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Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination

Edited by

Peter A. Kulakow
Valentina V. Pidlisnyuk

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Series C: Environmental Security

Application of Phytotechnologies for Cleanup of Industrial, Agricultural, and Wastewater Contamination

edited by

Peter A. Kulakow
Kansas State University
Manhattan, Kansas, U.S.A.

and

Valentina V. Pidlisnyuk
Kremenchug Technical University
Kremenchug, Ukraine

Springer

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PREFACE

The NATO Advanced Research Workshop, "Application of Phytotechnologies for Cleanup of Industrial, Agricultural, and Wastewater Contamination to Enhance Environmental and Food Security," took place 4–7 June 2007 in Kamenetz-Podilsky, Ukraine. The purpose of the workshop was to promote enhancement of environmental and food security through use of phyto-technologies for management of contaminated soil, surface water, groundwater and wastewater. Phytotechnologies represent a group of environmental management tools that are low-cost and require minimal advanced technology for implementation.

What do we mean by phytotechnologies? Phytotechnologies use vegetation to remediate, restore, remove, dissipate, or contain environmental contamination using natural biological processes to achieve environmental management objectives. Often the presence of vegetation alone can result in environmental benefits. In many cases, directed design, plant species selection, and management are needed to achieve a particular environmental management objective. Vegetation-based technologies comprise a subset of natural remediation or bioremediation technologies that often utilize many biological mechanisms, especially microbial activity, to achieve environmental benefits. In this book, we focus on technologies that utilize vegetation management realizing there is no clear dividing line with other bioremediation processes.

To address environmental issues, it is first necessary to understand potential environmental and human health risks and to focus on risk prevention whenever possible. It is important to evaluate the range of alternatives for reducing or preventing risk and determine which alternatives are scientifically effective and feasible to implement. Finally, acceptable alternatives need to be monitored and validated to determine if environmental management goals are achieved.

The workshop was held in a rural area of Ukraine because the multitude of pressing development issues limit resources in rural areas beginning with needs for basic economic development, provision of civil services, education, and healthcare, in addition to prevention or alleviation of environmental problems. Lack of information, awareness, and appreciation for ecological and human health risks posed by environmental contamination is a challenge for creating community concern and the political commitment needed to address environmental issues. Environmental management decision making should be based on reliable and scientifically valid information that is often not readily available, especially in rural areas.

Kamenetz-Podilsky is a small city of 100,000 in southwestern Ukraine near the border with Moldova. Kamenetz-Podilsky is developing its infrastructure in a rapid and progressive way that is exemplary for Ukraine outside of the major cities. The city is becoming a major tourist destination with many well-preserved historical and natural landscapes. The city administration has also taken an active interest in improving local environmental conditions and incorporating effective environmental management as part of public policy. This local support was demonstrated by media coverage of the workshop and active participation of local community members in a 1.5-h public session held on the third day of the workshop. Participants in the workshop included an environmental management specialist from a local national park and a representative of a local agricultural research station.

Research and development of effective phytotechnology applications has been limited, so knowledge to develop successful applications is incomplete. Sharing experiences and problems can help transfer good information to new locations and provide the synergy of shared experiences to advance our understanding of phytotechnologies. Addressing environmental management issues involves the interaction of many stakeholders including members of affected communities. Stakeholders include local community members, government authorities, businesses, providers of technical assistance, and nongovernmental organizations. All of these groups must work together to gather information, assess problems, identify alternatives, implement solutions, and monitor progress. The purpose of this NATO Advanced Research Workshop was to acknowledge the important roles for stakeholders involved in addressing environmental management issues.

Some phytotechnology applications have been well demonstrated and can be expected to achieve their intended purpose if designed and managed using established procedures documented in the literature and through technical guidance documents. Other phytotechnology applications are experimental with potential value if further research and monitoring demonstrates successful risk management. In all cases, implementation of phytotechnologies relies on site-specific considerations and will benefit from consultation with experienced professionals and local expertise.

This book begins with several introductory chapters before focusing on specific remediation applications. Chapter 1, by Vanek, Podlipna and Soudek, introduces factors that influence application of phytotechnologies. In Chapter 2, by Marmiroli and colleagues highlight recent activities to build capacity in phytotechnologies, focusing on initiatives in Europe that demonstrate opportunities for collaboration and networking. In Chapter 3, Pidlisnyuk, Sokol, and Stefanovska discuss public perceptions and understanding of sustainability based on surveys done in rural communities of

Ukraine to demonstrate understanding of green technologies and the need for outreach activities for rural communities.

Chapters 4–7 address use of phytotechnologies to reduce risk from persistent organic pollutants (POPs). This work is an excellent example of exploration of an experimental application that shows promise, if successful risk reduction can be demonstrated. A limited number of plant species have been shown to extract significant amounts of POPs chemicals from soil. Åslund and Zeeb review this evidence and describe additional work needed to demonstrate the potential for practical and economic application of POPs phytoremediation to field situations. Other chapters discuss efforts in Moldova, Kazakhstan, and Ukraine to describe the critical status of POPs issues and the potential for phytoremediation to address them.

Risks associated with inorganic contaminants associated with radionuclides, arsenic, and coal fly ash also have potential to be reduced with the use of phytotechnologies. In Chapter 8, Kalinin, Tsybulskaya, and Chubrik in Belarus review the work to address radionuclide contamination using soil amendments and vegetation. In Chapter 9, Zolnowski, Ciecko, and Najmowicz discuss research with organic soil amendments to reduce phytoavailability of arsenic from natural and anthropogenic sources. Although most research on environmental issues look at short-term effects, in Chapter 10 Ciecko, Zolnowski, and Chelstowski present results based on 19 years of coal fly ash applications and the effects of added organic soil amendments on carbon and nitrogen dynamics. Such long-term results are critical for understanding the impact of remediation technologies on soil health and plant growth.

The final three chapters focus on use of vegetation to manage organic contaminants. In Chapter 11, Zhu, Chen, and Nan, discuss emerging issues of soil contaminated from extraction of petroleum in Northwestern China and the need for development of integrated land management approaches that combine phytotechnologies with other remediation technologies to reduce risk from petroleum-contaminated soil. The final two chapters introduce two groups of phytotechnology approaches that have proven successful at numerous locations in the USA and provide examples of applications that are ready for use at appropriate locations. Newman discusses use of phytotechnologies to manage groundwater contamination in Chapter 12. Finally, in Chapter 13, Rock introduces evapotranspiration landfill covers as another group of validated phytotechnology applications to manage landfill leachate and to provide safe and economic cover for management of solid waste when the cover is designed on a site-specific basis. Although phytotechnologies are an emerging group of remediation options, sufficient progress has been made to demonstrate useful applications and promising avenues for further research.

We would like to acknowledge the financial support and critical assistance provided to make the NATO Advanced Research Workshop possible. The workshop and this publication were made possible with the financial support from the NATO Science for Peace Program. Our meeting in Kamenetz-Podilsky was made possible with cooperation and support from Oleksandr Mazurchak, former Mayor of Kamenetz-Podilsky and currently First Deputy Minister at the Ministry of Housing and Municipal Management of Ukraine. We would also like to thank Valery Klimenko, Head of the International Affairs Department at Kamenetz-Podilsky city administration, and all Kamenetz-Podilsky Administration. We also acknowledge the support of Kamenetz-Podilsky State University and the Sustainable Development and Ecological Education Center. Ellen Rubin, Steve Rock, and Barbara Zeeb provided critical support and encouragement to initiate this project. Larry Erickson, Blase Leven, and Mary Rankin provided important assistance from Kansas State University. Finally, Tatyana Stefanovska and Lesya Sokol provided critical organizational support in Ukraine. We thank Wil Bruins of Springer for assistance with preparation of the manuscript for publication.

PETER A. KULAKOW AND
VALENTINA V. PIDLISNYUK
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GENERAL FACTORS INFLUENCING APPLICATION OF PHYTOTECHNOLOGY TECHNIQUES

T. VANĚK*, R. PODLIPNA, AND P. SOUDEK
Laboratory of Plant Biotechnologies, Joint Laboratory of Institute of Experimental Botany AS CR, v.v.i. and Research Institute of Crop Production, v.v.i., Rozvojová 263, 165 02 – Prague 6, Czech Republic

Abstract The aim of this contribution is to give readers basic knowledge of phytoremediation methodology, including basic definitions, advantages, and potential drawbacks, as well as information about recent developments in this field of research and application.

Keywords: phytotechnologies, phytoremediation

1. Introduction

Plants are thought to be primarily a source of food, fuel, and fiber. However, it has been realized recently that plants may serve potentially as environmental counterbalance to industrialization processes, and not only as a sink for increased atmospheric CO_2. Indeed, during the last century, the content of xenobiotic compounds in ecosystems has increased considerably. Many organic synthetic substances, which include pesticides, solvents, dyes, and by-products of chemical and petrochemical industries, are eventually transported to natural vegetation and cultivated crops, where they can either be harmful to the plant itself; totally or partially degraded, transformed, or accumulated in plant tissues and organs. In the latter case, xenobiotics are concentrated in food chains and finally in man, with possible detrimental effects on his health. Such a situation also occurs with heavy metals. Actually, anthropogenic sources of toxic metals in the environment are numerous: metalliferous mining and smelting, electroplating, energy and fuel production, gas exhausts, agriculture, or waste disposal.

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* To whom correspondence should be addressed.

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Reports on plants growing in polluted areas without being seriously harmed indicate it may be possible to detoxify contaminants using agricultural and biotechnological approaches. Higher plants possess a pronounced ability to metabolize and degrade many recalcitrant xenobiotics and may be considered "green livers," acting as an important sink for environmentally damaging chemicals. On the other hand, different plant species are able to hyper-accumulate toxic metals in their tissues. It thus appears that crops and cultivated plants could be developed and used for the removal of hazardous persistent organic compounds and toxic metals from industrial wastewaters, and for phytoremediation purposes.

2. Phytoremediation

Phytoremediation has been defined as the use of green plants and their associated micro-organisms, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental contaminants (Chappell, 1997). These plants can be herbs, shrubs, or trees, and they may be able to accumulate organics and heavy metals high above the levels found in nature (Brown, 1995; Ma et al., 2000; EPA, 2000). Phytoremediation represents a typical in situ biological treatment with the main advantage that it allows for soil to be treated without being excavated and transported, resulting in potentially significant cost savings. However, in situ treatment generally requires longer time periods, and there is less certainty about the uniformity of treatment because of the variability in soil and aquifer characteristics, and because the efficacy of the process is more difficult to verify.

Phytoremediation is expected to be complementary to classical bio-remediation techniques based on the use of micro-organisms. It should be particularly useful for extraction of toxic metals from contaminated sites and treatment of recalcitrant organic pollutants like trinitrotoluene and nitroglycerin. Plant biomass could also be used efficiently for removal of volatile organic pollutants or different priority pollutants like pentachloro-phenol, other polychlorophenols, and anilines.

At present, phytoremediation is still a nascent technology that seeks to exploit metabolic capabilities and growth habits of higher plants: delivering a cheap, soft and safe biological treatment applicable to specific contaminated sites; wastewater is a relatively recent focus. In such a context, there is still a significant need to pursue both fundamental and applied research to provide low-cost, low-impact, visually benign, and environmentally sound decontamination strategies (Schwitzguebel and Vaněk, 2003).

One of the greatest forces driving increased emphasis on research in this area is the potential economic benefit of an agronomy-based technology. Growing a crop can be accomplished at a cost ranging from two to four orders of magnitude less than the current engineering cost of excavation and reburial. Expected applications will be in the decontamination of polluted soils and groundwater (phytoremediation), or in the cleanup of industrial effluents (plant cells, tissues, or biomass immobilized in appropriate containers; whole plants cultivated in constructed wetlands or under hydroponic conditions).

Five different technologies for phytoremediation are usually recognized in literature (ITRC, 2001; Salt et al., 1998; Chappell, 1997).

2.1. PHYTOREMEDIATION OF METAL CONTAMINANTS

At sites contaminated with toxic metals, plants are used either to stabilize or remove metals from the soil and groundwater through four mechanisms: phytoextraction, rhizofiltration, phytostabilization, and phytovolatization (Mulligan et al., 2001; Pulford and Watson, 2003). The same approach can be used for radionuclides (Dushenkov, 2003).

2.1.1. Phytoextraction

Phytoextraction describes uptake and translocation of metal contaminants in the soil by plant roots into the aboveground portions of the plants. Certain plants, called hyperaccumulators, absorb unusually large amounts of metals in comparison to other (Baker et al., 1994; Yang et al., 2005).

Such a plant or a combination of these plants is selected and planted at a particular site, based on the type of metals present and other site conditions. After the plants have been allowed to grow for some time, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as long as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of ash will be less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment. Limits of this approach are based on toxicity of metals to the plants, mechanisms of metal uptake, translocation and accumulation, and time requirements to clean the site to desired levels.

This approach has been described many times in the literature, both for metals and radionuclides (Lasat, 2002; Navari-Izzo et al., 2001; Shahandeh and Hossner, 2002; Fuhrmann et al., 2002; Gentry et al., 2004; Pilon-Smits and Pilon, 2002).

2.1.2. Rhizofiltration

In Rhizofiltration contaminants are adsorbed or precipitated on plant roots or absorbed into the roots from the zone surrounding the roots. Rhizofiltration is similar to phytoextraction, but the plants are used primarily to clean contaminated groundwater and wastewater instead of soil. Constructed wetlands of different size and flow arrangement are usually used for this purpose, although some greenhouse systems have also been described in the literature.

For example, sunflowers were used successfully to remove radioactive contaminants from pond water in a test at Chernobyl, Ukraine (Dushenkov et al., 1999) and many other examples have been published recently (Weis and Weis, 2004; Hinton et al., 2005; Williams, 2002; Tanner, 2001; Soudek et al., 2004).

2.1.3. Phytostabilization

Phytostabilization is use of selected plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (rhizosphere). This process reduces the mobility of the contaminant and prevents migration to the groundwater or air, and it reduces bioavailability for entry into the food chain. This technique can be used to reestablish a vegetative cover at sites where natural vegetation is lacking due to high metals concentrations in surface soils or physical disturbances to surficial materials. Metal-tolerant species can be used to restore vegetation to the sites, thereby decreasing potential migration of contamination through wind erosion and transport of exposed-surface soils and leaching of soil contamination to groundwater. For such purposes, utilization of low-accumulating plants is recommended to decrease potential food chain contamination. Selected examples of the abovementioned approach is noted in the literature (Panfili et al., 2005; Remon et al., 2005; Kucharski et al., 2005; Arthur et al., 2005; Simon, 2005; Rizzi et al., 2004; Shu et al., 2004; Petrisor et al., 2004; Wong, 2003; Davis et al., 2002).

2.2. PHYTOREMEDIATION OF ORGANIC CONTAMINANTS

Organic contaminants are common environmental pollutants. There are several ways the plants may be used for the phytoremediation of these contaminants: phytodegradation, rhizodegradation, and phytovolatilization. The main difference in comparison to metals and radionuclides is based on the fact that organic pollutants, on the contrary to metals, can be degraded and finally totally mineralised (Singh and Jain, 2003; Susarla et al., 2002; Gianfreda and Nannipieri, 2001).

2.2.1. Phytodegradation

Phytodegradation, also called phytotransformation, is the breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compounds, such as enzymes, produced by the plants (Schroder and Collins, 2002; Nepovím et al., 2004b; Gianfreda and Rao, 2004). Pollutants (complex organic molecules) are degraded into simpler molecules and incorporated into the plant tissues (Ji et al., 2004; Newman and Reynolds, 2004; Dominguez-Rosado and Pichtel, 2004; Hannink et al., 2002; Susarla et al., 2002; Schoenmuth and Pestemer, 2004; Coleman et al., 2002; Harvey et al., 2002; Collins et al., 2002; Nepovím et al., 2004a, 2005).

2.2.2. Rhizodegradation

Rhizodegradation, also called enhanced rhizosphere biodegradation, phytostimulation, or plant-assisted bioremediation/degradation, is the breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the root zone (Chaudhry et al., 2005) and is a much slower process than phytodegradation. Microorganisms, such as yeast, fungi, or bacteria, consume and digest organic substances for nutrition and energy. Certain microorganisms can digest organic substances such as fuels or solvents that are hazardous to human beings and break them down into harmless products in a process called biodegradation. Natural substances released by the plant roots (plant exudates) contain organic carbon that provides food for soil microorganisms and additional nutrients which enhance their activity (Miya and Firestone, 2001; Barea et al., 2005; Kuiper et al., 2004).

2.2.3. Phytovolatilization

Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant. Phytovolatilization occurs as growing trees and other plants take up water and organic contaminants. Some of these contaminants may be transported through the plants to the leaves and evaporate, or volatilize, into the atmosphere (Orchard et al., 2000). The same approach has been described for selected metals, such as mercury (Heaton et al., 1998, 2005; Rugh, 2001) and selenium (Tagmount et al., 2002; Berken et al., 2002). From a general point of view, this methodology is not real cleaning but dilution and transport of pollution to the atmosphere.

3. Advantages and Limitations of Phytoremediation

Based on recent research, phytoremediation technology is a promising cleanup solution for a wide variety of pollutants and sites, but it has its limitations. The following list reflects that many of phytoremediation advantages and disadvantages are a consequence of the biological nature of the treatment system, which depends mainly on year, season, and climatic conditions. Plant-based remediation systems can function with minimal maintenance once they are established, but they are not always the best solution to a contamination problem, mainly from the point of view of type of contaminant, its concentration and desired time for treatment (Chappell, 1997). The target pollutant must be bioavailable to a plant and its root system. If a pollutant is located in a deep aquifer, then plant roots may not be able reach it. If a soil pollutant is tightly bound to the organic portion of a soil, then it may not be available to plants or to microorganisms in the rhizosphere. On the other hand, if a pollutant is too water soluble, it might migrate passed the root system reducing potential for accumulation and/or degradation.

3.1. ADVANTAGES OF PHYTOTECHNOLOGIES

- *In situ*
- Passive
- Solar driven
- Costs 10–20% of mechanical treatments
- Transfer faster than natural attenuation
- High public acceptance
- Fewer air and water emissions
- Generates less secondary wastes
- Soils remain in place and are usable following treatment

3.2. LIMITATIONS TO PHYTOTECHNOLOGIES

- It is limited to shallow soils, streams, and groundwater.
- High concentrations of hazardous materials can be toxic to plants.
- It involves the same mass transfer limitations as other biotreatments.
- Climatic or seasonal conditions may interfere or inhibit plant growth, slow remediation efforts, or increase length of treatment period.
- It can transfer contamination across media, e.g., from soil to air.
- It is less effective for strongly sorbed (such as PCBs) and weakly sorbed contaminants.
- Phytoremediation often requires a large surface area of land for remediation.

- Toxicity and bioavailability of biodegradation products are not always known. Products may be mobilized into groundwater or bioaccumulated in animals. More research is needed to determine the fate of various compounds in the plant metabolic cycle to ensure that plant droppings and products manufactured by plants do not contribute toxic or harmful chemicals into the food chain, or increase risk exposure to the general public.

4. Performance

The most serious problem to overcome in new technologies is a lack of performance data. Phytoremediation is no exception, despite serious effort and progress during recent years. Barriers to performance data include the duration of phytoremediation projects and applications which are dependent on rate of plant growth, biological activity and climatic conditions. There are currently a number of pilot-scale projects in progress but conclusive performance data is not available at this time. These sites are being monitored and will report results in the next few years. Also, a number of companies have installed phytoremediation systems at polluted sites owned by private clients, so results from those sites are not publicly available. On the other hand, data from some basic research are available both from scientific literature and internet sites. For example, data from European research under the COST 837 and 859 programs are available at <http://lbewww.epfl.ch/ COST837/> and <http://www.gre.ac.uk/cost859/> or in "Phytoremediation Inventory" (Vanek and Schwitzguébel, 2003). Information more specific to the USA can be found at <http://clu-in.org/techfocus/>.

5. Cost

In addition to performance data, accurate cost data are often difficult to predict for new technologies. Most lab-, pilot-, and field-scale tests include monitoring procedures far above those expected at a site with a remediation goal. This inflates the costs of monitoring at these test sites. As a result, it is difficult to predict the exact cost of a technology that has not been established through years of use. However, since phytoremediation involves the planting of trees or grasses, then it is by nature a relatively inexpensive technology when compared to technologies that involve use of large-scale, energy-consuming equipment. Phytoremediation costs will vary depending on treatment strategy. For example, harvesting plants that bioaccumulate metals can drive up the cost of treatment when compared to treatments that do not require harvesting. Regardless, phytoremediation is often predicted to be cheaper than comparable technologies (Chappell, 1997).

Table 1 presents some estimates of phytoremediation costs in relation to conventional technologies. It should be kept in mind that costs of phytoremediation are highly site-specific, so numbers in these tables are rough estimates of potential costs. Many of these estimates are speculative based on laboratory- or pilot-scale data.

TABLE 1. Estimates of phytoremediation costs versus costs of established technologies.

Contaminant	Estimated phytoremediation cost	Estimated cost using other technologies
Metals	$80 per cubic yard	$250 per cubic yard
Site contaminated with petroleum hydrocarbons	$70,000	$850,000
Ten acres lead contaminated land	$500,000	$12 million
Radionuclides in surface water	$2–$6 per thousand gallons treated	None listed
1 ha to 15 cm depth (various contaminants)	$2,500–$15,000	None listed

Another example in Table 2 shows the cost and potential profit of land management of a radionuclide polluted area by phytostabilization using *Cannabis sativa* based on a 1-ha pilot experiment (Soudek and Vanek, 2005).

TABLE 2. Estimated cost calculation based on three seasons of experiments.

Cost/profit	Without support (Euro/ha)	With support (Euro/ha)
EU bonus for processing	0	364
Planting preparation	219	243
Seeds	117	117
Fibres	749	749
Cultivation and harvest	−625	−625
Seed for sowing	−125	−125
Profit	335	723

6. Recent Developments in Phytotechnologies

Phytoremediation belongs to the fastest growing areas of research and application. From a literature survey from the Web of Science based on 1,500 research papers using the keyword "phytoremediation" this development is clearly visible – from 43 papers (2.8%) listed in 1996 to 151 papers (10.0%) in 2001 to 291 (19.2%) in 2004. Results from September 2005 – 266 papers (17.6%) – confirm this trend.

Concerning countries of origin, among of 1,500 papers published in 1990, 43.6% were published in USA; 8.8% in China; 6.5% in the UK and Germany; following by Spain, 4.7%; Canada, 4.3%; France, 4.2%; and India and Japan, 3.3%. Participation of Asian countries (mainly China) is growing very fast and reflects growing concern about environmental problems. Surprisingly, this research area is still not too popular in new EU-member countries with seriously polluted environments. Czech Republic is in the first position of new EU countries with 2.3% (14th position among all countries). Others new EU-member countries are below 1% of all publications.

7. Conclusions

For efficient utilization of phytoremediation as an environmental cleanup technology for general application, it is still necessary to better understand the technology at the level of basic research and practical application. Some important topics are mentioned below (Schwitzguebel, 2004):

- Delineation of pathways employed in the uptake and metabolism of organic pollutants by plants
- Identification of metabolites produced and study of their ecotoxicological behaviour
- Appropriate selection of plants able to hyperaccumulate toxic metals, understanding the physiological and biochemical mechanisms leading to their uptake, translocation, and accumulation
- Production of a databank of genes/enzymes that will improve the rate and extent of detoxification of organic pollutants and toxic metals
- Evaluation of prospects for using metabolic engineering tools to enhance capacity of higher plants for phytoremediation and clean-up of industrial effluents
- Generation/evaluation of plants adapted to phytoremediation of specifically contaminated sites or wastewater

- Execution of pilot studies to scale-up selected plants with increased capacity for biodegradation of xenobiotics and accumulation of toxic metals

At present, phytotechnology techniques are available for practical application, provided individual optimization studies are carried out for each site.

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CAPACITY BUILDING IN PHYTOTECHNOLOGIES

N. MARMIROLI[1*], B. SAMOTOKIN[2], M. MARMIROLI[1], E. MAESTRI[1], AND V. YANCHUK[2]

[1]*Department of Environmental Sciences, University of Parma, Viale G.P. Usberti 11A, 43100 Parma, Italy*

[2]*Zhytomyr State Technological University, Cherniakhovsky str 103, Zhytomyr, Ukraine*

Abstract Phytoremediation and phytotechnologies exploit plants for decontamination of polluted environments. European scientists are engaged in innovative research on basic biological mechanisms and possible applications. Networking activity and education are very important for the progress of phytotechnologies. The paper illustrates some European initiatives for stimulating exchanges and innovation in the field of phytoremediation.

Keywords: European research, COST action, phytotechnologies, permanent education, university degrees, NATO, networking

1. European Research in Phytoremediation

Phytoremediation and phytotechnologies are a series of technologies applying higher plants and associated microorganisms to environmental cleanup and in situ treatment of contaminated soils, water, and sediments. Phytotechnology mechanisms are based on properties of plants in uptaking and mobilizing environmental contaminants, leading to degradation, accumulation, sequestration, volatilization, or stabilization of organic and inorganic substances (ITRC, 2001).

Application of phytoremediation is constrained and limited by several factors including public acceptance, regulatory restrictions, competition with conventional techniques, and lack of investments (Marmiroli and McCutcheon, 2003).

At the international level, the International Phytotechnology Society (www.phytosociety.org) is the worldwide association connecting all scientists

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[*]To whom correspondence should be addressed.

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and researchers studying the application of plants to environmental problems. The recent Fourth International Phytotechnologies Conference in Nanjing, China, October 2009, has shown the range of disciplines and applications contributing to phytoremediation: from molecular biology to chemistry, from monitoring to biofuels. The society is officially connected to the International Journal of Phytoremediation (http://www.informaworld.com/smpp/title~content=t713610150), the first journal for publication of laboratory and field research on phytotechnologies for environmental decontamination.

Research is needed to provide impulse and substance to the application of phytotechnologies. European research on phytoremediation is proceeding at a slower pace than in the USA and Canada; nevertheless, several research initiatives have been financed in the last few years by European Community and national programmes. The Website http://cordis.europa.eu lists major European research programmes financed in recent years on phytoremediation topics.

A survey carried out on the recent scientific literature and conferences of the phytotechnology sector has revealed that the European research scene concerns 29 countries and about 350 research groups. These groups are distributed approximately 60% in academic institutions, 30% in research centres and institutes, and 10% in private companies.

Research efforts in Europe are mainly focused on elucidation of the basic mechanisms underlying phytotechnologies, with expertise including plant genetics, physiology, biochemistry, and metabolism. Understanding these basic mechanisms is considered a prerequisite for rational application of phytotechnologies to decontamination. Less attention seems to be paid to applied research, with few examples of large-scale phytoremediation projects in the field. In this sense, constructed wetlands are the most frequent applied examples in the European scene. A review of the European research scene has been published in 2006 (Marmiroli et al., 2006).

1.1. COST ACTIONS

The European research scene for phytoremediation has organized networking activities within the framework of coordinated actions financed by "COST." Since 1971, COST has provided a framework for European cooperation in the field of scientific and technical research (www.cost.esf.org). Dealing with interdisciplinary research, COST has anticipated the objectives of the European Research Area (ERA), stimulating collaboration and increasing mobility of researchers. COST currently includes 34 member states, and 28 additional countries with institutions participating in COST initiatives. The scientific secretariat is administered by the European Science Foundation.

The main COST initiatives are the COST Actions. COST Actions are interdisciplinary research networks of research teams whose activities are financed at national level. COST financial contributions cover expenses for organization of meetings and conferences, as well as short scientific missions and dissemination initiatives. Through 2007, more than 200 COST Actions had been performed in different fields.

1.1.1. COST Action 837

From 1998 to 2003, a first important initiative was COST Action 837 "Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites," coordinated by Jean-Paul Schwitzguebel (EPFL, Switzerland). Twenty-four countries participated. The COST837 Website lists all participating scientists and the main publications produced (lbewww.epfl.ch/COST837/).

The main topics chosen for COST Action 837 were as follows:

- Selection of appropriate plants for uptake and metabolism of organic pollutants
- Delineation of metabolic pathways and enzymes involved
- Identification of metabolites produced and study of their ecotoxicological behaviour
- Selection of plants able to hyperaccumulate toxic metals
- Understanding physiological and biochemical mechanisms leading to the uptake, translocation and accumulation of metals
- Production of a databank of genes/enzymes that will improve rate and extent of detoxification of organic pollutants and toxic metals
- Evaluation of the prospect of using metabolic engineering tools to improve the capacity of higher plants for phytoremediation and cleanup of industrial effluents
- Generation, cultivation, and evaluation of plants adapted to decontamination of specifically polluted sites or wastewaters
- Execution of pilot studies in the scale-up of selected plants with an increased capacity for the biodegradation of xenobiotics and accumulation of toxic metals

At the end of the Cost Action a collection of the main results was published as a booklet with contributions by participant researchers (Vanek and Schwitzguébel, 2003). The booklet provides a picture of the lively scene in European research for phytoremediation.

1.1.2. COST Action 859

In 2004, COST Action 859 was been launched with the title, "Phytotechnologies to promote sustainable land use management and improve food safety," coordinated by Jean-Paul Schwitzguebel (EPFL, Switzerland). Twenty-nine countries are currently involved, some from outside the European Union: Switzerland, Norway, Turkey, and Israel. A total of 258 participants are currently involved, of which 39% are women. The project Website lists publications, meeting materials, and lists of participants (w3.gre.ac.uk/cost859).

The main objective of COST Action 859 is to provide a sound understanding of absorption/exclusion, translocation, storage, or detoxification mechanisms of essential or toxic mineral elements, as well as organic contaminants, at physiological, biochemical, and molecular levels, and to prepare guidelines for the best use of plants for sustainable land use management and improved food safety (Schröder and Schwitzguébel, 2004).

COST Action 859 is organized into four working groups:

- WG1: Plant uptake/exclusion and translocation of nutrients and contaminants
- WG2: Exploiting 'genomics, proteomics, and metabolomics' approaches in phytotechnologies
- WG3: Improving nutritional quality and safety of food crops
- WG4: Integration and application of phytotechnologies

COST Action 859 has organized several meetings since 2004 with abstracts available on the Website. As the end of the project approaches, the final conference has been scheduled for October 2009 (http://www.phyto2009.ch/index.html).

2. Dissemination and Education in Phytoremediation

2.1. DISSEMINATION

The application of phytoremediation requires public acceptance, and it is therefore essential to take appropriate measures for informing people about advantages and features of this technology. It is important that scientists, economists, lawyers, and managers from public and private agencies and institutions are able to share their own needs, experiences, and results. PHYTONET is a thematic network created with the purpose of addressing all of these issues and links with other networks operating on similar or complementary subjects. Hosted at the website www.dsa.unipr.it/phytonet, it was developed to allow easy worldwide communications between scientists who work on problems related to phytoremediation and application of plant systems for environmental control (Marmiroli and Monciardini, 1999).

The portal includes news and links relevant for phytotechnologies, news on conferences and books, and offers for jobs. It also includes an extensive list of members from more than 60 countries with details of contact address and current research interests. Finally, it is the entry point for a mailing list which distributes announcements and useful information to all subscribers, currently counting 621 members. The portal allows maintenance of links among researchers from different countries and from different backgrounds, offering the means for searching for potential partners in research projects.

2.2. EDUCATION AND CAPACITY BUILDING

The term “capacity” is used in different ways. Capacity is sometimes used to refer to

- Technical skills or knowledge of individuals
- The availability of sufficient human or financial resources or equipment
- The overall capability of an organization
- The way in which an organization uses available inputs to produce results

Sometimes capacity is described in quantitative terms (e.g. number of staff), but often it is expressed in terms of quality of performance or results achieved. Capacity development has been defined by the Organization for Economic Cooperation and Development (OECD) as “…the process by which individuals, groups, organizations, institutions, and societies increase their abilities to: (i) perform core functions, solve problems, define and achieve objectives, and (ii) understand and deal with their development needs in a broad context and in a sustainable manner.” This definition has been adopted by many institutions (UNESCO, 2006) and has three important aspects:

(i) Capacity is part of a continuing process.
(ii) Human resources and the way they are utilized are central to capacity development.
(iii) The overall framework (system) within which individuals and organizations undertake their functions is important.

Capacity building therefore encompasses a continuous process of improvements specific to existing capability and identified needs. It can occur at different levels (individuals, organizations, or the system in which they operate) and focus on different dimensions of capacity. Similarly, it can be targeted at different types of stakeholders such as government agencies, industry, consumers and their organizations, and others.

High quality in higher education is a priority in Europe, and all countries are involved in developing new activities within the framework of university activities or in other institutions. In Europe, each country still has competence at the national level for the content and organization of educational activities. For this reason, it becomes very important that trans-national initiatives be organized and maintained in a sustainable way, to pave the way towards mutual recognition of curricula. On the specific topic of environmental education, this is even more important due to the trans-national nature of environmental problems.

Higher education in environmental topics encompasses university degrees, such as those in environmental sciences, environmental engineering, or biotechnology, as well as, post-graduate courses, PhD courses.

2.2.1. The International University Master Course on Science and Technology for Sustainable Development of Contaminated Sites

The Universities of Parma in Italy, Mittweida in Germany, and Zhitomir in Ukraine formed a team more than 10 years ago in the framework of the TEMPUS (Trans European Mobility Programme for University Studies) JEP (Joint European Project) 10435 on "Environmental Sciences in Relation to the Implication of Radiation Exposure in Health Care," in order to develop common curricula for education linked to environmental technology and especially to the topic of radioactivity in the environment. In 2002, this initiative gave origin to an International University Master Course building on past experiences.

Five editions have been carried out since 2002, and the sixth is currently in progress. Students attending the course came from Italy, Ukraine, Venezuela, Romania, and Canada. In 2008, 51 students obtained their degrees, while seven are still attending the course.

The topics of lectures are the following:

- Structure and function of contaminated sites: biotic aspects (toxicology, ecology, geopedology, microbiology) and abiotic aspects (chemistry, radioactivity and radiation physics)
- Prevention and management methods (genetics and environmental mutagenesis, radiobiology and radioprotection, sanitary and environmental engineering, management of the nuclear emergencies)
- Remediation technologies (bioremediation, phytoremediation, and conventional technologies)
- Instruments (environmental monitoring, geographic information systems, environmental impact assessment)

- Economics and legislation fundamentals (fundamentals of environmental legislation and workplace safety)
- Industry organization elements (human resources management, project management, funding)

One of the most important aspects of the course is the practical work carried out by students under supervision of mentors in public administration, research institutions, or private companies. This internship introduces the student into a work environment. Following the internship the student discusses his or her work in front of a commission and obtains a university degree corresponding to 60 credits. Work placement of graduates has been excellent, thanks to cooperation among companies in Italy and other countries that have provided lecturers, internships, and jobs. Student results from the first master course (2002–2003) are illustrated in the 6th International Scientific Conference SATERRA, held in Mittweida in 2004 (Marmiroli et al., 2004).

3. NATO ASI School "Advanced Science and Technology for Biological Decontamination of Sites Affected by Chemical and Radiological Nuclear Agents"

Recently, the NATO Science for Peace and Security (SPS) Programme (www.nato.int/sps/index.html) sponsored important initiatives in education and information, organized by NATO and partner countries of Eastern Europe to bring new remediation technologies where they are needed for decontamination. Capacity-building activities are essential for application of advanced technologies in new countries.

In June 2005, a NATO Advanced Research Workshop (ARW) on "Viable Methods of Soil and Water Pollution Monitoring, Protection, and Remediation" was organized in Krakow, Poland (Twardowska et al., 2006). Participants reported on advanced systems for monitoring and decontamination.

A NATO Advanced Science Institute (ASI) activity organized by the authors in August 2005 in Zhytomyr, Ukraine, discussed "Advanced Science and Technology for Biological Decontamination of Sites Affected by Chemical and Radiological Nuclear Agents." This ASI also included phytotechnologies and their application. The main purposes were

(i) To train participants for principles of scientific and technology of biological decontamination, bioremediation, and phytoremediation, with particular emphasis on sites contaminated by radionuclides and chemical substances connected with explosives, ammunitions and fuels
(ii) To describe and discuss state-of-the-art developments and advances required for commercial applications and

(iii) To stimulate interactions and collaborations in this technologically important field of study

The ASI brought together international expert lecturers and facilitated interaction with interested stakeholders and end-users from academia, research institutions, public administration, military institutions, and private companies. Fifty-one participants ranged in age from 23 to 65, with 23 female scientist participants. This enhanced gender equality in the field of decontamination of radiological and chemical pollution. Participants came from 18 countries, representing Asia, Africa, most of Europe, and North America.

Site characterization procedures and related measures were addressed focusing in particular on problems connected with sampling and assessment of sites contaminated by radionuclides and explosives. Pollution problems generated after the Chernobyl accident were addressed, considering the contamination incurred to the forest ecosystems and the hazards to human health.

Biochemical mechanisms of phytoremediation were explained considering the differences between plant and microbial metabolism of contaminants together with a record of natural and cultivated plants frequently used for decontamination. Marmiroli et al. (2007) discussed the role of genetics and genetic engineering to increase understanding of detoxification processes, and to produce and obtain more specific types of decontaminating plants.

Speakers addressed experiences in scaling up from laboratory experiments to pilot studies and field applications. Gawronski talked about applications using plants, Diels discussed microbial applications, and Russell and Hebner addressed the development of constructed wetlands. Soudek also reported on phytoremediation of radionuclides.

Several examples of applications were provided by researchers and private company representatives for decontamination of explosives and radionuclides. Constructed wetlands were prominent among successful phyto-technology applications, and lecturers brought several examples. Analysis of case studies led to identification of advantages and limitations of con-structed wetlands technology.

In two thematic workshops, lecturers and participants discussed personal experiences. The first workshop on risk management and communication coordinated by Borys Samotokin (Ukraine) cited problems connected with risk assessment at contaminated sites, hindrances in communication with stakeholders, and public opinion regarding actions and solutions implemented at contaminated sites. The second workshop on regulatory issues coordinated by Wolf-Uwe Marr (Germany) addressed relationships between legislators and regulators on one side, and scientists and private companies acting in remediation on the other side.

The lectures of the NATO ASI School have been published in the NATO Science Series (Marmiroli et al., 2007).

Interaction of scientists and technicians with state and governmental agencies, regulators, economists, and evaluators is of paramount importance. Effective communication using understandable terminology is therefore a priority for successful implementation of biological decontamination practices. Many Eastern European participants and scientists are convinced that phytoremediation and bioremediation applications can be a more sustainable solution to environmental problems and are willing to learn and to apply them extensively in the field. It was also recognized that efforts should be devoted to an increase in capacity building of personnel, resources, and infrastructure, with a particular attention to young scientists and female scientists (Marmiroli et al., 2007).

