# The stimulation of nitrification in an organically enriched soil by zeolitic tuff and its effect on plant growth

Article · January 2009

2 authors:

Peter James Leggo
University of Cambridge
32 PUBLICATIONS   549 CITATIONS

SEE PROFILE

Beatrice Anne Ledesert
Université de Cergy-Pontoise
59 PUBLICATIONS   1,040 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

The Soultz sous Fprêts Enhaced Geothermal System View project

Geotref View project

# The Stimulation Of Nitrification In An Organically Enriched Soil By Zeolitic Tuff And Its Effect On Plant Growth.

Leggo, P.J* and Ledésert, B**

*Department of Earth Sciences, University of Cambridge, UK
**Departement des Sciences de la Terre et l'Environnement, Université de Cergy-Pontoise, France

The adsorption and ion-exchange properties of natural zeolite minerals such as phillipsite, clinoptilolite and mordenite are well studied and these microporous minerals are known to have a high selectivity towards the ammonium ion. Natural zeolites are often found as alteration products of volcanic glass, in deposits of volcaniclastic sediment. In such rocks they are very abundant and commonly exceed 80 percent of the mineral assemblage. Due to their high selectivity towards ammonium ions, rocks containing these minerals can be used to adsorb ammonia from sewage and organic farm waste. It has now been found that a composted mixture of the crushed zeolitic tuff and organic waste will enhance nitrification in soils, when added as an amendment. During composting ammonia, derived from the biological degradation of the organic waste, is adsorbed and ion-exchanged by the zeolite minerals present in the mixture. When added to a soil, nitrification occurs as a result of oxidation of the ammonium ions, first to nitrite and then further to nitrate, together with the production of protons. These reactions were studied in a time-course experiment using the analysis of aqueous leachates taken from untreated and amended soil substrates. Nitrification trends were observed and a linear relationship was found between the electrical conductivity (EC) and the nitrate concentration of the leachates; demonstrating how the ionic mobility of the substrate porewater increases with increase in the degree of nitrification. A comparison of plant growth in substrates without zeolitic tuff with those amended with the organo-zeolitic mixture, showed that plant growth in the amended soil is greatly increased.

## Introduction

Zeolite minerals found in volcaniclastic sedimentary deposits are known world wide and many papers have been published in the scientific journals on their occurrence, petrology and mineralogy [1 – 5]. Studies of the ion-exchange properties of zeolite minerals have shown that the ammonium ion $NH_4^+$ is highly selective in several of the commonly occurring natural zeolites [6, 7]. In this respect, rocks rich in clinoptilolite, have found use in removing ammonia from sewerage and organic farm waste [8]. It has since been found that crushed rock, containing an abundance of clinoptilolite, when mixed and composted with poultry manure will adsorb and ion-exchange ammonium ions produced during the biological degradation of the manure [9]. This work lead to a series of experiments in which an organo-zeolitic mixture was used to amend a neutral to slightly alkaline soil [10].This work was further extended to phytotoxic soils containing mine and refinery waste [11,12].

Organic fertilizers in the form of animal manures and composted plant waste are vulnerable to the loss of ammonia from volatilization and leaching, [13] and it is now clear that the addition of a zeolitic tuff will buffer the system to such losses. On the addition of an organo-zeolitic mixture to soil, having a porewater pH of between approximately 5.5 and 8.5, under aerobic conditions, ammonium oxidizing micro-organisms produce nitrite which is further oxidized to nitrate together with the production of protons [14].

In this paper a time-course experiment provides empirical evidence of the stimulation of nitrification in organo-zeolitic amended soils. An additional experiment was made to investigate the behaviour of ammonium ions on the clinoptiolite surface and a test was made for the presence of nitrifying micro-organisms in the amended substrate. Evidence of the effect of the amendment on plant growth is shown by *Brassica napus* grown under laboratory controlled conditions. Reference is also made to other growth studies involving a variety of plant types.

# Experimental Section

The ograno-zeolitic mixture was made by composting one volume of zeolitic tuff with two volumes of poultry manure. The zeolitic tuff came from a deposit of zeolitic tuff at Beli-Plast, Bulgaria that is rich in the zeolite mineral clinoptiolite; the average formula of the unit cell, based on 72 oxygen atoms, is ($K_{1..32}$ Ca $_{2.03}$ Na $_{0.4}$ Mg $_{0.32}$ Fe $_{0.05}$) [Al $_{6.6}$ Si $_{29.4}$ O$_{72}$] n.H$_2$0 [15]. The rock is typical of zeolitic tuffs, containing clinoptilolite, in many parts of the World.

