

Trinitrotoluene and Its Metabolites in Shoots and Roots of *Panicum maximum* in Nano-Phytoremediation

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Abstract—Phytoremediation is one of chemical removal methods but this is a long term process. Nanotechnology is a novelty method that can be used for toxic remediation. The objective of this study aimed to determine Trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) translocation in shoots and roots based on the nano-phytoremediation experiments. For methodology, the transplantation method of *Panicum maximum* (Purple guinea grass) were selected for this study. The plants were transferred and grown in the soil that was spiked with TNT with concentrations of 100 and 500 mg/kg and also added with nanoscale zero valent iron (nZVI) with concentrations of 100, 500, and 1000 mg/kg. The determination of TNT accumulation in *Panicum maximum* was carried out after harvesting at the end of 1st, 2nd, 3rd, and 4th months. The plants were divided into shoots and roots for the measurements of TNT and its metabolite residue concentrations. The present study can be concluded that the TNT uptake by roots in nZVI added soil was more effective than that without nZVI, particularly, the experiments with TNT concentration of 500 mg/kg. The results also showed that TNT was found in roots higher than that in shoots in all experimental groups. The 2-ADNT and 4-ADNT were only found in roots in all sets of the experiments. Both metabolites were undetectable in shoots.

Index Terms—Translocation, *Panicum maximum*, Trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), Nanoscale zero valent iron particles (nZVI), phytoremediation.

I. INTRODUCTION

Nano-phytoremediation involves nanotechnology and phytotechnology in remediation of the contaminated environment. *Panicum maximum* (Purple guinea grass) has been widely used as one of hyperaccumulators. The phytotechnology concerns four main processes. There are phytostabilization (the contaminants in soil are immobilized by means of plant roots), phytofiltration (plants take up contaminants by binding within their roots and shoots), phytodegradation or rhizodegradation (the contaminants are degraded and transformed to its metabolites within plants or

rhizosphere) and phytoextraction (the contaminants are taken up and stored in the aerial parts of the plant) [1], [2]. The combination of phytoremediation and nanoscale zero valent iron (nZVI) for 2,4,6-trinitrotoluene (TNT) removal in contaminated soil has been recently reported [3]-[5]. It was found that nano-phytoremediation for degradation and removal of TNT-contaminated soil has obviously more effective than either nano-remediation or phytoremediation [3]-[5]. The highest removal efficiency of nano-phytoremediation was found in soil with the TNT/nZVI ratio of 1/10 (100 mg/kg initial TNT concentration) in treated potting soil by *Panicum maximum* [4]. In the aspect of nano-phytoremediation, this current study was a continuation of previous research work [3]-[5]. Initially, the transplantation method was used and later the plants were grown in the TNT and nZVI-contaminated potting soil for a period of 4 months. Then, TNT, 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) translocation in shoots (leaves and stem) and roots was determined. This present study aims to measure concentrations of TNT and its metabolites (2-ADNT and 4-ADNT) in each part of plants in order to evaluate the uptake of the contaminants by *Panicum maximum* by means of nano-phytoremediation experiments.

II. MATERIALS AND METHODS

A. Materials

The commercial soil (Lumdol soil) was purchased from Taladthai market, Phatumtanee Province. Background soil was obtained from Prommanee sub-district, Muang district, Nakhon Nayok province. The chemical stocks of 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) (99.90% purity at 1000 mg/L concentration) were purchased from SUPELCO Co., USA. Seeds of *Panicum maximum* (*P. maximum* TD58) were purchased from Pakchong district, Nakhon Ratchasima province.

B. Nanoscale Zero Valent Iron (nZVI) Synthesis

The synthesis of nZVI particles was carried out by the reductive precipitation process using two chemicals, namely, sodium borohydride (NaBH₄) and iron (III) chloride (FeCl₃). This procedure followed the method of Wang and Zhang (1997) [6], Choe *et al.* (2001) [7], Sun *et al.* (2007) [8], Jiamjitrpanich *et al.* (2010) [5], and Jiamjitrpanich *et al.* (2012) [3], according to equation as below: [9]



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C. Preparation of the Potting Experiment for Nano-Phyto Remediation