4. Conclusions

It is necessary to understand the basic principles and interactions of microorganisms, plant physiology, biochemistry, and genetics because only from sound scientific knowledge can these phytotechnologies develop future applications. Interactions between plants and microorganisms in remediation must be studied with greater attention to discover contaminates that can benefit from synergies between remediation mechanisms. This holistic approach would consider interactions in the environment, as well as between organisms, contaminants, and other biotic and abiotic factors. Interaction of scientists and technicians with state and governmental agencies, regulators, economists, and evaluators is of paramount importance. The need to communicate and to make one other understandable is a priority for successful implementation of biological decontamination practices. Many Eastern European stakeholders and scientists are convinced that phytoremediation and bioremediation can be a more sustainable solution to their environmental problems. They are willing to learn more and to apply these technologies extensively in the field. There is a need for more cooperation between public and private sectors by integrating basic academic and private technological research into a common social goal. Open access to guidance materials and basic information on contaminated sites and previous decontamination attempts will promote a better understanding among countries, in particular Western and Eastern countries, in order to increase security through science and cooperation. It is necessary to support the increased capacity building of personnel, resources, and infrastructure, with particular attention to young scientists and female scientists.

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PERSPECTIVES ON SUSTAINABLE AGRICULTURE IN UKRAINE: THE PUBLIC VIEW

V. PIDLISNYUK[1], L. SOKOL[2*], AND T. STEFANOVSKA[2]
[1]*Kremenchug Technical University, 20 Pershotravneva, Kremenchug, 39614, Ukraine*
[2]*National University of Life and Environmental Sciences of Ukraine, 11 Geroev Oborony, Kiev, 03041, Ukraine*

Abstract A number of sociological surveys on sustainable development and sustainable agriculture were conducted in Central Ukraine from 2006 to 2008. Our purpose was to define understanding of new green technologies including phytoremediation. Two selected focus groups were researched: governmental officials and rural citizens. The results demonstrated a rather good understanding of the main terms and definitions of sustainable development and sustainable agriculture. Decreasing ecological pressure on the environment, improving the quality of drinking water, and providing for a better quality of life were selected as the main priorities for sustainable development and sustainable agriculture in Ukraine. Results also showed the low level of knowledge for either governmental officials or rural people about new ecologically based technologies including phytoremediation. Information campaigns and other educational activities should be provided in Ukraine in order to increase the level of understanding of green technologies including phytotechnologies.

Keywords: sustainable development, sustainable agriculture, public attitudes

1. Introduction

The only way to reduce the harmful effect of society on the environment is to promote and implement more sustainable approaches in which presentation, promotion and implementation of green technologies, including phyto-technologies, are among the priorities. (Gough and Scott, 2006; Melnychuk

*To whom correspondence should be addressed.

et al., 2003; Pidlisnyuk, 2008). Successful implementation of sustainable development and sustainable agriculture in Ukraine requires understanding of the idea and its acceptance by different stakeholders across the country.

Sustainable development is a modern concept of societal development based on the principles of interaction between society and nature that lead to harmonization of economic and social development with protection and preservation of the environment. The essence of sustainable development consists of the obligatory coordination of these three pillars from generation to generation to ensure that quality and safety of life do not decrease, state of the environment does not deteriorate, and social progress takes place recognizing every person's problems (Gough and Scott, 2006; Melnychuk et al., 2003). The definition of sustainable development given in the 1987 report of the UN Committee on Environment and Development, by the so-called Brundtland Commission, is considered to be the classic definition. Sustainable development is "development that meets the needs of the present without compromising the ability of future generations to meet their own needs." It contains within it two key concepts: the concept of "needs," in particular, the essential needs of the world's poor to which overriding priority should be given; and the idea of limitations imposed by the state of technology and social organization on the environment's ability to meet present and the future needs (Brundtland Commission, 1987).

Key challenges of sustainable development include the following:

- Coordination of the rate of economic development with the economic capacity of ecosystems
- Provision for solution of social and intellectual development problems and
- Preservation and protection of natural ecosystems with simultaneous maintenance of ecosystem abilities for resilience and self-renewal

The United Nations Agenda 21 is a primary document in sustainable development theory and practice. Chapter 14 of Agenda 21 describes basic principles of sustainable agriculture (Pidlisnyuk, 2008). Sustainable agriculture integrates three main components:

1. Environmental protection
2. Income and prosperity for farming communities and
3. Achievement of crop production capacity and economic returns without making unjustified impact on the environment

The extent existing farming systems can be recognized as sustainable agricultural practices depends on ecological and social considerations particular to the bioregion, the agricultural crops, and local cultural traditions (Norman et al., 1997).

Sustainable agriculture generally has the following main components (Tyburskiy et al., 2006):

- Constantly increasing role of natural-regulating mechanisms
- Treatment of soil that broadly uses soil-protecting technologies
- Application of multi-functional crop rotations that support biodiversity in agroecosystems
- Water quality control
- Raising of multifunctional crops including cover crops, trap crops, and border plant species such as riparian buffers and filter strips
- Support of nutrient balances in soil by applying microbiological and organic fertilizers
- Implementation of integrated pest management with maximal use of biological control
- Use of rotational grazing for livestock that does not cause soil destruction
- Utilization of legumes, grasses, and green manures
- Introduction of agricultural forest restoration
- Development and marketing of new technologies and their popularization among agricultural scientists, educators, managers and farmers

Many of these issues address development and possible implementation of phytotechnologies as new techniques for environmental remediation, restoration, and management in a sustainable manner.

Currently, implementation of sustainable development, including sustainable agriculture, is rather weak in Ukraine (Danilishin, 2002). Development of phytotechnologies as a scientific direction and practical use is little known in Ukraine compared to its intensive exploration and promotion abroad.

In order to understand attitudes toward sustainable development and sustainable agriculture in Ukraine and to determine the main priorities for future progress, a number of sociological surveys were conducted in Central Ukraine from 2006 to 2008 with special emphasis on sustainable agriculture and green technologies, including phytotechnologies. Research was accomplished for two selected focus groups: governmental officials and rural citizens who will play a crucial role in implementation of sustainable agriculture in Ukraine.

2. Materials and Methods

Research was accomplished in 2006–2007 for the first focus group, governmental officials in Central Ukraine, and in 2008 for the second focus group, citizens of rural communities in Central Ukraine.

In 2006–2007, two groups of governmental officials were interviewed: (1) members and staff of the Ukrainian Parliament, "Verkhovna Rada," and (2) local governmental representatives from four typical rural villages and the city of Boryspol in Kyiv oblast of Central Ukraine. Altogether, 270 persons were polled, among them 98 men and 172 women from 25 to 70 years old. All respondents had a higher education with 12 having a Ph.D. degree. The questionnaire included questions regarding attitudes toward sustainable development and sustainable agriculture, selection of priorities issues, and definition of responsibilities for promotion and implementation. There was also a specific question to survey understanding of new green technologies, including phytoremediation.

In 2008, another sociological survey was administered to rural citizens from five locations in Kyiv oblast of Central Ukraine. These included the villages of Mirne, Lubarci, Rogoziv, and Stare in Kyiv oblast, in addition to citizens of the city of Borispol. Altogether 444 persons were interviewed, among them 195 men and 249 women. Educational background included 157 respondents with a higher education, including one with a Ph.D. The questionnaire for rural citizens included three questions connected with sustainable development, sustainable agriculture, and phytotechnologies that were also in the first poll. The primary aim of the second poll was to define the attitude of rural people toward sustainable water management. All people interviewed were voluntary participants using a written form.

3. Results and Discussion

3.1. GOVERNMENTAL OFFICIALS

Governmental officials play an important role in sustainable development, in particular in its promotion and implementation (Pidlisnyuk and Gess, 2008). We interviewed two groups of governmental officials: members and staff of the Ukrainian Parliament, "Verkhovna Rada," and local governmental representatives from rural areas in the Central Ukraine.

Results of the sociological surveys are presented in Table 1. As one can see, results show that 68.2% of respondents had heard about sustainable development prior to the survey. Information about sustainable development and sustainable agriculture was received primarily from TV and newspapers (58%); however, a significant proportion of the respondents (16%) received information from their colleagues and friends. Regarding the understanding of sustainability ideas, people interviewed most commonly classified it as the "harmony of economy, ecology, and social aspects" (51.4%), "improving quality of life" (25.0%), and setting a "balance between economic growth and environmental protection" (17.4%).

Most respondents (226 people) regarded the idea of estimated sustainable development positively while only four people regarded the idea negatively and 28 people did not take the idea seriously.

Selection of priorities in sustainable development and sustainable agriculture in Ukraine resulted in 40% of respondents selecting "better quality of life," 24% selected economic growth, 21% selected elimination of ecological pressure on the environment, and 12% selected democracy development and access to information. These data showed those interviewed still relate future development more on economic growth than on preservation of nature or implementation of green technology.

Governmental officials considered special laws devoted to sustainable development and sustainable agriculture as a main legislative document in terms of promotion and implementation of sustainability. Fifty-three percent of respondents supported the importance of legislation. Almost an equal number of governmental officials regarded a decree of the Ukraine Cabinet of Ministers (19%) or a decree of the President of Ukraine (18%). Only a small amount of respondents (3%) suggested the Ministry of Ecology might play a key role in developing legislative support for sustainable development and sustainable agriculture in Ukraine.

Ninety-eight percent of polled persons were convinced of the necessity for a transition of Ukrainian agriculture toward sustainability, with only 2% denying this issue.

One-third of interviewed persons had heard about green technologies as elements of sustainable development and sustainable agriculture. This is a good result taking into account the weak state of legislative support for sustainability in Ukraine (Pidlisnyuk, 2008). Unfortunately, survey data showed knowledge of governmental officials regarding phytotechnology as rather low. Only a small number of respondents (8.9%) had heard about this new technology, while almost half of the respondents had not heard about this technology. Forty percent of people were not sure about the term. Results from this first survey stress the importance of dissemination of knowledge about phytotechnologies as a current key important issue for Ukraine.

3.2. CITIZENS OF RURAL COMMUNITIES

Citizens of rural communities are among the main actors for implementation of sustainable agriculture, including green technologies. This is why the second survey group included citizens from four villages located in Central Ukraine and from Borispol, a major city located nearby. Results from the second survey are shown in Table 2.

Respondents selected the following sustainable development priorities: 27.5% chose a better quality of life, 22.9% chose improved drinking water quality, 12.4% – elimination of ecological pressure to the environment, 10.8% – economic growth; 9.2% – development of organic farming, 7.6% – democracy development, 4.3% – sustainable waste management, and 5.3% – biodiversity preservation. Compared with governmental officials, it is interesting to mention that rural citizens place relatively more focus on quality of drinking water as a main priority in sustainability. Another difference is in the number of people who selected improving quality of life as a main priority, with 40.4% for governmental officials compared to only 27.5% for rural citizens.

It is also interesting that only 9.2% of rural people identified organic farming among priorities of sustainability. This fact can be connected with the weak information support regarding organic farming in Ukraine.

The necessity of putting agriculture in Ukraine on a path toward sustainability was favored by 64.9% compared to 29.3% who denied this.

Knowledge of rural citizens regarding the phytotechnology topic was similar to the observations for governmental officials. Only 10.7% of respondents had heard about phytotechnologies, while 43.7% had not heard about phytotechnologies. A large group (45.6%) experienced difficulty answering this question. Thus, interviews with rural citizens also confirmed the necessity for education and promotion of phytotechnologies in Ukraine.

4. Conclusions

Results of two surveys showed good understanding of sustainable development terms and definitions among selected stakeholder groups of governmental officials and rural citizens. The main sustainable development priorities for Ukriane include elimination of ecological pressure on the environment, improving drinking water quality, and providing for a better quality of life. Results also showed the low level of knowledge about newer green technologies, including phytoremediation. Information campaigns along with other educational activities should be provided in Ukraine to increase the level of understanding of phytotechnologies.

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TABLE 1. Results of survey of governmental officials from Central Ukraine taken in 2006–2007.

	Question	Answer choices	Percentage
1.	“Sustainable development” is:	1. Harmonization of economical, ecological, and social aspects	51.4
		2. Same as “economic growth”	4.2
		3. Balance between economic growth and environmental protection	17.0
		4. Improving the quality of life	25.0
		5. Other	2.4
2.	Have you ever heard about sustainable agriculture?	1. Yes	68.2
		2. No	31.9
3.	How did you first learn about sustainable development and sustainable agriculture?	1. TV	29.0
		2. Radio	8.0
		3. Press	29.0
		4. Colleagues, friends	16.0
		5. Other	18.0
4.	What is your attitude to the idea of sustainability?	1. It is positive	87.6
		2. It is negative	1.6
		3. Cannot define	10.9
5.	According to your own opinion which priorities of sustainable development are among the most important?	1. Better quality of life	40.4
		2. Economic growth	23.9
		3. Elimination of ecological pressure to the environment	21.0
		4. Democracy development and access to information	12.5
		5. Implementation of green technologies	2.2

6.	Which piece of legislation should define the main base for implementation sustainability in Ukraine?	1. Special law	53.2
		2. Decree of the President of Ukraine	18.4
		3. Decree of the Ukraine Cabinet of Ministers	18.9
		4. Order of the Ukraine Ministry of Ecology	3.0
		5. Other	5.5
7.	Are sustainable development reforms necessary for Ukrainian agriculture?	1. Yes	97.7
		2. No	2.3
8.	Are “green technologies” including phytoremediation important for sustainable development and sustainable agriculture?	1. Yes	28.9
		2. No	44.5
		3. Hard to say	26.6
9.	Have you ever heard about phytotechnology?	1. Yes	8.9
		2. No	50.5
		3. Hard to say	40.6

TABLE 2. Results of surveys for rural citizens from Central Ukraine, 2008.

Questions	Answers	Response (%)	Response differentiation by village (%)				
			Mirne	Lubarci	Rogoziv	Stare	Borispol
What priorities are most important in sustainable development and sustainable agriculture?	1. Better quality of life	27.5	29.4	33.7	24.6	26.8	21.0
	2. Economic growth	10.8	13.7	5.3	12.0	13.4	4.8
	3. Democracy development and access to information	7.6	3.3	7.4	4.9	13.4	14.5
	4. Elimination of ecological pressure to the environment	12.4	10.5	8.4	15.5	8.9	22.6
	5. Better quality of drinking water	22.9	24.8	32.6	20.4	18.8	16.1
	6. Biodiversity preservation	5.3	5.9	4.2	8.5	2.7	3.2
	7. Developing of organic farming	9.2	8.5	6.3	8.5	14.3	8.1
	8. Sustainable waste management	4.3	3.9	2.1	5.6	1.8	9.7
	9. Another	0	0	0	0	0	0
Is it important to put Ukrainian agriculture on the way to sustainability?	1. Yes	64.9	65.5	52.0	64.4	90.5	48.1
	2. No	5.8	17.25	4.0	0	0	3.7
	3. Hard to say	29.3	17.25	44.0	35.6	9.5	48.1
Have you ever heard about phytotechnologies?	1. Yes	10.7	1.7	22.0	17.8	7.1	11.1
	2. No	43.7	41.4	54.0	37.8	45.2	37.0
	3. Hard to say	45.6	56.9	24.0	44.4	47.6	51.9

A REVIEW OF RECENT RESEARCH DEVELOPMENTS INTO THE POTENTIAL FOR PHYTOEXTRACTION OF PERSISTENT ORGANIC POLLUTANTS (POPS) FROM WEATHERED, CONTAMINATED SOIL

M. WHITFIELD ÅSLUND AND B. A. ZEEB*
Royal Military College of Canada, Kingston, ON, Canada K7K 7B4

Abstract This chapter provides a summary of recent research exploring the potential of phytoextraction as a remediation strategy for soils contaminated with persistent organic pollutants (POPs). Evidence is first provided to show that plants in the species *Cucurbita pepo* ssp *pepo* (which includes zucchini and pumpkins) have the ability to mobilize significant concentrations of highly hydrophobic POPs from the soil and translocate them to their shoots, while many other plants do not. Current hypotheses regarding the mechanisms by which *C. pepo* ssp *pepo* plants achieve these high concentrations of POPs are then discussed. Next, a summary is given of research which has investigated use of soil amendments and other treatments to increase the efficiency of POPs phytoextraction by *C. pepo* ssp *pepo* and other plants. Finally, some of the impediments to the practical application of this technology are discussed and suggestions are made for future research to help make phytoextraction a feasible remediation strategy for POPs-contaminated soil.

Keywords: persistent organic pollutants (POPs), phytoextraction, bioaccumulation factors (BAFs), uptake pathway, phytoremediation, field study, soil amendments, composting

1. Introduction

Persistent organic pollutants (POPs) are a well known group of environmental contaminants including industrial chemicals such as polychlorinated biphenyls (PCBs); chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT),

*To whom correspondence should be addressed.

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chlordane, and dieldrin; and unintentional industrial by-products such as dioxins and furans (PCDDs/PCDFs). These contaminants are grouped together based on their long half-lives in the environment and their potential to bioaccumulate through the food chain. POPs have been linked to diverse health effects including endocrine disruption, cancer, immune system effects, and reproductive and developmental defects in both animals and humans (Li et al., 2006).

POPs that have been released to the environment tend to accumulate in soils and sediments due to their strong preference for organic matter over water or air (Northcott and Jones, 2000). Traditional approaches used to remediate POPs-contaminated soils and sediment have relied on physical and chemical processes, including *in situ* containment with physical barriers (e.g. paving over contaminated soil) and *ex situ* methods such as incineration, chemical extraction, and/or disposal in a landfill (Arthur et al., 2005). These processes themselves are often very costly, and in the case of *ex situ* treatments, can involve substantial additional costs for excavation and transportation of the contaminated material.

As a result of the high cost and energy requirements of many traditional remediation processes, there is a growing interest in phytoremediation technologies that use plants to remove, reduce, degrade, or immobilize contaminants from soil, sediment, or water. Because phytoremediation can be applied *in situ* and has low equipment costs, it is anticipated that it may be two to ten times less expensive than conventional remediation (Pilon-Smits and Freeman, 2006). Presently, phytoremediation has been adapted for a number of contaminants other than POPs (Raskin and Ensley, 2000; McCutcheon and Schnoor, 2003; Pilon-Smits and Freeman, 2006). However, phytoremediation of POPs is still at an early stage of development.

This chapter summarizes recent developments in research which suggest that a type of phytoremediation known as phytoextraction may be adapted for remediation of POPs-contaminated soil.

2. Why Phytoextraction?

The objective of phytoextraction is for plants to accumulate significant amounts of contaminant from the soil and store it in the plant shoot, which can then be harvested and treated as contaminated waste (Cunningham and Ow, 1996). Although this process relies on traditional treatment methods, it leaves the soil on site intact and can reduce the volume of contaminated solids for transport and treatment when compared to the excavation of contaminated soil. In this case, disposal of contaminated vegetation or recovery of the contaminant from the plant matter can be more cost effective than direct disposal of the contaminated soil (Arthur et al., 2005).

In order for phytoextraction to function successfully, plants must be able to accumulate high concentrations of the contaminant in their shoots while simultaneously maintaining high biomass production (Koopmans et al., 2007). The shoot bioaccumulation factor (BAF, where BAF = $[PCB_{plant\ part}]/[PCB_{soil}]$) can provide an indication of whether the shoot concentration of the contaminant is high enough for phytoextraction purposes. Most commonly, the goal of phytoextraction is to reduce the volume of contaminated solids for transport and further treatment off site. This is accomplished when the phytoextraction-generated waste has a higher contaminant concentration than the soil. If the shoot BAF is greater than one, this goal has been met and disposal of contaminated vegetation directly will be more cost effective than disposal of the contaminated soil. It follows that the higher the shoot BAF, the more cost effective this process would become. However, shoot BAFs of less than one may be acceptable in certain circumstances. For instance, additional on-site steps such as composting could be applied prior to transportation of the waste off site to further reduce the volume of contaminated plant matter and thereby increase the contaminant concentration in the final phytoextraction-generated waste (Sas-Nowosielska et al., 2004). The potential of these types of treatments has not been extensively researched (Sas-Nowosielska et al., 2004), so it is difficult to estimate a minimal shoot BAF less than one for which phytoextraction would be practical. For instance, Lazzari et al. (1999) noted that up to 50% of dry weight could be lost as CO_2 during composting, which would mean that a shoot BAF of 0.5 and higher would be sufficient. Shoot BAFs of less than one may also be appropriate in circumstances where contaminated soil is being used for agricultural purposes, and the funds for direct soil remediation are not available. For instance, many countries in the former Soviet Union accumulated large stockpiles of persistent pesticides during the Soviet era (Fedorov, 1999; Chaudhry et al., 2002). Many of these stockpiles are now in disrepair and are a source of POPs contamination to surrounding areas, including local farmland (Fedorov, 1999; Chaudhry et al., 2002). The cost to transport and incinerate this contaminated soil is often prohibitively high (Chaudhry et al., 2002). In this situation, contaminant removal from the soil using phytoextraction, followed by safe storage and/or disposal of the contaminated plant waste, might reduce transfer of the contaminants to the food chain without having to end the agricultural use of the soil.

To date, most phytoextraction research has focused on plant uptake of metals and metalloids (Baker et al., 1994; Cunningham et al., 1995; Koopmans et al., 2007). This research area has had considerable success in identification of 'hyperaccumulating' plants (Ma, 2001; McGrath and Zhao, 2003; Zhao et al., 2003) as well as in identification of soil amendments that increase contaminant bioavailability to plants (Blaylock et al., 1997; Huang et al., 1997, 1998). Additional research has also examined the physiological and molecular mechanisms of metal hyperaccumulation in plants (Lasat et al., 1996; Macnair et al., 1999; Lombi et al., 2001), and attempts have been made to engineer tolerance and accumulation of metals in plants through breeding and genetic manipulation (Magher, 2000; Dhankher et al., 2002). As a result, phytoextraction is now considered a promising technology for the remediation of moderately contaminated soils for some metal and metalloid contaminants (Robinson et al., 1998; Zhao et al., 2003).

Conversely, since phytoextraction requires root uptake of the contaminant from the soil and subsequent translocation to the shoot, POPs have been considered unlikely candidates for this technology due to their highly hydrophobic nature. Typically, models have predicted that little to no root uptake and translocation of these contaminants from soil will occur (Ryan et al., 1988; Cousins and Mackay, 2001; Collins et al., 2006), and early investigations into the uptake pathways of POPs into plants appeared to confirm these models (Suzuki et al., 1977; Bacci and Gaggi, 1985; McCrady et al., 1990; Hulster and Marschner, 1993; Muller et al., 1993).

However, in the mid-1990s, Hulster et al. (1994) observed zucchini fruits (*Cucurbita pepo* ssp *pepo* cv. *giromontiina*) with dioxin (PCDD) and furan (PCDF) concentrations that were approximately two orders of magnitude greater than those of other fruits and vegetables grown in the same PCDD/PCDF-contaminated soil. Whereas previous studies had determined that the main uptake pathway of POPs into plant shoots was via atmospheric deposition, Hulster et al. (1994) demonstrated that the primary uptake pathway of PCDD/PCDF into zucchini fruits was via uptake and translocation from the root. This finding, of a plant that could 'hyperaccumulate' dioxins and furans as compared to other plants, was the first to indicate that it might be possible to develop a phytoextraction approach for dealing with POPs-contaminated soils. Soon other researchers decided to explore this possibility, using a similar approach to that used in development of metal phytoextraction research.

3. Researching the Potential of POPs Phytoextraction from Soil

3.1. THE SEARCH FOR POPS HYPERACCUMULATING PLANTS

In the context of metal phytoextraction, hyperaccumulation has traditionally been defined based on threshold concentrations, i.e. the concentration of the metal in the shoot by dry weight must be higher than 10,000 μg/g for Zn and Mn; 1,000 μg/g for Al, As, Se, Ni, Co, Cr, Cu, and Pb; and 100 μg/g for Cd (Foy, 1984; Baker and Brooks, 1989; McGrath and Zhao, 2003;

Branquinho et al., 2007). It is difficult to adapt these concentration-based definitions to POPs phytoextraction research. However, two operational definitions from McGrath and Zhao (2003) for metal hyperaccumulation might be appropriate with respect to POPs phytoextraction. First, shoot concentration of the contaminant must be two or more orders of magnitude higher than shoot concentration observed in normal plant species, and shoot BAF must be greater than one.

The original observation by Hulster et al. (1994) of zucchini fruits with PCDD/PCDF concentrations that were two orders of magnitude greater than other fruits and vegetables grown in the same contaminated soil provided evidence that zucchini might qualify as a hyperaccumulator of dioxins and furans according to the first characteristic listed above. Given the expectation of little to no uptake for POPs via roots to shoots due to their hydrophobic nature (Ryan et al., 1988; Cousins and Mackay, 2001; Collins et al., 2006), this finding warranted further research, even though the second characteristic was not met (i.e., fruit and leaf BAFs were much lower than one). Therefore, in the early stages of POPs phytoextraction research, the focus was simply to identify plants that accumulated significantly higher POPs concentrations in their shoots than other plants.

Using this basic criterion, researchers subsequently compared the performance of numerous varieties of *Cucurbita pepo* ssp *pepo* (which includes zucchini and pumpkins) with other plants for their ability to take up and translocate significant concentrations of a number of POPs, including DDT and its metabolites (White, 2001, 2002; Lunney et al., 2004), chlordane (Mattina et al., 2004), PCBs (White et al., 2006a; Zeeb et al., 2006; Whitfield Åslund et al., 2007), and dieldrin and endrin (Otani et al., 2007). The ability to take up concentrations of the contaminant into the plant shoot that were two or more orders of magnitude higher than the concentrations of the contaminant in other plants was found almost exclusively in varieties of the plant species *Cucurbita pepo* ssp *pepo*, which includes pumpkins and zucchini. The only exceptions were observed by Zeeb et al. (2006) and Whitfield Åslund et al. (2007), who also noted some uptake into shoots of the sedge *Carex normalis* and the grass *Festuca arundinacea* (tall fescue). Even close relatives to *Cucurbita pepo* ssp *pepo*, such as *Cucumis sativa* (cucumber) and *Cucurbita pepo* ssp *texana*, were not found to exhibit this capability (White, 2002; White et al., 2003a). Similarly, noticeable variations were observed between different varieties of the *Cucurbita pepo* ssp *pepo* species (White et al., 2003a).

Encouragingly, some of these studies observed BAFs for the shoot or parts of the shoot of *C. pepo* ssp *pepo* that were greater than one for specific POPs. For instance, stem BAFs greater than one were observed for chlordane (Mattina et al., 2000) and p-p′-DDE (White, 2001, 2002; White et al., 2003a), although fruit and leaf BAFs were less than one. In fact, White (2001) observed stem BAFs of greater than eight in a pumpkin variety and greater than 20 in a zucchini grown in p,p′-DDE-contaminated soil ([p,p′-DDE] = 155–397 ng/g). In addition, Lunney et al. (2004) reported whole shoot BAFs of 1.2 and 2.4 for pumpkin plants grown in soil contaminated with a mixture of DDT, DDD, and DDE at concentrations of 3,700 and 150 ng/g, respectively. However, contaminant-specific differences became apparent when phytoextraction of PCBs was assessed. No whole-stem or whole-shoot BAFs greater than one were observed in any of the studies using PCB-contaminated soil. In these studies, maximum BAFs observed for whole stems or shoots ranged from 0.14 to 0.53 (White et al., 2006a; Zeeb et al., 2006; Whitfield Åslund, 2008; Whitfield Åslund et al., 2007, 2008) for *C. pepo* ssp *pepo* plants. However, Whitfield Åslund et al. (2008) observed PCB concentrations in the lower stem and leaves equal to or greater than the soil PCB concentration (i.e., partial-shoot BAFs were greater than one). In addition, shoot BAFs of 0.29–0.45 for PCBs were observed in *Carex normalis* (sedge) (Zeeb et al., 2006; Whitfield Åslund et al., 2007). No BAFs were calculated in the Otani et al. (2007) study of dieldrin and endrin uptake.

Despite the large variation observed in whole-shoot and shoot-compartment BAFs, as well as contaminant-specific differences, these studies conclusively demonstrated that highly recalcitrant, hydrophobic POPs can be taken up from soil and mobilized within plants, particularly certain varieties of *Cucurbita pepo* ssp *pepo.* Since this result was unexpected based on existing models (Ryan et al., 1988; Cousins and Mackay, 2001; Collins et al., 2006), it became clear that *Cucurbita pepo* ssp *pepo* likely possesses a unique mechanism for mobilizing significant amounts of POPs into the plant shoot. Further research then focused on identifying this mechanism, as a better understanding of the mechanism would assist in development of contaminant-specific phytoremediation strategies for POPs-contaminated soils.

3.2. UNDERSTANDING THE MECHANISMS OF POPS UPTAKE INTO *CUCURBITA PEPO* SSP *PEPO*

Hypothetically, POPs can accumulate in plant shoots via the following pathways, (i) root uptake and subsequent transport to the shoot, (ii) volatilization of POPs from soil, (iii) deposition of atmospheric PCBs, and (iv) contamination by soil particles (Hulster et al., 1994). Only pathway (i), root uptake and transportation to the shoot, is well suited to the purposes of phytoextraction. The other three pathways depend primarily on the physical-chemical properties of the contaminant, and therefore offer little opportunity to manipulate uptake efficiency through selection of specific plant varieties or soil amendments. Also, research has shown that these abiotic pathways generally only result in the transfer of very low POP concentrations to plant shoots (Suzuki et al., 1977; Bacci and Gaggi, 1985; McCrady et al., 1990; Hulster and Marschner, 1993; Muller et al., 1993).

Evidence suggests that the primary uptake pathway of POPs into the shoots of *Cucurbita pepo* ssp *pepo* is root uptake and translocation to the shoots. One way this has been illustrated has been by the comparison of shoot-contaminant concentrations between plants grown in the same POP-contaminated soil at the same time. If the main PCB transfer pathways were volatilisation, atmospheric deposition, or direct soil contact, it would be expected that PCB accumulation in plant shoots would be driven by growth habit (i.e. height, leaf surface area). For instance, plants with large leaves that grow close to the ground may be expected to accumulate higher concentrations of POPs in their shoots via volatilisation or soil contact. However, for *Cucurbita pepo* ssp. *pepo*, even closely related plants with very similar morphology and growth habits such as those in the *Cucumis* genera or different subspecies of *Cucurbita pepo* showed significantly lower POP concentrations in their shoots than *Cucurbita pepo* ssp *pepo* species when grown in the same soil (White, 2002; White et al., 2003a).

Evidence for a root uptake and translocation pathway of POPs into *Cucurbita pepo* ssp *pepo* shoots has also been provided by the patterns of POP contamination in different parts of the shoot. If uptake from air (via volatilisation or atmospheric deposition) or direct soil contact was the primary uptake pathway, the plant's leaves would be expected to accumulate the highest concentrations of the contaminant due to their large surface area and the lipophilic nature of plant cuticles (Riederer, 1990). This has been shown to be the case for the accumulation of PCBs in corn (Buckley, 1982) and tomato plants (Ye et al., 1992). However, for *Cucurbita pepo* ssp *pepo*, POP concentrations in the shoot were commonly found to be highest in the stem, followed by the leaves, and then the fruit. For instance, White (2001, 2002) and Lunney et al. (2004) found that the concentrations of DDT and its metabolites were generally an order of magnitude greater in the stems than in the leaves of *C. pepo* ssp *pepo* plants. Mattina et al. (2000) observed a similar pattern for chlordane. Reports of contaminant distribution patterns of PCBs in *C. pepo* ssp *pepo* shoots conflict, with White et al. (2006a) reporting higher PCB concentrations in the plant stem than leaves, while Whitfield Åslund et al. (2007, 2008) reported similar concentrations in plant stems and leaves, with differences in concentration being driven primarily by the length of the shoot from which the sample was taken. Since none of these studies reported the leaves to have the highest concentration of POPs, these findings all support the hypothesis that

C. pepo ssp *pepo* plants are actually phytoextracting POPs via uptake and translocation from the roots.

The ability of a plant to take up significant levels of weathered POPs from soil into its shoots requires successful completion of two distinct processes: mobilisation of POPs from the soil into the roots, and subsequent translocation of these highly hydrophobic compounds within the plant from the root to the shoot. Currently, research into these processes has focused on the mechanisms behind POP uptake in *C. pepo* ssp *pepo* species. For this species, research suggests that enhanced extraction of POPs from soil into the roots of *C. pepo* ssp *pepo* is likely related to substances released into the soil by the plant root. Plant roots are known to produce a wide variety of compounds such as organic acids, sugars, amino acids, and enzymes that mediate complex interactions between both abiotic and biotic components of the rhizosphere (Dakora and Phillips, 2002). These root exudates are known to contribute significantly to the accumulation of metals in plants (Mench and Martin, 1991; Salt et al., 1995a; Krishnamurti et al., 1997; Lin et al., 2003; Wenzel et al., 2003; doNascimento and Xing, 2006), so it is possible they might be involved in plant uptake of POPs as well. This hypothesis was first investigated by Hulster and Marschner (1995) and later by Campanella and Paul (2000), who demonstrated that root exudates of *C. pepo* ssp *pepo* increased the apparent aqueous solubility of PCDDs/ PCDFs in abiotic desorption tests. They hypothesized that root exudates contained a substance that could bind reversibly with POP molecules in soil, creating a more hydrophilic complex which could then be absorbed by the root and transported throughout the plant more readily than the POP molecule alone. Campanella and Paul (2000) provided evidence that this substance might be of a proteinic nature, but conclusive purification and identification of this substance was never performed.

More recently, it has been hypothesized that root exudates affecting POPs mobilization from soil to the root in *C. pepo* ssp *pepo* might be low-molecular-weight organic acids (LMWOAs) such as citric, malic, and oxalic acids (White et al., 2003b). These compounds are known to be exuded by some plants in order to facilitate nutrient acquisition. Specifically, the acids are able to chelate inorganic micronutrients (Fe, Zn, Cu, Mn) from the soil structure, making the ions available for plant use (Godo and Reisenauer, 1980). Accordingly, these LMWOAs are also considered extremely influential in the phytoextraction of metals (doNascimento and Xing, 2006). Interestingly, *Cucurbita pepo* ssp *pepo* plants have been observed to exude numerous LMWOAs including malate, succinate, and citrate (Richardson et al., 1982), while cucumber plants (proven non-accumulators of POPs) exuded only minor amounts of organic acids (Vancura and Hovadik, 1965). It is now hypothesized that chelation of metals in the soil by LMWOAs exuded by the roots of *C. pepo* ssp *pepo* as part of a nutrient acquisition strategy may result in a partial dissolution of the soil matrix and subsequent release of any anthropogenic chemicals (e.g. POPs) that were previously bound within the soil solids (White and Kottler, 2002; White et al., 2003b; Luo et al., 2006). Evidence for this hypothesis was provided by Yang et al. (2005), White and Kottler (2002), and White et al. (2006b), who observed that amendments of LMWOAs increased the abiotic desorption both of metal ions such as Fe, Mn, and Al, and organic contaminants such as PAHs, p,p′-DDE, chlordane, and PCBs from weathered, contaminated soil.

In contrast, the mechanism by which *C. pepo* ssp *pepo* translocates POPs from the roots to shoots is presently unknown. Once the contaminant has been taken up into the roots, it must be transferred via the xylem to the shoots. Normally, once inside the plant it is expected that highly lipophilic compounds would become progressively sorbed to stem components (Briggs et al., 1983; McCrady et al., 1987; Collins et al., 2006). However, Mattina et al. (2004) observed significant levels of chlordane and p,p′-DDE within the xylem sap of *C pepo* ssp *pepo* species and Whitfield Åslund et al. (2007, 2008) have reported mobilization of highly chlorinated PCB congeners to the end of 5-m-long pumpkin shoots. It does not appear this mobilization within the *C. pepo* ssp *pepo* plant can be explained simply by the root exudation of LMWOAs, as other plant species which are known to exude large quantities of LMWOAs such as lupin, pigeonpea, peanut, and canola were observed to be poor accumulators of p,p′-DDE (White et al., 2005a). Instead, the uptake of POPs into *C. pepo* ssp *pepo* shoots appears to be dependent on some characteristic of the plant root. Both Otani and Seike (2006, 2007) and Mattina et al. (Mattina et al., 2007) observed this for dieldrin and endrin and chlordane, respectively, in studies where roots and shoots of *C. pepo* ssp *pepo* and *Cucumis sativus* were both homografted and heterografted. In this work, live plants of both species were separated into roots and shoots and then new root/shoot pairs were grafted back together, creating pairs with roots and shoots of the same species (homografted) and pairs with roots and shoots from different species (heterografted). In these studies, the transfer of POPs into aboveground tissues was observed to be driven solely by plant roots, i.e. plants with *C. pepo* ssp *pepo* roots had significantly higher POPs concentrations in their shoots than plants with *Cucumis sativus* roots. Given the importance of root-to-shoot transfer in phytoextraction, research into identifying and understanding this mechanism could prove extremely valuable in the development of POPs phytoextraction.

3.3. IDENTIFICATION OF SOIL AMENDMENTS AND OTHER TREATMENT PROCESSES THAT COULD INCREASE CONTAMINANT BIOAVAILABILITY TO PLANTS

Although shoot BAFs or partial shoot BAFs of greater than one have been observed in certain *C. pepo* ssp *pepo* varieties for specific POPs, they are still generally significantly lower than shoot BAFs reported in metals phyto-extraction research. The maximum aboveground BAF for POPs observed for *C. pepo* ssp *pepo* thus far was a stem BAF of greater than 20 observed in a zucchini variety grown in p,p′-DDE-contaminated soil ([p,p′-DDE] = 155–397 ng/g) (White, 2001). Most stem BAFs were more conservative. For example, the maximum whole-stem BAF reported for PCBs was 0.5 (Whitfield Åslund et al., 2008). Estimates of whole-shoot BAFs tend to be even lower (0.14–2.4), since leaf BAFs are generally less than one (Lunney et al., 2004; Zeeb et al., 2006; Whitfield Åslund et al., 2007). In comparison, some metal hyperaccumulator plants have been identified with total shoot BAFs as high as 50–100 (Ma, 2001; McGrath and Zhao, 2003; Zhao et al., 2003). Therefore, there is an interest in identifying treatments that might increase the transfer of POPs from soil into shoots of *C. pepo* ssp *pepo* and/or other plants, since this would increase the rate of POPs phytoextraction.

Treatments that have been considered are based on the preliminary understanding of the mechanisms for POP uptake into *C. pepo* ssp *pepo*. A summary of these treatments and an assessment of the current research into their effects is provided below.

3.3.1. Soil Amendment with Low Molecular-Weight Organic Acids (LMWOAs)

Given the hypothesized role of LMWOAs in the hyperaccumulation of POPs by *C. pepo* ssp *pepo*, it was reasonable to expect that soil amendments with LMWOA's might increase the POP uptake by *C. pepo ssp pepo* and other plants. This approach has been tested with some success in metal phyto-extraction, where it has been observed that the addition of chelators such as EDTA can induce Pb hyperaccumulation in a range of non-hyperaccumulating plant species (Blaylock et al., 1997; Huang et al., 1997, 1998). Similarly, citric acid treatments have been used to increase uranium accumulation in plants (Huang et al., 1998).

White and Kottler (2002) tested the effects of citrate amendments on clover, mustard, hairy vetch, and rye grass (known non-accumulators of POPs) grown in p,p′-DDE-contaminated soil. They observed the citrate treatment was able to increase the concentration of p,p′-DDE in the plant roots by nearly 40%. However, the citrate treatment had no effect on the concentration of p,p′-DDE in the plant shoot. This suggests that although the ability of *C. pepo* ssp *pepo* to mobilize POPs from the soil into the root may be mediated by root exudation of LMWOAs, an additional mechanism is most likely involved in the consistent translocation of high concentrations of the contaminants to the plant shoots.