Composting was carried in a rotating drum under warm wet conditions. The fermenting material generally reaches a temperature of between 50 to 60 °C, after which the temperature declines. When the temperature declines to ambient air temperature the mixture is ready for use. To prepare an amended soil substrate the mixture is added to soil in the ratio of one part mixture to 5 parts soil. The amendment used for substrate SM, defined below, was made by mixing one volume of Garden soil with two volumes of poultry manure. It should be noted that in this respect the zeolitic tuff was replaced by an equal volume of Garden soil.

The time-course experiment and plant growth studies were performed under controlled greenhouse conditions using soil from the Botanic Garden, University of Cambridge. The soil at the Garden is derived from alluvial sand and silts from underlying beds of gravel, chalk and flint. The top soil is essentially structureless owing to a long history of cultivation and the characteristic low rainfall of the area has preserved its calcareous nature. This soil can be described as a dark grey clayey silt containing fine to medium sand together with some fine to medium flint gravel. The pH values of aqueous extracts from the garden soil were found to vary between 7.0 and 8.0.

The substrates used for the experiments are designated as follows;

GS.  Garden soil.

SM. Garden soil amended with a mixture of soil and poultry manure, as stated above.

OZS. Garden soil amended with a mature organo-zeolitic mixture made more than three years previously.

FOZS. Garden soil amended with freshly made organo-zeolitic mixture.

Each substrate was prepared in triplicate using 1 litre pots standing in plastic dishes to collect aqueous leachates; care was taken to thoroughly wash the dishes before sample collection.

The collection of the leachate samples is dependant on a systematic treatment involving regular watering to maintain the water potential of the substrates to sustain soil nitrifying micro-organisms [16]. This approach was adopted rigorously and although leachate is unavoidably lost, from the free draining pots, it is thought that the samples can be used for the comparison of pore-water from the respective substrates. Thus, the samples collected under identical conditions, were taken at intervals over a period of 180 days. At the time of collection the substrates were watered to field capacity, using de-ionized water, after which a 100 ml was taken from each substrate for analysis. The

sampling of each substrate was made in triplicate. All samples were centrifuged before measurement to remove any soil particulate matter. Blank analysis of the de-ionized water showed it to contain less that 0.10 mg.L$^{-1}$ of any of the nitrogen species analysed.

The pH and EC of each triplicate leachate sample was measured and standard deviations were calculated. A Cranwell CR 95 digital pH/mV meter using an Amagru S5 electrode was used to make the pH measurements and EC was measured with an Electronics Instruments Ltd., conductivity meter Model MC-1, mark 5.

To provide a large enough sample for the analysis of the nitrogen species, each triplicate set was bulked. Ammonium nitrogen was determined by colorimetric analysis using its reaction with alkaline hypochlorite and phenol to form an indolphenol coloured complex. The nitrite determination is based on the formation of a diazo compound between nitrite and sulphanilamide. This compound is coupled with N-l-Napthylethylenediamide dihydrochloride to give a red azo dye which is then quantified spectrophotometrically. Total Oxidized Nitrogen (TON) is determined by reducing nitrate to nitrite using a cadmium reactor followed by the formation of the red azo dye with any nitrite present. The nitrite and reduced nitrate are both measured as TON and nitrate is determined by subtraction of the nitrite from the TON values. As a much used standard procedure the concentrations have been measured to an accuracy of 9 per cent.

Apart from the time-course experiment the FOZS substrate was also used to examine the substrate for the presence of nitrifying micro-organisms. The method applied employs acetylene to inhibit ammonium mono-oxygenase, the enzyme that catalyses the ammonium oxidation reaction [17]. The acetylene was industrial compressed gas as supplied by the British Oxygen Company. 12.5 ml was taken by hypodermic syringe from a rubber bladder filled from the gas cylinder and injected into a 200 ml flask, through a rubber stopper, containing 20g of the FOZS substrate, designated FOZS-A, moistened with de-ionized water. The same amount of moist FOZS substrate, designated FOZS-B, was placed in a similar 200 ml flask, under air, and used as the control sample. The samples were held in these conditions for 30 days after which both samples were separately leached with 20 ml of de-ionized water. The leachates were then measured for pH, EC ($\mu$S. cm$^{-1}$), and $NH_4^+$, $NO_2^-$ and $NO_3^-$ concentrations (mg. L$^{-1}$).

In order to measure the concentration of $NH_4^+$ ions absorbed to the zeolite surface, samples of zeolitic tuff were separated from the organo-zeolitic mixture by rapidly washing and settling in a water column. The difference in density between the organic material and the zeolitic tuff enabled a clean separation after a few washings. The sample was then dried at 70 °C and any extraneous material removed by hand picking. Repetition of this technique over many years, in our laboratory, has shown that measurable loss of $NH_4^+$ does not occur as a result of the washing procedure. The analytical method adopted involves the ion-exchange of lattice bound $NH_4^+$ ions by $K^+$ ions achieved by repeated centrifuging at 3000 rpm in a 10% KC1 solution [18]. A selective ammonium ion electrode , manufactured by Russell (model 95-5 129) together with an Orion 720A ion meter, were used to make the concentration measurements. Calibration standards were prepared from Analar NH$_4$CI in the range of $10^{-1}$ to $10^{-4}$ M $NH_4^+$. Concentrations are expressed as m.mol $NH_4^+100g^{-1}$ zeolitic luff and standard mean deviations were calculated from quadruplicate analyses.

