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Fungal elimination of 2,4,6-trinitrotoluene (TNT) from the soils

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Abstract

The analysis of microscopic fungi collection created at theDurmishidze Institute of Biochemistry and Biotechnology revealed 107 strains assimilating 2,4,6-TNT (2,4,6-trinitrotoluene) belonging to the different fungal genera. The strains have been isolated from the polluted areas adjacent to the military grounds and industrial waste waters. It has been shown TNT is degraded most actively by strains belonging to the following genera: *Trichoderma*, *Aspergillus, Mucor* and *Trichoderma.* Optimal cultivation conditions for highly active strains -the destructors of TNT have been revealed.

It has been established that the carbon skeleton of TNT being utilized by the mentioned strains undergoes biotransformation. The existence of radioactive intermediates of biotransformation, organic acids (70-90%) and amino acids (10-30%) have been detected in liquid culture. Radioactive label of 1-^{14}C-TNT is mostly found in fumaric acid, which is known as one of the main products of benzene biotransformation and further conversion into succinic acid.

Remediation level of TNT-contaminated red and black soils treated by the most active strains *Aspergillus niger*N2-2 and *Mucor* sp. T1-1 have been studied under laboratory and field conditions. Cultivation of the above mentioned strains under laboratory conditions in sterile, black and red soils for 30 days at 30°C allowed decreasing the content of TNT in black soil to the residual, and in red soil – to 15%; cultivation of *Aspergillus niger* N2-2 decreased the amount of TNT in black soil to 11 and in red soil – to 21%. Under field conditions, TNT degradation level in contaminated soils by naturally existing micro flora during 100 days was equal to 40-50%, and in the case of additional introduction of both fungal strains, TNT-destructors reached 80%.

Keywords: microscopic fungi, destructor strains, organic toxicant, 2,4,6-trinitrotoluene (TNT), bioremediation

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Introduction

Nitroaromatic, polycyclic and polychlorinated compounds of dibenzo-dioxin group are characterized by high and long-term toxicity (1). TNT (2,4,6-trinitrotoluene),an explosive listed in all countries military arsenal, belongs to nitro aromatic compounds and has clearly defined toxic effects on all biological objects and prolonged stability under natural conditions; penetrates into the human organism via gastrointestinal tract, skin and lungs and accumulates in the liver, kidneys and adipose tissues (2). TNT belongs to carcinogenic toxicants. Due to low water solubility, TNT occurs in soil mainly in the form of crystals and is washed gradually into ground waters. To create the strategy of low water solubility compounds elimination from the soil first of all dioxins having extremely low water solubility and high sorption properties should be mentioned. Compounds similar to dioxins are not capable to migrate vertically even in sandy soils; however, according to many years' investigations, it has been established that dioxins are able to penetrate into the certain layers and maintain toxicity of soil for a rather long time (3, 4).

Microorganisms are considered to play an important role in cleaning of soils and waters contaminated with organic toxicants. Unlike non-biological technologies of environmental cleaning, bioremediation is recognized as a cost-effective and comprehensive

technology. By applying this method, maximal cleaning and long-term protection of the environment without breach of ecological balance can be achieved.

Nowadays, strains-destructors of organic toxicants mainly belonging to bacteria and basidial fungi (white-rot fungi) have been studied (5, 6). In comparison with other taxonomic groups of microorganisms, the detoxification potential of microscopic fungi is investigated in a less extent; however, according to the data of last decade, representatives of some genera of microscopic fungi, in particular, zygo- and deuteromycetes also displayed an ability to decompose 2,4,6-TNT and other toxic compounds and mineralize them (7).

The production and use of TNT for military purpose has led to its wide distribution. Being one of the most toxic explosives TNT is classified as a carcinogenic substance of group C. Microbial transformation of TNT begins with reduction of one of the nitro groups. The goal of the present work is the revelation of potential of some microscopic fungal strains to assimilate TNT and on the base of these data creation of ecobiotechnology for their application in remediation of soils polluted by TNT.

Materials and Methods

For the selection of microscopic fungi strains-destructors of 2,4,6-TNT, from the collection of microscopic fungi representativesof the following genera: *Aspergillus, Penicillium, Mucor, Fusarium, Trichoderma, Rhizopus, Botrytis, Altenaria, Cladosporium* and *Trichothecium*, isolated from different polluted regions and available at the Durmishidze Institute of Biochemistry and Biotechnology of Agricultural University of Georgia have been used. To evaluate the degradation potential of the above mentioned genera, previously chosen cultures have been studied (8, 9).

After primary isolation of microflora from the soils and their purification, strains were identified according to the manuals of microscopic fungi (10, 11, 12).

