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BIOREMEDIATION METHODS FOR TREATMENT OF TNT CONTAMINATED SOILS

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U S ARMY BIOMEDICAL RESEARCH & DEVELOPMENT LABORATORY
Fort Detrick
Frederick, MD 21702-5010

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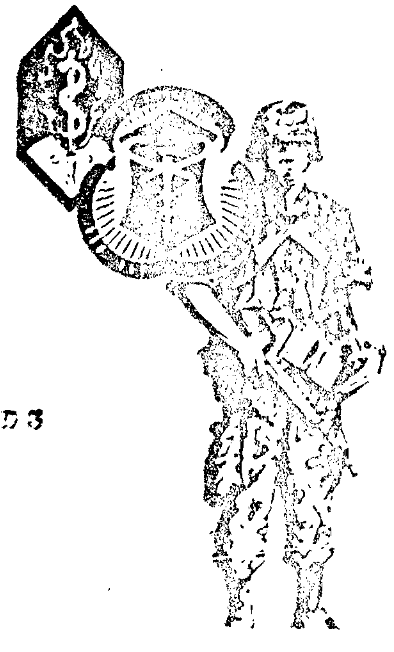

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TNT and its metabolites are the most important munition derived pollutants encountered at military installations. Remediation of sites contaminated with explosives are currently accomplished by incineration of the soil or by composting processes. The costs of incineration is too great to enable its widespread utilization and questions remain about the effectiveness of composting methods. Although biodegradation of the ring structure of TNT is not currently feasable, a novel method of binding TNT metabolites to soil may provide an effective and low-cost means of clean up.

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BACKGROUND:

TNT and its metabolites are the most important munition-derived pollutants encountered at military installations. Large amounts of this compound have been released into the environment during the synthesis and during manufacturing and demilitarization of ordinance. Remediation of sites contaminated with explosives is required by current statutes and cleanup criteria have often been set on the order of 25 ppm for TNT. The Department of Defense (DOD) may ultimately become responsible for remediation of up to one billion tons (author's estimate) of soil, in which TNT concentration exceeds 25 ppm. Some areas of contamination (AOCs) have soils that contain explosives in excess of 30% by weight, but the majority are contaminated at less than 2000 ppm. Successful remediation has been accomplished by "burning" explosive-contaminated soils with on-site portable incinerators. However, the total cost of excavation, incineration, and disposal of the burned residue ranges from $450 to $1000 per ton. Remediation of grossly contaminated soils will continue to require incineration in the foreseeable future, but less contaminated soils may soon be remediated by biological methods currently under development.

DEGRADATION OF TNT IN NATURAL SYSTEMS

TNT is degraded by natural processes in many environments, but the rates of decay are normally slow and the *meta*-substituted aromatic ring structure is modified, rather than destroyed, by these reactions (1-7). TNT can be transformed either by oxidation of the methyl group or reduction of nitro groups (1,2,5,7) (Fig. 1). However, the suite of compounds generated in this manner are themselves quite toxic (8). TNT contaminants that reside in aerobic environments at the surface of the soil are often degraded by oxidation of the methyl carbon. This oxidation is probably a multi-step process by which the methyl group is oxidized first to an alcohol, then to an aldehyde, and finally to a carboxylic acid. The carboxylic acid function may then leave the ring as CO_2.

yielding trinitrobenzene. Indication of such a pathway is substantiated by Spanggord et al. (9), who report the formation of trinitrobenzealdehyde and trinitrobenzoic acid during the degradation of TNT to trinitrobenzene. It has been reported that oxidation of the methyl group of TNT is mediated by surface catalysis on soil minerals (10), by ozonation (11), and possibly, by the action of sunlight (9). At sites where the TNT contamination is localized to the soil surface, the concentration of trinitrobenzene may often exceed that of TNT (12).

Nitroaromatics are commonly reduced to amino-nitro compounds in a multi-step process involving nitroso (N=0) and hydroxyl amine (HNOH) intermediates (7) (Fig. 1). Reduction of the nitro group that resides *para* to the methyl group (at the #4 position) is thermodynamically preferable to reduction of those at the #2 and #6 positions, as predicted by the work of McCormick et al. (6). It is known that the 4-amino reduction product is the predominant reduced form of TNT in aerobic environments and 2,4-diaminonitrotoluene predominates under mildly acidic (pH 6) anaerobic conditions (8). It has also been reported that 2,4-diamino-6-nitrotoluene can also be generated under aerobic conditions by reaction with certain minerals associated with clays. This compound is stable in the surface soil environment (13). The tri-amino reduction product of TNT is not formed at the oxygen levels encountered in the majority of natural soil environments (1,8).

Reduction of the nitro groups of TNT has pronounced environmental impact because it is predicted that this process will increase the rate of leaching of the molecule into groundwater. Leaching is increased because hydroxylamine and the amino functional groups can be linked by a hydrogen bond with water. This property makes these compounds more water soluble and, therefore, more leachable than the parent compound. In addition, hydroxylamines are reactive compounds that may react with the nitroso intermediate to link aromatic rings through azoxy linkages (1,2,6,7,8). Hydroxylamines may also undergo disproportionate reactions in which two hydroxylamines react to yield a nitroso and an amine. The latter compounds may then react abiotically to yield an azo compound (1,2,7).

