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Phytoremediation of 2,4,6-Trinitrotoluene (TNT) by *Tagetes patula*

Lakshmi Hooda[1, 2], S Mary Celin[1]and SK Singh[2]

[1]*Center for Fire, Explosive and Environment Safety, DRDO, Timarpur, Delhi, India*
[2] *Center of Excellence for Energy and Environmental Studies,*
Deenbandhu Chhotu Ram University of Science and Technology, Sonepat, Haryana, India
celinkumaran@yahoo.com

ABSTRACT

Explosives are an important category of environmental contaminants. 2,4,6- trinitrotoluene (TNT), the most widely used high explosive is a hazardous compound and also is a possible human carcinogen. TNT contamination is common in areas adjacent to manufacturing sites/firing/training ranges. Thus it is imperative to remediate the soil and wastewater contaminated with TNT. Phytoremediation using ornamental plant is a promising technique of removing soil pollutants. In the present study Tagetes patula (Marigold) an ornamental plant was used for the removal of TNT from soil. We investigated the potential use of marigold for effective removal of high concentrations of TNT i.e up to 1000 mg/ Kg. Different concentrations of TNT were prepared and used for pot culture experiments (100 – 1000 mg/Kg) in soil. After 3 months duration, the average soil degradation of TNT by marigold ranged from 87.6 to 98.6%. Contents of nitrate and organic matter in soil were increased. Biometric observation also showed some toxic effects of TNT on the growth of marigold.The overall plant growth decreased with respect to control. Thus, marigold could be a potential ornamental plant for effective phytoremediation of soils contaminated with TNT.

Key words: Phytoremediation, 2,4,6-trinitrotoluene, Tagetes patula, Ornamental, Degradation, Contamination

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INTRODUCTION

High explosive 2,4,6-trinitrotuolene, is one of the most widely used explosive due to its low melting point, chemical and thermal stability and high temperature. Due to its wide application in military and related industrial activities, contamination of TNT is encountered. TNT is toxic to all organisms; exposure to humans causes hyperplasia of the bone marrow, toxic hepatitis, and anaemia.

Therefore, remediation of TNT from soil is necessary to protect the organisms and the environment. TNT-contaminated soils have traditionally been treatedbyincineration, open detonation, chemical oxidation [1].These techniques often are extremely expensive and do not result in complete degradation, and cause secondary contamination.

Phytoremediation is an emerging technique, which uses plants to clean up the contaminant from soil, water and air. Phytoremediation is a cost effective, environmentally friendly and aesthetically pleasing alternate to clean up explosives on contaminated land [2].

Many plants are identified and applied for phytoremediation of TNT contaminated soil. Fallahi et al [3]shows the evidence that *Medicago sativa* remove TNT from soil. Varderford et al [4] stated that *Myriophyllum aqueticum* and *Catharanthus roseus* could transform TNT into amino derivatives. However, there is less literature available on ornamental plants, especially on the remediation of TNT contaminated soil.

Ornamental plants can be used in phytoremediation, as they can grow in polluted areas, can tolerate contaminant toxicity. In addition, ornamental plants may beautify the environment of contaminated areas. Ornamental plants are not edible plants so the risk of entering contaminants in food chain is reduced. *Tagetes patula* (French marigold), a popular ornamental plant flourishes in varied agro-climatic conditions. Marigold has been reported to takeup Copper from contaminated soil [5]. Sun et al [6] reported that *T patula* can be used for the phytoremediation of

Benzo[a]pyrene (B[a] P) and B[a] P–Cd contaminated sites. Reports also indicate that *T. patula* is a novel Cd accumulator and able to tolerate with Cd-induced toxicity by activation of its antioxdative defence system [7].

The objective of the present study was to explore the phytoremediation potential of *Tagetes patula* for the removal of TNT, through pot culture experiments.

MATERIAL AND METHODS

Chemicals

2,4,6- Trinitrotoluene (TNT) was used in pure form. All other chemicals used throughout this study were of analytical grade and were obtained from standard manufacturers.

Plants and Soil

Seeds of *Tagetes patula* were obtained from Biocarve seeds, India. Seeds were sown in garden soil, after 3 weeks, 2 seedlings in each pots (18 pots) were transferred in TNT contaminated soil. 1.5 kilogram of sieved (2mm) soil was artificially contaminated with different concentrations of TNT. Due to low aqueous solubility of TNT (130mg L^{-1} at 20ºC; [8]), TNT was dissolved in acetonitrile which was added to the soil to obtain final concentrations of TNT in range of 100- 1000 $mgKg^{-1}$. This soil was air dried for 3-4 days so that acetonitrile, which was a carrier solvent to prepare TNT, evaporate from soil [9].Dried soil pulverized prior to being used in pots. Pots were filled in Triplicates, pots without TNT and without plants were used as controls.

Soil sampling and Preparation

With the help of soil auger and kurpi soil samples from surface (1-10 cm) and sub-surface (10-40 cm) were taken from the pots. Soil samples were air-dried, crushed gently in ceramic pestle and mortar and were passed through 2 mm stainless steel sieve. Soil samples were then stored in zip lock polyethylene bags. The ground samples were mixed well before weighing them for analysis [10].

