

## Performance of three Dicotyledon Species on Lead/Zinc Mine Spoil with Various Nitrogen Sources

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**Abstract:** Rapid leaching of nitrogen fertilizers is a factor limiting the long term success of revegetation on the Trelogan mine spoil due to the physical structure of mine waste material, with its almost total lack of organic matter. A slow release nitrogen fertilizer was examined, in addition to fast release sources, to assess its performance. Single and split doses of conventional nitrogen fertilizers were also used to compare their effects. Shoot and root dry weight of *Rumex acetosa* was greater than control at a single and split application of  $\text{NaNO}_3$  and only root dry weight of *Minuartia verna* was improved at the single dose of  $\text{NaNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ . In contrast there was no effect of single and split doses of urea and  $\text{NH}_4\text{NO}_3$  and the single application of slow release source (Golden-N) on the growth characters of *Plantago lanceolata* when compared to control. Split doses of conventional fertilizers were more effective than single doses.

**Key words:** Mine spoil, nitrogen sources, single, split, doses, species

### Introduction

Deficiencies in essential plant nutrients are almost universal features of derelict land, and probably the single greatest constraint on the vegetation establishment (Johnson and Bradshaw, 1979). Abandoned non ferrous metal workings in England and Wales and other heavy metal polluted areas of the world have attracted considerable attention especially where adjacent land and water resources are subjected to serious contamination by trace metals, in particular heavy metals such as lead and zinc. The impact of mine drainage water, dispersal of air borne particles and spoil tip erosion, on receiving ecosystems has stimulated investigations into potential hazards to human health (Davies and Roberts, 1975 and Thomas *et al.*, 1977), and agriculture (Davies, 1971; Thornton, 1975) in affected areas. Polluted soils have toxic concentrations of heavy metals, low nutrient status and unfavourable physical conditions (Bremmer, 1965; Johnson and Bradshaw, 1977). These problems can be overcome by avoiding or countering the factors which inhibit growth. This is based on countering toxicity of the substrate by sowing metal tolerant ecotypes which have evolved on mine wastes and supply of nitrogen in its different sources.

In order to obtain plant growth on metal contaminated wastes, slow release chemical fertilizers has been advocated (e.g., sulphur coated urea, (Gold-N) and urea formaldehyde) and their value has been proven in several mine waste situations (Johnson and Bradshaw, 1979; McNeily and Johnson, 1981). Such materials are nevertheless expensive and a single application at seeding, even at higher rate ( $100 \text{ kg N ha}^{-1}$ ) is rarely satisfactory by itself for sustainable growth (Gemmell, 1975). For the establishment of the vegetation on mine spoils, considerably more valuable slow acting sources of nitrogen are organic amendments such as sewage sludge, mushroom compost and farmyard manure.

### Materials and Methods

**Seedlings:** Seeds of three dicotyledon plant species, *Plantago lanceolata*, *Rumex acetosa*, and *Minuartia verna* collected from their indigenous Trelogan mine populations were sown in growth room conditions ( $22 \pm 2^\circ\text{C}$ , 16 h photoperiod ( $45 \text{ lmoles m}^{-2} \text{ s}^{-1}$ ) and 65% relative humidity). The seeds were sown in seed trays on moist paper towel.

**Nitrogen fertilizer treatments:** Three weeks old seedlings (as uniform in height and size as possible) were transplanted, five per pot into mine waste in 130 mm diameter plastic pots. The seedlings were thinned after two weeks to leave only three seedlings per pot. P and K were added as superphosphate (18% P) and potassium sulphate (50% K) respectively. They were incorporated into the surface 10 mm of the mine spoil to provide uniform P and K nutrient contents. The superphosphate and potassium sulphate fertilizers were applied at rates equivalent to  $44 \text{ kg ha}^{-1}$  ( $7.92 \text{ kg P ha}^{-1}$ ) and  $42 \text{ kg ha}^{-1}$  ( $21 \text{ kg K ha}^{-1}$ ) respectively.

Four nitrogen fertilizers, urea, (04%N), sodium nitrate, (16% N), ammonium sulphate, (21% N), and ammonium nitrate, (34.5% N) and slow-release sulphur coated urea (Gold-N, 32%N), were incorporated  $100 \text{ Kg N ha}^{-1}$ . They were added either as a single treatment prior to seedling transplantation for the conventional and slow release types, or as a split application for conventional types only, equivalent to  $50 \text{ N ha}^{-1}$  at the beginning of the experiment and  $50 \text{ Kg N ha}^{-1}$  4 weeks after seedling transplantation. Only P and K were applied to the control (no nitrogen) treatment.