At first, in order to select concentrations of organic toxicants TNT content was determined in experimental soil sampling sites. Organic compounds from soil samples have been extracted by methanol. TNT content in the extract was determined by reversible-phase high effective liquid chromatography on the column $BondaPacC_{18}$ (15 cm 4.6 mm, size of particles –5 μm) under the following conditions: system of solvents – methanol : water, at the volume ratio 90 : 10, flow rate – 1.0 ml/min. Detection – at 254 nm, retention time of TNT – 12.5 min. It was found that the content of TNT in soils much exceeded permissible limit and was equal up to 5 mg/kg, Ecotoxicological index of TNT by means of quantitative assessment of ecotoxicological risk of contaminated soils is equal to 2 mg/kg (13).

For the cultivation of microscopic fungi, the following nutrient media were used:

1. Czapek's modified medium #1, % glucose – 2,0; $NaNO_3$ – 0,91; KH_2PO_4 – 0,1; $MgSO_4 \times 7H_2O$ – 0,05; KCl – 0,05; $FeSO_4 \times H_2O$ – 0,002, agar –2,0 (pH-6.0)
2. Czapek's modified medium #1*, % $NaNO_3$ – 0,91; KH_2PO_4 – 0,1; $MgSO_4 \times 7H_2O$ – 0,05; KCl – 0,05; $FeSO_4 \times H_2O$ – 0,002, TNT (different concentration); agar –2,0 (pH-6.0).
3. Czapek's modified liquid medium #2, % glucose – 3,0; $NaNO_3$ – 0,91; KH_2PO_4 – 0,1; $MgSO_4 \times 7H_2O$ – 0,05; KCl – 0,05; $FeSO_4 \times H_2O$ – 0,002, malt seedlings – 2g/100 ml, (pH-6.0 –6.5).
4. Czapek's modified liquid medium #2*, % $NaNO_3$ – 0,91; KH_2PO_4 – 0,1; $MgSO_4 \times 7H_2O$ – 0,05; KCl – 0,05; $FeSO_4 \times H_2O$ – 0,002, TNT (different concentration); malt seedlings – 2g/100 ml, (pH-6.0 –6.5).

In order to receive biodegradable mass of microorganisms, cultures were grown on solid agar medium #1 and medium #1* containing different concentrations of TNT (100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l).

Conidial suspension of cultures grown on solid nutrient medium were used an inocula Cultivation was conducted at 28-30°C for 10 days. Growth of microscopic fungi was analyzed on 3rd, 5th, 7th and 10th days. The capability of microscopic fungi to apply TNT from the nutrient medium as a sole source of carbon and nitrogen has been studied. TNT was introduced into the medium in concentration 200 mg/l. Growth intensity of cultures on solid nutrient medium was estimated visually by 3 point system: + poor growth, ++ moderate growth, +++ good growth.

In order to determine the amount of 2,4,6-TNT assimilated by fungi, deep cultivation of selected strains in Czapek's liquid modified medium #2 and medium #2* was conducted in 750 ml Erlenmeyer flasks. The cultures were incubated on a rotary shaker at 200 rpm, at 30°C for 72 hours and the amount of residual TNT was determined (14). Intermediates and final products of TNT conversion was determined by introducing into nutrient medium (1-^{14}C)-trinitrotoluene as a component of nutrient medium #2*.

In order to establish the efficacy of TNT conversion highly active strains of microscopic fungi were inoculated into the liquid medium with (1-^{14}C)-trinitrotoluene as a sole source of carbon. Labeled (^{14}C-TNT) was introduced in Czapek's modified liquid medium under sterile conditions (concentration 200 mg/l; specific activity – 500 Bq/min). Cultivation was conducted on temperature controlled shaker (180 rpm) for 5 days at 30-40°C in 750 Erlenmeyer flasks. In parallel, surface growth of cultures was carried out for two weeks under the same conditions. After completion of cultivation, biomass was removed by centrifugation (5000 rpm for 10 minutes). To remove adsorbed labeled (^{14}C) compounds ,the pellet was washed with distilled water, dried at 60 °C and weighed. For qualitative determination of certain compounds from culture liquid paper chromatography method was used. For isolation of organic acids solvent system – sulphuric ether [diethylether] : formic acid : water, at the ratio 140: 2 : 18, and for separation of sugars and amino acids – pyridine : ammonia : acetone, at the ratio – 70 : 30 : 20 were used.

The radioactivity of biomass, culture liquid and their fractions was determined on scintillation counter – LKB 1211 Rackbeta at efficiency 95%.

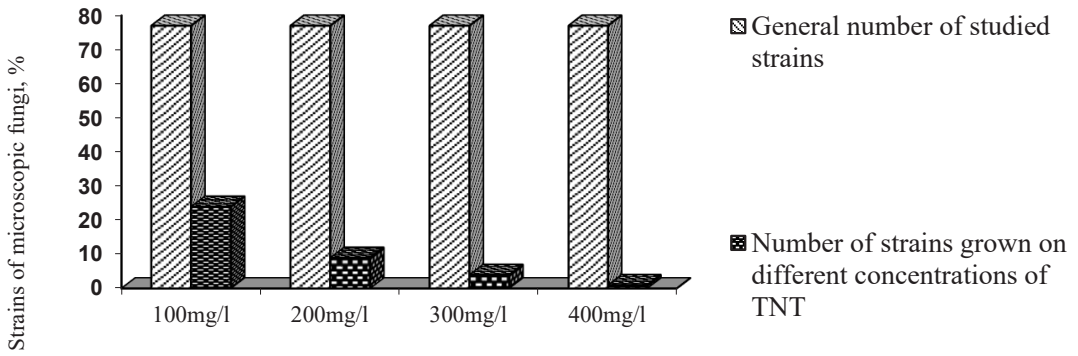


Figure 1. Microscopic fungi growth potential on the media containing different concentrations of TNT.

