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Organzo-zeolitic treatment of mine waste to enhance the growth of vegetation

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Abstract: Zeolitic tuff containing an appreciable abundance of clinoptilolite was composted with animal waste to produce a dry friable non-odorous material that can easily be mixed with soil to produce an amended soil substrate. In earlier work it was found that leachate samples taken from organzo-zeolitic treated substrates had nitrate concentrations an order of magnitude greater than those without the amendment. Comparative analysis of separate batches of organic- soil mixtures had shown that those containing zeolitic tuff had a very much higher concentrations of nitrate, in their leachate samples, than those containing only animal manure. Clinoptilolite has an ion-exchange property that is highly selective towards ammonium and it appears that the clinoptilolite component in adsorbing and ion-exchanging ammonia, as it is liberated from the decomposing animal waste, “buffers” its loss from volatilization and leaching. As a result, ammonium oxidizing micro-organisms are stimulated, which leads to enhanced nitrification and increasing ionic mobility of the substrate pore water. These functions allow plant growth to be stabilized on acid mine waste in such a way as to favour luxuriant growth on such sites, which normally are devoid of vegetation. The chemical analysis of plant leaf and stem tissue has identified trends in nutrient uptake and an attempt is made to understand the mechanisms responsible for this behaviour.

Key-words: clinoptilolite, aqueous leachate, ammonia, nitrification, nutrient uptake.

1. Introduction

Earlier research work had shown that organzo-zeolitic bio-fertilizers, when used as soil amendments, considerably enhance plant growth (Minato, 1968; Leggo, 2000) and in this respect this paper gives an example of its use on barren mine waste sites. Evidence is now available which strongly indicates that the presence of the zeolite mineral increases the activity of nitrifying and other micro-organisms which are beneficial to plant growth (Andronikashvili *et al.*, 2008).

Referred to as open framework silicates, zeolites are characterised by pore structures that form continuous channels throughout the mineral lattice. The pore channels can be present in one, two or three dimensions and it is the pore architecture that is most specific to individual zeolite minerals (Tsitsishvili *et al.*, 1992). The basic building unit is a tetrahedral arrangement of oxygen atoms with a silicon or aluminium atom at its centre. Again, in all such minerals, the substitution of aluminium for silicon produces a charge discrepancy which is satisfied by the adoption of an extra cation.

In nature, zeolite pore structures always contain water which behaves in a way to complete the coordination of the extra-framework cations and minimise the electrostatic

repulsion between the framework oxygen atoms. Cations, diffusing into the water filled pore space, are only loosely held in the anionic framework and some are easily ion-exchanged. Among the most selective cations is the ammonium ion NH_4^+ which has an ion-exchange behaviour that is reversible (Ames, 1967) and can be used, indirectly, to provide a source of available nitrogen to plants.

In our work the preferred approach is to charge clinoptilolite with NH_4^+ ions by composting a mixture of crushed zeolitic tuff and animal wastes (Minato, 1968). The fermentation yields NH_4^+ ions, some of which are ion-exchanged into the zeolite pore structure or adsorbed to the zeolite surface. The analysis of aqueous leachates show that soils amended with the organzo-zeolitic mixture have nitrate concentrations two orders of magnitude higher than those without the amendment (Leggo, 2000). The lattice bound NH_4^+ ions were shown to decrease exponentially over time and that most of these ions had left the zeolite within 2 months; presumably oxidized by nitrifying soil microbes. After eighty days from the time of entering the soil environment the nitrate levels in aqueous leachates, taken from the amended soil, had increased considerably (Leggo & Ledésert, 2009). The hypothesis put forward is that when an organzo-zeolitic material is added to soil, ammonium ions on the zeolite surface are available for

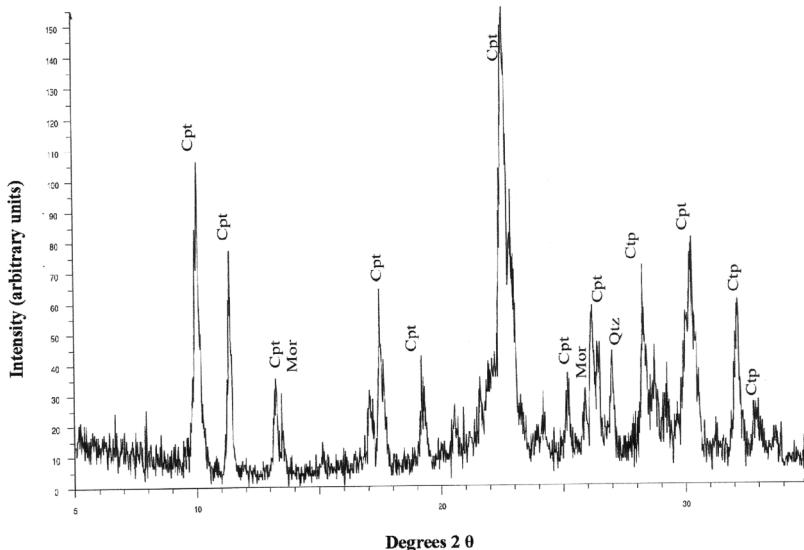


Fig. 1. X-ray diffraction data ($\text{CuK}\alpha 1$) of the Beli-Plast zeolitic tuff.

Note: Peaks are labelled Cpt for clinoptilolite, Mor for mordenite and Qtz for quartz.

