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Production of the Forage Halophyte *Atriplex amnicola* in Metal-Contaminated Soils

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Abstract

Cleanup of contaminated soils is a costly and slow process that requires long periods of time to be effective. Therefore, direct use of contaminated sites with appropriate management is often likely to be a more efficient use of such land. Consequently, the production of safe animal forages from contaminated soils was the aim of this research. Field studies were conducted to evaluate the growth and elemental composition of river saltbush (*Atriplex amnicola*) grown on a metal-contaminated soil. The soil was amended with compost at rates of 0, 15 and 30 t/ha to assess its role on plant growth and metal uptake. Compost application significantly ($P<0.05$) increased biomass yield, crude protein (CP), and ash content of river saltbush; in contrast it decreased the Zn and Pb concentrations in shoot tissues. When 30 t/ha of compost was added, the Pb concentrations in the stems and leaves decreased by 32% and 38%, respectively. Despite the large total and extractable content of metals in the studied soil, shoot concentrations of these metals in *A. amnicola* were always maintained below potentially toxic levels. The biomass material of *A. amnicola* had a high nutritive value compared to conventional forage crops and could safely be used as animal forage. This work demonstrates that an *Atriplex* spp, *A. amnicola*, has significant potential for use as a safe forage crop in a sustainable on-site management of contaminated soils.

Keywords: Metals, *Atriplex amnicola*, Yield, Toxicity, Compost, Phytostabilization, Phytoremediation.

Introduction

Production of traditional forage crops in contaminated soils is not acceptable due to sensitivity to metal stress and a tendency in these species to translocate metals to the edible portions and thus introduce metals into the food chain. Most current remediation methods are effective over small areas, but are expensive and cause soil disturbances (Martin & Ruby, 2004;

Saifullah *et al.*, 2009). A more practical solution may be the stabilization of metal-contaminated sites by cultivation with plants that are tolerant but do not translocate appreciable quantities of the metals to their edible parts, a practice known as phytostabilization. Some halophytic plants may be suitable for use in phytostabilization; especially those belonging to the genus *Atriplex* which may be of special interest because of a deep root system and ability to tolerate extreme conditions. In particular, *Atriplex* species have a high tolerance to metal stress (Lutts *et al.*, 2004; Sai Kachou *et al.*, 2009). They can be irrigated with saline water and can also tolerate drought, freezing and heat (Thomas *et al.*, 1998; Jordan *et al.*, 2002; Lutts *et al.*, 2004).

Organic amendments form insoluble complexes with metals, reducing their solubility (Bernal *et al.*, 2007; 2009). However, there is little available information about the response of halophytes to organic amendments. Only a few studies have investigated the accumulation of metals in the shoot tissues of *Atriplex* grown on contaminated soils (Sabey *et al.*, 1990; Jordan *et al.*, 2002). Furthermore, only one single study focused on metal accumulation in *Atriplex* spp. when grown in mine tailings with organic amendments (Sabey *et al.*, 1990). Recently Martinez Fernandez & Walker (2012), Pérez-Esteba *et al.* (2010), and Mendez *et al.* (2007) studied the effect of organic amendments on growth and uptake of metals by some halophytes. Most of these studies concentrated on *Atriplex halimus* and only Mendez *et al.* (2007) studied the accumulation of metals in *Atriplex lentiformis*. Lutts *et al.* (2004) suggested that *A. halimus* may be suitable for use in phytoextraction of metals but their experiments were conducted in hydroponic solutions. Other studies were carried out in pots using artificially contaminated soils (Manousaki & Kalogerakis, 2009) and therefore likely to have higher bioavailability than long-term contaminated soils. Thus, field studies using contaminated soils are needed to evaluate these plants. In addition, the genus of *Atriplex* contains more than 100 species, but most of the above mentioned studies have used *A. halimus* and therefore a broader range of species need investigation. To our knowledge, the only field studies on the phytostabilization potential of *Atriplex* spp. Was by Eissa (2014; 2015) to evaluate the impact of compost on the metal phytostabilization potential of *A. lentiformis*, *A. undulata* and *A. amnicola*. However, the experimental site contained small concentrations of metals and could not be considered to be a contaminated soil.

The current study focused on the use of contaminated soils for forage production and explored the role of compost on the growth and metal uptake by river saltbush (*Atriplex amnicola*), a halophyte native to Australia and considered to be among the most useful natural forages for animal feeding (Masters *et al.*, 2007; 2010; Fancote *et al.*, 2009). This study aimed to determine (i) the growth and the nutritive value of river saltbush grown on metal-contaminated soil; (ii) the toxicity of metals in the stems and leaves of river saltbush and (iii) the impact of compost on reducing metal toxicity.