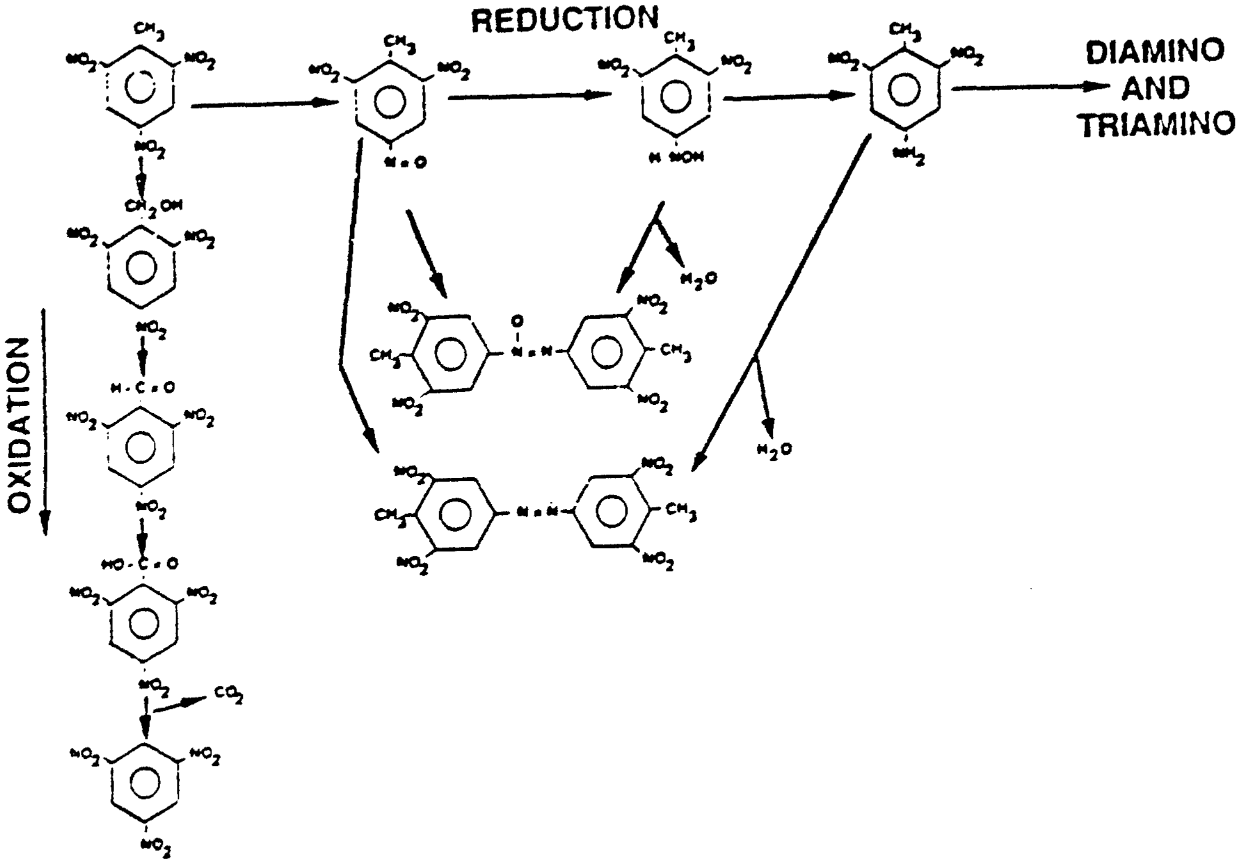

REDUCTION
DIAMINO AND TRIAMINO
OXIDATION
CH3
NO2
N=O
H NOH
NH2
CH2OH
H-C=O
HO-C=O
CO2
H2O
N=N
CH3

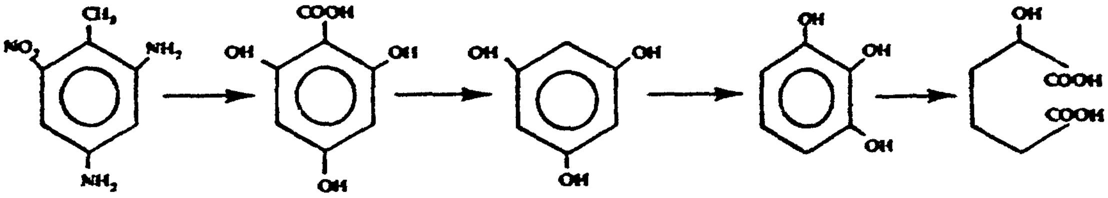
Fig. 1

CLEAVAGE OF THE AROMATIC RING

Biological mineralization of the carbon ring structure of TNT is made difficult because of the orientation of the nitro groups. During synthesis of TNT, the initial nitration of the toluene ring occurs preferentially at the ring positions *ortho* or *para* from the methyl substituent. Additional nitrations of the ring become more difficult because of the "deactivation" of the ring caused by the electron-withdrawing power of the nitro group. The result of this deactivation is that additional nitro groups are added *ortho* and *para* to the methyl group and *meta* to each other. It is this *meta* substitution of the nitro groups that makes mineralization of the TNT ring difficult to achieve by biological processes. Biological cleavage of aromatic rings requires emplacement of phenolic substituents that are oriented *ortho* or *para* to each other (14,15). However, the *meta* substitution of TNT ensures that any additional substituents also be entered *meta* to each other. Thus, the required *ortho* or *para* addition of hydroxyl groups cannot be achieved directly.

Despite the above limitations, experiments conducted with isolated microorganisms in laboratory settings have shown degradation of the ring structure of TNT. Traxler et al. (16) contend that a single gram-negative organism isolated from an enrichment culture was able to use TNT as its sole source of carbon and nitrogen. However, experiments with ring-labeled ^{14}C-TNT did not yield radioactive carbon dioxide. Experiments with the white rot fungus *Phanerochaete chrysosporium* have yielded evidence of mineralization of TNT to CO_2 (17). However, TNT in concentrations greater than 20 ppm was sufficiently toxic to inhibit fungal growth (18), and reports of fungal mineralization of TNT in natural environments are lacking.

