

# Polyacrylate polymers as immobilizing agents to aid phytostabilization of two mine soils

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## Abstract

We evaluated the effect of polyacrylate polymers as immobilizing agents to aid phytostabilization of two mine soils. One soil had a very low pH (3.7) and a large Pb content, while the other was less acidic but had a greater content of Cu and Zn. Growth of perennial ryegrass (*Lolium perenne* L. cv. Victorian) was stimulated in polymer-amended soils. After ryegrass had been growing for 35 days, the amounts of water-extractable Cu, Zn and Pb (one soil only) present in the polymer-amended soils were smaller than those from soil without polymer. The number of culturable heterotrophic bacteria and the activities of dehydrogenase and  $\beta$ -glucosidase increased following polymer application. In contrast, the urease activity was impaired by polymer application, presumably because of the presence of ammonium as a counter ion. In another experiment, the acidic soil was limed to pH 6.5 before growth of perennial ryegrass took place. Liming the soil greatly enhanced plant growth, but by the third cut, differences between treatments became apparent, with plants from polymer-amended limed soil accumulating a greater biomass compared with limed soil without polymer. After ryegrass had been growing for 119 days (five cuts), the amount of water-extractable Pb and the urease activity in the polymer-amended soil were smaller than those from limed soil without polymer. The numbers of culturable heterotrophic bacteria and the activities of dehydrogenase,  $\beta$ -glucosidase and acid phosphatase increased following polymer application. The results are consistent with phytostabilization being achieved by the application of polyacrylate polymers, improving soil chemical and biological properties. In very acidic soils, the use of both a liming material and polymer together appears to give a considerable advantage.

**Keywords:** Amendments, enzymatic activity, perennial ryegrass, soil quality, trace elements

## Introduction

Soil contamination with trace elements has toxic effects on plants and soil organisms and impairs biochemical processes. The addition of various amendments into soils precipitates metals or increases metal sorption, therefore decreasing the proportion of the total element in soil solution. Some of the amendments studied include additives such as lime (Geebelen *et al.*, 2003), organic matter as biosolids, compost and manure (Farfel *et al.*, 2005) as well as industrial products such as zeolites (Friesl *et al.*, 2003), steel shots (Geebelen *et al.*, 2003), birnessite (Mench *et al.*, 2000) and beringite (Boisson *et al.*, 1999). An alternative technique, based on insoluble

polyacrylate polymers, was proposed by Torres & de Varennes (1998). Polyacrylate polymers are based on acrylic acid, and are composed of chains with regularly distributed carboxylic groups:  $(-\text{CH}_2\text{CHCOOH})_n$ .

The degree of ionization of these groups depends on pH, and the negative charges formed are neutralized by  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{NH}_4^+$ .

Large molecular weight insoluble polyacrylates swell to form gels that contain many times their weight in water. They are used in diapers, paper towels and feminine products. It is estimated that over 130 Gg of polyacrylates are used annually in such products (Martin, 1996), and there is therefore a great knowledge on the lack of toxicity of these products.

A previous study showed that the application of polyacrylate polymers to a mine soil heavily contaminated with Pb enhanced growth of orchard grass and improved the quality

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of the soil as evaluated by the activities of dehydrogenase, phosphatase, protease, cellulase and  $\beta$ -glucosidase (Guiwei *et al.*, 2008). This seemed to result from a decrease in the bioavailable Pb and greater water content in amended soil, compared with soil without polymer.

In this work, we investigated whether polyacrylate polymers could be used in two mine soils contaminated with several trace elements. We also compared the use of polymers with or without simultaneous application of lime to one of the soils.

Several parameters related to soil quality can be used to monitor rehabilitation of contaminated sites (Pascual *et al.*, 2000; de Mora *et al.*, 2005; de Varennes & Queda, 2005; Hernández-Allica *et al.*, 2006). In this study, we chose to measure the effects of polymer application on plant biomass accumulation, levels of metals in soil solution, culturable bacteria and fungi, and activities of dehydrogenase and several hydrolytic enzymes. Dehydrogenase is an intracellular enzyme used as an index of overall microbial activity (Nannipieri *et al.*, 2003), while hydrolytic enzymes exist both within viable cells and outside living cells (the assays carried out usually do not distinguish between these two groups). The hydrolytic enzymes tested in the present work are related to the C, N and P cycles and are commonly used to assess soil quality (Gil-Sotres *et al.*, 2005). Their activity represents an integration of the accumulated changes that have occurred as a result of the remediation.