Materials and methods

Field experiment

The field experiment was conducted at Arab Elmadabegh, Assiut Governorate, Egypt (27° 12' N latitude and 31° 09' E longitude). Untreated sewage waste water had been used to irrigate the soils for more than 50 years. The physical and chemical characteristics of the field site are presented in Table 1. This soil was classified as a Typic Torripsamments according to Soil Taxonomy (Soil Survey Staff, 2010).

Compost was made from cattle manure and ground maize straw (<10 mm) at a ratio of 1:4 manure to straw. Composting process continued for two months. Data in Table 2 show selected properties of the compost. Compost was mixed with the top soil layer (0-30 cm) during the preparation of the field and before transplanting in the two growth seasons. The compost was applied to the soil at rates of 0, 15 and 30 t/ha in a randomized complete block design (RCBD) with three replications of each compost rate.

Seeds of *Atriplex amnicola* Paul G. Wilson (river saltbush) were soaked in tap water for three days with daily renewal of the water. Soaked seeds were placed in pots filled with washed sand and irrigated with tap water as needed. After a period of 30 days, the height of the seedlings was approximately 15 cm. Thirty-day-old seedling was transplanted to the field at 1×1 m spacing in plot areas of 2×5 m² (a total of 10 plants per plot) on May 15, 2012 and May 25, 2013.

. The plants were irrigated every 20 days by surface irrigation. Some chemical characteristics of the irrigation water are summarized in Table 3. No inorganic fertilizers were added. The ten plants from each plot were harvested on September 1, 2012 and September 10, 2013. The shoots

were cut, separated into stems and leaves, and fresh weights were recorded. The roots, stems, and leaves were washed thoroughly with tap water, then with 0.1% HCl and then rinsed with distilled water before being oven-dried at 70 °C until a constant weight. Dry weights were recorded and the dried roots, leaves and stems were ground and kept for analysis.

Soil, water, compost, and plant analysis

To determine the physical and chemical properties of the experimental site, a composite soil sample (0–30 cm) was collected before compost application and plant cultivation. After harvest, composite soil samples (0–30 cm) were collected from each treatment to study the effect of compost on soil properties and availability of metals. The soil was air dried and ground to pass through a 2mm sieve and mixed thoroughly. Analysis of soil (texture, total carbonate, cation exchange capacity (CEC), electric conductivity (EC), pH, total organic carbon, total and available nitrogen, and available phosphorus and potassium were determined according to the standard methods described by Burt (2004) and Cottenie *et al.* (1982). To determine total metals, the soil samples were digested according to the procedure given by the US EPA (1996) then the metals in the extract were measured. Zinc, Cu, Pb, and Cd were extracted from the studied soil samples using a 0.005 M DTPA (diethylene triamine penta acetic acid) solution buffered at pH 7.3 as described by Lindsay & Norvell (1969).

Four wastewater samples were collected from the irrigation source during the study period and chemically analyzed. The pH of water samples was measured by a digital pH meter, while the total soluble salts was estimated using the salt bridge method (Rhoades, 1982). To determine the total metals content of the wastewater samples, 50 ml of each sample were digested with 10 ml concentrated HNO₃ at 80 °C according to APHA (2005).

Compost and plant samples were digested in H₂SO₄ and H₂O₂ as described by Parkinson & Allen (1975). Nitrogen in plant samples was measured by Kjeldhal distillation method (Cottenie *et al.*, 1982). Crude protein was calculated as % nitrogen × 6.25. The ash content was determined by dry-ashing in a muffle furnace at 450 °C for 6 h.

The pH of compost was measured in a 1:5 suspension of compost to water ratio by a digital pH meter and electrical conductivity (EC) was measured in 1:5 extract by the salt bridge method.

The suspension was shaken for 10 minutes at 25 °C, then the pH was measured, then the EC was measured in the extract.

Zinc, Cu, Cd, and Pb in the soil, plant, water, and compost samples were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES, thermo iCAP 6000 series)

Statistical analysis of data

Analysis of Variance (ANOVA) and Duncan multiple range tests were used to assess the level of significance (confidence level of 95%) of the differences in response to treatments. The statistical analyses were performed using the SPSS statistical software, version 15.

