Water Air Soil Pollut (2009) 197:15–22
DOI 10.1007/s11270-008-9787-8

Use of Germination and Seedling Performance Bioassays for Assessing Revegetation Strategies on Bauxite Residue

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Received: 28 March 2008 / Accepted: 22 June 2008 / Published online: 11 July 2008

Abstract Bauxite residues from the extraction of alumina from bauxite ore are stored in residue disposal areas. These areas require revegetation and the major constraints and suitable plant species will differ with each site. Germination bioassays were used on bauxite residue from the Aughinish Alumina Ltd. refinery to determine properties inhibitory to seed germination and seedling development. Unamended residue was characterised as having high pH, sodicity, salinity and Al content. These properties had negative tests on seed germination and performance. Amendment of the residue improved chemical properties and greatly increased seedling performance in four test species. Decreased sodicity content in the residue extract resulted in seedling growth greater than achieved in the control. *Lolium perenne* and *Trifolium pratense* were identified as useful species for revegetation of amended bauxite residue.

Keywords Germination index (GI) · *Lolium perenne* · Relative root growth · Sodicity · *Trifolium pratense*

1 Introduction

An estimated 70 million metric dry tons of bauxite residue is produced globally per year; much of which is disposed on land in large residue disposal areas (The Aluminium Association 2000). Restoration of a vegetation cover on mine residues (tailings) can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Wong 2003).

Revegetation of bauxite residue has been demonstrated to be difficult because of the high alkalinity, salinity, sodicity, high Al content, and poor soil permeability (Wong and Ho 1993, 1994; Meecham and Bell 1977; Fuller et al. 1982; Fortin and Karam 1998).

Grasses tolerant of saline and sodic soils are often used in the revegetation of bauxite residue; *Distichlis spicata* (Fuller et al. 1982; Bucher 1985); *Sporobolus airoides* (Fuller et al. 1982); *Agropyron smithii* (Fuller et al. 1982; Bucher 1985); *Agropyron elongatum* (Fuller et al. 1982; Bucher 1985; Wong and Ho 1993); *Cynodon dactylon* (Wong and Ho 1993), and *Chloris gayana* (Meecham and Bell 1977).

Chemical characteristics of bauxite residue can be improved by amending with gypsum (Wong and Ho 1993; Courtney and Timpson 2005; Ippolito et al. 2005), copperas (Ho et al. 1985), seawater neutralisation (Menzies et al. 2004), sowing with tolerant species (Fuller et al. 1982), or bioremediation (Hamdy and Williams 2001).

Properties of bauxite residue are determined by the chemical and mineralogical composition of the processed bauxite and by the applied variant of the Bayer process, chiefly by digestion parameters. (Solymar and Bujdoso 1973). The differences in residue composition

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and characteristics observed between refineries ultimately affect the ease with which the residue can be revegetated (Wehr et al. 2006). Furthermore, the chemical composition can vary vertically and laterally in the same tailings pond (Shu et al. 2005) and temporal variation due to changes in process may also occur. Consequently, residue composition and texture, quantities of ameliorant used, candidate species for revegetation, and climatic conditions will vary between refineries.

Identification of the major constraints and determination of target values is a crucial step in developing strategies for the revegetation of each bauxite residue site. Seed germination and root elongation tests have been documented as techniques for detection of toxicity in heavy metals (Wong and Bradshaw 1982), lead/zinc tailings (Yang et al. 1997; Ye et al. 2002), coal fly ash/compost (Lau and Wong 2001), municipal waste (Marchiol et al. 1999), and metal engraving effluent (Wang and Keturi 1990).

It is proposed to use similar techniques to enhance revegetation strategies for the bauxite residue produced at the Aughinish Alumina Refinery (Ireland). With the intention of using a new range of candidate species for revegetation, parameters of bauxite residue inhibitory to plant growth were investigated using the test species *Lolium perenne*, *Lattuca sativa*, *Trifolium pratense* and *Lepidium sativum.* Growth performance in different bauxite residue treatments was assessed by seed germination and root elongation tests.