The level of TNT degradation was studied in black and red soils under laboratory sterile and modeling conditions. 200 mg of TNT was artificially introduced per kg of soil. The fungi were incubated in Petri dishes, at 35-40°C for 30 days, containing 25 g of sterile soil and 5 mg of TNT. Under field conditions, experiments were conducted on no sterile soil, on the area of 0.5 m^2, starting from the end of March till July.

Residual TNT was removed from soils by 3-fold extraction by methanol and according to residual amount the level of TNT transformation/biodegradation was determined.

Results

Selection of strains actively degrading TNT

For the selection of fungi strains the most actively degrading TNT, 107 cultures of microscopic fungi being isolated from chemically polluted military grounds and industrial wastewaters, have been used.

Based on the 20 years experience of the authors, freshly isolated and collection strains representing the following genera *Aspergillus, Trichoderma* and *Mucor* were recognized by ability for TNT degradation. Additionally ,according to the data of recent years, some strains of these genera have feature of TNT assimilation (6). At first, screening was conducted on Czapek's solid modified medium #1 with different concentrations of TNT (100 mg/l; 300 mg/l, 400 mg/l) as a sole source of carbon (these concentrations exceed TNT permissible concentration).

According to the experimental data, it has been shown that 24 among initially taken 107 strains grew well at low concentration of TNT (100 mg/l), 9 cultures – at 200 mg/l, 4 cultures at higher TNT concentration (300 mg/l) and only 1 culture grew at the concentration 400 mg/l (**Fig. 1**).

As the purpose was the quantitative assimilation of TNT by fungal strains it was found that 9 cultures expose moderate and good growth in the presence of TNT, namely: *Mucor* sp. SH 6-3,*Mucor* sp. T1-1, *Aspergillus niger* N2-2; *Aspergillus niger* K3-5, *Trichoderma viride* N1-9,*Trichoderma* sp. N2-6,*Trichotecium* sp.S1-6,*Penicillium* sp. N-2,*Fusarium monoliforma* S2-6.

In case of TNT increased concentrations (from 200 mg/l – 400mg/l0), morphological characters of selected strains had the following changes: *Mucor* sp. T1, cotton-like colony gradually transformed into the leather-like one. At high concentration of TNT, circumferential rings (supposedly, induced mutation) were formed around the colonies of *Fusarium monoliforma* S2-6, and the pigmentation was bright; colony growth was hindered in the case of *Aspergillus niger* K3-5 and *Trichoderma viride* N1-9; however, in both cases, color and sporogenesis were maintained.

In order to select active strains, destructors of TNT, toxicant was introduced into the solid medium together with glucose and as a sole source of carbon. In the nutrient liquid medium, glucose together with TNT was added with the aim to expose initial growth activity of strains. Deep cultivation