## Material and methods

### Experiment 1

Two different soils were taken from the S. Domingos mine. This is a pyrite mine located in the Iberian Pyrite Belt that was exploited during the XIX and XX centuries for Cu, until exploration was discontinued in 1966.

One soil was less acidic and had a greater content of Cu and Zn (soil 1), while the other had more total Pb and a lower pH (soil 2) (Table 1). Soils were passed through a 2-mm sieve and received a basal dressing of 100 mg N, 100 mg P, 210 mg K and 30 mg Mg per kg of soil. The nutrients were supplied as ammonium nitrate, calcium dihydrogen phosphate, potassium sulphate and magnesium sulphate. In

**Table 1** Characteristics of the soils used in the experiment

	Soil 1	Soil 2
Texture	Sand	Loamy sand
Organic C (g/kg)	6.0	3.3
pH water (1:2.5)	5.2	3.7
Total Cu (mg/kg)	583	288
Total Zn (mg/kg)	1230	85
Total Pb (mg/kg)	7600	33500

treatments with polymers, N and K were omitted from the basal dressing as these were supplied by the counter ions present in the polymers.

Polyacrylate polymers (supplied by Hoechst Marion Roussel Lda) with  $K^+$  (210 mg K per gram of polymer) or  $NH_4^+$  (100 mg N per gram of polymer) as counter ions were added at 0.2% (0.1% of each polymer) to half of the soil.

For each treatment, three replicate pots (upper diameter: 16 cm; height: 14 cm) were filled with 2 kg of each soil. Perennial ryegrass (*Lolium perenne* L. cv. Victorian) was sown and plant number was adjusted to 45 per pot 2 weeks later. The pots were kept in a glasshouse (minimum temperature: 8 °C; maximum temperature: 25 °C) and watered daily. All the pots were supplied with the same amount of water at each irrigation, to maintain 70% of the water-holding capacity of the unamended soil.

The shoots were cut 35 days after sowing, washed with de-ionized water, dried at 65 °C and weighed. The soils were passed through a 2-mm sieve. Fresh sub samples of soil were analysed for culturable heterotrophic bacteria by plating in a 'Nutrient' and yeast extract agar, and for culturable fungi by plating in a malt and yeast extract with rifampicin, according to Pochon & Tardieux (1962), with incubation periods of 48 h at 27 °C for bacteria and 7 days at 27 °C for fungi. Other fresh soil sub samples were analysed for dehydrogenase activity according to Tabatabai (1994). The substrate used in the assay is inhibited by Cu (Chander & Brookes, 1991) and although the results may reflect that effect, the relative values are still valid.

Other soil sub samples were frozen until analysed for several enzymatic activities.

Acid phosphomonoesterase (EC 3.1.3.2) and  $\beta$ -glucosidase (EC 3.2.1.21) were measured by incubating the soil with a substrate containing a *p*-nitrophenyl moiety according to Eivazi & Tabatabai (1977, 1988). Acid phosphomonoesterase (acid phosphatase) catalyses the hydrolysis of organic P esters and  $\beta$ -glucosidase is involved in the final step of cellulose degradation catalysing the hydrolysis of carbohydrates with  $\beta$ -D-glucoside bonds.

Urease (EC 3.5.1.5) was determined according to Kandeler & Gerber (1988). Urease catalyses the hydrolysis of urea to  $CO_2$  and  $NH_3$ .

A sub-sample of the soil from each pot was air-dried and analysed for pH in water (1:2.5) and water-extractable trace elements. Soil samples (40 g) were shaken with 60 cm<sup>3</sup> of de-ionized water for 2.5 h. The extract was filtered through Whatman No. 6 paper and analysed for Cu, Zn and Pb by atomic absorption spectrophotometry.

