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# EFFECTS OF TANNERY WASTE ON LEGUME-*RHIZOBIUM* SYMBIOTIC PROCESS IN SOIL

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*Key words :* Tannery waste, Bacterial population, Nitrogen fixation

**Abstract**

Experiments conducted to investigate the effects of tannery waste on bacterial population, especially on legume-*Rhizobium* symbiotic process showed that in both contaminated and uncontaminated soils, bacterial population and growth were higher at low rate (2.5 t/ha) tannery waste treatment. Nitrogen fixation by cow pea (*Vigna sinensis*) was also higher at low rate tannery waste treatment.

## Introduction

Tannery industry is one of the most important industries in Bangladesh. It is the fourth largest foreign exchange earner of the country, contributing to about 6% of the total export earnings.(1) Of the 214 tanneries in Bangladesh, 200 are concentrated in Dhaka city. One hundred forty nine of them are located at Hazaribagh area near river Buriganga.(1) In the Hazaribagh area, about 260 MT of hides and skins are processed per day. These tannery wastes (TW) are discharged and dumped (solid and effluent) without treatment into nearby soil and water bodies and thus pollutes those areas.

Although TW are rich in organic matter, yet, these contain huge amount of Na, S and other inorganic compounds and various heavy metals such as Cr, Cd, Pb etc.(2) These have effect on various biological activities of soil. Brook(3) observed that yield and $N_2$-fixation capacity of Clover *Rhizobium liguminosarum-Biover trifoli* association was hampered by applying such heavy metals. This study reflects the effect of TW on bacterial population especially the legume- *Rhizobium* symbiotic process.

## Materials and Methods

Samples were collected from four sites of Hazaribagh tannery area. One was taken from outside the embankment and three from inside the embankment. The 1st spot was near the main outfall area of Rajib Leather Factory, 2nd spot was about

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500 meter and 3rd spot was 1 km away from the 1st spot, respectively. The 4th spot was outside the embankment. Soil and waste samples were collected from first three spots but from the 4th spot only soil samples were collected. The surface (0 - 15 cm) soil of Chandina series (loamy mixed, non-acid, Aeric Haplaquept) was used as control. The compositions of the collected samples are presented in Tables 1 - 3.

**Table 1. Some physico-chemical and chemical characteristics of the soil samples.**

| Parameters | Chandina soil | Tannery soil | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| pH | 6.53 | 6.00 | 4.50 | 6.60 | 5.60 |
| EC (dS/m) | 0.05 | 5.67 | 1.96 | 5.49 | 0.76 |
| Moisture (%) | 30.54 | 17.42 | 32.32 | 24.65 | 31.89 |
| Particle size analysis : | | | | | |
| Sand (%) | 27.80 | 50.30 | 42.80 | 35.30 | 40.30 |
| Silt (%) | 52.50 | 35.00 | 45.00 | 45.00 | 45.00 |
| Clay (%) | 19.70 | 14.70 | 12.20 | 19.70 | 14.70 |
| Texture | Silt loam | Sandy loam | Loam | Loam | Loam |
| CEC (meq/100 g) | 37.90 | 14.18 | 17.25 | 51.39 | 32.95 |
| OC (%) | 0.67 | 0.77 | 0.34 | 1.12 | 0.40 |
| OM (%) | 1.15 | 1.33 | 0.58 | 1.93 | 0.69 |
| Total N (%) | 0.11 | 0.14 | 0.06 | 0.24 | 0.05 |
| Exchangable (ppm) : | | | | | |
| $NH_4$ | 10.00 | 300.00 | 160.00 | 330.00 | 80.00 |
| $PO_4$ | 5.00 | 31.26 | 4.94 | 0.82 | 19.74 |
| K | 330.00 | 202.00 | 78.00 | 220.00 | 85.00 |
| $SO_4$ | 5.00 | 350.00 | 290.00 | 410.00 | 390.00 |
| Ca | 640.00 | 731.00 | 718.00 | 787.00 | 627.00 |
| Na | 150.00 | 1750.00 | 500.00 | 1550.00 | 200.00 |

Wastes were mixed with Chandina soil at the rate of 2.5 t/ha and 5 t/ha. The treatments were $T_0$ (Unpolluted soil or control), $T_1$ (Soil with waste of 1st spot @ 2.5 t/ha), $T_2$ (Soil with waste of 2nd spot @ 2.5 t/ha), $T_3$ (Soil with waste of 3rd spot @ 2.5 t/ha), $T_4$ (Soil with waste of 1st spot @ 5 t/ha), $T_5$ (Soil with waste of 2nd spot @ 5 t/ha), $T_6$ (Soil with waste of 3rd spot @ 5 t/ha), $T_7$ (Soils of 1st spot of tannery area), $T_8$ (Soils of 2nd spot of tannery area), $T_9$ (Soils of 3rd spot of tannery area), $T_{10}$ (Soils of 4th spot of tannery area).