1 nitrification. As a result, nitrate and protons are produced
2 as end-products of the microbial reaction; the protons
3 reacting with the substrate porewater to cause intensive
4 ionic mobilization. Due to this reaction and other microbial
5 activities, occurring in the rhizosphere, both cations and
6 anions are made available to the plant root for uptake.

7 In this study particular attention has been given to the
8 growth and sustainability of plants grown on phytotoxic
9 mine waste as soil contamination, due to the discard of
10 metalliferous waste from mining, metallurgical and other
11 contiguous industries, poses a most difficult environmental
12 problem. As chemical elements these pollutants cannot be
13 reduced to less harmful forms unlike the phytoremediation
14 and biodegradation of many anthropogenic xenobiotic sub-
15 stances (Burken, 2003; Hutchinson *et al.*, 2003). In this
16 respect the use of an organo-zeolitic amendment is now
17 emerging as an important tool to improve the phytostabil-
18 ization of mine-waste sites in order to alleviate the constant
19 problem of transport of toxic particles by aeolian dispersal
20 and aqueous leaching (Brooks *et al.*, 1998; Tordoff *et al.*,
21 2000; Mendez & Maier, 2008). A successful approach to
22 phytostabilisation must consider not only the problem of
23 phytotoxicity, caused by elevated metal concentrations,
24 but also the general unavailability of plant nutrients, the
25 abnormal soil structure and highly stressed microbial com-
26 munity, all of which add to the difficulty of sustaining plant
27 growth in such hostile substrates (Southam & Beveridge
1992; Moynahan *et al.*, 2002; Mendez *et al.*, 2007). Mine
28 waste deposits often exhibit acidic characteristics that are
29 known to have a detrimental effect on nitrification (Paul &
30 Clark, 1996). However, a review of this topic (De Boer &
31 Kowalchuk, 2001) reveals that soil acidity alone does not
32 exclude the activity of nitrifying micro-organisms and it is
33 accepted that nitrification can occur over a wide range of
34 acid soils. Perhaps more important is the presence of auto-
35 trophic iron and sulphur-oxidizing bacteria which, in

acidic sulphide tailings, dominate the microbial commu-
nity and lead to plant death (Schippers *et al.*, 2000).

37
38 Although there is no unique solution to the problem of
39 environmental damage, caused by abandoned mine waste
40 sites, it well known that the formation of a protective
41 vegetative cover limits the distribution of wind blown
42 particles and leaching. In this work we attempt to show
43 that by utilizing the mineral properties of natural zeolite a
44 biofertilizer can be easily prepared that, acting as an
45 amendment, enables plant growth to be sustained on
46 waste containing high metal concentrations.
47

2. Starting materials and experimental methods

2.1. Source and properties of the zeolitic tuff used in this work

The zeolitic tuff used in this work comes from an extensive
51 open-cast mining operation in the classic type locality at
52 Beli-Plast, Bulgaria. The rock has a fine grained texture, a
53 light colour and is composed mostly of zeolitized glass
54 shards with few quartz grains and occasional crystals of
55 biotite, presumably of pyroclastic origin. It is of mineralog-
56 ical interest to note that the Beli-Plast zeolitic tuff, like
57 many others, retains the original outlines of the glass shards,
58 which form a large part of the assemblage. Rocks of this
59 type are found worldwide, commonly occurring as thick
60 deposits of volcaniclastic sediment (Mumpton, 1973).

This rock, used in previous studies (Leggo, 2000; Leggo
62 & Ledésert, 2001; Leggo *et al.*, 2006), has been charac-
63 terised by X-ray powder diffraction (XRD) and scanning
64 electron microscopy (SEM). For the XRD analysis, the
65 sample was crushed to a fine powder and further ground
66 to below $10\ \mu\text{m}$ to improve powder statistics. The sample
67 was run on a Bruker D8 diffractometer with a primary
68

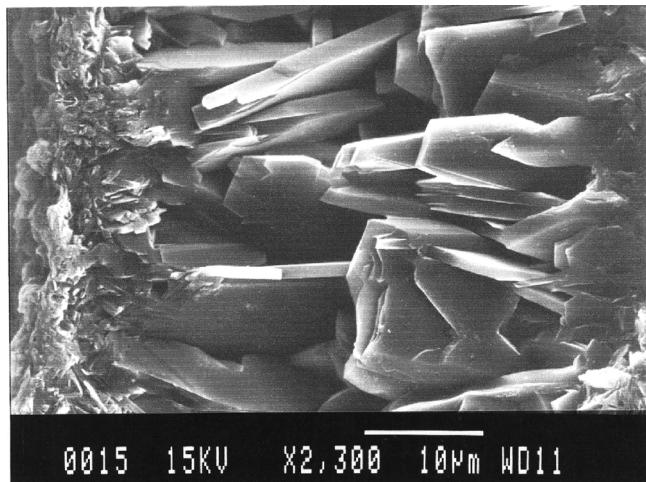


Fig. 2. SEM image of part of a zeolitized glass shard. Notice exterior coating of clay minerals. The inner part of the shard is altered to clinoptilolite with opal C-T as an accessory mineral.

beam monochromator that enabled $\text{CuK}\alpha_1$ radiation to be used. The recording was made at a step scan of $0.02^\circ(2\theta)$. In the range from 5° to $34^\circ(2\theta)$ 12 peaks occur, which correspond to clinoptilolite reflections. A discrete peak was recorded at $26.6^\circ(2\theta)$ which is taken to be due to the presence of quartz. Clay minerals, mostly montmorillonite and celadonite are seen to coat the surface of the shards (Burlet, 1997) and, as shown in Fig. 2, the remaining glass has been altered to highly crystalline clinoptilolite, of monoclinic habit. Very similar alteration textures were found in zeolithic tuffs from the Maramures Basin, Romania (Cochemé *et al.*, 2003) where micro-beam studies showed the clay mineral coating is a mixed assemblage varying from montmorillonite to muscovite.