A possible pathway for TNT ring cleavage under laboratory conditions is reported by Selivanovskaya et al. (19) as referenced by Walsh. The mechanism of cleavage is a multi-step process in which a *meta*-substituted diaminonitrotoluene is converted to phloroglucinol carboxylic acid. The latter compound is decarboxylated to yield phloroglucinol which undergoes rearrangement to pyrogallol. Pyrogallol has the phenol functional groups on adjacent ring carbons and is therefore suitable as a substrate for ring cleavage enzymes. A probable mechanism for this pathway involves oxidative deamination (biological transamination) of the two primary amines. The *meta* configuration of these groups would preclude the formation of a di-keto tautomer and the reaction product exists in the di-phenol form. In a subsequent step, the methyl group is oxidized, the remaining nitro group is also replaced with a phenol, and the phenol groups are moved to adjacent carbons. The resultant phloroglucinol carboxylic acid could then be decarboxylated and the phenolic substituents rearranged by the action of isomerases.



Although the mineralization of the ring carbons of TNT has been demonstrated in the laboratory, it appears that TNT is not utilized as a nutrient for microorganisms in natural environments. It is probable that the sequence of reduction, transamination, and oxidation is "disfavored" as a nutritive process if other food sources are available.

REDUCING SYSTEMS

Reduction of one of the three nitro substituents of TNT may occur biotically (8) or abiotically (13), in both aerobic and anaerobic environments (8). Although reduction of nitro groups occurs under aerobic conditions, the speed of the reaction and the equilibrium ratio of amino reduction products to the more oxidized nitro, nitroso, and hydroxylamine forms increase under reducing conditions (1,7,8). It is also known that anaerobic conditions and low pH favor the rapid formation and stability of amino substituents. Rapid formation of amines is beneficial for remediation purposes, because the quick completion of the reduction sequence limits the generation of unwanted condensation products that arise from the reactive hydroxylamine intermediate (8).

Conversion of TNT to 2,4,6-triaminotoluene is known to occur only under extreme reducing conditions (1,8) and negative redox potentials (> - 0.200 mV). It has also been reported that reduction processes leading to the formation of triaminotoluene require the presence of gaseous hydrogen (1). Conditions such as these would destroy the natural soil environment by killing many aerobic organisms, reducing soil humus, and inhibiting humification reactions.

Current research at the University of Texas and the University of Idaho, focuses on the utilization of reducing environments for bioremediation of soil systems contaminated with a variety of nitroaromatic residues (8,20,21). In these experiments, severe reducing conditions are established by the action of facultative anaerobes on added nutrients, in the absence of oxygen and light. Under these conditions, the nitro groups of TNT are utilized as the final electron acceptors for microbial electron transport systems, with the resulting formation of diaminonitrotoluene and triaminotoluene. Reduction of nitro groups occurs rapidly in such anaerobic systems, with little formation of unwanted condensation products. This system is quite effective in generating nitro-reduction products from a variety of compounds and has reportedly succeeded in destroying the ring structure of a dinitrophenol pesticide (21). However, to date this system has proven unsuccessful in mineralization of the aromatic ring of TNT (8,20).

BINDING CONTAMINANTS TO SOIL

Although TNT is difficult to mineralize, evidence is accumulating that natural processes may bind chemically modified TNT molecules to reactive centers in the soil (Fig. 2). This process offers the potential of bioremediating TNT-contaminated sites by effectively preventing pollutants from entering the biosphere. Thus, binding nitroaromatic residues to soil could be an acceptable means of remediation if it were proven that the binding is quantitative and of sufficient stability to prevent leaching to groundwater and accumulation in plants and soil invertebrates. Prevention of bioaccumulation is dependent on the ability of the munition/soil complex to resist dissolution in rainwater and cleavage by biotic and abiotic mechanisms encountered in the soil environment. Biotic systems can release covalently bound compounds from soil by producing localized changes in the pH or redox potential of the soil environment, or by secretion of extracellular enzymes. In addition, vertebrates may be directly exposed to toxic residues if these compounds are released from inhaled or ingested soil particles by pulmonary macrophages or by digestive processes.

POSSIBLE REACTIONS OF AMINO NITROTOLUENES WITH SOIL ORGANICS TO YIELD IMINES, 2 AMINES, AND AMIDS.

