

ECOLOGICAL INDICATION, BIOACCUMULATION, AND PHYTOREMEDIATION AS TOOLS FOR ENVIRONMENTAL QUALITY MANAGEMENT

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The DoD currently has an estimated 12,000 sites that require some form of remediation related to production activities, field usage, and disposal of military unique compounds (MUCs). Chemical methods to explore types and levels of contamination, and chemical/physical and mechanical methods for cleanup of contaminated soils and sediments exist, are very costly, but do not give insight in environmental toxicity. We explore biological methods that can: (1) indicate toxicity (level and spatial extent), bioaccumulation and potential for trophic transfer; and (2) remove contaminants by root uptake, subsequent transport to shoots, and degradation, or prevent contaminants from leaving the site in whatever form, such as leachate, runoff, trophic transfer (phytoremediation). We use risk assessment to evaluate the toxicity and need for cleanup. Cleanup costs are expected to greatly exceed the cost of evaluation, but to decrease with a decrease in uncertainty to reach the cleanup targets. At present, strategies are under development for evaluating the potential hazards of MUCs to terrestrial organisms/wildlife (risk assessment). These strategies require exposure-based effects data that can be used for defining criteria or reference values, and can also be used as a basis for environmental management.

Studies were conducted to provide data for predicting exposure-based effects of explosives, spread via representative components of increasing trophic levels of a relevant, terrestrial food chain. The goal of these studies is to determine if concentrations at a site might be harmful to indigenous species. The trophic levels studied include plants, soil invertebrates, and mammals.

Long-term exposure tests were conducted to evaluate chronic sublethal toxicity and transfer of aged soil-based explosives, with 2,4,6-trinitrotoluene (TNT) as the main contaminant. In these tests, plants were exposed for 55 days in the greenhouse to soil-TNT concentrations up to 18 mg kg⁻¹ dry weight, biomass was determined and residues of explosives parent compounds and TNT metabolites were analyzed using HPLC techniques. Worms were exposed for 28-42 days in the laboratory, biomass and number was determined, and tissues were analyzed for explosives compounds. Plants were less sensitive than worms to TNT. Among the plants, *L. perenne* (perennial ryegrass) was far more tolerant towards TNT than *M. sativa* (alfalfa). An effective concentration causing a 20% decrease in plant biomass (EC20) of 2.4 mg TNT kg⁻¹ soil-DW was derived for *L. perenne* from linear regression. TNT-metabolites (2ADNT, 4ADNT), RDX and HMX

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 00 DEC 2004		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Ecological Indication, Bioaccumulation, And Phytoremediation As Tools For Environmental Quality Management				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Army Engineer Research And Development Center, Environmental Laboratory, 3909 Halls Ferry Road, Vicksburg, Ms 39180,				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001736, Proceedings for the Army Science Conference (24th) Held on 29 November - 2 December 2005 in Orlando, Florida.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 2	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

were recovered in *L. perenne* shoots and roots. The TNT-metabolite concentrations in shoots increased significantly with soil-TNT concentration. Among the worms, *E. fetida* (earthworm) tolerated far higher TNT-concentrations than *E. crypticus* (enchytraeid). A 28d-EC20 of 1.2 mg TNT kg⁻¹ soil-DW was derived for *E. fetida* from linear regression. No explosives metabolites were recovered in the worms. Because only the effects of soil-TNT concentration on the biomass of *L. perenne* and *E. fetida* were significant, the toxicity of the soil was attributed mainly to the contamination by TNT. However, the other explosives may have contributed also. Clay amendment did not significantly affect the plant and worm responses.

Similar tests to those on TNT-contaminated soil were conducted on RDX-contaminated soil. In these tests plants and worms were exposed to RDX concentrations up to 15,000 mg kg⁻¹ dry weight. The test organisms survived exposure, and were far less sensitive to RDX than to TNT. RDX accumulated in both, plants and worms up to concentrations ranging from 3300 to 3900 mg kg⁻¹, but the RDX-metabolite MNX accumulated only in plants. The test data are currently being analyzed.

The trophic transfer of TNT and RDX, respectively, and their effects on male *Peromyscus maniculatus* (deer mouse) are being determined. The mice were exposed for 24 days to food contaminated with 'cold' + ¹⁴C-labeled TNT, or 'cold' + ¹⁴C-labeled RDX, metabolized into the tissues of perennial ryegrass and earthworms. Gross effects resulting from the uptake of explosives and metabolites into rodent tissues were evaluated relative to food ingestion rates, animal weight, and behavioral pattern. These data will provide quantitative effects of trophic transfer within a relevant food chain.

The use of vegetation to remediate large contaminated areas by containment and/or destruction, called 'phytoremediation', has been suggested as a relatively low-cost, aesthetically-pleasing method with wide public acceptance. However, vegetation may add to the risk of exposure also by its' contents of parent contaminants and degradation compounds because of its' role in food chains. Methods to evaluate potential catabolic pathways of explosives in vegetation, combined with verification of these pathways in selected relevant plant species are, therefore, needed. Identification of the predominant phytocatalytic pathways and evaluation of the distribution of these pathways in terrestrial plants will greatly diminish the risk currently perceived for these compounds. Therefore, several studies are currently underway to elucidate these pathways. A most recently initiated study explores fundamental capabilities of computational chemistry techniques including ab initio and QSAR techniques as predictors of properties and reactivities of explosives, in plants, and verify the predictions by *in vitro* and *in vivo* experimentation. The knowledge gained from this work will reduce risk and greatly advance the development and application of green cleanup technologies.

Examples from on-going research will be presented.