Results

Effect of compost on some soil properties and availability of metals

The values of DTPA–extractable Zn in the soil with 15 and 30 t/ha compost increased significantly ($P=0.02$) by 17 and 25%, respectively, compared to levels in unamended soil (Figure 1). In comparison, the levels of DTPA–extractable Cu, Pb, and Cd were significantly reduced due to compost application (Figure 1). The application of the high rate of compost (30 t/ha) reduced the availability of Cu, Pb, and Cd by 6, 17 and 28%, respectively, compared to the unamended soil (Figure 1). Compost application had no effect on soil pH ($P=0.15$), but led to significant increases in soil OC ($P=0.001$) and CEC ($P=0.02$). Compost application at a rate of 30 t/ha increased the soil OC and CEC by 36 and 16% compared to the unamended soil (Figure 2).

Effect of compost on metal uptake by *Atriplex amnicola*

Compost application had significant effects on the Pb concentrations in the roots, stems, and leaves of river saltbush (Table 4). The concentrations of Zn in the roots and leaves were affected significantly ($P=0.002$ and $P=0.005$, respectively) by the addition of compost. When 30 t/ha of compost was applied to the soil, the Zn and Pb concentrations in the roots increased by 5 and 12%, respectively, whereas concentrations in the stems were decreased by 11% and 32%, respectively, and in the leaves were decreased by 6 and 38%, respectively. The concentrations of Cu and Cd in the roots, stems, and leaves of river saltbush were not affected by compost application.

Effect of compost on yield and nutritive value of *Atriplex amnicola*

Compost application at 30 t/ha increased the FW and DW of the stems by approximately 9% and 14% and the FW and DW of the leaves by approximately 15 and 6% respectively (Table 5). During the 3-months trial, *A. amnicola* plants produced a total FW yield (stems + leaves) of between 880–1000 kg/ha and a total DW yield of between 500–570 kg/ha. The FW of the leaves accounted for about 38% of the total FW of the plants but the DW of the leaves accounted for about 32% of the total DW of the plants.

The leaves of *Atriplex amnicola* contained more crude protein (CP) and ash than the stems (Table 5). Increasing the application rate of compost to 30 ton/ha significantly increased the crude protein (CP) in the stems and leaves by about 34 and 24% and ash content by about 45 and 13%, respectively. Cynodon hay, big saltbush (*Atriplex lentiformis*), corn (*Zea mays*), alfalfa (*Medicago sativa*), and Sudan grass (*Sorghum spp*) are commonly used as forage crops in many countries of the world. The CP and ash content of these forages are low compared to the leaves of *A. amnicola* in the current study (Table 6).

Discussion

Yield and nutritive value of *Atriplex amnicola*

A. amnicola plants produce more than 500 kg/ha of dry yield (stems and leaves) during three months. The plant material had a high nutritive value (crude protein and ash). Irshad *et al.* (2000) found that *A. amnicola* gave more fresh and dry (stems and leaves) compared to *A. lentiformis* and *A. undulata*, and they reported that the fresh biomass of *A. amnicola* ranged between 3–4 t/ha and the dry biomass yield ranged between 1.1–1.5 t/ha for 9-months old plants.

Ash content reflects the amount of nutrients in the forage and crude protein (CP) is a critical parameter for animal feed. The crude protein (CP) and ash in the stems ranged between 5–7% and 8–12%, respectively and in the leaves they ranged between 20–25% and 25–29% (Table 5). Khalil *et al.* (1986) found that the ash content of leaves of *Atriplex* species grown in uncontaminated soils ranged between 18–27% and the CP ranged between 16–25%. Thus the

plants in the current study were able to maintain their nutritive value when grown in contaminated soil, showing potential to be used as forage in these situations.

Application of compost improved the nutritive value of the forage, in agreement with studies on other *Atriplex* spp.: Martinez-Fernandez & Walker (2012) studied the effect of compost on the growth of *A. halimus* grown on metal contaminated soils and found that the shoot biomass increased significantly with compost application. *Atriplex lentiformis*, *A. undulata*, and *A. amnicola* were also reported to show a similar behavior when compost was added to uncontaminated soils (Eissa, 2014; 2015). It is likely that the organic amendment led to increased availability of nitrogen, and phosphorous in the soil (Jakobsen, 1995; Joshua *et al.*, 1998).

Toxicity of heavy metals for plants and animals

Despite the large value of total and available metals concentrations in the studied soil, the concentration of elements in the shoots was below toxic levels. The toxicity levels of Zn, Cu, Pb, and Cd range from 100–400, 20–100, 30–300, and 5–30 mg/kg dry weight respectively (Kabata-Pendias, 2001). Toxicity levels are related to plant health but metal concentrations are also important for animal health. Permissible limits of metals in the forage of animals are 10 mg/kg for Cd, 10–100 mg/kg for Pb, 20–100 mg/kg for Cu, and 250–1000 mg/kg for Zn (National Research Council, 2005). The concentrations of metals in the aboveground biomass of river saltbush plants were below these limits, and therefore could be safely used as a source of forage for livestock production. Our findings are in agreement with Sai Kachout *et al.* (2009) who studied the uptake of metals (Cd, Cu, Zn, Pb, and Ni) by *A. hortensis* and *A. rosea*, and found that the *Atriplex* spp. accumulated the metals in the roots but there was little translocation of these elements to the shoots. Thus it appears that the *Atriplex* genus is able to exclude metals from the aboveground portions.