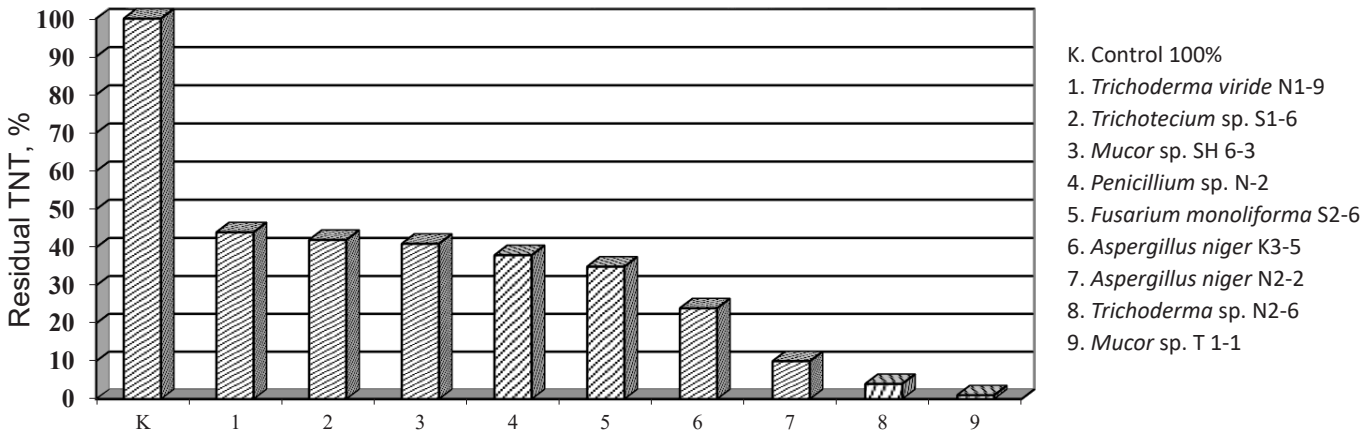
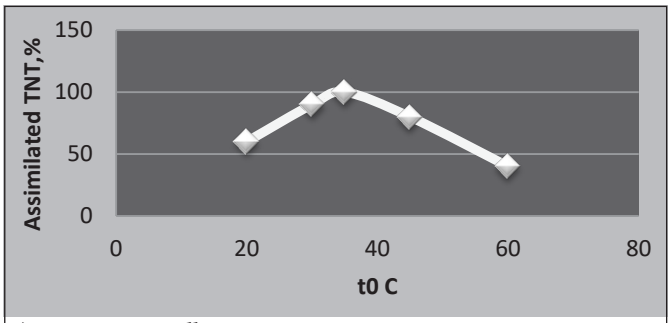
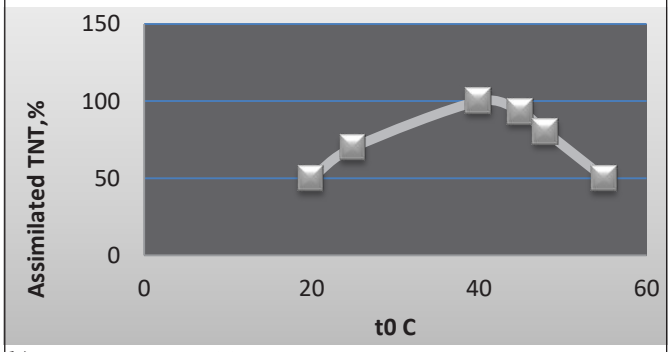


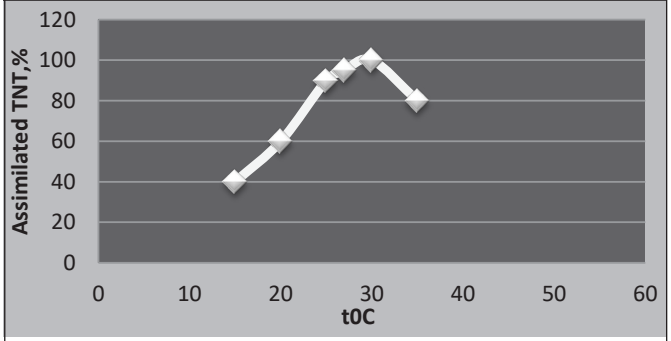
Figure 2. Strains potential to transform TNT.



a) Strain *Aspergillus niger* N2-2

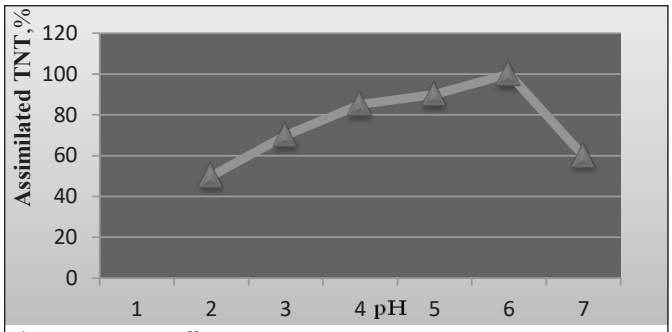


b) *Strain Mucor* sp. T1-1

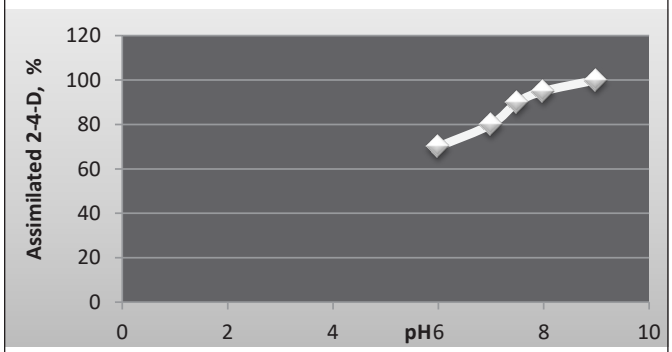


c) *Strain Trichoderma* sp. N2-6

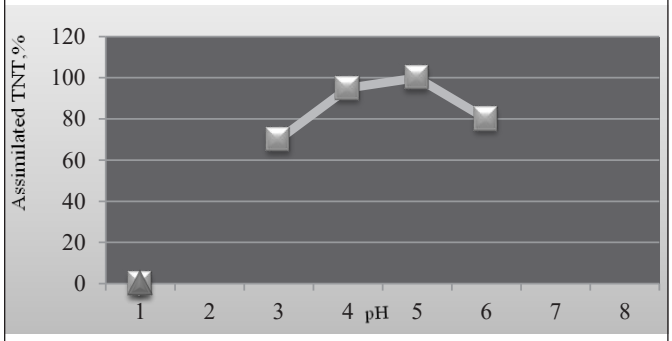
Figure 3. Degradation of TNT at different temperatures by microscopic fungi.