### Experiment 2

Sieved ( $\leq 2$  mm) soil 2 was limed with 1.5 g  $CaCO_3$  per kg of soil and incubated wet during 2 weeks to reach a pH in

water of 6.5. Limed soil received a basal dressing and polyacrylate polymers with  $K^+$  (210 mg K per gram of polymer) or  $NH_4^+$  (100 mg N per gram of polymer) as counter ions were added at 0.2 % to half of the soil, as before.

For each treatment, three replicate pots (upper diameter: 16 cm; height: 14 cm) were filled with 2 kg of soil, and perennial ryegrass was grown as before. The shoots were cut 35, 49, 63, 77 and 119 days after sowing. After each cut a solution of ammonium nitrate containing 0.1 g N per kg of soil was applied. The plant material was washed with de-ionized water, dried at 65 °C and weighed.

At the end of the experiment the soil was passed through a 2-mm sieve and analysed as before.

### Statistics

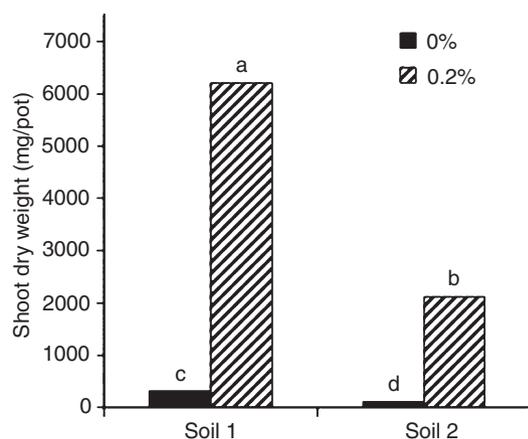
All data were analysed for variance by the General Linear Model (GLM) and mean separation was performed using the Newman-Keuls test at  $P \leq 0.05$ .

## Results

### Experiment 1

Plants from unamended soil grew poorly with thin chlorotic leaves that became progressively necrotic. The incorporation of the polymers into the soil resulted in increased growth of ryegrass. Plant dry weight in both amended soils was 21 times greater compared with biomass of plants from each corresponding soil with no polymer (Figure 1).

The pH increased slightly following polymer application to soil 2, but the polymers had no effect on pH in soil 1 (Table 2). The amounts of water-extractable Cu and Zn present in the unamended soil 1 were 2.8 and 2.0 times greater, respectively, than those from amended soil. The water-



**Figure 1** Shoot biomass of perennial ryegrass grown for 35 days in two mine soils, with or without polyacrylate polymers. Columns with the same letter are not significantly different as estimated by the Newman-Keuls test at  $P < 0.05$ .

**Table 2** Soil pH, number of culturable heterotrophic bacteria and levels of water-extractable Pb, Zn and Cu in two mine soils, with or without polyacrylate polymers, after growth of perennial ryegrass for 35 days

Soil	Level of polymer	pH	Bacteria (CFU*10 <sup>-4</sup> per gram)	Trace elements (mg/kg)		
				Pb	Zn	Cu
1	0%	5.2a	261b	<0.1	37a	0.4a
1	0.2%	5.2a	1174a	<0.1	13b	0.2b
2	0%	3.8c	1d	25a	0.8c	0.2b
2	0.2%	4.0b	3c	16b	0.4d	0.1c

Values in a column followed by the same letter are not significantly different as estimated by the Newman-Keuls test at  $P < 0.05$ . CFU, colony forming units.

extractable Pb was below the detectable limit in this soil. In amended soil 2, water-extractable Pb was reduced to 63% and Cu and Zn to 50% of the amounts present in unamended soil (Table 2).

At the end of the experiment, the acid phosphatase activity was similar in both soils and treatments (1.45 mmol *p*-nitrophenol per gram DW soil per hour), while the activity of dehydrogenase increased at least 60% (Figure 2a) and that of  $\beta$ -glucosidase at least 46% following polymer application (Figure 2b). In contrast, the urease activity was lower in both soils with polymer than in unamended soils (Figure 2c).

