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# Response of *Azadirachta Indica* and *Eucalyptus tereticornis* to Bioinoculants (VAM, *Phosphobacterium* and *Azospirillum*) in Sewage Sludge Amended Soil

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## ABSTRAK

*Ujian kultur pot telah dijalankan pada tanah peroi merah bagi menilai tindak balas pertumbuhan dan pengertian nutrien* Azadirachta indica *dan* Eucalyptus tereticornis *yang tumbuh dalam tanah yang berbentuk asal dan tanah berlumpur yang telah diubah. Penggunaan lumpur telah mempertingkat pertumbuhan dan pengeluaran biojisim* A. indica *berbanding* E. tereticornis. *Gabungan inokulan terhasil dengan peningkatan signifikan pertumbuhan tanaman serta pengambilan nutrien. Keberkesanan inokulan lebih ketara pada spesies pokok yang tumbuh dalam tanah berlumpur. Ujian masa kini jelas menunjukkan kemungkinan penggunaan inokulasi sesuai bagi baja konvensional dalam memantapkan pokok-pokok hutan.*

## ABSTRACT

*Pot culture experiment was conducted in red loamy soil to evaluate the growth response and nutrient uptake of* Azadirachta indica *and* Eucalyptus tereticornis *grown in non-amended and sludge amended soil. Sludge application enhanced growth and biomass production of* A. indica *compared to* E. tereticornis. *Combined inoculants resulted in a significant increase in plant growth and nutrient uptake. Inoculant effectiveness was higher in tree species grown in sludge-amended soil. The present experiment clearly indicates the feasibility of using inoculation as suitable for conventional fertilizers in the establishment of forest trees.*

## INTRODUCTION

Urbanization and industrialization have lead to the generation of large volumes of wastewater. Huge quantities of wastewater and sludge produced from wastewater treatment plants pose problems of environment friendly disposal in many of the metros, corporations and municipalities. Often the heavy metals and pathogens present in the sludge represent a potential hazard to human if not properly handled. Therefore, it is always the interest of regulatory agencies as well as the public to search for an economic and environmentally sustainable option for sewage sludge disposal. Soil and its vegetation acts as a filter to clean the waste through uptake, accumulation and stockpiling of pollutants within their biomass (Perttu and Kowalik 1997).

Land application of sewage sludge is considered to be the best alternative, which provides scope not only to dispose the solid waste but also to exploit the nutrients in the sewage-sludge for productive purposes. Application of sewage sludge to land may be desirable in that it can improve the physical chemical and biological properties of the soils (Sauerbeck 1987), which in turn enhanced the plant growth and biomass production (Couillard and Grenier 1989; Labrecque *et al.* 1977). Sewage sludge often contained significant amounts of nitrogen, phosphorus, calcium, organic matter and trace elements such as Zn, Cu, and Mn and hence a good source of nutrients for plant growth (Tester 1990). In some bases, the biomass productivity of sludge-amended plots can be two to three times higher than that of control plots (Labrecque *et al.* 1995).

Numerous studies have been carried out on the utilization of sludge for agricultural crops. However, using sludge for forest species is more advantageous because it has the capabity to accumulate the toxic elements in its non-edible parts. As a result it diminishes the risk of human exposure to the harmful elements. In recents years, research has been focused on the use of sludge as a fertilizer with the aim of maximizing biomass production and natural recycling of nutrients. The use of tree plantations for the treatment of sludge and simultaneous production of forest produce (biomass) could become a viable method for waste management (Chakrabarti and Nashikkar 1994).