In contrast, citric and oxalic acid treatments were shown to significantly increase the p,p′-DDE concentration in the stems of *C. pepo* ssp *pepo*, but did not change the contaminant concentration in either the leaves or the roots (White et al., 2003b). In addition, these LMWOA treatments had either no effect or a positive effect on plant biomass. As a result, plants receiving citric or oxalic acid treatments removed slightly more of the contaminant from the soil (2.1% and 1.9% of the p,p′-DDE from the soil, respectively) than the water-only treatment (1.7%). However, a second crop of *C. pepo* ssp *pepo* grown in the same soil with the same treatments (water only, citric acid, and oxalic acid) exhibited markedly different results. The p,p′-DDE concentrations in the tissues of the ‘water-only’ treatment plant tissues increased significantly (by 1.9–4.3 times) in the second crop as compared to the first, resulting in the transfer of 2.5% of the p,p′-DDE from the soil into the plant. In contrast, p,p′-DDE concentrations in plants that received LMWOA treatments were either unchanged or reduced in the second planting, while their biomass was also slightly reduced in comparison to the plants in the water-only treatment. Therefore, the advantage of the LMWOA amendments for total p,p′-DDE extraction was lost in the second planting.

The effect of LMWOA amendments on bioavailability of POPs to plants was investigated again by White et al. (2006a), who observed that citric acid treatments increased the stem and leaf PCB concentrations by up to 330% and 600%, respectively, in each of *C. pepo* ssp *pepo*, *C. pepo* ssp *ovifera*, and *Cucumis sativa*.

In metals phytoextraction research, use of LMWOA amendments has been found to be problematic as the addition of chelators can lead to an increased risk of contaminant leaching to groundwater. For instance, Wenzel et al. (2003) observed that soil treatment with EDTA caused leaching losses of metals that far outweighed the amount taken up by plants. White et al. (2003b) postulated that this would not be the case in relation to POPs in the presence of chelators due to the indirect mechanism of mobilisation. Kelsey and White (2005) provided support for this theory by demonstrating that the presence of *C. pepo* ssp *pepo* in DDE-contaminated soil did not increase the bioavailability of DDE to earthworms. Also, White et al. (2006a) demonstrated that although citric acid amendments more than doubled apparent aqueous solubility of PCBs in an abiotic desorption test, the same citric acid treatment had no impact on PCB accumulation in two earthworm species.

In summary, it appears that treatments with LMWOA may increase the bioavailability of POPs to *C. pepo* spp *pepo* plants. However, further research is required in order to identify the optimal concentrations to apply, as well as the effects of these treatments on multiple plantings. In addition, the impact of these treatments on the mobility of POPs in soil-water systems and the potential for risk to nontarget organisms must be assessed.

3.3.2. Nutrient Amendments

In many phytoremediation approaches, it is assumed that the application of sufficient or abundant macronutrients will result in larger, healthier plants and will therefore increase the efficiency of the phytoremediation. For instance, Hutchinson et al. (2001) observed that additions of N and P significantly increased petroleum hydrocarbon degradation by Bermuda grass and tall fescue. This increased phytodegradation rate appeared to be directly linked to the increased total biomass of both plant species. Likewise, fertilizer amendments were observed by White et al. (2003c) to increase plant biomass and enhance the phytodegradation of crude oil in soil.

However, since the process of phytoextraction of POPs by *C. pepo* ssp *pepo* appears to be linked to the exudation of LMWOAs as part of a nutrient-acquisition strategy, it is possible that availability of easily accessible nutrients may reduce the rate of POPs phytoextraction. Specifically, it has been hypothesized that *C. pepo* ssp *pepo* produces LMWOA root exudates to assist in the acquisition of phosphorus from soil. Evidence for this hypothesis was provided by Gent et al. (2005), who observed that when grown hydroponically, *C. pepo* ssp *pepo* exuded more citric acid under phosphorus depletion than *C. pepo* ssp *ovifera* (a known non-accumulator of POPs). Therefore, it was hypothesized by White et al. (2005b) that in the presence of abundant phosphorus, *C. pepo* ssp *pepo* cultivars would have reduced exudation of LMWOAs and would therefore accumulate lower amounts of POPs. Similarly, if the plant was supplied with abundant nitrogen or subjected to low phosphorus conditions, it might increase the rate of LMWOA exudation in order to extract phosphorus from the soil and consequently take up more POPs from the soil. Based on this hypothesis, White et al. (2005b) tested the effect of nitrogen and phosphorus amendments on the uptake of p,p′-DDE from soil by *C. pepo* ssp *pepo*. As expected, nutrient amendments with increased nitrogen or decreased phosphorus significantly increased the total amount of p,p′-DDE extracted from the soil (by 1.9 times), while treatments where both nitrogen and phosphorus were elevated simultaneously or where only phosphorus was increased had no significant effect on the total amount of contaminant extracted. In conclusion, results of this experiment suggest that POP uptake by *C. pepo* ssp *pepo* can be increased by judicious application of nutrient amendments.

3.3.3. Planting Density

It has also been hypothesized that increased planting density might increase POP uptake into plants by increasing root-to-soil contact and/or because the increased nutrient competition between plants under crowded conditions could induce increased production of root exudates associated with the mobilization of POPs from soil into plant roots (Wang et al., 2004; Kelsey et al., 2006). Some research has supported this hypothesis. Kelsey et al. (2006) observed that in treatments with one, two, or three plants grown in 500 g of p,p′-DDE-contaminated soil, the treatment with three plants had significantly higher stem and leaf BAFs than the less-crowded treatments. However, other studies have shown increased planting density to decrease the total amount of POPs extracted (Wang et al., 2004; White et al., 2006a; Whitfield Åslund et al., 2008). Wang et al. (2004) observed that stem, leaf, and fruit BAFs for zucchini plants grown in p,p′-DDE-contaminated soil were significantly reduced in crowded treatments compared to uncrowded treatments. Similarly, an increased density of zucchini plants in PCB-contaminated soil resulted in a similar total plant biomass, but decreased contaminant concentrations in plant tissues and therefore decreased total PCBs extracted (White et al., 2006a). Most recently, Whitfield Åslund et al. (2008) observed that increased planting density decreased both total plant biomass and PCB concentration in pumpkin plant shoots grown in the field (Whitfield Åslund et al., 2008). Therefore, it may be necessary to establish a site-specific optimum planting density for each plant and contaminant combination before POP phytoextraction activities are carried out.

Planting density has also been shown to affect the performance of *C. pepo* ssp *pepo* plants in comparison to other plant species. White et al. (2006a) observed that in small pot conditions (one plant in 400 g soil), there were no significant differences in the root or stem uptake of PCBs between known POPs accumulator *C. pepo* ssp *pepo* and known non-accumulators of POPs *C. pepo* ssp *ovifera* and *Cucumis sativa*. However, under large pot conditions (one plant in approximately 70 kg of soil), *C. pepo* ssp *pepo* outperformed the other two species as expected. This suggests that the full potential of the hyperaccumulating properties of *C. pepo* ssp *pepo* are best observed when plants are allowed to grow in non-dense, field-like conditions. This may explain why the Kelsey et al. study (2006), which took place in the greenhouse in 500 g pots, observed crowding to have a different effect than other studies which included uncrowded treatments placed in much larger pots (70 kg) or in the field (Wang et al., 2004; White et al., 2006a; Whitfield Åslund et al., 2008). Future POPs phytoextraction research should be carried out at a realistic field scale whenever possible, as important differences in POP uptake appear to occur between greenhouse and field scale.

3.3.4. Surfactants

Surfactants are amphiphilic molecules with both a hydrophobic and hydrophilic region. At low concentrations in aqueous solutions, surfactants exist as monomers, but as the concentration increases, surfactant molecules with a hydrophilic 'head' region and a hydrophobic 'tail' region will group together to form 'micelles' with a hydrophilic outer shell and a hydrophobic centre. This type of surfactant can enhance the apparent aqueous solubility of hydrophobic organic compounds by encapsulating the hydrophobic molecules within the hydrophobic core of the micelles (Edwards et al., 1994). For example, surfactants have been demonstrated to increase the solubility of PCBs in a water-soil system (Park and Boyd, 1999). These properties are shared by both synthetically produced surfactants and bio-surfactants, which are produced by living organisms. Since one of the main barriers to plant uptake and translocation of POPs is their low aqueous solubility, it has been hypothesized that application of surfactants to POPs-contaminated soil could increase the mobility of POPs in soil and therefore increase the bioavailability of POPs to plants.

White et al. (2006b) observed that rhamnolipid biosurfactant amendments significantly increased the bioavailability of p,p′-DDE to both *C. pepo* ssp *pepo* and *C. pepo* ssp *ovifera* (a known non-accumulator of PCBs). The surfactant amendments significantly increased all tissue BAFs (including those of leaves and fruits) for both subspecies. However, the final biomass of ssp *ovifera* was significantly reduced (by 60%) by the surfactant treatments and therefore, there was no difference in the final total amount of p,p′-DDE extracted by this plant between the surfactant treatment and a water-only control. However, the surfactants did not affect the biomass of ssp *pepo* plants, so for these plants the surfactant treatment resulted in a significant increase in the total amount of contaminant extracted. In contrast, Lunney (2007) observed that soil amendments of three synthetic surfactants to growing *C. pepo* ssp *pepo* plants resulted in no significant difference in plant $\sum$ DDT concentration or plant fresh weights. Lunney (2007) hypothesized that applications of higher concentrations of surfactants may have been required to achieve an increase in $\sum$ DDT uptake. Therefore, although surfactants appear to have the potential to increase the rate of POP uptake by plants, further work is required in order to identify ideal surfactants for phytoremediation purposes and to determine the optimal concentration and application schedule to maximize plant uptake of various POPs.

The ecological impact of surfactant applications to soil must also be researched before surfactants can be applied in the field for phytoextraction purposes. Some synthetic surfactants have been shown to be toxic to soil bacteria and therefore may be poor choices as soil amendments for the purposes of phytoextraction (Tiehm, 1994; Roch and Alexander, 1995). Biosurfactants may be more appropriate as they are generally non-toxic and biodegradable. In addition to the direct toxicity of surfactant treatments, the possibility that the increased aqueous solubility of PCBs might lead to increased PCB exposure to local organisms or to groundwater must also be addressed.

3.3.5. Mycorrhizal Fungi

Mycorrhizas are widespread associations between soil fungi and roots of higher plants. The roots provide a carbon substrate to the fungi and in turn the fungi are able to solubilise soil nutrients that are taken up by the plants (Smith and Read, 1997). As a result, mycorrhizal associations generally have a beneficial effect on plant growth. The most common type of these associations are arbuscular mycorrhizas (AM), which are non-specific with respect to host species. The AM fungi function by penetrating the root and then emitting their hyphae from within the root to the soil, effectively increasing the surface area of soil in contact with the plant (Smith and Read, 1997). It has been hypothesized that this increased surface area might enhance POP bioavailability to *C. pepo* ssp *pepo* plants. This hypothesis has been investigated by White et al. (2006b, c) and Lunney (2007). White et al. (2006c) investigated the effect of a commercially available mycorrhizal inoculant on the uptake of p,p′-DDE in varieties of both *C. pepo* ssp. *pepo* and *C. pepo* ssp. *ovifera* and observed that the mycorrhiza increased root and stem BAFs of all cultivars from 1.1 to 14 times. The fungal inoculation did not significantly alter the biomass of plants in subspecies *pepo*, but it significantly decreased total plant biomass in *ovifera* cultivars. As a result, mycorrhizal inoculant treatment had no effect on the total amount of contaminant removed by the subspecies *ovifera*, but resulted in a significant increase in the total amount of contaminant removed by the subspecies *pepo*. In a subsequent study, White et al. (2006b) tested three commercially available mycorrhizal inoculants on three cultivars of zucchini. In this study, the effect of the mycorrhizal inoculants varied at the cultivar level. In general, the mycorrhizal inoculants increased the total amount of p,p′-DDE extracted by 30–60%. However, there were some cultivars for which certain fungal inoculations either did not affect or significantly reduced the total contaminant uptake. Similarly, Lunney (2007) observed that a mycorrhizal inoculant had no significant effect on the uptake of $\sum$ DDT by a pumpkin cultivar of *C. pepo* ssp. *pepo*. Therefore, it seems that AM fungi may offer a useful amendment for increasing the POP uptake by *C. pepo* ssp *pepo*. However, preliminary research performed in site-specific soil may be required to identify optimal plant/mycorrhiza pairings before these amendments are applied in the field.

3.3.6. Other Growth Conditions (Fruit Prevention, Soil Moisture Content, and Intercropping)

In general, studies have reported that POPs concentration in *C. pepo* ssp *pepo* fruits is an order of magnitude lower than concentrations observed in the stem (White, 2001, 2002; White et al., 2003a). Preventing the fruits from developing might therefore encourage the production of a larger stem biomass, thereby increasing both overall shoot concentration and total amount of contaminant removed. White et al. (2006b) observed that when the fruits of *C. pepo* ssp *pepo* plants were prevented from developing by removing female flowers, the stem and leaf biomass increased significantly. In addition, although stem BAFs were decreased by 14%, leaf BAFs increased by 14 times. Overall, the biomass increase combined with the leaf BAF increase resulted in a 41% increase in the total amount of contaminant extracted. Therefore, future POPs phytoextraction research using *C. pepo* ssp *pepo* plants should consider fruit prevention as a method for increasing phytoextraction efficiency. This technique would also reduce the opportunity for POPs to be introduced to the local food chain through the consumption of contaminated fruits by local animals.

Kelsey et al. (2006) investigated the effect of soil moisture content on POPs accumulation in ssp *pepo*. Soil moisture content had previously been shown to influence both biomass production and bioaccumulation of nutrients and contaminants by plants. For instance, Angle et al. (2003) observed that increased soil moisture content resulted in increases in plant biomass and plant metal accumulation, while Tennant and Wu (2000) observed that increased soil moisture decreased selenium uptake by tall fescue. In the context of POPs phytoextraction, Kelsey et al. (2006) observed that increased soil moisture content increased root BAF in *C. pepo* ssp *pepo* varieties, but did not affect translocation of p,p′-DDE to the shoot. Therefore, soil moisture content does not appear to affect the efficiency of POPs phytoextraction into the shoots of *C. pepo* ssp *pepo* varieties.

Another growth condition unexpectedly found to affect the efficiency of POPs phytoextraction in *C. pepo* ssp *pepo* varieties was intercropping *C. pepo* ssp *pepo* varieties with non-accumulating plant species. White et al. (2006b) observed that intercropping *C. pepo* ssp *pepo* and ssp *ovifera* increased the tissue p,p′-DDE content of cultivars in both subspecies. Because of the hypothesized relationship between ssp *pepo* root exudates and the ability of *C. pepo* ssp *pepo* to hyperaccumulate POPs, one might hypothesize that the presence of *C. pepo* ssp *pepo* plants would increase the general bioavailability of the POPs to other plants. However, the mechanism that caused the POPs concentration in *C. pepo* ssp *pepo* plants to increase in the presence of a nonaccumulator species is unclear. It is possible that this may be due to enhanced root exudation to maximize nutrient acquisition under the crowding stress of being grown together with a different plant variety.

3.4. IMPEDIMENTS TO THE PRACTICAL APPLICATION OF THIS TECHNOLOGY

It has now been conclusively demonstrated that varieties of *C. pepo* ssp *pepo* are able to mobilize significant concentrations of POPs from the soil and translocate them to their shoots, and that certain soil amendments and other treatment processes are capable of influencing the extent of POPs accumulation in *C. pepo* ssp *pepo* shoots. However, a number of issues require further research before this technology can be practically implemented at real contaminated sites.

For instance, the long-term POPs phytoextraction potential of pumpkin and zucchini plants after multiple plantings in the same soil is not known. This information is required in order to provide an accurate estimate of the length of time required to meet remediation goals, which will be a critical determinant of the commercial applicability of POPs phytoextraction. Some studies have attempted to address this issue by estimating percent of the contaminant that was removed from the soil into the plant (White, 2002, White et al., 2003a). In greenhouse conditions, these estimates were based on the known mass of soil the plant was grown in. For plants grown in field conditions, the amount of soil accessed by each individual plant was estimated based on the observed volume of soil containing the bulk of the root system and the density of the soil (White, 2002). For instance, White (2002) estimated that *C. pepo* ssp *pepo* cv Howden (a pumpkin) removed approximately 2.4% of the p,p′-DDE from 270 kg of soil in less than 3 months under field conditions. This estimate compares reasonably well with rates of contaminant removal that have been deemed promising in terms of metal contamination. For instance, Lasat et al. (1998) estimated that radiocesium-contaminated soil at a specific contaminated site could be successfully remediated within 15 years at a rate of phytoextraction of only 3% per crop. However, further research is required to better understand this issue. For instance, current estimates generally assume a constant rate of contaminant uptake into plant shoots throughout the remediation process (Koopmans et al., 2007). However, it has been hypothesized that as phytoextraction activities decrease contaminant concentration in the soil, it may become progressively more difficult for plants to access and extract the remaining fraction of the contaminant from soil, thereby significantly increasing the projected time period for successful remediation (Koopmans et al., 2007). Thus far, this has not been shown to be the case for phytoextraction of POPs using *C. pepo* ssp *pepo* plants. For DDT and its metabolites, the contaminant concentration in zucchini shoots was observed to either increase or remain stable while the soil concentration decreased significantly after two or three plantings (White et al., 2003b; Kelsey et al., 2006). For PCBs, shoot biomass remained constant while the contaminant concentration in pumpkin shoots was observed to increase significantly in the field in a second season, and this concentration was maintained in the third field season, although no decreases in soil PCB concentration were observed (Whitfield Åslund, 2008; Whitfield Åslund et al., 2008). Further research is required to determine the effects of more than three plantings, and to determine the rate of POPs phytoextraction over the range of soil concentrations to be encountered throughout the phytoextraction process.

Another obstacle to commercial implementation of any type of phytoextraction (metals or POPs) is the need for a strategy for disposing of contaminated crop material at the close of the study (Sas-Nowosielska et al., 2004). Due to the persistent nature of POPs, it is expected that the phytoextraction-generated plant waste will ultimately need to be disposed of via a traditional treatment method, such as incineration. However, pretreatment steps to reduce the volume of the contaminated plant matter and remove excess water could significantly reduce the costs of transportation and treatment. One pre-treatment step that might easily be adapted to POPs phytoextraction is composting (Kumar et al., 1995; Salt et al., 1995b, 1998; Garbisu and Alkorta, 2001). It has been reported that up to 50% of plant dry weight could be lost as CO_2 during composting (Lazzari et al., 1999). Therefore, composting of phytoextraction-generated plant waste would reduce the mass of the contaminated plant matter, thereby increasing the contaminant concentration and further decreasing transportation and treatment costs (Sas-Nowosielska et al., 2004). In addition, preliminary studies suggest that composting of PCB-contaminated soil may actually result in degradation and/or dechlorination of individual PCBs congeners (Michel et al., 2001; Braendli et al., 2007). PCB congeners with greater than four chlorines can be dechlorinated anaerobically, and those with less than or equal to four chlorines can be biodegraded aerobically (Abramowicz, 1990). Generally, these bioremediation processes have been investigated in soil slurry formats. However, in the last decade, investigations have begun into the use of composting strategies for the bioremediation of organic pollutants (Semple et al., 2001). At this point, further research is required as the effects of composting processes on PCB congeners are poorly understood (Braendli et al., 2007).

4. Conclusions

Research into POPs uptake and transfer by plants has been carried out since the 1970s. However, early research generally focused on the environmental fate of POPs or the likelihood of POPs introduction to the food chain through contaminated food or fodder crops. The possibility that POP uptake and transfer by plants might be harnessed as part of a remediation strategy for POPs-contaminated soil has only recently been considered. The first articles specifically addressing the possibility of using plants to 'phytoextract' POPs were not published until 2000 (Mattina et al., 2000; White, 2000). Since the publication of these initial studies, further work has investigated the POP uptake potential of various plant species (White, 2002; Lunney et al., 2004; Mattina et al., 2004; White et al., 2006a; Zeeb et al., 2006; Otani et al., 2007), the effects of soil amendments and growing conditions on POP uptake by plants (White et al., 2005b, 2006 b, c; Kelsey et al., 2006), and the mechanisms of POP uptake by plants (Gent et al., 2007).

Overall, this research suggests that phytoextraction of POPs is possible, and that plants in the species *Cucurbita pepo* spp. *pepo* offer an excellent study species for further research in this area. The primary disadvantage of this technology is that it is likely to be much slower than traditional remediation processes. However, as a result of the prohibitively high costs of traditional POPs remediation techniques, many sites that are contaminated with POPs (e.g. urban brownfields and locations in developing countries) are currently not likely to be remediated at all in the foreseeable future. The longer time period required for successful phytoextraction may be acceptable in these cases, while the lower cost of phytoextraction would be highly advantageous. Future research should focus on optimizing the conditions of POPs phytoextraction and methods for on-site treatment of POPs-contaminated plant waste. Also, research must be done to better understand the long-term behaviour of POPs phytoextraction during repeated field seasons.

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ELIMINATION OF ACUTE RISKS FROM OBSOLETE PESTICIDES IN MOLDOVA: PHYTOREMEDIATION EXPERIMENT AT A FORMER PESTICIDE STOREHOUSE

O. BOGDEVICH * AND O. CADOCINICOV
Institute of Geology and Seismology, Chisinau, Moldova

Abstract The objectives of this investigation were to determine risk from old pesticide storehouses and to assess phytoremediation technology for potential implementation to reduce risk in the Republic of Moldova. A risk assessment method was proposed and 16 storehouse sites were evaluated in the Hînceşti district. More than 60% of the sites showed middle to high risk levels from POPs (persistent organic pollutants) pesticides. One site with a high risk level was chosen for a phytoremediation case study under field conditions. Several cultivated plants were evaluated for determination of pesticide extraction efficiency: maize (*Zea mays* L.), zucchini (*Cucurbita pepo* L. var. *pepo*), pumpkin (*C. pepo* L. var. *pepo*), carrot (*Daucus carota* L.), and sorghum (*Sorghum bicolor* L. Moench). Analytical measurements of POPs in soil, plants and other environmental media determined these old storehouse sites remain highly polluted after the removal of obsolete chemical stockpiles, and additional remediation actions are needed. Phytoremediation can be used for remediation of polluted sites; however, it needs to be designed based on local conditions. The best phytoextraction efficiency was shown by zucchini and pumpkin. Time required for agricultural phytoremediation might be long and require utilization of complex approaches that consider use of biotechnology as well as native and perennial plants to develop a successful strategy.

Keywords: POPs, phytoremediation, site assessment, environmental risk assessment, gas chromatography

*To whom correspondence should be addressed.

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1. Introduction

The Moldova National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants indicates that from the 1950s to 1990s a total of 560,000 t of pesticides were used in Moldova, including 22,000 t of persistent organochlorine compounds (OCPs) (Isac et al., 2008). Past absence of controls on pesticide manufacture, imports, transportation, storage, and use have resulted in stockpiling of now banned and useless pesticides which constitute an acute environmental hazard. In order to find a solution for the ever-increasing amount of obsolete pesticides accumulated in the country, a pesticide landfill was built in 1978 in an area adjacent to Cişmichioi village in the south of Moldova. During a period of 10 years (1978–1988), 3,940 t of pesticides were buried there, including 654.1 t of DDTs.

By the early 1990s, when Moldova became independent, more than 1,000 warehouses for pesticide storage were built on kolkhozes (collective farms). Between 1991 and 2003, about 60% of these were destroyed or dismantled. Only 20% of the remaining warehouses remained in satisfactory condition. Deterioration of the warehouses resulted in significant amounts of obsolete pesticides stored in the open without security. Deteriorated storage facilities with exposed pesticides and packaging materials constitute a human health and ecological risk, especially in those situated close to residential areas. Currently, the total amount of obsolete pesticides in Moldova is approximately 5,650 t, including about 3,940 t buried at the pesticide dump in Cişmichioi and 1,712 t stored in 344 deteriorating facilities that lack proper monitoring and security.

The National Implementation Plan calls for actions related to capacity building, remediation measures, and measures to increase public awareness, training, and education. Several governmental and NGO-funded projects have been initiated to support this activity including a project managed by the NGO Milieudontakt Oost-Europa to eliminate acute risks of obsolete pesticides (Iordanov and Molenkamp, 2008). Initially this project focused on repackaging and removal of obsolete pesticides from former storehouses in the Hînceşti district. As a follow-up to facilitate eventual reuse of the agricultural land surrounding the former storehouses, a project was developed to assess pollution levels of soil and other components of the environment near the foundations of the old pesticide storehouses and to determine impacts to the surrounding agriculture landscape. A more important task is the determination of cleanup alternatives for the polluted territory.

A participatory research approach was used to develop objectives to plan the site investigations and experimental methods for a site cleanup strategy based on phytoremediation. Participants in the project inception discussions included local governmental authorities, specialists from the ecological agency, scientists, and the NGO. A 2-day workshop was held to introduce concepts on phytoremediation and to discuss principal approaches and milestones for a phytoremediation study. Roles and responsibilities of each team were determined at this meeting.

Objectives of this project were to determine human health risk from old pesticide storehouses and to assess phytoremediation technology for potential implementation to reduce risk in Moldova. To address these objectives the following tasks were developed:

- Investigate pesticide pollution levels following pesticide stockpile repackaging at old pesticide storehouses in the Hînceşti district
- Conduct a risk assessment of pesticide pollution of the surrounding agriculture territory
- Select a site for a detailed site investigation and phytoremediation experiment
- Design and implement a phytoremediation experiment and
- Evaluate phytoextraction efficiency of several cultivated plant species

Phytoremediation is the name given to a set of technologies that use different plants as a containment, destruction, or extraction technique. Phytoremediation has been receiving attention as results from field trials indicate potential cost savings compared to conventional treatments that require soil excavation and transportation (USEPA, 2000). General approaches of this technology were used for design of the phytoremediation experiment. As reviewed in the earlier chapter of this volume by Whitfield and Zeeb, previous studies showed a good extraction of DDTs, PCBs, and other chlororganic compounds from soil by zucchini and pumpkin plants (Iwata and Gunther, 1976; Smith and Jones, 2000; Webber et al., 1994). Design of a phytoremediation system varies according to the contaminants, conditions at the site, level of cleanup required, and plants used. A thorough site characterization should provide the needed data to design any type of remediation system. The source of the pollution may need to be removed if phytoremediation is the chosen technology for remediation. Clearly, phytoextraction has different design requirements than phytostabilization or rhizodegradation. Nevertheless, it is possible to specify a few design considerations that are a part of most phytoremediation efforts. These include contaminant levels; plant selection; treatability; irrigation, agronomic inputs (P, N, K, salinity, zinc, etc.), and maintenance; groundwater capture zone and transpiration rate; contaminant uptake rate; and cleanup time.

2. Materials and Methods

2.1. INITIAL SITE CHARACTERIZATION AND SITE SELECTION

For initial site characterization and determination of the location of the phytoremediation trial, soil was sampled systematically at 16 former storehouse sites in the Hînceşti district of Moldova (Figure 1). The number of composite soil samples for each site characterization varied from three to seven samples. At each sample point, two depth intervals of 0–20 cm and 20–40 cm were collected. Based on results from these samples, two sites were selected for a more detailed investigation. For these two sites, soil was sampled at four locations along each of four transects oriented in different directions from the center of the storehouse foundation. These samples were taken only from top soil, 0–20 cm. Lengths of transects ranged from 275 to 500 m. Based on these results, one site was selected for the phytoremediation trial. At this location, four boreholes were made to determine the groundwater level.

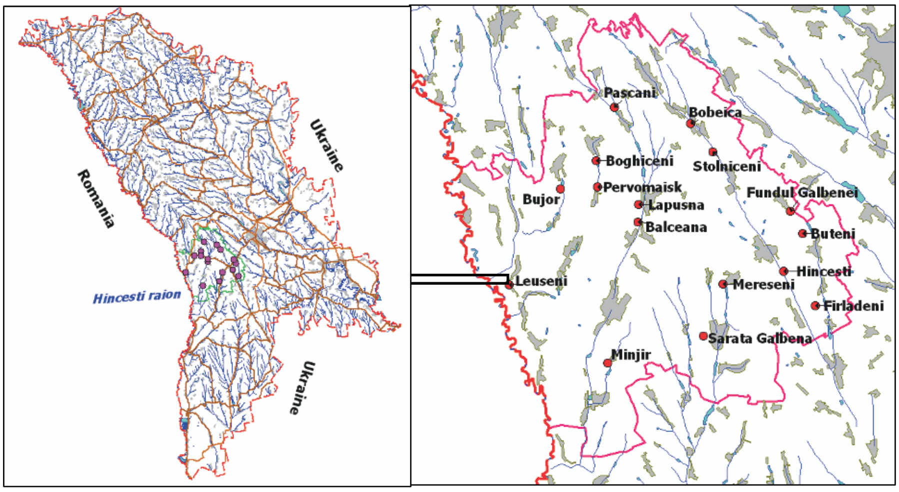

Figure 1. Location of 16 former pesticide storehouses in the Hînceşti district of Moldova.

Soil samples were air-dried under laboratory conditions of about 20°C. Samples were then sieved through a 1.0-mm screen and homogenized. Plant samples were dried in a drying box at 60°C. Ten gram subsamples of soil and 1–2 g subsamples of plant tissue were extracted with duplicates using a Soxhlet system for 14 h. The solvent was a mixture of hexane and dichloromethane in the proportion 1:1 with a total volume of 150 ml. Extracts were concentrated to 1 ml and cleaned by silica column chromatography and by solid-phase extraction silica cartridges. All analytical determinations of

POPs' content in soil, plants, and other environmental media were made by gas chromatography using an Agilent 6890 equipped with μECD (micro-electron-capture detector). In this paper, DDTs refer to the sum of concentrations of all DDT metabolites (DDT, DDE, DDD) and HCHs refer to the sum of concentrations of HCH isomers (α-, β-, and γ-HCH).

2.2. METHOD OF RISK ASSESSMENT

Performance of an environmental risk assessment (ERA) for old pesticide storage areas is an important task to determine which polluted sites have priority for remediation action. The methodology for the ERA in this study was developed based on several environmental agency recommendations and a local case study (FAO, 2000; Scottish EPA, 2008; Ministry Ecol. and Nat. Res., 2005; USEPA, 1989; USNPS, 1999). The proposed risk indices are presented in Table 1. The level of pesticide contamination is a principal factor which impacts the risk level of polluted sites. Two levels of maximal admissible concentrations were used for the pollution ranking: 100 μg/kg, the maximal admissible level for polluted soil (higher concentration is determined to be polluted soil); 50,000 μg/kg, the maximal admissible level for toxic waste (higher concentration is determined to be toxic waste). The integrated risk was calculated as a sum of risk indices.

The storehouse survey included the following tasks:

- Binding the site in the system of geographical coordinates WGS84
- Geomorphologic characteristics
- Description of buildings and grounds
- Sampling and testing of soil and construction debris
- Expert assessment of depth of groundwater
- Identifying distances to objects at risk, human settlements, agriculture, and water bodies and
- Photographing the site

Three important factors in the risk assessment are concentration of POPs chemicals, condition of the warehouse, and distance to objects of risk.

Additional environmental samples were taken for assessment of risk for the environment and public health near the two sites evaluated in detail for the phytoremediation experiment. These samples included groundwater, dairy products, and poultry. Milk samples were taken from villages in the investigated area. These results were compared with two principal dairy manufacturers and two private farmers from other regions of Moldova.

TABLE 1. Indices for the risk assessment procedure.

Risk index	0	1	2	3	4	6
Total HCHs concentration (μg/kg)	<100	100–1,000	1,000–3,000	3,000–10,000	10,000–50,000	>50,000
Total DDTs concentration (μg/kg)	<100	100–1,000	1,000–3,000	3.000–10,000	10,000–50,000	>50,000
Volume of polluted soil (t)		<1	1–5	>5		
Polluted area (ha)		<0.5	0.5–1.5	>1.5		
Access to the territory	Close	Limited	Open			
Groundwater level (m)	<20	10–20	3–10	<3		
Distance to settlement (km)		>1.0	0.3–1.0	<0.3		
Distance to agricultural land (m)		>200	50–200	10–50	<10	
Distance to water bodies (km)		<1.0	0.3–1.0	0.1–0.3	>0.1	

2.3. ANALYTICAL DETERMINATION

Organochlorine pesticide concentrations were determined by gas chromatography using an Agilent 6890 equipped with a μECD detector using USEPA Method 8081A. The calibration interval was from 0.02 to 0.5 μg/ml. Samples with pesticide concentrations higher than the calibration interval were diluted to the appropriate concentration. Eleven pesticides or pesticide metabolites were checked: α-, β-, and γ-HCH isomers, hexachlorbenzene (HCB), heptachlor, aldrin, dieldrin, endrin, chlordane, DDE, DDD, and DDT. Each extracted sample was analyzed twice (two chromatograms) for quality assurance and quality control. The detection limit was 10.0 μg/kg for DDTs, aldrin, dieldrin, endrin, and chlordane. The detection limit was 5.0 μg/kg for HCHs, hexachlorbenzene, and heptachlor. For this research, the maximal admissible concentration (MAC) for pesticides in soil was considered to be 100 μg/kg based on typical norms for Russia and the former USSR (Ministry of Health of USSR, 1991).

2.4. SPATIAL ANALYSIS

A local coordinate system was used to locate sample points using the Moldref system and GIS software ArcView 3.2a. The interpolation of pesticide concentrations in soil was done using a krieging method from ArcView software. Corrections for the spatial distribution of pesticides for land outside the experimental site were made by taking into consideration natural and artificial landscape barriers.

2.5. PHYTOREMEDIATION STUDY

The Balceana site was selected for the phytoremediation field experiment based on two primary considerations: (1) a sufficiently large cultivatable area surrounding the site was polluted by obsolete pesticides, primarily by DDTs and (2) there was good potential for local community involvement for planning and conducting the study. Situated in the lower Lapusna River Valley, the soil at the Balceana site is classified as a "chernozem" with a pH of 7.6 and an organic carbon content of 3.2%. The depth-to-groundwater depth is 3.0–3.5 m. An area around the building foundation was highly contaminated by triazine herbicides. This area was not used for the vegetation study. The foundation area also was not used for the phytoremediation study.

The trial was managed by a local farmer who won a tender and conformed to special requirements. This decision demonstrated the feasibility of managing a phytoremediation application using local expertise in cooperation with government and institute consultation.

The experimental plot was established using typical agricultural techniques for soil preparation and planting. No additional soil treatments or soil amendments were used. The field experiment was designed using five vegetation treatments on plots planted with the following plant species: maize (*Zea mays* L.), zucchini (*Cucurbita pepo* L. var. *pepo*), pumpkin (*C. pepo* L. var. *pepo*), carrot (*Daucus carota* L.), and sorghum (*Sorghum bicolor* L. Moench).

The site plan is illustrated in Figure 2. Due to differences in the area available for each of the five plots, different numbers of vegetation were planted in some plots. Three plots included the three vegetation treatments of maize, zucchini, and pumpkin. One plot utilized all five plant species, and one plot was limited to two plant species: corn (*Zea mays* L.) and pumpkin (*C. pepo* L. var. *pepo*).

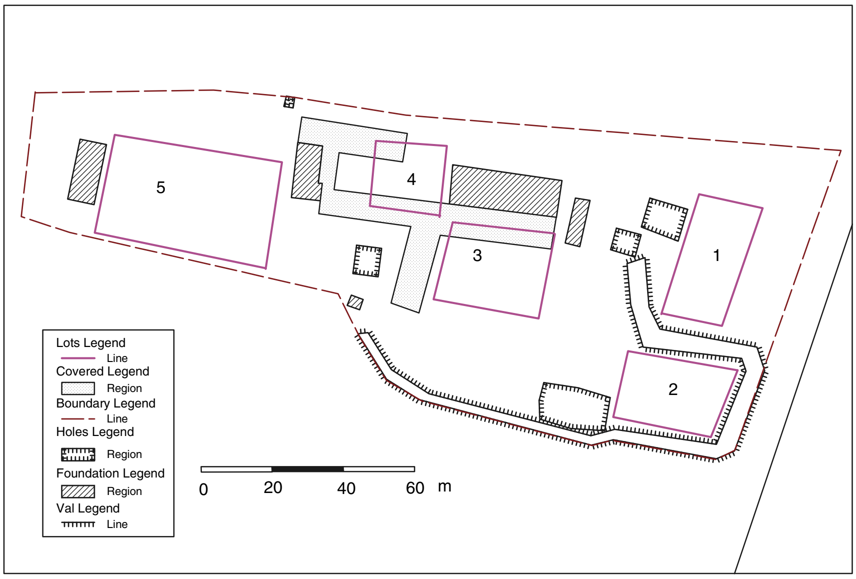

Figure 2. The design of plots within the foundation area of the former Balceana pesticide storage facility (plots are numbered 1–5).

Planting was started at the beginning of April 2007. The site was cleaned to remove garbage and old building materials. All polluted objects were stored on the site, within the building foundation. Seeding was carried out according to recommendations for each plant species. Supplemental irrigation was utilized for plant growth due to the impact of the dry season in the summer of 2007. Irrigation water came from a local well with sufficient water quality (mineral content of not more than 1.0 g/l). Water was transported to the site by a specially equipped horse cart. Every day two or three runs were made with 1m^3 of water.

Plant samples were taken for each species after a vegetative growth period. Principal plant parts were sampled for assessment of pesticide accumulation (roots, stems, petioles, leaves, and fruit). Three repetitions of each plant species were sampled from every plot. Average-sized plants were selected for sampling. Individual plants were weighed and plant parts separated. All plant parts and rhizosphere soil samples were transported to the laboratory.

3. Results

3.1. SITE SELECTION AND RISK ASSESSMENT

The range in pesticide pollution for the 16 surveyed sites is shown in Table 2, with sites ordered from lowest to highest concentrations based on the sum of total HCHs and total DDTs. Final site selection criteria for the phytoremediation trial were based on pesticide concentration in soil, soil quality, presence of a building foundation, and consultations with local community leaders and government authorities. Two primary site selection criteria were pesticide pollution levels and feasibility of setting up and managing a field experiment.

TABLE 2. Total HCHs, total DDTs, and the sum of both for 16 former storehouses in the Hînceşti district of Moldova (sites are listed in increasing order of total pesticide concentration).

Site No.	Site name	Total HCHs (μg/kg)	Total DDTs (μg/kg)	ΣHCHs and DDTs (μg/kg)
1	Lapusna	40	68	107
2	Minjir	43	77	120
3	Fundul Galbenei	82	79	161
4	Boghiceni	611	188	799
5	Mereseni	414	668	1,082
6	Hincesti	338	843	1,182
7	Bobeica	1,560	389	1,949
8	Pascani	1,037	1,122	2,159
9	Leuseni	1,918	313	2,231
10	Sarata Galbena	258	2,268	2,525
11	Firladeni	2,381	1,876	4,257
12	Pervomaisk	130	11,821	11,952
13	Balceana	7,880	40,560	48,440
14	Bujor	279	217,845	218,123
15	Stolniceni	928,858	116,814	1,045,672
16	Buteni	1,217,219	233,535	1,450,754

High pesticide concentrations observed at the storehouses were explained by the spread of contamination from the remaining obsolete chemical stockpiles and absence of sufficient actions for their removal. The principal volume of the residual waste was composed of a mixture of construction material mixed with chemicals and soil. Every site was different and would require an individual site characterization and design for appropriate cleanup actions, including bioremediation and phytoremediation technologies.