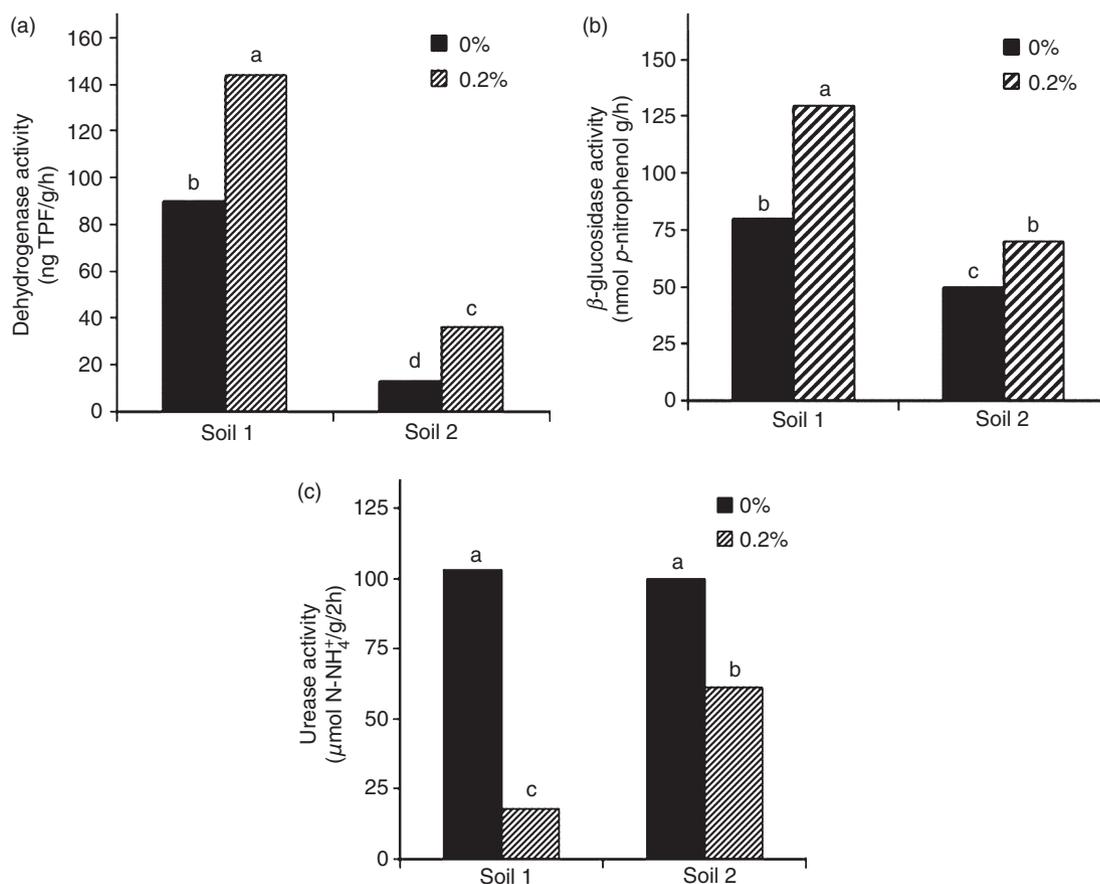
The numbers of culturable bacteria were very small in soil 2 compared with soil 1, but in both cases the numbers were greater in amended soil than in soil without polymer (Table 2). The treatment had no effect on numbers of culturable fungi (32000 and 58000 colony forming units per gram of soils 1 and 2, respectively).

### Experiment 2

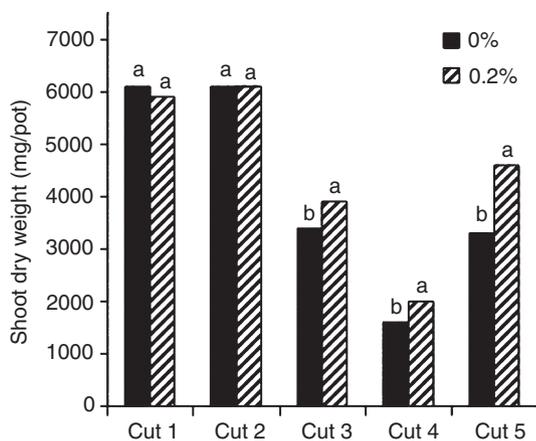
Plants from both treatments (limed soil with or without polymer) accumulated a similar biomass in cuts 1 and 2, but later on plants grew better in polymer-amended soil. By cut 4, plant dry weight in polymer-amended limed soil was 40% greater compared with biomass of plants from soil with no polymer (Figure 3).