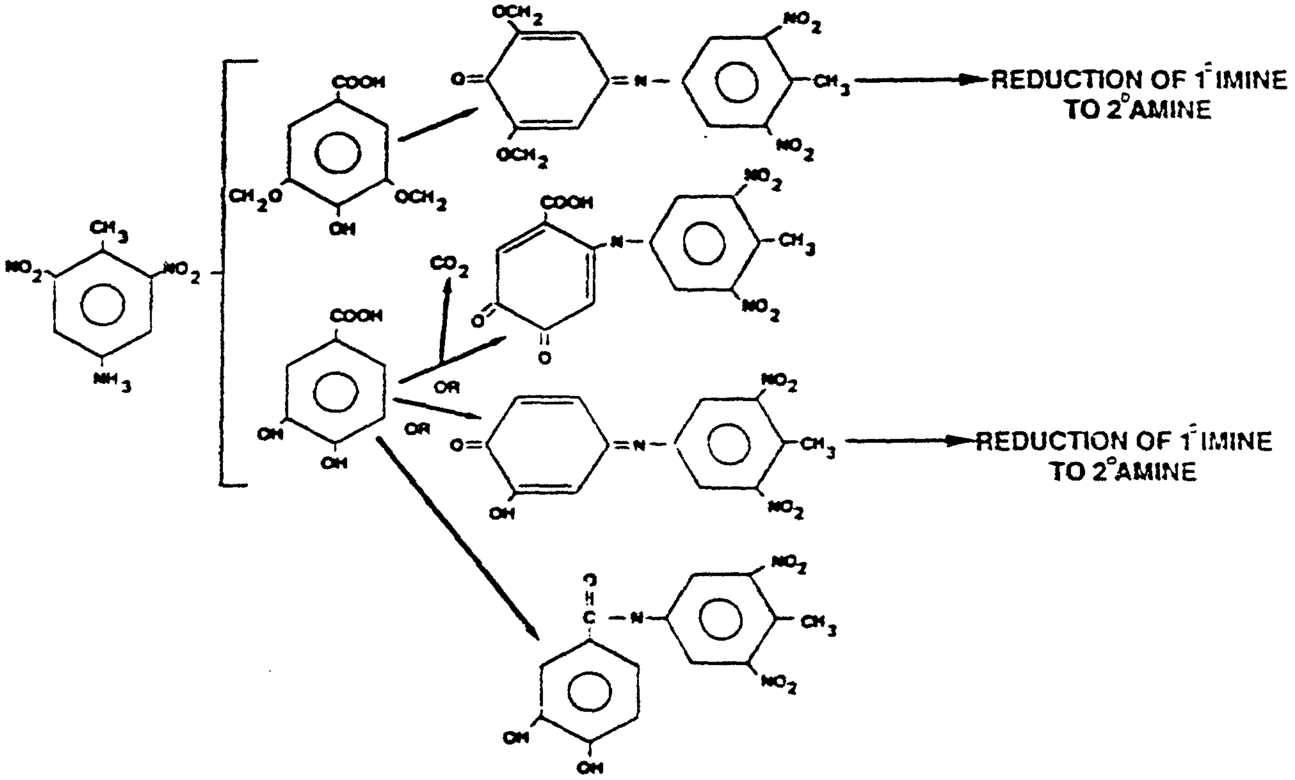


Fig. 2

Little is known of the covalent binding forces between explosives and soils, but it is likely that reduction of the nitro substituents must occur prior to generation of covalent linkages. Greene et al. (22) found that TNT was retained at higher levels in soil columns that were supplemented with glucose. This experiment supports the contention that reduction of nitro groups is needed for binding because addition of glucose to the leaching mixture would tend to promote anaerobic conditions within the column and thus, increase the rate of reduction.

It is likely that aromatic amino-nitro compounds formed in reduction processes may be bound to soil in a manner similar to that reported for anilines. Work with anilines demonstrates the existence of several types of covalent linkages that may bind these compounds to organic compounds in the environment.

COMPOSTING

Composting is currently the only bioremediation method for explosive-contaminated soils that has progressed through the field trial stage and enjoys acceptance as a means of remediation of TNT-contaminated sites. In these procedures, explosive-contaminated soil is mixed with straw, manure, and nutrients in a ratio of approximately one part soil to three or four parts compost. The mixture is augmented with a "digestable organic nutrient" and maintained at approximately 50% moisture content with mixing and aeration for several months (23,24). It is hoped that the composting process will destroy or bind the nitroaromatic component to the soil and the finished compost can be reintroduced into the AOCs. The composting process does indeed remove the majority of the available TNT from contaminated soils but questions concerning the effectiveness of this process as a remediation method remain.

Composting, like other bioremediation methods, is effective in converting TNT to an assortment of other nitroaromatic compounds. As with other biological field methods, reliable mineralization of the aromatic ring has not been demonstrated (25,26). Composting methods may nonetheless become acceptable for remediation, if it can be demonstrated that this procedure has the potential of quantitatively and irreversibly binding nitroaromatic residues to insoluble complexes in the soil/compost mixture.

An examination of the reaction conditions produced by this process reveals some areas of concern. Composting is an oxidative process. Therefore, the reducing conditions required to rapidly convert nitro to amino groups are not established and a build-up of hydroxylamine intermediates may be expected. In addition, active composts tend to become alkaline. Isbister et al. (2) reported that the pH of their composts rose from 6.9 to 8.8 in only six weeks. Under conditions of relatively high pH and high oxygen pressure, the persistence of hydroxylamine intermediates is of special concern. It is known that hydroxylamines spontaneously condense to yield dimeric and polymeric azoxy compounds (2,5,6,7) and that alkaline oxidizing conditions are optimal for these reactions (8). Kaplan and Kaplan (4) reported that azoxy compounds can become the principal reservoir of TNT metabolites under simulated composting conditions.

Azoxy-linked dimers and higher level polymers may be bioremediation "dead ends" because they have very low aqueous solubility and are, therefore, theoretically less reactive toward more stable binding processes. Moreover, azoxy compounds are susceptible to hydrolysis and have the potential to become "slow-release" sources of toxic monomers, which may make composted soils hazardous for years.

The presence of unbound reduction products of TNT in active composts is subject to debate. Studies that used very low concentrations of TNT in the compost (26) report little accumulation of TNT metabolites. This is consistent with the work of Isbister et al. (2) who reported that metabolites were largely absent even with initial TNT concentrations as high as 10,000 ppm. Other studies show that concentrations of nitro reduction products often rise early in the composting process and decrease with time (23,24).

Current work by Palmer et al. (27) also indicate possible problems with composting as a remediation method. In these studies, composts of ^{14}C ring-labeled TNT were administered to rats by intratracheal instillation. Fully 1/3 of the administered radioactivity was excreted in the urine in 160 days, indicating that the current composting process is not effective in limiting the bioavailability of TNT-based pollutants from contaminated soil. In addition to the above problem, these studies revealed a marked tendency for radiolabel from TNT composts to slowly accumulate in the liver and kidney. These results are in contrast to studies with pure TNT which exhibited little tissue accumulation. A slow accumulation of radiolabel in the liver and kidney is consistent with the conversion of TNT to a more hydrophobic form and supports the contention that composting can generate unwanted azoxy compounds.

The Department of the Army is currently proceeding with composting as a means of remediating TNT-contaminated soils. This work should be supported with laboratory experiments using pure compounds. Laboratory experiments are necessary to determine the TNT metabolites generated under various composting conditions and also to determine how variations in composting conditions affect the binding reactions which accomplish the remediation.