TABLE 3. Risk indices and integrated risk data for 16 sites in the Hînceşti district of Moldova.

Site name	Total HCH	Total DDTs	Volume of polluted soil	Polluted area	Access to territory	Ground water level	Distance to settlements	Distance to agriculture lands	Distance to water bodies	Integral risk index	Risk level	ΣHCHs and DDTs (μg/kg)
Lapusna	0	0	2	2	3	3	2	2	4	18	Low	107
Minjir	0	0	3	2	3	1	3	4	1	17	Low	120
Fundul Galbenei	0	0	2	1	3	2	3	4	3	18	Low	161
Boghiceni	1	1	3	1	2	2	3	4	3	20	Middle	799
Mereseni	1	1	3	3	3	1	1	4	2	19	Low	1,082
Hincesti	1	1	3	2	3	2	1	3	3	19	Low	1,182
Bobeica	2	1	3	2	2	2	3	3	3	21	Middle	1,949
Pascani	1	2	3	2	2	3	3	4	4	24	Middle	2,159
Leuseni	2	1	3	3	3	3	2	3	3	23	Middle	2,231
Sarata Galbena	1	2	2	3	2	1	2	3	2	18	Low	2,525
Firladeni	3	2	3	2	3	1	2	4	2	22	Middle	4,257
Pervomaisk	1	4	3	2	3	3	3	4	4	27	High	11,952
Balceana	3	4	3	3	3	3	2	4	2	27	High	48,440
Bujor	1	6	1	2	3	2	2	4	3	24	Middle	218,123
Stolniceni	6	6	2	2	1	2	1	2	3	25	High	1,045,672
Buteni	6	6	3	3	3	1	1	4	2	29	High	1,450,754

Integrated risk indices (Ir) obtained at the 16 sites are presented in Table 3 and Figure 3. The sites were separated into three ranges: low risk with Ir < 20, middle risk with Ir from 20 to 25, and high risk with Ir > 25. Thirty-eight percent of the sites had low risk, 38% had middle risk levels, and 25% had high risk levels. The integrated risk index results were mainly due to the POPs pesticide contaminant concentrations, the extent of the contaminated area, and the volume of contaminated material. Hence, more that 60% of the investigated sites had middle or high risk levels from POPs pesticide pollution.

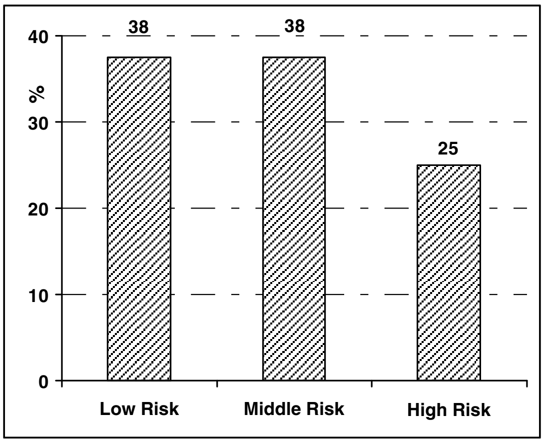

Figure 3. Results of the risk assessment procedure for 16 sites in the Hînceşti district of Moldova.

The integrated risk index correlated with the POPs pesticide concentrations in soil. Four sites with POPs concentrations greater than 10,000 μg/kg (from Table 1) had a high risk level (Balceana, Buteni, Pervomaisk, and Stolniceni). The Bujor site showed a middle integrated risk index due to the small volume of contaminated soil, despite having high POPs concentrations.

The Balceana site was selected for more detailed investigation because conditions for managing a phytoremediation experiment were more favorable and pesticide pollution levels were high. The Bujor site was investigated in more detail for the assessment of utilization of naturally occurring wild plants for phytoextraction. These two former pesticide storehouse sites will be referred to as Balceana and Bujor in the remainder of this paper.

3.2. CHARACTERIZATION OF SOIL POLLUTION IMPACT ON SURROUNDING AGRICULTURAL LAND AT THE BALCEANA AND BUJOR SITES

3.2.1. Balceana Site

The impact to agricultural land surrounding the Balceana site was assessed by estimating pollution in the top soil layer along four transects originating from the foundation of the former storehouse. The spatial distribution of DDTs is illustrated in Figure 4. All territory surrounding the Balceana site was highly polluted by DDTs with concentrations more than ten times the 100 μg/kg MAC. Two anomalous samples were identified with the unusually high pesticide concentrations greater than 50,000 μg/kg. Higher soil concentrations of DDTs were observed in the lower part of the site. Distribution of total HCHs is presented in Figure 5. The pollution level of HCHs was lower in comparison with DDTs. In the two anomalous samples, high HCHs correlated with the DDTs results. The principal problem with this site was contamination by DDTs.

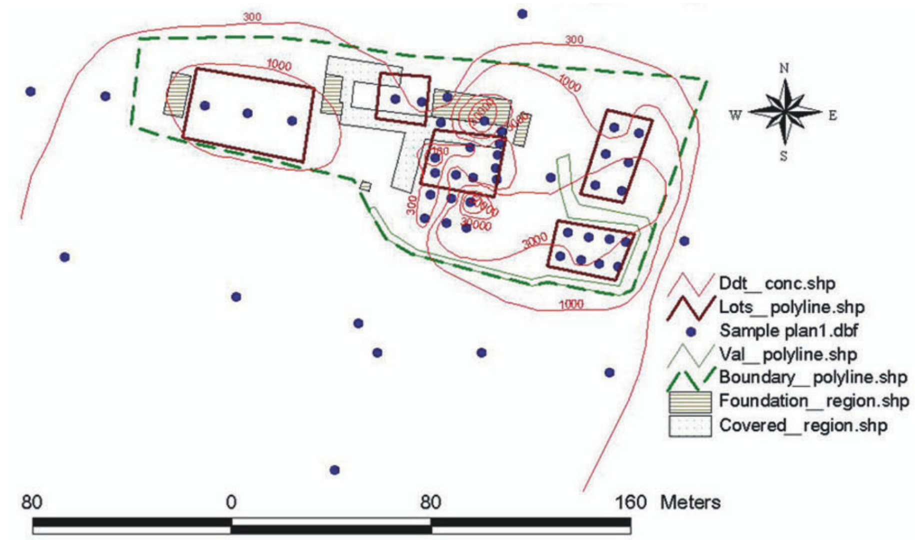

Figure 4. Total DDTs concentration in surface soil of the Balceana site (contours of equal concentration are given in μg/kg).

The pattern of surface soil contamination along the four transects showed the principal method of migration for DDTs as wind transport from dust material blown in a southwest direction. Soil pesticide concentrations in this direction varied from 300 to 1,000 μg/kg (Figure 6). One anomalous sample showed a 14,690 μg/kg concentration of DDTs situated in a northeast direction. It is likely that anomalous samples with high DDTs outside the building foundation area can be explained by pesticide spills during the time pesticides were used at the site.

Pesticide distribution within the soil profile was investigated by sampling four boreholes at six depth intervals. Results showed limited downward migration of pesticides.

These results provided information to estimate the approximate area and volume of polluted soil. These parameters were calculated by ArcView software and are presented in Tables 4 and 5.

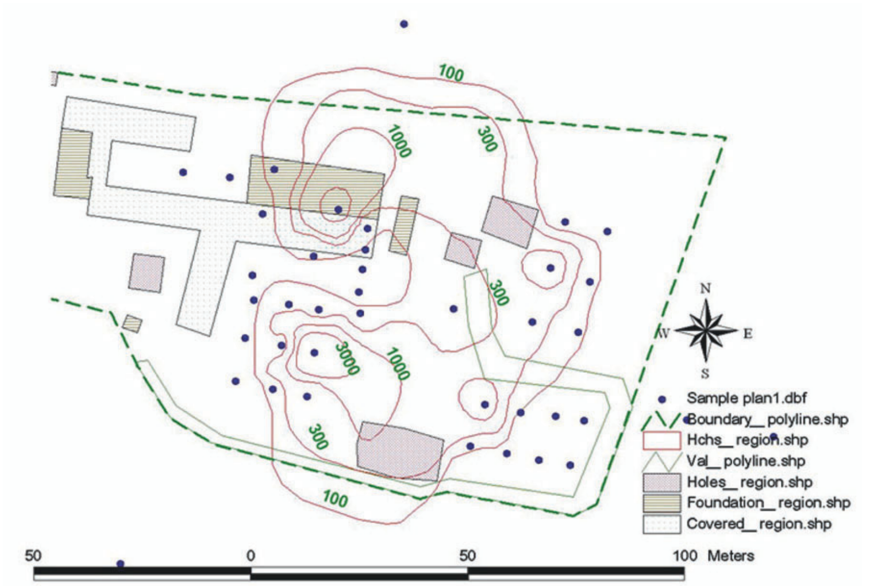

Figure 5. HCHs concentration in surface soil of the Balceana site (contours of equal concentration are shown in μg/kg).

TABLE 4. Spatial distribution for concentrations of total DDTs and HCHs expressed as area covered in the Balceana study area.

Concentration interval for total DDTs (μg/kg)	Area (m^2)	Area (%)	Concentration interval for total HCHs (μg/kg)	Area (m^2)	Area (%)
100–300	14,982	9.8	100–300	2,397	41.4
300–1,000	114,320	75.0	300–1,000	2,513	43.4
1,000–3,000	16,994	11.2	1,000–3,000	731	12.6
3,000–10,000	4,857	329	>3,000	151	2.6
10,000–30,000	1,054	079	–	–	–
30,000–50,000	177	0.1	–	–	–
>50,000	52	0.03	–	–	–
Total polluted area	152,435	100.0	–	5,792	100.0

The mass of contaminated soil with DDTs greater than 50,000 μg/kg was estimated at 41.3 t. This soil would be considered toxic waste. The estimated mass of soil with the DDTs in the interval 30,000–50,000 μg/kg was 141.7 t. Other intervals with lower concentrations had greater volume and were also dangerous for the environment and public health. The area at the Balceana site with a pollution level greater than the MAC for DDTs was larger than 15.2 ha.

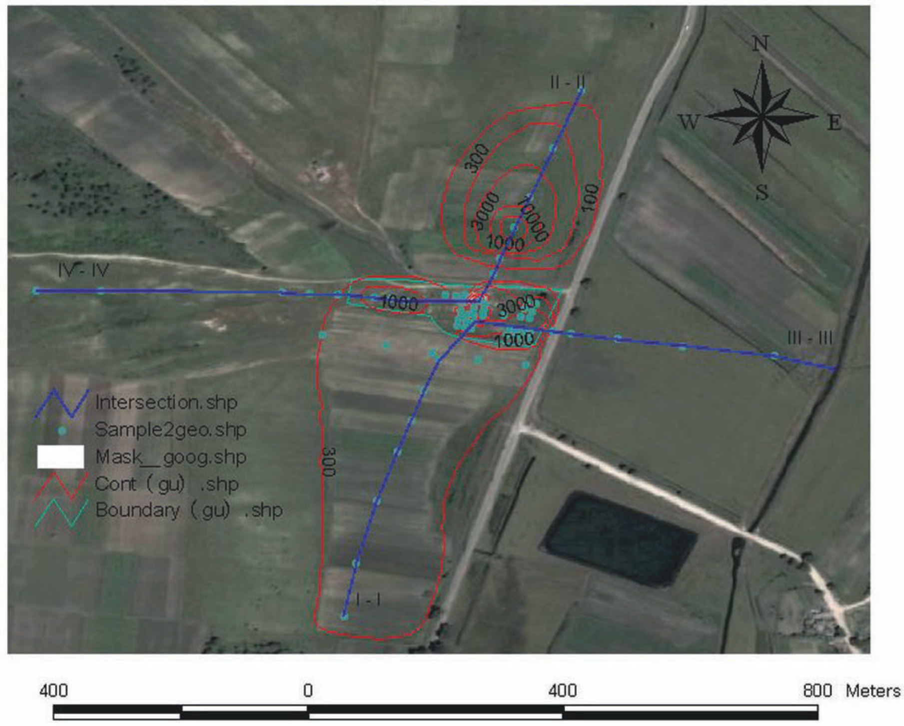

Figure 6. Total DDTs concentration in surface soil along four transects originating from the Balceana site (contours of equal concentration are shown in μg/kg).

TABLE 5. Estimated soil volume and mass with different concentrations of DDTs.

Interval DDTs concentration (μg/kg)	Area (m^2)	Volume (m^3) to 0.5 m depth	Weight (t) to 0.5 m depth[a]
100–300	14,982	7,491	11,986
300–1,000	114,320	57,160	91,456
1,000–3,000	16,994	8,497	13,595
3,000–10,000	4,857	2,428	3,885
10,000–30,000	1,054	527	843
30,000–50,000	177	89	141
>50,000	52	26	41

[a]Average density of soil 1.6 g/cm^3.

3.2.2. Bujor Site

The Bujor site was investigated in more detail because high pollution levels were found during the first step of investigation. Soil samples from the storehouse territory showed very high concentrations of DDTs. In two samples, DDTs exceeded 50,000 μg/kg, the lower limit to classify the pesticide-contaminated soil as hazardous waste.

Spatial distribution or spread of pesticide contamination covered a smaller area in comparison to the Balceana site (Figure 7). This fact can be explained by two observations. First, the contaminated soil was primarily located within the foundation of the Bujor site; and second, all area surrounding the site was covered by natural vegetation that minimized pesticide migration by movement of soil, water, and air.

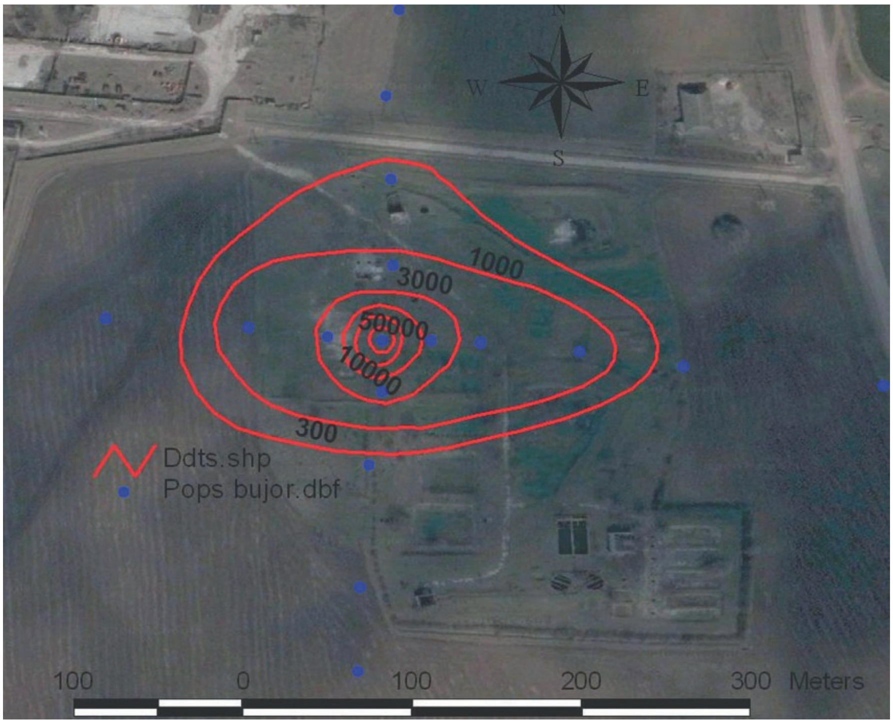

Figure 7. Total DDTs concentration in surface soil along four transects originating from the Bujor site (contours of equal concentration are shown in μg/kg).

Potential for phytoremediation at this site was limited since the principal pollution was located within the construction foundation in a relatively high concentration. A better option for this location would be to apply bio-remediation approaches before phytoremediation.

The Bujor site was used to investigate pesticide extraction by local wild plants that had colonized the site. Wild carrot was the dominant plant species assessed.

3.3. PHYTOREMEDIATION EXPERIMENT

The dry growing season limited growth of the wild carrot treatment, allowing assessment of only four plant species for phytoremediation. In this paper, only results for two treatments, zucchini (*C. pepo* L. var. *pepo*) and pumpkin (*C. pepo* L. var. *pepo*), are presented.

3.3.1. Zucchini

Zucchini plants were grown in three of the plots at the Balceana site. Each plot had different initial soil pesticide concentrations with DDTs concentration in the rhizosphere varying from 117 to 4,022 µg/kg. A total of nine zucchini plants were harvested.

Zucchini accumulated a high level of DDTs and the amount of accumulation depended on pesticide concentration in the soil near each plant (Figure 8). Harvested plants were divided into roots, stems, petioles, leaves, and fruit. Total accumulation of DDTs decreased from the roots to the fruit. Relatively high levels of accumulation were indicated in the roots and stems. Accumulation in the stem is the most important result for phytoremediation potential because it indicates translocation of DDTs from the roots to aboveground stems that are most easily harvested and removed from the site.

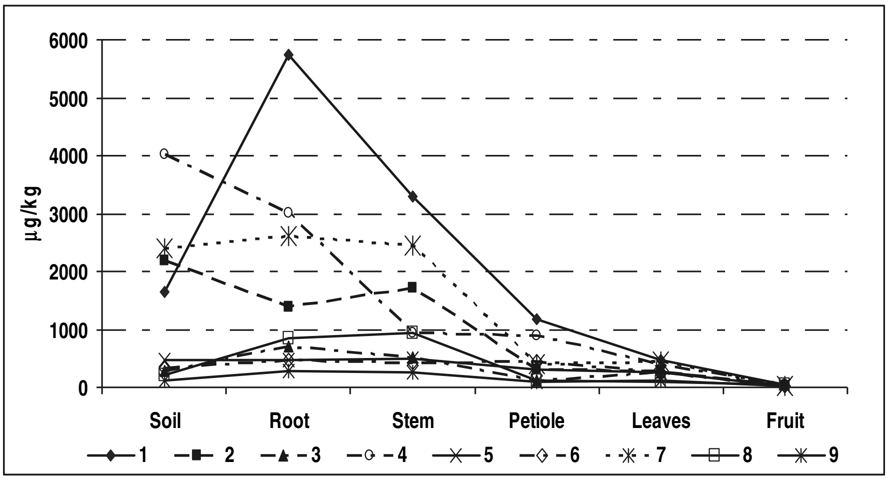

Figure 8. Accumulation of DDTs in zucchini by different plant parts (each line represents one of nine zucchini plants that were harvested).

The bioaccumulation factor (BAF), an important indicator of phyto-remediation potential, is calculated as the ratio of pesticide concentration in plant parts to pesticide concentration in the soil. Observed BAF values for all zucchini plants fluctuated from 0.5 to 4.0 for roots and from 0.3 to 4.3 for stems (Figure 9). Other plant parts showed lower bioaccumulation potential with ranges of 0.14–1.32 for flowers, 0.10–0.91 for leaves, and 0.01–0.28 for fruits. A BAF greater than 1.0 shows evidence of accumulation potential since the concentration in plant tissue is greater than the concentration in soil. The mean and sample standard deviation of BAF values for different zucchini plant tissues are presented in Figure 10. The BAF for roots and stems averaged more than 1.0 with means of 1.90 and 1.64, respectively.

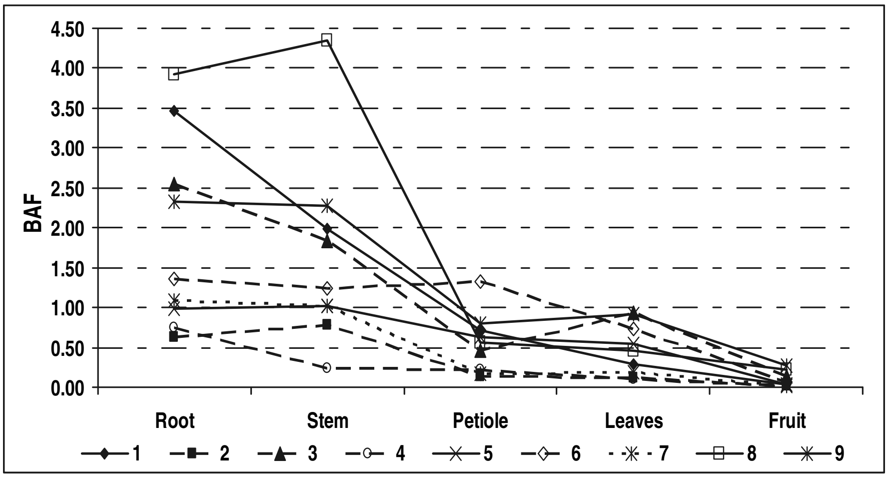

Figure 9. Bioaccumulation factor for DDTs for different tissues of nine harvested zucchini plants (each line represents one of nine harvested zucchini plants).

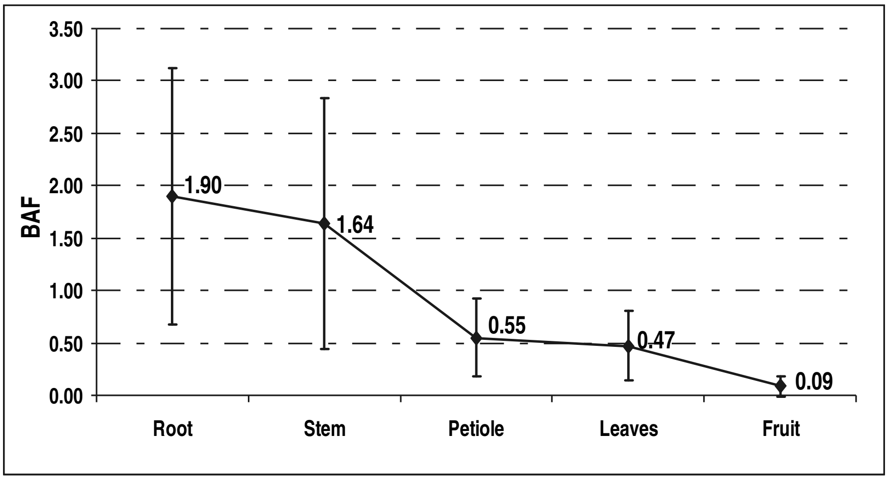

Figure 10. Mean and standard deviation of BAF for DDTs in different plant tissues for nine zucchini plants.

3.3.2. Pumpkin

The pumpkin vegetation treatment also showed good phytoextraction capacity. Results from ten pumpkin plants are presented in Figure 11, with six plants showing higher pesticide concentrations in the roots and stems compared to the soil. Four plants showed lower DDTs concentrations in roots and stems compared to soil.

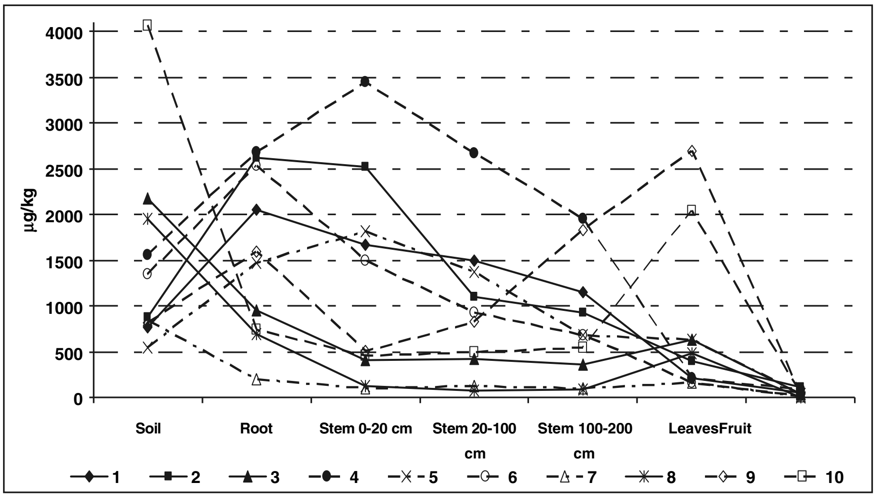

Figure 11. Accumulation of DDTs in different plant tissues of pumpkin (each line represents results from one of ten harvested plants).

Pumpkin biomass was greater than zucchini biomass. Pumpkin stems reached up to 3–5 m in length. Stems were separated into groups by the distance from plant roots. Stems harvested near plant roots showed higher concentrations of DDTs compared to stems farther from plant roots. Bioaccumulation factors for DDTs in plant tissues were very similar to observations for zucchini (Figures 12 and 13). Range of BAF for stem concentration varied from 0.45–3.01. The average decreased from 2.05 for roots to 0.82 for leaves. The fruit accumulated much lower amounts of DDTs with BAF 0.05. The weighted average value of BAF for zucchini was 1.10, and 1.42 for pumpkin.

3.3.3. Total Pesticide Accumulation

Total amount of pesticides extracted by pumpkin and zucchini can be calculated after analyzing plant weigh data (Table 6). Zucchini plant density was about 50 plants per 0.01 ha and pumpkin plant density was about 30 plants per 0.01 ha (10 × 10 m).

Total green mass (stem and roots) for zucchini was about 40.0 kg per 0.01 ha and about 50.0 kg per 0.01 ha for fruits. Pumpkins produced about 60 kg of green mass and about 85 kg for fruit. Root green mass ranged from 1–2% of the total green mass for zucchini and 3–5% for pumpkin. The proportion of stem green mass was about 40% for zucchini and 90% for pumpkin. Leaves (and petioles for zucchini) produced less green mass compared to other plant parts.

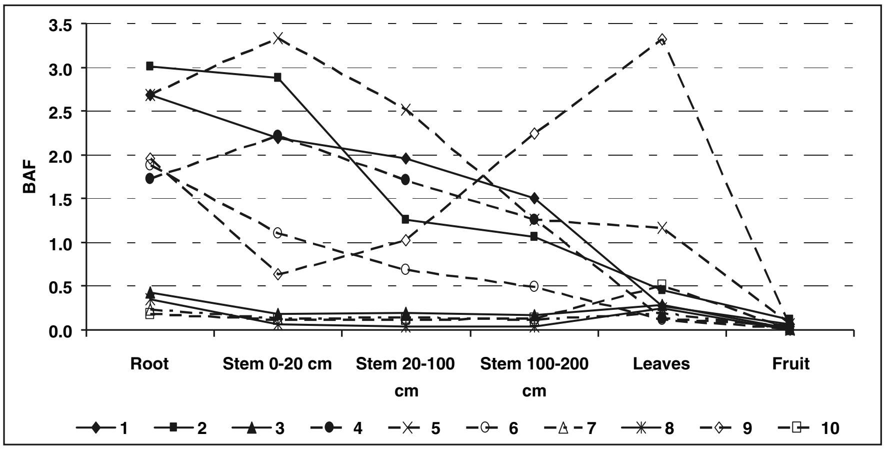

Figure 12. Bioaccumulation factors for accumulation of DDTs different plant parts of pumpkin (each line represents results from one of nine harvested plants).

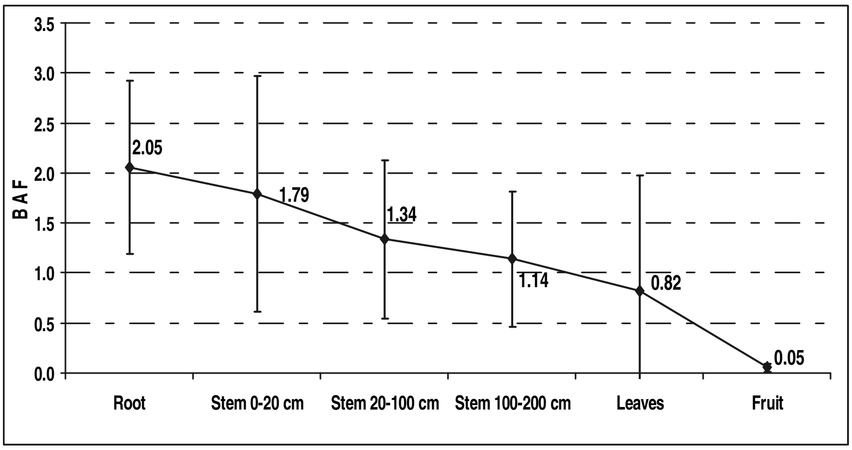

Figure 13. Mean and standard deviation of the bioaccumulation factor for DDTs in different plant tissues from ten pumpkin plants.

TABLE 6. Plant biomass data for zucchini and pumpkin. Data shown are wet weights.

Pumpkin	Length (m)	Plant weight (kg)		Zucchini	Plant weight (kg)	
		Stem	Fruit		Stem	Fruit
1	1.10	0.85	2.00	1	0.40	0.90
2	0.42	0.50	1.80	2	1.50	2.00
3	1.10	1.20	1.80	3	0.40	0.60
4	3.80	1.00	3.60	4	2.00	2.20
5	3.60	1.70	3.80	5	0.80	1.40
6	4.90	4.60	7.60	6	0.80	0.80
7	3.00	5.60	2.00	7	0.40	0.50
8	0.90	0.50	1.20	8	0.30	0.30
9	0.70	0.50	1.20	9	0.40	0.45
10	0.01	1.00	1.60	10	0.65	0.50
11	1.20	0.60	1.60	11	0.95	1.00
12	0.88	0.80	1.80	12	1.15	1.25
13	5.00	5.20	6.00	13	0.50	0.60
14	4.10	2.20	3.80	14	0.80	1.20
Mean	2.19	1.88	2.84	Mean	0.79	0.98
St. dev.	1.78	1.84	1.93	St. dev.	0.49	0.58

We estimated a total BAF of 1.1 for zucchini and 1.42 for pumpkin, based on the soil pesticide concentrations sampled at the location of each harvested plant. For zucchini, soil concentrations of DDTs ranged from 117 to 4,023 μg/kg with a mean value of 1,300 μg/kg. For pumpkin, soil concentrations of DDTs varied from 764 to 2,177 μg/kg with a mean value of 1,343.

Plant moisture content was about 90%. Overall mean concentration of DDTs for both zucchini and pumpkin was 1,300 μg/kg. In 1 year, estimated mass of pesticide extracted by zucchini and by pumpkin from 0.01 ha was calculated using the following formula:

$$X = (C_{DDT} \times W_{plant} \times W_{dry}) \times BAF$$

where C_{DDT} is the mean pesticide content in soil; W_{plant} is the total plant wet mass; W_{dry} is the proportion of dry matter; and BAF is the bioaccumulation factor.

For zucchini, mean soil pesticide concentration was 1,300 μg/kg with 160 kg of harvested wet biomass from approximately 200 plants. The dry matter proportion of 0.1 and BAF of 1.1 gives 20,800 μg or 20.8 mg or pesticide extracted from 0.01 ha.

For pumpkin, the mean soil pesticide concentration was 1,343 μg/kg with 440 kg of harvested wet biomass from approximately 200 plants. The dry matter proportion of 0.1 and BAF of 1.42 gives 83,910 μg or 83.9 mg of pesticide extracted from 0.01 ha.

The approximate weight of soil in 0.01 ha was calculated using the following formula:

$$W_{soil} = 100\ m^2 \times 0.3\ m\ \text{soil depth} \times 1.5\ t/m^3 = 45\ t$$

where 100 m^2 is the area of 0.01 ha, 0.3 m is the depth of polluted soil; and 1.6 t/m^3 is the soil bulk density.

The mass of DDTs in this soil volume can be calculated as follows: 45,000 kg soil × 1.30 mg/kg concentration of pesticide in soil = 58,500 mg. This is the amount of pesticide that could be extracted from the soil in 0.01 ha. If pumpkin can extract 83.9 mg pesticide per year, then at a constant rate of extraction, we would need more than 700 years for all DDTs to be removed from the polluted soil with this pollution level. This calculation makes numerous assumptions, but it illustrates that a long time would be necessary to reach acceptable cleanup levels with the rate of phytoextraction observed in this study.

Phytoextraction ability might be improved by intensification of planting with higher plant densities, multiple crops per year, or by increasing the bioaccumulation factor. More likely, combining phytoextraction with other mechanisms like microbiological destruction of pesticides can increase phytoextraction efficiency. Repeated tillage of pesticide-contaminated soil also might result in dilution of soil concentrations by mixing. The fate of pesticide residues during the remediation process and potential exposure risks need to be understood. At a minimum, use of vegetation at the contaminated site could stabilize contamination, reducing spread by erosive forces. Plant biomass destruction by composting at the cleanup site might be a good solution for utilization of harvested pesticide containing plant biomass.

Further investigation is needed to confirm these estimates and to study other processes of pesticide destruction on the site, especially microbiological activity in the soil or by composting.

3.3.4. Pesticide Uptake by Wild Carrot

Four wild carrot plants were analyzed from the Bujor site (Figure 14). The principal pollutant here was DDTs. These plants were collected from the center of the polluted area with DDTs concentration of greater than 50,000 μg/kg. Wild carrot grew very well over the entire polluted area in spite of high POPs pollution levels. We observed both plant roots and stems with high DDTs concentration. The extraction efficiency of wild carrot was limited in this condition with a BAF of 0.67 for roots and 0.18 for stems.

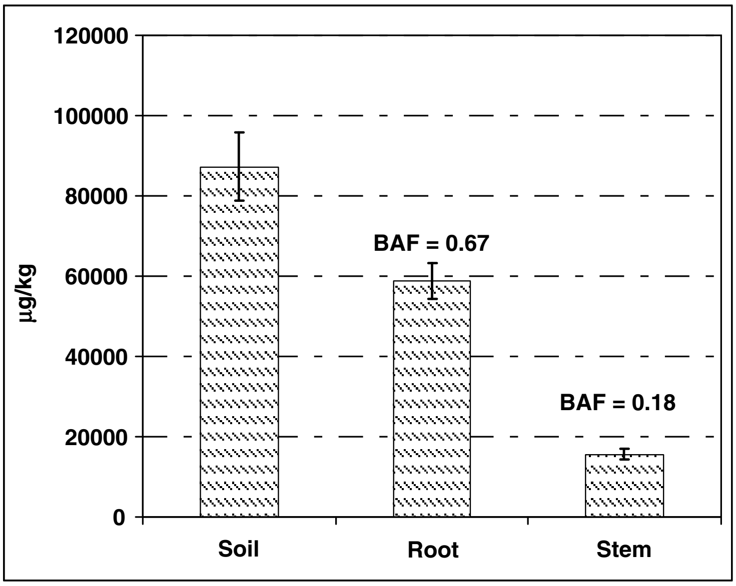

Figure 14. Bioaccumulation of DDTs by wild carrot at the Bujor site.

Although the plant limited uptake of toxic substances under this very high pollution level with a low BAF, the relatively low water content or moisture percentage for wild carrot biomass resulted in uptake of a greater volume of POPs compared with zucchini and pumpkin.

Samples for a risk assessment to public health and the environment were taken during summer field trips to the Balceana and Bujor sites. Groundwater, dairy products, and poultry samples were collected in regions near the polluted sites.

Milk samples were taken from a village near the Balceana site and from a market in the capitol city Cishinau. Samples from two principal dairy manufacturers and two private farmers were analyzed from other regions of Moldova for background determinations. Two samples of local sheep cheese, "brinza," also were taken from the Bujor and Balceana regions.

The principal DDTs isomer in all milk and cheese samples was DDE. Total concentration of DDTs was below the MAC value for dairy products (100 µg/ml), but the pollution level was still high (Table 7).

The pesticide concentration was below the MAC but high for consumption of this dairy product. Pesticide accumulation in the fat matrix was higher in regions of Moldova with higher environmental impact from these toxic substances. Control of pesticides in dairy products is important for Moldova and other regions with high levels of pesticide pollutants in the environment.

TABLE 7. Estimated pesticide concentrations in milk samples.

Pesticide name	CAS number		Pesticide concentration					
		Sample name	MB1	MB2	ML	MA	MS	Lap.
α_HCH	319-84-6	Product (μg/l)	0.9	7.1	4.0	<0.1	<0.1	1.1
		Milk fat (μg/kg)	37.6	71.2	160.0	<1.0	<1.0	27.1
HCB	118-74-1	Product (μg/l)	<0.1	0.5	0.3	<0.1	0.3	0.7
		Milk fat (μg/kg)	<1.0	4.8	11.2	2.0	6.1	17.0
β_HCH	319-85-7	Product (μg/l)	0.4	4.1	1.8	<0.1	<0.1	<0.1
		Milk fat (μg/kg)	16.0	40.8	73.6	<1.0	<1.0	<0.1
γ_HCH	319-86-8	Product (μg/l)	<0.1	1.1	0.8	<0.1	<0.1	<0.1
		Milk fat (μg/kg)	<1.0	11.2	33.6	<1.0	<1.0	<1.0
DDE	72-55-9	Product (μg/l)	4.0	17.0	9.6	3.6	8.8	14.7
		Milk fat (μg/kg)	158.4	170.4	384.0	90.0	219.0	368.5
DDD	72-54-8	Product (μg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	0.6
		Milk fat (μg/kg)	<1.0	<1.0	<1.0	<1.0	<1.0	14.7

Milk sample description:
Milk from factory B: MB1 – milk with 2.5% fat; MB2 – milk cream with 10% fat
Milk from factory L: ML – milk with 2.5% fat
Milk from private persons: MA – milk with 4.5% fat; MS – milk with 4.0% fat
Lap – milk from Lapusna village with 4.5% fat

Poultry samples were taken from private housekeeping for two hens. The first hen was represented by four samples labeled K1F – fat; K1L – liver; K1E – eggs; K1S – skin. The second hen was also represented by four samples: K2F – fat; K2L – liver; K2E – eggs; K2S – skin. Results are presented in Table 8. Analysis of poultry samples showed very similar pesticide content in poultry fat compared with milk fat samples. The fate of pesticides in the human food was similar for dairy products and poultry.

TABLE 8. Estimated pesticide concentrations in fat of poultry samples.

Name	CAS number	Concentration (μg/kg)							
		K1F	K2F	K1E	K2E	K1L	K2L	K1S	K2S
α_HCH	319-84-6	7.9	9.8	13.5	16.1	29.5	12.7	34.6	20.2
HCB	118-74-1	3.8	8.6	4.7	5.3	3.0	2.0	8.7	5.4
β_HCH	319-85-7	1.2	2.3	2.7	3.0	7.2	4.5	31.5	4.1
γ_HCH	319-86-8	6.5	4.8	5.2	5.7	7.3	2.9	10.0	9.1
DDE	72-55-9	125.6	220.4	106.3	79.3	91.2	84.6	180.4	111.7
DDD	72-54-8	29.2	15.9	5.1	14.1	9.3	11.8	30.8	13.7
DDT	50-29-3	1.6	2.8	2.4	3.1	2.8	1.2	4.8	5.0

4. Conclusions

1. Old pesticide storehouses remain highly polluted sites after repackaging and removal of obsolete pesticide stockpiles. Repacking was carried out only for old chemicals, while other polluted objects such as construction materials with soil and chemicals were not removed from old storage houses. Huge areas of soil near old storehouses also remain a serious danger for environmental and public health. Additional remediation actions are necessary in these areas. Every polluted site needs a site-specific remediation plan dependent on local conditions. An inventory of old storehouses is needed for the entire territory of the Moldovan Republic.

