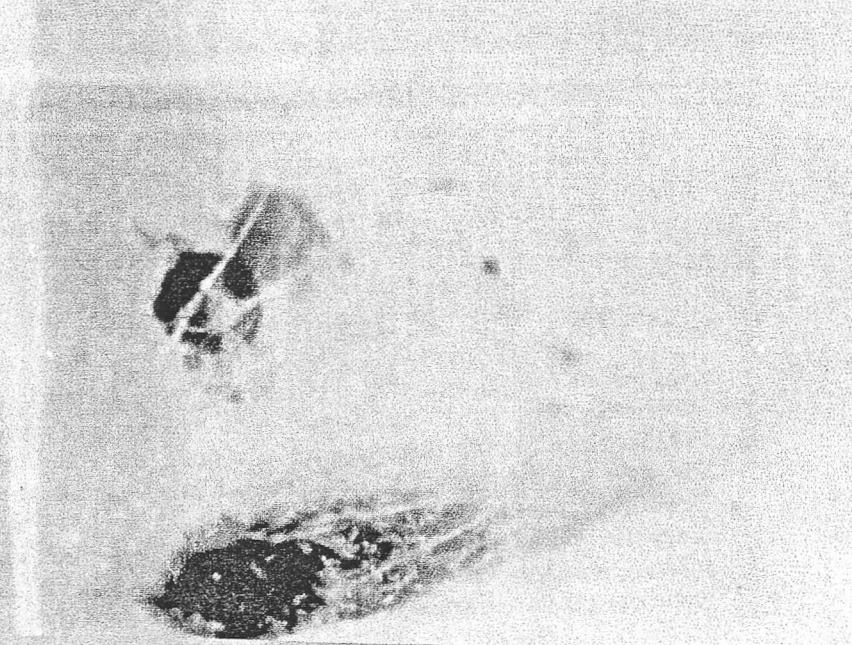


MYCORRHIZA

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EFFECT OF HEAVY METALS ON MYCORRHIZAL FORMATION AND GROWTH OF *Pinus kesiya* SEEDLINGS

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ABSTRACT

An experiment was carried out under field conditions to study the effect of heavy metals on ectomycorrhizal system of pine seedlings. Aseptically germinated seedlings were grown and inoculated with *Boletus* sp., *S. aurantium*, *S. luteus*, and *C. graniforme*. The seedlings were then treated with heavy metals (Cd, Cu, Zn, Ni, Pb and Al). The ectomycorrhizal symbionts enhanced the growth of pine seedlings. However, plants treated with heavy metals (50 ppm and above) had a deleterious effect on the growth and survival of pine seedlings.

INTRODUCTION

The knowledge of ectomycorrhizal function is being applied to improve the success of reforestation programme throughout the world. Ectomycorrhizae constitute an important element of forest fungal communities (Vogt *et al.*, 1991). Many tree species, including conifers are able to grow in poor soil condition due to their extensive mycorrhizal root system (Koide and Li, 1995). *P. kesiya*, an early colonizer among tree species is highly ectomycotrophic (Kumar, 1990; Jha *et al.*, 1991). Ectomycorrhiza helps in the growth, establishment and survival of pine seedlings. Though studies on mycorrhizae

have received enough attention during the past few years, yet, their interaction in relation to environmental factors further needs careful investigation, particularly with respect to the role of heavy metals. Atmospheric input of heavy metals often leads to their accumulation in the forest soil ecosystem and reduce mycorrhizal infection (Dixon *et al.*, 1988). Mycorrhizal fungi affect the survival of plants on metal contaminated soils by influencing the uptake and accumulation of toxic ions. Several ectomycorrhizal fungi can protect their host plant against toxicity of heavy metals (Jones and Hutchinsons, 1986). The interaction between these contaminants have been studied with respect to the toxicity.

