3±0.5	17.5±2.1	8.2±0.3	51.0±2.3
PM	–	7.2±0.0	31.5±1.0	–	120.0±2.5	–	5.0±0.1
SM	–	7.5±0.3	23.2±0.5	–	250.0±2.7	–	9.5±0.3
UA	0	---------------------(see parent soil, this served as the control)----------------------------					
	100	6.5±0.1	5.7±0.1	35.5±1.4	115.1±2.7	30.7±0.9	153.4±3.2
	200	6.2±0.0	5.2±0.0	60.2±2.0	214.3±3.5	52.4±1.7	255.2±2.9
	300	6.0±0.0	5.0±0.0	85.4±2.0	315.2±2.9	68.7±1.8	350.5±3.4
	400	5.8±0.0	4.5±0.1	110.8±1.9	416.3±3.5	90.3±1.9	448.6±4.0
	500	5.7±0.0	4.2±0.0	138.2±1.5	513.7±3.9	110.3±2.3	549.1±4.1
PMA	0	7.1±0.0	13.9±0.2	6.0±1.0	27.0±1.8	4.8±0.0	46.4±1.0
	100	6.8±0.1	12.8±0.1	25.4±0.9	115.6±3.2	22.4±0.5	138.6±2.1
	200	6.7±0.1	12.5±0.0	43.3±0.5	204.9±2.3	35.2±0.7	230.2±2.0
	300	6.5±0.0	12.0±0.0	50.3±1.5	295.7±3.0	50.3±1.2	316.0±2.2
	400	6.4±0.0	11.8±0.3	55.6±2.5	386.7±3.1	55.5±1.3	404.3±3.1
	500	6.2±0.2	11.3±0.2	65.3±2.0	474.4±3.3	60.3±0.9	494.7±3.0
SMA	0	6.7±0.1	10.4±0.0	8.5±0.4	40.7±2.2	6.4±0.5	46.9±2.6
	100	6.5±0.1	9.8±0.1	30.3±1.1	128.6±2.4	27.3±1.0	139.0±2.5
	200	6.2±0.1	9.2±0.2	46.8±1.5	217.9±4.0	46.3±2.8	230.6±3.0
	300	6.0±0.2	9.0±0.0	60.3±2.0	308.7±4.2	63.3±3.0	316.4±3.1
	400	5.9±0.2	8.7±0.0	75.3±2.4	399.7±3.6	70.3±2.1	404.7±4.0
	500	5.6±0.0	8.6±0.0	82.1±3.0	487.4±4.6	75.2±2.3	495.2±3.7

*Mean of triplicate determinations ± s.d; UA=unamended soil; PMA=poultry manure-amended soil; SMA=swine manure-amended soil; OM=organic matter; Q_e=available metal; Q_T=pseudototal metal

Growth attributes

The effect of different degrees of metal-stress and manure amendments on the growth of *A. hybridus* was studied by monitoring changes in plant appearance, height, leaf breadth and above-ground biomass for up to 49 days. This length of time was justifiable since most *Amaranthus* cultivars grow rapidly and may be harvested from 30 to 55 days from sowing (DAFF, 2010; Bigaliev *et al.*, 2012). Most plants were stunted with yellow leaves and reduced biomass especially in soils with higher metal doses. Growth profiles (Fig 1a-c) were sigmoid (S-shaped) in soils with 0 – 300 mg kg^{-1} Cu/Pb and plateau-like (L-shaped) in soils loaded with 400 – 500 mg kg^{-1} of the metals. *A. hybridus* height and leaf breadth appeared to decrease in an approximate order of the degree of metal contamination. At the elapse of the 49-day pot study, maximum heights (3.3 – 13.3 cm) and leaf breadths (2.1 – 6.9 cm) were attained in the unamended soil (UA). Consequent upon manure-amendment, however, maximum *A. hybridus* heights and leaf breadths ranged from 8.9 – 16.7 cm and 5.0 – 7.3 cm, respectively in PMA; with corresponding ranges in SMA as 7.1 – 9.2 cm and 4.9 – 6.9 cm. Under normal conditions *Amaranthus* species may reach a height of 60 cm when fully matured (DAFF, 2010). Figure 2 illustrates that the above-ground *A. hybridus* dry biomass harvested after 49 days of growth decreased with increasing metal dose and ranged as UA

(0.2 – 1.7 g pot^{-1}), PMA (0.77 – 4.58 g pot^{-1}) and SMA (0.31 – 2.74 g pot^{-1}). Poultry manure enhanced the growth attributes of *A. hybridus* to a greater extent than swine manure, thus, justifying its frequent land application by farmers.