The aim of the present study was to assess the influence of bioinoculants and their interaction with sludge on growth response and nutrient uptake behaviour of two important forest tree species viz., *Azadirachta indica* and *Eucalyptus tereticornis.*

## MATERIALS AND METHODS

A pot culture experiment was conducted in a factorical randomized block design with three replications in a nutrient deficient red sandy loan soil. The pots were divided into two groups. One group was not treated with this sewage sludge (non-amended soil), whereas the other group received sewage sludge at the rate of 100 kg/ha (dry weight basis) (sludge amended soil). The sludge was mixed throughly with soil before filling into the pots (11 kg/pot). The experiment consisted of two types of tree seedlings vic., *Azadirachta indica* and *Eucalyptus tereticornis* and eight treatments viz., vesicular-arbuscular mycorrhiza (VAM) (TI) (25 g/pot), *Phosphobacterium* (PB) (T2) (5g/pot), *azospirillum* (AZO) (T3) (25g/pot), VM+PB (T4) (25.5g/pot), VAM+AZO (T5) (25,5g/pot), PB+AZO (T6) (5,5g/pot), VAM+PB+AZO (T7) (25,5,5g/pot) and control (T8). The bioinoculants were purchased from Tamil Nadu Agricultural University, soil and sludge were analyzed for their lhysico-chemical properties by adopting the standard procedure of Hesse (1971).

The initial plant height and stem diameter was measured at the first day of planting. These parameters were monitored at monthly intervals. Seedlings were allowed to grow for ten months and then harvested. At harvest, the plants were separated into leaves, stems and roots. Biomass production (dry weight), leaf area, chlorophyll and nutrient contents were determined. The leaf area was measured using a LI-COR area meter. Chlorophyll was extrated using acetone and determined by spectrophotometric analysis (Arnon 1949). Leaf materials were dried, ground and digested for the determination of nutrients. Nitrogen was estimated by Kjeldah's method. Hesse (1971) methods were followed to estimate phosphorus, potassium and calcium and magnesium using colorimetry, flame photometry and titrimetry. Data on plant growth, dry weight, leaf area, total chlorophyll and leaf nutrient content were subjected to analysis of variance (ANOVA) and the means separated using Duncan's multiple range test at P = 0.05%.

TABLE 1

| Parameters | Soil | Sludge |
|---|---|---|
| Textural group | Red sandy | - |
| Bulk density | 1.47 | - |
| Ph | 6.30 | 7.6 |
| Ec (m.mohs $cm^{-1}$) | 0.47 | 1.8 |
| Available N (mg $kg^{-1}$) | 38 | 347.81 |
| Available P (mg $kg^{-1}$) | 17.00 | 290.00 |
| Available K (mg $kg^{-1}$) | 21.50 | 129.00 |
| Extractable Ca (%) | 0.24 | 1.20 |
| Extractable Mg (%) | 0.09 | 0.64 |

## RESULTS

*Plant Growth*

Plant height and basal width of *E. tereticornis* on 290th day after planting were greater in sludge-amended soil (Table 2). Sludge amended significantly increased growth of *E. tereticornis* while in *A. indica* the growth rate was slightly higher than the non-amended soil. In *Azadirachta indica*, sludge amendment also resulted in significant differences among the treatments. All the treatments except VAM+PB and VAM+PB+AZO combination increased plant height in sludge-amended soil. In non-amended soil there were significant differences in plant height between treatments, with maximum height recorded from the control (152 cm). The results obtained for *E. tereticornis* contrast the results obtained for *Azadirachta indica.* The combination of VAM+PB+AZO significantly influenced plant growth of *E. tereticornis* (Table 2).

The increase in basal width was similar to that of plant height. In general, plants grown in sludge-amended soil had higher value than plants grown in non-amended soil. Maximum

TABLE 2

Effect of inoculants on growth and biomass production of *Azadirachta indica* and *Eucalyptus tereticornis* grown in sludge amended and non amended soils