It has been suggested that the clay coating is developed in acidic conditions during the transport of glass in pyroclastic ash clouds before deposition (Leggo *et al.*, 2001). The zeolitized tuffs are thought to accumulate below water, as in many cases they are associated with shallow marine faunas. On entering water the ash would be in a neutral or slightly alkaline environment which would favour the further alteration of the glass to tectosilicate. It is thought that once in the aqueous environment the bulk of the glass alters to zeolite (Cochemé *et al.*, 2003).

As in most cases zoning of the zeolite mineralogy occurs vertically and laterally in the Beli-Plast deposit, which is thought to be due to the different conditions under which glass alteration occurs; both the depth of water and the pH conditions playing a dominant role. A description of the geology and mineralogy of the Bulgarian zeolitized tuff is reported in the scientific literature (Alexiev & Djourova, 1988).

The rocks used in this study contain predominantly clinoptilolite with K and Ca as the predominant extra-framework cations, as estimated by scanning electron microscopy (SEM) involving energy dispersive X-ray spectrometry (EDS). The instrument used was a Jeol 820 SEM with a Link Systems EDS. The clinoptilolite abundance of the tuff is estimated to be between 75 and 85 vol%

with mordenite only occurring in trace amounts. The mean composition of the clinoptilolite, based on 72 oxygen atoms, is:



where n is in the order of 22 (Burley, 1997).

Measurements of the Cationic Exchange Capacity (CEC) for NH_4^+ , made in our laboratory using the method of Minato (Minato, 1994), were found to vary from 92 ± 7 to 200 ± 9 meq/100 g. The variation of the CEC value is thought to be mainly due to the nature of the samples used, as these were rock particles of 0.5–2.0 mm in diameter rather than an homogenous finely ground powder. It was decided to use this particle size range as it is representative of the material used in the preparation of the bio-fertilizer.

2.2. Preparation of the organo-zeolitic biofertilizer

The organo-zeolitic amendment (biofertilizer) was prepared by composting a mixture of crushed zeolitic tuff with chicken manure in a ratio of one part zeolitic tuff to two parts manure, by volume. This is best achieved in a closed vessel that can be heated. Water is added, at about 5 vol% and the mixture is rotated regularly. This procedure will allow the mixture to be thoroughly aerated, under which conditions ammonifying micro-organisms quickly produce ammonia. After a few days the temperature of the fermenting mixture increases to a maximum of about 50°C after which the temperature falls to ambient air temperature. To sterilize the mixture the content of the reactor is raised to 70°C after fermentation has ceased. On cooling to ambient air temperature the mixture is ready to use, however it is advisable to allow the mixture to out-gas volatiles that produce a malodour and is best handled in a dry condition that occurs after a few weeks.

2.3. Waste site location, description and characteristics of the mine waste

Acidic metalliferous mine waste from an abandoned site at Fron Goch in Central Wales, approximately 14 statute miles South East of Aberystwyth at UK Ordnance Survey grid reference 272314, 274405UK, has been used. Sites of this nature are common in this part of mid-Wales, as in the 19th and 20th centuries the area was extensively mined for base metal sulphides. Since 1984 the northern section of the site has been used as a sawmill with waste material being dumped in the northern open-working. In the centre of the main site, covering a total area of several hectares, is a shallow flat-bottomed depression that appears to have been used previously as a tailings lagoon. The material used in this study, is a grey to green fine grained silt, and was taken from this area using a bucket and spade to collect approximately 25 kg. The sample site chosen was close to that sampled by the British Geological Survey (BGS) and the analyses of As, Cd, Pb and Zn concentrations of the waste used in this study were found

Table 1. Element concentrations (mg kg^{-1}) of the Fron Goch mine waste used in this study.

Al	As	B	Ba	Ca	Cd	Co
35120 ± 12	9.34 ± 14	25.1 ± 27	175 ± 8	158 ± 14	1.23 ± 28	8.10 ± 91
Cr	Cu	Fe	K	Li	Mg	Mn
23.9 ± 19	19.1 ± 9	19692 ± 6	9069 ± 12	165 ± 12	3140 ± 19	206 ± 10
Mo	Na	Ni	P	Pb	S	Se
$<7.46 \pm \text{n/a}$	1890 ± 13	13.3 ± 16	133 ± 7	4345 ± 11	$1446 \pm \text{n/a}$	$<7.46 \text{ n/a}$
Sr	V	Zn				
25.0 ± 23	43.5 ± 8	1049 ± 10				

1 to be very similar to those quoted in the BGS Report
 2 (Palumbo & Klinc, 2002). Replicate analyses of the mine
 3 waste pore-water gave a pH measurement of 4.0 ± 0.02 . A
 4 chemical analysis of the mine waste used in this study is
 5 given in Table 1.

