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Environmental Contamination and Toxicology

Accelerated Tetramethylthiuram Disulfide (TMTD) Degradation in Soil by Inoculation with TMTD-utilizing Bacteria

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The use of microorganisms for the decontamination of pollutants in the environment is only a recently acknowledged feasibility. Only in the past decade have investigators (Kearney et al. 1969; Clark and Wright 1970 a,b; McClure 1972; Daughton and Hsieh 1977) shown that soil undesirably contaminated with a pesticide could possibly be decontaminated by inoculation with specifically adapted microorganisms. Much of the work that has been done has concerned insecticides and herbicides- presumably because of the much greater usages of these agrochemicals- but there is growing interest in the fate of fungicides (Woodcock 1978).

High concentrations of TMTD in seed bed soils has been shown to be quite persistent (Duffield and Eide 1962), and the effectiveness of microbial inocula towards degrading TMTD concentrations greater than 50 ppm has not been investigated. Our results, reported here, concern the use of highly acclimated TMTD -utilizing bacterial culture for accelerating the degradation of large concentrations of TMTD in soil, such as those found at disposal and spill sites.

We have reported on the characteristics and maintenance in liquid culture, of TMTD- utilizing bacteria (Shirkot, 1983). The bacterium was identified as Pseudomonas aeruginosa and was capable of rapidly degrading TMTD, utilizing it as a sole carbon and energy source.

MATERIAL AND METHODS

Pseudomonas aeruginosa active in degrading TMTD was isolated by enrichment and adaptation technique using TMTD as sole carbon source.

Composite soil samples of alluvial sandy loam (pH 7.3), typical of Northern Region of India were obtained from one to six inch surface layer and seived through a 2 mm wire mesh, mixed to ensure uniformity and 10 g aliquots were placed into conical Pyrex screwcap centrifuge tubes. Fortification with a proper amount of technical tetramethylthiuram disulfide (TMTD, Fluka AG,

Switzerland, Purity 99%) was done with either neat material or with a solution of 0.5-1.0 ml of dimethyl sulfoxide (DMSO; BDH). The TMTD was then evenly dispersed by using a vortex mixer. Other treatments included autoclaving (20 min. for 2 successive days), inoculation with the acclimated culture (at most 2.5×10^6 CFU g^{-1} soil), bringing to 40% saturation with water (2 ml) and finally mixing to ensure homogeneity. All samples were then incubated at room temperature (22-26°C) and ambient water content.

TMTD was extracted from the samples with chloroform after addition of 10 ml of water by shaking for one minute in the incubation centrifuge tube. The resultant emulsion was broken down by centrifugation. TMTD in chloroform layer was estimated according to the method of Rangaswamy et al (1970).

RESULTS AND DISCUSSION

Since our TMTD- utilizing culture was enriched for and maintained in liquid culture, we first determined whether this organism could function (i.e., degraded TMTD) in the soil environment. Samples were fortified to 300 ppm (wt/dry wt soil) with TMTD and subjected to various treatments. The per cent TMTD degraded in triplicate samples was determined after 0,4,8,16 and 20 days of incubation. Figure 1 shows results from autoclaved and non-autoclaved soils, respectively. The per cent TMTD degraded was calculated on the basis of the average amount of TMTD recovered from all soil samples on day 0, which were then normalized to 100%.

Results in Figure 1 indicate that 85% of TMTD was recoverable 24 days after fortification to non-autoclaved and non-inoculated soil. The high recovery indicates that little TMTD disappeared due to autochthonous microbiota, chemical hydrolysis, volatilization and time dependent absorption to soil colloids. In comparison, 40% TMTD was degraded only 4 days after TMTD fortification and inoculation with P. aeruginosa to non-autoclaved soil; only 14% remained 24 days after fortification and inoculation to either autoclaved or non-autoclaved soil. The TMTD degradation was reduced by 7% in non-inoculated, autoclaved soil, indicating the limited role of autochthonous microbiota in TMTD degradation. The TMTD degradation capacity of P. aeruginosa was not affected by autoclaving the soil. These results indicate that a selective organism like P. aeruginosa in sufficient number is required for this accelerated degradation of TMTD in soil. To our knowledge this is the first report of accelerated TMTD degradation in soil by inoculation with adapted bacterial culture.

As the organism was found highly effective in degrading TMTD in alluvial sandy loam, another experiment was designed to determine its capacity to degrade 200 ppm TMTD in different soils, as soils differ in their physical, chemical and biological properties. These properties might also influence the inoculant in terms of survival and capacity to degrade the chemical.