| | Non amended | | | | | | | Sludge amended soil | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Plant Height (cm) | Basal Diameter (cm) | Biomass Leaf ($g\text{-}plant^{-1}$) | Biomass Stem ($g\text{-}plant^{-1}$) | Biomass Root ($g\text{-}plant^{-1}$) | Leaf area ($cm^2$) | Chlorophyll (a+b) ($mgg^{-1}$) | Plant Height (cm) | Basal Diameter (cm) | Biomass Leaf ($g\text{-}plant^{-1}$) | Biomass Stem ($g\text{-}plant^{-1}$) | Biomass Root ($g\text{-}plant^{-1}$) | Leaf area ($cm^2$) | Chlorophyll (a+b) ($mgg^{-1}$) |
| a) *Azadirachta indica* | | | | | | | | | | | | | | |
| VAM | 123.7 d | 1.62 ab | 21.0 a | 63.3 bc | 38.8 a-c | 17.4 a | 2.05 a | 152.0 a | 2.16 a | 27.3 a | 90.7 bc | 34.0 c | 18.4 a | 2.63 a |
| PB | 116.0 e | 1.47 ab | 17.7 c | 57.0 c | 35.0 ab | 17.1 a | 2.38 a | 144.3 b | 2.09 ab | 31.3 a | 88.3 b-d | 45.7 bc | 16.6 a | 2.85 a |
| AZO | 133.0 c | 1.59 ab | 17.0 a | 68.7 a-c | 20.7 c | 17.9 a | 1.95 a | 147.3 ab | 2.17 a | 30.3 a | 106.0 a | 57.0 ab | 18.8 a | 2.48 a |
| VAM+PB | 147.3 b | 1.64 ab | 19.3 a | 64.3 a-c | 7 a-c | 16.6 a | 1.90 a | 136.0 d | 2.08 ab | 29.0 ab | 98.0 ab | 69.7 a | 19.0 a | 2.57 a |
| VAM+AZO | 129.7 c | 1.40 b | 20.0 a | 63.3 bc | 21.7 bc | 17.2 a | 1.92 a | 138.3 cd | 1.91 a-c | 27.0 a | 91.3 bc | 60.0 a | 15.6 a | 3.04 a |
| PB+AZO | 118.0 e | 1.39 b | 16.7 a | 56.3 c | 27.7 a-c | 16.5 a | 2.02 a | 143.0 bc | 1.74 c | 24.3 a | 93.3 b | 58.0 ab | 17.6 a | 2.70 a |
| VAM+PB+AZO | 148.0 b | 1.80 a | 21.7 a | 76.7 a | 37.3 a | 17.2 a | 2.24 a | 121.7 e | 1.70 c | 24.3 a | 80.3 cd | 40.7 c | 18.2 a | 2.71 a |
| Control | 153.0 a | 1.72 ab | 23.3 a | 72.3 ab | 28.7 a-c | 15.8 a | 1.91 a | 123.0 e | 1.80 bc | 23.3 a | 76.7 d | 39.3 c | 15.3 a | 1.84 b |
| b) *Eucalyptus tereticornis* | | | | | | | | | | | | | | |
| VAM | 215.0 b | 1.80 a | 41.0 a | 86.0 a | 54.3 a | 41.9 ab | 1.61 a | 212.3 d | 1.75 a-c | 46.3 b | 89.0 a | 51.3 ab | 50.1 bc | 1.98 a |
| PB | 192.0 d | 1.72 b | 41.7 b | 77.7 a-c | 52.7 ab | 44.7 a | 1.26 a | 215.3 d | 2.01 a | 32.3 c | 90.0 a | 56.7 a | 43.6 cd | 1.80 ab |
| AZO | 197.0 d | 1.78 b | 47.3 a | 77.3 a-c | 40.0 bc | 35.9 bc | 1.28 a | 185.0 f | 1.57 c | 34.7 c | 67.3 a | 38.0 bc | 47.9 bc | 1.47 a-c |
| VAM+PB | 161.0 e | 1.49 b | 32.0 b | 70.0 bc | 37.0 c | 40.9 ab | 1.41 a | 171.3 g | 1.51 c | 29.0 c | 65.7 a | 40.7 bc | 38.5 d | 1.22 bc |
| VAM+AZO | 217.0 b | 1.71 b | 26.7 b | 71.7 bc | 47.3 a-c | 30.9 cd | 1.04 a | 192.7 e | 1.17 d | 49.3 b | 83.0 a | 41.7 bc | 55.8 ab | 1.72 a-c |
| PB+AZO | 221.0 b | 1.79 b | 43.7 a | 83.0 ab | 43.7 a-c | 43.4 ab | 1.68 a | 240.3 c | 1.97 ab | 61.3 a | 87.3 a | 47.3 ab | 48.8 bc | 1.80 ab |
| VAM+PB+AZO | 228.0 a | 1.70 b | 28.0 b | 75.7 a-c | 33.7 c | 39.8 ab | 1.32 a | 268.3 a | 1.73 a-c | 48.0 b | 91.0 a | 28.7 c | 58.3 a | 1.37 a-c |
| Control | 205.0 c | 1.59 b | 29.3 b | 67.3 c | 36.3 c | 27.2 d | 1.34 a | 247.3 b | 1.65 bc | 50.3 b | 81.7 a | 33.3 c | 46.3 c | 1.13 c |