The experiment was laid out in randomized complete block design. The potted seedlings were grown in a glasshouse maintained at  $22 \pm 2^\circ\text{C}$  with an 18 h photoperiod at  $230 \text{ lmoles m}^{-2} \text{ s}^{-1}$ , photosynthetically active radiation are maintained at field capacity using tap water from above regularly every day.

**Harvest and growth measurements:** The three species, *P. lanceolata*, *R. acetosa*, and *M. verna* were harvested 3 months after transplanting. The plants with roots were removed from the pots and excess moisture on the plants were removed. Roots and shoots of each plant in each pot were separated, and transferred into an oven at  $60^\circ\text{C}$  to dry. Shoot and root dry weights were measured after drying for 5 days (Dancer *et al.*, 1977; McNeily and Johnson, 1981).

The data for root and shoot dry weight of 3 species were transformed into  $\log_{10}$  and two way analysis of variance was computed from PROC ANOVA by SAS package (SAS Institute Inc., 1989). LSD was also determined for treatment mean comparison (Steel and Torrie, 1980).

## Results

**Mean shoot dry weight per plant:** The mean square values and their significance from the analysis of variance for mean shoot dry weight is presented in Table 1. There was a significant ( $p < 0.001$ ) difference between species, reactions to different nitrogen fertilizer sources. There was no significant difference between replicates.

***Plantago lanceolata:*** *P. lanceolata* produced maximum and significantly greater shoot dry weight (Fig.1) in the control treatment in which only phosphorous and potassium were applied as superphosphate and potassium sulphate than in any of the other conventional nitrogen sources. But urea and ammonium nitrate seedlings did not survive, while treatment with sodium nitrate produced more significant ( $p < 0.05$ ) shoot dry weight than ammonium sulphate.

Transplanted seedlings did not survive the split application of both urea and ammonium nitrate. Sodium nitrate responded slightly less than ammonium sulphate when given as split application. Split doses did not enhance shoot dry weight compare control. A single application of sodium nitrate produced more shoot dry matter than a split application. Gold-N (sulphur coated urea) failed to produce significant ( $p < 0.05$ ) increase in shoot dry weight. In comparison with other nitrogen treatments, lower dry weight was recorded in response to Gold-N than any other treatment except ammonium sulphate. However, Gold-N and ammonium sulphate in its single dose responded equally.

***Rumex acetosa:*** Under single sodium nitrate treatment, this species produced significantly ( $p < 0.05$ ) greater shoot dry weight than control as well as from other single applications of nitrogen (Fig.2). However, seedlings did not survive under single dose of urea, ammonium sulphate or ammonium nitrate. The split application of sodium nitrate gave a significantly ( $p < 0.05$ ) greater response than the other split doses of other N sources. Seedlings failed to survive in the split dose of urea. The split application of ammonium nitrate gave greater shoot growth in comparison with ammonium sulphate.

There was no survival in both single and split applications of urea. The split doses of ammonium sulphate and ammonium nitrate resulted in considerably less shoot growth than the control. Urea, ammonium sulphate, and ammonium nitrate, were toxic to plants of *R. acetosa* at the amount supplied (100 kg N ha<sup>-1</sup>) as a single application.

When Gold-N was added as single application, the shoot dry weight yield was significantly ( $p < 0.05$ ) less than the control treatment. Thus the supply of nitrogen was apparently too high even with Gold-N, a slow-release formulation.

***Minuartia verna:*** *M. verna* seedlings only produced shoot growth in the single applications of sodium nitrate and ammonium sulphate (Fig.3). These two treatments produced significantly less shoot growth than control. Seedlings were killed by applying urea and ammonium nitrate.

When split applications of nitrogen sources were added, seedlings only survived after addition of ammonium nitrate. Shoot dry weight after this split dose was significantly less than the single doses of sodium nitrate and ammonium sulphate and less than the control. There was no survival at the once application of Gold-N. The results demonstrated that, with the exception of single was no effect of nitrogen source in increasing the shoot dry mass of the other 4 species examined in the experiment (Table 2).