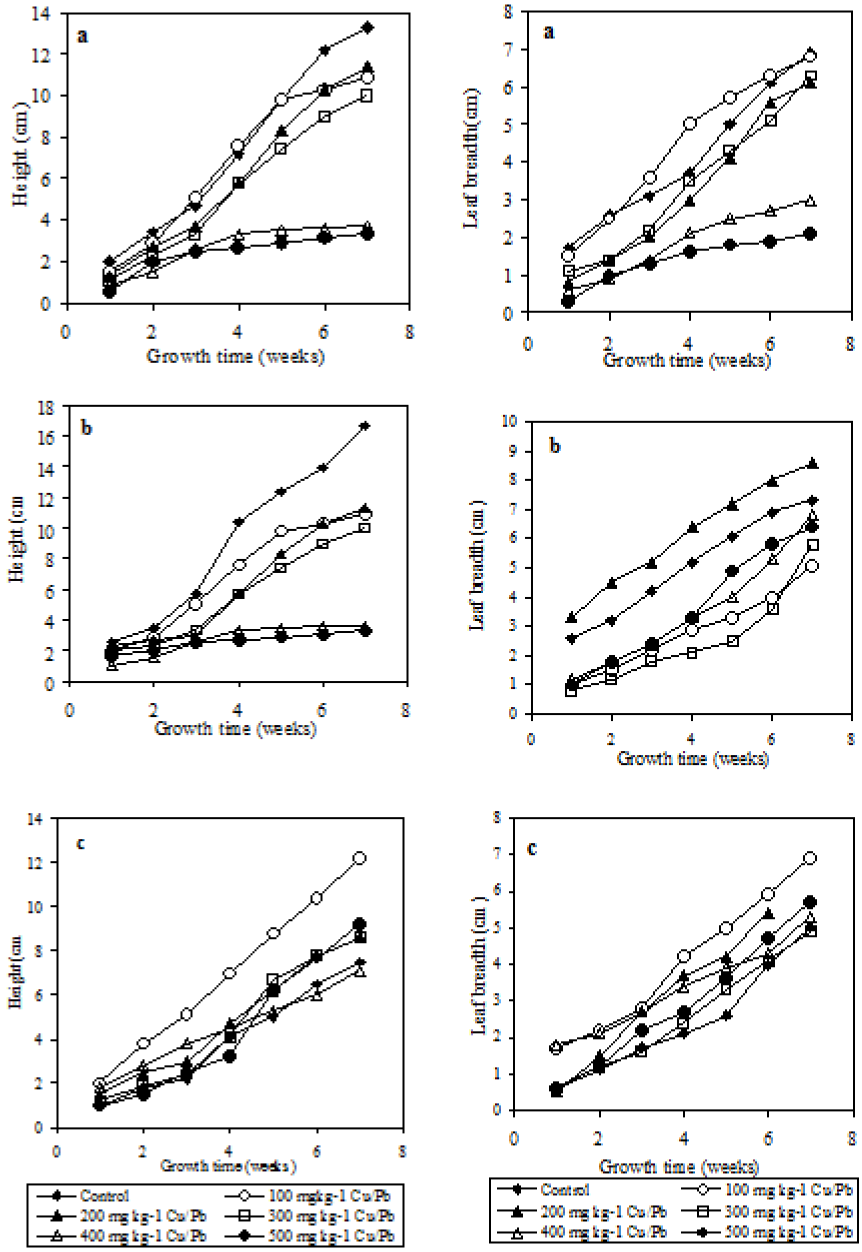

Fig. 1. Growth rate of A. hybridus in (a) unamended, (b) poultry manure-amended and (c) swine manure-amended soils contaminated with various doses of Cu/Pb.

Cu and Pb phytoavailability

The phytoavailability of Cu and Pb to *A. hybridus* in response to increasing levels of metal contamination and manure amendments in soil was also investigated. An index of metal phytoavailability called the soil-to-plant transfer factor, *f* (%) was calculated as the ratio of the above-ground tissue metal concentration, Q_P (Fig. 3) to pseudototal metal content in soil, Q_T (Table 1).