Mean followed by the same letter are nor significantly ($p<0.05$) different as determined by Duncan's Multiple Range Test in table and figure 1.

diameter increments were recorded in single inoculation of AZO on *A. indica* and PB inoculated pots on *E. tereticornis,* both in sludge-amended soils.

*Dry Weights of Leaf, Stem and Roots*

Inoculation did not show any pronounced effect on left dry weight when compared to the control in *A. indica.* The effect of inoculants was on par with control both in non-amended and sludge-amended soil. However, *E. tereticornis* in inoculant significantly increased leaf dry weight when compared to control. Higher leaf dry weight was recorded from plants inoculated with PB+AZO in sludge-amended soil, followed by VAM+AZO in the same group (Table 2).

The effect of inoculants on stem dry weight of *A. indica* showed significant variation among the treatments both in sludge and non-amended soil. VAM treatment significantly increased stem dry weight when compared to all the other inoculants in both soil amendments. In *E. tereticornis* higher stem dry weight was recorded in VAM inoculated pots grown in non-amended soil, while in the sludge amended soil the effect of inoculants were similar to that of control. In A. *indica,* association with VAM+PB recorded higher root dry weight in sludge amended soil than the other combinations. PB treated plants showed pronounced effect when compared to the other treatments in *E. tereticornis* (Table 2).

*Leaf Area*

There was a significant increase in the leaf area of both the tree species in sludge amended soil. In *A. indica* the trends were similar to that of chlorophyll. In *E. tereticornis* the effectiveness of the treatment VAM+PB+AZO increased leaf area of *E. tereticornis* (Table 2).

*Total Chlorophyll*

The total chlorophyll (a+b) content of *A. indica* was higher than *E. tereticornis.* Application of sludge increased chlorophyll content of both tree species. However, the inoculants did not influence the total chlorophyll content of *A. indica* but influence the chlorophyll content of *E. tereticornis.* Higher chlorophyll (1.98) was recorded in sludge amended soil inoculated with VAM. The lowest value was from the control the plants.

*Leaf Nutrients*

The nutrient contents in leaves of *A. indica* and *E.tereticornis* grown in non-amended and sludge amended soils showed that sludge application facilitated higher uptake of nutrients (N, Ca and Mg). Comparison between the two species revealed that *A. indica* benefited more than *E. tereticornis* with respect to phosporus, potassium and calcium. However, there is no significant variation in uptake of Mg between the two species. The inoculants showed variable effects on tree species and sludge amendment (Fig. 1).