***Plantago lanceolata:*** A single application of sodium nitrate

Table 1: Mean squares and significances from the analysis of and split applications of sodium nitrate in *R. acetosa* there variance of shoot dry weight of three dicotyledons growing in Trelogan mine spoil with various nitrogen sources

Species. →	<i>P. lanceolata</i>		<i>R. acetosa</i>		<i>M. verna</i>	
	df	M.S.	df	M.S.	df	M.S.
N. Sources	9	0.01780***	9	0.1035***	9	0.00022***
Replicates	3	0.00017 <sup>ns</sup>	3	0.0108 <sup>ns</sup>	3	0.000008 <sup>ns</sup>
Error	27	0.00019	27	0.000818	27	0.000008
Total	39	-	39	-	39	-

\*\*\* = Significant at 0.001 NS = Non Significant

gave significantly greater root growth in comparison with all the other nitrogen sources (Fig. 4). There was no seedling survival in urea and ammonium nitrate when added as a single or split treatment. The control treatment had significantly ( $p < 0.05$ ) highest root growth, of all the nitrogen sources.

In the split treatments of urea and ammonium nitrate seedlings did not survive. Root growth in the split sodium nitrate and ammonium sulphate treatments was significantly less than the control. Root dry weight in the single application of Gold-N (sulphur coated urea) was significantly less than the control. Differences in root dry weight between the nitrogen treatments in which seedlings of *P. lanceolata* survived were not great and all were less than 20% control treatment root biomass.

***Rumex acetosa:*** Seedlings of *Rumex acetosa* survived only in sodium nitrate treatment (single dose). There was no significant difference between root weight with this treatment and the control (Fig. 5). Seedlings did not survive in single treatments of urea, ammonium sulphate, and ammonium nitrate.

The split application of sodium nitrate produced a root dry weight not significantly different from the single sodium nitrate application, or control. With split applications of ammonium sulphate and ammonium nitrate, there was some seedling survival, and root growth, but it was very small as compared to control. Application of Gold-N suppressed root growth very substantially in comparison with the control ( $p < 0.05$ ).

Overall comparison single and split application of sodium nitrate produced the highest root dry weight yield but it was not significantly greater than control. In contrast single applications of urea, ammonium sulphate and ammonium nitrate were sufficiently toxic kill all seedlings of *R. acetosa*. The same applied to split application of urea.

***Minuartia verna:*** Plants survived only when grown in single applications of sodium nitrate ammonium sulphate, and split application of ammonium nitrate. Root dry weight yield was significantly ( $p > 0.05$ ) greater than control treatment after single dose of sodium nitrate (Fig.6) the seedlings did not survive in the split applications of urea, sodium nitrate and ammonium sulphate Root dry weight from split application of ammonium nitrate was not significantly from control. Gold-N did not allow any plant growth.

Over all effect of nitrogen sources on the root dry biomass of five plant species and the result indicated that root dry matter of *R. acetosa* increased after single and split doses of sodium nitrate (Table 4). Similarly a single application of sodium nitrate and ammonium sulphate allowed to increase the root dry mass of *M. verna* over control.

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Table 2: Mean shoot dry weight (% of control) of three plan species in response to various sources of Nitrogen growing on Trelogan mine spoil

Species	Treatments									
	U single 100 kg ha <sup>-1</sup>	SN single 100 kg ha <sup>-1</sup>	AS single 100 kg ha <sup>-1</sup>	AN single 100 kg ha <sup>-1</sup>	U split 50+50 100 kg ha <sup>-1</sup>	SN split 50+50 100 kg ha <sup>-1</sup>	AS split 50+50 100 kg ha <sup>-1</sup>	AN split 50+50 100 kg ha <sup>-1</sup>	GN single 100 kg ha <sup>-1</sup>	
<i>P. lanceolata</i>	0.0	30.8	10.5	0.0	0.0	18.0	19.4	0.0	10.0	
<i>R. acetosa</i>	0.0	194.0	0.0	0.0	0.0	140.0	17.8	47.7	14.0	
<i>M. verna</i>	0.0	62.6	52.1	0.0	0.0	0.0	0.0	13.8	0.0	

Table 3: Means squares and significances from the analysis of variance of root dry weight of three dicotyledons growing in Trelogan mine spoil with various nitrogen sources