2. Residual pollution levels at old pesticide storage sites depend on the human element and previous history of the facility. The better equipped sites generally have lower pollution levels. Migration of pesticides from polluted sites occurs locally by transportation of soil dust by wind and water, as well as by human influence. Unguarded sites are subject to unapproved actions by the local population, and better constructed facilities with more security will have lower impact from pollution.

3. Phytoremediation can be used for the remediation of polluted sites; however, it needs to be designed based on local conditions. Investigators must take into consideration all advantages and limitations of this technology. This is important for plant selection, design of optimal plant density, and appropriate soil fertilization for the improvement of BAFs.

4. Time required for agricultural phytoremediation might be long and require utilization of complex approaches like biotechnology for the highest polluted soils. Utilization of native and perennial plants may prove to be useful for complex polluted sites.

5. Composting of crops harvested at a phytoremediation site is an appropriate solution for the handling of contaminated plants to reduce the quantity of hazardous material that must be disposed of.

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OBSOLETE PESTICIDES POLLUTION AND PHYTOREMEDIATION OF CONTAMINATED SOIL IN KAZAKHSTAN

A. NURZHANOVA[1*], P. KULAKOW[2], E. RUBIN[3], I. RAKHIMBAYEV[1], A. SEDLOVSKIY[1], K. ZHAMBAKIN[1], S. KALUGIN[1], E. KOLYSHEVA[4], AND L. ERICKSON[2]
[1]*Institute of Plant Biology and Biotechnology, 45 Timiryazev St., Almaty, 050040, Kazakhstan*
[2]*Center for Hazardous Substance Research, 104 Ward Hall Kansas State University, Manhattan, KS 66506 USA*
[3]*USEPA, 1200 Pennsylvania Ave, NW, (1400F), Washington, DC 20460 USA*
[4]*Biomedpreparat, Biomonitoring Laboratory, Micro District 9, Building 3, Stepnogorsk, Akmolinsk, 021500, Kazakhstan*

Abstract In Kazakhstan, a deepening ecological crisis has been caused by contamination of the environment with obsolete and expired pesticides. Large-scale physical and chemical technologies for managing pesticide-contaminated soils are expensive and unacceptable for Kazakhstan because of limited financial resources. Phytoremediation is a promising innovative technology for managing pesticide-contaminated soils. Pesticide contamination is common on land surrounding destroyed warehouses that were part of the official plant protection service of the former Soviet Union.

We surveyed substances stored in 76 former pesticide warehouses in Almaty and Akmola oblasts of Kazakhstan to demonstrate an inventory process needed to understand the obsolete pesticide problem throughout the country. The survey areas were within 250 km of Almaty (the former capitol of Kazakhstan) and within 100 km of Astana (the new capitol). In Almaty oblast, a total of 352.6 t of obsolete pesticides and 250 pesticide containers were observed. In Akmola oblast, 36.0 t of obsolete pesticides and 263 pesticide containers were observed. Persistent organic pollutants (POPs) pesticides contaminated soil around 26 of the former storehouses where the concentration of POPs exceed the Kazakhstan MAC (maximum allowable

[*]To whom correspondence should be addressed.

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concentration) for soil contaminated by tens to hundreds of times. The POPs pesticides include metabolites of DDT (dichlorodiphenyltrichloroethane) and isomers of HCH (hexachlorocyclohexane).

We studied plant community structure at six "hot points" contaminated sites with three located in Almaty oblast and three in Akmola oblast. From these studies, 17 pesticide-tolerant plant species were selected from colonizing plants that grew near the centers of the hot points.

A greenhouse experiment using the pesticide-tolerant species showed some plant species have the ability to change plant growth characteristics when grown in contaminated versus uncontaminated soil. These characteristics include biomass production, rate of phenological development, peroxidase activity in roots and leaves, ratio of chlorophyll a to chlorophyll b, rate of evapotranspiration, and phytoaccumulation of organochlorine pesticides and their metabolites (4,4 DDE, 2,4 DDD, 4,4 DDT, α-HCH, β-HCH and γ-HCH).

We observed pesticide accumulation was influenced by plant species, plant biomass, and soil pesticide concentrations. Among the investigated species, four accumulated metabolites of DDT and isomers of HCH in plant tissue concentrations exceeding the Kazakhstan MAC (maximum acceptable concentration) for plant tissue by 400 times. The Kazakhstan MAC for DDT and HCH metabolites in plant tissue is 20 μg/kg. Species in this category included: *Artemisia annua* L., *Kochia sieversiana* (Pall.) C.A. Mey. *Kochia scoparia* (L.) Schrad., and *Xanthium strumarium* L. Three species exceeded the MAC by up to 90 times including *A. annua*, *Ambrosia artemisiifolia* L., and *Erigeron canadensis* L. Most pesticides accumulated in the root systems; however, among the species investigated, *K. scoparia*, *A. annua*, *Barbarea vulgaris* W. T. Aiton, and *A. artemisiifolia* demonstrated capabilities to translocate pesticides from roots to aboveground tissues.

To help identify the location of accumulated pesticides within plant tissue, we employed histological analysis whereby a few species indicated pesticides were distributed unevenly within different plant tissues. If a species had a dorsiventral and isolateral leaf type, then pesticides appeared to accumulate in palisade mesophyll tissue. If a species had homogeneous mesophyll, then pesticide appeared to accumulate in mesophyllous cells around conducting bunches. For example, *X. strumarium*. has a dorsiventral type of leaf; thus, pesticides collected in the palisade mesophyll. In the stem, pesticides accumulated in walls of xylem cells. In root tissue, pesticides accumulated in parenchymous cells and xylem walls.

We investigated cultivation methods to enhance plant uptake of pesticides. Use of mineral fertilizers resulted in stimulation of growth and biomass accumulation that increased phytoextraction. The concentration of DDT metabolites and isomers of HCH in soil and the application of

fertilizers lengthened the rate of phenological development increasing plant height and biomass. In a greenhouse experiment using fertilizer applications to pesticide-contaminated soil, tolerant species showed increased phytoextraction of pesticides. Phytoextraction by *X. strumarium* increased from 0.3% to 0.6%, *A. annua* from 0.5% to 0.7%, and *Cucurbita pepo* L. *pepo* from 0.4% to 0.7%. *K. scoparia and Amaranthus retroflexus* L. showed high bioaccumulations factors but showed low biomass compared to other species and thus weak phytoextraction. *A. annua*, *K. scoparia*, *A. retroflexus*, and *X. strumarium* decreased pesticide concentration of rhizosphere soil 11–24% more in treatments with fertilizer compared to treatments without fertilizer. Field experiments using selected wild species demonstrated reduction of pesticide concentrations in soil in excess of reductions observed without plants and without fertilizers. Additional work is needed to determine if practically useful phytotechnology applications can effectively manage pesticide-contaminated soil at former storehouse sites.

Keywords: obsolete pesticides, phytoremediation, DDT, HCH, pesticide tolerance, inventory

1. Introduction

Kazakhstan became independent from the former Soviet Union in 1991; however, many of the impending environmental problems were not anticipated. Within 5 years of independence, pesticide storage warehouses that used to be managed by the official plant protection service of the former Soviet Union were destroyed, leaving obsolete pesticides and their containers unattended and open to the environment. Most of the bulk obsolete pesticides have been moved to other storage areas, taken by citizens for individual use, resold, or released into the surrounding environment with no indication of their potential danger to local residents. Much of the obsolete pesticides that were resold were first repackaged in unlabeled or mislabeled containers. People living around the warehouse sites often use the land for pasture, kitchen gardens, play areas for children, and as a source of construction materials. Pollution of soil and water by obsolete pesticides is a serious ecological problem. Many of these former warehouses have become hot points of contamination and represent a serious ecological danger.

The Republic of Kazakhstan government has developed laws to address this situation; however, it is necessary to implement these laws. Official data on the number of warehouses, their location, the fate of bulk pesticides, and quantities of buried or unburied pesticides are inconsistent for different regions and for Kazakhstan as a whole. For example, the Ministry for

Environmental Protection estimates the Almaty area has burial places with more than 87 t of pesticides, while the Ministry of Agriculture estimates this area has about 126 t of buried pesticides. Bismildin (1997) stated that Kazakhstan accumulated 574 t of obsolete pesticides, while Nazhmetdinova (2001) estimated accumulation of 1 million tons of pesticides.

Kazakhstan signed the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2001 and ratified the treaty in 2007. In 2004, a Global Environment Facility sponsored project to provide initial support for the performance of Kazakhstan's obligations under the Stockholm Convention estimated there were 1,500 t of obsolete pesticides and pesticide mixtures. The project suggested that many of the mixtures contained POPs pesticides (UNEP, 2004). This initial inventory of obsolete pesticides described only the condition of pesticide storehouses and quantities and conditions of pesticide containers. There has been insufficient scientific study to estimate the danger to public health and the environment from these sites. Mass media within Kazakhstan has not given sufficient attention to the problem of chemical contamination of the environment.

Many different methods can be used for remediation of pesticides in soil. Some large-scale and expensive remediation technologies that may be effective for treatment of pesticide-contaminated soil and water are likely to be unacceptable in Kazakhstan due to limited financial resources. Phytotechnologies use vegetation to accumulate, degrade, or stabilize environmental contaminants. Innovative natural remediation technologies like phytoremediation are promising if they can be shown to address cleanup requirements and can be effectively managed at an acceptable cost. The strategy for this project was to identify pesticide-tolerant plant genotypes which can be used for phytoremediation of pesticide-contaminated soil in the Almaty and Akmola oblasts of Kazakhstan.

In this study, pesticide analysis was limited to the organochlorine pesticides DDT (p,p′-dichlorodiphenyltrichloroethane) and HCH (hexachlorocyclohexane), along with their associated metabolites and isomers: 2,4 DDD (p,p′-dichlorodiphenyl dichloroethane); 4,4 DDD; 4,4 DDT; 4,4 DDE (p,p′-dichlorodiphenyldichloroethylene); α-HCH; β-HCH; and γ-HCH. While these pesticides represent only a subset of all obsolete pesticides, they are important due to their status as persistent organic pollutants and as compounds that represent a much larger problem.

To investigate potential use of phytoremediation, we delineated the following seven tasks:

Task 1: Inventory former obsolete pesticide warehouses to document obsolete pesticide stockpiles and to characterize levels of soil contamination.

Task 2: Study genotoxicity of organochlorine pesticides.

Task 3: Identify pesticide-tolerant plant species using surveys of plant community structure at selected “hot points”.

Task 4: Describe physiological and biochemical characteristics of pesticide-tolerant plants grown in pesticide-contaminated soil.

Task 5: Document pesticide accumulation patterns in pesticide-tolerant plants.

Task 6: Study the fate and transport of pesticides in soil and plants in the greenhouse using soil collected from hot points.

Task 7: Study the effect of fertilization on phytoremediation potential in the greenhouse and field.

2. Methods and Results

2.1. TASK 1: INVENTORY FORMER OBSOLETE PESTICIDE WAREHOUSES TO DOCUMENT OBSOLETE PESTICIDE STOCKPILES AND TO CHARACTERIZE LEVELS OF SOIL CONTAMINATION

To address problems associated with obsolete pesticides in Kazakhstan, it is critical to understand the scope of the problem and the location of affected areas. Since Kazakhstan is a very large country, we chose to initially survey two regions to demonstrate an inventory process that could be applied more widely when sufficient resources are available. The largest warehouses of the Soviet plant protection service in Kazakhstan were located in Almaty and Akmola oblasts because of the administrative importance and level of agricultural development in these regions. We surveyed obsolete pesticide storehouses in 10 of 14 rayons or districts in Almaty oblast and five rayons of Akmola oblast. In each rayon, the Ministry of Agriculture Department of Plant Protection was contacted to obtain locations of former pesticide storehouses and permission to access the sites. Local government authorities were contacted to receive further information on locations and permission to survey and sample each site.

In this paper, we refer to the former storehouse sites where we have observed pesticide contamination as “hot points.” Based on the history of agriculture in these areas, we assumed the hot points were chemically heterogeneous, and probably contained not only organochlorine pesticides, but also other classes of pesticides and fertilizers. Our study focused on analysis of organochlorine pesticides as markers of field contamination due to their status as persistent organic pollutants and their prevalence. We took more than 800 soil samples to determine residual pesticide concentrations using standard methods adopted by the United States Environmental

Protection Agency. All soil samples were extracted using the solvent dichloromethane that was boiled and cycled for several house using a Soxhlet apparatus. Soil extracts were analyzed using an HP6890 gas chromatograph equipped with an electron capture detector and a capillary column using EPA method 8081 (USEPA, 2007).

A total of 76 former storehouses were surveyed in Almaty and Akmola oblasts. All storehouse buildings were either partially or completely destroyed. The inventory included descriptions of conditions of the storehouse structures; estimation of bulk obsolete pesticide stockpiles and pesticide containers, inspection of storehouses and surrounding areas for pesticide contamination, assessment of vegetation growing at the sites, and public outreach. An inventory worksheet was developed to provide a systematic description of each location.

In Almaty oblast, a total of 352.6 t of obsolete pesticides and unidentified stockpile material were observed. We also observed 250 pesticide containers. In Akmola oblast, a total of 36.0 t of obsolete pesticides and unidentified stockpile material were observed, along with 263 pesticide containers (Table 1).

In Almaty oblast, several different classes of substances were identified. Much of the bulk chemical substances did not have readable labels and remain unidentified. The following classes of pesticides were observed: triazine herbicides (atrazine, protrazine, propazine, simazine), organophosphate insecticides (metaphos or methyl parathione), organochlorines (nitrophen and illoxan or diclofop-methyl), dinitroanaline herbicides (treflan), carbamate (temik or aldicarb), and a pesticide mixture including compounds labeled Thiram and Hataonyag.

Total amount of identified obsolete pesticides was 36,620 kg. The amount of identified pesticides that are forbidden or banned was 350 kg. In Almaty oblast, the quantity of unidentified mixtures of obsolete pesticides was 315,980 kg or 89.6% of the total obsolete pesticide stockpiles. In Akmola oblast, 100% of the 36,045 kg of obsolete pesticide stockpiles were unidentified chemical mixtures.

Soil samples were collected from each pesticide storehouse site to examine migration and expansion of pollution. Sites where soil contamination was observed in excess of maximum acceptable concentrations (MAC) for the Republic of Kazakhstan (1996, 2001, 2003) were called hot points. Twenty-six of the storehouse sites showed soil concentrations in excess of MACs. The MAC for Kazakhstan for soil is 100 μg/kg for the DDT metabolites (4,4 DDT; 4,4 DDE) and HCH isomers (β-HCH; γ-HCH). Three compounds we analyzed did not have MAC for Kazakhstan 2,4 DDD, 4,4 DDD, and α-HCH.

TABLE 1. Quantities of obsolete, forbidden, and unidentified pesticides in former warehouses in Almaty and Akmola oblasts of the Republic of Kazakhstan.

Rayon	No. sites	Identified obsolete pesticides (kg)	Banned pesticides (kg)	Unidentified substances (kg)
Almaty Oblast				
Karasajsk	6			1,150
Talgar	7	30,600		
Dzhambul	5	200		100,500
Enbekzhi-Kazakh	9	2,950	350	4,570
Uigur	3	970		2,860
Balkazh	7			500
Ulisk	7	1,450		105,700
Eskeldinsk	8	50		100,700
Kerbulak	12	0	0	0
Koksuisk	0			
Total	**64**	**36,620**	**350**	**315,980**
Akmola Oblast				
Atbasar	3			26,430
Buladinsk	2			5,345
Enbekshilder	2			900
Zharkain	1			700
Shortandi	4			2,670
Total	**12**			**36,045**

The most polluted storehouses were four sites located in Almaty oblast in the rayons of Eskeldinsk, Talgar, Karasajsk, and Enbekzhi-Kazakh where concentrations of organochlorine pesticides exceeded MAC up to 114 times (Figure 1). The most common pollutants were α-HCH, β-HCH, 4,4 DDE, and 4,4 DDT. For example, in the village of Aldabergenova in Eskedinsk rayon, concentrations of 4,4 DDT exceeded MAC by 19 times (1,955 ± 69 μg/kg), 4,4 DDE by 28 times (2,867 ± 68 μg/kg), and β-HCH by 17 times (1,731 ± 117 μg/kg).

In the village of Kyzyl-Gairar in Talgar rayon, α-HCH was observed to be 1,239 ± 136 μg/kg; 2,4 DDD; 398 ± 8 μg/kg; and 4,4 DDD, 1,899 ± 42 μg/kg. In Balkhazh, Uigur and Ilijsk rayons, insignificant amounts of HCH isomers were observed. Although α-HCH has no MAC, since isomers of HCH are known to be highly toxic and mutagenic (Medved, 1977), there is cause for concern about soil contaminated with this compound.

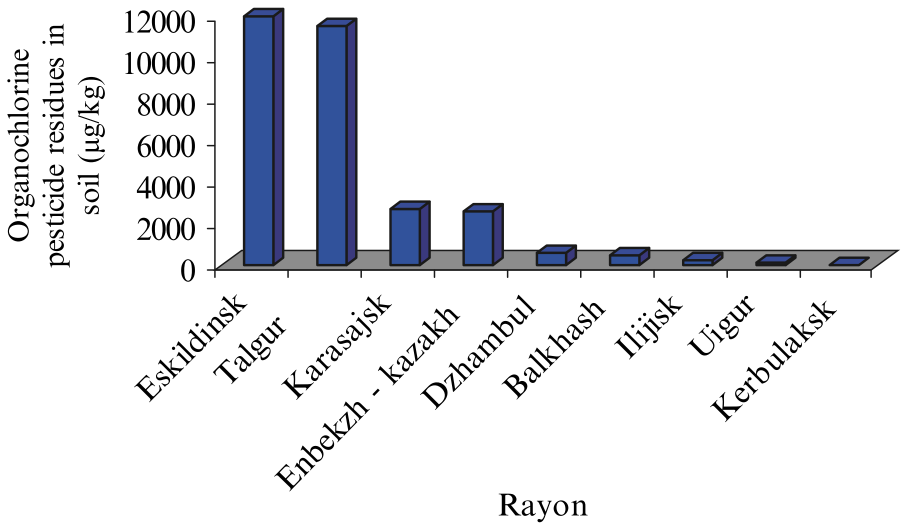

Figure 1. Total soil concentrations of isomers of HCH and metabolites of DDT from former pesticide storehouses in Almaty oblast.

Residual metabolites of DDT and HCH isomers we observed in soil do not depend on the presence or absence of bulk, obsolete pesticide stockpiles at the storehouses. For example, in the village of Belbulak in Karasajsk rayon, 500 kg of unidentified white powders were observed open to the air. Observed soil concentrations of 4,4 DDT exceeded MAC by 16 times (1,670 ± 66 µg/kg) and 4,4 DDE exceeded MAC by eight times (852 ± 18 µg/kg). In the village of Kyzyl-Gairar in Talgar rayon, no pesticide stockpiles were observed but soil concentrations of 4,4 DDT exceeded MAC by 65 times (6,584 ± 207 µg/kg) and 4,4 DDE by 20 times (2,097 ± 54 µg/kg).

Control soil batches were sampled at least 800 m from each hot point in Karasajsk rayon. The control samples contained α-HCH and some metabolites of DDT, primarily 4,4 DDE and 4,4 DDT, but these did not exceed MAC.

In Almaty oblast, several lakes are located near former storehouses in Talgar and Dzhambul rayons. Lake water was sampled from one lake in each of these areas. Two water samples from a lake located 100 m from a storehouse in the village of Beskanar in Talgar rayon contained an average of 114 µg/l 4,4 DDE. Maximum concentration of pesticides observed in soil around the storehouse in this area was 1,660 µg/kg. Chemical exposure to humans could result from contact or consumption of water, or fish from the lake.

These data demonstrate the potential ecological danger and health risk posed by the former pesticide storehouses, especially those located near populated areas. Resolution of this risk will require elimination of obsolete pesticide stockpiles and pesticide containers, including locations where pesticides have been buried. Further priorities include remediation of soil polluted by organochlorine pesticides. Screening pesticide polluted sites will provide a basis for development of an action plan to prevent or minimize ecological risk from pesticide pollution in Kazakhstan. Results of inventories and inspection of former pesticide storehouses provide an additional source of data for official inventory of obsolete pesticide stocks, and for development and conduct of public and state programs and projects on preservation of the environment and maintenance of ecological safety.

2.2. TASK 2: STUDY GENOTOXICITY OF ORGANOCHLORINE PESTICIDES

To analyze genotoxicity of organochlorine pesticides, we analyzed chromosome structural mutations observed during the metaphase stage of mitosis in meristem cells of barley seed. Seeds of *Hordeum vulgare* L. variety Odessa-100 were treated using pesticide concentrations observed in soil around former storehouses. To treat barley seed with pesticides, air-dried seeds were immersed for 4 h in hexane solutions used to dissolve HCH isomers and DDT metabolites. Two control treatments included seeds wetted with only distilled water and seeds wetted with only hexane. Seeds were washed, slightly dried, and germinated on filter paper moistened with distilled water at 25 ± 1°C. Prior to fixation of cells for staining, seeds were transferred to a solution of 0.01% colchicine. Fixation of chromosomes was accomplished by placing macerated roots in a solution of 0.002 M 8-oxyquinoline for 1 h at 13–15°C. Cytogenetic preparations were made using standard techniques (Paucheva, 1974).

More than 300 metaphase cells were examined for each treatment. Analysis of chromosomal reorganizations for different fixings did not show significant differences; therefore, analysis of results was based on all data. Analysis of chromosome structural mutations took into account not only the total of all abnormalities, but also types of chromosomal and chromatid aberrations including isolocus chromosome breaks and micro fragments (Figure 2). Control observations recorded spontaneous mutations in seed that was not exposed to pesticides.

Results of cytogenetic analysis showed that not all tested concentrations of HCH isomers and DDT metabolites resulted in chromosome aberrations significantly exceeding control treatments (Table 2). Frequency of chromosome aberrations for the water control was 2.9% ± 0.9% and 3.4% ± 0.2% for the hexane control. Aberrations observed in the water control were limited to terminal deletions (1.4% ± 0.4%) and isolocus breaks (1.5% ± 0.5%).

Aberrations for the hexane control were microfragments (1.2% ± 0.3%), isolocus breaks (1.7% ± 0.2%), and single fragments (0.5% ± 0.1%).

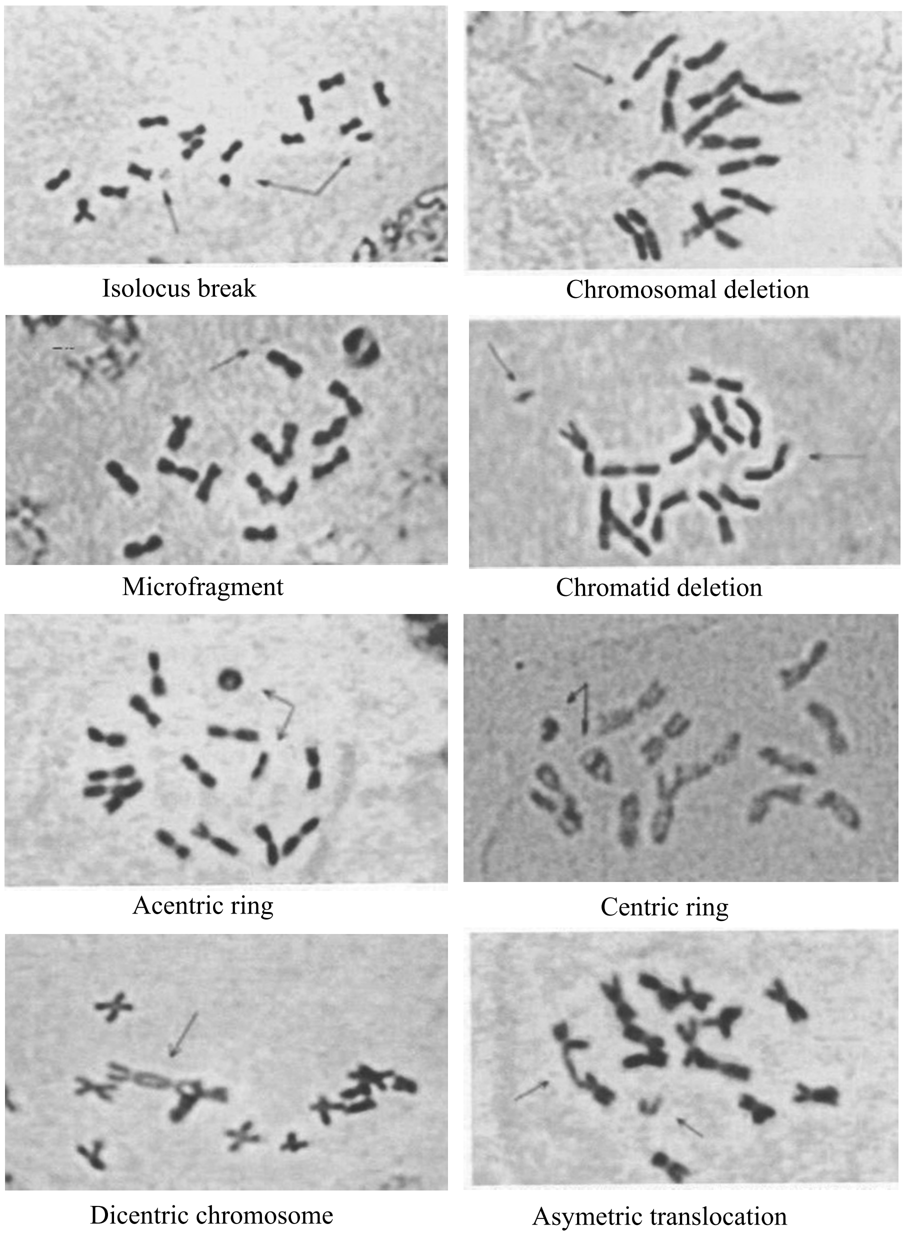

Figure 2. Structural chromosome aberrations Hordeum vulgare L. induced by seed treatment with HCH isomers and DDT metabolites.

Significant excess chromosomal mutations were observed in treatments by HCH isomers. Aberrations from γ-HCH treatments were 13.9% ± 1.9% for concentrations of the MAC and 15.6% ± 2.0% for concentrations of two times the MAC. For β-HCH, the treatment of eight times the MAC resulted in excess aberrations of 11.7% ± 1.7%. For α-HCH, aberration frequency was 8.5% ± 1.6% for the treatment concentration of 50 µg/kg and 15.5% ± 2.0% for the treatment concentration of 200 µg/kg. The main types of aberrant chromosomes for pesticide treated barley seed were centric and acentric rings, as well as dicentric and isolocus breaks.

TABLE 2. Frequency of chromosome aberrations in barley seed treated with HCH isomers and DDT metabolites compared to control treatments with water and hexane.

Treatment	Pesticide conc. (µg/kg)	No. cells	Metaphase cells with aberrant chromosomes		Total aberrations	Aberrations per 100 metaphases
			No.	%		
Control (water)		344	10	2.9 ± 0.9	10	2.9 ± 0.9
Control (hexane)		415	13	3.2 ± 0.7	14	3.5 ± 0.7
α-HCH	3	312	15	4.8 ± 1.2	15	4.8 ± 1.2
	50	304	25	8.2 ± 1.6	26	8.6 ± 1.6[a]
	200	302	45	14.9 ± 2.1	47	15.6 ± 2.1[a]
β-HCH	100	305	14	4.6 ± 1.2	14	4.6 ± 1.2
	200	318	14	4.4 ± 1.2	16	5.0 ± 1.2
	800	323	37	11.5 ± 1.8	38	11.8 ± 1.8[a]
γ-HCH	5	316	15	4.8 ± 1.2	17	5.4 ± 1.3
	100	301	39	13.0 ± 1.9	42	14.0 ± 2.0[a]
	200	314	47	15.0 ± 2.0	49	15.6 ± 2.1[a]
4,4 DDT	200	318	15	4.7 ± 1.2	17	5.4 ± 1.23
	1,000	302	17	5.6 ± 1.3	20	6.6 ± 1.4
	5,000	324	49	15.1 ± 2.0	52	16.1 ± 2.0[a]
2,4 DDD	5	317	16	5.1 ± 1.2	17	5.4 ± 1.3
	50	302	32	10.6 ± 1.8	37	12.3 ± 1.9[a]
	150	306	39	12.8 ± 1.9	40	13.1 ± 1.9[a]
4,4 DDE	100	381	48	12.6 ± 1.7	48	12.6 ± 1.7[a]
	800	312	27	8.7 ± 1.6	29	9.3 ± 1.6[a]
	1,800	321	45	14.0 ± 1.9	46	14.3 ± 2.0[a]

[a] $p < 0.001$ in comparison with control values.

For treatments with DDT metabolites, excess aberrations were observed for several treatments: 4,4 DDT in concentrations of five MAC (16.0% ± 2.0%); 2,4 DDD in concentrations of 50 μg/kg (12.2% ± 1.8%) and in concentrations of 100 μg/kg (13.07% ± 1.9%); 4,4 DDE at the MAC (12.5% ± 1.7%); and 18 times the MAC (14.3% ± 1.9%). DDT metabolites induced all types of chromosomal aberrations including centric and acentric rings, dicentric rings, single fragments, micro fragments, isolocus breaks, and asymmetric chromatid translocations.

Observations of chromosomal mutations from pesticide-treated barley seed using concentrations similar to those found in the soil at hot points suggest health risk from potential exposure to contaminated soil around former warehouses.

2.3. TASK 3: IDENTIFY PESTICIDE-TOLERANT PLANT SPECIES USING SURVEYS OF PLANT COMMUNITY STRUCTURE AT SELECTED HOT POINTS

To identify pesticide-tolerant plant species, plant community structure was investigated at five former storehouse sites (three in the Almaty oblast and two in Akmola oblast). At each location, plant species were identified along 400 m transects originating from the center of each site. Plant community structure was described by the Tahtadjan technique (1987). In the field, the following parameters of plant community structure were recorded: plant species identity, botanical family, patchiness, vegetative coverage, frequency, and distribution (Bykow, 1978). Additional parameters included stratification of plant species with distance from the center of the hot point, phenological stages, and species vigor at monthly intervals during the growing season from April to August.

Observations of plant diversity at these sites show that each site had a different plant community structure. Plant species diversity in the zone of influence of pesticide-contaminated sites included more than 100 species of flowering plants (not including seasonal ephemeral species).

Other observations of plant community structure included the following:

- Center of sites were dominated by annual and biannual plants.
- Sites varied in number of species and quantitative growth characteristics.
- In general, there was less diversity toward the center of sites.
- Centers of sites exhibited suppression of plant vigor.
- Plants of the same species often differed in phenological stages.

Genetic heterogeneity of plant populations growing at the hot points allowed identification of likely pesticide-tolerant species. In Almaty oblast, 75 plant species from 26 families were documented at the first hot point;

83 species from 23 families were identified at the second point; and 87 species from 22 families at the third point. Seventeen pesticide-tolerant species were identified, including *Artemisia annua* L., *Artemisia absinthium* L., *Agropyron pectiboformis* L., *Artemisia proceraeformis* L., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Barbareae vulgaris* W. T. Aiton, *Bromus tectorum* L., *Erigeron canadensis* L., *Kochia scoparia* (L.) Schrad, *Kochia sieversiana* L., *Lactuca tatarica* (L.) C.A. Mey, *Onopordon acanthium* L., *Polygonum aviculare* L., *Rubus caesius* L., *Rumex confertus* Willd., and *Xanthium strumarium* L.

In Akmola oblast, 82 plant species from 13 families were documented with identification of five likely pesticide-tolerant species including *A. proceraeformis*, *Agropyron pectiboformis* L., *Artemisia absinthium* Willd., *Kochia sieversiana* L., and *Solanum dulcamara* L.

2.4. TASK 4: DESCRIBE PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF PESTICIDE-TOLERANT PLANTS GROWN IN PESTICIDE-CONTAMINATED SOIL

The influence of variable pesticide concentrations on plant growth was studied in the greenhouse. Characteristics examined included rate of phenological development, peroxidase activity in roots and leaves, ratio of chlorophyll a to chlorophyll b, and transpiration rate (Gavrilenko et al., 1975). Fifteen plant species from eight families were grown: *Artemisia annua*, *Ambrosia artemisiifolia*, *Xanthium strumarium*, *Erigeron canadensis*, *Onopordon acanthium*, and *Artemisia absinthium* (Asteraceae), *Amaranthus retroflexus*, *Amaranthus tricolor* L. (Amaranthaceae), *Kochia scoparia*, *Kochia sieversiana* (Chenopodacea), *Solanum dulcamara* (Solanaceae), *Barbareae vulgaris* (Brassicaceae), *Rumex confertus* (Polygonaceae) *Aegilops cylindrica* Host (Poaceae), and *Medicago sativa* L. (Fabaceae).

The greenhouse experiment included five soil treatments applied to containers with 3 kg of soil. A control treatment utilized clean soil. One treatment used artificial contaminated soil from a solution of α-HCH, β-HCH, 4,4 DDE, 2,4 DDD, and 4,4 DDD. Average total pesticide concentration in this treatment was 145 μg/kg. Three treatments utilized soil collected from hot points 1, 2, and 3 from Karasajsk rayon in Almaty oblast. Average total pesticide concentration in soil from hot point 1 was 734 μg/kg; hot point 2, 6,270 μg/kg; and hot point 3, 343 μg/kg.

Results from observations of greenhouse-grown plants showed most plants completed a full life cycle despite high pesticide concentrations in soil. Phenological development and plant height of plants grown in pesticide-contaminated soil varied. Reduction of duration of the vegetative period and earlier flowering for pesticide-tolerant plants appeared to demonstrate adaptation to stressed environments.

2.4.1. Ratio of Chlorophyll a to Chlorophyll b

Transformation of light energy to chemical energy in photosynthesis is the basis of life on earth, and the process of photosynthesis is very sensitive to changes in environmental conditions. Suppression of photosynthesis under the influence of anthropogenic factors is confirmed by many authors (Bauer and Grill, 1977). It is known that chlorophyll a content is usually two to three times higher than chlorophyll b content, with the high ratio demonstrating adaptation of plants to light. Figure 3 shows the concentration of chlorophyll a and chlorophyll b in leaves during flowering for seven plant species grown in soil from hot point 1 and hot point 2. Chlorophyll b was approximately in two times higher than chlorophyll a. For example, leaves from *Artemisia annua* grown in soil from hot point 1 had chlorophyll a concentration of 0.21 ± 0.1 mg/g and chlorophyll b concentration of 0.59 ± 0.2 mg/g; and from hot point 2, chlorophyll b was 0.18 ± 0.2 mg/g while chlorophyll a was 0.32 ± 0.1 mg/g. The change in the ratio of chlorophyll a to chlorophyll b demonstrated changes in response of the photosynthetic mechanism to growth in pesticide-contaminated soil.

2.4.2. Transpiration Rate

When water in plant tissue is subjected to toxic substances, it can have different physiological effects. On one hand, increased water saturation of tissue can dilute toxic substances like pesticides, resulting in decreased toxicity. On the other hand, uptake of plant nutrients through transpiration can amplify exposure to toxic substances (Sandermann, 1992). It is known that photosynthetic processes are regulated by stomatal mechanisms that affect transpiration of the supply of carbon dioxide to mesophyll cells. Transpiration rate is cited in the literature as an adaptive response of an organism to prevent water loss under stress. In this study, transpiration rates of pesticide-tolerant plants varied with the concentration of pesticides in the soil. Higher pesticide concentrations in soil were associated with lower transpiration rates. For example, *Artemisia annua* growing in soil from hot point 2 with higher pesticide concentration exhibited transpiration rates six to ten times lower than plants growing in soil from hot point 1 and hot point 3. Transpiration rate for hot point 2 was 0.24 g/g/h compared to 1.44 g/g/h for hot point 1 and 2.52 g/g/h for hot point 3. Pesticide-tolerant plants are capable of adjusting the water balance of cells to support normal physiological functions.

From this greenhouse trial, we concluded plants can demonstrate adaptation to pesticide-contaminated soils through changed plant growth, phenological development, ratio of chlorophyll a to chlorophyll b, and transpiration rates. These characteristics can be useful as biological indicators of stressful effects of obsolete pesticide-contaminated soil.

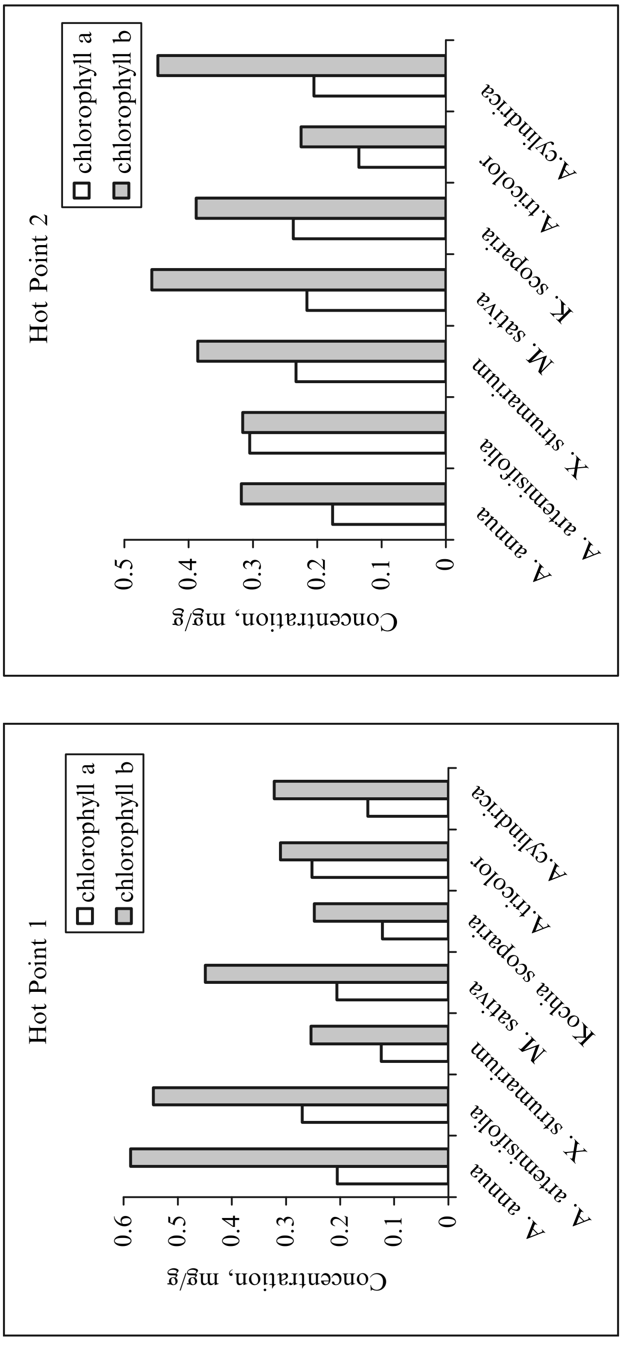

Figure 3. Concentrations of chlorophyll a and chlorophyll b in leaves of seven species growing in pesticide-contaminated soil from hot point 1 and hot point 2.

2.5. TASK 5: DOCUMENT PESTICIDE ACCUMULATION PATTERNS IN PESTICIDE-TOLERANT PLANTS

Pesticide-tolerant species were used to study the pattern of accumulation of pesticides in a greenhouse pot study. Sixteen plant species were grown in three soil treatments using soil from hot point 1, hot point 2, and a control soil. All treatments were grown in triplicate. Thirteen of the species established sufficiently to use for analysis. Soil was sampled at the beginning of the experiment. Plant tissue and soil were sampled at the time of flowering to estimate plant biomass production and content of HCH isomers and DDT metabolites in soil, plant root tissue, and aboveground plant tissue.