After plant growth for 119 days, the amount of water-extractable Pb present in the unamended limed soil was 1.8 times greater than that from polymer-amended soil (Table 3). The water-extractable Cu and Zn were similar in both treatments (0.1 mg/kg soil).

The activity of dehydrogenase increased 60%, that of  $\beta$ -glucosidase at least 33% and that of acid phosphatase 82%, following polymer application to limed soil (Table 3). In contrast, the urease activity was again smaller in soil with polymer than in unamended soil (Table 3).



**Figure 2** Activities of dehydrogenase (a),  $\beta$ -glucosidase (b) and urease (c) in two mine soils, with or without polyacrylate polymers, after growth of perennial ryegrass for 35 days. Columns with the same letter are not significantly different as estimated by the Newman-Keuls test at  $P < 0.05$ . TPF, triphenylformazan.



**Figure 3** Shoot biomass in five cuts of perennial ryegrass grown in a limed mine soil, with or without polyacrylate polymers. For each cut, columns with the same letter are not significantly different as estimated by the Newman-Keuls test at  $P < 0.05$ .

The numbers of culturable bacteria were greater in polymer-amended soil than in limed soil without polymer (Table 3). The treatment had no effect on numbers of cul-

**Table 3** Soil pH, number of culturable heterotrophic bacteria, levels of water-extractable Pb, and enzymatic activities in a limed mine soil, with or without polyacrylate polymers, after growth of perennial ryegrass for 119 days

Soil characteristics	Level of polymer	
	0%	0.2%
pH	5.0a	5.1a
Bacteria (CFU* $10^{-4}$ per gram)	143b	371a
Pb (mg/kg)	3.4a	1.9b
Dehydrogenase ( $\mu\text{g TPF}$ per gram per hour)	149b	239a
$\beta$ -glucosidase (nmol p-nitrophenol per gram per hour)	90b	120a
Urease ( $\mu\text{mol N-NH}_4^+$ per gram 2 per hour)	117a	71b
Phosphatase ( $\mu\text{mol p-nitrophenol}$ per gram per hour)	0.49b	0.89a

CFU, colony forming units; TPF, triphenylformazan.

Values within a line followed by the same letter are not significantly different as estimated by the Newman-Keuls test at  $P < 0.05$ .

turable fungi (65000 colony forming units per gram of soil).

## Discussion

Natural background concentrations of Cu, Zn and Pb in soils range from 2–200, 10–300 and 10–200 mg/kg, respectively (Srivastava & Gupta, 1996). Soil 1 can therefore be considered as contaminated with Pb, Cu and Zn, and soil 2 contaminated with Pb and Cu.

To examine the use of polymers for land rehabilitation, it is important to try to differentiate its different effects on plant growth and soil quality. Hydrophilic polymers may enhance plant growth by increasing the water-holding capacity of the soil (Boatright *et al.*, 1997; Johnson & Piper, 1997; de Varennes *et al.*, 1999) and by supplying the counter ions present (Silberbush *et al.*, 1993; de Varennes *et al.*, 1999). To make sure that plant growth would not be stimulated by increased supply of K and N, the same amount of these nutrients was applied to the unamended soil as present in the polymer added to the amended soil. We assumed that the nutrients in the polymers had similar bioavailability, compared to those directly applied to soil, as already shown in previous work (de Varennes *et al.*, 2006). We also excluded the possibility of enhanced plant growth in amended soil being explained by additional water supply by the polymer because all the pots were supplied with the same amount of water throughout the experiment. As water was supplied daily, the plants never suffered from water stress. This contrasts with the experiment of Guiwei *et al.* (2008) where water was applied according to the water-holding capacity of each amended soil.