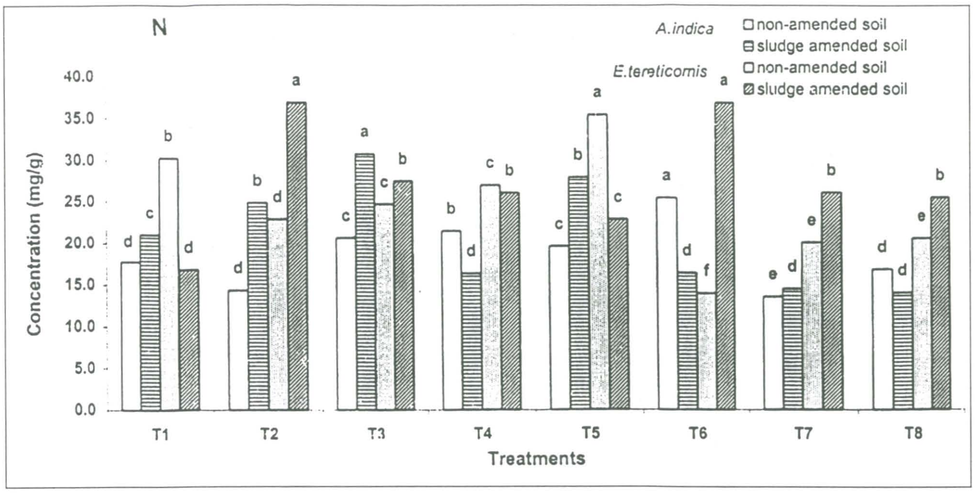
Dual inoculation significantly increased leaf nutrient concentrations. Significant increase in N, K and Ca was observed in plants inoculated with PB+AZO and VAM+PB. Combination of PB+AZO recorded higher uptake of Mg in *A. indica* grown in sludge-amended soil than the other treatments. Higher Mg uptake was observed in *E. tereticornis* sludge amended soil. (Fig. 1).

VAM inoculation recorded higher concentration of phoshorus in non-amended soil, but in sludge amended soil inoculation with PB resulted in higher levels of phosphorus concentration in the leaves. The plants in the control treatment recorded the least amount of P with an exception of *E. tereticornis* is non-amended soil (Fig. 1)

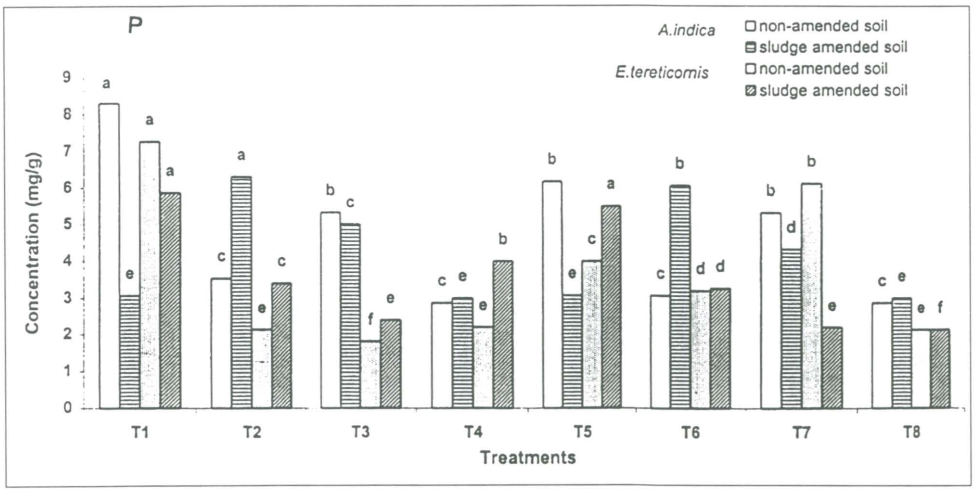
## DISCUSSION

Results on the growth response revealed variability in plant height and increment for both *A. indica* and *E. tereticornis.* This probably indicates the dominance of inoculam species variability. In *A. indica,* maximum height was observed in VAM inoculated pots, while in *E. tereticornis,* it was in the VAM+PB+AZO treatment. The enhanced plant growth in sludge-amended soil was probably the result of increased availability of plant nutrients in the soil.