MATERIALS AND METHODS

The study was conducted at the pine forest stand in Permanent Campus of North Eastern Hill University, Mawkyroh, Shillong (altitude 1,500m MSL, latitude 25.34° N, longitude 91°54'E) in the East Khasi hills district of Meghalaya. Two sites were selected on a gentle slope. Thirty six microplots were prepared and irrigated, as needed for both sites. The seeds of *P. kesiya* were sterilised and sown in December. *S. luteus* and *C. graniforme* were collected and their sporocarps were air dried and mixed with distilled water. After one month of germination of seedlings, plants were inoculated with slurry of mixed ectomycorrhizal inoculum. After confirming the mycorrhizal establishment, seedlings were treated with different concentration of heavy metals such as 0, 10, 50, 100, 200 and 500 ppm of Cd, Pb, Cu, Zn, Ni and Al. A controlled set was maintained in the same manner, except that the seedlings were not inoculated with mycorrhizal fungi. Seedlings were harvested from each plot on the 4th, 8th and 12th month after treatment with heavy metals. At each harvest, shoot height, needle length, number of needles, seedlings volume, root collar diameter, root length and mycorrhizal infection were measured and the percentage of mycorrhizal colonization was calculated as follows:

$$\text{Ectomycorrhizae (\%)} = \frac{\text{Number of mycorrhizal lateral rootlets}}{\text{Total number of lateral rootlets}} \times 100$$

Seedling volume was calculated as $D^2 H$ or

$$(\text{root collar diameter})^2 \times \text{height (Marx, 1982)}.$$

Percentage of survival of seedling was determined by:

$$\text{Survivability (\%)} = \frac{\text{Total number of seedlings at the time of harvesting}}{\text{Total number of seedlings at the time of germination}} \times 100$$

The data was processed by the analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Ectomycorrhizal infection was visible after two months of inoculation. After 4 months, the percentage of infection ranged between 25-40% in seedlings treated with heavy metals. In untreated seedlings, the mycorrhizal infection was 45%. After 8 months, the infection increased to 40-60% in seedlings treated with heavy metals and the controlled seedlings showed 73% of mycorrhizal infection. Highest percentage of mycorrhizal infection was observed in Pb treated seedlings after one year, whereas in others the mycorrhizal infection decreased as concentration of heavy metals increased. Untreated controlled seedlings showed 90% of mycorrhizal infection after one-year (Table 2).

Stem height was maximum at Zn and Ni treated seedlings on the 4th month and minimum in Cd treated seedlings. It increased by 1-2 cm in each seedlings of different treatments after 8 months. After one year, the highest shoot height was observed in Cd treated seedlings and lowest in Pb and Ni (Table 3), treated seedlings. In case of non-mycorrhizal plants, the shoot height was maximum in Ni treated seedlings and minimum in Cu treated seedlings (Table 2).

Needle length was maximum and minimum in Pb and Zn treated non-mycorrhizal seedlings. In case of mycorrhizal plants, Pb treated showed maximum and minimum in Ni treated seedlings. Number of needles were highest in Pb treated mycorrhizal seedlings and minimum in non-mycorrhizal ones. Root length was maximum in mycorrhizal seedlings treated with Cu and Zn. The untreated mycorrhizal seedlings showed better root than heavy metal treated ones. The elongation of primary and lateral roots was reduced by addition of heavy metals, especially at the concentration of 50 ppm and above. Seedlings volume was maximum and minimum with Cd and Pb treated mycorrhizal seedlings. Highest percentage of survival was observed with Ni and Zn treated mycorrhizal seedlings.

Table 1. Effect of heavy metals on establishment and growth of One-year old pine seedlings under field conditions