Impact of compost on reduction of metal toxicity

The application of compost increased the concentrations of available Zn in the soil, but reduced the availability of Cu, Pb, and Cd (Figure 1). The reason for the increased Zn concentrations may be due to the presence of Zn in the compost (Table 2), as each 15 t/ha of compost added 2.28 kg of Zn to the soil. Although compost application has no effects on the soil pH, it reduced the DTPA-extractable Cu, Pb, and Cd. This finding is in agreement with Walker

et al. (2003) and Martinez Fernandez & Walker (2012) who found that the application of compost to metal-contaminated soils reduced the availability of metals without a significant effect on soil pH. The highly humified OM (e.g. humic acids) in mature composts has a great affinity for metals such as Cu, Cd, and Pb, forming stable, insoluble complexes (Bernal *et al.*, 2007; 2009) which may explain the reduction of Cu, Pb, and Cd availability in the studied soil.

It is clear that the application of compost increased the accumulation of Zn and Pb in the roots but reduced the concentrations of the two metals in the shoots (leaves and stems) (Table 4). A larger root system following compost application may have led to greater uptake of Zn and Pb. Adewole & Ilesanmi (2011) found that the application of compost to a soil significantly increased the concentrations of Zn in the roots of okra (*Abelmoschus esculentus*).

The decline in the Zn and Pb concentrations in the leaves and stems may be explained by: i) the increase in the shoot growth as a result of compost application diluting the Zn and Pb concentrations in the leaves and stems, ii) reduction of Pb availability in the soil by redistribution of metal from soluble and exchangeable forms to fractions associated with organic matter or carbonates and the residual fraction, adsorption and by forming stable complexes with humic substances (Shuman, 1999; Walker *et al.*, 2004; Bernal *et al.*, 2007, 2009), and iii) minimization of the root-to-shoot transfer of Zn and Pb by precipitation of the metals in the roots. Martinez-Fernandez & Walker (2012) and Cao *et al.* (2002) suggested that the root-to-shoot transfer of Pb by *A. halimus* may have been reduced by its precipitation as $Pb_{10}(PO_4)_6Cl_2$ on the root surface. Eissa (2015) recently found that root phosphorus and chloride reduced the transfer of Zn and Pb to the shoots of *A. undulata* and *A. lentiformis* in uncontaminated soil and so it is likely that a similar effect occurred in *A. amnicola* grown in contaminated soil in the current study.

Conclusions

The current study showed the ability of *A. amnicola* to restrict the accumulation of metals in aboveground tissues. Biomass yield and nutritive value of this species increased when compost was applied and, in addition, compost reduced the concentration of metals in the harvestable tissues. *A. amnicola* contained more crude protein and ash content than traditional forage crops and produced plant material suitable as safe animal forage under contaminated

conditions. Thus, *A. amnicola* together with compost amendments could be used to provide low risk forage for animals on metal-contaminated soils.

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Table 1 Physical and chemical properties (0–30 cm) of the field soil from Arab Elmadabegh, Assiut Governorate, Egypt used in the study

Soil properties	Value	Permissible limits*
Clay (g/kg)	90	
Silt (g/kg)	140	

Sand (g/kg)	770	
Texture	Sandy loam	
CaCO ₃ (g/kg)	65	
Cation exchange capacity (cmol/kg)	20	
pH (1:2)	7.50	
Electrical conductivity (1:1) (dS/m)	1.6	
Organic carbon (g/kg)	5.4	
Total nitrogen (mg/kg)	200	
Available nitrogen (mg/kg)	20	
Available P (mg/kg)	12	
Available K (mg/kg)	200	
Available Zn (mg/kg)	6.5	
Available Cu (mg/kg)	4.9	
Available Pb (mg/kg)	6.2	
Available Cd (mg/kg)	0.54	
Total Zn (mg/kg)	650	200–300
Total Cu (mg/kg)	303	50–140
Total Pb (mg/kg)	306	300
Total Cd (mg/kg)	7.4	3.0

*European Union Standards (EU, 2002) and United States Environmental Protection Agency (USEPA, 1997).