6 Relatively high concentrations of Fe, Pb and Zn were
 7 found to be present together with high concentrations of K
 8 and Na which are not typical of sulphide waste. It was later
 9 found that other waste, deposited on top of the sulphide
 10 waste, had a pore-water pH value of 8.2 but this overlying
 11 material was not used in the present work. However, there
 12 remains the possibility that alkalis may have been trans-
 13 ported, in some way, into the underlying sulphide waste.
 14 But, for the purpose of this work the origin of its chemical
 15 composition is unimportant.

2.4. Plant types and substrate compositions

17 The plants used in this study were *Salix viminalis* (Ossier
 18 Willow) and *Brassica napus* (Oil Seed Rape). *S. viminalis*
 19 was grown from cuttings and planted into 4 kg (13 cm soil
 20 depth) substrates containing one plant per pot and *B. napus*
 21 was grown from seed in 1 kg (8 cm soil depth) substrates
 22 and thinned to four plants per pot. These plants were
 23 chosen for their metal tolerability and high biomass pro-
 24 duction in soils (Hammer *et al.*, 2003., Marchiol *et al.*,
 25 2004). It was of interest to know how these plants would
 26 behave in untreated and treated metalliferous mine waste
 27 to define their usefulness for phytoremedial purposes. Both
 28 plants, in each substrate, were replicated three times.

29 The garden soil used was from the University of
 30 Cambridge Botanic Garden and can be described as a dark
 31 grey clayey silt containing fine to medium sand together
 32 with some fine to medium sized flint gravel. In this area
 33 glacial alluvial sands and silts overly gravel beds composed
 34 of chalk and flint. Cultivation has left a top soil lacking in
 35 structure and the calcareous nature of the underlying rocks is
 36 preserved in the soil due to the relatively low rainfall of the
 37 area. The content of soil organic matter was not measured
 38 but from close inspection appears to be very low.

39 The substrates used to compare plant growth, are
 40 defined as follows:

- 41 S.1 Untreated, un-polluted, garden soil (Leggo, 2000).
- 42 S.2 Untreated mine waste.
- 43 S.3 Mine waste amended with 16.7 vol% organo-
 44 zeolitic mixture.

45 S.4 Mine waste amended with 16.7 vol% organo
 46 zeolitic mixture + 100 wt% excess un-ammoniated
 47 zeolitic tuff. It should be noted that in a former
 48 study this substrate was shown to enhance growth
 49 above that of the S.3 substrate (Leggo *et al.*, 2006).

50 As the mine waste has a pore-water pH of close to 4.0 it was
 51 thought necessary to increase the pH to a value of about 6.5
 52 to protect nitrifying micro-organisms from the acidic con-
 53 ditions. Both mine waste and amended mine waste sub-
 54 strates were thus modified by the addition of 5 g of CaCO_3 /
 55 kg substrate to achieve this value.

2.5. Sampling of plant material

56 The plants were harvested at different times depending on
 57 growth stage. *B. napus* was harvested after 3.5 months
 58 whereas *S. viminalis* was harvested after 12 months. Shoots
 59 were cut at 2 cm above the surface of the substrate. The plant
 60 material was washed thoroughly in water until it was con-
 61 sidered that all traces of the substrate had been removed and a
 62 final washing in de-ionized water was performed before
 63 drying. Dry weights were recorded after the plant material
 64 (leaves + stems) was dried at 70°C for 24 h. After weighing,
 65 the dry plant material was crushed in an agate mortar to a fine
 66 homogenous powder before analysis.

2.6. Chemical analysis of mine waste and plant 69 material

70 The analysis was made in the analytical geochemistry
 71 laboratory of the British Geological Survey where a sam-
 72 ple of the waste was milled to $<50 \mu\text{m}$ under ultra clean
 73 conditions prior to analysis. To obtain the total major and
 74 trace cation concentrations this sample was digested in a
 75 PTFE tube with a mixture of hydrofluoric, perchloric and
 76 nitric acids. The analyses were made with a Varian/Vista
 77 AX CCD simultaneous Inductively Coupled Plasma –
 78 Atomic Emission Spectrometer with a dedicated Varian
 79 SPS-5 Autosampler. The instrument views the plasma
 80 along its axis and is equipped with a high resolution echelle
 81 polychromator with a 70,000 pixel charge coupled device
 82 (CCD) detector with continuous wavelength coverage
 83 from 167 to 785 nm. Combined uncertainty is expressed
 84 as relative standard deviation (RSD) together with a con-
 85 centration range of standards used to calculate uncertainty.

The (RSD) varied between 6 and 28 %. No reference data were available for B or Li, so uncertainty was estimated assuming a 10 % error in recovery. It was not possible to estimate the uncertainty associated with Mo, S and Se.

The plant material was measured at the Department of Earth Sciences using an ICP-MS technique. Samples in the order of 100 mg were carefully weighed into teflon (PTFE) tubes and digested on a hot block, in ultra clean nitric acid (69 wt%) over night and were treated with hydrogen peroxide (30 wt%) for a further 12 h (Rodushkin *et al.*, 1999). Element concentrations were corrected for the reagent blanks which were several orders of magnitude below those of the measured concentrations and in this respect were considered to be negligible.

Analyses were carried out on a Perkin Elmer Elan DRC II and calibrated using standard plant materials, Peach, Tomato, Ryegrass and Spinach; achieving an internal precision of 2–5 %. Accuracy is seen, from the multiple analysis of the Spinach Standard Material, to be within 5–15 % of the values of the certified elements. The % Relative Standard Deviation (RSD) of the repeats is typically 5–10 % within each batch run.