| Metal conc. (ppm) | Shoot height (cm) | Needle length (cm) | No. of needles | Seedlings vol. (cm) ³ | Root collar dia. (cm) | Root length (cm) |
|-------------------|-------------------|--------------------|----------------|----------------------------------|-----------------------|------------------|
| 0 | 14.0 | 5.3 | 167 | 0.590 | 0.25 | 30.5 |
| Zn(10) | 6.6 | 3.8 | 113 | 0.319 | 0.22 | 27.1 |
| Zn(50) | 5.5 | 3.5 | 89 | 0.266 | 0.22 | 20.8 |
| Zn(100) | 5.4 | 2.5 | 78 | 0.194 | 0.19 | 16.1 |
| Zn(200) | 5.2 | 2.5 | 57 | 0.168 | 0.18 | 14.8 |
| Zn(500) | 5.0 | 2.4 | 54 | 0.162 | 0.18 | 10.6 |
| Ni(10) | 9.5 | 3.8 | 146 | 0.418 | 0.21 | 26.0 |
| Ni(50) | 8.2 | 3.0 | 112 | 0.296 | 0.19 | 21.1 |
| Ni(100) | 7.0 | 2.7 | 102 | 0.252 | 0.19 | 15.8 |
| Ni(200) | 6.1 | 2.6 | 92 | 0.156 | 0.16 | 15.1 |
| Ni(500) | 5.9 | 2.4 | 87 | 0.132 | 0.15 | 14.8 |

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| Metal conc. (ppm) | Shoot height (cm) | Needle length (cm) | No. of needles | Seedlings vol. (cm) ³ | Root collar dia. (cm) | Root length (cm) |
|----------------------|----------------------|-----------------------|-------------------|-------------------------------------|--------------------------|---------------------|
| Al(10) | 9.1 | 3.8 | 136 | 0.481 | 0.23 | 16.1 |
| Al(50) | 8.5 | 3.6 | 78 | 0.411 | 0.22 | 16.0 |
| Al(100) | 6.0 | 2.9 | 77 | 0.290 | 0.22 | 15.6 |
| Al(200) | 5.8 | 2.8 | 74 | 0.209 | 0.18 | 15.0 |
| Al(500) | 5.5 | 2.7 | 58 | 0.178 | 0.18 | 14.5 |
| Cd(10) | 9.5 | 3.5 | 120 | 0.419 | 0.21 | 16.3 |
| Cd(50) | 8.5 | 3.0 | 119 | 0.255 | 0.21 | 16.0 |
| Cd(100) | 5.4 | 2.7 | 91 | 0.138 | 0.16 | 15.4 |
| Cd(200) | 5.0 | 2.5 | 83 | 0.128 | 0.16 | 14.8 |
| Cd(500) | 4.5 | 2.3 | 66 | 0.064 | 0.12 | 14.6 |
| Cu(10) | 8.2 | 3.5 | 116 | 0.361 | 0.21 | 26.4 |
| Cu(50) | 6.8 | 3.4 | 106 | 0.174 | 0.16 | 23.0 |
| Cu(100) | 6.0 | 3.1 | 105 | 0.150 | 0.14 | 16.0 |
| Cu(200) | 5.5 | 3.1 | 105 | 0.079 | 0.12 | 15.0 |
| Cu(500) | 5.4 | 2.5 | 85 | 0.077 | 0.12 | 13.8 |
| Pb(10) | 7.0 | 4.5 | 111 | 0.308 | 0.21 | 18.9 |
| Pb(50) | 6.5 | 3.7 | 98 | 0.286 | 0.21 | 18.0 |
| Pb(100) | 6.1 | 3.2 | 81 | 0.156 | 0.16 | 17.1 |
| Pb(200) | 5.8 | 2.8 | 76 | 0.148 | 0.16 | 17.0 |
| Pb(500) | 5.7 | 2.7 | 59 | 0.082 | 0.12 | 15.9 |

Table 2. Effect of heavy metals on survivability and mycorrhizal infection in pine seedlings inoculated with mixed mycorrhizal fungi under field conditions