The polymers slightly increased the pH of the very acidic soil 2, presumably by exchange of counter ions with acidic ions in soil solution. This may have contributed to a smaller bioavailability of the metals in experiment 1; however, soil pH was not the main factor responsible for the differences observed in plant growth and soil quality due to polymer application, as the polymers had no effect on the pH of soil 1 and of limed soil 2.

A plant cover could not be established in the unamended unlimed soils as plants were necrotic 35 days after sowing. The incorporation of the polymers in both soils protected plants from excessive levels of the metals. As a result, plant growth in polymer-amended soils was significantly increased, compared to the original soils.

The polymers used had a similar effect on plants grown in both soils, suggesting that they can be applied to aid phytostabilization of mine soils contaminated with a broad range of trace elements. This extends the results of the previous study, where Pb was the main contaminant present (Guiwei *et al.*, 2008).

Soil 2, with a lower pH and more bioavailable Pb, exhibited a greater toxicity to plants and micro-organisms than

soil 1, which contained more Cu and Zn. It is well known that Pb can cause significant damage to plants and soil biota if present in large amounts (Broos *et al.*, 1999; Guiwei *et al.*, 2008), and that a very acid pH greatly limits plant growth. It was therefore pertinent to test if liming soil 2 to pH 6.5 could substitute for polymer application. A pH of 6.5 was chosen as the target acidity for the amended soil because this is the optimum value for most crops (Alloway, 1995) and it is commonly recognized that, at this pH, nutrient availability is at a maximum while the likelihood that the presence of many elements at toxic levels is at a minimum (Wong, 2003).

Plant growth was greatly stimulated in the limed soil. The accumulated biomass after 35 days of growth was more than 60 times greater than the dry weight of plants from unlimed soil without polymer, and three times greater than the dry weight of plants from polymer-amended unlimed soil (Figures 1 and 3). Nevertheless, by cut 3 (63 days after sowing) the additional positive effect of polymer application became apparent and by 119 days after sowing plant dry weight in polymer-amended limed soil was 40% greater compared with biomass of plants from limed soil without polymer. There are several possible explanations for this effect. First, increasing soil pH could actually enhance the sorbing effect of the polymer, as it increases the ionization of the polymer chains, and thereby facilitates the formation of bonds with trace elements. Second, plant exudates tend to acidify the soil, partially neutralizing the effect of lime. Third, it is known that controlling metal availability by liming is insufficient in sites containing pyrite as these have the potential to generate further acidity (Scullion, 2006). The fact that polymer application complements the effect of lime is an important result, as the latter has a more immediate effect and the polymer extends the period available for the establishment of a plant cover.

Most soil enzymes are inhibited by high levels of trace metals (Kandeler *et al.*, 1997; Huang & Shindo, 2000; Belén-Hinojosa *et al.*, 2004). Very little is known on the effect of soil amendments on soil micro-organisms and enzymatic activities, except when organic matter is concerned. Applying compost, biosolids or other organic amendments will naturally increase microbial populations and the activities of dehydrogenase and hydrolytic enzymes, as these amendments supply organic C to soil organisms (de Mora *et al.*, 2005; Sastre-Conde *et al.*, 2007; Renella *et al.*, 2008).

De Mora *et al.* (2005) reported an increase in the activities of dehydrogenase, arylsulphatase and  $\beta$ -glucosidase following application of sugar beet lime (70–80% CaCO<sub>3</sub>) to a heavy-metal contaminated soil, showing that an increase in soil pH can enhance biological indicators of soil health.

In the present experiment, liming the very acidic soil enhanced the activity of dehydrogenase and the number of culturable heterotrophic bacteria, compared with unlimed soil (data not shown), but applying polymer to limed soil led to the greatest values for these indicators. The establishment of a plant cover (that produces root exudates) and the lower

concentration of trace elements in soil solution should contribute to a more favourable environment for soil micro-organisms, and stimulate the activity of soil enzymes. Polymer application enhanced the activity of  $\beta$ -glucosidase, in both limed and unlimed soil, suggesting that increased production of root exudates stimulated hydrolytic enzymes related to the C cycle. It had no effect on the activity of acid phosphatase in experiment 1, with unlimed soil and plant growth for only 35 days, but its activity was enhanced in polymer-amended limed soil after perennial ryegrass had been grown for 119 days. These results agree with those reported by Guiwei *et al.* (2008) and confirm that the main reason for enhanced enzymatic activity and greater number of culturable bacteria is the decrease in bioavailable pools of trace elements.