Detection limits for Cd, As, Co, Ni are respectively; 12, 100, 15 and 300 ng g⁻¹. These were calculated as three times the blank to give minimum detectable levels (MDL).

Shoot samples, from plants growing in triplicate, in their respective substrates were analysed separately for 20 elements; Al, As, B, Ca, Cd, Co, Cs, Cu, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr and Zn. The median values of the concentrations (mg kg⁻¹) are reported together with their relative standard deviations (Table 3a, b).

2.7. Substrate aqueous leachate analysis

Due to the fact that the substrates were watered regularly to maintain a pore-water potential that would allow the microbial community to function it should be realised that the substrates unavoidably loose nitrate and other ions. Thus, the pH and EC measurements are “apparent” rather than the values that would have been obtained if regular watering, allowing free draining conditions, had been omitted. However, as the leaching was controlled in respect to the amount of water regularly added, when required, to keep the substrates moist, it is thought that comparisons of these measurements made between the respective substrates are valid. Aqueous leachates were collected systematically from the substrates (S.1) to (S.4) by watering with de-ionized water to field capacity after which a further 100 ml of de-ionized water was added and a sample was collected for the analysis of pH and electrical conductivity (EC). All samples were centrifuged before measurement to remove any substrate particulate matter. Leachate samples from each of the triplicate substrates were measured in this way and standard deviations are reported for each set. A Cranwell CR95 digital pH/mV meter using an Amagru S5 electrode was used to make the pH measurements and EC was measured with an Electronics Instrument Ltd., conductivity meter Model MC-1, mark 5.

3. Results

3.1. Aerial growth of plants in un-amended and amended substrates

Both the *B. napus* and *S. viminalis* were sustainable in the mine waste. However, in both cases the growth enhancement in the amended mine waste, relative to plants grown in untreated mine waste, was very large. Plants growing in the untreated mine waste have stunted growth and leaves that show clear signs of chlorosis. The opposite is true of plants growing in the amended substrates, see Fig. 3a, b, as these have much taller stems and much larger, green to blue-green, leaves which is characteristic of plants receiving an adequate nutrient supply.

In relation to plants growing in the unamended mine waste (S.2), the difference in dry weight, of the aerial parts of the plants, growing in the amended mine waste (S.3), is three to four times greater in the case of the *S. viminalis* and over thirty times greater in the case of *B. napus* (Table 2). If the zeolitic tuff content is increased by 100 %, by the addition of un-ammoniated tuff, a smaller but significant increase in plant growth also occurs; further addition has little or no effect.

3.2. pH and EC measurements of the leachates

The pH values of the leachate from the amended mine waste substrates (S.3 and S.4), shown in Table 2, are seen to be a little lower than those of the garden soil (S.1) and mine waste (S.2). The lower values are thought to result from active nitrification as the ensuing enzyme reactions produce protons which, in reacting with the soil porewater, to form hydronium ions (H_3O^+) would tend to decrease the leachate pH values.

Differences in the EC of leachates from un-amended and amended substrates are found to be very large. In the case of *S. viminalis* the leachate from unamended garden soil



Fig. 3a. Growth differences in *B. napus*. Plants growing in mine waste (left) and organo-zeolitic amended mine waste (right).



Fig. 3b. Growth differences in *S. viminalis*. Plant growing in mine waste (left) and organo-zeolitic amended mine waste (right).

(S.1) and the unamended mine waste (S.2) have values of 123 ± 8 and $124 \pm 12 \mu\text{S cm}^{-1}$, whereas those of the amended substrates (S.3 and S.4) are over an order of magnitude higher at 3520 ± 726 and $3498 \pm 295 \mu\text{S cm}^{-1}$ respectively. The same difference occurs in the case of *B. napus*. The EC leachate values from substrates (S.1) and (S.2) are 236 ± 45 and $228 \pm 85 \mu\text{S cm}^{-1}$ whereas those from (S.3) and (S.4) have values of 7650 ± 2933 and $7183 \pm 1898 \mu\text{S cm}^{-1}$. The high variability of the measurements is thought to be due to the heterogeneous nature of the substrates. Although the replicated measurements gave large standard deviations, close to 40 % in some cases, the very large difference between un-amended and amended substrates is clear.

3.3. Chemical analysis of aerial growth

In this study the chemical analysis of bulk leaf and stem was initially performed on the two plant species to measure the

degree of uptake of the base metals, Cu, Zn, and Pb together with As, Cd, Co and Ni, as these elements are known to have detrimental effects on the biosphere (Fudge, 2005). The number of elements analysed was subsequently increased to 20 to include those essential and beneficial to plant growth. Analyses of leaves and shoots from plants growing in both unamended and amended substrates were conducted on both plant species, see Table 3a, b. It is of interest to note that in the majority of cases the concentration of a particular element found in plants growing in the amended mine waste trend towards those of plants growing in the garden soil. In the case of *S. viminalis* the elements seen to decrease in plants growing in the amended substrates, relative to those growing in the mine waste, are: Al, As, B, Co, Na, Ni, Pb, and Si; whereas those seen to increase are: Ca, Cd, Cs, Cu, K, Mg, Mn, Mo, P, S, Sr, and Zn. Whereas with *B. napus* the elements involved behave somewhat differently. Elements seen to decrease their concentrations are: Al, As, Cd, Co, Cs, Cu, Ni, P, Pb, S, Si, Sr and Zn. Elements that increase are: Ca, K, Mo and Na. Examples illustrating these trends in both plant species are shown in Fig. 4.