The metabolites of TNT and the means by which these compounds are linked to compost are currently unknown because of the extreme complexity of compost and the resultant difficulty in detecting specific bonds. Knowledge in this area is required before the effectiveness of composting as a remediation method can be fully determined. Current research demonstrates that a variety of bonds may link TNT residues to the composts. Although some of these associations are quite strong, the majority of these linkages are liable to hydrolytic processes (23,26) and may, therefore, lack sufficient stability to resist degradation by environmental forces. Caton et al. (26) report that 74% of the ^{14}C in labeled TNT becomes bound on the first day of composting and this value increases to 99% by day 90. However, they also report that the majority of the ^{14}C-labeled material is bound to the soil by semi-stable bonds that can be broken by alkaline hydrolysis. More stable (non-hydrolyzable) linkages are also formed, but these are generated much more slowly and involve only a small portion of the added TNT. The Caton et al. (26) study showed that only 5% of the ^{14}C label was bound stably at the completion of the 90-day study. This behavior indicates that weaker binding interactions are established more quickly than stronger ones during composting. Hsu and Bartha (27) report similar results in their investigations with binding of chloroaniline to soil. In these studies binding of anilines was most efficient in the early stages and only approximately 40% of the bound anilines could be released by alkaline hydrolysis.

A more through understanding of the chemistry of the binding process is important because, if composts prove unable to bind explosive residues under all environmental conditions, the composts may require incineration at some future date. It is apparent that specific information on the covalent bonds formed between composts and nitroaromatics is required to adequately assess this process as a method of TNT remediation. The hydrolysis experiments described above indicate a potential vulnerability of this procedure as a long-term remediation method. Identification of all compounds released during hydrolysis would provide better insight as to the nature of the binding reactions. To date, systematic research has not been conducted to determine the specific chemical reactions involved in the composting process.

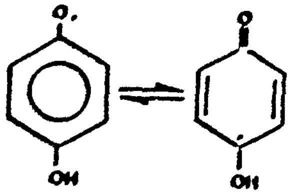
AMIDES

Although the existence of amide linkages between aromatic amines and soil have not been reported, the existence of extracellular microbial enzymes that are capable of forming amide bonds has been demonstrated in activated sludge systems (3) and such enzymes may also be present in soil systems. Earlier research in an experimental activated-sludge system for treatment of explosive-contaminated wastewater indicates that multiple amide linkages (polyamides) are formed between reduced forms of TNT and the carboxyl functions of fatty acids and proteins (3). Similar linkages may be generated in soil systems where free carboxylic acids are found extensively both in humic acid-forming phenolic monomers and in the structure of "finished" (polymerized) humic materials. The presence of natural systems capable of generating amide linkages and the resistance of polyamides to degradation supports the theory that such complexes may be formed and persist in natural soil systems.

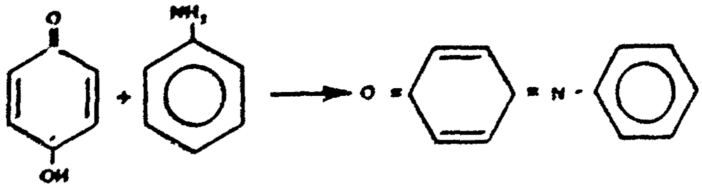
It is likely that the stable binding of anilines with amide bonds requires bond formation at more than one amino group. Moreover, it is probable that the production of tri-substituted polyamides does not depend on the *a priori* formation of triaminotoluene. Instead, formation of amide linkages to the diamino TNT reduction product would substantially reduce the electron density of the aromatic ring and potentiate the subsequent reduction of the third nitro group.

HUMIFICATION

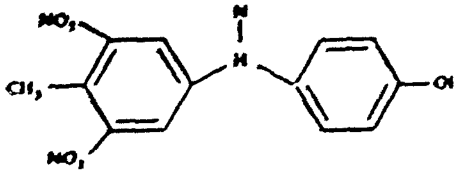
The most likely mechanism for the covalent binding of anilines and aminonitrotoluenes to soil is through the humification process (natural process for conversion of aromatic compounds to the humic and fulvic acid portions of soil). During this process, natural polyphenols are polymerized in an oxidative process to form fulvic or humic acid. It is likely that the rate-limiting step in this process is the conversion of the polyphenol to a semiquinone radical (a highly reactive free radical generated with some *ortho* or *para*-substituted polyphenols) (29), an example of which is illustrated below.



Semiquinone radicals react to form ether linkages with natural polyphenols or phenolic acids during the polymerization reactions of the humification process. They may also react with other aromatics to link them to the developing humic polymer (30,31). It has also been reported that phenolic free radicals (perhaps semiquinones) react with anilines to form imine bonds (32,33), as illustrated below.



Imine bonds would probably fail to provide stable linkages between aromatics and soil in many environments, because their chemical properties make them susceptible to hydrolysis (34). Hydrolysis of imine bonds is known to occur spontaneously in aqueous solution under even mildly acidic or alkaline pH conditions. Moreover, the bond may be susceptible to one or more of a number of common pyridoxal phosphate-type enzymes that are known to generate imines as transition-state intermediates in transamination reactions. A more stable bond may be generated by reduction of the imine bond to a secondary amine, as shown below.

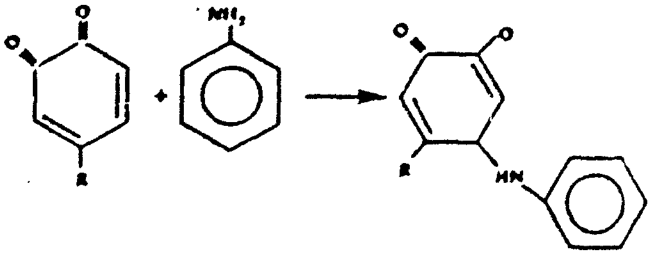


Reduction of imine bonds is more likely with anaerobic (reducing) conditions, but the formation of semiquinone radicals is an oxidative process (31). Therefore, well aerated soils would be theoretically ideal for formation of imines but poor environments for reduction of imines to the more stable secondary amines that are probably required to produce binding of sufficient stability for remediation. A theoretically superior method of binding TNT residues to soil would incorporate a mechanism for producing reactive semiquinone radicals in a medium with sufficient reducing potential to convert nitro compounds to anilines and imines to amines at a rapid rate.