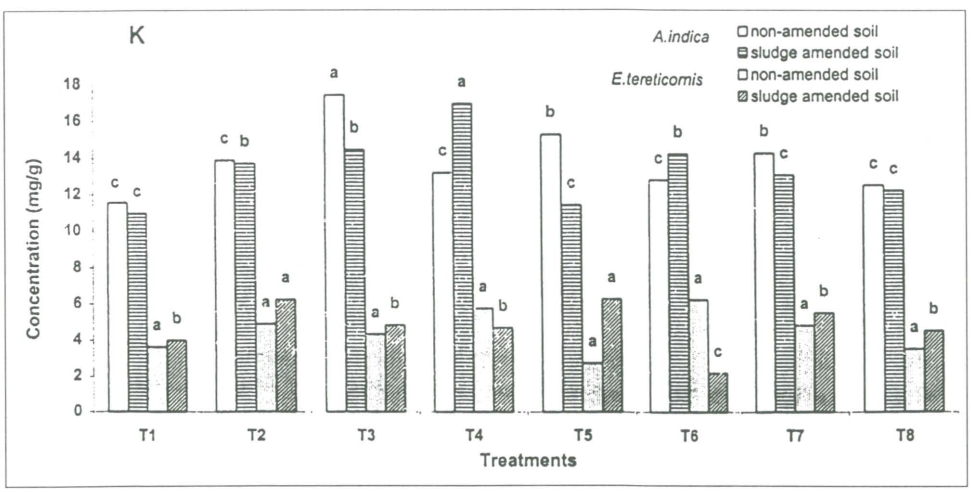
Schneider *et al.* (1991) reported that the survival, growth and vigor of hard wood and pine seedlings increased after sewage sludge application and that the growth of the hard wood and pine were not uniform. Danielson (1990) reported that addition of sewage sludge had a positive effect on growth and mycorrhizal development in Picea and Pinus spp. This effect however could not be wholly attributed to the amendment. Sopper (1992) reported that sludge


N
A.indica
E.tereticornis
non-amended soil
sludge amended soil
non-amended soil
sludge amended soil
Concentration (mg/g)
Treatments
T1 T2 T3 T4 T5 T6 T7 T8

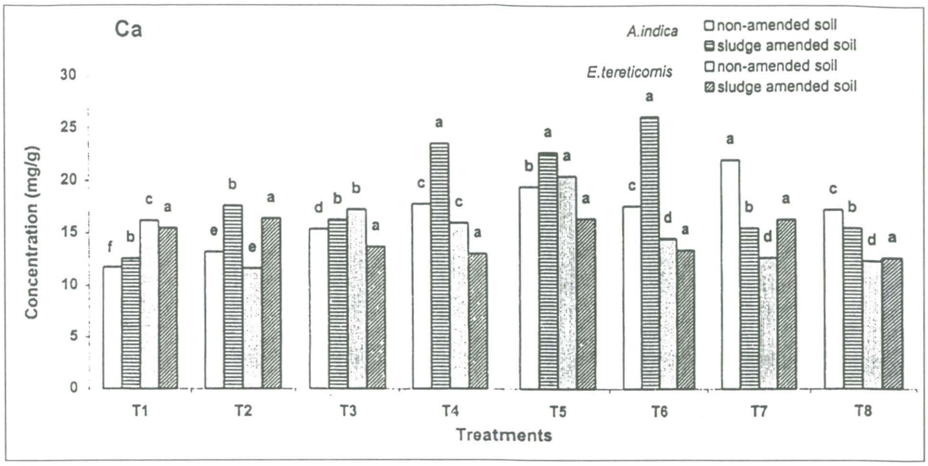
1(a)


P
A.indica
E.tereticornis
non-amended soil
sludge amended soil
non-amended soil
sludge amended soil
Concentration (mg/g)
Treatments
T1 T2 T3 T4 T5 T6 T7 T8

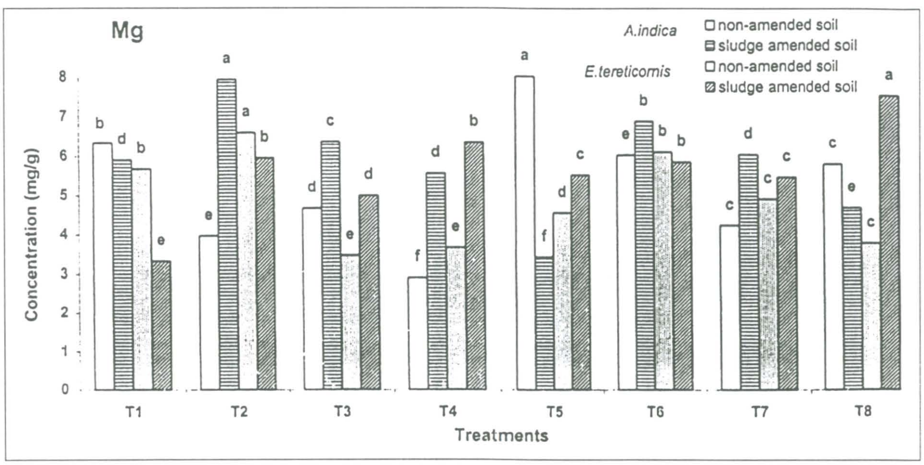
1(b)