2 Materials and Methods

2.1 Study Site

The Bauxite Residue Disposal Area (BRDA) is situated on Aughinish Island on the south side of the Shannon estuary, 32 km downstream from Limerick City, Ireland. The Bayer process produces approximately 1.2 Mt of bauxite residue annually, this is stored in a 103 ha BRDA. A series of revegetation field trials have been ongoing since 1997.

Bauxite residue samples were taken from seven separate trial areas, each representing a different stage of the revegetation process. Residue types (B–E) had all undergone residue improvement using varying amounts of amendment (May–September 2006). Other than gypsum amount indicated, all received application of process sand fraction, compost application, rotavation and a period of leaching. Site types and history are indicated (Table 1).

Samples of the residues were randomly collected from within the selected sites (0–15 cm) in September 2006. All samples were air-dried and passed through a 2-mm sieve.

2.2 Seed Germination and Root Elongation Tests

Seeds from four plants: red clover (*T. pratense* L.), perennial ryegrass (*L. perenne* L.), lettuce (*Lactuca sativa* L.), and cress (*L. sativum* L.) were purchased locally. Before being used for assays, their germination potential was examined at 25±1°C in darkness, and germination over 90% guaranteed the viability of the seeds (Płaza et al. 2005).

Seed germination and root length tests were performed on water extracts prepared by shaking the prepared residue samples with deionized water at a residue/water=1:2 (*w*/*v*) ratio at 180 rpm for 1 h followed by centrifugation at 3,000 rpm and then filtered through a 0.45 µm membrane filter. Ten seeds were placed in a 100×15 mm, plastic petri dish containing 6.0 ml of the residue extract. Deionized water was used as the control treatment. All treatments were in triplicate and were germinated at 25±1°C in the dark. The numbers of seed germinated were counted, and the lengths of roots were measured at 2, 4 and 7 days. Germination was defined as a primary root of ≥5 mm and the test was terminated

Table 1 Treatments for bauxite residue used in the study

Treatment name	Treatment received
A	Unamended
B	Amended, residue has received no gypsum
C	Amended, residue amended with gypsum (22 t/ha) 6 months previously
D	Amended, residue amended with gypsum (45 t/ha) 6 months previously
E	Amended, residue amended with gypsum (90 t/ha) 6 months previously
F	Amended, residue revegetated in 1997 and currently supports vegetation
G	Amended, residue revegetated in 1999 and currently supports vegetation

Amended 25% *w*/*w* process sand and SMC at 90 t/ha when control seed for all test species had developed roots at least 20 mm long (USEPA 1992).

Seedling performance was assessed using the following relative seed germination (RSG), relative root growth (RRG), and germination index (GI) tests (Tiquia et al. 1996);

$$\text{Relative seed germination (RSG) (\%)} = \frac{\text{number of seeds germinated in residue extract}}{\text{number of seeds germinated in control}} \times 100$$

$$\text{Relative root growth (RRG) (\%)} = \frac{\text{mean root length in residue extract}}{\text{mean root length in control}} \times 100$$

$$\text{Germination index (GI) (\%)} = \frac{\text{RSG} \times \text{RRG}}{100}.$$

2.3 Residue Extract Properties

Residue extracts were measured for pH and electrical conductivity (EC). Levels of Ca, Mg, Na and Al were determined in the extract by ICP emission spectrometery. Indices of sodicity of the extracts were expressed in terms of the residue solution concentration in relation to other cations through the sodium absorption ratio (SAR)

$$\text{SAR} = C_{\text{Na}} \Big/ \left[\left(C_{\text{Ca}} + C_{\text{Mg}}\right)/2\right]^{1/2}$$

where C represents concentration in millimoles$_c$ per liter of the cations identified as subscripts (Qadir and Schubert 2002) and the exchangeable sodium percentage (ESP) by the following equation;

$$\text{ESP} = \frac{[-1.26 + (1.475\,\text{SAR}]}{[0.9874 + (0.0147\text{SAR}]}.$$

The relationship between germination bioassays and residue extract at 7 days was determined using Pearson's bivariate correlations analyses on SPSS, version 11.0 (SPSS 2002). Graphs were constructed using GraphPad Prism, Version 4.