Analytical Methods

TNT were extracted from soil samples at beginning (0 day)and at interval of 30 days up to 90 days and analysed for residual TNT by high-performance liquid chromatography (HPLC) by using (EPA) method 8330A [11]. For this 2g of soil was suspended in 10 ml of acetonitrile, vortexed for 1min and sonicated for 18hrs at 18ºC. Then the supernatant was combined with 5ml calcium chloride solution, the mixture was filtered through a 0.45 µm Teflon filterto be made ready to load. Flexar HPLC of Perkin Elmer Inc. equipped with a C-18 column (15cm x 4.6mm, 5µm) was used. The mobile phase consisted of a 1:1 methanol (HPLC grade) and Water (HPLC grade) filtered through solvent filtration unit. Theflow rate, sample injection volume and run rate of the chromatograph were 1ml min^{-1}, 100µl and 15 min, respectively. Photodiode array detector at a wavelength of 254 nm was used for TNT detection.

Statistical Analysis

All experiments were carried out in triplicates, the means and standard deviations (SD) were calculated. Results were expressed as mean ± SD.

RESULT AND DISCUSSION

Uptake of TNT by marigold was carried out by analysing soil samples for 90 days by HPLC. The changes in the concentrations of TNT under different treatments and the controls were depicted in figure 1. *T. Patula* could promote the removal of TNT from the contaminated soil during the growth. *T. Patula* exhibited a high affinity for TNT, with high removal (up to 98.6 %) of TNT from the soil. There is also some degradation (28.33 %) of TNT in control treatment. Degradation of TNT can be caused by physical, chemical and biological actions. In control, treatment i.e. unplanted pot, degradation often caused by volatilization, eluviations and photolysis.

Soil conditions before and after experiment for 1000 mg/ Kg (Table 1.) shows the increase in nitrate concentration in soil, which confirms the oxidation of nitrite group from TNT to nitrate. An increase in nitrate concentration confirms the TNT transformation and a positive evidence of TNT mineralization. Detection of nitrate in medium without external source of nitrogen other than TNT is an indicator of TNT catabolism [12].

Biometric observation revealed that the *T. Patula* has shown endurance up to a concentration of 1000 mg/ Kg of TNT (Table 2.). Marigold plant showed comparable growth up to 200 mg/Kg of TNT with control. Retardation in growth of marigold plant was observed in treatments after 200 mg/Kg of TNT. These results show toxicity of TNT on growth using *T. patula.* In 1000 mg/ Kg reduced growth (shoot length- 20, root length- 5.83) was observed when compared to control (plant without TNT) (shoot length- 36, root length- 9.8).

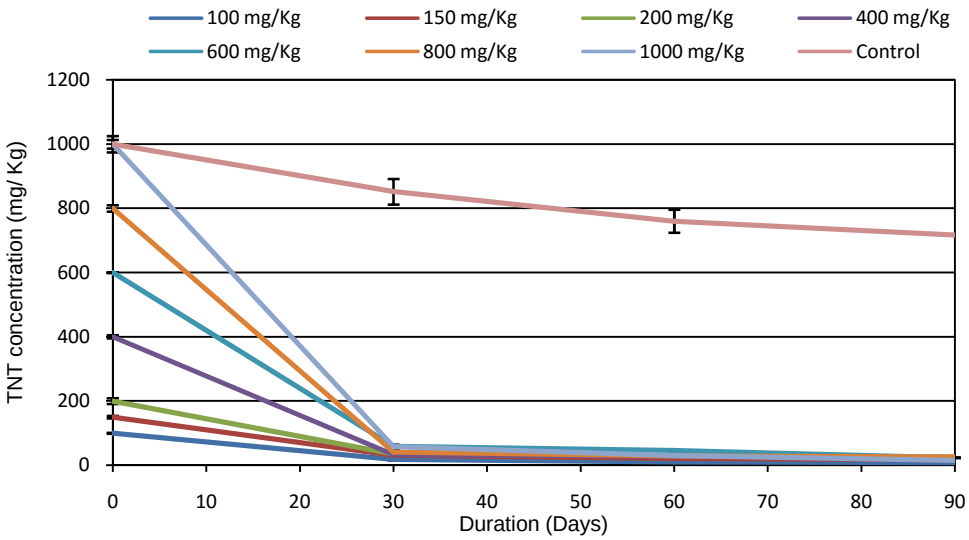

Fig. 1 TNT removal from soil planted with *T. patula* and control

Table- 1 Physiochemical Parameter Before and After Treatment

Parameters	Before treatment	After 90 days of treatment
pH	8.35	8.27
Water holding capacity (%)	41.1	40
Nitrate	52	169.77
Potassium (mg/ L)	90.5	193.5
Ash (%)	2.68	35.6
Organic Carbon (%)	56.69	51.64
Organic matter (%)	127	115.75

Table -2 Biometric Observation of *T. Patula* with TNT

Treatments (mg/ Kg)	Shoot Length (inch)	Root Length (inch)
100	34.81 ±1.09	9.93 ±0.25
150	34 ±1	9.46 ±0.23
200	34.76 ±1.06	8.54 ±0.30
400	30.6 ±1.5	8.13 ±0.25
600	28 ±2	7.43 ±0.321
800	24.33 ±1.52	6.93 ±0.15
1000	20 ±1	5.83 ±0.15
Control	36 ±1	9.8± 0.15

CONCLUSION

The present study explored the potential of phytoremediation of TNT contaminated soil with *T. patula.* However, retardation in plant growth with respect to shoot and root length in plants growing TNT contaminated soil beyond concentration of 200 mg/Kg was observed. These plants could able to produce flowers which indicated the application potential for soil contaminated with TNT upto 1000 mg/Kg. *T. Patula* affectively removed upto 98.6 % of TNT from the contaminated soil. Hence, *T. patula* could be used for phytoremediation of TNT contaminated site.

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