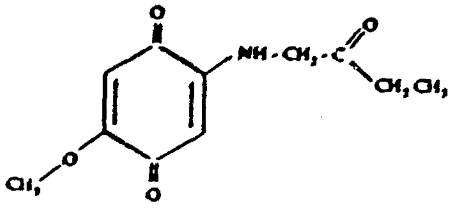
A possible means to create these reactions is by introduction of metal oxides into reducing environments. Several researchers have reported mechanisms that used oxides of iron and manganese to oxidize aromatic phenols and amines (35-41). It is probable that these reactions, like the enzyme-induced oxidations described above, proceed via the formation of semiquinone radicals. Evidence of the similarity of the metal-oxide and enzymatic reactions is seen in the similarity of their quinonic reaction products and in the apparent similarity of the "humic-like" polymers generated by both reaction mechanisms. It is, therefore, possible that semiquinones are produced under reducing conditions in which they would not normally exist. Generation of semiquinone radicals in a reducing environment enriched in aminonitrotoluenes would create theoretically ideal conditions for generation of amine linkages between explosive residues and soil. Such a system would require sufficient reducing power to rapidly reduce TNT to aminodinitrotoluene and diaminonitrotoluene while avoiding the severe reducing conditions required to produce triaminotoluene. Avoidance of severe anaerobic conditions would prevent the complete reduction of soil functional groups, this is subsequently required for the polymerization of humus constituents.

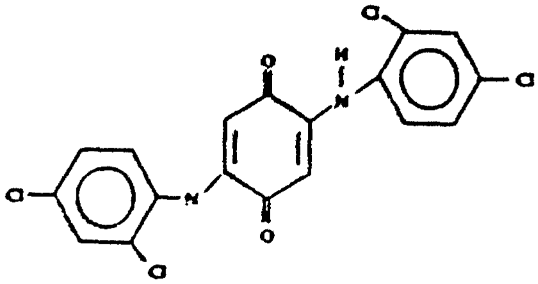
Alternatively, the needed reduction and oxidation reactions could be achieved by rotating the soil environment through anaerobic and aerobic phases. In the first phase, the soil could be made anaerobic by the procedure of Roberts (described above). When soil analysis reveals that the majority of the TNT is reduced to diaminonitrotoluene, the soil environment could then return to the natural (aerobic) state and reaction conditions optimized for humification to proceed. An additional transition to anaerobic conditions would then stabilize the binding process by the reduction of imine bonds to the more stable secondary amines. This system would quickly bind TNT residues to soil and might prove potentially useful as a means of bioremediation. However, the relationship between the reduction of imine bonds and the establishment of reducing conditions has not been adequately researched; additional work is required to assess the feasibility of such a procedure.

A second possible method for *in situ* formation of linkages between contaminants and soil is described by Parris (34). In these studies, aromatic amines reacted spontaneously with quinones, resulting in the formation of a secondary amine linkage between the aniline nitrogen and the ring carbon *para* to the carbonyl group, as shown below.



A similar product is seen in the work of Liu et al. (42,43), who demonstrated formation of a bond between the primary amino group of glycine-ethyl ester and a ring carbon of syringic acid and between the ring carbon of 2-chlorobenzoquinone and the amino group of 2,4-dichloroaniline to generate the products below.





In addition, both Parris (34) and Hsu et al. (44) report mechanisms whereby substituted anilines could be transformed into heterocyclic rings. The proposed reaction mechanisms involve formation of a secondary amine linkage between the aniline nitrogen and a carbon of a substituted aromatic ring; subsequent formation of an additional bond between other functional groups or between the secondary amine nitrogen and reactive centers on adjacent molecules also then occurs.

The above methods offer the following advantage: generation of the semi-stable imine is avoided, thus eliminating the necessity for a subsequent reductive step. In addition, these methods have increased potential for producing environmentally stable complexes due to the considerable thermodynamic stability of the heterocyclic ring. Formation of a heterocyclic ring by direct formation of a tertiary amine is a likely subsequent reaction for secondary amines (Fig. 3) because these compounds do not form imines (34). Inclusion of one or more of the aniline groups (after TNT reduction) into heterocyclic rings would essentially make the residue a part of the soil humus complex and would very probably eliminate the compound as a "free" pollutant.