3 Results and Discussion

3.1 Properties of Residue Extract

Chemical properties of the different residue extracts are given in Table 2. Due to entrained residual caustic, high pH levels and ESP are inherent characteristics of bauxite residues. Unamended residue (treatment A) can be characterized as having high salinity (EC), Na content, sodicity (ESP) and pH. With little or no gypsum amendment, but a period of weathering, EC values have decreased in treatments B and C. ESP and pH values remain high in these treatments.

Gypsum amendment at rates ≥45 t/ha reduced pH, Al content and ESP of the residue and increased Ca and Mg content. Sodium remained the dominant cation in extracts A–E with marginally more Ca in the treatments F and G. Gypsum amended residue had EC values ranging from 2.1–4.5 mS cm^{-1} with higher levels recorded in the treatments with higher application rates.

A range of 9.2 to 12.1 for residue pH, was cited by Fuller et al. (1982), as being 'above levels considered harmful to plant growth'. They also reported a high

Table 2 Selected properties of extracts from the residue treatments

	pH	EC (mS cm^{-1})	Al (cmol kg^{-1})	Na	Ca	Mg	ESP (%)
A	11.3	14.1	0.070	36.5	0.02	0.005	92
B	10.0	2.14	0.089	3.8	0.02	0.005	85
C	10.0	2.66	0.081	4.9	0.05	0.005	78
D	8.3	4.58	0.007	6.5	3.47	0.013	11
E	8.1	4.56	0.004	6.1	4.37	0.034	8
F	8.0	0.61	0.064	0.30	0.34	0.051	3
G	7.9	0.57	0.027	0.35	0.42	0.056	3

correlation between residue pH and Na content. ESP values for treatments A, B and C are typical for the range reported for bauxite residues 70.4% (Wong and Ho 1994) and 90.9% (Fuller et al. 1982). Bernstein (1974) suggested critical ESP values of 10% for fine, and 20% for coarse textured soils. ESP and SAR values suggested for soils may not be appropriate for mine residues due to other factors such as clay and salinity (Munshower 1994) although the values may serve as a guide. Treatments A, B and C exhibit pH and ESP values above those suggested for plant growth, additionally EC values for treatment A are exceptionally high and are above values for normal plant growth.

Reduction in residue substrate ESP and pH upon application of gypsum have been reported on (Wong and Ho 1994). Findings in the current study show the marked reduction in ESP as a result of the increase in Ca and Mg levels and the accompanying decrease in Na levels. ESP and pH values for treatments D–G are within levels reported for normal plant growth. Slightly higher EC values were recorded for treatments receiving high application rates of gypsum. Wong and Ho (1994) obtained similar levels and expressed concern on the effect of the salinity on sensitive species.

3.1.1 Germination Bioassays

All plant species failed to germinate in treatment A. By day 7, all other treatments had relative seed germination (RSG) of ≥75% with 95–100% achieved in D, E, F and G (Fig. 1). Significant negative correlations were obtained for RSG with pH, EC, Na, SAR and ESP content (Table 3) for all test species.

Delayed or poor seedling emergence occurs in bauxite residue (Wong and Ho 1993; Bucher 1985). Germination in saline and sodic soils is usually inhibited by excess salts, which lower the soil water potential preventing water uptake by plants, and by toxic effects of the ions (Waisel 1972; Bucher 1985). Increasing NaCl concentration in solution decreased final seed germination and germination rate of prosopis and acacia seeds (Catalan et al. 1994; Rehman et al. 1996).

Due to the high EC and Na content exhibited by unamended residue (treatment A) no test species germinated in this treatment. All species germinated