# Results and analysis

Aqueous leachate samples, taken after thirty days, showed little difference in pH but a very large difference in their EC. The EC of the leachate from the sample under the acetylene-air mixture, FOZS-A, was found to be 160 $\mu$S.cm$^{-1}$, whereas that taken from the sample under air, FOZS-B, was 1,500 $\mu$S.cm'. This result indicated that the pore-water of the sample under air had undergone

considerable ionic mobilization suggesting that nitrification had been active. Analysis of the nitrogen species revealed that the nitrate concentration of the leachate from the sample under air (139 mg.L$^{-1}$) was an order of magnitude greater than that of the sample under the acetylene-air mixture (16.8 mg.L$^{-1}$). It was also found that the ammonium concentration of the leachate from the sample under air (5.00 mg .L$^{-1}$) was far lower than that of the leachate from the sample under the acetylene-air mixture (30.6 mg.L$^{-1}$). These results demonstrate that the ammonia in the substrate under air had been substantially oxidized. By comparison it is clear that nitrification has occurred in the sample under air and, although not totally inhibiting the activity of the ammonia mono-oxygenase, the presence of the acetylene had a most noticeable effect. It is therefore apparent that nitrification takes place in the organo-zeolitic amended substrate, under aerobic conditions.

The time-course experiment, investigating the aqueous leachate chemistry of garden soil and garden soil with organic and organo-zeolitic amendments, was conducted over a 180 day period. Leachates samples taken from the garden soil, had a value of 3.40 mg.L$^{-1}$ two days from the starting date. This value increased to 8.50 mg.L$^{-1}$ after 30 days and then decreased to 2.20 mg.L$^{-1}$ at the end of a 90 day period.

Leachate samples from the garden soil amended with poultry manure were found to have generally higher nitrate concentrations. After 2 days the leachate was found to have a very low value of less than 0.10 mg.L$^{-1}$. However, after 14 days this had increased to a value of 38.10 mg.L$^{-1}$ and on further sampling the concentration reached 159.2 mg.L$^{-1}$ after 30 days. At 90 days the value had fallen to 42.40 mg.L$^{-1}$.

At 30 days the leachate from the FOZS substrate had a nitrate concentration of 277.0 mg.L$^{-1}$. whereas that of the leachate taken from the SM substrate was found to be 159.2 mg.L$^{-1}$ . At 60 days the nitrate concentration of the leachate, from the FOZS substrate had reached 291.0 mg.L$^{-1}$ and the value of the leachate from the SM substrate had dropped to 99 mg.L$^{-1}$. A similar difference between the two substrates was recorded at 90 days when each were found to have declined to 112.0 and 42.4 mg.L$^{-1}$, respectively. (Fig 1, Table 1).

In contrast the nitrate concentration of leachate from the OZS substrate was found to be anomalously high, measuring 1099 mg.L$^{-1}$ on day 2; falling to a concentration of 493.6mg.L$^{-1}$ after 30 days and further declining to 264.7 mg.L$^{-1}$ at 60 days. On cessation of regular watering, after 60 days, the concentration of the leachate reached a plateau at between 264.7 and 280 mg.L$^{-1}$ until dropping to 189.0 mg.L$^{'}$ at 180 days.

The pH and EC measurements are shown together with the analyses of the nitrogen species in Table I and although there is only slight systematic variation in pH the EC measurements vary over an order of magnitude. Those of the garden soil have values varying between 117± 15 and 193 ± 12 µS.cm$^{-1}$ whereas the leachates from the amended garden soil, containing the mature organo-zeolitic mixture, vary between 2,347±411 and 8000 ± 200 µS.cm$^{-1}$.

As part of this experiment the OZS substrate was prepared by amending the garden soil with an organozeolitic mixture that had matured over several years. At the start of the experiment the leachate nitrate concentration was found to be 1099 mg.L$^{-1}$, whereas the ammonium concentration was 15.l mg.L$^{-1}$.

This very high nitrate concentration can not be due to nitrification taking place contemporaneously as the measurement was made only two days after preparation of the amended garden soil. We therefore think that during the long period of maturation (more than three years), under aerobic conditions, ammonium ions were liberated from the decomposing manure at various rates, depending on the type of molecule undergoing bio-degradation. The ammonium ions on the zeolite surfaces are thought to have been repeatedly oxidized and re-adsorbed in a number of stages over time. It is also likely that nitrogen fixing bacteria have contributed to the build-up of the nitrate concentration over the years that the material was stored, as it has been reported that the introduction of clinoptilolite to an organic substrate promotes favourable conditions for the

4

development of the nitrogen-fixers such as blue-green algae, actinomycetes and azotobacter under weakly alkaline conditions, [19.20].