Species →	<i>P. lanceolata</i>		<i>R. acetosa</i>		<i>M. verna</i>	
	df	M.S.	df	M.S.	df	M.S.
N. Sources	9	0.030118***	9	0.0563***	9	0.000006***
Replicates	3	0.00002 <sup>NS</sup>	3	0.00345 <sup>NS</sup>	3	0.0000001 <sup>NS</sup>
Error	27	0.00014	27	0.00422	27	0.000008
Total	39	-	39	-	39	-

\*\*\* = Significant at 0.001 NS = Non Significant

Table 4: Mean root dry weight (% of control) of five plant species in response to various sources of nitrogen growing on Trelogan mine spoil

Species	Treatment									
	U Single 100 kg ha <sup>-1</sup>	SN single 100 kg ha <sup>-1</sup>	AS single 100 kg ha <sup>-1</sup>	AN single 100 kg ha <sup>-1</sup>	U split 50+50 100 kg ha <sup>-1</sup>	SN split 50+50 100 kg ha <sup>-1</sup>	AS split 50+50 100 kg ha <sup>-1</sup>	AN split 50+50 100 kg ha <sup>-1</sup>	GN single 100 kg ha <sup>-1</sup>	
<i>P. lanceolata</i>	0.0	18.1	5.7	0.0	0.0	12.6	11.3	0.0	6.5	
<i>R. acetosa</i>	0.0	132	0.0	0.0	0.0	111	7.5	40.0	4.7	
<i>M. verna</i>	0.0	160	12.5	0.0	0.0	0.0	0.0	80.0	0.0	

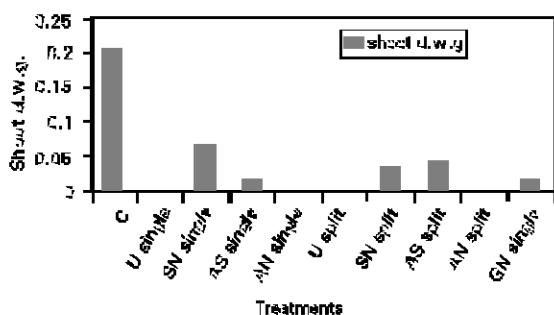


Fig. 1: Shoot dry weight of *Plantago lanceolata* in different N. sources (LSD 5% = 0.022)

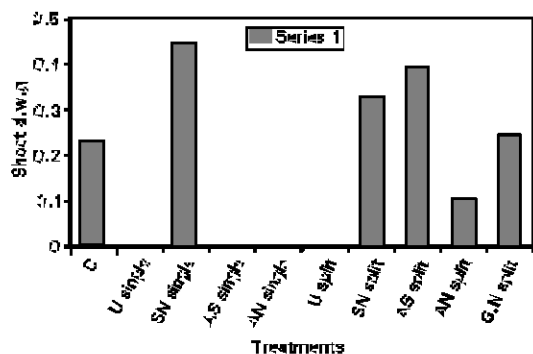


Fig. 2: Shoot dry weight of *Rumex acetosa* in different N. sources. (LSD 5% = 0.10)

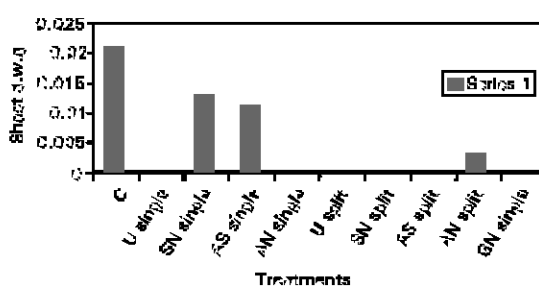


Fig. 3: Shoot dry weight of *Minuartia verna* in different N. sources (LSD % 0.004)

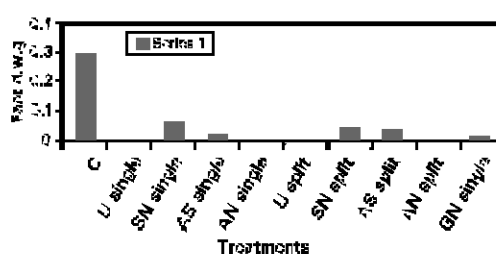


Fig. 4: Root dry weight of *P. lanceolata* in different nitrogen sources. (LSD 5% = 0.0189)