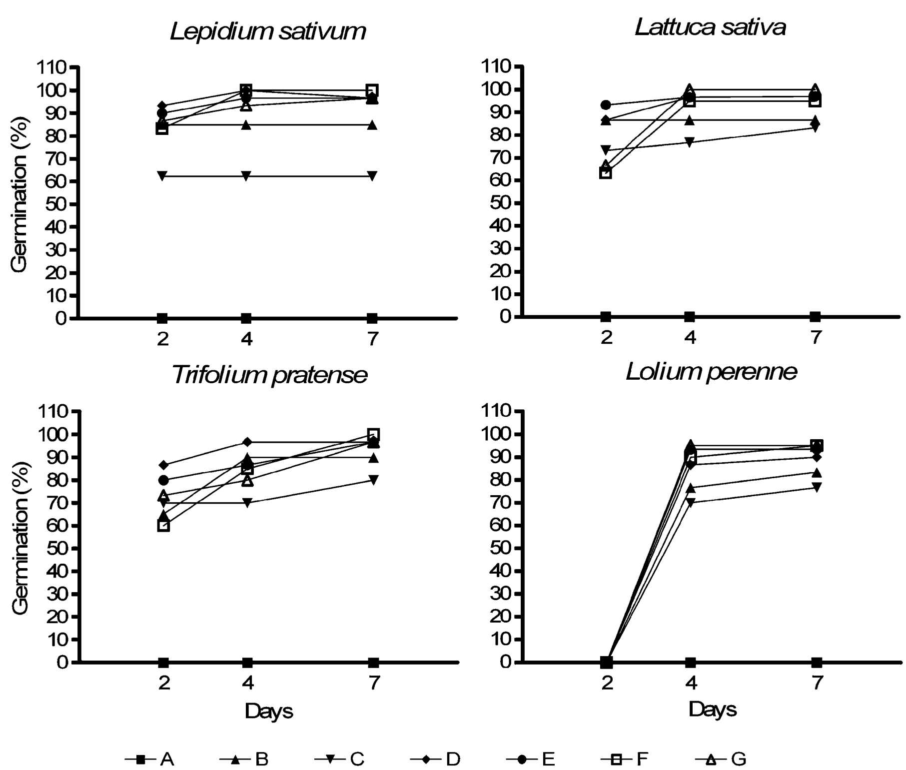

Fig. 1 Relative seed germination (RSG) percentages of four plant species in bauxite residue extract

Table 3 Correlation coefficient values between residue extract properties and germination assays

	pH	EC	Al	Ca	Mg	Na	SAR	ESP
Relative seed germination (RSG)								
T. pratense	−0.582b	−0.868a	−0.062	0.356	0.458	−0.932a	−0.957a	−0.806a
L. perenne	−0.555b	−0.866a	−0.039	0.355	0.464	−0.927a	−0.949a	−0.794a
L. sativa	−0.533b	−0.860a	−0.023	0.345	0.418	−0.932a	−0.966a	−0.775a
L. sativum	−0.560b	−0.896a	0.006	0.314	0.446	−0.958a	−0.983a	−0.795a
Relative root growth (RRG)								
T. pratense	−0.785a	−0.316	−0.581a	0.869a	0.475b	−0.471b	−0.636a	−0.812a
L. perenne	−0.856a	−0.622a	−0.478b	0.610a	0.661a	−0.719a	−0.802a	−0.951a
L. sativa	−0.685a	−0.256	−0.560b	0.871a	0.308	−0.418	−0.596a	−0.728a
L. sativum	−0.890a	−0.431	−0.684a	0.646a	0.654a	−0.536b	−0.641a	−0.890a
Germination index								
T. pratense	−0.631a	−0.183	−0.538b	0.854a	0.293	−0.343	−0.523b	−0.655a
L. perenne	−0.862a	−0.563b	−0.532b	0.591a	0.692a	−0.654a	−0.734a	−0.929a
L. sativa	−0.712a	−0.260	−0.581a	0.881a	0.338	−0.423	−0.600a	−0.752a
L. sativum	−0.901a	−0.424	−0.704a	0.642a	0.670a	−0.527b	−0.632a	−0.897a

Correlation is significant at the 0.01 level (a) or at the 0.05 level (b).

in the other treatments indicating that the high inhibitory levels of EC and ESP content in bauxite residue can be effectively lowered with amendment and leaching to levels that promote germination.

Relative root growth (RRG) decreased over the 7 days for all tested species in treatments B and C with no root development in treatment A (Fig. 2). By day 7 RRG of over 100% was achieved for treatments D and E for all species. High RRG was also obtained in *L. perenne* in treatments F and G, with lower values recorded for the other test species.