In the greenhouse study, the amount of pesticide accumulated in plant tissue depended on the plant species, plant biomass production, and initial level of pesticide contamination in soil. Figures 4 and 5 show the total concentration of pesticide in plant tissue for the 13 species grown in soil from hot point 2.

Five groups of plant species were identified based on the observed pattern of pesticide accumulation.

- Pesticide-accumulating plants: The concentration of pesticides in plant tissue exceeds MAC up to 400 times. MAC for plant tissue in Kazakhstan is 20 μg/kg. Species in this category include *Xanthium strumarium*, *Kochia scoparia*, *Artemisia annua*, and *Kochia sieversiana*.
- Accumulators of HCH isomers: The concentration of HCH isomers in plant tissue exceeds MAC up to 90 times. Four representatives of family *Asteracea* in this category include *Artemisia annua*, *Ambrosia artemisifolia*, *Xanthium strumarium*, and *Erigeron canadensis*.
- Accumulators of metabolites 2,4 DDD and α-HCH: These compounds do not have MAC for plants or soil. These species accumulate trace metabolites of DDT and α-HCH in plant tissues in which residual concentration of pesticides exceeds MAC for other compounds. These species include *Ambrosia artemisifolia*, *Xanthium strumarium*, *Artemisia annua*, *Solanum dulcamara*, *Medicago sativa*, and *Barbarea vulgaris*.
- Ability to accumulate and translocate pesticide from roots to aboveground plant tissue: Most pesticide accumulated is in the root system; however, some species demonstrated capability to translocate pesticides from roots to aboveground tissues. These included *Kochia scoparia*, *Artemisia annua*, *Barbarea vulgari*, and *Ambrosia artemisifolia*. For these plants, concentration of pesticide in aboveground tissue exceeded concentration in root tissue, giving a translocation factor of greater than one.

- Non-accumulators: Two species, *Solanum dulcamara* and *Rumex confertus*, did not accumulate significant concentrations of pesticides in plant tissues despite growing in the most contaminated areas of the hot points. These species may have practical value for phytostabilization or phyto-degradation technologies that seek to stabilize or enhance degradation of organochlorine pesticides in soil.

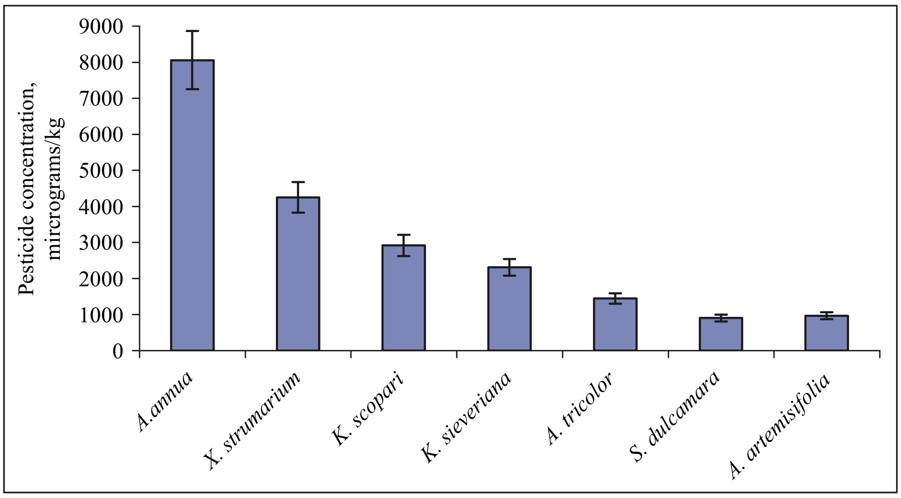

Figure 4. Total pesticide residuals accumulated in plant tissue for seven annual plant species grown in soil from hot point 2.

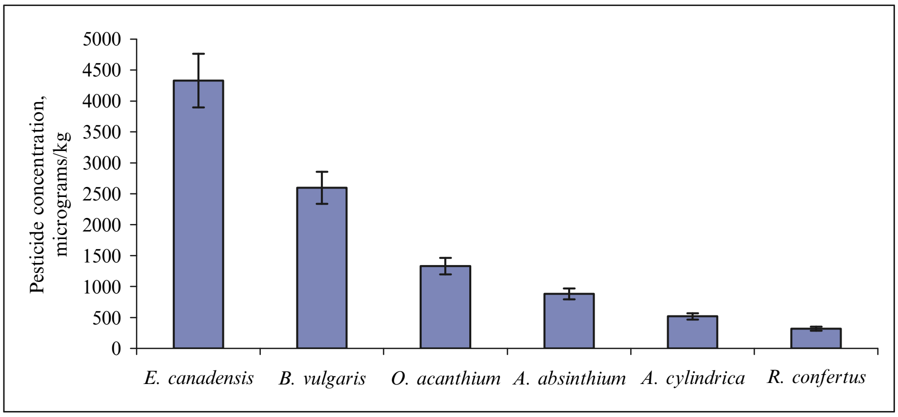

Figure 5. Total pesticide residuals accumulated in plant tissue for six biannual plant species grown in soil from hot point 2.

Table 3. Localization of pesticide residues in plant tissues based on histological analysis.

Species	Mosophyll type	Location of pesticide residues in plant tissue		
		Root	Stem	Leaf
Xanthium strumarium L.	Dorsiventral	Parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
Ambrosia artemisiafolia L.	Isolateral	Parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
Erigeron canadensis L.	Dorsiventral	Parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
Artemisia annua L.	Homogeneous	Parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
Kochia scoparia L.	Homogeneous	Parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
Barbarea vulgare L.	Dorsiventral	Parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll

2.5.1. Histological Analysis to Locate Pesticides in Plant Tissue

Histological methods were used to locate organochlorine pesticide residues in plant tissue (Prozina, 1960; Esau, 1977; Karthikeyan et al., 2004). The results of this analysis demonstrated pesticides were distributed unevenly within different plant tissues. If a species had a dorsiventral and isolateral leaf type, then pesticides appeared to accumulate in the palisade mesophyll. If the species had homogeneous mesophyll, then pesticide residues collected in mesophyllous cells around conducting bunches. For example, *Xanthium strumarium* L. has a dorsiventral type of leaf; thus, pesticides collected in the palisade mesophyll. In the stem, pesticides collected in walls of xylem cells. In root tissue, pesticides collected in parenchymous cells and xylem walls (Table 3).

2.6. TASK 6: STUDY THE FATE AND TRANSPORT OF PESTICIDES IN SOIL AND PLANTS IN THE GREENHOUSE USING SOIL COLLECTED FROM HOT POINTS

Task 1 had established the extent of the obsolete pesticide problem by documenting locations of pesticide storage in two oblasts of Kazakhstan. The problem was manifested by leftover stockpiles of highly toxic materials and residual contamination of containers, construction debris, soil, and water. Task 2 described potential genotoxicity of pesticide residues to barley seed using concentrations found at contaminated sites. Chromosomal mutations rates in barley appeared to be elevated when exposed to pesticide residues at concentrations found at contaminated sites. Task 3 documented plant community structure at the hot points and identified plant species that grow in pesticide-contaminated soil near the center of the sites. The type of vegetation was characteristic of early successional plant species dominated by annuals and biannuals. Many of the species would typically be considered weeds. Task 4 evaluated selected pesticide-tolerant plant species to document growth characteristics in pesticide-contaminated soil compared to clean soil. Task 5 described interaction of pesticides and selected plant species to identify species that might accumulate pesticides in plant tissue and translocate pesticides to aboveground plant tissue.

Can plant species that naturally colonize abandoned storehouse sites play a role in restoration and recovery of these sites? Can vegetation reduce risk of human or ecological exposure to toxic compounds? Dissipation of pesticide contamination in soil likely occurs through numerous mechanisms including adsorption of pesticides to plant roots, translocation of pesticides in plant tissue, migration of pesticides through the soil structure, pesticide runoff by wind and water erosion, volatilization, photochemical decomposition, and biological decomposition. Plant species' involvement in site recovery might occur by several mechanisms. First, do plants help stabilized the site and reduce further spread of contamination? Second, do plants promote conditions that will increase the breakdown of contaminants to less harmful compounds? Third, can some plants remove a significant amount of toxic compounds from the soil by accumulating the compounds in plant tissue that can be harvested and removed?

The final set of tasks from this project utilized selected plant species to describe how phytoextraction might practically function and what management tools might be useful for improvement of phytoextraction performance. Selected plant species were grown in pesticide-contaminated soil in containers in a greenhouse, in small field plots, and at a pesticide-contaminated site at a former storehouse. By tracking pesticide residuals in soil and plant tissue, issues that will impact development of phytotechnology applications were identified.

Any technology to reduce risk from pesticide-contaminated soil must track the fate of toxic compounds using a mass balance approach. The purpose of this study was to study the fate of pesticides in soil with and without plants. Thirteen pesticide-tolerant plant species were grown in greenhouse containers along with a control treatment without vegetation. Each experimental unit was a container with 3 kg of clean or contaminated soil that had been placed above a layer of ceramzite clay and sand to facilitate drainage. Five soil treatments were used including a clean soil control; three soils collected from hot points 1, 2, and 3 in Almaty oblast; and one artificially contaminated soil. Each treatment was grown in three replications (13 species × 5 soils × 3 replications). Soil was sampled at the beginning of the study and after harvest of plants at flowering, or approximately 6 months.

For treatments with no plants, overall soil pesticide concentrations decreased 41–44% for contaminated-soil treatments from hot points 1, 2, and 3. This decrease in pesticide concentrations was due to a combination of possible natural breakdown of pesticide compounds and migration of compounds. This result illustrates the difficulty in tracking pesticide fate in these studies. Some of the compounds migrated into the sand layer of the containers. The sand was clean at the beginning of the study.

For the 13 plant species tested, reduction of soil pesticide concentrations ranged from about 30–80%. The amount of pesticide accumulated in plant tissues was a small proportion of the total dissipation. Of the 13 species tested, *Solanum dulcamara* accumulated the largest amount of pesticide, 177 µg or 1.21% of the original soil pesticide content from hot point 2. The percentage of pesticide reduced from phytoextraction in this experiment ranged from 0.01% to 0.04% for plants growing in hot point 1 soil; 0.01% to 1.2% for plants growing in hot point 2 soil; and 0.01–0.1% for plants growing in hot point 3 soil. The experiment resulted in the following useful observations:

- The amount of pesticide taken up in plant tissue varies with initial soil pesticide concentrations and plant biomass produced.
- Plant species appear to vary in the amount of pesticide residues they accumulate.
- Some plant species are more useful for stabilization of pesticides in soil than for accumulation of pesticides in plant tissue.
- Although soil pesticide concentrations in this study declined about 30% to 80% with different plant species treatments, only a small proportion of this decline was due to phytoextraction.
- Good control of pesticide mass balances is needed to advance development of phytoextraction technologies.

2.7. TASK 7: STUDY THE EFFECT OF FERTILIZATION ON PHYTOREMEDIATION POTENTIAL IN THE GREENHOUSE AND FIELD

Low phytoextraction percentage is in part connected to slow growth of plants and limited biomass production. Several experiments were conducted using mineral fertilizers to increase plant biomass and monitor its effect on phytoextraction potential. Three experiments were conducted under greenhouse conditions, experiment field plot conditions at a research station, and under field conditions at a former pesticide warehouse site.

2.7.1. Greenhouse Study

A greenhouse study was used to examine the effect of added fertilizer on phytoaccumulation of five plant species. Two soil treatments included an artificially contaminated soil and a clean soil control. Vegetation treatments included five select plant species and a no-vegetation treatment. Plant species included four of the locally occurring pesticide-tolerant species, *Artemisia annua*, *Amaranthus retroflexus*, *Kochia scoparia*, *Xanthium strumarium*, and the known DDT-accumulating species, *Curcurbita pepo* ssp. *pepo* (White, 2002; Zeeb et al., 2003). Two fertility treatments included a control with no added fertilizer and a fertilizer treatment with 500 mg of ammonium phosphate and 250 mg of potash chloride added to each 3 kg soil container. Response variables of this experiment included phenological development measured by days to flowering; plant height; root biomass; aboveground biomass; and pesticide concentration of root tissue, aboveground tissue and soil.

Results demonstrated that added fertilizer extended the plant vegetative period and resulted in increased biomass production. Pesticide concentrations in soil decreased for all treatments included fertilized and unfertilized controls without plants. Mean initial concentration of pesticides in the soil was 145 μg/kg for all pots. Most pots showed a reduction in pesticide concentrations. Soil with no plants and no fertilizer showed a final pesticide concentration of 68 μg/kg compared to an initial concentration of 147 μg/kg for a reduction of 27%. Soil with no plants and added fertilized had an initial concentration of 155 μg/kg before the experiment and 112 μg/kg at the end of the study for a reduction of 37%. Treatments with vegetation also showed overall decreases in pesticide concentrations ranging from 32% to 45% without added fertilizer and 41–76% with added fertilizer. Plant uptake of pesticides accounted for a small proportion of the overall reduction in soil pesticide concentrations, although added fertilized increased plant biomass and increased the amount of pesticide taken up by plants. Among the five plant species included in the study, *Artemisia annua* and *Xanthium strumarium* showed the highest pesticide accumulation ability including all plant biomass. *Cucurbita pepo* ssp. *pepo* and *Kochia scoparia* showed the highest translocation factors for accumulating pesticides in aboveground plant tissue.

Application of fertilizers resulted in increased plant biomass and increased percentage phytoextraction of pesticides. *Xanthium strumarium* phytoextraction percentage increased 0.3–0.6%, *Artemisia annua* increased from 0.5% to 0.7%, and *Cucurbita pepo* ssp. *pepo* increased from 0.4% to 0.7%. *Kochia scoparia and Amaranthus retroflexus* had low biomass production in this study and did not increase phytoextraction with added fertilizer. The proportion of changes in pesticide concentrations explained by plant uptake is small in this study.

2.7.2. Field Plot Study

Pesticide-contaminated soil was transported from hot point 1 to an experimental field site to form 1 by 1-m field plots. Two hundred kilograms of soil was used to form each plot. Initial soil pesticide concentrations in the field plots varied from 332 to 593 μg/kg. Total mass of pesticides in the field plots varied from 60,400 to 126,600 μg per plot. In this study, 20 g of ammonium phosphate and 20 g of potash chloride were applied to each fertilized field plot. Two control treatments included the contaminated soil without fertilizers and without plants, and the contaminated soil with fertilizer and without plants. The same five plant species were used in the field plot study as the previous greenhouse study.

Results from the field plot study demonstrated that added fertilizer generally extended the vegetative period and usually increased plant biomass. Three of the five species showed a decrease in soil pesticide concentrations with the added fertilizer and a relatively high accumulative ability (*Xanthium strumarium*, *Cucurbita pepo* ssp. *pepo*, and *Artemesia annua*). Residual pesticide concentrations in plant tissue were 1,435 ± 202 μg/kg for *Artemisia annua*, 948 ± 89 μg/kg for *Xanthium strumarium*, and 194 ± 16 μg/kg for *Cucurbita pepo* ssp. *pepo*. The bioconcentration factor was 2.4 for *Artemsia annua* and 2.6 for *Xanthium strumarium*. Despite some improvement of phytoextraction with added fertility, this study did not show phytoextraction as a significant contributor to reduction of pesticide concentration in the soil. For example, *Cucurbita pepo* ssp. *pepo* extracted 0.01% of the soil pesticides without fertilizer and 0.1% with added fertilizer. *Xanthium strumarium* extracted 0.02% without fertilizer and 0.1% with added fertilizer.

Although plant uptake of pesticides was not responsible for reducing soil pesticide concentrations, final soil pesticide concentrations were still reduced by 73% for *Cucurbita pepo* ssp. *pepo*, 60% for *Artemesia annua*, and 61% for *Xanthium strumarium* in the fertilizer treatments. This compared to 40% without plants and without fertilizer, and 49% without plants and with fertilizer. These reductions in soil pesticide concentrations are quite high and processes responsible for the reduction need to be investigated further.

2.7.3. Phytoremediation Field Test Trial at Hot Point 2

In a final field test, two 1 by 1 m test plots were set up at hot point 2 in Almaty oblast to study the effect of added fertilizer on phytoextraction by *Xanthium sturmarium*, and changes in soil pesticide concentration after one growing season. *Xanthium sturmarium* was chosen because it is one of the dominant species occurring at the former warehouse sites with high biomass production, a short vegetative period, and demonstrated ability to accumulated metabolites of DDT and isomers of HCH. It is also poisonous and not consumed by livestock. One plot included *Xanthium strumarium* with added fertilizer (20 g ammonium phosphate and 20 g potash chloride) and the other plot included *Xanthium strumarium* with no added fertilizer. Table 4 summarizes the soil pesticide concentrations, biomass produced in each plot, total pesticide mass, and amount of pesticides accumulated in plant tissue.

Table 4 shows the initial mass of pesticide in the soil was reduced by more than one-half in a single growing season in both plots. Plants accumulated significant concentrations of pesticide into plant tissue compared to the initial concentrations in soil; however, the mass of pesticide taken up into plant tissue represents a very small fraction of the total pesticide mass in the soil. Therefore, the reduction of pesticide concentrations in soil was not due to plant uptake of pesticides. Other processes are mostly responsible for changes in pesticide concentrations in the soil. Additions of fertilizer appeared to increase plant biomass production and increase the amount of pesticide accumulated in plant tissue.

The decline observed in soil pesticide concentrations suggests practically useful soil remediation processes may be functioning; however, mechanisms other than phytoextraction are apparently responsible for this change. Prior bioremediation and phytoremediation studies with DDT and HCH have reported that transformations take place in soils under favorable conditions (Karthikeyan et al., 2004; Kamanavalli and Ninnekar, 2004; Huang et al., 2007; Gao et al., 2000; Phillips et al., 2005; Langenhoff et al., 2002; Raina et al., 2008; Wu et al., 1997; Quintero et al., 2006). Further research is needed to understand the fate and transport of pesticides in these contaminated soils. This work should be accomplished to advance development of pesticide remediation technologies for obsolete pesticide-contaminated sites in Kazakhstan.

TABLE 4. Pesticide concentrations and mass in soil and *Xanthium strumarium* plants from two test plots at hot point 2; one test plot had no added fertilizer and one test plot was fertilized with ammonium phosphate and potash chloride.

	Soil or plant mass (kg)	Pesticide concentration (μg/kg)	Pesticide mass (μg)
Contaminated soil without fertilizer			
Soil before experiment	402	489	196,598
Aboveground plant biomass	1.3	60	78
Root biomass	0.1	182	18
Soil after experiment	402	227	91,274
Contaminated soil with fertilizer			
Soil before experiment	402	420	168,840
Aboveground plant biomass	3.3	101	334
Root biomass	0.3	474	142
Soil after experiment	402	113	45,426

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PHYTOREMEDIATION OF SOIL POLLUTED WITH OBSOLETE PESTICIDES IN UKRAINE

L. MOKLYACHUK*, I. GORODISKA,
O. SLOBODENYUK, AND V. PETRYSHYNA
Institute of Agroecology of Ukrainian Academy of Agrarian Sciences, Metrologichna, 12, Kyiv, Ukraine, 03143

Abstract The problem of disposal of obsolete pesticides in Ukraine has two aspects: utilization of bulk substances accumulated in storehouses and remediation of areas polluted with residual toxic compounds associated with these former pesticide storehouses. Soil contamination surrounding 11 former pesticide storehouses was investigated. In many cases, land around the former warehouses was not fenced or secured, which has increased the likelihood of contaminant exposure to local populations. Soil in these areas was found to contain residual metabolites of the persistent organic pollutant pesticide DDT and the pesticide lindane. Observed contaminants included 4,4′-DDT; 2,4′-DDT; 4,4′-DDE; 4,4′-DDD; α-HCH; β-HCH; and γ-HCH. Phytoremediation offers potential ecologically safe and economically viable alternative methods to restore these sites. Soil phytotoxicity might limit the success of phytotechnologies in some locations. To estimate the potential of phytoremediation of pesticide-contaminated soils, it will be necessary to check soil phytotoxicity. In this study, soil phytotoxicity was studied according to international and Ukrainian standards for determination of the effects of pollutants on soil flora. This study demonstrated that phyto-remediation of pesticide-contaminated soil using pesticide-tolerant wild plants offers a promising technology.

Keywords: phytoremediation, phytoextraction, phytostabilization, phytodegradation, persistent organic pollutants, organochlorine pesticides, persistent herbicides

*To whom correspondence should be addressed.

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1. Introduction

1.1. OBSOLETE PESTICIDE PROBLEM IN UKRAINE

Sustainable development of agroecosystems is impossible without clean air, water, and soil to produce ecologically safe food. Large quantities of pesticides were used for agriculture in Ukraine since the 1950s. From 1953 to 1986, about 40,000 t of DDT were manufactured in Ukraine each year. In 1972, a decision was made to end use of DDT for agriculture, but its manufacture in Ukraine continued until 1986. One of the pesticide manufacturing facilities was the “Radical” plant located in Kyiv. Continued production of pesticides in excess of amounts used resulted in extensive accumulation of unused pesticides. Each collective farm in Ukraine had a storehouse for storage of pesticides and mineral fertilizers. The quantity of obsolete pesticides that accumulated in these storehouses differs among different regions of Ukraine. The following four regions accumulated the largest quantities of obsolete pesticides: Sumy (2,426 t), Kyiv (1,933 t), Kirovograd (1,310 t), and Zaporizhia (1,214 t) (Tsyguleva and Korsunscaya, 2005). In an effort to manage obsolete pesticides, most obsolete materials were transported to central storehouses, but some amounts were left in local storehouses.

In addition to accumulation of obsolete pesticides, activities of repackaging, loading, and mixing pesticide preparations took place around pesticide storehouses. This resulted in pesticide contamination from spillage (Moklyachuk et al., 2005). The number of pesticide-contaminated sites in Ukraine that require cleanup actions exceeds 5,000. Without management, polluted soil is a source for transfer of toxic compounds by air, water, erosion, and exposure to living organisms. Organochlorine compounds, including persistent organic pollutants DDT, HCH, and PCBs, are among the most widespread contaminants in agroecosystems (Golovlyova, 1991). In many locations, contaminated soil around pesticide storehouses and burial places is now used for cultivation, further increasing the risk of exposure to toxic compounds.

Chemical composition and extent of soil contamination surrounding pesticide storehouses varies with each location. Unfavorable soil conditions slow or limit microbial degradation processes. Degradation of DDT in soil can proceed in two ways, depending conditions of the environment. The result is appearance of two DDT metabolites, DDD (1,1-(bis-4-chlorophenyl)-2,2-dichloroethane) and DDE (1,1-(bis-4-chlorophenyl)-2,2 –dichloroethene). These substances also occur as impurities in DDT products. Their physical and chemical properties are similar to DDT, but they are more persistent in the environment (Wania and Mackay, 1996).

In aerobic conditions DDE is the predominant metabolite produced. Further degradation of DDE is very slow. In anaerobic conditions, DDD is the main degradation product. It is less persistent than DDE. To decontaminate any polluted site, it is necessary to characterize the soil to develop appropriate remediation plans.

1.2. CLEANUP TECHNOLOGIES FOR PESTICIDE-POLLUTED SOIL

Cleanup or dissipation of pesticide residuals in soil is a complex process. The complexity is caused by many factors including soil type, chemical and physical properties of the pesticides, and pesticide concentration (Felsot et al., 2003).

The most effective method, but also the most expensive and laborious, is excavation of the polluted soil and replacement with clean soil. Excavated soil must be disposed of in a safe manner such as a hazardous waste landfill. Depending on availability of replacement soil, this method can lead to decreases of soil fertility.

Thermal processing is another cleanup method for soil contaminated with organic pollutants. Thermal processing involves heating soil to temperatures that volatilize contaminants so they can be separated from the soil. For organochlorine compounds, temperatures of 900–1,200°C can lead to formation of extremely dangerous polychlorinated dibenzodioxins (PCDDs). To avoid formation of these compounds, higher temperatures in the range of 3,000–4,000°C are needed, along with appropriate monitoring for PCDDs. Unfortunately, this process is not currently available in Ukraine.

To stabilize or localize soil pollution, several methods can be used to immobilize contaminants or to reduce their bioavailability. Some of these technologies include establishing hydraulic control to reduce migration of soluble contaminants, encapsulation, and sorption using materials like zeolite or glauconite (Smetanin, 2004). These immobilization methods are expensive and laborious with danger of backward desorption.

Soil flushing is another cleanup method when soil is either excavated or processed in place with special reagents that effectively wash the soil. This method is also expensive and can cause pollution problems from the derived materials.

Biological methods of soil cleanup or bioremediation are typically based on use of microorganisms that can decompose dangerous pollutants to less toxic forms. Many known species of bacteria, actinomycetes, and fungi are capable of degrading organic compounds. In laboratory conditions, microorganisms can degrade almost all modern pesticides, including DDT (Palgunov and Sumarkov, 1990). Many bioremediation methods are extremely slow or do not taking place readily in nature. This is caused either by absence of required microorganisms in the soil or lack of conditions favable for this process to take place. Using bioremediation, residual persistent pesticides might remain in the soil for an unacceptably long time.

1.3. PHYTOREMEDIATION – A PROMISING SOIL REMEDIATION METHOD

Biological remediation methods are often considered to be safer and less expensive than other soil cleanup methods. Phytoremediation has received attention as an innovative and cost-effective alternative bioremediation method. Prasad estimated the cost of phytoremediation for soil contaminated by heavy metals, radionuclides, petroleum, or pesticides as only 5% of other methods (Prasad, 2007). American researchers estimated the cost for conventional remediation for 0.4 ha of mercury-contaminated soil to a depth of 50 cm as \$0.4 to \$1.7 million compared to phytoremediation costs from \$60,000 to \$100,000.

2. Materials and Methods

To characterize the residual amount of pesticides in soil near pesticide warehouses, we studied organochlorine pesticides and their metabolites (α-HCH, β-HCH, γ-HCH, 4,4′-DDT, 4,4′-DDE, 4,4′-DDD, and 2,4′-DDT). Eleven pesticide storehouses and the surrounding land were sampled. Identity and quantities of pesticides stored at each location was unknown.

Soil and plant sampling were conducted according to engineering specifications and state standards for Ukraine (Ministry of Health, USSR, 1979; ISO 10381; ISO 6498). Soil samples were taken along four transects in each direction from a warehouse (north, south, east, and west). Six samples were collected on each transect at distances of 1, 5, 10, 15, 25, and 50 m from the warehouse. Soil was stored in paper bags prior to transfer to labeled plastic bags for transport to the laboratory. Soil samples were stored frozen until analysis.

At each location, plant species' identity and coverage were determined within four 50 by 50 cm quadrats using Roshensky grids (Grigora and Solomakha, 2000). Common plant species were selected from each site to estimate plant uptake of DDT metabolites. Sampled plants were carefully cleaned and separated into roots and shoots for analysis.

Organochlorine pesticides were quantified by gas chromatography (GC) using an electronic-capture detector (ECD) according to accepted engineering specifications and state standards for Ukraine (Klisenko et al., 1992; ISO 10382; ISO 14181). Soil phytotoxicity was studied according to international and Ukrainian standards ISO 11269-22004 (ISO 11269-2).

3. Results and Discussion

3.1. SITE CHARACTERIZATION

Site characterization was conducted according to the following three stage methods developed to guide implementation of the Basel Convention on the Transboundary Movement of Hazardous Wastes and Their Disposal (UNEP, 2002).

Stage 1. Initial assessment of potential hazardous compounds was determined by inclusion on lists of hazardous waste. Obsolete pesticides are considered potentially hazardous, because they are included in the list of hazardous wastes according to Appendix VIII of the Basel Convention (UNEP, 2002).

Stage 2. Estimation was based on the concentration of toxic compounds. The main soil pollutants are POPs pesticides: 4,4′-DDT; 4,4′-DDE; 4,4′-DDD, 2,4′-DDT, α-, β-, and γ-isomers of HCH. Pesticides hexachlorobenzene, aldrin, dieldrin, endrin, chlordane, and mirex were not found in soil samples from this research (Table 1). The *de minimis* level of HCH and DDT has been designated as 50 mg/kg for each compound. If soil is polluted with several compounds, then the *de minimis* level is taken from the sum of their concentrations with corresponding coefficients according to the Basel Convention. If the total concentration of all persistent organic pollutants in

TABLE 1. Concentrations of POPs compounds in the surface layer of soil from contaminated zones near pesticide warehouses (units in μg/kg).

Location Oblast, Rayon	Range for DDTs[a]	Range for HCHs[b]	Range for PCBs	Year of analysis
Kyiv, Vasilkyvskiy	15–667	7–826	No data	2001
Chernivtsi, Sokyrnya	140–550	16–405	No data	2001
Kherson, Askaniya–Nova[c]	19–1,696	16–617	3–692	2002
Kherson, Dniprovsk	24–234	12–89	25–211	2002
Donetsk, Yasynuvata	2–58	1–17	44–286	2002
Khmelnitsky, Starokostyantyniv	27–1,909	2–2,031	No data	2003
Vinnitsa, Vinnitsa	2–175	2–17	No data	2004
Kyiv[d], Makarivsky	2–633	182–352	No data	2005
Poltava[d], Kobylyaky	417–510	691	No data	2005
Zhitomir, Korosten	3–938	2–18	No data	2005
Kyiv, Borispol	9–4,754	1–4	No data	2007

[a] DDTs denotes the sum of DDT metabolites: 4,4′-DDT, 4.4′-DDE, 4.4′-DDD, and 2.4′-DDT.

[b] HCHs denotes the sum of HCH isomers: α-HCH, β-HCH, and γ-HCH.

[c] Biosphere Reserve.

[d] Soil sample from single point 5 m from storehouse at 0–20 cm depth.

soil exceeds *de minimis* level, then the soil is considered toxic waste and should be excavated.

Plots with concentrations of POPs exceeding *de minimis* level were located at two sites of the 11 sites studied, Makarivsky and Kabylyaky. For the other sites, total concentration of DDTs and HCHs did not exceed *de minimis* levels. Therefore, *in situ* technologies, such as phytoremediation, can be used for remediation of these sites.

3.2. TESTING PHYTOTOXICITY OF DDT-CONTAMINATED SOIL

Stage 3. To test for phytotoxicity of DDT-contaminated soil, ecotoxicological tests were conducted according to the standard ISO 11269-1,2:2004 for monocotyledons (category 1) and dicotyledons (category 2). Pesticides that typically do not show herbicidal activity might be phytotoxic to plants in high concentrations. To determine the influence of high DDT concentration in soil on the development of seedlings, a pot experiment was conducted. Plants were seeded in dernovopodzolic sandy-loam soil that had been artificially contaminated with 1,500 µg/kg of DDT. Comparison of root length and stem length for seedlings after 30–35 days did not indicate strong phytotoxicity for the species assayed (Table 2). None of the seedlings in this trial showed chlorosis symptoms.

TABLE 2. Phytotoxicity test of DDT-contaminated soil (1,500 µg/kg) on seedling growth; control was uncontaminated soil.

Plant species		Root length		Stem length		Day[a]
		cm	% control	cm	% control	
Barley	*Hordeum vulgare* L.	6.7 ± 2.4	99.3	20.0 ± 2.4	100	35
Wheat	*Triticum vulgare* L.	7.3 ± 2.5	136.0	26.5 ± 6.4	106.0	35
Pumpkin	*Cucurbita pepo*	8.8 ± 1.4	89.3	15.0 ± 1.3	120.0	32
Squash	*Cucurbita pepo*	6.3 ± 1.2	92.0	18.0 ± 1.0	102.1	32
Bean	*Phaseolus vulgaris*	12.5 ± 2.8	105.0	40.7 ± 2.9	107.2	31
Soybean	*Glycine max* L.	10.2 ± 1.7	158.0	19.6 ± 1.0	121.0	30

[a] Days from seedling emergence to harvest.

Similar phytotoxicity experiments were conducted using collected contaminated soil with multicomponent pollution with DDT and herbicides (Table 3). The concentration of DDT in this soil averaged 937.7 ± 39.5 µg/kg. Estimated concentration of herbicides in the soil was atrazine – 0.21 µg/kg; prometryn – 0.51 µg/kg; simazine – 0.14 µg/kg; and total triazine herbicide derivatives – 855 µg/kg.

Plants were collected 29 days after germination of control treatments, after appearance of first signs of leaf chlorosis as a result of chlorophyll destruction, a typical symptom caused by xenobiotics. Stem length of some experimental plants was greater then the control in some cases (such as *C. pepo*), but the mass was generally less than the control. Root length was less than the control in all cases but varied from 15% for *Glycine max* to 54% for *Cucurbita pepo* var. *pepo*.

TABLE 3. Phytotoxicity test of multi-component DDT and triazine herbicide-contaminated soil; control was uncontaminated soil. Seedlings were grown for approximately 30 days.

Plant species	Root length		Stem length		Chlorosis, days	Day[a]
	cm	% control	cm	% control		
Hordeum vulgare L.	7.8 ± 1.3	80	23.3 ± 2.4	100	28	35
Triticum vulgare L.	4.7 ± 1.4	78	25.4 ± 2.4	83	28	35
Cucurbita pepo	4.7 ± 1.5	46	16.5 ± 3.7	123	29	32
Cucurbita pepo	5.6 ± 1.4	74	25.6 ± 1.4	131	29	32
Phaseolus vulgaris	8.0 ± 1.6	81	32.7 ± 1.4	93	28	31
Glycine max L.	8.0 ± 1.6	85	18.9 ± 2.3	71	27	30

[a] Days from planting to harvest.

3.3. PLANT UPTAKE OF DDT

To estimate potential use of phytotechnology applications, we tested DDT accumulation of *Cucurbita pepo* var. *pepo* on a typical Ukrainian dernovopodzoilic soil. Vegetation from *C. pepo* var *pepo* was sampled to

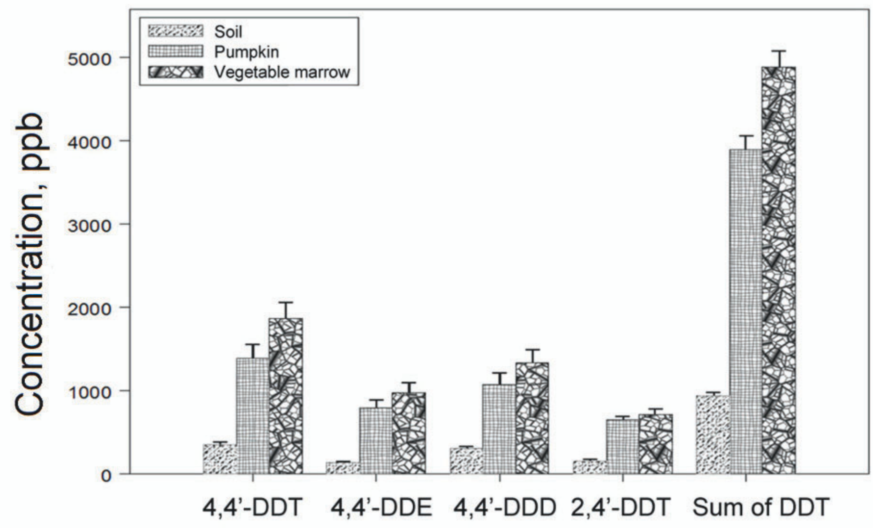

Figure 1. Concentration of DDT and its metaolites in the soil and plants from two varieities of *C. pepo* var. *pepo*.

determine the concentration of DDT and its metabolites in plant tissue. Results showed *C. pepo* actively accumulated DDT and its metabolites. Figure 1 illustrates the relationship between the concentration of DDT in soil and the concentration of DDT in plants growing in the same soil. For all DDT metabolites, the concentration in plant tissue exceeded the concentration in soil for two varieties of *C. pepo*.

3.4. INDENTIFICATION OF PESTICIDE-TOLERANT PLANT GENOTYPES

Use of cultivated plant species for phytoremediation might be limited due to potential high soil phytotoxicity. Studies of plant communities that colonize obsolete pesticide-contaminated sites can help identify pesticide-tolerant plants. We studied plant community structure at two contaminated sites. Total DDTs' concentration in the soil varied from 4,753.6 ± 510.2 to 6,377.0 ± 45.7 at the first site, and from 158.0 ± 5.6 to 389.0 ± 1.7 µg/kg at the second site. Total concentration of sim-triazine-derived herbicides was 321 µg/kg at the second site.

Both number of plant species and vegetation coverage increased with increasing distance from the storehouse. The number of botanic families increased with the distance from the source of pollution (Table 4).

Plant community structure of the area within 50 m of the two warehouses was represented by 54 species from 21 families, with the domination of Asteraceae and subdomination of Poaceae (Table 5).

The dominant species were *Aillea millefolium*, *Artemisia absinthium*, *Artemisia vulgaris*, *Elytrigia repens*, *Poa pratensis*, *Spergula arvensis*, and *Taraxacum officinalis*. In these areas with contamination from both organochlorine pesticides and herbicides, the plant community was composed of a combination of perennial and annual/biannual species. (Figure 2).

TABLE 4. Plant community structure expressed as species richness per m^2 (number of species) and plant density per m^2 along transects in four directions from two former pesticide storage sites; data from the two locations has been pooled.

Transect	0–1 m				1–5 m				5–15 m			
Direction	2006		2007		2006		2007		2006		2007	
–	R[a]	D[b]	R	D	R	D	R	D	R	D	R	D
East	8	409	8	506	11	466	16	596	17	554	18	687
South	10	335	8	344	33	478	20	540	16	212	16	384
West	15	449	14	260	16	517	21	448	20	716	21	708
North	7	327	7	228	20	687	20	392	12	491	25	560
Mean	10	380	9	335	20	537	19	494	16	493	20	585

[a] R – species richness per m^2.

[b] D – plant density per m^2.

TABLE 5. Plants species tolerant to pesticide-contaminated soil.