a) Strain *Aspergillus niger* N2-2



b) *Strain Mucor* sp. T1-1



c) *Strain Trichoderma* sp. N2-6

Figure 4. Transformation of TNT at different pH values by microscopic fungi.

was conducted in 750-ml Erlenmeyer flasks, on a shaker at 200 rpm, for 72 hours. Microscopic fungi were grown in the liquid medium #2, conidial suspension of 10-day cultures were taken as inocula. The amount of residual TNT in culture liquid was determined under the strong alkaline conditions (pH>11). The appropriated nutrient medium in the presence of 2,4,6-TNT without inoculation of culture was considered as control containing 100% of initial TNT. Pure nutrient medium was control sample.

The amount of residual TNT indicates degradation potential of microscopic fungi carried out at these particular experimental conditions. Results are given in **Fig. 2**.

Selection of growth conditions

Since the metabolic activity of microorganisms significantly depends on cultivation conditions, optimal temperature, pH and duration of cultivation. From the point of view of TNT assimilation the optimal temperature for the deep cultivation of selected strains – *Aspergillus niger* K 3-5, *Mucor* sp. T1-1, *Trichoderma sp.* N2-6 and *Aspergillus niger* N2-2 was conducted between 20°C and 50°C, at intervals of 5°C. All three strains are typical mesophiles with the optimum of growth and TNT degradation at temperatures between 30-40°C. The results are shown in **Fig. 3a, 3b** and **3c**.

Maximal degradation of TNT (decomposition) is achieved by cultivation of microscopic fungi *Aspergillus niger* K3-5 and *Aspergillus niger* N2-2 at 35°C (**Fig. 3a**). The strain *Mucor* sp.T1-1 utilizes maximal amount of TNT at 40°C (**Fig. 3b**),*Trichoderma* sp.N2-6 – at 30°C (**Fig. 3c**). Acidity of reaction mixtures significantly determines metabolic activity of microscopic fungi strains. *Aspergillus niger* K3-5and *Aspergillus niger* N2-2 maximally assimilate TNT at pH 6.0 (**Fig. 4a**), *Mucor* sp.T1-1 at pH 9.0 (**Fig.4b**) (almost by 100%) and *Trichoderma* sp.N2-6 – at pH 5.0 (**Fig 4c**).

Table 1. The amount of (1-^{14}C)-TNT detected in fungi biomass, in%

Culture	Nutrient medium #2	Biomass, mg	Total radioactivity of biomass, in %
Mucor sp. T1-1	Czapek's medium	29	53.6
Trichoderma sp. N2-6	Czapek's medium	25	51.9
Aspergillus niger N2-2	Czapek's medium	27	52.9

Table 2. Main products of biotransformation of (1-^{14}C)-Trinitrotoluene in culture liquid

Strain	Radioactivity, in%	
	Organic acids	Amino acids
*Mucor sp.*T1-1	72.2	27.8
Trichoderma sp. N2-6	77.4	22.6
Aspergillus niger N2-2	89.8	10.2

Assimilation of (1-14C)-TNT by microscopic fungi

In spite of some datathe mechanism of explosive degradation by fungi has not been completely investigated (5). In our turn order to investigate the products of (1-^{14}C)-trinitrotoluene transformation and distributionthe strains of the different genera – *Mucor* sp.T1-1, *Aspergillus niger* N2-2 and *Trichoderma* sp. N2-6, were applied. Surpassingly, strains of various genera microscopic fungi convert 1-^{14}C TNT with different intensities. Under the conditions of 5-day exposition with radioactive label, more than half of radioactivity of assimilated by strains 1-^{14}C-TNT remained in the biomass. Results are given in **Table 1**. Content of 14C in soluble fractions is presented in **Table 2**. Distribution of radioactivity in formed organic acids (**Table 3**) and amino acids (**Table 4**) has been established.

Radioactive organic acids containing 70-90% of total radioactivity and amino acids with 10-30% of radioactivity have been detected in culture liquid.

Laboratory and field experiments

The two active fungi strains of different genera – *Aspergillus niger* N2-2 and *Mucor* sp. T1-1 were chosen for remediation of TNT-contaminated soils under the natural modeling conditions.

Laboratory experiments were conducted in Petri dishes containing 25 g of black and red soils (widely spread in western and eastern Georgia) and contaminated with 200 g/kg TNT. The strain *Aspergillus niger* N2-2 was introduced in the amount of 8.5×10^6 CFU (Colony Forming Unit) in terms per 1 g of soil, and *Mucor* sp. T1-1 – in the amount of 7.5×10^5CFU. Black and red soils contaminated with the same amount of TNT without introduction of microorganisms served as control. Cultures were incubated at 30°C, with duration of the experiment for 30 days. Every 10th day quantitative changes in CFU and the amount of residual TNT were tested. The results are presented in **Table 5** and **Fig. 5**.