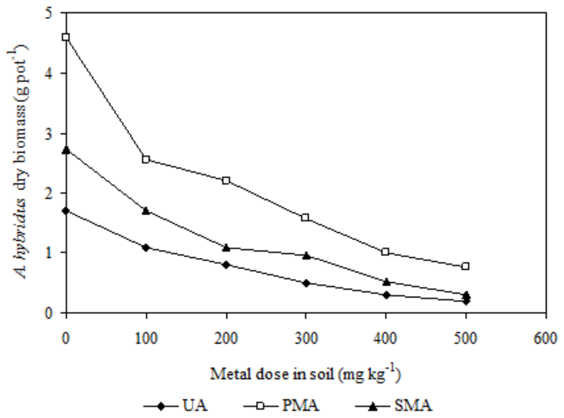

Fig. 2. Above-ground dry biomass yield of A. hybridus in soil contaminated with various doses of Cu/Pb before (UA) and after manure (PMA = poultry manure; SMA = swine manure) amendment.

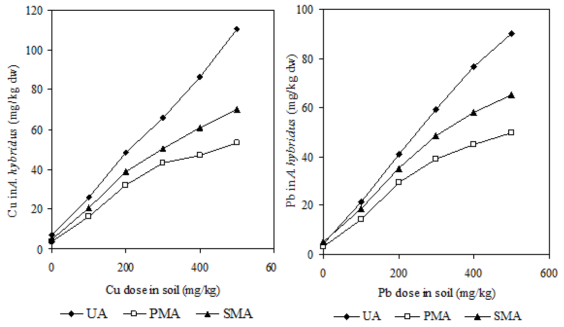

Fig. 3. Cu and Pb concentration (mg kg-1 dw) in above-ground tissue of A. hybridus as a function of metal dose in soil before (UA) and after manure (PMA = poultry; SMA = swine) amendment.

In UA, *A. hybridus* tissue Cu and Pb concentrations, Q_p increased linearly with increasing metal dose. In other words, as the concentration of Cu and Pb increased in the soil, there was an increase in the metal concentration in plant tissue. This observation corresponds to the phenomenon of the *salt effect* since the metals were added as the nitrates (Cunningham *et al*., 1975). In the unamended soil, *A. hybridus* Cu concentrations ranged from 7.2 – 110.3 mg kg^{-1} (20.7 ≤ T_c ≤ 41.1 %); while the concentration of Pb ranged from 4.3 – 90.3 mg kg^{-1} (8.0 ≤ T_c ≤ 17.1%). These observations show that the transferability of Cu to *A. hybridus* was higher than Pb possibly due to the latter's tendency to associate with insoluble weathering products formed by the oxidation of sulphides and insoluble soil fractions (Pueyo *et al*., 2008).

In the presence of manure amendments in soil, non-linear responses of plant tissue concentrations of Cu and Pb to increasing metal doses were observed, interpretive of the *plateau effect* (Cunnigham *et al*., 1975; McBride 1995, Basta *et al*., 2005; Silveira *et al*., 2003). In PMA, Q_p for Cu ranged from 4.0 – 53.2 mg kg^{-1} (11.2 ≤ T_c ≤ 15.8%); while those for Pb were 3.0 – 50.0 mg kg^{-1} (6.5 ≤ T_c ≤ 12.7%). In SMA, as much as 5.0 – 70.2 mg kg^{-1} Cu (12.3 ≤ T_c ≤ 17.8%) and 5.0 – 65.3 mg kg^{-1} Pb (10.7 ≤ T_c ≤ 15.4%) bioaccumulated in *A. hybridus*. This observation suggests that manure application can reduce the solubility and risk of heavy metal contaminants in the soil. The manures, however, need to be applied at such doses that would not trigger short-term leaching pulses of the heavy metal in the soil after eventual degradation of the manure.

Modeling Cu and Pb phytoavailability from soil parameters

Table 2 presents three step-wise multiple regression models (Eqs.1 – 3) which were generated using the SPSS® 17.0 statistical software in order to forecast *A. hybridus* tissue Cu and Pb concentrations from soil parameters. The models assummed soil pH, % OM, plant available metal, Q_e and soil pseudototal metal content, Q_T (Table 1) as covariates determining Q_p (Römkens *et al*., 2004; Brus *et al*., 2005; de Vries 2007).

$$\log Q_p = \alpha \log Q_e + \beta \quad \text{...(1)}$$

$$\log Q_p = \alpha_1 \log Q_e + \beta_1 \text{pH} + \gamma_1 \log \text{OM} + \delta_1 \quad \text{...(2)}$$

$$\log Q_p = \alpha_2 \log Q_e + \beta_2 \text{pH} + \gamma_2 \log \text{OM} + \delta_2 \quad \text{...(3)}$$

In the models, α, β, γ, δ are constants.

Table 2. Step-wise regression models for predicting *A. hybridus* tissue Cu and Pb concentrations from soil parameters.