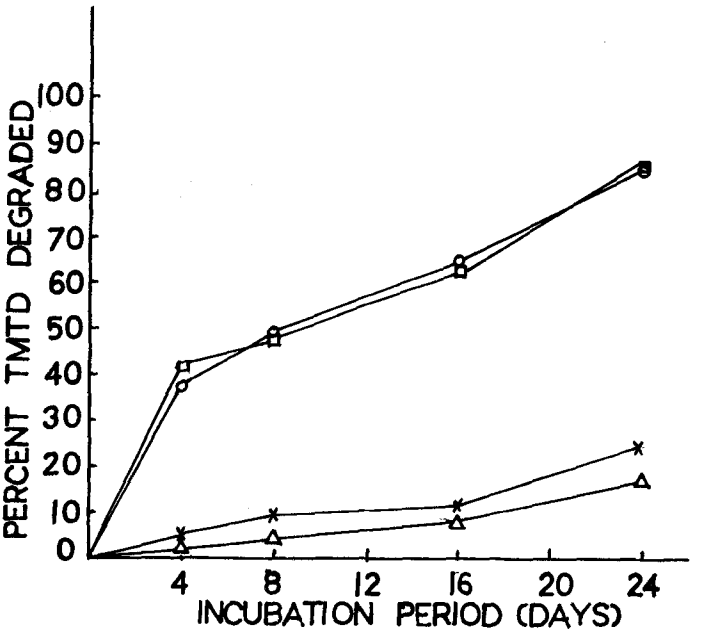

Figure 1. Degradation of TMTD in autoclaved and non-autoclaved soil inoculated and uninoculated with Pseudomonas aeruginosa.

Treatments: TMTD degradation in non-autoclaved inoculated (○-○) and in autoclaved inoculated (□-□) and uninoculated non-autoclaved (x-x) soil; autoclaved uninoculated (△-△) soil.

The results in Figure 2 indicate that this strain of P. aeruginosa can degrade TMTD in various types of soils i.e, alluvial, laterite, peat, black and red. The maximum degradation of TMTD was observed in peat (88.5%) followed by black soil (86.5%), laterite (78.86%), alluvial (75%), and red soil (57.85%) after 24 days of TMTD fortification and inoculation with P. aeruginosa. This may be attributed to the difference in the chemical composition and physical structure of soils, which might be influencing the growth and population of P. aeruginosa or affecting the production and activity of the enzymes responsible for TMTD degradation by way of absorption or protection against environmental conditions. The autochthonous biota does not have much influence because of the minor difference in the rate of TMTD depletion in autoclaved and non-autoclaved soils.

Another experiment was designed to determine the time period during which this strain of P. aeruginosa could retain its TMTD degrading ability in autoclaved and non-autoclaved soil when TMTD was not present as a growth and energy substrate.