As in Guiwei *et al.* (2008), the urease activity was impaired by polymer application in the present experiments, presumably because one of the polymers had ammonium as counter ion. In unamended soil, the basal dressing with ammonium nitrate, although providing the same amount of total N, supplied only half in the  $\text{NH}_4^+$  form.

It is not easy to choose the most appropriate remediation method for a particular site. In the case of severely contaminated soils, a vegetation cover needs to be established to prevent wind erosion and reduce raindrop impacts, thereby protecting the soil from structural degradation that stimulates surface run-off, while it also increases soil water deficits that will decrease run-off and leaching of both beneficial and toxic elements. The application of amendments becomes necessary for the development of the plant cover that will also improve the physical, chemical and biological properties of these soils.

Application of composts or biosolids is common. They may initially increase plant growth by providing nutrients and adsorbing metals, but after degradation of the organic matter the metals will again become available to plants. They might even increase the bioavailability of some metals (Clemente *et al.*, 2003). In contrast, polyacrylate polymers are more stable in soils, although they can be slowly degraded by white-rot fungi (Cameron & Aust, 1999).

Zeolites have also been used for *in situ* remediation of metal-contaminated soils, but they can adversely affect soil structure and therefore the application rates have to be small (Geebelen *et al.*, 2002). Liming is not sufficient in many pyrite soils, as already discussed above. Given the limitations of each amendment, the best results will probably be obtained with a combination of several substances. For example, Legg & Ledésert (2001) used an organo-zeolite to remediate a metal-contaminated soil. As shown in the present work, polymers can be applied simultaneously with liming materials in very acidic soils, further improving the quality of soils and potentially allowing the establishment of a plant cover over a period of at least four months.

The main advantage of polyacrylate polymers over other amendments is that they seem to provide a microcosm, rich in water and nutrients, and with small concentrations of

trace elements, where roots and micro-organisms can proliferate. In fact, the polymer particles in amended soil were completely penetrated and enmeshed with roots. This means that the effects observed in bulk soil are probably only a poor representation of the conditions in the rhizosphere, as polymer particles may act as screens around roots. This is supported by the fact that a polyacrylate polymer did not decrease water-extractable Cu in a Cu-contaminated vineyard soil in the absence of plants. Plant exudates solubilized Cu and polymer particles competed with plants for Cu uptake (de Varennes & Torres, 1999).

In the present work we used analytical grade  $\text{CaCO}_3$  and polymers that were synthesized at our request. Once it is established that liming with simultaneous application of polyacrylate polymers can be used to remediate mine soils contaminated with trace elements, alternative sources of liming materials and polymers can be used. Commercial polyacrylate polymers cost about 2 Euros per kilogram, corresponding to 4 Euros per tonne of soil when a rate of 0.2% is used. Agricultural lime at the rate applied will add a few cents to that cost. Although the total value is still substantially smaller than that of conventional engineering approaches, which cost at least 40 Euros per t of soil (Cunningham & Ow, 1996), cheaper alternatives should be sought when extensive areas of land are considered.

Sugar beet sludge has been successfully used instead of commercial lime to correct acidity of a pyrite-derived soil (Alvarenga *et al.*, 2008). A coarse-textured lime could be obtained as a by-product from quarries. This would act as a slow release source of carbonate.

Many diapers contain polyacrylate polymers and these could become a potential local source of polymers at zero cost. The use of diapers to remediate mine soils is presently under investigation.

## Conclusions

We have shown that the application of insoluble polyacrylate polymers, alone or in combination with lime, allowed the establishment of a plant cover in two mine soils contaminated with Zn, Cu and Pb and enhanced soil quality. Together with previous results dealing with a long-term Cu-contaminated vineyard soil (de Varennes & Queda, 2005) and a Pb-contaminated mine soil (Guiwei *et al.*, 2008), it supports the view that the application of insoluble polyacrylate polymers provides a new method for *in situ* remediation of sites contaminated with trace elements.

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