Plant species		Family
Yarrow	*Achillea millefolium*	Asteraceae
Wormwood	*Artemisia vulgaris*	Asteraceae
Wormwood bitter	*Artemisia absinthium*	Asteraceae
Couch-grass	*Elytrigia repens*	Poaceae
Erigeron	*Erigeron canadensis*	Asteraceae
Lamb's-quarters	*Chenopodium album*	Chenopodiaceae
Rye brome	*Bromus secalinus*	Poaceae
Kentucky bluegrass	*Poa pratensis*	Poaceae
Dandelion medicines	*Taraxacum officinalis*	Asteraceae
Violet field	*Viola arvensis*	Violaceae
Wild-oat	*Avena persica*	Poaceae
Corn spurry	*Spergula arvensis*	Caryophyllaceae

Extraction and analysis of plant root and shoot tissue from selected wild plants showed all samples contained measurable quantities of DDT metabolites. Table 6 shows root and shoot concentrations of total DDTs from two locations. Bioconcentration factors (BCFs) were calculated for each tissue compartment (root and shoot) by determining the dry-weight ratio of total DDTs' concentration in plant tissue to that in the soil using the higher estimate of soil concentration of DDTs at the location (White et al., 2005). All wild

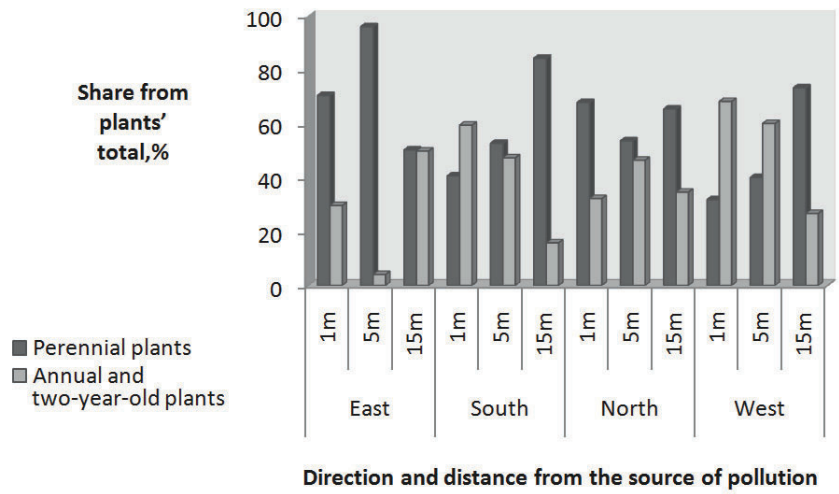

Figure 2. Relative proportions of plant species at two contaminated sites represented by perennial versus annual or biannual life cycles.

plants had significant concentrations of contaminant associated with their root systems; however, BCFs depended on DDT concentration in soil. For example, for *Taraxacum officinalis*, BCF values were higher for the location with lower DDT concentration in soil.

For most wild plants growing on soils with long-term pesticide contamination, DDT concentration in roots is higher than in shoots, but lower than in soil. Although plants with lower translocation factors might be less suited for phytoextraction of pesticides from soil, DDT absorption or adsorption to plant roots might help prevent pesticide migration to air or water.

TABLE 6. Distribution of DDT between root and shoot tissue for DDT-tolerant plants grown at contaminated sites.

Name	Soil or tissue	Total DDT, μg/kg dry matter			BCF[a]	Translocation factor[b]
Location 1	Soil	6,377	±	46		
Poa pratensis	Root	2,253	±	113	0.35	
	Shoot	101	±	8	0.01	0.045
Artemisia absinthium	Root	1,328	±	36	0.20	
	Shoot	1,373	±	27	0.20	1.00
	Root	3,872	±	110	0.60	
Artemisia vulgaris	Shoot	466	±	10	0.07	0.12
Elytrigia repens	Root	3,353	±	114	0.50	
	Shoot	200	±	3	0.03	0.06
	Root	1,606	±	2	0.25	
Taraxacum officinalis	Shoot	730	±	52	0.10	0.45
Location 2	Soil	389	±	2		
Spergula arvensis	Root	54	±	3	0.14	
	Shoot	101	±	3	0.26	1.90
Achillea millefolium	Root	1,016	±	7	2.60	
	Shoot	613	±	6	1.60	0.60
Taraxacum officinalis	Root	313	±	3	0.80	
	Shoot	179	±	5	0.50	0.57

[a] Biological concentration factor represents the concentration of contaminant in plant tissue as a proportion of the soil concentration.

[b] Translocation factor represents concentration in the shoots as a proportion of the root concentration.

4. Conclusions

To reach sustainable development of the agroecosystems, pollution of the environment and food with persistent organic pollutants has to be prevented.

Contaminated sites are major environmental and human health problems, especially in countries of the former Soviet Union like Ukraine. More than 5,000 pesticide-contaminated sites require restoration in Ukraine. To restore these sites, ecologically safe and economical remediation methods like phytotechnologies are needed. Many of the pesticide-contaminated sites are polluted with multiple compounds including persistent herbicides. This might increase phytotoxicity for potential phytoremediation plant genotypes. To estimate potential phytoremediation applications for cleaning pesticide-contaminated soils polluted with pesticides, it is necessary to first check phytotoxicity. We observed that territories with long-term pesticide pollution sustained vegetative communities dominated by pesticide-tolerant plant species. Perennial plants with large root systems commonly dominated plant communities formed at these sites. In this study, we observed pesticide-tolerant wild plants that accumulated DDT metabolites mainly in their roots. This may help stabilize soil contaminants and facilitate their further degradation or removal. We recommend creating conditions to enhance vegetation coverage of pesticide-contaminated sites by pesticide-tolerant wild plants, as this will minimize the spread of contaminants and contaminated soil off site through soil erosion and volatilization.

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BELARUS EXPERIENCE IN REDUCTION OF RADIONUCLIDES AND HEAVY METALS CONTENT IN PLANTS FOLLOWING THE CHERNOBYL DISASTER

M. KALININ*, Y. TSYBULSKAYA, AND N. CHUBRIK
Central Research Institute for Complex Use of Water Resources, 1/2, Slavinskogo Str., 220086 Minsk, Belarus

Abstract This paper presents results of field and laboratory experiments to investigate use of soil ameliorants to minimize environmental contamination in soil by reducing the transfer of radionuclides and heavy metals to plant tissue. This is achieved by application of effective ameliorant complexes to the soil to reduce mobility and bioavailability of radionuclides and heavy metals. This research represents only one applied solution to this important challenge.

Keywords: ameliorant, soil amendments, radionulicides, contamination, caustic lime, phosphorus gypsum, dolomite, phyto-filter

1. Introduction

Progress in high-level global development leads to the great achievements in meeting human demands and at the same time, essentially affects the environment.

The large-scale exploitation of nonrenewable resources including deforestation, withdrawal of potable water, industrial development has resulted in production of many synthetic substances, fertilizers, pesticides, and agricultural wastes that have turned some regions of the planet into environmental disaster areas.

Anthropogenic contamination affects atmosphere, water, and land resources. People breath polluted air and in many cases, ingest water and food of low quality. Pollution is transferred from one region to another, either naturally or under anthropogenic influence.

*To whom correspondence should be addressed.

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Accumulation of carbon dioxide, ozone, nitrogen-oxide compounds, and other non-indigenous agents results in air pollution from numerous sources including emissions from industry, transportation, and waste combustion. The most widespread and dangerous air pollutants include carbonic oxide and carbon dioxide, oxygen compounds of sulfur, nitrogen oxides, hydrocarbons, and suspended particles. Under the influence of solar radiation, these substances of toxic origin can interact with each other and create new toxic combinations, for example peroxyacetyl nitrates (PAN) which constitute photo-chemical smog.

Nearly all elements of the Mendeleyev Periodic Table can be found in atmospheric air in various combinations. Some of the compounds, such as dioxins, are more toxic than snake poisons such as curare, strychnine, and cyanides.

Nuclear weapons testing, as well as exploitation and disasters of thermal and nuclear power stations, have led to the radionuclide contamination of the environment and human living space. This challenge is very acute for Belarus after the Chernobyl nuclear disaster.

Air pollution within specific administrative regions of Belarus varies over a wide ranging scale both in qualitative and quantitative components. In 2007, the general volume of pollution from stationary and mobile sources in Belarus constituted more than 1,500,000 t (National Environmental Monitoring System of Belarus, 2008).

2. Soil Rehabilitation Technology

Plants which absorb and neutralize considerable amounts of pollutants play the role of an industrial phyto-filter. Plant species differ regarding tolerance and selectivity to various atmospheric pollutants. In this regard, selection of specific plant genotypes, which combine high tolerance to toxic pollutants with high filtering characteristics, is one of the most important issues in developing green plantations that can effectively filter the air from toxic gases and pollutants. In terrestrial ecosystems, soils are the main sink for storage of air pollutants.

On the basis of research carried out in different countries, a selection of coniferous as well as herbaceous plants and bushes has been suggested for planting in regions of Belarus with anthropogenic pollution. A selection of plants was developed for revegetation of industrial areas depending on the type of pollutant (Sergeichik, 1997).

Fertilizer and pesticide application, as well as decay of plants with high content of pollutants has led to soil contamination. Soil contamination often is not initially obvious due to substantial buffering capacity that might limit observations of increased groundwater pollution or impact on plant growth.

Growing plants in contaminated soil may lead to degradation of the contaminant or storage of toxic pollutants that could be dangerous for people and animals. Various measures are being developed to prevent contamination and to ensure production of clean food. Phytorehabilitation is one of these land treatment measures. Phytorehabilitation aims at growing plants with high tolerance to specific pollutants, accumulating the pollutants in aboveground plant tissue, and decreasing pollutant levels in the soil. Brooks et al. (1977) first used the term hyperaccumulator to describe plants in which nickeliferous compound concentrations exceed 1,000 mg/kg in harvested plant tissues. In general, hyperaccumulators are plants grown in natural environments and accumulate more than 100 mg/kg of Cd; more than 1,000 mg/kg of As, Co, Cu, Ni, or Pb; and more than 10,000 mg/kg of Mn and Zn (Baker and Brooks, 1989; Reeves, 1992). Selection and application of these plants for phytoaccumulation has been the study focus of many researchers. One recent successful example addresses the ability of certain fern species to reduce arsenic concentrations in soil (Wang et al., 2006).

Another method for production of clean food in contaminated areas is application of ameliorants or soil amendments to change the mobility or bioavailability of contaminants in soil. This method has been used very efficiently in Belarus, especially after the Chernobyl disaster.

The overall territory of the Republic of Belarus is more than 20,759,000 ha. Agricultural land constitutes 43.9% of the area; forests, 42.1%; marshes, 4.4%; water resources, 2.3%; development areas and roads, 4.1%; and unused land, 3.2%.

The most dangerous type of soil pollution in Belarus is radionuclide contamination caused by the Chernobyl Nuclear Power Station accident. Twenty-three percent of the country, including 1.3 million hectares of agricultural land and 1.6 million hectares of forest land, has been contaminated by radionuclides. Due to the high level of land contamination and the inability to produce clean agricultural products, 265,000 ha of land in Belarus has been excluded from use. High-level contaminants include cesium (Cs^{137}) at 1,480 kBq/m², strontium (Sr^{90}) at 111 kBq/m², and plutonium ($Pu^{238,\ 239,\ 240}$) at 3.7 kBq/m². Further horizontal migration of radionuclides causes additional secondary soil pollution.

Rehabilitation technologies for soil contaminated by radionuclides have been developed by the Central Research Institute for Complex Use of Water Resources. This technology was based on the application of soil amendments or chemical ameliorants consisting of two components (caustic lime and phosphorus gypsum).

An experimental field of 100,000 m^2 in the area with soil contamination of 20 Ku/km^2 was created in the Loev Region of Gomel Oblast of Belarus in 1993. The experimental field was divided into three parts to carry out three experimental treatments. In the first treatment (I), the control, no ameliorant was applied to the soil. In second treatment (II), a mixture of 0.5 kg/m^2 caustic lime and 0.5 kg/m^2 phosphorus gypsum were introduced to the soil. In the third treatment (III), 0.5 kg/m^2 of dolomite powder was introduced to the soil. It should be noted that dolomite powder is recommended as an ameliorant, which reduces the level of radiation accumulated in plants (Reduction of Radionuclide Content in Plant Production, 1989).

Before tillage of the soil amendment, samples were taken for laboratory analysis from the three treatment areas to define water-physical, physical-chemical, and agro-chemical soil characteristics. Nine samples were taken from each treatment, with sampling depths of 0–20 and 20–40 cm.

The experimental field soil was clay sand with clay content varying from 18% to 20% and sand content from 80% to 82%. At the beginning of the experiment, soil texture was identical for each treatment. Soil organic matter content for the field was classified as medium. The cation exchange capacity averaged 23 mg/100 g of soil, with nearly 80% of the exchange sites represented by calcium. The soil pH of a water and salt extract was nearly neutral. Soil nitrogen and potassium content were considered medium.

Pea (*Pisum sativa* L.) was the first crop sown in the experimental field. At harvest, six samples of plants and soil were sampled at the same location for each treatment. Levels of radionuclides, lead (Pb), and cadmium (Cd) were determined in the plant and soil samples. Results of the analyses are presented in Tables 1 and 2.

The content of Cs-137 in green pea was reduced 4.5 times in treatment II and 1.5 times in treatment III, compared to the control. Thus the combination of caustic lime and phosphorus gypsum was three times more effective then dolomite powder in reducing Cs-137 uptake by green pea.

Results for Pb and Cd content in green pea and soil are presented in Table 2. Under the influence of caustic lime and phosphorus gypsum, the Pb content of green pea was reduced to 1.1 and Cd to about 2.0, compared to the control. When dolomite powder was introduced into the soil, the content of Pb increased 1.2 times and Cd content decreased 1.3 times. Thus, caustic lime and phosphorus gypsum application decreased plant uptake of Pb and Cd 1.35, and 1.5 more than dolomite powder.

In addition to the positive influence of reducing plant uptake of radioactive pollutants and heavy metals, caustic lime and phosphorus gypsum also increased productivity of green pea by 49%, compared to the control.

In autumn of 1993 and 1994, winter rye was sowed in the experimental field. Results of the radionuclide and heavy metal content assessment in the soil samples and winter rye seed and soil are presented in Tables 3 and 4.

Results in Table 3 show the ameliorants lowered Cs-137 content in the winter rye seed. Caustic lime and phosphorus gypsum reduced Cs-137 by two times in 1994 and by 1.8 times in 1995, compared to the control. In 1995, dolomite powder reduced Cs-137 content by only 12%, compared to the control.

In regard to Pb and Cd uptake, winter rye seed showed a tendency to lower uptake under the influence of caustic lime and phosphorus gypsum application. In 1994, Pb was 1.1 times less and Cd 2.8 times less than the control. In 1995, these values were 1.25 times for Pb and 1.35 times for Cd. In 1995, winter rye productivity was 20% higher with the lime and gypsum ameliorant compared to the control.

Observations of soil characteristics from all three treatments during the 3 years did not show differences among treatments except pH in the lime and gypsum treatment rose from 7.07 to 7.6 with the water extract and from 6.82 to 7.45 for the salt extract. Ph values remained within acceptable ranges for crop growth.

Application of ameliorants to reduce introduction of radionuclides and heavy metals into crop plants is a logical continuation of previous research carried out in contaminated soils, where increases in the filter coefficient demonstrated improvement in soil microbiological activity, reduction in the level of Pb and Cd in aboveground plant parts, as well as an increase in crop productivity.

Theoretical and field experiments show the mechanism of ameliorant influence involves changes in soil quality characteristics. One cause for reduced radionuclide and heavy metal movement from soil to plants is the increase in the energy of their interaction with the solid-phase surfaces of soil colloids and the corresponding decrease in their mobility. Results from field and theoretical experiments demonstrate decreasing diffusion coefficients for these ions in soil under the influence of caustic lime and phosphorus gypsum complexes (Olodovsky, 1996).

TABLE 1. Radionuclide content in green pea and soil sampled in on 20 July 1993; numerical values are radionuclide content expressed in Ku/kg times 10^{10}.

Treatment	Plant or soil	Radionuclide content (Ku/kg)							Index of accum. Cs-137	Prop. of control
		Ce-144	Ru-106	Cs-134	Cs-137	Sb-125	K-40	$\Sigma\gamma$ activity		
I – Control	Plants	6.09	16.3	9.93	186.6	7.5	140	364	0.307	1.00
	Soil	8.13	16.05	34.48	608.6	24.15	39.6	732		
II – Lime and gypsum	Plants	3.04	7.6	3.0	45.9	1.94	229	290	0.068	0.22
	Soil	12.94	9.22	36.64	671	12.96	51.5	794		
III – Dolomite	Plants	3.07	3.98	4.55	113.4	2.99	229	349	0.207	0.67
	Soil	3.74	11.21	29.38	549	9.22	260.3	833		

TABLE 2. Lead (Pb) and cadmium (Cd) content in green pea and soil sampled on 20 July 1993.

Treatment	Lead				Cadmium			
	M_c – Pb plants (mg/kg)	M_s – Pb soil (mg/kg)	Index of accumulation (M_c/M_s)	Proportion of control	M_c – Cd plants (mg/kg)	M_s – Cd soil (mg/kg)	Index of accumulation (M_c/M_s)	Index as proportion of control
I – Control	5.0	4.3	1.16	1.00	8.2	0.14	58.6	1.0
II – Lime and gypsum	5.0	4.8	1.04	0.90	4.8	1.16	30.0	0.51
III – Dolomite	5.8	4.1	1.42	1.22	5.7	1.13	43.8	0.75

M_c – Mean Pb content in plant tissue.

TABLE 3. Radionuclide content in samples of winter rye ears, and soil sampled in experimental field in 1994 and 1995.

Treatment	Year	plant versus soil	Radionuclide content (Ku/kg)			Index of accumulation Cs-137	Index as proportion of control
			Cs-134 × 10^{10}	Cs-137 × 10^{10}	K-40 × 10^{10}		
I – Control	1994	Plants	0.68	14.62	44.3	0.029	1.0
		Soil	20.0	509	56.5		
II – Lime and gypsum		Plants	0.28	6.99	55.4	0.015	0.52
		Soil	19.5	478	57		
I – Control	1995	Plants	0.33	10.34	47.3	0.018	1.0
		Soil	11.9	572	60.0		
II – Lime and gypsum		Plants	0.154	6.28	46.3	0.010	0.56
		Soil	13.4	588	51.8		
III – Dolomite		Plants	0.26	8.34	49.4	0.016	0.89
		Soil	11.1	508	54.7		

TABLE 4. Lead (Pb) and cadmium (Cd) content in samples of winter rye ears, and soil sampled in experimental field in 1994 and 1995.

Treatment	Year	Lead				Cadmium			
		M_c – Pb plants (mg/kg)	M_s – Pb soil (mg/kg)	Index of accum. (M_c/M_s)	Prop. of control	M_c – Cd plants (mg/kg)	M_s – Cd soil (mg/kg)	Index of accum. (M_c/M_s)	Prop. of control
I – Control	1994	2.40	1.66	1.31	1.0	1.33	0.046	30.07	1.0
II – Lime and gypsum		2.53	1.93	1.44	0.91	0.69	0.063	10.95	0.36
I – Control	1995	2.53	3.48	0.73	1.00	0.95	0.113	8.41	1.0
II – Lime and gypsum		2.11	3.66	0.58	0.75	0.66	0.128	5.15	0.61
III – Dolomite		2.57	3.35	0.77	1.05	0.63	0.124	5.08	0.60

3. Conclusions

Research conducted in Belarus using chemical ameliorants to reduce the content of radionuclides and heavy metals in plants proved to be effective. However, scientists in Belarus would prefer to use selected plant species such as bushes and trees with the same capacities to restrict uptake of radionuclides and heavy metals. Further research is planned using similar conditions to compare the functions of chemical ameliorants and selected plants. Some gains have been made, but a major effort is still required.

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ARSENIC CONTENT IN AND UPTAKE BY PLANTS FROM ARSENIC-CONTAMINATED SOIL

A. C. ZOLNOWSKI*, Z. CIECKO, AND T. NAJMOWICZ
Department of Environmental Chemistry, University of Warmia and Mazury, Plac. Lodzki 4; 10-718 Olsztyn, Poland

Abstract Environmental pollution from arsenic may affect crop yield and its quality. Its increased content in soils may come from natural minerals from which the soil was formed; on the other hand, unfortunately, the increase may have an anthropogenic background. Arsenic may be released to the environment in a variety of ways, but usually with wastewater, sludge, or some pesticides. It may also appear in soil as a result of irrigation with water from reservoirs in which bottoms may contain elevated concentrations of arsenic. Plants' reactions to increased amounts of arsenic in soil may be varied. Reactions may include changes of the concentration of the metalloid, both in the overground mass and in roots. In the years 2001–2002, two pot experiments were conducted with the aim to assess the sensitivity of two crop species, maize (*Zea mays* L.) and orchard grass (*Dactylis glomerata* L.), to arsenic soil contamination with 0, 25, 50, 75, and 100 mg As/kg of soil. A second objective of the investigation was to determine the possibility of reducing arsenic phytoavailability in the contaminated soil by the application of compost, charcoal, clay, lime, and synthetic zeolite. The study demonstrated the plants' reaction by simulating the soil contamination with arsenic. Arsenic concentration in soil and its uptake by the test plants was determined, as well as the effect of the applied inactivating additives on the features mentioned above.

Keywords: arsenic, phytoavailability, accumulation, compost, charcoal, clay, lime, zeolites. *Dactylis glomerata* L., *Zea mays* L.

*To whom correspondence should be addressed.

P.A. Kulakow and V.V. Pidlisnyuk (eds.), *Application of Phytotechnologies for Cleanup of Industrial, Agricultural, and Wastewater Contamination*,

1. Introduction

Contamination of the natural environment with arsenic may have a natural as well as an anthropogenic background. Although it occurs endemically in many parts of the world, it is becoming a global issue. Arsenic concentrations in many regions exceed acceptable concentrations. This is the case in Thailand (Visoottiviseth et al., 2002) Argentina, Chile, Mexico, the USA, Greece, Hungary, and Mongolia (O'Neill, 1995; Smedley and Kinniburgh, 2002). Epidemiological data show a distinctly negative effect of the metalloid on human health. Higher than normal concentrations of arsenic have been recorded in contaminated soils, sludge, and wastewater, which are the main sources of arsenic incorporated in the food chain (Frankenberger and Arshad, 2002). Important sources of arsenic in the environment include chemical agents used in agriculture, such as pesticides and insecticides, and wood protection agents and soil disinfectants (Woolson et al., 1971; Azcue and Nriagu, 1994; Murphy and Aucott, 1998). One method for reducing availability of heavy metals, including arsenic, is the addition of neutralising substances to soil (USEPA, 2002). Such substances are added to soil to bind a toxic element to form insoluble, metalomineral, or metaloorganic compounds, which in favourable conditions may remain in the soil for a long time in an unharmful form. Such materials include bentonite, zeolites, silts, lime, and organic additives (straw, sawdust, ground tree bark, compost, manure, and green fertilisers).

2. Materials and Methods

The study was based on two pot experiments, conducted in 2001–2002 in the vegetation hall of the University of Warmia and Mazury in Olsztyn, Poland. Two-factorial experiments were conducted in a randomized block design with three replications. The tested plants were two species of gramineous crops: maize (*Zea mays* L.) and orchard grass (*Dactylis glomerata* L.).

The first factor included materials which neutralise the effect of arsenic: compost, charcoal, clay, lime, and natural zeolite, type MHZ. The composition of the additives is presented in Table 1. The second factor was simulated soil arsenic contaminations: 0, 25, 50, 75, and 100 mg As/kg. Arsenic was applied as an aqueous solution of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) (POCh SA).

The experiments were carried out in Kick-Brauckamnn's pots, filled with 9 kg of soil. Before plants were sown, mineral fertilisers were added to the soil at 1 g N, 0.44 g P, and 1 g K/pot, as urea 46% N (Zakłady Azotowe "Puławy" S.A.), triple superphosphate 46% P_2O_5 (Szczecińskie Zakłady Nawozów Fosforowych SUPERFOSFAT SA), and potassium salt 60% K_2O

(Bialchem Group Sp. z o.o.). Compost, charcoal, clay, and natural zeolite were applied in rates of 392 g/pot (3% of the soil in a pot), and lime was applied at 8.19 g CaO/pot (in an amount sufficient to neutralise 1 mM H^+/kg soil).

TABLE 1. Chemical properties of amendments used for arsenic inactivation.

Additives	Elements									
	P	K	Mg	Ca	Na	As	Fe	Mn	Cu	Zn
	g/kg of dry mass					mg/kg of dry mass				
Compost	2.71	1.58	1.56	18.21	0.14	2.55	168	72	15.22	129.82
Charcoal	0.72	9.33	2.58	7.29	0.81	–	125	325	8.22	31.25
Clay	0.41	21.60	17.30	23.87	8.00	3.20	38,000	451	43.20	98.13
Lime	0.16	0.67	2.32	421.16	0.13	1.92	630	295	2.15	11.17
Zeolite (MHZ)	0.11	23.21	0.32	15.28	16.12	1.33	7,950	342	5.52	32.22

Soil used in the experiments was light mineral soil, classified as proper brown soil, with granulometric composition of light loamy sand (BN-78/9180-11, soil classification, Polish Society of Soil Science). Soil taken from humus level A_p was used in the experiment. The chemical properties of the soil are presented in Table 2.

TABLE 2. Chemical properties of soil used in experiments.

pH and soil acidity		
pH measured in H_2O	pH measured in 1 M KCl	Hh (mM $H^+ \cdot 100$/g gleby)
6.07	5.91	1.95

Macrelements content (% of soil dry mass)						
C	N	P	K	Mg	Ca	Na
0.501	0.061	0.043	0.052	0.041	0.110	0.009

Microelements content				
(mg/kg of soil)				(g/kg of soil)
As	Cu	Zn	Mn	Fe
2.21	1.58	24.34	72.22	10.70

A constant concentration of humidity (60% of the full-field water capacity of the soil) was maintained in the pots.

Maize and orchard grass were sown on 17 July 2001 and 20 June 2002, respectively, and were harvested on 10 September 2001 and 15 October 2002. Mass of the aboveground plant tissue was determined after harvest of stems, and mass of roots after separation and washing of soil under running water. Dry-matter content was determined after drying the material at 60°C and grinding the samples. The prepared samples were then wet-mineralised in nitric acid (60% HNO_3) (Merck) with hydrogen peroxide (30% H_2O_2) (POCh-Gliwice). Microwave-assisted mineralization was done in a MARS 5 microwave accelerated reaction system (CEM Corporation Matthews, North Carolina, United States) equipped with HP500 teflon vessels. Arsenic content in the samples was determined by AAS with hydride generation using a Solar 939 QZ atomic absorption spectrometer (Unicam, Great Britain).

Results of the study were verified statistically by ANOVA test, at a significance level of $\alpha = 0.05$ using Statistica software v. 6.0 (StatSoft, 2001).

3. Results and Discussion

According to reports by many authors, plants may vary in terms of their sensitivity to soil contamination with arsenic (Paliouris and Hutchinson, 1991; Meharg, 1994; Fitter et al., 1998; Sharples et al., 2000; Meharg and Hartley-Whitaker, 2002). The authors associate the fact with various forms of arsenic and the mechanisms of its transport in plants. According to Alloway and Ayres (1999) and Caussy (2003), bioavailability of arsenic depends mainly on soil conditions such as its pH value, and humus and mineral colloid content. Arsenic concentration in plants is dynamic and may be different for different species, cultivars, and plant-growth phases, as well as the applied dose and chemical form in which the analysed xenobiotic occurs in soil. Discussions of arsenic concentration in plant tissues should take into account presence of other elements which may boost or hinder its uptake (Kabata-Pendias and Pendias, 1999; Siedlecka et al., 2001). The literature provides data which indicate that arsenic concentration in plant tissue is positively correlated with its content in soil (Helgesen and Larsen, 1998; Paivoke and Simola, 2001). Kabata-Pendias and Pendias (1999) associate it with a lack of the plants' ability to assimilate arsenic suggesting plants absorb arsenic passively by mechanical transfer of ions with the transpiration of water current.

According to results of our study, an increase in arsenic concentration in soil was accompanied by gradual increase of its concentration in plants. It grew significantly both in aboveground parts and roots. The arsenic concentration determined in maize (*Zea mays* L.), grown without neutralising agents, was equal on average to 6.10 mg As/kg of dry matter, whereas it was 22 times higher in the root and was 138.04 mg As/kg of dry matter (Table 3).

Additives applied in the experiment contributed to reduction of arsenic concentration in both aboveground parts and the roots of maize. The most beneficial effect on the feature was recorded with silt and lime, which reduced arsenic concentration in the overground part by 1.51 and 1.34 mg As/kg d.m., respectively. The two additives also influenced the concentration of arsenic in roots – by 41.53 and 46.3 mg As/kg d.m., respectively.

TABLE 3. Arsenic content in aboveground mass and roots of maize (*Zea mays* L.).

Arsenic soil contamination (mg As/kg)	Type of neutralising additives					
	Without additives	Compost	Charcoal	Clay	Lime	Zeolite
Above ground mass (mg/kg of dry mass)						
0	0.32	0.65	0.57	0.39	0.41	0.30
25	5.55	3.93	3.15	3.46	2.97	3.68
50	8.57	6.98	6.01	6.77	6.00	8.24
75	8.70	6.99	7.40	6.23	7.14	7.75
100	7.36	6.81	7.06	6.09	7.26	6.70
Average:	6.10	5.07	4.84	4.59	4.76	5.33
LSD $_{p = 0.05}$ for:	First factor – neutralising additives = 0.11; second factor – arsenic contamination = 0.10; interaction – first × second = 0.24					
Roots (mg/kg of dry mass)						
0	6.11	3.94	5.81	4.57	5.49	8.02
25	104.18	91.13	48.36	55.17	88.58	58.65
50	155.15	150.05	129.27	113.19	96.20	125.74
75	182.49	170.42	178.05	152.11	137.21	134.86
100	242.29	171.87	224.97	157.49	131.20	235.00
Average:	138.04	117.48	117.29	96.51	91.74	112.45
LSD $_{p=0.05}$ for:	First factor – neutralising additives = 2.16; second factor – arsenic contamination = 1.97; interaction – first × second = 4.84					

Soil pH value has a decisive effect on the mobility of trace elements contained in it, including arsenic (Spiak, 1996). By raising soil pH, such as by liming, the mobility of trace elements in soil is reduced (Curyło and Jasiewicz, 1998; Gorlach and Gambuś, 2000). Reduction of soil pH results in formation of insoluble compounds in the soil, which in consequence reduces the bioavailability of xenobiotics, including arsenic (Patorczyk-Pytlik and Spiak, 2000). According to Goldberg (2002), arsenic adsorption by silty minerals increases with soil pH, reaching a maximum between 3 and 7. Caution should be exercised not to exceed 7 towards alkaline values, as the adsorptive potential of silt decreases above that value.

Compared to other substances applied in the study, lime and clay contained high concentrations of calcium, 421.16 and 23.87 g Ca/kg, respectively, (Table 1); and magnesium, 2.32 and 17.30 g Ca/kg of dry mass of the soil. Reduction of arsenic concentration in the aboveground tissue and roots of the plants included in the study may be associated with a change in the chemical properties of the soil, mainly its pH value, which, in this case, was a deciding factor affecting the mobility of arsenic ions.

The soil used in the study had a slightly acidic pH (Table 2) and, consequently, the application of silt in all the experiments may have effectively reduced arsenic uptake by plants. The difference between arsenic concentration in the aboveground mass and in the roots of orchard grass (*Dactylis glomerata* L.) was greater than in maize (Table 4). Soil contamination with arsenic also caused the concentration of arsenic to grow in orchard grass, especially in its roots. Arsenic concentration in the series without additives was equal to 2.17 As/kg of aboveground dry matter and 144.56 mg As/kg of dry-root matter. This means arsenic concentration in roots was 66 times higher than in green mass.

TABLE 4. Arsenic content in aboveground mass and roots of orchard grass (*D. glomerata* L.).

Arsenic soil contamination (mg As/kg)	Type of neutralising additives					
	Without additives	Compost	Charcoal	Clay	Lime	Zeolite
Above ground mass (mg As/kg of dry mass)						
0	0.71	0.60	0.66	0.41	0.44	0.62
25	2.31	1.66	2.04	2.25	1.60	1.68
50	2.51	1.64	2.10	2.26	1.92	1.70
75	2.60	1.89	2.12	2.24	2.02	1.98
100	2.70	1.95	2.40	2.16	1.78	1.89
Average:	2.17	1.55	1.86	1.86	1.55	1.57
$LSD_{p=0.05}$ for:	First factor – neutralising additives = 0.15; second factor – arsenic contamination = 0.13; interaction – first × second = 0.33					
Roots (mg As/kg of dry mass)						
0	2.37	2.82	2.07	2.60	3.20	2.13
25	73.94	30.24	55.75	69.40	57.32	44.36
50	154.70	81.90	128.50	134.20	116.00	102.40
75	227.50	154.15	203.10	142.80	174.25	163.85
100	264.30	167.60	214.90	165.05	196.40	194.00
Average:	144.56	87.34	120.86	102.81	109.43	101.35
$LSD_{p=0.05}$ for:	First factor – neutralising additives = 10.49; second factor – arsenic contamination = 9.26; interaction – first × second = 23.45					

Following application of neutralising additives, the concentration of arsenic was found to decrease both in the roots and in leaves. Compost and lime were found to reduce the arsenic concentration to the greatest extent. In this case, arsenic content in the aboveground parts was reduced on average by 0.65 mg As/kg of dry matter. Zeolite, applied in the study, also significantly reduced the arsenic concentration by 0.60 mg As/kg. The application of compost also had a beneficial effect on the arsenic concentration in the roots of orchard grass, reducing it by 57.22 mg As/kg as compared to the concentration determined in the roots of control plants. The effect of silt and zeolite was also found to be positive. In these cases, the concentration of As was found to be reduced by 43.21 and 41.75 mg As/kg of dry matter for silt and zeolite, respectively.

According to Kabata-Pendias and Pendias (1999), a number of plant species reduce the transport of arsenic from the roots to the aboveground parts by application of a specific biological barrier, which may be indicated by higher accumulation of the element in the roots than in aboveground parts, with only insignificant amounts reaching the generative parts.

Results of this study have confirmed the hypothesis suggested above. The concentration of arsenic in the roots of all the plants was higher than in their aboveground parts. This means the species included in the study were among those plants which have a system of blocking arsenic translocation towards the aboveground organs.

According to Brogowski et al. (1979), Gworek (1993), Kroczyński et al. (1996), and Gorlach and Gambuś (2000), the addition of zeolites to soil may reduce the availability of trace elements to plants. Zeolite substances improve the sorptive and ion-exchange properties of soil. There are no reports in the literature exploring the usability of zeolites in reclamation of soils contaminated with arsenic; however, other papers discuss their action on other elements. A study by Gworek (1993) into neutralisation of cadmium, lead, zinc, copper, and nickel provides grounds for expecting positive results in a study with arsenic. The effects in this experiment confirm earlier speculations that application of zeolites reduced arsenic concentration in all the plants, regardless of the analysed plant organ compared to the control series with no additives applied. Therefore, it can be concluded that zeolites immobilised arsenic in the soil.

Considerations regarding plant resistance to soil arsenic contamination and determination of the effectiveness of various neutralising substances include not only the concentration of the examined element in various plant organs, but also its total uptake. According to Tyksiński (2002), uptake is an important feature affecting properties of a plant as a phytoaccumulator. The amount of arsenic taken up from soil was dependent upon the plant species, contamination of substrate with arsenic, and the neutralising agent applied in the experiment. These factors directly affected the concentration of arsenic and the yield achieved (Tables 5 and 6). Both in maize and in orchard grass, yield decreased in a linear manner due to increasing soil arsenic contamination.

Arsenic uptake by test plants was associated with yield of the experimental plants and the arsenic concentration. Arsenic concentration was dependent on the soil contamination with arsenic and the additives applied.

Assessment of the plants in terms of their usability in the phytoremediation procedures indicates that the highest arsenic uptake by maize (*Zea Mays* L.) was recorded in pots where soil was contaminated with 25 mg As/kg, where it was equal to 1.063 mg As/pot (Figure 1). Higher contamination of soil resulted in a decrease in the yield caused by the toxic effect of arsenic on the plant development.

TABLE 5. Yield of aboveground mass and roots of maize (*Zea Mays* L.).

Arsenic soil contamination (mg As/kg)	Type of neutralising additives					
	Without additives	Compost	Charcoal	Clay	Lime	Zeolite
Above ground mass (g of fresh mass/pot)						
0	563	571	564	564	533	514
25	509	533	478	514	521	451
50	229	406	323	382	323	230
75	110	197	195	262	124	103
100	23	105	118	125	67	57
Average:	287	362	336	369	314	271
LSD $_{p=0.05}$ for:	First factor – neutralising additives = 29.0; second factor – arsenic contamination = 26.5; interaction – first × second = 64.9					
Roots (g of fresh mass/pot)						
0	53.9	56.2	57.8	58.7	53.0	54.0
25	23.9	48.4	43.7	52.3	35.4	29.4
50	11.6	40.5	35.5	31.6	29.6	22.3
75	7.9	28.5	22.5	22.7	15.0	15.9
100	2.9	13.1	13.2	15.8	6.8	12.4
Average:	20.0	37.3	34.5	36.2	28.0	26.8
LSD $_{p=0.05}$ for:	First factor – neutralising additives = 2.4; second factor – arsenic contamination = 2.2; interaction – first × second = 5.4					

TABLE 6. Yield of aboveground mass and roots of orchard grass (*Dactylis glomerata* L.).

Arsenic soil contamination (mg As/kg)	Type of neutralising additives					
	Without additives	Compost	Charcoal	Clay	Lime	Zeolite
Above ground mass (g of fresh mass/pot)						
0	186.0	196.6	158.0	210.0	228.6	177.3
25	161.3	176.0	139.3	207.3	216.0	172.6
50	155.6	152.6	124.0	191.3	201.3	168.0
75	118.0	149.3	123.3	139.3	191.3	153.6
100	90.0	127.3	123.3	135.3	160.0	169.3
Average:	142.2	160.4	133.6	176.6	199.4	168.2
LSD $_{p=0.05}$ for:	First factor – neutralising additives = 29.0; second factor – arsenic contamination = 26.5; interaction – first × second = 64.9					
Roots (g of fresh mass/pot)						
0	92.5	93.0	91.1	102.5	126.8	91.1
25	69.0	86.6	72.8	86.5	91.3	83.1
50	50.6	59.8	62.6	65.3	84.6	61.6
75	46.5	48.0	44.8	62.8	57.6	40.6
100	27.0	31.0	39.1	58.5	31.6	38.3
Average:	57.1	63.7	62.1	75.1	78.4	62.9
LSD $_{p=0.05}$ for:	First factor – neutralising additives = 2.4; second factor – arsenic contamination = 2.2; interaction – first × second = 5.4					

Properties of orchard grass (*Dactylis glomerata* L.) in this respect were much better. The highest arsenic uptake of 2.177 mg As/pot was recorded in combination with soil contamination of 75 mg As/kg (Figure 2).

All neutralising additives applied in maize cultivation in combinations with >50 mg As/kg of soil resulted in increased arsenic uptake as a consequence of the yield increase. In the case of orchard grass, arsenic uptake was found to increase in the soil with 100 mg As/kg after the addition of clay and charcoal. Compost and zeolite generally reduced arsenic uptake from soil, which may be proof of arsenic binding in the soil by the additives.

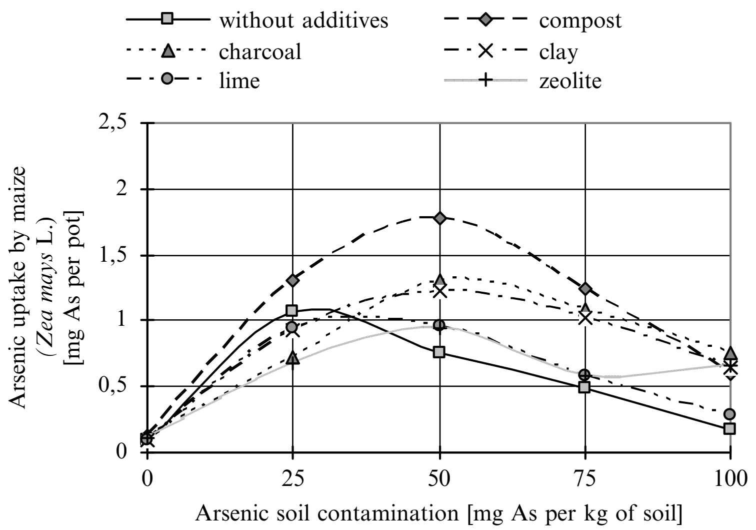

Figure 1. Arsenic uptake by maize (*Zea mays* L.).