Table 3. Distribution of radioactivity of (1-^{14}C)-trinitrotoluene transformation by fungi strains among organic acids

Strain	Total radioactivity in %, of low molecular compounds fraction	Distribution of radioactivity in organic acids, in %					
		Fumaric acid	Succinic acid	Glycolic acid	Citric acid	Malic acid	Unknown
Mucor sp.T1-1	72.0	86.6	4.1	2.8	2.7	1.8	2.0
Trichoderma sp. N2-6	77.4	91.8	4.0	1.5	1.1	0.8	0.8
Aspergillus niger N2-2	89.8	88.8	3.3	3.7	1.2	0.7	2.3

Table 4. Radioactivity of amino acids products of (1-^{14}C) TNT transformation by fungi strains

Strain	Radioactivity of amino acids in %	Distribution of radioactivity in individual amino acids, in %					
		Phenylal-anine	Glutamic acid	Tyrosine	Arginine	Asparag-inic acid	Serine
Mucor sp.T1-1	27.8	27.4	19.3	17.5	13.6	11.5	10.7
Trichoderma sp. N2-6	22.6	24.3	16.6	18.7	18.5	11.5	10.4
Aspergillus niger N2-2	10.2	38.9	14.7	12.8	11.4	11.8	10.4

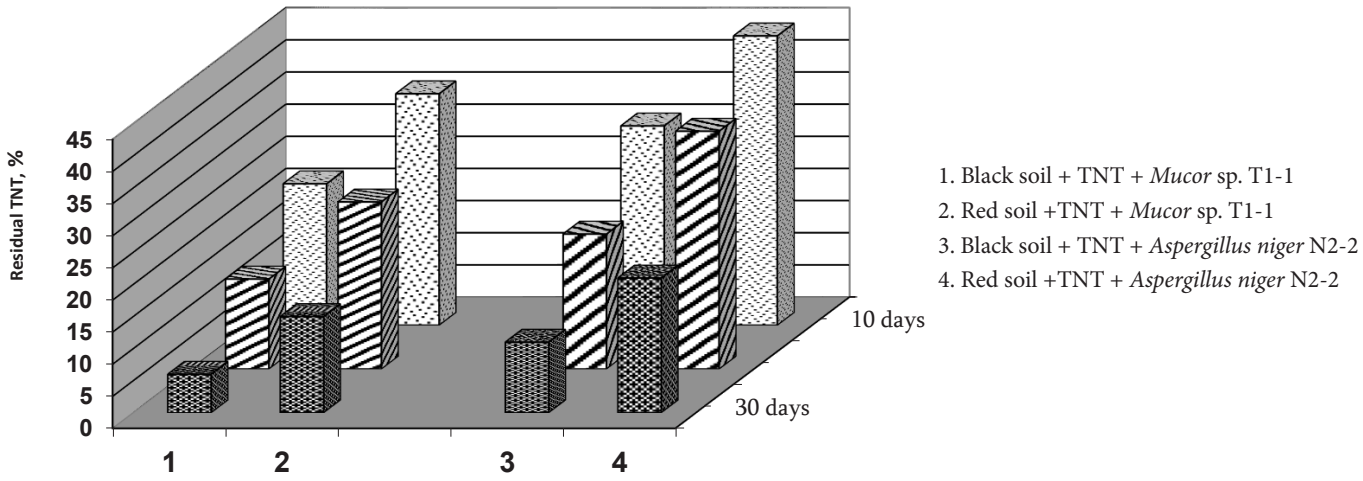


Figure 5. Degradation of TNT in black and red soils by microscopic fungi.

Table 5. Changes of CFU of fungi introduced into TNT-contaminated soils

Strain	Soil types	CFU per g of soil			
		Initial amount	10 days later	20 days later	30 days later
Mucor sp. T1-1	Black soil	$7,5\times10^5$	$9,5\times10^6$	$6,5\times10^5$	$3,5\times10^4$
	Red soil	$7,5\times10^5$	$5,5\times10^5$	$3,5\times10^4$	$2,3\times10^4$
Aspergillus niger N2-2	Black soil	$8,5\times10^6$	$4,5\times10^7$	$5,5\times10^5$	$6,5\times10^4$
	Red soil	$8,5\cdot10^6$	$4,5\times10^6$	$3,5\times10^5$	$4,5\times10^4$

Development of fungi introduced in black and red soils proceeds differently. In black soils, CFUs of introduced cultures are increased by single-order on the 10th day of cultivation, and then are gradually reduced. In red soils, CFUs of introduced strains are reduced on the 10th day. It might be explained that black soils are richer by organics and presumably the existing organic matter promotes to overcome the stress caused by action of the toxicant. Maximal decrease of TNT takes place during the first 10 days (by 64-72%) and the amount of residual TNT makes up only 6-15% for the last 20 days. It should be mentioned that degradation of TNT is more intensive in black soils. Experiments conducted under laboratory conditions showed that introduction of active strains effectively decreases the concentration of TNT in soil. To compare this data with field conditions 0.3 m^2 of black and red soils were artificially contaminated by 1mM TNT per kg of soil; the depth of contamination – 30 cm.