A pot experiment was set up with cowpea (*Vigna sinensis*) to assess the effects of tannery waste on bacterial population and $N_2$-fixation. Three replications of each treatment were taken and arranged in a completely randomized way. The soils were sterilized at 120°C for 48 hours in an oven after the

**Table 2. Some chemical characteristics of wastes.**

| Parameters | Waste-1 | Waste-2 | Waste-3 |
|---|---|---|---|
| pH | 7.90 | 7.10 | 7.80 |
| EC (dS/m) | 6.76 | 5.50 | 6.83 |
| OC (%) | 4.69 | 4.32 | 5.00 |
| OM (%) | 8.10 | 7.43 | 8.60 |
| CEC (meq/100 g) | 83.94 | 54.64 | 25.81 |
| Total N (%) | 1.26 | 1.63 | 1.39 |
| Exchangeable (ppm) : | | | |
| $NH_4$ | 970.00 | 210.00 | 300.00 |
| $PO_4$ | 300.00 | 70.00 | 60.00 |
| K | 3320.00 | 960.00 | 168.00 |
| $SO_4$ | 470.00 | 700.00 | 820.00 |
| Ca | 813.00 | 796.00 | 844.00 |
| Na | 13500.00 | 7500.00 | 4000.00 |

**Table 3. Some total (ppm) heavy metals content of soils and wastes.**

| Sample name | | Fe | Mn | Zn | Cu | Cr | Cd | Pb |
|---|---|---|---|---|---|---|---|---|
| Chandina soil | | 22128 | 382.00 | 229.00 | 145.00 | - | - | 47.04 |
| Tannery soil | 1 | 21989 | 422.00 | 219.36 | 63.70 | 20907 | 1.67 | 141.12 |
| | 2 | 22684 | 482.00 | 168.85 | 70.98 | 17422 | 3.34 | 33.60 |
| | 3 | 22323 | 520.00 | 158.47 | 85.54 | 38329 | 6.67 | 53.76 |
| | 4 | 22879 | 518.00 | 15.62 | 76.44 | 13938 | 4.17 | 53.76 |
| Waste | 1 | 33360 | 333.30 | 562.82 | 91.00 | 394910 | 8.34 | 123.20 |
| | 2 | 29931 | 290.00 | 309.09 | 75.83 | 145187 | 2.78 | 78.40 |
| | 3 | 32294 | 340.00 | 302.17 | 69.77 | 81305 | 4.07 | 134.40 |

treatments were applied. Then the soils were cooled down and soaked with sterilized deionized water. Surface sterilized (with 0.5% potassium hypochlorite) seeds of cow pea were sown in the pots. Seven days after germination, the purified *Rhizobial* strain was added to rhizosphere of the seedlings. Each pot was watered with sterile deionized water. Plants were harvested manually after 60 days by uprooting the plant carefully from the pot. Soil samples were also collected at harvest. Plant samples were dried in an oven at 80 ± 5°C for 48 hours and ground to pass through a 0.2 mm sieve. Soil samples after drying was ground and passed through a 0.5 mm sieve.

Plant samples were digested with $H_2SO_4$ and N content in the digest was determined by micro-kjeldahl method.(4) The amount of N fixed by plants was calculated by deducing the N content of 100 plants at harvest from nitrogen content of 100 grains.(5)

Plant samples were extracted with ternary acid mixture for the determination of P and other heavy metals.

Series dilution technique was used for plate count of total bacteria in soil. Colonies were counted by a colony counter and the results were expressed as number of colony forming units per gram of soil (cfu/g).(6)

## Results and Discussion

*Effect on bacterial population :* The highest bacterial population was found in $T_3$ and the lowest in $T_5$ and $T_8$ (Fig. 1). Tannery waste decreased microbial population because of high salt and heavy metal concentration in tannery waste. The bacterial population was less in tannery soil than unpolluted soil (Fig. 1). Lowest bacterial population was also found where pH of the soil was low. Adverse metal toxicity occurs at low pH and hence affects the bacterial population.(7)

Islam(8) reported that the total bacterial population declined from $13.5 \times 10^7$ cfu/g soil (colony forming unit) to $2.14 \times 10^7$ cfu/g soil at Sonatola series and from $10.3 \times 10^7$ cfu/g soil to $3.18 \times 10^7$ cfu/g soil in the soil of Savar Bazar series because of adding tannery wastes on those soils.

Bacterial population decline as a consequence of heavy metals toxicity is thought to be due to metals binding to selected sites on enzymes essential for micorbial growth and to the exclusion of other required element such as Mg, Mn and Fe.(9)

*Effect on $N_2$-fixation :* The cowpea did not show any visual symptoms of toxicity after 20 days of germination. However, at the later stage, plant growth rate decreased and chlorosis was observed due to high rate of waste application.

Fresh and dry matter contents of cow pea were also lower in contaminated soils than in control.