Significant negative correlations were obtained for RRG and pH, EC, Al, Na, SAR and ESP content in the residue extract. Conversely, RRG was positively

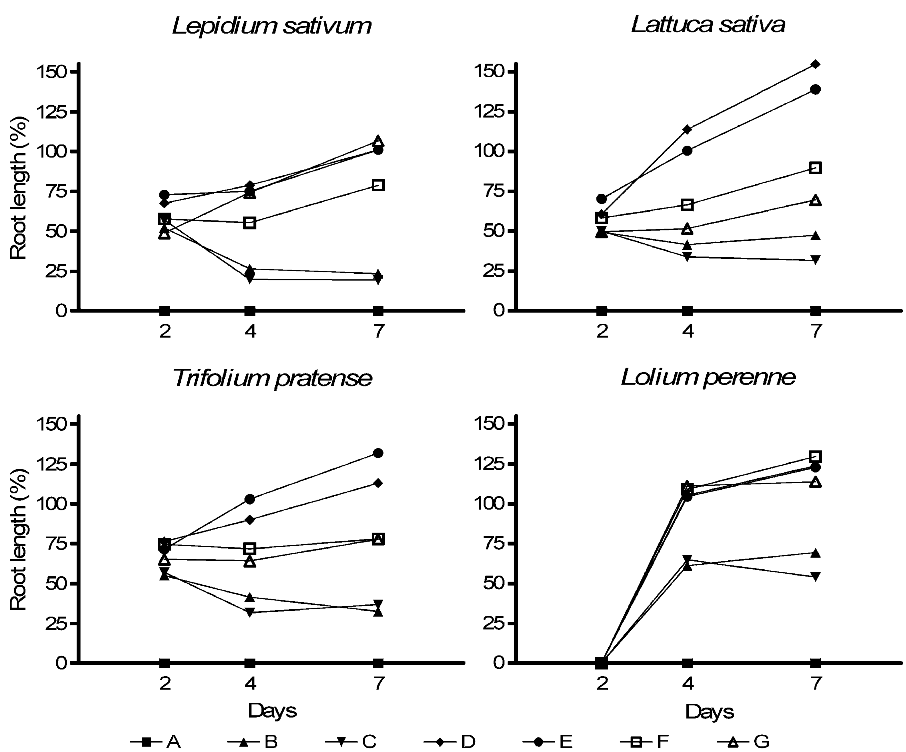

Fig. 2 Relative root growth (RRG) percentages of four plant species in bauxite residue extract

correlated with Ca and Mg content in the residue extract (Table 3).

Seed germination has been regarded as a less sensitive method than root elongation when used as a bioassay for the evaluation of phytotoxicity (Wang and Keturi 1990). Relative root growth was a more sensitive indicator than relative seed germination for evaluating the inhibitory effects of spent litter extract (Tiquia et al. 1996) and metal toxicity (Wong and Bradshaw 1982) on plant growth.

Wong and Ho (1993) found poor seedling emergence of *Agropyron* and *Cynodon* in bauxite residue with no correlation between seedling growth and EC content. Improved seedling emergence in amended residue was likely to have resulted due to the lowering of pH, Na and Al contents and ESP of the soil solution. In the current study, only *L. perenne* root growth was correlated with residue EC. Root development for the four test species was negatively affected by pH, Al and Na content.

Increased levels of Ca and Mg in the residue extract significantly improved RRG for the test species. Supplemental Ca in saline solutions has increased the growth of cotton (*Gossypium hirsutum* L.) (Kent and Lauchli 1985) and tomato (*Lycopersicon esculentum*) (Al-Harbi 1995). Kinraide (1999) reported no inhibition of root elongation in *Triticum* in high Na concentration if Ca content was >2 mM in solution.

Bucher (1985) found no significant correlations for Ca and Mg content in residue with *Agropyron* seedling emergence. Results for the current study are in contrast with this and are possibly due to less tolerant species being used in the study. *Agropyron* is known for its tolerance of salinity and alkalinity (Wong and Ho 1994).

Germination index (GI) was poorest in treatments A–C and optimum results obtained for treatments D and E (Fig. 3). GI at day 7 in treatments B and C were below 40% for all treatments except *L. perenne*, which were 41% and 46% respectively. Lowest GI's were recorded for *L. sativum*.