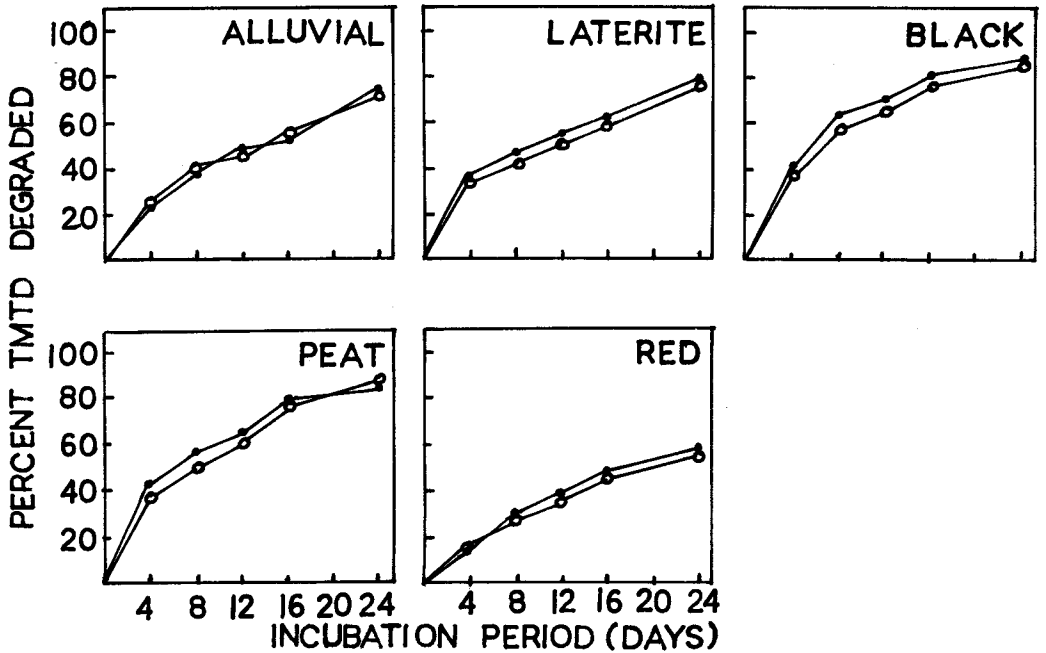

Figure 2. Degradation of 300 ppm. TMTD by Pseudomonas aeruginosa in different soils autoclaved (O–O) and non-autoclaved (●–●).

24 hour old culture of P. aeruginosa (O.D.=0.5; 1 ml) was inoculated to 10 g samples of autoclaved or non-autoclaved soil in centrifuge tubes. After inoculation (day 0), soil samples were fortified to 300 ppm TMTD on days 0,4,8,12,16 and 20 for each of the two treatments autoclaved and non-autoclaved. The retention of TMTD degradative activity of P. aeruginosa in each of these groups was determined by extracting the TMTD remaining in triplicate samples 0,4 and 8 days after fortification with TMTD.

The retention of degradative activity in the absence of TMTD is shown in Figure 3. The per cent TMTD degraded was calculated on the basis of average amount of TMTD recovered on day 0 after TMTD fortification for all the samples, of six groups, which were then normalized to 100%.

The results indicate that P. aeruginosa when inoculated into autoclaved soil, maintained full activity for up to 20 days in the absence of TMTD. After 12 days of incubation in the absence of TMTD, the P. aeruginosa in non-autoclaved soil was less able to degrade TMTD; TMTD degradation decreased by 6% i.e., 51.7% (0 day) to 45% at 12 days incubation in the absence of TMTD. After 20 days incubation in the absence of TMTD in non-autoclaved soil, about 11% decrease in TMTD degradation was observed at the end of the 10 day incubation. This phenomenon could perhaps be explained by assuming that the P. aeruginosa cells when forced to compete with the autochthonous microbiota for low levels of carbon source, begin to die or lose their ability to synthesize enzymes required for TMTD metabolism. A similar type of observation has been made by Daughton and Hsieh (1977), while studying the parathion degradation by P. stutzeri under entirely different conditions.

Finally, we determined the range of TMTD concentration in the soil which the P. aeruginosa could effectively degrade.

After TMTD fortification, the samples were mixed and allowed to equilibrate for 8 hours prior to inoculation. Five TMTD concentrations in the soil were tested: 100, 250, 500, 1500 and 2500 ppm. Each of these treatments were performed in groups of 18, one set of triplicates was extracted on day 0, (all per cent recoveries were greater than 90%) and other groups of triplicates were extracted on 4,8,12,16 and 24 days of incubation.

The results shown in Figure 4 indicate that within each of the treatments, the P. aeruginosa was effective in degrading TMTD. P. aeruginosa could degrade nearly 70% of TMTD concentration as high as 500 ppm. At 1500 and 2500 ppm, the amount of metabolism of TMTD was reduced to nearly 52%. This may probably be due to toxicity of higher TMTD concentration towards P. aeruginosa as the population has been observed to decrease gradually with increase in initial TMTD concentrations. It may also be due to