Earnst[10] observed that metal stress could reduce the growth of plants. Growth reduction with visible symptoms such as chlorosis and necrosis was also osberved due to metal toxicity. The plasma membrane of root may be the first target of metal toxicity. Due to delay of metal translocation from root to shoot, the shoot seems to be still healthy when roots were already heavily affected by the heavy metals.

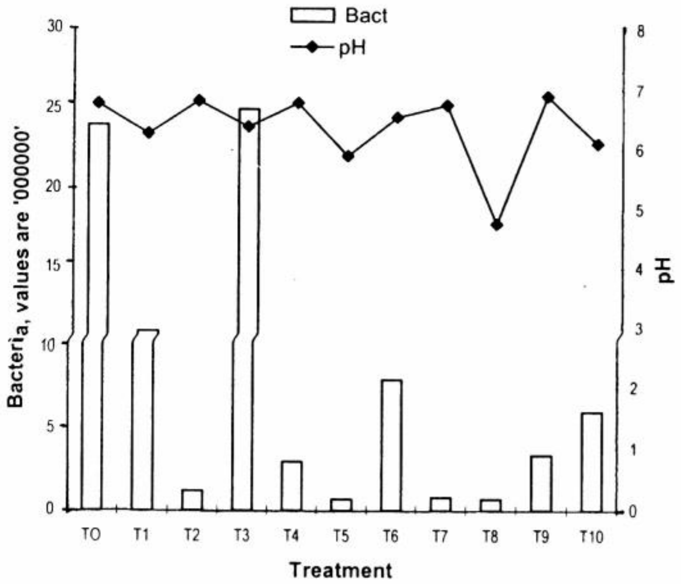


Fig. 1. Microbial population (total count of bacteria) of the soils as affected by various treatments.

Amount of nitrogen fixation by cow peas was higher in control (Table 4). Amount of nitrogen fixation by cow peas was higher in $T_2$ than $T_1$ and $T_3$. It may be because of low heavy metal content of waste of 2nd spot. However, at higher waste treatment the amount of nitrogen fixation was highest in $T_6$ treatment. This may be due to high organic matter content of waste of 3rd spot. Nitrogen fixation of $T_{10}$ was higher than $T_7$, because soils of $T_7$ treatment was taken from vicinity of the discharging site of the tannery industry, while soil of $T_{10}$ was farthest from the site. This indicated that soil pollution by tannery waste decreases with increasing distance from the discharging site. Tannery waste decreased nitrogen fixation because of high metal content of the waste. High content of Zn, Cu, Cd and Cr reduces nitrogen fixation.[11] Moreover, soluble salts of tannery waste also inhibit the growth of *Rhizobium*.[12]

Islam[8] observed that due to application of tannery effluents to soils of differing textures, yield of Mung bean was reduced with enhanced uptake of Na, Zn, Cd and Pb. The adverse effect was more pronounced in light soil than in heavy soils. The effluent was also found to have negative effect on growth, nodulation and performance of this crop.

Tannery wastes not only contain heavy metals but also various salts. It has been well documented that nitrogen accumulation by the symbiotic system of legume is reduced by salinity. Salinity may limit the symbiosis by (i) affecting survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere, (ii) inhibiting the infection process, (iii) directly affecting root nodule function, or (iv) reducing plant growth, photosynthesis and demand for nitrogen.(13)

**Table 4. Amount of fixed nitrogen (mg/100 plants), fresh and dry matter content of cowpea at different level of treatment.**

| Treatments | Amount of N in100 plants (mg) | Amount of N* fixed by 100 plants (mg) | Fresh weight (gm/plant) | Dry weight (gm/plant) |
|---|---|---|---|---|
| $T_0$ | 2813 | 2433 | 8.08 | 0.97 |
| $T_1$ | 1155 | 775 | 2.36 | 0.33 |
| $T_2$ | 2019 | 1639 | 5.07 | 0.66 |
| $T_3$ | 1260 | 880 | 3.46 | 0.45 |
| $T_4$ | 1180 | 800 | 3.08 | 0.37 |
| $T_5$ | 392 | 12 | 0.21 | 0.07 |
| $T_6$ | 2856 | 2476 | 6.0 | 0.84 |
| $T_7$ | 400 | 20 | 0.23 | 0.07 |
| $T_8$ | 1320 | 950 | 3.08 | 0.4 |
| $T_9$ | 414 | 34 | 0.23 | 0.07 |
| $T_{10}$ | 2025 | 1645 | 5.92 | 0.77 |
| LSD 5% | 1.69 | 2.23 | 0.51 | 0.13 |

*Amount of N fixed by 100 plants = amount of N in 100 plants – amount of N in 100 grains. Amount of N in 100 grains = 380 mg.

The results of the study revealed that tannery waste reduced the growth and nitrogen fixation of cow pea and also has negative impact on bacterial population.

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