This current study followed the transplantation method that was described in the previous study [3], [4]. The transplantation involved the seeds that germinated in commercial soil for 2 weeks. Two week-old healthy plants with similar height and biomass were transplanted into TNT and nZVI-contaminated soil. With regard to the preparation of the TNT and nZVI-contaminated soil, this involved the surface layer of background soil that was processed as previously described by Jiamjitpanich *et al.* (2012) [3], [4]. The soil was spiked with TNT with concentrations of 100 and 500 mg/kg. After that, nZVI were added at concentrations of 100, 500, and 1000 mg/kg. The plants were transferred and grown in the TNT and nZVI-contaminated soil in pots for a period of 4 months. The size of pots was 15 cm width and 15 cm height containing 1 kg of soil. The plants were kept in a greenhouse with sun light at average temperature of 30 °C in the daytime and 27 °C at night. The growing plants in the background soil without TNT and nZVI were also carried out and served as controls. Finally, the set of the experiments was divided into 9 groups as follows: control soil, 100 mg/kg TNT soil, 100 mg/kg TNT +100 mg/kg nZVI soil, 100 mg/kg TNT+500 mg/kg nZVI soil, 100 mg/kg TNT+1000 mg/kg nZVI soil, 500 mg/kg TNT soil, 500 mg/kg TNT+100 mg/kg nZVI soil, 500 mg/kg TNT+500 mg/kg nZVI soil, and 500 mg/kg TNT+1000 mg/kg nZVI soil.

D. Analytical Method of TNT and Its Metabolites in Plants

The plants were grown and exposed to TNT concentrations at 100 and 500 mg/kg with varied concentration of nZVI for a period of 4 months. Dosage of nanoparticles varied in 100, 500, and 1000 mg/kg. A number of plants from each set of the experiments and each time point including controls were collected and separately processed for analysis of TNT and its metabolite concentrations at the 1st, 2nd, 3rd, and 4th months of the exposure. The plant height and biomass were observed for abnormality. *Panicum maximum* was divided into two parts to determine TNT and its metabolites translocation. There were shoots (leaves and stem) and roots. The amount of TNT, 2-ADNT and 4-ADNT in each part of plants was measured at all experimental time points including the starting time point.

After harvesting, the plants were dried for 24–48 hr. The dry weight of each part of plants was recorded before grinding. The supernatant was obtained by extraction in acetonitrile for 18 hr. It was later filtered through a 0.45 µm PTFE filter prior to analysis by using gas chromatography with an electron capture detector (GC-ECD: Hewlett Packard 5890 series II, USA.) based on U.S.EPA method 8095 [10, 11]. The TNT and its metabolite concentrations were calculated and presented in terms of µg/gDW. The average concentration and standard deviation (S.D.) was later performed for each set of the experiments and each time point.

The stock solutions were obtained from TNT, 2-ADNT and 4-ADNT standards (purity of 99.90 %) in pure acetonitrile. The calibration curves were consisted of five standard points (ranging from 0–20 mg/L). These calibration curves presented linearity with correlation coefficients (R^2) greater than 0.99.

III. RESULTS AND DISCUSSION

It is known that the nZVI particles react with TNT under reducing conditions. In the presence of water, oxygen of the nitro group is removed and replaced with hydrogen. Following this reaction, TNT was transformed to the compounds less toxic and more amenable to biological breakdown such as 2-ADNT and 4-ADNT. According to the present study, the determination of TNT and its metabolite accumulation at the end of 1st, 2nd, 3rd, and 4th months was carried out in *Panicum maximum* that was grown in the TNT and nZVI-contaminated potting soil. The results were described as below:

The Figs. 1–4 showed TNT residues in roots and shoots of the plants in relation to time points, an initial amount of TNT and nZVI in soil. Generally, TNT concentration of each treatment in the roots was higher than in the shoots of all months measured and TNT concentration in roots of treatments with nZVI was found higher than that without nZVI, especially, the experiments with 500 mg/kg TNT. The TNT concentrations in roots were found at all time points. The highest TNT concentration (Almost 35 µg/gDW) was found in roots at 4th month in the experiment of 500 mg/kg TNT+1000 mg/kg nZVI soil. The TNT concentrations (less than 5 µg/gDW) were found in shoots only in 1st and 2nd months in all sets of the experiments. This indicated that *Panicum maximum* took up TNT from soil by the roots and translocated to the shoots for the first two months. In the later months (3rd and 4th months), the TNT concentrations was undetectable within the shoots. It was possible that TNT was degraded to its metabolites or conjugated with other molecules. According to this study, it was suggested that the shoots (stem and leaves) of plants with the ages less than 3 months should not be used for animal feeding. In this study, there was no detection of TNT found in controls at all time points.