Over the 180 day period of the experiment the nitrate concentration of the leachate samples from the OZS substrates had decreased to 189mg.L$^{-1}$ whilst the ammonium concentrations varied irregularly between 0.1 and 15.1 mg.L$^{-1}$. As a consequence of regular watering nitrate losses must have occurred. To test this assumption watering of the OZS substrates was stopped after 60 days. During the following 120 days the nitrate concentration remained relatively constant (Table.1) suggesting that the rapid loss of nitrate was due to leaching as a result of the regular watering.

It was found that the ammonium and nitrite concentration of the leachate from the garden soil have very low values and the low nitrate concentration recorded appears to be a residual inherited from a former period of cultivation. Leachates from the SM substrates (poultry manure amended soil) have an appreciable ammonium value at the beginning of the experiment. This appears to have been oxidized to nitrite and nitrate and after 60 days the nitrate concentration is reduced to a low level. In the case of the FOZS substrates (freshly made organo-zeolitic amended soil) the ammonium concentrations of the leachates are comparable to those from the SM substrate but the nitrate concentrations are considerably higher (Fig. 1, Table. 1). It appears therefore, that the zeolite surface in providing a reservoir for ammonium ions has increased the concentration of nitrate in the FOZS substrate relative to that of the, zeolite free, SM substrates.
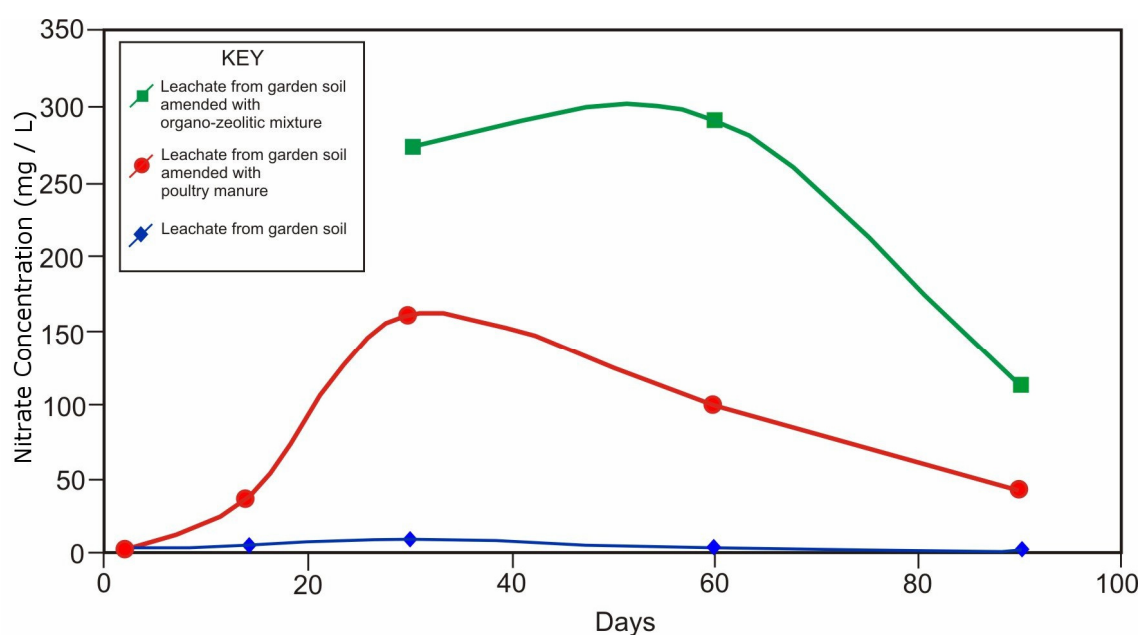


**Fig. 1.** Change of nitrate concentration of leachates with time.

Measurement of the pH and EC of the aqueous leachates provided more evidence of the effect of enhanced nitrification. Although the pH values do not change appreciably their variation was found to be systematic. It was found that the pH was higher in the untreated garden soil and we think this is related to the lack of nitrification taking place in these substrates. The lower values in the amended soils appear to be due to proton activity. This observation appears to be in agreement with our hypothesis of nitrification, as protons are released as a consequence of the activity of the nitrifying micro-organisms [14]. Under moist soil conditions the protons would be expected to react with the soil porewater to form hydroxonium ions that would be rapidly neutralized by

5

oxy/hydroxide complexes and polymers present in the soil so liberating cations, as is the case with Al and Fe [21].