GI performance was positively correlated with Ca content for all species and Mg content for *L. perenne* and *L. sativum*. Similarly to RRG results, GI was negatively correlated with pH, Al, SAR and ESP (Table 3).

Inhibition of seedling performance ranking, expressed by GI, was treatments A > B = C > D = E = F = G for *L. perenne*. Similar trends were observed for the other species (Fig. 3).

Germination index is the most sensitive parameter that is able to account for low toxicity affecting root

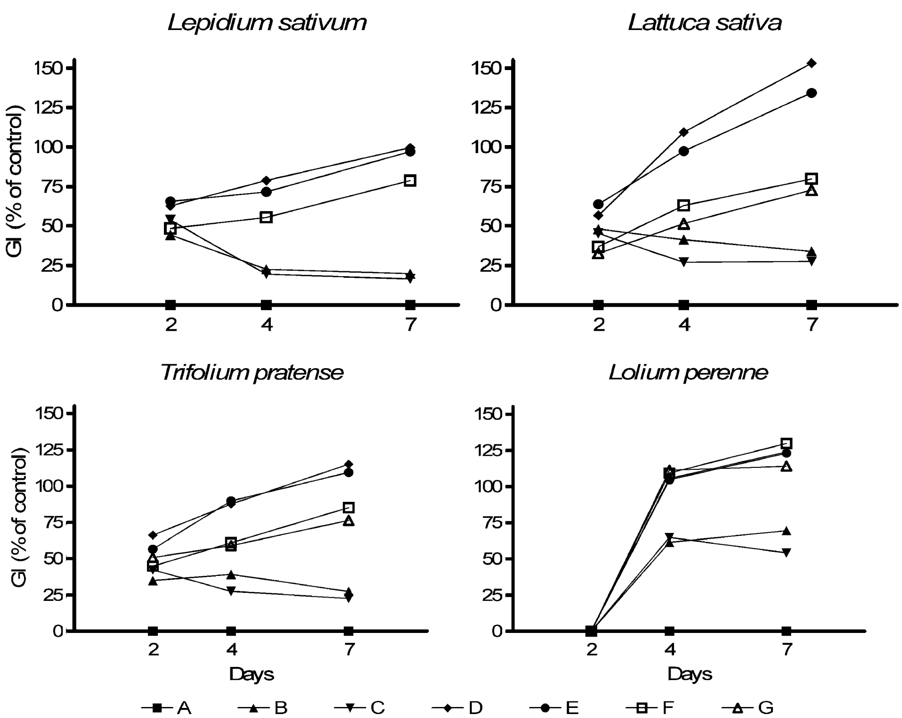

Fig. 3 Germination indices (GI) of four plant species in bauxite residue extract

growth and seed germination (Zucconi et al. 1981). Of the species tested, this shows a greater tolerance for *L. perenne* to grow in bauxite residue following amendment.

GI on day 7 for all species in treatments D, E, F and G were above 80%, indicating the disappearance of phytotoxicty (Zucconi et al. 1981). Treatments D and E had GI's of ≥100% with Ca content positively correlated to GI performance indicating a growth stimulating effect on plant growth.

Lower GI's were recorded for *T. pratense*, which may have importance due to the inclusion of legumes within seeding mixtures for mine waste (Bradshaw and Johnson 1992). *L. perenne* achieved highest GI's for all treatments; *L. perenne* is an important component of seeding mixes used in the BRDA revegetation programme (Courtney et al. 2008) and has a higher salinity tolerance than *T. pratense* or *L. sativa* (Rhoades and Miyamoto 1990).

4 Conclusions

Unamended bauxite reside is characterized by high pH, sodicity and salinity. Without sufficient amendment these properties are inhibitory to seed germination and/or seedling emergence.

Amendment of residue with gypsum and a period of weathering improves seed germination and seedling performance in bauxite residue extract. High Ca content in the residue extract resulted in RRG and GI higher than those achieved in the control treatment.

L. sativum and *L. sativa* are most sensitive to the inhibitory properties exhibited by the residue. *L. perenne* is the most tolerant of the tested species.

Acknowledgements The author would like to thank Aughinish Alumina Ltd. for their financial support for this research. Thanks are also extended to Dr. John Breen of University of Limerick for his assistance with Graphpad Prism.

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