One of the main goals of the project was testing TNT transformation potential of *Aspergillus niger* N2-2 and *Mucor* sp. T1-1 through their introduction directly into contaminated non sterile soils. Certain amount of TNT was introduced into both types of soils (sterile and non sterile). In order to exclude utilization of TNT by local indigenous microorganisms the same amount of TNT was introduced into the sterile soils In addition, culture liquid of selected strains with biomass (grown under deep cultivation conditions), together with the TNT were introduced into the soil (each strain was grown under optimal growth conditions). Thus, the following variants were tested for each type of soil and each fungi strain:

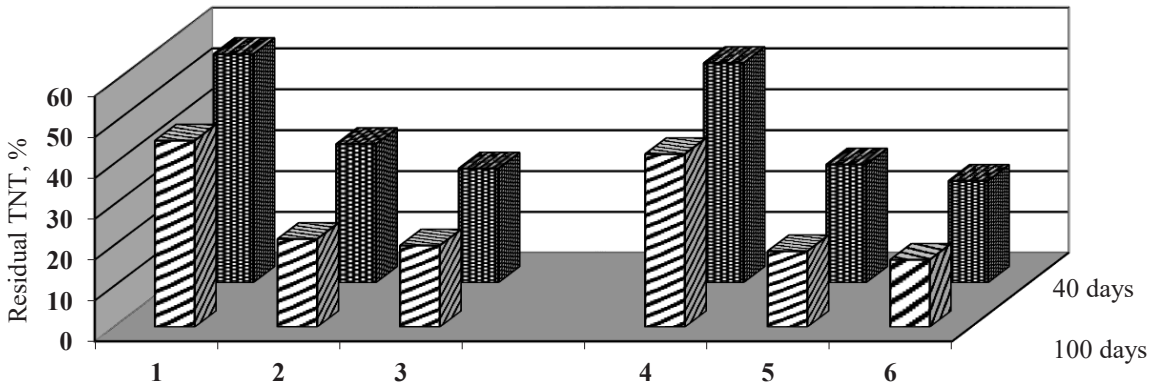
- Sterile soil + TNT (considered as control)
- Nonsterile soil + TNT
- Nonsterile soil +TNT + microorganism

After 40 and 100 days of incubation, both the amount of residual TNT and that of CFU in terms of 1 g of dry soil were determined in each sample. The experimental data showed that the number of local (indigenous) microorganisms reduced after 40 and 100 days; it was caused by the fact that part of local microorganisms could not undergo adaptation to the introduced toxicant. While at the first stage(introducing selected strains), the amount of microorganisms existing in the soil slightly decreased, but later, returned again to the initial amount or in some cases, even insignificantly exceeded it (**Table 6** and **Fig. 6**).

As seen from the above presented data, the level of TNT assimilation in soil by local microorganisms makes 40-50%, and in case of additional introducing of strains-destructors reaches 80%.

Discussion

In spite of some suppositions on different genera strains activities directed to TNT degradation experimental results showed a picture. As shown in **Fig. 2**, strains of microscopic



1. Red soil (nonsterile soil) + TNT
2. Red soil (nonsterile soil) + TNT + *Aspergillus niger* N2-2
3. Red soil (nonsterile soil) + TNT + *Mucor* sp. T1-1
4. Black soil (nonsterile soil) + TNT
5. Black soil (nonsterile soil) + TNT +*Aspergillus niger* N2-2
6. Black soil (nonsterile soil) + TNT + *Mucor* sp. T1-1

Figure 6. Degradation of TNT by microorganisms under natural modeling conditions.

Table 6. Changes of CFU of introduced and indigenous fungi in TNT contaminated soils under natural modeling conditions

Test variant	CFU of fungi per 1g of soil		
	At the moment of inoculation	40 days later	100 days later
Red soil (sterile soil) + TNT	0	0	0
Red soil (nonsterile soil) + TNT	3.8×10^6	1.2×10^6	1.3×10^5
Red soil (nonterile soil) + TNT + *Aspergillus niger* N2-2	5.4×10^6	3.9×10^5	4.9×10^6
Red soil (nonsterile soil) + TNT + *Mucor* sp. T1-1	1.9×10^6	7.2×10^5	1.3×10^6
Black soil (sterile soil) + TNT	0	0	0
Black soil (nonsterile soil) + TNT	6.5×10^6	4.7×10^5	3.2×10^5
Black soil (nonsterile soil) + TNT + *Aspergillus niger* N2-2	5×10^6	8.8×10^5	5.7×10^6
Black soil (nonsterile soil) + TNT + *Mucor* sp. T1-1	1.9×10^6	7.0×10^5	2.4×10^6