# Table. 1

*Leachate data for the time-course experiment.*

Leachate measurements at 2 days from the start of experiment.

| Substrate | Electrical Conductivity ($\mu S.cm^{-1}$) | pH | $NH_4^+$ | $NO_2^-$ (mg . $L^{-1}$) | $NO_3^-$ |
|---|---|---|---|---|---|
| GS / 01 | 193 ± 12 | 8.31 ± 0.04 | <0.10 | <0.10 | 3.40 |
| SM / 01 | 1,633 ± 322 | 7.49 ± 0.07 | 114.9 | <0.10 | <0.10 |
| OZS / 01 | 8, 000 ± 200 | 7.52 ± 0.02 | 15.1 | 4.77 | 1099 |

Leachate measurements at 14 days

| | | | | | |
|---|---|---|---|---|---|
| GS / 02 | 120 ± 33 | 8.94 ± 0.08 | <0.10 | <0.10 | 5.00 |
| SM / 02 | 1,363 ± 112 | 7.67 ± 0.15 | 55.7 | 72.1 | 38.1 |
| OZS / 02 | 4,613 ± 443 | 7.79 ± 0.12 | 16.5 | 0.50 | 729.0 |

Leachate measurements at 30 days

| | | | | | |
|---|---|---|---|---|---|
| GS / 03 | 166 ± 34 | 9.56 ± 0.03 | 0.10 | < 0.10 | 8.50 |
| SM / 03 | 1,650 ± 269 | 8.07 ± 0.17 | 2.00 | 22.0 | 159.2 |
| OZS / 03 | 3,743 ± 84 | 7.84 ± 0.01 | 0.10 | 0.20 | 493.6 |
| FOZS / 03 | 2,200 | 7.17 | 1.10 | <0.10 | 277.0 |

Leachate measurements at 60 days

| | | | | | |
|---|---|---|---|---|---|
| GS / 04 | 117 ± 15 | 8.99 ± 0.01 | <0.10 | <0.10 | 3.30 |
| SM / 04 | 980 ± 17 | 7.94 ± 0.17 | 0.20 | < 0.10 | 99.0 |
| OZS / 04 | 2,387 ± 475 | 7.87 ± 0.09 | 0.18 | <0.10 | 264.7 |
| FOZS / 04 | 2,700 | 7.46 | 1.00 | 0.90 | 291.0 |

Leachate measurements at 90 days

| | | | | | |
|---|---|---|---|---|---|
| GS / 05 | 176 ± 25 | 8.00 ± 0.04 | <0.10 | <0.10 | 2.20 |

| Sample | | | | | |
|---|---|---|---|---|---|
| SM / 05 | 1,347 ± 219 | 7.57 ± 0.05 | 0.10 | <0.10 | 42.4 |
| OZS / 05 | 2,347 ± 411 | 7.51 ± 0.04 | 0.20 | <0.10 | 280.0 |
| FOZS / 05 | 850 | 8.00 | 0.70 | < 0.10 | 112.0 |

Leachate measurements at 140 days

| | | | | | |
|---|---|---|---|---|---|
| SM / 06 | 860 ± 213 | 7.62 ± 0.01 | 1.20 | < 0.10 | 84.5 |
| OZS / 06 | 3,250 ± 356 | 7.62 ± 0.04 | 1.40 | <0.10 | 267.7 |

Leachate measurements at 180 days

| | | | | | |
|---|---|---|---|---|---|
| SM / 07 | 628 ± 521 | 7.89 ± 0.03 | 1.30 | <0.10 | 131.0 |
| OZS / 07 | ND | 7.84 ± 0.02 | 1.40 | <0.10 | 189.0 |

[ ND refers to No Data ]

2. Ammonium mono-oxygenase inhibition experiment

| | | | | | |
|---|---|---|---|---|---|
| FOZS-A ( Air + Acetylene) | 160 | 7.17 | 30.6 | <0.10 | 16.8 |
| FOZS-B (Air) | 1,500 | 7.17 | 5.0 | < 0.10 | 139.0 |

3.  Measurement of surface adsorbed  $NH_4^+$ ions

| Time ( Days) | $NH_4^+$ ion concentration  (mmols / 100g zeolitic tuff) |
|---|---|
| Start of the experiment | 13.20 ± 5.0 |
| 16 | 22.8 ± 1.58 |
| 38 | 36.44 ± 2.84 |
| 68 | 12.22 ± 0.46 |
| 104 | 16.6 0 ± 1.02 |
| 148 | 13.80 ± 0.08 |
| 179 | 9.89 ± 0.03 |

[Note:  The start of this experiment was made 40 days after the fermentation of the mixture had ceased].

Again, if our hypothesis is correct a direct relationship should be found between the EC, as a measure of the proton activity in the leachate solutions and their nitrate concentrations. This is the case as a linear relationship was found to between these two variables (Fig.3).