K
A.indica
E.tereticornis
non-amended soil
sludge amended soil
non-amended soil
sludge amended soil
Concentration (mg/g)
Treatments
T1 T2 T3 T4 T5 T6 T7 T8

1(c)




1(d)




1(e)

*Fig. 1. Influence of bio-inoculants on nutrient (N P K Ca Mg) status of A. indica and E. tereticornis*

application enhanced the growth of woody vegetation and yield compared to application of inorganic fertilizers. Labrecque *et al.* (1997) further supported the above conclusions.

The data on biomass production (leaf, stem and root) clearly indicated that leaf and stem biomass production was greater in sludge-amended soil for both the tree species. However, in *E. tereticornis* the root biomass was higher in non-amended soil. Sludge application has resulted in enhanced leaf (27%) stem (28%) dan root biomass (41.25%) in *A. indica*. In *E. tereticornis* it was 17.5% for leaf biomass and 7% for stem biomass. The results have been reported in *Larix laricina* (Couillard and Grenier 1989), *Populus sp.* (Mc Inotosh *et al.* 1984), *Pinus aiba* (Brockway 1983) and *Salix species* (Labrecque *et al.* 1995).

Leaf area forms the basis of the photosynthetic activity and hence attainment of optimum leaf area is essential in maximizing productivity (Wittwer and Stringer 1985). The increased leaf area indices suggest that sludge application enhanced photosynthetic activity, which in turn is reflected on biomass production. As a result biomass production was higher in sludge-amended soil. Labrecque *et al.* 1995 reported similar results.

The nutrient status of the soil is one of the factors influencing the chlorophyll contents in the leaf (Lewandowska and Jarvis 1977). In the present study, cholorophyll levels were higher in sludge-amended soil, which could be attributed to the influence of available nutrients. Shrive *et al.* (1994) observed increased photosynthetic rate in res maple (*Acer rubrum L.*) and hybrid popular (*Populus sp. Nigra x maximowiczii*) irrigated with wastewater. They have concluded that vegetation species selection and the quantity of the leachate at landfills are the important factors to be considered for forest plantations established for the purpose of land application of municipal solid waste.

Sopper (1992) in his review has indicated that an increase in the major plant nutrients (N, P, K, Ca and Mg) is usually observed in crops grown in sludge-amended soils. Results from the present study revealed wide variations in uptake of N, P, K, Ca and Mg among the tree seedlings, with or without sludge amendments. Similar results were reported by many authors (Michelsen and Rosendhal 1990; Sanginga *et al.* 1995; Whitbread-Abrutat 1977). Increase in accumulation of nutrients was observed in plants inoculated with VAM+PB and PB+AZO compared to single inoculation. Piccini *et al.* (1988) and Greep *et al.* (1997) who observed that inoculation of VAM and *rhizobium spp* in sterilized soils increase the nutrient content in *Medicago sativa* L., (alfalfa) and *Macroptilium atropurpureum* Urb (siratro), respectively. The increase in elemental contents of Ca, Mg, k and P were reported in certain studies after the establishment of the fungal association with the various host plants and trees (Schwab *et al.* 1991; Osonubi *et al.* 1995; Goicoechea *et al.* 1997).

## CONCLUSION

The present study clearly demonstrates that combination of sewage sludge and microbial and inoculants could be used for the establishment of forest species wastelands or nutrient deficient soil.

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