| Metal conc. (ppm) | Survivability (%) | | | Mycorrhizal infection (%) | | |
|----------------------|-------------------|----------|----------|---------------------------|----------|----------|
| | 120 days | 240 days | 360 days | 120 days | 240 days | 360 days |
| 0 | 100 | 93 | 93 | 45 | 73 | 90 |
| Zn(10) | 100 | 94 | 86 | 41 | 64 | 72 |
| Zn(50) | 86 | 86 | 80 | 40 | 61 | 70 |
| Zn(100) | 80 | 80 | 73 | 35 | 53 | 69 |
| Zn(200) | 80 | 76 | 66 | 30 | 50 | 62 |
| ZN(500) | 73 | 73 | 50 | 30 | 42 | 53 |
| Ni(10) | 100 | 90 | 86 | 43 | 62 | 73 |
| Ni(50) | 93 | 86 | 80 | 39 | 55 | 64 |
| Ni(100) | 90 | 86 | 80 | 36 | 51 | 61 |
| Ni(200) | 86 | 80 | 73 | 36 | 43 | 59 |
| Ni(500) | 80 | 80 | 73 | 30 | 43 | 56 |
| Al(10) | 100 | 90 | 90 | 38 | 58 | 70 |
| Al(50) | 90 | 80 | 86 | 36 | 57 | 63 |
| Al(100) | 80 | 80 | 74 | 30 | 49 | 60 |
| Al(200) | 73 | 76 | 73 | 30 | 42 | 52 |
| Al(500) | 73 | 60 | 60 | 28 | 41 | 48 |
| Cd(10) | 93 | 93 | 86 | 40 | 61 | 71 |

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| Metal conc. (ppm) | Survivability (%) | | | Mycorrhizal infection (%) | | |
|----------------------|-------------------|----------|----------|---------------------------|----------|----------|
| | 120 days | 240 days | 360 days | 120 days | 240 days | 360 days |
| Cd(50) | 90 | 90 | 80 | 38 | 59 | 65 |
| Cd(100) | 86 | 86 | 80 | 32 | 51 | 63 |
| Cd(200) | 86 | 80 | 73 | 31 | 43 | 60 |
| Cd(500) | 80 | 76 | 66 | 25 | 40 | 51 |
| Cu(10) | 100 | 93 | 86 | 41 | 59 | 68 |
| Cu(50) | 90 | 90 | 80 | 37 | 56 | 66 |
| Cu(100) | 86 | 80 | 80 | 37 | 51 | 63 |
| Cu(200) | 80 | 80 | 76 | 31 | 43 | 55 |
| Cu(500) | 273 | 73 | 66 | 26 | 39 | 50 |
| Pb(10) | 93 | 91 | 86 | 42 | 60 | 67 |
| Pb(50) | 88 | 86 | 85 | 39 | 58 | 63 |
| Pb(10) | 81 | 77 | 80 | 36 | 50 | 56 |
| Pb(200) | 76 | 73 | 71 | 33 | 42 | 51 |
| Pb(500) | 71 | 70 | 62 | 278 | 36 | 48 |

Inoculation of ectomycorrhizal fungi consistently stimulated the shoot height, number of needles, needle length, seedlings volume, root length and root collar diameter as compared to non-mycorrhizal ones. Macfall and Slack (1991) also noted significant increase in pine (*Pinus resinosa*) seedlings root and shoot dry weights inoculated with ectomycorrhizal fungi as compared to non-mycorrhizal ones. Similarly, Gbdegesion (1990) observed better survival and growth of pine (*Pinus oocarpa*) seedlings with *P. tinctorius* in field condition after a period of four years.

In this study, the pine root were most susceptible to heavy metal damage than other growth parameters and all the ectomycorrhizal fungi appeared to ameliorate this damage. Initially roots became dark, short and thick; symptoms attributed to metal toxicity (Menge *et al.*, 1982). However, after fungal inoculation and mycorrhizae formation, root grew normally when compared with dark brown, short and stubby roots observed in non-mycorrhizal plants. The presence of growing fungus appeared to reduce the toxicity of Cd, Ni, Zn, Cu, Pb and Al. The toxicity symptoms remained in uninoculated plants. It appeared that either the plant itself or the inoculated fungus was able to bind the metals, mediate the rhizosphere pH, or that the root exudates form complex with the available metals so that the element was no longer a problem. The ectomycorrhizal fungi increased the tolerance of pine seedlings to heavy metals. Although, the fungi did not prevent completely the effect of metals in reducing the seedlings growth, but ectomycorrhizal fungi grew better than non-mycorrhizal ones at all level of metals, which could be due to their inoculum potential. The strong impact of mixed inoculum on host plant and fungal growth rate may have major ecological implications and will have a competitive advantage in infecting more root tips. Enhancement of host growth and mycorrhizal colonization due to mixed inoculum was also observed in jack pine (Gardes *et al.*, 1990; De la Bastide *et al.*, 1995).