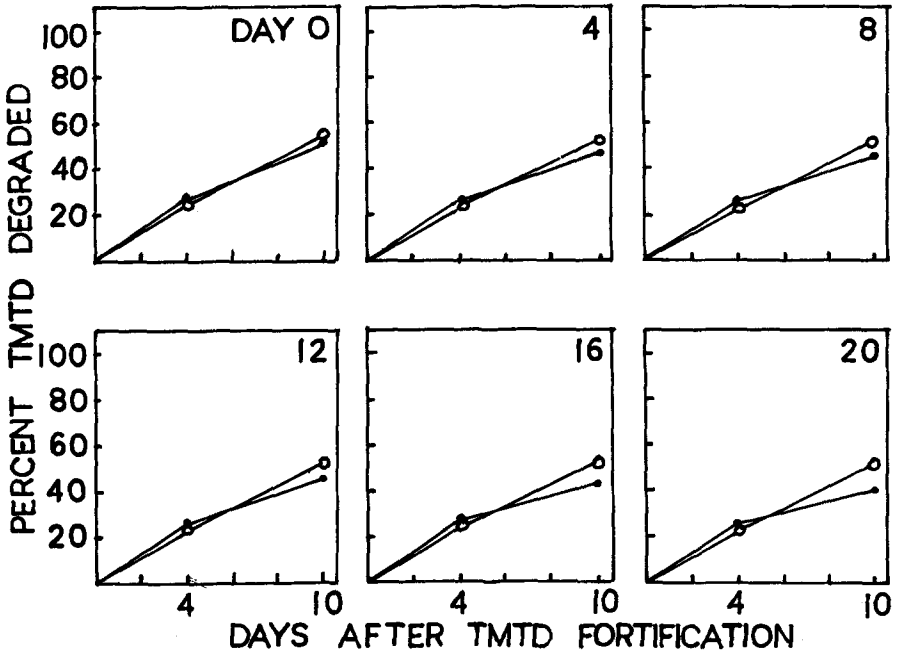

Figure 3. Functional longavity of Pseudomonas aeruginosa in soil. TMTD degraded (% of initial 300 ppm) in soil versus days after TMTD fortification. Each of the graph labels, day 0,4,8,12,16 or 20, indicates the days of incubation with Pseudomonas aeruginosa prior to fortification with TMTD. Treatments: (●-●) non-autoclaved soil, inoculated with P. aeruginosa. (O-O) autoclaved soil, inoculated with P. aeruginosa.

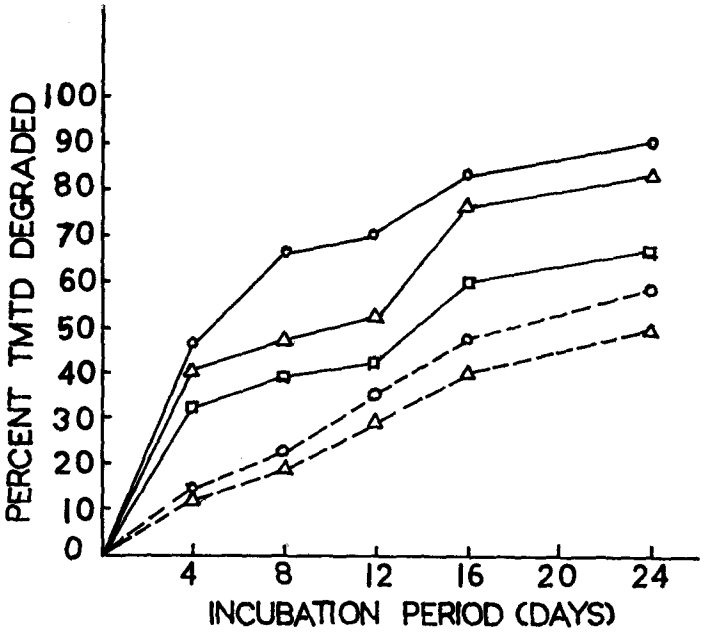

Figure 4. Effectiveness of Pseudomonas aeruginosa towards degradation of higher concentrations of TMTD.

Treatment: 100 ppm TMTD (O-O) 250 ppm TMTD (Δ-Δ), 500 ppm TMTD (□-□), 1500 ppm TMTD (O--O), 2500 ppm TMTD (Δ--Δ).

the increase in inhibition of depletion factors. Raghu et al. (1974) and Radwan (1965) have reported marked decrease in TMTD degradation by their isolates when the concentration was increased beyond 100 ppm in nutrient broth and 300 ppm in soil, respectively.

In conclusion, the TMTD degrading P. aeruginosa was extremely effective in rapidly degrading concentration of TMTD of at least 300 to 500 ppm in different soils within 3 weeks under laboratory conditions. The TMTD degradation capacity of P. aeruginosa was not affected by autoclaved soil. Soil type had significant effect on the capacity of P. aeruginosa to degrade TMTD. This strain of P. aeruginosa could maintain its TMTD degrading ability in sterilized soil for at least 20 days and in non-sterilized soil for at least 8-12 days while its effectiveness after 20 days in non-sterilized soil without exposure to TMTD was diminished by 11%. These results warrant a study under the field conditions to determine if P. aeruginosa can accelerate TMTD degradation and maintain activity under fluctuating temperature extremes and water content.

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