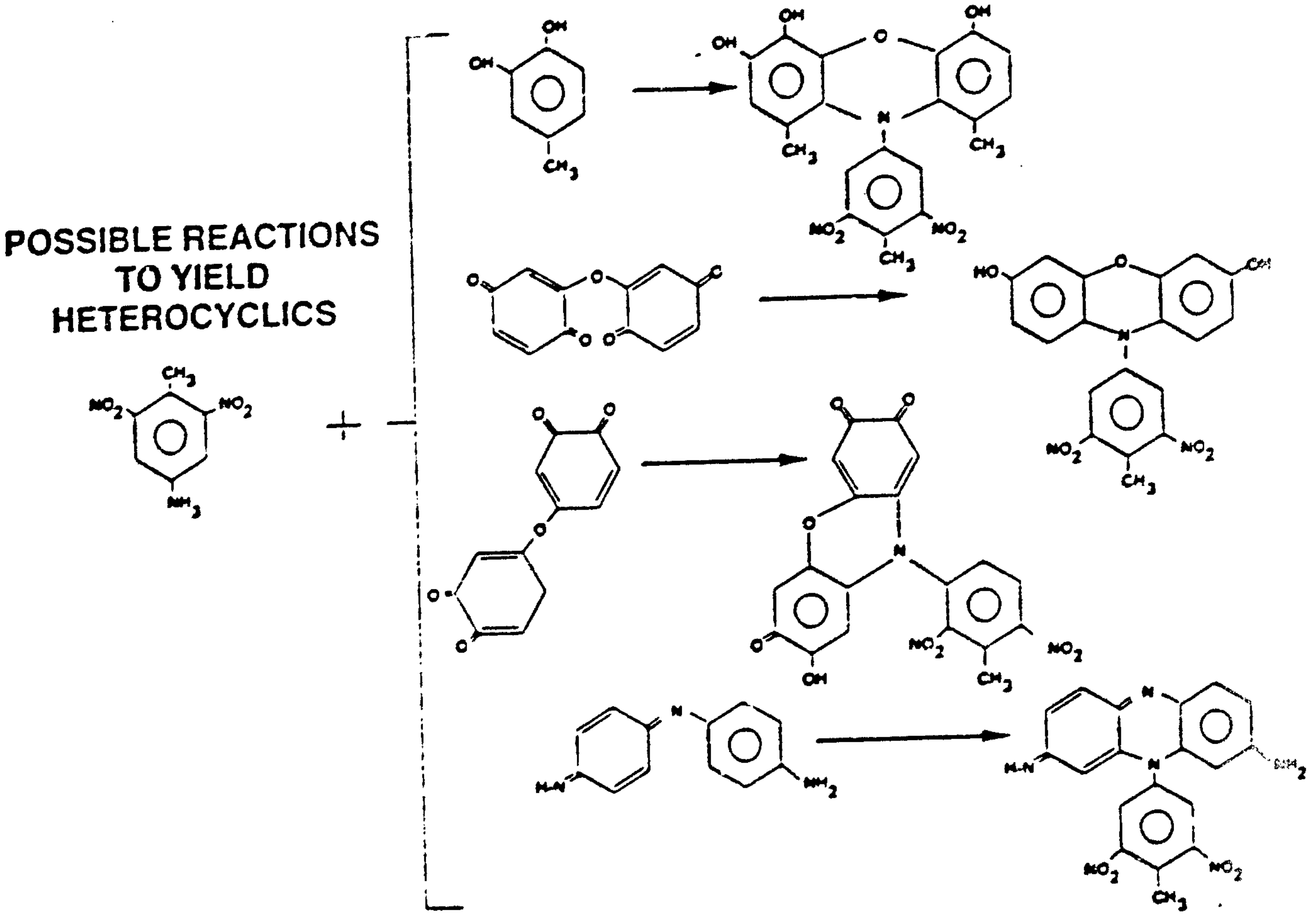

POSSIBLE REACTIONS TO YIELD HETEROCYCLICS

Fig. 3

REFERENCES:

1. Kaplan, D. L. 1990. Biotransformation pathways of hazardous energetic organonitro compounds, p. 155-182. In D. Kamely, A. Chakrabarty and G.S. Omenn (eds.), Advances in applied biotechnology, 4: Biotechnology and Biodegradation, Portfolio Publishing Company, Texas.

2. Isbister, J.D., G.I. Anspach, J.F. Kitchens, and R.C. Doyle. 1993. Composting for decontamination of soils containing explosives. Microbiological. 7: 47-73.

3. Carpenter, D.F., N.G. McCormick, J.H. Cornell, and A.M. Kaplan. 1978. Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. Applied and Environmental Microbiology. 35: 949-954.

4. Kaplan, D.L., and A.M. Kaplan. 1982. 2,4,6-Trinitrotoluene-surfactant complexes: Decomposition, mutagenicity and soil leaching studies. Environmental Science and Technology. 16: 566-571.

5. Kaplan, D.L. and A.M. Kaplan. 1982. Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Applied and Environmental Microbiology. 44: 757-760.

6. McCormick, N.G., F.E. Feeherry, and H.S. Levinson. 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. Applied and Environmental Microbiology. 31: 949-958.

7. Walsh, M.E. 1990. Environmental transformation products of nitroaromatics and nitramines. U.S. Army Corps of Engineers Cold Regions Research & Engineering Laboratory, Special Report 90-2.

8. Funk, S.B., D.J. Roberts, D.L. Crawford, and R.L. Crawford. 1993. Initial phase optimization for the bioremediation of munition-contaminated soils. 1993. A Publication of the Idaho Agricultural Experiment Station. Moscow, ID.

9. Spanggord, R.J., T. Mill, T. Chou, W.R. Mabey, J.H. Smith, and S. Lee. 1980. Environmental fate studies on certain munition wastewater constituents lab studies. Menlo Park, California: SRI international. ADA099256.

10. Checkai, R.T., M.A. Major, R.O. Nwanguma, J.C. Amos, C.T. Phillips, R.S. Wentsel, and M.C. Sadusky. 1993. Transport and fate of nitroaromatic and nitramine explosives in soils from open burning open detonation operations at Anniston Army Depot. ERDEC Technical Report # (number not yet assigned).

11. Kearney, P.C., Q. Zeng, and J.M. Ruth. 1983. Oxidative pretreatment accelerates TNT metabolism in soils. Chemosphere. 12: 1583-1597.

12. Checkai, R.T., M.A. Major, R.O. Nwanguma, J.C. Amos, C.T. Phillips, R.S. Wentsel, and M.C. Sadusky. 1993. Transport and fate of nitroaromatic and nitramine explosives in soils from open burning open detonation operations at Radford Army Ammunition Plant. ERDEC Technical Report # (number not yet assigned).

14. Dagley, S. 1975. Microbial degradation of organic compounds in the biosphere. American Scientist. 63: 681-689.

15. Simpson, J.R. and W.C. Evans. 1953. The metabolism of nitrophenols by certain bacteria. The Biochemical Journal. 55: 24.

16. Traxler, R.W., E. Wood, and J.M. Delaney. 1974. Bacterial degradation of Alpha-TNT. Developments in Industrial Microbiology. 16: 71-76.

17. Tudor, F., J.A. Bumpus, and S.D. Aust. 1990. Biodegradation of TNT (2,4,6-Tinitrotoluene) by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology. 55: 1666-1671.