In order to better understand the function of the zeolite, the concentration of ammonium ions bound to the zeolite surface were measured by taking fractions of zeolitic tuff from an organo-zeolitic mixture; beginning 40 days after fermentation had ceased. It was found that the concentration reached a peak after 38 days from the start of the experiment and then declined, reaching a minimum after 180 days (Fig 2). It is suggested that the ammonification of uric acid, in the poultry manure, accounts for the initial build up of surface bound ammonium ions as this relatively simple compound would require less energy to biodegrade that an amino acid or protein [22]. As the amount of uric acid must be finite the ammonium ion concentration would be expected to decrease after the initial adsorption had reached its peak due to oxidization by nitrifying micro-organisms.
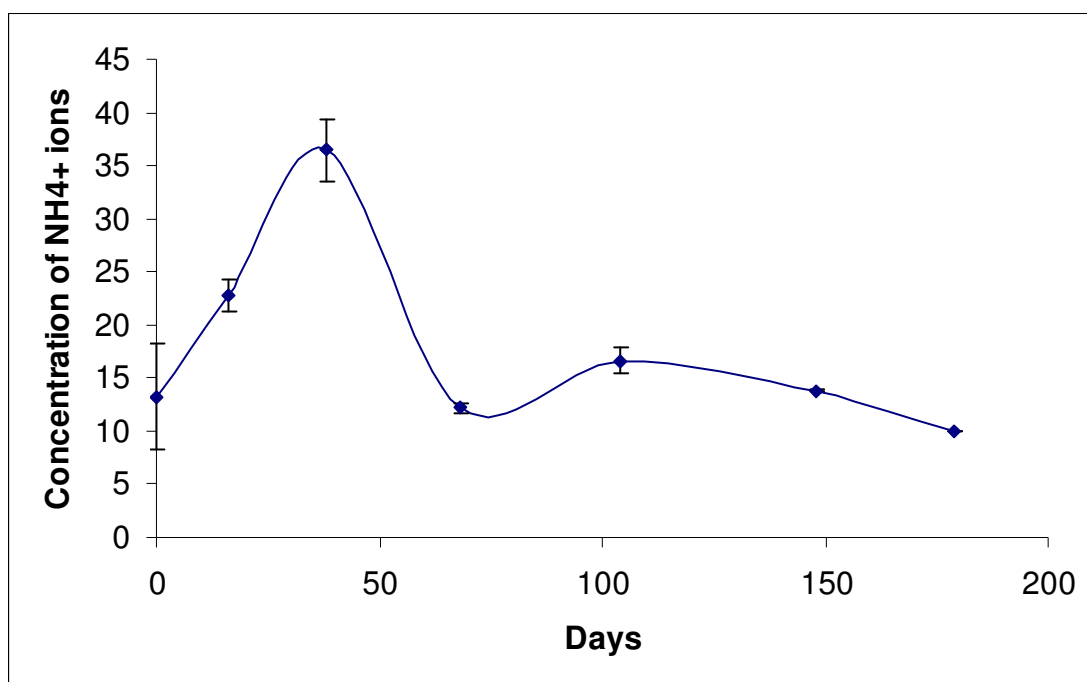


**Fig. 2.   Change of surface adsorbed NH$_4^+$ ions with time**

It is thought that the ammonium ions adsorbed to the exterior surface of the zeolite crystals are mostly involved in the microbiological reactions that take place, as the size of the clinoptilolite pore channels of are in the order of angstrom units (10'° m); the largest of which measure 3.3 x 4.6 A [23].  Thus, it would appear unlikely that ammonium oxidizing bacteria such as *Nitrosolobus* or *Nitrosospira* could enter the zeolite pores to obtain access to any ammonium ions adsorbed on the interior surfaces, as these organisms are larger than the pore size by almost an order of magnitude [24].  However, this does not preclude the ion-exchange of ammonium by potassium ions, which have a higher selectivity [25] from displacing ammonium ions to the external surfaces from which they could be oxidized by nitrifying micro-organisms.