The 2-ADNT residue concentration in *Panicum maximum* showed Figs. 5–6. The 2-ADNT concentration of each treatment in roots was detected differently depending on the sets of the experiments and time points. However, the 2-ADNT residue concentration was undetectable in some sets of the experiments and time points. By contrast, there was no detection of 2-ADNT residue in shoot parts in all sets of the experiments and all time points. There was also no detection of 2-ADNT residue found in controls at all time points.

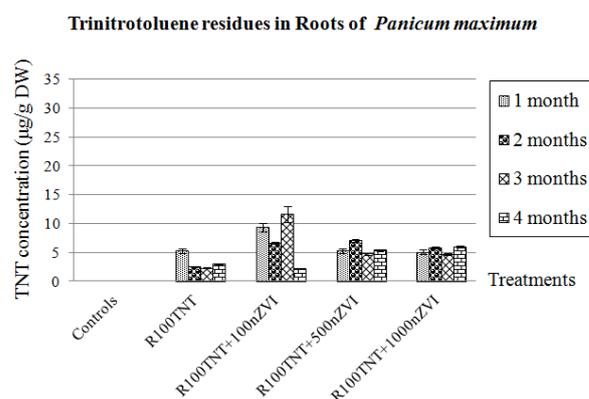


Fig. 1. Trinitrotoluene residues in roots of *Panicum maximum* in treatments with 100 mg/kg TNT.

Trinitrotoluene residues in Roots of *Panicum maximum*

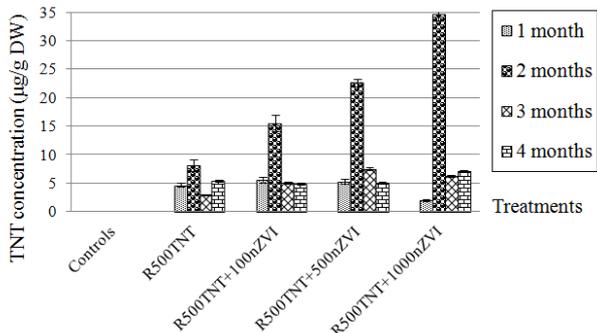


Fig. 2. Trinitrotoluene residues in roots of *Panicum maximum* in treatments with 500 mg/kg TNT

2-ADNT residues in Roots of *Panicum maximum*

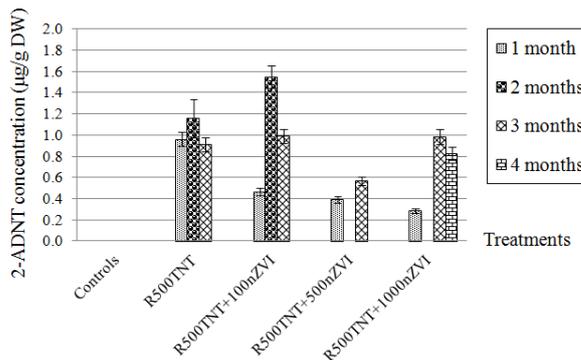


Fig. 6. 2-ADNT residues in roots of *Panicum maximum* in treatments with 500 mg/kg TNT

Trinitrotoluene residues in Shoots of *Panicum maximum*

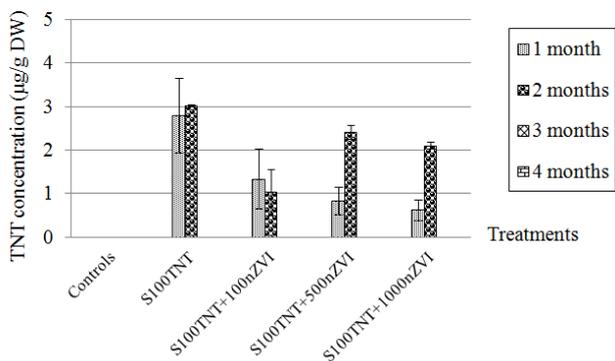


Fig. 3. Trinitrotoluene residues in shoots of *Panicum maximum* in treatments with 100 mg/kg TNT