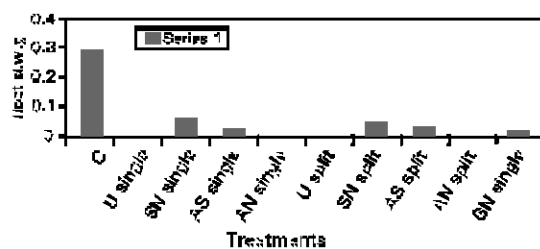


Fig. 5: Root dry weight of *R. acetosa* in different N sources (LSD 5% = 0.0189)

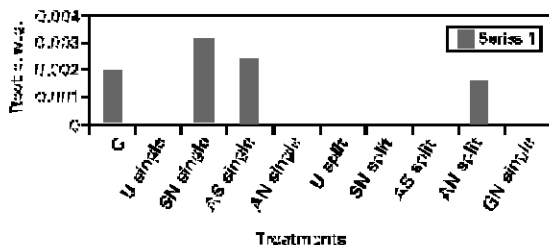


Fig. 6: Root dry weight of *M. verna* in different N sources (LSD 5% = 0.0078)

### Discussion

It is evident that all the three species were killed by a single and split application of urea. A similar depressing effect was also found by Davies (1973), where urea application gave a total dry yield of grass, that was less than that from ammonium nitrate and sulphur coated urea. Johnson (1977) found that urea gave the lowest shoot dry yield response on fluor spar mine tailings from those of  $\text{NaNO}_3$ ,  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , sulphur coated urea, hoof and horn, and dried blood. This may have been due to the loss of ammonia from urea which is often more marked than from ammonium nitrate and ammonium sulphate (Volk, 196; Hamid and Mahler, 1994).

The three species in the experiment responded differently to the single and split doses of  $\text{NaNO}_3$  in terms of shoot and root dry weight.  $\text{NaNO}_3$  was the least inhibitory source of nitrogen assessed and the split application usually gave greater dry weight response than the single dose. These results were in accordance in part with the results of McNeilly and Johnson (1981), who reported that shoot dry matter yield per pot of *F. rubra* from  $\text{NaNO}_3$  was greater on acidic Y Fan mine spoil than on the calcareous Minera mine waste.

In all species nitrogen sources depressed yield whether applied as a single or split application. In case of *P. lanceolata*, *R. acetosa*, and *M. verna* a single application of ammonium nitrate was lethal. A split application of ammonium nitrate or ammonium sulphate often allowed survival but nevertheless depressed plant yield. McNeilly and Johnson (1981) found that ammonium nitrate and urea treatments had significantly greater overall plant dry matter yield than ammonium sulphate on calcareous Minera Pb/Zn mine spoil which was applied at the same rate as in this experiment. In contrast ammonium nitrate gave relatively better growth than urea, similar to the findings of Pollett *et al.* (1995). Lower production in urea may have been due to greater loss from urea than ammonium nitrate by volatilization in the normal soil (Hamid and Mahler, 1994).

Split doses generally appeared to be less damaging to the growth of three species examined in comparison with single

applications. Similar results were reported by McNeilly and Johnson (1981), when split doses of urea and ammonium nitrate, gave significantly greater shoot dry matter yield than a single application. This is due to low cation exchange capacity of both wastes, and the consequent inability of the material to retain ammonium ions when leached due to lack of organic matter. Johnson (1977) also resulted the lower shoot dry yields from plots treated with single applications of  $\text{NaNO}_3$  than 2 and 3 applications of  $\text{NaNO}_3$  probably therefore reflect losses of the nutrient by volatilisation and leaching.

On the calcareous Trelogan mine spoil single and split applications of nitrogen fertilizers did not improve the dry weight yield production of any of the species except *R. acetosa*, where both applications of sodium nitrate gave a higher yield than control treatment. However, *M. verna* root dry weight was also found greater at single doses of sodium nitrate and ammonium sulphate over the control. This suggests that addition of nitrogen was in excessive amounts either causing depression of plant growth due to high conductivity, or toxic concentrations of ammonium ions. It is almost certain that nitrogen is not the critical limiting soil mineral in the Trelogan spoil. Similar results had been reported by Smith (1973) who showed that nitrogen was not an important limiting factor for plant growth on Trelogan mine spoil from the lower mine site because application of nitrogen in the form of slow release Nitroform, did not result any increase in growth over the control. Smith argued that phosphorous was the limiting factor in Trelogan mine waste. But McNeilly and Johnson (1981) found that nitrogen was indeed a limiting plant growth factor on both the calcareous Minera mine and acidic Y Fan mine spoils. The different results of the two investigators may be due to different types of mine spoil used and the species undertaken were originally from different mines. The importance of adequate nitrogen supply had been shown to be a major cause of sward regression in restored derelict land and many substrates by Bradshaw and Chadwick (1980), lack of nitrogen lead ultimately, to the deterioration of even the most successfully established ground cover vegetation.