Table 2 Chemical composition of the corn straw and farm manure compost used in the study

Organic Carbon (%)	Electrical Conductivity 1:5	pH 1:5	N (%)	P (%)	K (%)	Zn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
37.7	8.42	8.51	2.21	1.58	2.55	152	42	0.10	0.55

Dry weight basis

Table 3 Selected chemical properties of the untreated wastewater used in the irrigation of *Atriplex amnicola*

TSS	pH	Zn	Cu	Pb	Cd
1700	7.31	0.50	0.32	1.0	0.05

TSS refers to total soluble salts (mg/L). The concentrations of Zn, Cu, Pb, and Cd are in (mg/L). All values are means of four replicates.

Table 4 Concentrations of metals in the roots, stems, and leaves of *Atriplex amnicola* grown under a range of compost amendments

Compost rates (t/ha)	Zn mg/kg	Cu mg/kg	Pb mg/kg	Cd mg/kg
Roots				
0	195 ± 3.5 b	55 ± 2.4 a	60 ± 1.1 c	3.2 ± 0.21 a
15	200 ± 4.8 ab	53 ± 2.1 a	63 ± 1.3 b	3.4 ± 0.24 a
30	205 ± 6.5 a	54 ± 3.0 a	67 ± 2.1 a	3.3 ± 0.30 a
Stems				
0	45 ± 1.2 a	11 ± 0.70 a	3.1 ± 0.51 a	0.10 ± 0.04 a
15	43 ± 2.1 a	10 ± 1.0 a	2.2 ± 0.12 b	0.09 ± 0.04 a
30	40 ± 2.4 a	10 ± 1.2 a	2.1 ± 0.10 b	0.10 ± 0.05 a
Leaves				
0	88 ± 2.1 a	24 ± 1.1 a	5.2 ± 0.08 a	0.12 ± 0.02 a
15	85 ± 2.0 b	23 ± 1.1 a	3.2 ± 0.09 b	0.11 ± 0.01 a
30	83 ± 1.2 c	23 ± 1.0 a	3.2 ± 0.10 b	0.12 ± 0.05 a

Data represent the average across the two years. The means ($\pm$ standard deviation, n=6) of each metal followed by the same letter are not significantly different at $P < 0.05$.

Season of 2012									
Compost	<i>Atriplex amnicola</i>	Cynodon hay		Big saltbush	Corn	Alfalfa	Sudan grass		
CP	23.2	12.3		12.2	7.2	16.9	10.8		
(t/ha)	FW	DW	CP	Ash	FW	DW	CP	Ash	
0	520 ± 10 c	320 ± 8.4 c	5.3 ± 0.54 b	8.7 ± 1.20 b	360 ± 3.5 c	180 ± 5.2 b	20.1 ± 1.2 b	25.1 ± 2.0 b	
15	550 ± 12 b	350 ± 7.2 b	6.5 ± 0.54 a	10.2 ± 0.98 a	380 ± 5.5 b	180 ± 4.5 b	24.1 ± 1.6 a	27.1 ± 2.5 a	
30	580 ± 13 a	380 ± 8.5 a	6.8 ± 0.55 a	12.2 ± 1.00 a	420 ± 6.4 a	190 ± 3.7 a	25.2 ± 1.5 a	28.8 ± 3.5 a	
Season of 2013									
0	580 ± 13 b	340 ± 10 c	5.0 ± 0.25 b	8.0 ± 1.0 b	340 ± 5.5 b	160 ± 3.5 c	20.3 ± 1.0 b	24.5 ± 2.2 b	
15	590 ± 14 b	390 ± 11 a	6.7 ± 0.24 a	11.2 ± 1.1 a	380 ± 6.5 a	180 ± 4.2 a	24.5 ± 1.2 a	27.0 ± 1.9 a	
30	620 ± 22 a	370 ± 12 b	7.0 ± 0.15 a	12.0 ± 1.2 a	380 ± 7.8 a	170 ± 4.0 b	25.0 ± 2.4 a	27.3 ± 1.8 a	

Table 5 Yield and nutritive value of *Atriplex amnicola*

Where FW and DW refer to fresh and dry weight in kg/ha; CP refers to crude protein concentration (%) and Ash to ash concentration (%). The means (± standard deviation, n=3) followed by the same letter are not significantly different at $P < 0.05$.

Table 6 Crude protein (CP) and ash content of *Atriplex amnicola* and traditional forage plants

All the data as % based on dry weight. Cynodon hay values are from Swingle *et al.* (1996); big saltbush values are from Soliz *et al.* (2011); corn, alfalfa, and Sudan grass values are from Dann *et al.* (2008); *Atriplex amnicola* values are from the current study (means are overall treatments and seasons).

Fig. 1.

Fig. 2.

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