Trinitrotoluene residues in Shoots of *Panicum maximum*

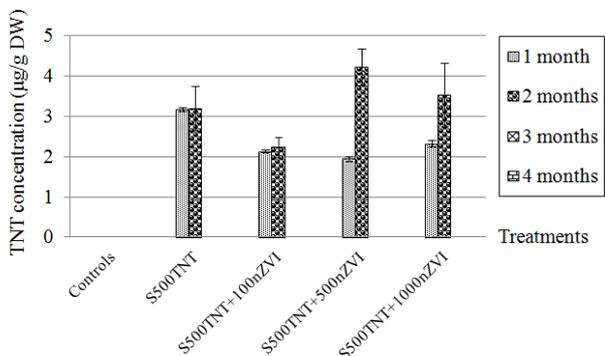


Fig. 4. Trinitrotoluene residues in shoots of *Panicum maximum* in treatments with 500 mg/kg TNT

2-ADNT residues in Roots of *Panicum maximum*

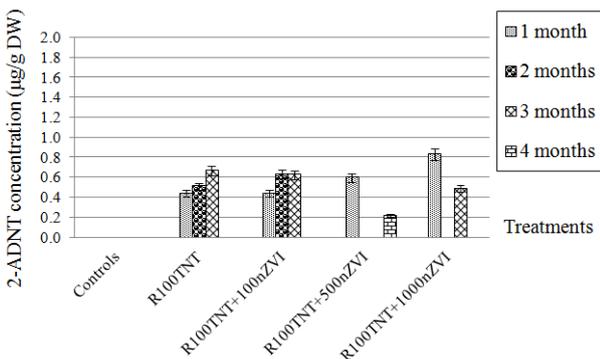


Fig. 5. 2-ADNT residues in roots of *Panicum maximum* in treatments with 100 mg/kg TNT

4-ADNT residues in Roots of *Panicum maximum*

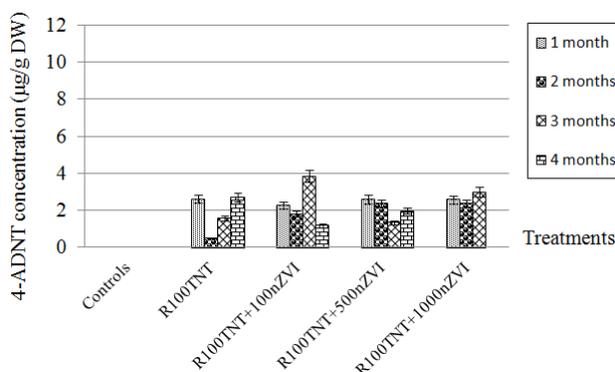


Fig. 7. 4-ADNT residues in roots of *Panicum maximum* in treatments with 100 mg/kg TNT

4-ADNT residues in Roots of *Panicum maximum*

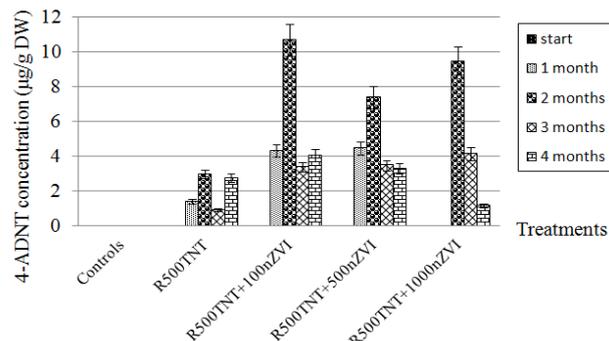


Fig. 8. 4-ADNT residues in roots of *Panicum maximum* in treatments with 500 mg/kg TNT

With regard to the present study, TNT accumulation was found in roots higher than that in shoots in all experimental groups. The results indicated that *Panicum maximum* took up TNT from soil by roots but a little amount was translocated to shoots. This may be due to the fact that TNT was degraded to its metabolites or conjugated with other molecules such as sugar compounds. The 2-ADNT and 4-ADNT in roots were found in most of experimental time points. By contrast, both metabolites in shoots were undetectable in all sets of the experiments. The translocation was not occurred to the shoots because their structure may easily conjugate with other molecules. Another possibility is that no absorption through the roots because of their negative charge.

IV. CONCLUSION

The current study was to determine TNT and its metabolites (2-ADNT and 4-ADNT) translocation in the nano-phytoremediation experiments. The transplantation method of two week-healthy *Panicum maximum* (Purple guinea grass) with similar height and biomass were selected for this study. The plant was divided into two parts for the measurement of TNT and its metabolites translocation.

This study indicated that the combination between nanotechnology and phytotechnology for TNT remediation in contaminated soil resulted in higher accumulation of TNT in plant roots, particularly, the experiments with TNT concentration of 500 mg/kg. The present study can be concluded that the TNT uptake by roots in nZVI added soil was more effective than that without nZVI.

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