Inspection of Table 3a, b reveals that, in the case of *B. napus*, such trends are exhibited by 71 % of the elements analysed and in *S. viminalis* these trends are shown by 90 % of the elements analysed.

4. Discussion

4.1. Effect of the organo-zeolitic amendment on nitrification

The phenomenon of enhanced plant growth in a soil amended with an organo-zeolitic mixture has been known for over a decade. During this time experimental work has indicated that microbial oxidation of zeolite bound NH_4^+ ions, by nitrifying soil micro-organisms, is responsible for greatly increasing the ionic mobility of the substrate porewater, as during the second stage of nitrification, in which nitrite is oxidized to nitrate, protons are released (Paul & Clark, 1996) that in a wet soil would react with the soil pore water to form hydronium ions (H_3O^+). These ions would be expected to be rapidly neutralized by oxy/hydroxy complexes and polymers present in the soil so liberating cations, as is the case with Al and Fe (Süsser & Schwertmann, 1991). If nitrification is responsible for the high ionic mobility found, and it is difficult to see what other mechanism could be involved, the EC of the aqueous

Table 2. Bulk plant (leaves and stems) dry weight; pH and EC of substrate leachates.

Substrate	<i>Salix viminalis</i>			<i>Brassica napus</i>		
	Weight (g)	pH	EC	Weight (g)	pH	EC
S 1.	14.5 ± 0.7	7.5	123 ± 8	5 \pm 3.1	8.0	236 ± 45
S 2.	10.9 ± 0.6	8.0	124 ± 12	0.6 \pm 0.1	7.7	228 ± 85
S 3.	37 ± 4	7.7	3520 ± 726	18.5 ± 1.4	6.9	7650 ± 2933
S 4.	45.5 ± 5	7.7	3498 ± 295	19.9 ± 2.2	6.8	7183 ± 1898

Note: EC units ($\mu\text{S cm}^{-1}$). Analytical variation expressed as standard mean deviation, $n = 3$.

Table 3a. Element concentrations (mg kg^{-1}) in *B. napus* leaves.

	B	Na	Mg	Al	Si	P
S.1	54.1 ± 3.4	444 ± 8.7	3996 ± 178	34.3 ± 19.6	1311 ± 241	5004 ± 342
S.2	22.7 ± 2.5	392 ± 72.8	3340 ± 260	2900 ± 150	2491 ± 16.3	8504 ± 255
S.3	33.5 ± 3.1	1537 ± 173	3603 ± 172	21.3 ± 10.2	889 ± 100	2658 ± 95.1
S.4	16.5 ± 0.87	548 ± 91.1	1265 ± 21.8	8.6 ± 3.5	922 ± 119	2428 ± 60.3
	S	K	Ca	Mn	Co	Ni
S.1	4273 ± 336	27323 ± 911	26182 ± 1114	29 ± 2.3	0.16 ± 0.01	0.82 ± 0.06
S.2	7719 ± 192	8007 ± 885	6595 ± 364	63 ± 0.6	0.74 ± 0.02	2.8 ± 0.12
S.3	5643 ± 279	18912 ± 630	13521 ± 700	78 ± 3.6	0.24 ± 0.01	1.2 ± 0.06
S.4	3632 ± 245	15044 ± 530	9639 ± 84.9	45 ± 1.2	0.08 ± 0.01	0.34 ± 0.03
	Cu	Zn	As	Sr	Mo	
S.1	2.8 ± 0.12	47 ± 1.0	0.85 ± 0.07	154 ± 2.65	18 ± 1.1	
S.2	35 ± 0.23	795 ± 40.3	0.72 ± 0.04	31 ± 1.0	0.4 ± n.d.	
S.3	3.5 ± 0.05	43 ± 1.6	0.22 ± 0.01	14 ± 1.0	2.1 ± 0.10	
S.4	3.0 ± 0.64	39 ± 1.4	0.05 ± n.d.	8.3 ± 0.60	1.4 ± 0.04	
	Cd	Cs	Pb			
S.1	0.21 ± 0.01	0.04 ± 0.01	0.86 ± 0.08			
S.2	1.7 ± 0.03	0.79 ± 0.01	3219 ± 9.22			
S.3	0.34 ± 0.01	0.16 ± 0.01	5.10 ± 0.66			
S.4	0.44 ± 0.02	0.16 ± n.d.	3.53 ± n.d.			

Note: Analytical variation expressed as in Table 2.

Table 3b. Element concentrations (mg kg^{-1}) in *S. viminalis* leaves.