18. Spiker, J.K., D.L. Crawford, and R.L. Crawford. 1992. Influence of 2,4,6-trinitrotoluene (TNT) concentrations on degradation of TNT in explosive-

contaminated soils by the white rot fungus Phanerochaete chrysosporium. Applied and Environmental Microbiology. 58: 3199-3202.

19. Selivanovskaya, S.Yu., D.Z. Akhmetcvs, and R.P. Naumova. 1986. Terminal steps in preparatory metabolism of 2,4,6-trinitrotoluene in Pseudomonas fluorescens. Mikrobiologia. 56: 1040-1041.

20. Roberts, D.J., S. Funk, D.L. Crawford, and R.L. Crawford. 1993. Anaerobic biotransformation of munition wastes. Presented at the EPA Symposium on Bioremediation of Hazardous Wastes: Research, Development and Field Evaluations. May 4-6 Dallas TX.

21. Kaake, R.H., D.H. Roberts, T.O. Stevens, R.L. Crawford, and D.L. Crawford. 1992. Bioremediation of soils contaminated with the herbicide 2-sec-butyl-4,6-dinitrophenol (Dinoseb). Applied and Environmental Microbiology. 58: 1683-1689.

22. Greene, B., D.A. Kaplan, and A.M. Kaplan. 1985. Degradation of pink water compounds in soil-TNT, RDX, HMX. U.S. Army Natick Research and Development Center Technical Report # 85/046. AD-A157954.

23. Williams, R.T., P.S. Ziegenfuss, and W.E. Sisk. 1992. Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. Journal of Industrial Microbiology. 9, 137-144.

24. Woodward, R.E. 1990. Evaluation of composting implementation: A literature review. U.A. Army Toxic and Hazardous Materials Agency Report # TCN 89363. AD-A243 908.

25. Osmon, J.L., C.C. Andrews, A. Tatyrek. 1978. The biodegradation of TNT enhanced soil and compost systems. U.S. Army Armament Research and Development Command Technical Report ARLCD-TR-77032, ADA054375.

26. Caton, J.E., R.T. Williams, and W.H. Griest. 1993. Bound fractions of explosives transformed by composting. Environmental Toxicology and Chemistry, In Press.

27. Palmer, W.G., Beaman, J., Walters, and D.., Creasia, D.A. 1993. Bioavailability of TNT in composts of TNT-contaminated soil. Abstract, submitted to the Society of Toxicology, Annual Meeting, March 1994.

28. Hsu, T.S., R. Bartha. 1973. Interaction of pesticide-derived chloroaniline residues with soil organic matter. Soil Science. 116: 444-452.

29. Bartha, R. 1980. Pesticide residues in humus. American Society for Microbiology News. 46: 356-360.

30. Whelan, G. and R.C. Sims. 1992. Oxidation of recalicitrant organics in subsurface systems. Hazardous Waste and Hazardous Materials 9: 245-265.

31. Schnitzer, M. 1982. Quo vadis, soil organic matter research. Whither soil Research Publications of the 12th Int. Congr. Soil Sci. New Delhi India, 5: 67-78.

32. Bollag, J.-M., W.B. Bollag. 1990. A model for enzymatic binding of pollutants in the soil. International Journal of Analytical Environmental Chemistry. 39: 147-157.

33. Bollag, J.-M., R.D. Minard, and S.-Y. Liu. 1983. Cross-linkage between anilines and phenolic humus constituents. Environmental Science and Technology. 17: 72-80.

34. Parris, G.E. 1980. Covalent binding of aromatic amines to humates. 1. Reactions with carbonyls and quinones. Environmental Science and Technology. 14: 1099-1106.

35. Stone, A.T., and H.J. Ulrich. 1989. Kinetics and reaction stoichiometry in reductive dissolution of manganese (IV) dioxide and Co(III) oxide by hydroquinone. Journal of Colloid and Interface Science, 132:2, 509-522.

36. Lakind, J.S. and A.T. Stone. 1989. Reductive dissolution of goethite by phenolic reductants. Geochimica et Cosmochimica Acta. 53: 961-971.

37. Interactions of Soil Minerals with Natural Organics and Microbes. Special Publication Number 17 of the Soil Science Society of America Inc. 1986. Editors: Huang, P.M. and M. Schnitzer. Madison Wisconsin, USA.

38. Laha, S. and R.G. Luthy. 1990. Oxidation of aniline and other primary aromatic amines by manganese dioxide. Environmental Science and Technology, 24: 363-373.

39. Shindo, H. and P.M. Huang. 1982. Role of Mn(IV) oxide in abiotic formation of humic substances in the environment. Nature. 298: 363-365.

40. Shindo, H. and P.M. Huang. 1984. Catalytic effects of manganese (VI), iron III, aluminum, and silicon oxides on the formation of phenolic polymers. Soil Science of America Journal. 48: 927-934.

41. Littler, J.S. 1970. The mechanisms of oxidation of organic compounds with one-equivalent meta-ion oxidants: Bonded and non-bonded electron transfer. In "Essays on Free-Radical Chemistry; London Chemical Society." London UK.

42. Liu, S.-Y., A.J. Freyer, R.D. Minard, and J.-M. Bollag. 1985. Enzyme-catalyzed complex-formation of amino acid esters and phenolic humus constituents. Soil Science Society of America Journal. 49: 337-342.

43. Liu, S.-Y., R.D. Minard, and Bollag, J.-M. 1981. Coupling reactions of 2,4-dichlorophenol with various anilines. Journal of Agriculture and Food Chemistry. 29: 253-257.

44. Hsu, T.S. and R. Bartha. 1976. Hydrolyzable and nonhydrolyzable 3,4-dichloroaniline-humus complexes and their respective rates of biodegradation. Journal of Agriculture and Food Chemistry. 24: 118-122.

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