Table 3. Effect of heavy metals on establishment and growth in One-year old pine seedlings inoculated with mixed mycorrhizal fungi in field condition

| Metals conc. (ppm) | Shoot height (cm) | Needle length (cm) | No. of needles | Seedling vol. (cm) ³ | Root collar dia. (cm) | Root length (cm) |
|--------------------|-------------------|--------------------|----------------|---------------------------------|-----------------------|------------------|
| 0 | 16.7 | 5.8 | 219 | 1.00 | 0.25 | 48.7 |
| Zn10 | 13.6 | 4.7 | 201 | 0.783 | 0.24 | 44.0 |
| Zn50 | 12.2 | 4.2 | 190 | 0.702 | 0.24 | 41.0 |
| Zn100 | 11.0 | 3.6 | 189 | 0.581 | 0.23 | 38.0 |
| Zn200 | 10.3 | 3.2 | 172 | 0.498 | 0.22 | 20.0 |
| Zn500 | 10.0 | 3.1 | 156 | 0.484 | 0.22 | 18.0 |
| Ni10 | 12.6 | 4.5 | 173 | 0.725 | 0.25 | 32.0 |
| Ni50 | 12.2 | 3.7 | 168 | 0.624 | 0.21 | 31.0 |
| Ni100 | 11.9 | 3.6 | 153 | 0.575 | 0.22 | 26.0 |
| Ni200 | 8.7 | 3.2 | 115 | 0.383 | 0.21 | 25.0 |
| Ni500 | 7.9 | 3.1 | 111 | 0.285 | 0.19 | 21.0 |
| Al10 | 13.1 | 4.7 | 204 | 0.754 | 0.24 | 39.5 |
| Al50 | 11.2 | 4.3 | 169 | 0.592 | 0.23 | 37.0 |
| Al100 | 11.0 | 4.2 | 146 | 0.581 | 0.23 | 33.0 |
| Al200 | 10.0 | 4.0 | 140 | 0.484 | 0.22 | 30.0 |
| Al500 | 9.8 | 3.3 | 131 | 0.432 | 0.21 | 29.0 |
| Cd10 | 15.3 | 4.8 | 198 | 0.809 | 0.23 | 30.0 |
| Cd50 | 15.1 | 4.5 | 183 | 0.798 | 0.23 | 22.0 |
| Cd100 | 14.0 | 4.4 | 169 | 0.677 | 0.22 | 18.0 |
| Cd200 | 13.3 | 3.9 | 143 | 0.643 | 0.22 | 13.9 |
| Cd500 | 12.0 | 3.8 | 121 | 0.388 | 0.18 | 12.9 |
| Cu10 | 15.5 | 4.4 | 201 | 0.892 | 0.24 | 41.0 |
| Cu50 | 12.1 | 4.2 | 151 | 0.698 | 0.24 | 40.0 |
| Cu100 | 10.6 | 4.0 | 139 | 0.513 | 0.22 | 39.0 |
| Cu200 | 10.1 | 3.4 | 133 | 0.445 | 0.21 | 32.0 |
| Cu500 | 9.6 | 3.2 | 119 | 0.423 | 0.21 | 28.0 |
| Pb10 | 12.0 | 5.1 | 207 | 0.691 | 0.24 | 31.0 |
| Pb50 | 11.3 | 4.3 | 206 | 0.650 | 0.24 | 26.0 |
| Pb100 | 10.0 | 4.2 | 183 | 0.529 | 0.23 | 23.0 |
| Pb200 | 9.8 | 3.8 | 178 | 0.432 | 0.21 | 19.0 |
| Pb500 | 9.4 | 3.7 | 169 | 0.240 | 0.16 | 16.9 |

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