	B	Na	Mg	Al	Si	P
S.1	83 ± 8.1	989 ± 89	1689 ± 215	158 ± 52.7	953 ± 113	3808 ± 253
S.2	90.2 ± 10.6	1575 ± 258	2626 ± 176	296 ± 40.6	1019 ± 37.3	3203 ± 525
S.3	83.7 ± 17.5	656 ± 14	3126 ± 201	139 ± 27.7	878 ± 30.3	4991 ± 328
S.4	76 ± 12	623 ± 33	2903 ± 741	145 ± 60.2	943 ± 23.0	4215 ± 650
	S	K	Ca	Mn	Co	Ni
S.1	4561 ± 734	18352 ± 2373	14436 ± 801	104 ± 7.1	0.41 ± 0.02	0.92 ± 0.09
S.2	3815 ± 363	16796 ± 2878	9597 ± 1317	80 ± 6.0	0.34 ± 0.06	0.95 ± 0.09
S.3	5955 ± 377	20636 ± 3798	20444 ± 3564	297 ± 77	0.26 ± 0.07	0.62 ± 0.12
S.4	5937 ± 152	16953 ± 2255	21218 ± 3557	311 ± 88	0.20 ± 0.05	0.71 ± 0.14
	Cu	Zn	As	Sr	Mo	
S.1	5.6 ± 0.22	95.8 ± 11.8	0.22 ± 0.10	76.8 ± 4.75	3.00 ± 0.64	
S.2	4.1 ± 0.50	50.2 ± 10.5	0.38 ± 0.04	59.1 ± 4.39	0.63 ± 0.08	
S.3	6.7 ± 0.30	322 ± 64.4	0.15 ± 0.04	108 ± 20.5	1.6 ± 0.24	
S.4	7.6 ± 0.53	282 ± 79.3	0.11 ± 0.04	90.2 ± 27.6	2.4 ± 0.77	
	Cd	Cs	Pb			
S.1	1.2 ± 0.19	0.03 ± 0.01	6.12 ± n.d.			
S.2	0.35 ± 0.08	0.06 ± 0.01	30.8 ± 14.6			
S.3	1.7 ± 0.35	0.13 ± 0.01	16.8 ± 6.90			
S.4	1.5 ± 0.36	0.12 ± 0.01	8.13 ± 3.25			

Note: Analytical variation expressed as in Table 2.

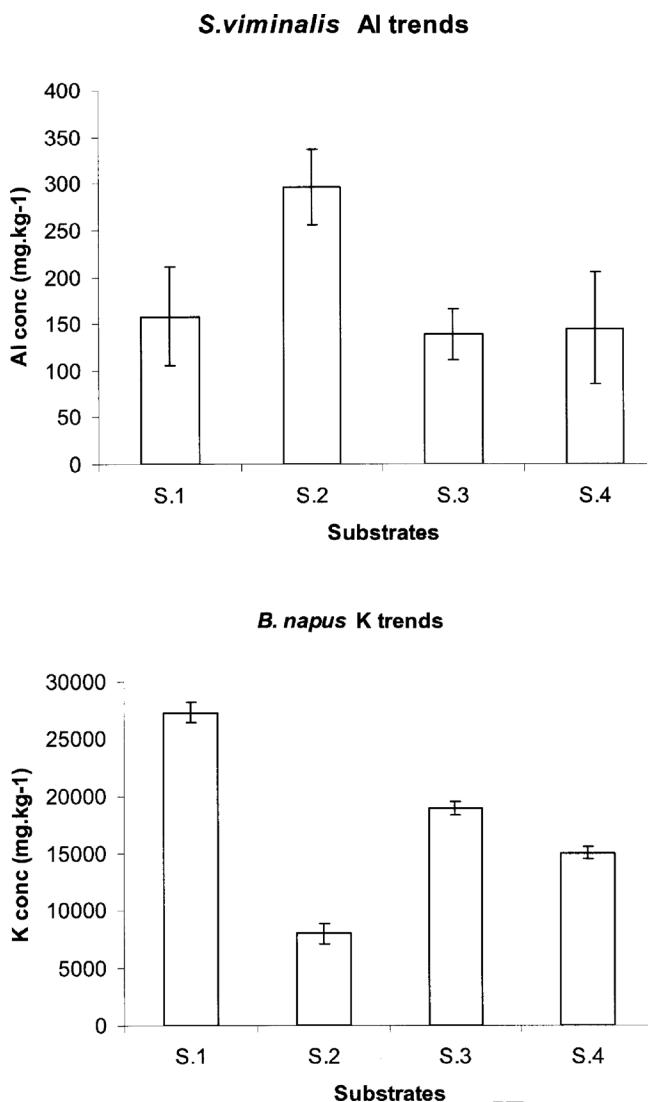


Fig. 4. Example of two typical uptake trends.

leachates would be expected to bear a relationship to the concentration of nitrate ions in the leachate samples; such a linear relationship has now been found (Leggo & Ledésert, 2009).

The difference in plant growth and quality is obvious from the pictorial representations shown in Fig. 3a, b. In both the garden soil and untreated mine waste leachates the EC values were one order of magnitude lower than those of the amended substrates. Thus, the relationship between plant growth and the EC of the respective leachates suggests that the ionic mobility of the soil pore water has a direct bearing on the supply of nutrients. However, this feature cannot entirely account for the availability of all nutrients as other studies have shown the importance of the reaction of root exudates with soil organic material by which chelation of elements makes them available for plant uptake (Brady & Weil, 1996). In the present study chemical analysis of the root systems were not performed as it was considered very difficult to remove all traces of the substrate mine waste which would have caused serious error.