Growing plants in organo-zeolitic amended substrates over the last decade has shown that a large variety of plants respond in a similar way. Controlled experiments have demonstrated that large increases in plant growth, as expressed by shoot dry weight, occur in organo-zeolitic amended substrates that have received no treatment other than regular watering [11, 12,  26]. We have shown in these studies that apart from an increase in the nitrate concentrations other cations and anions have elevated concentrations in leachate from the amended substrates.
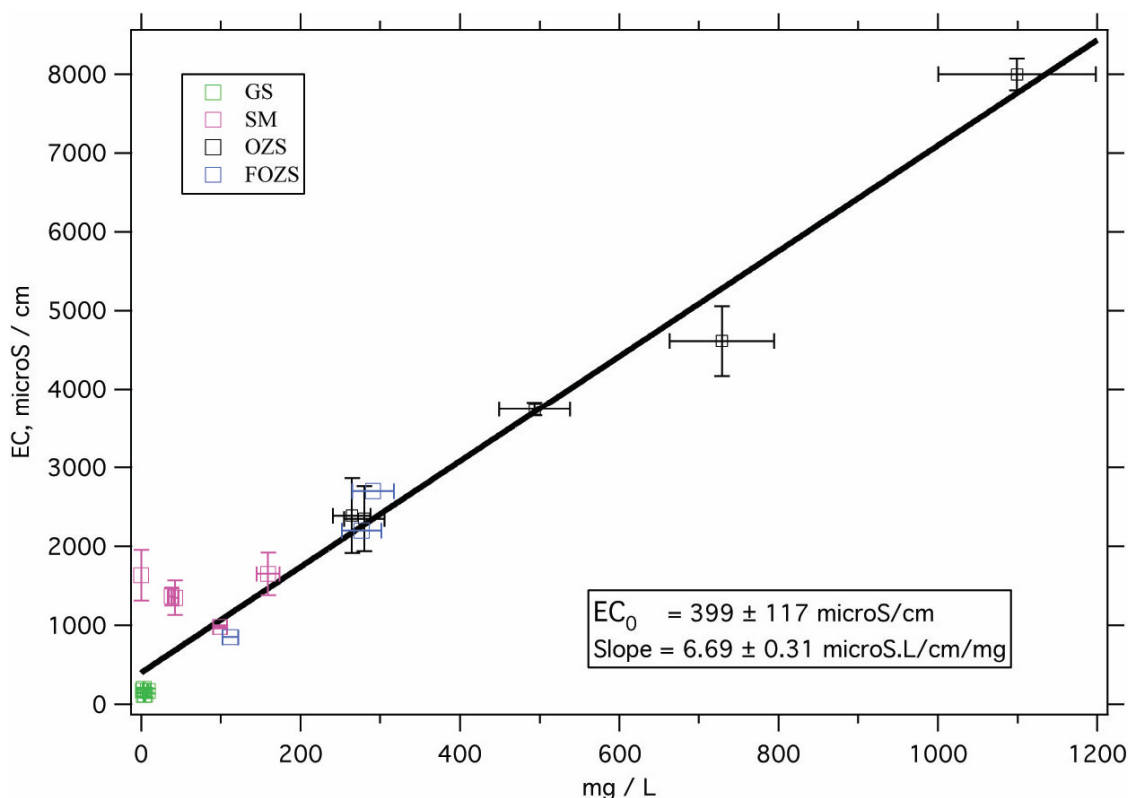
8

**Fig.3    Relationship of EC and nitrate concentration of aqueous leachates.**

As an example of enhanced growth *Brassica napus* is shown growing in garden soil and amended sulphide mine waste (Fig 4).  It should be noted that the mine waste was amended in exactly the same way  as that of garden soil in former studies [10, 11], apart from the fact that the pH of the mine waste pore-water was adjusted to approximately 6.5 by the addition of calcium carbonate to provide an environment which would support the function of nitrifying micro-organisms. Seeds were sown at the same time and germinated within a week to ten days. The plants were grown under identical greenhouse conditions and the photos were taken after a period of three months.

**Fig. 4   Growth of *B.napus*.   Left: Garden soil substrate. Right: Amended mine waste substrate**.

# Conclusions

On the work we have done with non leguminous plants it has been observed that the incorporation of an organo-zeolitic soil amendment greatly enhances growth. The present work shows that the absorption of ammonium ions on the zeolite surfaces buffers the loss of ammonia by volatilization to the atmosphere and leaching through the soil. In maximising the concentration of ammonium ions available for oxidation nitrification is highly stimulated. The proton supply, due to nitrification, increases the mobility of the soil pore-water as shown by the greatly increased EC of leachates from the amended substrates. The enhanced plant growth, in soils amended with the bio-fertilizer, appears to be directly related to an increase in nitrate together with the high degree of ionic mobilization of the soil pore-water which is thought to make other nutrient elements freely available to the growing plant.

# References

1. Coombs, DS The nature and alteration of some Triassic sediments from Southland. New Zealand. Trans. Roy. Soc. New Zealand 82: 65-103, 1954.

2. Mumpton, FA First reported occurrence of zeolites in sedimentary rocks of Mexico. Amer Miner, 58, 1973.

3. Alexsiev B, Djourova, E.G   On the origin of zeolitic rocks. C  R Acad Bulg Sci.,  28,  1975.

4. Hay, RL, Sheppard, RA Occurrences of Zeolites in sedimentary Rocks: An Overview. In: Bish, DL, Ming, DW (eds) Natural Zeolites: Occurrences, Properties, Applications. Reviews in Mineralogy and Geochemistry, Min .Soc. Amer., 45, 2001.