Soil	Metal	Models	R^2
UA	Cu	$\log Q_p = 1.124 \log Q_e – 0.349$	0.998
		$\log Q_p = 0.857 \log Q_e – 0.621pH – 0.211 \log OM + 3.984$	0.996
		$\log Q_p = 0.608 \log Q_T – 0.211pH – 0.393 \log OM + 1.839$	0.999
	Pb	$\log Q_p = 0.892 \log Q_e – 0.610$	0.998
		$\log Q_p = 0.630 \log Q_e – 0.607pH + 3.621 \log OM + 4.507$	0.910
		$\log Q_p = 0.769 \log Q_T – 0.124pH – 0.359 \log OM + 0.890$	0.920
PMA	Cu	$\log Q_p = 1.097 \log Q_e – 0.284$	0.994
		$\log Q_p = 0.871 \log Q_e – 0.328pH – 0.379 \log OM + 2.522$	0.998
		$\log Q_p = 0.899 \log Q_T + 0.156pH – 0.807 \log OM – 0.788$	0.987
	Pb	$\log Q_p = 0.898 \log Q_e – 0.408$	0.990
		$\log Qp = 1.233 \log Q_e – 0.596pH – 1.233 \log OM + 3.190$	0.982
		$\log Q_p = 0.407 \log Q_T – 0.583pH – 0.026 \log OM + 4.300$	0.867
SMA	Cu	$\log Q_p = 1.118 \log Q_e – 0.314$	0.994
		$\log Q_p = 1.120 \log Q_e – 0.020pH + 0.134 \log OM – 0.286$	0.996
		$\log Q_p = 0.898 \log Q_T + 0.062pH – 0.698 \log OM – 0.286$	0.976
	Pb	$\log Q_p = 0.914 \log Q_e – 0.293$	0.993
		$\log Q_p = 1.059 \log Q_e – 0.031pH + 0.035 \log OM – 0.014$	0.983
		$\log Q_p = 0.750 \log Q_T – 0.175pH – 0.405 \log OM + 1.282$	0.912

UA = unamended soil; PMA = poultry manure-amended soil; SMA = swine manure-amended soil; OM=organic matter; Q_p=tissue metal; Q_e=available metal; Q_T=pseudototal metal

Model I (Eq.1) assumes a log-linear relationship between *A. hybridus* tissue concentrations of Cu and Pb, Q_p (mg kg^{-1}) and plant available concentrations ($CaCl_2$-extracted), Q_e (mg kg^{-1}) (Watanabe *et al.*, 2009). Apparently, Q_e values were consistently higher than corresponding Q_p values in all the experimental soils, implying that the chemical extractant ($CaCl_2$), somewhat exaggerated the amount of plant available metals or that not all potentially phytoavailable fractions were taken up. Model I, however, gave good correlations between Q_e and Q_p as evident from the R^2 values. The R^2 values for Cu and Pb in UA, PMA and SMA fell in the range $0.990 \le R^2 \le 0.998$ (Table 3). Model II (Eq. 2) which aimed at predicting Q_p from Q_e, pH and OM gave R^2 values in the range $0.910 \le R^2 \le 0.998$ for in all the soils. Model III (Eq. 3) attempted to predict Q_p by assuming that Q_T, pH and OM are co-variates. The R^2 values appeared to fell in the range $0.867 \le R^2 \le 0.999$ across the experimental soils. It appeared that this model predicted Cu tissue concentrations in *A. hybridus* better than it did for Pb. The effectiveness of the model varied as Model II > model I > model III. The soil parameters, particularly pH and OM showed narrow ranges implying that these models may be valid particularly within this range of soil properties used to generate them. Application of the models to other soils having a wide distribution of these parameters will require model re-parameterization.

Conclusion

In this study, signs of Cu/Pb intolerance - yellowish appearance, stunted growth and decreased biomass were visible in *A. hybridus* at elevated metal doses with reduced severity upon manure (especially poultry) amendment in soil. Growth profiles were either sigmoid (0 – 300 mg kg^{-1} metal dose) or plateaux (400 – 500 mg kg^{-1} metal dose). Poultry manure enhanced the growth attributes of *A. hybridus* to a greater extent than swine manure. Tissue Cu and Pb concentrations increased linearly as metal doses in unamended soil, while non-linear responses were observed in manure-amended soil. Step-wise regression models for forecasting *A. hybridus* tissue metal concentrations from soil parameters (pH, organic matter, plant available and pseudototal metal) indicated that plant available metal is more reliable as a covariate than pseudotototal metal content.

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