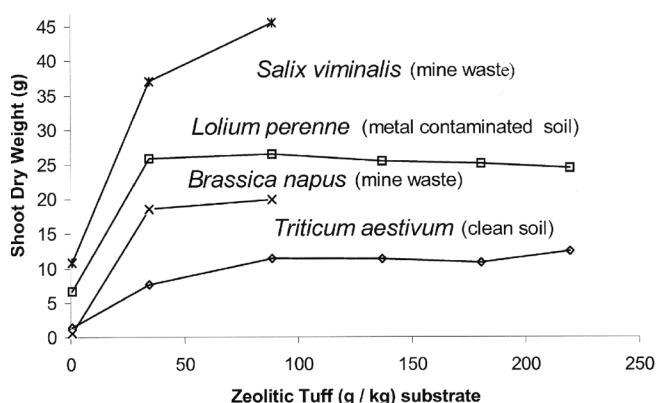


Fig. 5. Growth trends expressed as dry weight of bulk plant, leaf and stem, growing in organo-zeolitic amended substrates.

Note: *B. napus* and *S. viminalis* data is reported in this study. Data for *T. aestivum* and *L. perenne* is from Leggo *et al.* (2006).

4.2. Plant uptake trend

Plant uptake of mineral elements, in the ionic form, is a much studied topic (Marshner, 1995; Mengal & Kirkby, 2001) which together with plant physiology and biochemistry forms the basis of plant nutritional studies and it is well known that nutrient uptake is commonly reflected in a plant's shoot chemistry. The analytical results of the current study show pronounced differences in shoot chemistry between plants growing in the substrates amended with an organo-zeolitic mixture (S.3 and S.4) and those growing in the substrate without amendment. As the performance of the plants growing in the amended substrates far exceeds those growing in the unamended substrates it is logical to assume that the ionic mobility of the substrate porewater is a governing factor in the uptake trends and resulting growth behaviour.

As already mentioned an excess of zeolitic tuff when added to the amendment causes an additional increase in plant growth (Leggo *et al.*, 2006). This effect is shown, to various degrees in Fig 5, by four plant species two of which are reported in our earlier research (Leggo *et al.*, 2006). The dry weight measurements in the current study also show that plants growing in the substrates amended with the organo-zeolitic mixture plus excess zeolitic tuff exhibit the greatest growth. Assuming that the nitrification hypothesis is correct it appears that the extra zeolitic tuff would provide additional surface area for the collection of NH_4^+ ions and in so doing would maximise nitrification; although the factors which cause this activity are, at present, unknown. However, it has also been suggested that in the case of the heavy metals Pb, Zn, Cd and Cu, zeolite cationic exchange prevents access of these ions to a plant (Damian & Damian, 2007) and is thus a benefit to its growth.

Although it appears that the accessibility and degree of uptake of the chemical elements present are controlled, to a large extent by the ionic mobility of the substrate porewater, it is well known that the supply of plant nutrients depends on many variable functions operating in the plant rhizosphere. In this study no mention has been made of the source of

1 phosphorus and sulphur which are essential for plant
 2 growth. The inspection of Table 3a, b shows that phosphorus
 3 uptake in *B. napus* shoots decrease in plants growing in the
 4 amended substrates whereas the reverse is true in the case of
 5 *S. viminalis*. This observation is not understood and apart
 6 from saying that the organic component of the amendment
 7 would undoubtedly be the source of this element and that
 8 mycorrhizal fungi and bacteria are thought to play an impor-
 9 tant role in its mineralization and solubilization (Richardson
 10 *et al.*, 2009) no more can be said at this time.

11 The ecology of the plant rhizosphere has recently been the
 12 subject of several detailed reviews. Subjects such as soil
 13 biophysics and biochemistry (Hinsinger *et al.*, 2009), soil
 14 microbial diversity (Buée *et al.*, 2009) and interaction
 15 between soil micro-organisms and plant growth (Lambers
 16 *et al.*, 2009) point to the complexity of the plant rhizosphere
 17 and reveal how much still remains unknown. In this respect
 18 our work remains ill defined but the presence of the organo-
 19 zeolitic material results in nutrient elements being made
 20 available for plant uptake and it is the unique mineralogical
 21 properties of the zeolite mineral that makes the organo-zeo-
 22 litic-soil system viable.

23 5. Conclusions

24 This study has confirmed the growth behaviour of plants in an
 25 organo-zeolitic amended mine waste that was observed in
 26 earlier work (Leggo & Ledésert, 2001). The enhanced growth
 27 and quality of the plants appears to occur, to a large extent, in
 28 response to an increase in nitrification that takes place in such
 29 substrates. In this study we report the observed nutrient uptake
 30 trends in the two different plant species but make no claims in
 31 understanding the phenomenon other than to say that our
 32 experimental data show a high soil pore-water ionic mobility,
 33 apparently induced by the stimulation of nitrifying micro-
 34 organisms in the amended substrates. The high degree of
 35 ionic mobility of the soil pore water, that develops in the
 36 amended substrates, appears to allow plants under phytotoxic
 37 stress to regulate their uptake. As a result of this behaviour
 38 growth is enhanced and plants species, that otherwise would
 39 not be sustainable in the phytotoxic conditions of the metalli-
 40 ferous waste, can survive. Provided attention is paid to the pH
 41 of the untreated waste and the amended substrate is kept near
 42 neutral by an application of lime, to allow the soil nitrifying
 43 and other micro-organisms to function, it is foreseen that
 44 many mine waste sites could be re-vegetated by a surface
 45 amendment of an organo-zeolitic mixture without the addi-
 46 tion of a clean soil overburden or other treatment. It is hoped
 47 that new studies will reveal further details that will increase
 48 our understanding of this behaviour.

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