5. Cochemé, J-J., Leggo, PJ., Damian G., Fulop, A., Ledésert, B., Grauby, O The mineralogy and distribution of zeolitic tuffs in the Marainures Basin, Romania. Clays and Clay Minerals 51, 2003.

6. Nastro, A, Colella, C Colunm ion exchange data for ammonium removal from water by phillipsite tuff.  Ing. Chim Ital., 19, 1983.

7. Mumpton, FA. La roca magica: Uses of natural zeolites in agriculture and industry. Proc.Nat.Acad.Sci.USA, 96, 1999.


8. Kalló, D. Applications of Natural Zeolites in Water and Wastewater Treatment.
In: Bish, D.L, Ming, D.W(eds) Natural Zeolites: Occurrence, Properties, Applications. Reviews in Mineralogy and Geochemistry., Min. Soc Amer 45., 2001.

9. Minato, H. Occurrence and Application of Natural Zeolites in Japan. In: D.Kalló ,H.S.Sherry (eds.,).Occurrence, Properties and Utilization of Natural Zeolites,Akadémiai Kiadó, Budapest,1988.

10. Leggo, P.J. An investigation of plant growth in an organo-zeolitic substrate and its ecological significance. Plant and Soil. 219, 2000.

11. Leggo, P.J, Ledésert, B. Use of organo-zeolitic fertilizer to sustain plant growth and stabilize metallurgical and mine-waste sites. Min. Mag 65, 2001.

12. Leggo, PJ, Ledésert, B, Christie, G. The role of clinoptilolite in organo-zeoliticsoil systems used for phytoremediation. Sci. Total Environ., 363, 2006.

13. Witter, E ,Lopez-Real, J. Nitrogen losses during the composting of sewage sludge and the effectiveness of clay soil, zeolite and compost in adsorbing the volatilized ammonia. Biological wastes 23, 1988.

14. Paul, E.A, Clark, F.E. In: Soil Microbiology and Biochemistry, 2nd ed., Academic Press Ltd., London, UK, 1996.

15. Passiglia, E, Sheppard, R. The crystal chemistry of zeolites. In: Bish, D.L, Ming, D.W (eds) Natural Zeolites, Occurrences, Properties, Applications. Reviews in Mineralogy and Geochemistry, Min. Soc Amer., 45, 2001.

16. Stark, J.M., Firestone, M.K. Mechanisms for Soil Moisture Effects on Activity of Nitrifying Bacteria. Appl.Environ. Microbiology, 61, 1995.

17. Hynes, R.K., Knowles, R. Effect of acetylene on autotrophic and heterotropbic nitrification. Can .J. Microbiology. 28, 1982.

18. Minato, H. Method for measurement of cation exchange capacity (C.E.C). In: No.111, Zeolite sub-committee (ed.) Natural Zeolite and its Utilization. Japan Society for the Promotion of Science, 1994.

19. Andronikashvili, T.G., Tsitsishvili, G., Kardava M., Gamisonia, M.. The effect of organic-zeolite fertilizers on the microbial landscape of soil. Izvestiya Akademii Nauk Gruzii, Seriya Khimicheskaya, 25, 1999.

20. Andronikashvili T.G., Urushadze T., Eprikashvili, L., Gamisonia, M., Nakaidze, E. Towards the Biological Activity of the Natural Zeolite-Clinoptilolite-Containing Tuff. Bull.Georgian National Academy of Science 2 , 2008.

21. Schwertmann, U, Süsser, P. Nätscher, L. Proton buffer systems in soils. Z.Pflanzenernähr. Bodenk, 1987

22. Mayer, L.M., Rice, D.L. Early diagenesis of protein: A seasonal study. Amer Soc, Limnology and Oceanography, 37, 1992.
23. Armbruster T., Gunter, M.E. Crystal Structures of Natural Zeolites. Reviews of Mineralogy and Geochemistry 45, 2001.

24. Takahashi, B., Ohishi, M., Ohshima, M., Saitoh, M., Omata, K., Tokuyama,T. Characteristics of an ammonia oxidizing bacterium with a plasmid isolated from alkaline soils and its phylogenetic relationships. J. of Bioscience and Bioenguneering, 92, 2001.

25. Tsitsishvili, G.V., Andronikashvili, T.G., Kirov, G.N, Filizova, L.D. Ion Exchange Properties. In:Williams, P.A (translation ed) Natural Zeolites, Ellis Horwood Series in Inorganic Chemistry, Ellis Horwood Ltd., UK, 1992.

26. Leggo, P.J., Ledésert, B. Organo-Zeolitic-Soil Systems: A New Approach to Plant Nutrition. In: L.R.Elsworth and W.O.Paley (eds), Fertilizers: Properties, Applications and Effects, Nova Science, Hauppauge, NY, USA, 2008.

14