From the results of this experiment it can be concluded that all fertilizer additions did not bring about any significant yield increase over the control except both doses of  $\text{NaNO}_3$  added to *R. acetosa* and single doses of  $\text{NaNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  increasing only the root dry weight of *M. verna* than control. This also may have been due to the adaptation to low soil nitrogen in plants on sites very low in nitrogen (Bradshaw *et al.*, 1964).

### References

- Bradshaw, A. D. and M. J. Chadwick, 1980. The restoration of land. Oxford: Blackwell Scientific Publications.
- Bradshaw, A. D., M. J. Chadwick, D. Jowett and R. W. Snaydon, 1964. Experimental investigations into the mineral nutrition of several grass species. IV. Nitrogen level. *J. Ecol.*, 52: 665-676.
- Bremner, J. M., 1965. Total nitrogen. *In: Method of Soil Analysis* (Ed by Black, C. A.). Madison, Wisconsin Amer. Soc. Agron, pp: 1149-76.
- Dancer, W. S., J. F. Handley and A. D. Bradshaw, 1977. Nitrogen accumulation in kaolin mining wastes in Cornwall. I. Natural communities. *Plant and Soil*, 48: 153-167.
- Davies, B. E., 1971. Trace metal content of soils affected by base metal mining in the west of England. *Oikos*, 22: 366-373.
- Davies, L. H., 1973. Two grass field trials with a sulphur coated urea to examine its potential as a slow release nitrogen fertilizer in the U.K. *J. Sci. Food Agric.*, 24: 63-67.

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- Davies, B. E. and L. J. Roberts, 1975. Heavy metals in soils and radish in mineralized area of Wales, Great Britain. *Sci. Tot. Env.*, 4: 249-261.
- Gemmell, R. P., 1975. Establishment of grass on waste on iron smelting. *Environ. Pollut.*, 8: 35-44.
- Hamid, A., and R. L. Mahler, 1994. The potential for volatilization losses of applied nitrogen fertilizers from northern Idaho soils. *J. Comm. in Soil Sci. and Plant Analysis*, 25:361-373.
- Johnson, M. S., 1977. The establishment of vegetation on metalliferous fluorspar mine tailings. Ph.D. Thesis, Univ. of Liverpool.
- Johnson, M. S. and A. D. Bradshaw, 1977. Prevention of heavy metal pollution from derelict mine sites by vegetative stabilization. *Trans. Inst. Mining and Metall*, 86 A: 47-55.
- Johnson, M. S. and A. D. Bradshaw, 1979. Ecological principles for restoration of disturbed and degraded land. *Appl. Biology*, 4: 141-200.
- McNeilly, T. and M. S. Johnson, 1981. Performance of Pb/Zn tolerant *Festuca rubra* on metalliferous spoil in relation to nitrogen source. *Fertilizer Res.*, 2: 135-146.
- Pollett, R. H., D. G. Westfall, J.F. Shanahan and D.W. Lybecker, 1995. Nitrogen fertilization of mountain meadows. *J. Production Agric.*, 8: 239-243.
- SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1, Cary, N.C: SAS Institute Inc.
- Smith, R. A. H., 1973. The reclamation of old metalliferous mine workings using tolerant plant populations. Ph.D. thesis, Univ. of Liverpool.
- Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. Second Edition.
- Thomas, H. F., F. Moor, E. Welsby, P.C. Elwood, and J.N.M. Firth, 1977. The hazards of old lead mines in Wales. *Brit. J. Soc. Medicine*, 31: 624-668.
- Thornton, I., 1975. Applied geochemistry in relation to mining and the environment. *In: Minerals and the Environment*. Jones M.J., ed., London. Institute of Mining and Metallurgy, pp: 1-16.
- Volk, G.M., 1961. Gaseous losses of ammonia from surface applied nitrogenous fertilizers. *J. Agric. Food Chem.*, 9: 280-283.