fungi – *Trichoderma* sp. N2-6, *Trichotecium* sp.S1-6 and *Mucor* sp. T1-1, maximally utilized trinitrotoluene.A bit less activity was expressed by the strain *Trichotecium* sp.S1-6, which also should be related to highly active TNT degrading strains. The strains Aspergillus niger N2-2 and *Aspergillus niger* K3-5 also utilized TNT by average intensity, as compared with 9 selected strains. In spite of higher activities expectations, strains *Trichoderma* sp. N2-6 and *Mucor* sp.T1-1exposed less but still significant activities of TNT utilization. The strainsdistinguished by the best growth in the presence of high concentrations of TNT have the best TNT transformation potential. It should be underlined that the 4 strains – *Mucor* sp. T1-1, *Aspergillus niger* K3-5, *Trichoderma* sp. N2-6 and *Aspergillus niger* N2-2 were isolated from adjacent to military grounds and their activity comparatively could be explained by long time adaptation of strains to the toxicants containing environment**.**

The strains – *Aspergillus niger* K3-5, *Aspergillus niger* N2-2 and *Trichoderma* sp. N 2-6 maximally conducted biotransformation of TNT for 72-78 h of cultivation. For the strain Mucor sp. T1-1, optimal duration of cultivation time was 96-104 hours.Such a long time transformation of TNT is required by the complicated metabolism pathway of this contaminant. As it has been shown the initial microbial conversion of TNT undergoes reducing reaction in particular microorganisms, conduct transformation of TNT into the following intermediate metabolites: azoxytetranitrotoluene, aminodinitrotoluene and hydroxylaminodinitrotoluene (15).

The identification of individual low molecular weight compounds formed as a result of biodegradation of (1-14C)- TNT by the strains of microscopic fungi having a high detoxification ability are shown in **Table 2**. Formation of labeled amino acids, directly indicate on TNT carbon skeleton utilization by selected fungi strains. Carbon atoms of 1-14C-TNT are converted by microscopic fungi in a way that first carbon atom of TNT mainly participates in synthesis of organic acids. Radioactive label of 1-14C-TNT is mostly found in fumaric acid which is one of the

main products of benzene microbial transformation and easily undergoes further transformations.

As a result of consecutive enzymatic reactionsof TNT proceeding under the action of microscopic fungi, individual carbon atoms of this compound are involved in intracellular processes of metabolic and energy exchange. It can be concluded that the carbon skeleton of the TNT molecules adsorbed by the test cultures undergoes deep degradation. The initial stage of this process must be the reduction of the nitro groups, after which the aromatic ring of the TNT molecule is used in the biosynthesis of aromatic amino acids. After reduction of the main part of the assimilated toxicant molecules, their oxidation follows which leads to removal of the amino groups and cleavage of the aromatic ring, and as a result organic acids are formed as standard cell metabolites. As a result it might be concluded that TNT is completely decomposed under theactionof microscopic fungi and carbon atoms are involved in metabolic process typical for these taxonomic group of microorganisms (2). The introduction of strains- *Aspergillus niger* N2-2 and *Mucor* sp. T1-1 improves the potential of soils directed to TNT degradation that confirms high destructive activity of these strains in natural conditions.

Final Remarks

As a result of selection microscopic fungi kept in the collection at Durmishidze Institute of Biochemistry and Biotechnology, 107 strains assimilating 2,4,6-TNT belonging to the different genera – *Aspergillus, Penicillium, Mucor, Fusarium, Trichoderma, Rhizopus, Botrytis, Alternaria, Cladosporium* and *Trichothecium* have been revealed. The strains have been isolated from the polluted areas adjacent to the military grounds and industrial waste waters. The most actively TNT is degraded by strains representing genera *Aspergillus, Mucor* and *Trichoderma.* Optimal cultivation conditions for highly active strains, destructors of TNT have been revealed.

In order to establish degradation of TNT carbon skeleton, transformation products of (1-^{14}C)-TNT by fungi strains *Mucor* sp. T1-1, *Trichoderma* sp. N2-6 and *Aspergillus niger* N2-2 have been studied. Formation of radioactive organic and amino acids indicates on deep degradation of TNT. It was found that carbon atoms of 1-^{14}C-TNT assimilated by microscopic fungi mainly participate in synthesis of organic acids (70-90%) and amino acids (10-30%).

Remediation level of TNT-contaminated soils treated by the most active strains *Aspergillus niger* N2-2 and *Mucor* sp. T1-1 have been studied under laboratory and field conditions. Cultivation of the above mentioned strains under laboratory conditions in sterile black and red soils for 30 days at 30°C allowed to decrease the content of TNT in black soil to the residual, and in red soil – to 15%; cultivation of *Aspergillus niger* N2-2 decreased the amount of TNT in black soil to 11 and in red soil – to 21%. Under the field conditions, TNT degradation level in contaminated soils by naturally existing microflora during 100 days was equal to 40-50%, and in case of introduction additionally fungal strains, TNT-destructors, reached 80%. Finally, the data analysis indicate that TNT is deeply degraded under the action of fungal strains and carbon atoms of this toxic compound are involved in regular metabolic process of fungal strains.

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