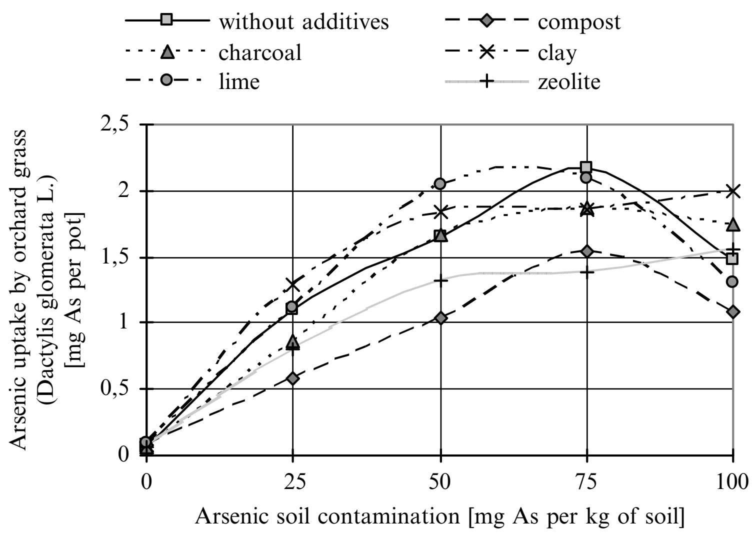

Figure 2. Arsenic uptake by orchard grass (*Dactylis glomerata* L.).

4. Conclusions

This study revealed a varied reaction of plants to simulated contamination of soil with arsenic. Arsenic significantly reduced yields of both maize and orchard grass. Contamination of soil with arsenic resulted in linear growth of its concentration in aboveground parts and in roots, with the values determined for the roots being several dozen times higher than those found in the overground parts. Maize accumulated about three times more of the metalloid in the aboveground plant tissue compared to orchard grass'; however, due to the orchard grass increased tolerance to arsenic in soil, it took up higher amounts of arsenic, especially from the soil with addition of more than 50 mg As/kg. Neutralising additives applied in the experiment reduced arsenic concentration in plants with the reducing effect achieved with silt and lime for maize and with compost, lime, and zeolite for orchard grass.

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LONG-TERM EFFECT OF COAL FLY ASH APPLICATION ON SOIL TOTAL NITROGEN AND ORGANIC CARBON CONCENTRATIONS

Z. CIECKO, A. C. ZOLNOWSKI*,
AND A. CHELSTOWSKI
Department of Environmental Chemistry, University of Warmia and Mazury, Plac. Lodzki 4; 10-718 Olsztyn, Poland

Abstract The study was performed on the basis of a field experiment established in 1984. Coal fly ash was applied at a rate of 0–800 t/ha with organic fertilizers – farmyard manure, straw, and tree bark. In the first years of research, only traditional crops were grown in trial plots. Since 1992, the field was used as permanent grassland, and no mineral amendments were applied. Nineteen years after fly ash application, soil samples were collected for analyses, which included determination of organic C and total N levels. It was found that coal fly ash applied in 1984 permanently changed the properties of the soil. Despite passage of a long period since their application, significant differences were still observed among the combinations, especially in the organic carbon content of the soil plough layer. The organic carbon concentration was also permanently affected by organic fertilizers. Tree bark had the most beneficial effect on the soil levels of organic carbon. The experimental factors had a less powerful influence on the total nitrogen concentration. The C:N ratio of the soil showed that coal fly ash considerably modified nitrogen values. This indicates a long-term effect of coal fly ash on nutrient immobilization and mineralization in the soil.

Keywords: coal fly ash, environmental utilization, total N, organic C, C:N ratio, soil properties

*To whom correspondence should be addressed.

P.A. Kulakow and V.V. Pidlisnyuk (eds.), *Application of Phytotechnologies for Cleanup of Industrial, Agricultural, and Wastewater Contamination*,

1. Introduction

Changes in regulations concerning air protection recently introduced in Poland resulted in a gradual reduction in dust-emission levels in the power industry sector. In 1990, emissions were about 570,000 t, whereas in 2000, they were about 56,000 t. These data concern dust emissions, excluding power engineering and industrial technologies. The above positive changes are a consequence of the installation of more and more modern and efficient dust-collection systems, capable of arresting even 98% of dusts generated during combustion. The dust-emission reduction is accompanied by increased amounts of fly ash at disposal sites located in the vicinity of power plants. It is estimated that in 2003, about 99.2% (approx. 13,600 t) of dusts generated at power stations and heating plants in Poland were arrested and deposited. It follows that more and more attention should be paid to the problem of the management and utilization of power plant dusts. Heaped power plant dusts may act as aggressive factors, changing the activity and productivity of ecosystems. Hard and brown coal dusts, although treated as waste, contain valuable elements that can be used for purposes of agricultural production and fertilization (Ciećko et al., 1993; Koter et al., 1983; Kabata-Pendias et al., 1987; Hermann and Sadowski, 1985; Terelak and Żórawska, 1979). Discharge of these wastes into the soil enables improvement of element balances in the natural environment, as well as reduction of the negative impacts of their excessive concentrations near disposal sites, i.e. waste dumps and heaps. Although the effects of fly ash on properties and yields of crops and on soil properties already have been described in detail, the literature provides scant information on long-term changes caused by the presence of these substances in the soil.

The aim of the present study was to determine the residual effect of coal fly ash on total soil nitrogen and organic carbon concentrations, as well as the soil C:N ratio. An attempt was also made to investigate interactions between coal fly ash and organic fertilizers, farmyard manure, straw, and tree bark applied to the soil together with fly ash.

2. Materials and Methods

The study was performed on the basis of a field experiment established in 1984 in the village of Łęg Starościński, located in the Lelis commune, Mazowsze Province, on luvisol soil with granulometric composition of slightly loamy silty sand – 63% sand, 30% silt, and 7% clay (BN-78/9180-11, soil classification, Polish Society of Soil Science). The soil was characterized by a mean abundance of available phosphorus (55 mg P/kg) and a high abundance of available potassium (152 mg K/kg) and magnesium

(55 mg Mg/kg). The adsorbing capacity of the soil was 12.5 cmol(+)/kg, and the soil reaction measured in water and in 1 M KCl was 6.5 and 5.6, respectively.

A two-factorial experiment was conducted in a randomized block design with four replications. Experimental factor I was increasing rates of fly ash (0, 100, 200, 400, 600, and 800 t/ha) from electrical precipitators of the power plant "Ostrołęka," Joint Stock Company. The ash used in the experiment contained, per kg dry matter, 491 g SiO_2, 1,700 mg P, 2,900 mg K, 1,500 mg Ca, and 7,100 mg Mg; and its pH measured in 1 M KCl was 9.2. Experimental factor II included organic amendments: farmyard manure, straw, and tree bark applied to the soil together with fly ash, in the amount of 10 t dry matter per ha. The area of each trial plot was 54 m^2.

Coal fly ash and organic fertilizers were applied before fall ploughing in 1984. The following crops were grown in consecutive years: 1985 – potatoes; 1986 – forage, oat + forage lupine; 1987 – forage rye + forage legume-grass mixture, 1988–1991 – forage legume-grass mixture. Mineral NPK fertilization was applied at equal rates during the entire experimental period, in accordance with relevant standards. Since 1992, the field has been used as permanent grassland, and no mineral fertilizers have been applied.

In 2003, 19 years after coal fly ash application, soil samples were collected in particular treatment, at depths of 0–25, 25–50, 50–75, and 75–100 cm. The samples were taken with a soil sampler, 50-mm in diameter, at four different sites in each plot and combined into one analytical sample. Fresh soil taken for analyses was air-dried and passed through a sieve of 1.0-mm mesh. The following determinations were made: total nitrogen content – by the Kjeldahl method (Ostrowska et al., 1991), after open sulfuric acid digestion of soil samples; organic carbon content – by the modified Tiurin method (Ostrowska et al., 1991).

Results of the study were verified statistically by ANOVA at a significance level $\alpha = 0.05$ using Statistica software v. 6.0 (StatSoft, 2001). The simple correlation coefficients between rates of fly ash, the concentrations of total N and organic C, and the soil C:N ratio were determined using Microsoft Excel 2000 software (Microsoft, 2000).

3. Results and Discussion

Fly ash contains no carbon or only small amounts of carbon. As reported by other authors, the carbon content of fly ash ranges from 0.02% to 1.5% (Kabata-Pendias et al., 1987; Lee et al., 1999). Despite such low quantities of this element, fly ash indirectly affects the humus level of the soil. This is easily noticeable on light soils, where the discharge of these wastes is followed by a substantial improvement in the adsorbing capacity, base saturation, air–water relations, and biological balance (Koter et al., 1984).

3.1. ORGANIC CARBON

Results of the present study showed that coal fly ash applied 19 years before increased the soil levels of organic carbon, especially in the plough layer, a depth of 0–25 cm. (Table 1). A similar effect of fly ash on soil organic carbon content was observed by Maciak (1981). In the study performed by this author the increase in soil organic matter levels caused by fly ash application ranged from 1.5 g/kg (fly ash from the power plant "Ożarów" – 100 t/ha) to 2.7 g/kg d.m. of soil (fly ash from the power plant "Siekierki" – 200 t/ha), compared with unfertilized treatments. This effect was also recorded 4 years after fly ash application (increase by 1.4 g of organic C/kg d.m. of soil, at a fly ash rate of 120 t/ha, in comparison with the control treatment). In our experiment, 19 years after fly ash application, significant differences in the soil organic carbon samples taken at a depth of 0–25 cm were observed for all fly ash rates and in all experimental series, including treatments where no organic amendments were applied. The organic carbon level was the lowest, 3.80 g/kg d.m. of soil, in the treatment where no fly ash or organic amendments were applied. A gradual increase in the organic carbon content, to 15.45 g/kg, was observed in the treatment where fly ash was applied in the amount of 400 t/ha. This increase strongly positively correlated with fly ash rates ($r = 0.77^{**}$). Organic amendments applied at a single rate 19 years before had a significant influence on soil organic carbon levels, compared with unfertilized treatments. In the treatments where no fly ash was applied, farmyard manure and straw caused an increase in organic carbon by 0.75 g/kg d.m. of soil, whereas tree bark increased the soil organic carbon concentration by as much as 2.32 g/kg. The increase in organic carbon affected by the above amendments confirms the humus-forming effects of organic fertilizers, such as manure, straw, and tree bark (Spiak and Piszcz, 2001; Wiater and Dębicki, 1993). The impacts of particular organic amendments on the soil carbon concentration were gradually neutralized as rates of fly ash were increased. At the highest fly ash rate, there were no differences in the soil organic carbon content; and in the treatments where manure and tree bark were applied, the carbon concentration decreased by 0.9 and 2.85 g/kg d.m. of soil, respectively, in comparison with treatment with no organic fertilizers.

TABLE 1. Organic C content of the soil 19 years after the application of coal fly ash and organic amendments (g/kg of dry soil).

Coal fly ash rate (t/ha)	Organic amendments			
	No amendments	Farmyard manure	Straw	Tree bark
Soil layer 0–25 cm				
0	3.80	4.55	4.55	6.12
100	10.50	11.25	12.75	11.25
200	10.65	10.65	11.10	9.60
400	15.45	13.65	13.35	10.80
600	12.45	12.30	12.00	11.10
800	14.85	13.95	14.85	12.00
$LSD_{(0.05)}$:	fly ash rate–0.055**; organic amend.–0.046**; fly ash × organic amend.–0.113**			
Correlation	0.77**	0.76**	0.70*	0.69*
Soil layer 25–50 cm				
0	1.65	2.10	3.00	3.30
100	7.80	6.00	6.60	3.60
200	2.10	2.10	2.40	2.55
400	3.45	3.45	2.25	1.05
600	0.75	3.90	2.25	0.45
800	1.35	3.00	3.60	0.60
$LSD_{(0.05)}$:	fly ash rate–0.027**; organic amend.–0.021**; fly ash × organic amend.–0.053**			
Correlation	0.47 n.s.	−0.02 n.s.	−0.30 n.s.	−0.93**
Soil layer 50–75 cm				
0	1.80	1.65	1.95	1.20
100	3.15	3.60	5.70	1.95
200	2.10	2.40	1.95	2.10
400	1.95	2.25	2.40	2.10
600	1.35	1.95	1.50	1.05
800	1.35	1.65	1.50	1.65
$LSD_{(0.05)}$:	fly ash rate–0.024**; organic amend.–0.018**; fly ash × organic amend.–0.044**			
Correlation	−0.67*	−0.44 n.s.	−0.50 n.s.	−0.16 n.s.
Soil layer 75–100 cm				
0	0.48	0.42	0.42	0.38
100	0.54	0.30	0.42	0.30
200	0.42	0.54	0.42	0.36
400	0.42	0.36	0.36	0.36
600	0.36	0.48	0.42	0.36
800	0.42	0.24	0.36	0.48
$LSD_{(0.05)}$:	fly ash rate–n.s.; organic amend.–0.004*; fly ash × organic amend.–0.011**			
Correlation	−0.69*	−0.33 n.s.	−0.62*	0.68*

Two-way ANOVA: ** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant.

In the 25–50 cm soil horizon, the organic carbon content was much lower than in the top layer and amounted to 1.65 g/kg of soil in the treatment where neither fly ash nor organic fertilizers were applied. Farmyard manure, straw, and tree bark significantly increased the soil organic carbon levels in treatments without fly ash to 2.10, 3.00, and 3.30 g/kg d.m. of soil, respectively. Data on the effects of fly ash on the organic carbon content of this soil horizon do not indicate explicitly the direction of changes in the concentrations of this element. The treatments differed significantly in this respect, but there was no clear correlation between fly ash rates and the organic carbon content of the soil in particular experimental series. At a depth of 25–50 cm, the correlation changed from positive to negative. This tendency was especially noticeable in deeper soil layers.

Much lower differences in organic carbon concentration, related to both organic fertilizers and fly ash rates, were recorded at depths of 50–75 cm. The highest mean level of this nutrient was observed in the experimental series with straw (2.50 g/kg) and the lowest in the series with tree bark application (1.93 g/kg). In treatments with no supplementary organic fertilization, there was a significant negative correlation between fly ash rates and organic carbon ($r = -0.67^{*}$). In the 75–100 cm horizon, the mean soil organic carbon concentration oscillated around 0.38–0.44 g/kg of soil and was not considerably modified by varying fly ash rates.

An increase in organic carbon content caused by increasing rates of fly ash was also reported by Wojcieszczuk et al. (1996) and Giedrojć and Fatyga (1985). Terelak and Żórawska (1979) observed a decrease in organic matter, from 2.97 to 2.4 g organic C/kg d.m. of soil, when fly ash was applied at a rate of 5–25 t/ha.

3.2. TOTAL NITROGEN

Power plant dusts contain small amounts of carbon and nitrogen. During combustion, nitrogen is emitted to the atmosphere in the form of oxides. Total nitrogen concentration in fly ash is as low as several tens of percent (Gaind and Gaur, 2003), so they cannot be treated as a source of this nutrient to plants. However, fly ash used in agriculture, especially on light soils, may contribute to nitrogen deficiency in the soil.

Nineteen years after fly ash application, no significant relationships were found between fly ash rates and total nitrogen content of the soil (Table 2).

TABLE 2. Total N content of the soil 19 years after application of coal fly ash and organic amendments (g/kg of dry soil).

Coal fly ash rate (t/ha)	Organic amendments			
	No amendments	Farmyard manure	Straw	Tree bark
Soil layer 0–25 cm				
0	0.89	0.95	1.03	1.01
100	1.17	1.29	1.23	1.26
200	1.20	1.22	1.26	1.17
400	1.32	1.40	1.21	1.19
600	1.15	1.17	1.12	1.07
800	1.13	1.17	1.20	1.04
$LSD_{(0.05)}$:	fly ash rate–0.024**; organic amend.–0.017**; fly ash × organic amend.–0.041**			
Correlation	0.35 n.s.	0.21 n.s.	0.15 n.s.	−0.34 n.s.
Soil layer 25–50 cm				
0	0.14	0.19	0.33	0.36
100	0.84	0.60	0.62	0.36
200	0.24	0.28	0.38	0.36
400	0.47	0.52	0.41	0.30
600	0.30	0.47	0.23	0.27
800	0.32	0.46	0.60	0.34
$LSD_{(0.05)}$:	fly ash rate–0.017**; organic amend.–0.013.; fly ash × organic amend.–0.032**			
Correlation	−0.15 n.s.	0.38 n.s.	0.09 n.s.	−0.55 n.s.
Soil layer 50–75 cm				
0	0.25	0.20	0.24	0.24
100	0.27	0.27	0.29	0.21
200	0.18	0.20	0.22	0.18
400	0.21	0.21	0.18	0.17
600	0.18	0.18	0.25	0.22
800	0.24	0.20	0.22	0.23
$LSD_{(0.05)}$:	fly ash rate–0.026**; organic amend.–n.s.; fly ash × organic amend.–0.048*			
Correlation	−0.24 n.s.	−0.43 n.s.	−0.28 n.s.	0.04 n.s.
Soil layer 75–100 cm				
0	0.19	0.18	0.17	0.18
100	0.17	0.18	0.18	0.15
200	0.18	0.21	0.23	0.21
400	0.17	0.19	0.18	0.15
600	0.22	0.20	0.19	0.20
800	0.25	0.19	0.15	0.19
$LSD_{(0.05)}$ for:	fly ash rate–n.s.; organic amend.–n.s.; fly ash × organic amend.–n.s.			
Correlation	0.56 n.s.	0.21 n.s.	-0.34 n.s.	0.24 n.s.

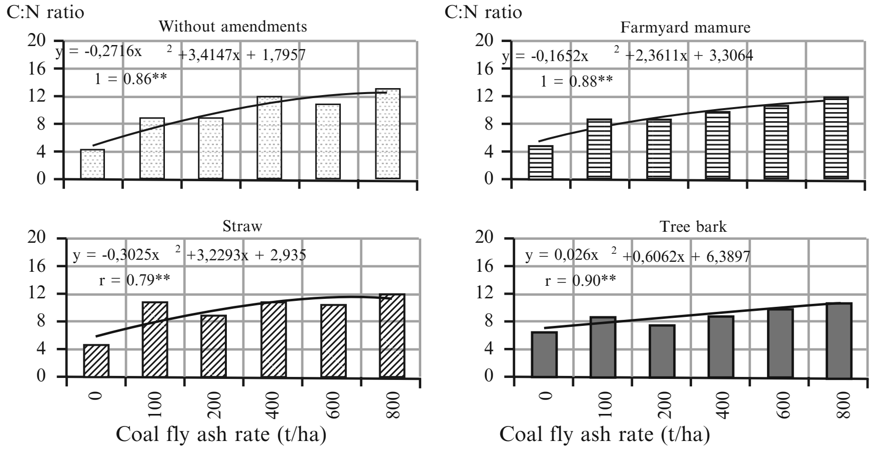
Two-way ANOVA: ** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant.

Despite considerable differences in total nitrogen concentrations between particular treatments, low coefficients of correlation between total soil nitrogen content and fly ash rates do not permit drawing conclusions about the direction of changes affected by experimental factors. Such a situation was observed in all horizons from which soil samples were taken. This could be caused by high mobility of nitrogen compounds in the soil, as well as their availability to the roots. The soil used in the study was light soil, which could also affect nitrogen leaching. During the 19-year period, the combined effects of the experimental factors neutralized the impacts of coal fly ash on the total soil nitrogen levels. Maciak (1981) demonstrated a distinct increase in total nitrogen concentrations 4 years after application of fly ash to light soil, compared with unfertilized treatments. Levels of nitrogen ranged from 1.5 to 1.8 g N per kg d.m. of soil, depending on the type (origin) of fly ash. In the control sample (no fly ash application) nitrogen concentration was 1.3 g N/kg d.m. of soil.

In the present experiment, total soil nitrogen content was affected by the organic amendments applied. In the treatments fertilized with farmyard manure and straw, mean nitrogen concentrations were 1.20 and 1.18 g/kg respectively. Tree bark caused a decrease in soil nitrogen level by 0.02 g/kg d.m. of soil in comparison with the experimental series without fertilizers. In deeper layers, there were no significant differences in total nitrogen concentrations between the experimental series.

3.3. C:N RATIO

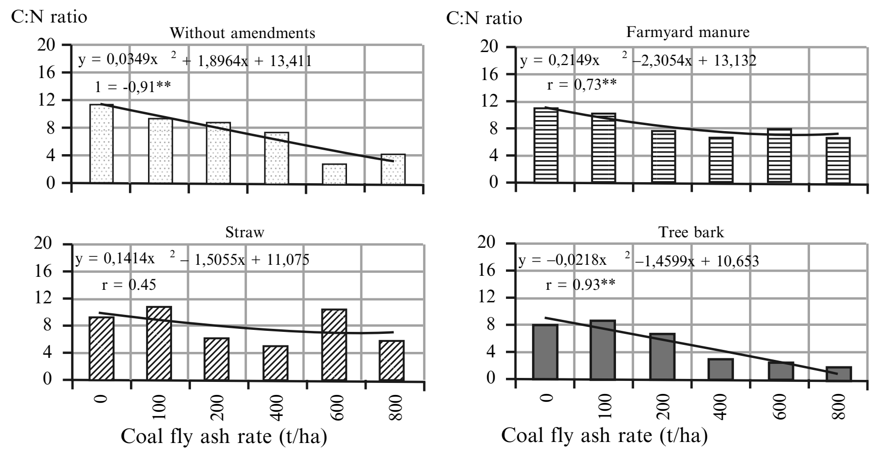
Total levels of soil nitrogen and organic carbon, the C:N ratio, provide valuable information about the processes of nutrient mineralization and immobilization occurring in soil. In the present study, increasing rates of coal fly ash widened the C:N ratio, but only in the soil plough layer of 0–25 cm (Figure 1), as indicated by trend lines generated for a particular experimental series. A highly significant correlation was also found between fly ash rates and the C:N ratio. Correlation coefficients calculated for the above parameters in this soil horizon varied from $r = 0.79^{**}$ for the series with fly ashes and straw as a source of organic matter (10 t d.m.), to $r = 0.90^{**}$ for the series with tree bark. In deeper soil layers, the C:N ratio was narrower in all treatments (Figure 2). In unfertilized treatments the decrease in the C:N ratio was observed to the fly ash rate of 600 t/ha, when it reached 2.60. Application of farmyard manure and straw without fly ash resulted in a slightly narrower C:N ratio. Applied together with high rates of fly ash (600 and 800 t/ha), these amendments had a stabilizing effect and enabled maintenance of the C:N ratio at a higher level than in the control series.



LSD for: coal fly ash rate – 0.49**
organic amendments – 0.43**,
coal fly ash rate x organic amendments – 1.06**

** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant

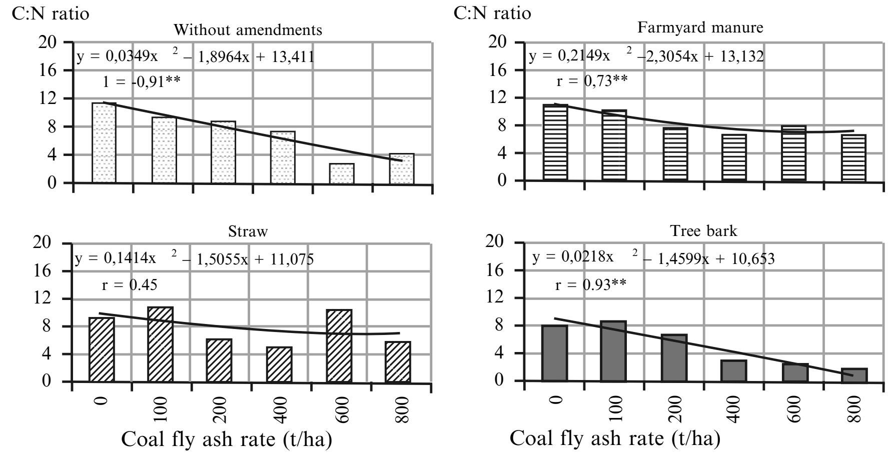
Figure 1. C:N ratio of the soil 19 years after application of coal fly ash and organic amendments – layer 0–25 cm.



LSD for: coal fly ash rate – 0.85**
organic amendments – 0.57**,
coal fly ash rate x organic amendments – 1.40**

** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant

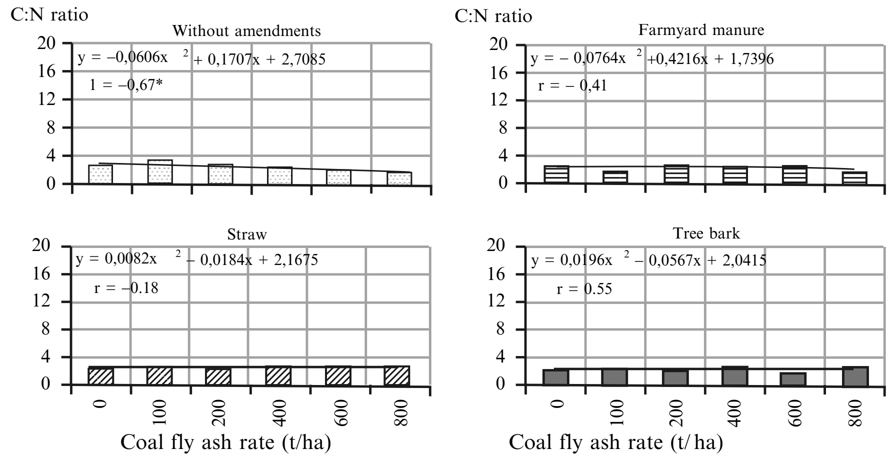
Figure 2. C:N ratio of the soil 19 years after application of coal fly ash and organic amendments – layer 25–50 cm.



LSD for: coal fly ash rate – 0.85**
organic amendments – 0.57**,
coal fly ash rate x organic amendments – 1.40**

** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant

Figure 3. C:N ratio of the soil 19 years after application of coal fly ash and organic amendments – layer 50–75 cm.



LSD for: coal fly ash rate – n.s.;
organic amendments – n.s.;
coal fly ash rate x organic amendments – 0.85*

** – significant at $\alpha < 0.01$, * – significant at $\alpha < 0.05$, ns – not significant

Figure 4. C:N ratio of the soil 19 years after application of coal fly ash and organic amendments – layer 75–100 cm.

Tree bark contributed to the narrowing of the soil C:N ratio, both in treatments without organic amendments and those fertilized with manure and straw. In deeper soil layers, values of the C:N ratio increased in response to fly ash application, on average to a rate of 200–400 t/ha, and the changes were parabolic. Higher fly ash rates resulted in a narrower C:N ratio (Figure 3). In the 50–75-cm horizon, no significant differences were recorded in the C:N ratio, whose mean values ranged from 2.0 in the series with tree bark to 2.35 in the series with straw (Figure 4).

4. Conclusions

1. An analysis of the direction of changes in organic C content, total N content, and C:N ratio in the soil plough layer and in the 25–50-cm horizon suggests that coal fly ash induces primarily changes in the upper layers of the soil, thus differentiating conditions of plant growth and development.
2. Fly ash applied to light soils is conducive to plant growth since it improves such soil properties as adsorbing capacity, texture, and nutrient concentrations.
3. Favorable soil conditions contribute to development of root systems and aboveground mass, which with time become organic carbon and humus providers.

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PHYTOREMEDIATION OF LOESS SOIL CONTAMINATED BY ORGANIC COMPOUNDS

K. ZHU[1*], H. CHEN[2], AND Z. NAN[3]
[1]*Department of Environmental Engineering, Lanzhou Jiaotong University, Lanzhou, Gansu 730070, P.R. China*
[2]*College of Chemistry & Chemical Eng., Northwest Normal University, Lanzhou, 730070, P.R. China*
[3]*College of Resources & Environment, Lanzhou University, Lanzhou, Gansu 730000, P.R. China*

Abstract Loess soils extensively cover Central Asia and North China topography where oil fields are widely exploited; thus, organic contamination has become a critical environmental issue in these regions. As China faces severe land and eco-environmental deterioration, phytoremediation has been considered as a priority remedial alternative for reclamation of contaminated cultivated land. This article provides a framework to understand phytoremediation applications in loess land contaminated by organic compounds, particularly by petroleum pollutants. Mechanisms of phytoremediation in the soil matrix are introduced and discussed. Experimental study at field test plots demonstrates selected plants are capable of growing well in the arid and semi-arid loess plateau and effectively dissipate oil pollutants from loess soil. A combined approach including phytoremediation, surfactant flushing and microbial degradation is suggested for restoration of petroleum-contaminated agricultural land.

Keywords: soil contamination, organic compounds, phytoremediation, loess soil, restoration

1. Introduction

With continuing economic development of double-digit annual growth in the last 3 decades, China is facing three depressive issues: population growth, water scarcity, and land desertification. For example, a large area of

[*]To whom correspondence should be addressed.

agricultural land has vanished in arid and semi-arid Northwest China where desertification threatens and oil fields are widely explored. This district has a typical topographic structure with loess soils and Gobi-desert, with loess soils covering 450,000 km^2 in the arid and semi-arid regions of northern China. Although China's fossil fuel production first exceeded 100 million tons in 1978, unceasing growth has resulted in annual production maintained between 150 and 185 million tons since 2000 (MLRC, 2008a). Thus, China has become one of the main petroleum production countries of the world. As with any large-scale industrial process, petroleum production can lead to contamination of soil and groundwater. Major causes of crude oil-contaminated soil include spills and leakage of oil products. Moreover, large areas of fertile farming land have been damaged in the oil fields, while the environment in urban areas is threatened by petroleum pollution through leakage from petrol stations, pipelines, vehicles, and other sources. Currently, no specific regulation or standard specifically limits soil contamination by petroleum in China. This increasing environmental problem threatens cultivatable land, causes increased air pollution, and surface water and groundwater contamination.

The objective of this article is to highlight serious land issues in China emphasizing the role for remediation technologies in reclamation of polluted land. Due to natural and topographic conditions in the loess soil regions of northern China, a special concern is to certify available cleanup techniques applicable for *in situ* remediation of petroleum-polluted soils, with priority given to phytoremediation combined with soil excavation, soil flushing, and microbial degradation.

2. Land Issues in China

China, as an over-populated nation, has an area of 9.6 million square kilometers with high land in the west and low land along the east coast. Mountains, hills, and plateaus account for two-thirds of the total area. Out of the total area, arable land is around 122 million hectares or 12.7%; forests, 236 million hectares or 23.3%; natural grassland, 261 million hectares or 27%; fresh water bodies, 18 million hectares or 2%; and land used for construction, 27 million hectares or 3%. The remaining land, 32%, is composed of Gobi-deserts, glaciers, and rocky mountains, which are unsuitable for agricultural use (MEPC, 2008).

China is one of the countries suffering from the most serious soil and water loss. A government investigation reveals that almost 37% of China's territory, 3,569,200 km^2, has been seriously impacted by soil erosion, including 46% by water erosion and 54% by wind erosion. Soil erosion has already caused at least $29.4 billion in economic losses to China since 2000. The population of China is equal to 22% of the total population of the world, but China owns only 7% of world's arable land. Only 40% of China's arable land has abundant water sources and irrigation facilities, while medium- and low-yield arable land accounts for 60% of the total. During 2007, arable land in the country decreased by 42,000 ha due to occupation or destruction by non-agriculture construction, ecological restoration, agri-culture restructuring, or natural disasters. Taking into account the difference between increases and decreases of arable land, per capita arable land actually reduced from 0.26 ha to less 0.106 ha in the last half century, which equals only 43% of the world average per capita arable land area (MEPC, 2008).

Particularly noteworthy are China's geographical features that put its land security under increasing threat from drought, flooding, and desertification. A paramount reason for this concern is ecological deterioration in the arable land area. Ecological and environmental challenges seriously damage China's land resources, with climate change representing only one of many reasons for desertification. Destruction of natural vegetation, excessive cultivation, and water shortages are also contributing factors.

Decline in soil quality has become one of the most worrisome by-products of China's economic growth. The major culprit behind the worsening soil quality is industrial pollution. Non-point source pollution is also a growing danger. As the world's biggest fertilizer and pesticide user, only 30% of manure and fertilizer chemicals are actually utilized by crops, compared to 60% utilization in all other industrial countries. This low-nutrient-use efficiency means most nutrients are swept away by runoff causing eutrophication of surface water, such as the algae choking of Dianchi Lake, Taihu Lake, and Chaohu Lake, or pollution of groundwater aquifers. Pollution from livestock production is also a huge problem; while about 2.7 billion tons of livestock manure was generated in China in 2007, only 20% of rural livestock farms had adequate pollution treatment facilities (MEPC, 2008). In some regions, pollution from livestock has become an important factor causing deterioration of water sources (Hu, 2008). Additionally, 280 million tons of household garbage, 9 billion tons of domestic sewage, and 260 million tons of human waste were generated in 2007, with most dispersed on-site resulting in heavy metal accumulation of soil, hardening of the soil surface, and reduction of soil fertility (MEPC, 2008). Currently, about 10 million hectares of China's cropland has been contaminated, most of which is located in more affluent regions (MEPC, 2008).

To resolve the growing crisis of soil quality in its northern regions, China has invested more than $72 billion in the last decade to conduct the Natural Forest Conservation program and Grain to Green program (Xinhua, 2008). The forest conservation program was designed to rectify damage caused by years of unfettered logging, which has led to soil erosion, contamination of agricultural land, devastation of habitat, and other environmental problems. The Grain to Green program works to convert cropland on steep slopes to forest and grassland by providing farmers with grain and cash subsidies. The program was enacted by the federal government in early 2003, with about \$20 billion planned to support the program for 5 years from 2006 to 2010 (FPGC, 2005).

China is facing a sharp conflict between land supply and demand, with arable land shrinking from 121.80 to 121.72 million hectares in 2007, slightly above the minimum of 120 million hectares set by the government. In early 2008, the Chinese government intensified the protection of arable land and applied strict systems for economizing land use to keep the total amount of arable land above the red line of 120 million hectares (FPGC, 2008). According to a recent report by the Ministry of Land and Resources of China, total arable land area in China maintained at 121.71 million hectares suggesting progress in control of the decrease of arable land (MLRC, 2008b). Variability in the total agricultural land of China from 2000 is shown in Figure 1 (MLRC, 2008b; Wang, 2009).

Although soil quality has become a major challenge and continues to deteriorate, China has made efforts on land and soil protection (Xinhua, 2009a; Wei, 2009). A large 3-year soil pollution survey project funded by the Chinese central government with \$325 million was initiated in 2007 (Hu, 2008; Xinhua 2007). The survey reportedly concentrates on key regions near heavily polluting factories, industrial sites, solid-waste disposal sites, oil fields, mining areas, and major vegetable-growing areas. Remediation of petroleum-contaminated soil is included as a sub-project of the survey to be researched (Hu, 2008). It is expected that a soil environmental quality monitoring and management system will be established by the end of the survey period. According to an official report, 4.77 million hectares of forest were planted in 2008, of which 3.29 million were afforested by manpower (FPGC, 2008). By the end of 2008, there were 2,538 natural reserves, including 303 national reserves. About 47,000 km^2 of eroded land were put under comprehensive treatment programs, and 26,000 km^2 were closed for restoration and protection in areas suffering water and soil erosion (NBSC, 2009). During the current international financial crisis, China has approved a massive \$586 billion, 2-year investment plan to curb the economic downturn, but this precludes boosting economic growth at the expense of environmental protection. It is therefore an unavoidable task for China to find ways that will effectively improve the environment and allow for “green growth” of the national economy at the same time (Xinhua, 2009b).

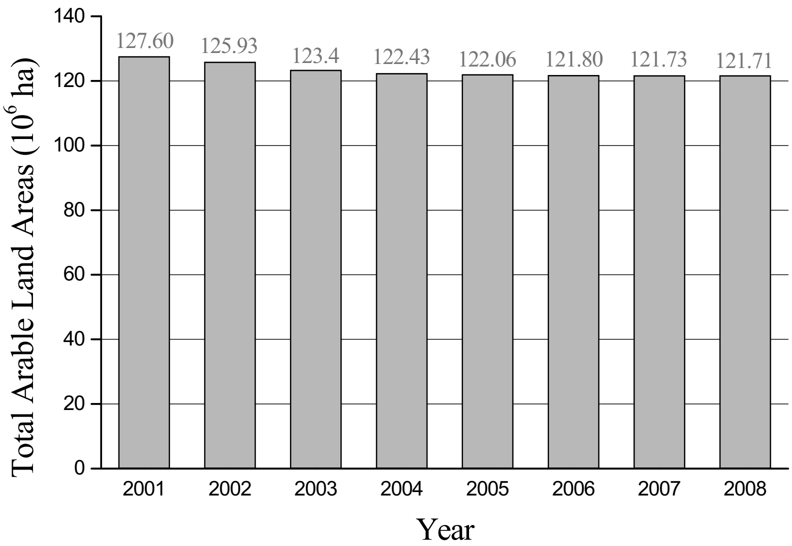

Figure 1. Decreasing tendency of total arable land in China after 2000.

3. Availability of Phytoremediation for Cleanup of Soils Contaminated with Organic Pollutants

3.1. REVIEW OF PUBLISHED LITERATURE

Phytoremediation is the use of plants to partially or substantially remediate contaminants in contaminated soil, sludge, sediment, groundwater, surface water, and wastewater. Phytoremediation encompasses a number of different methods that can lead to contaminant degradation or removal through accumulation, dissipation, or immobilization (Suresh and Ravishankar, 2004). Phytoremediation is potentially applicable to a variety of contaminants, including some of the most significant contaminants such as petroleum hydrocarbons, chlorinated solvents, metals, radionuclides, nutrients, pentachlorophenol (PCP), and polycyclic aromatic hydrocarbons (PAHs) (Pivetz, 2001).

Several projects examined the interaction between plants and organic contaminants such as trinitrotoluene (TNT), total petroleum hydrocarbons (TPH), pentachlo-rophenol (PCP), and polycyclic aromatic hydrocarbons (PAH) and BTEX (Benzene, toluene, ethylbenzene, and xylenes) (USEPA, 2000). Soil from the rhizosphere of poplar trees had higher populations of benzene-, toluene-, and o-xylene-degrading bacteria than did non-rhizosphere soil. Root exudates contained readily biodegradable organic macromolecules

(Jordahl et al., 1997; Haakrho and Crowley, 2007). A field test showed a comparable decrease of soil total PAHs concentrations were obtained for three plots, reaching a maximum value of 26% of the initial PAHs concentration. (Denys et al., 2006). Ryegrass has been shown to increase microbiological biomass carbon and other enzyme activities, resulting in improved degradation rates of benzo[a]pyrene in soil while the shoot of ryegrass accumulated only trace amounts of benzo[a]pyrene (Liu et al., 2007). For phytoremediation of polyaromatic hydrocarbons, the relative amount of degradation decreases as the number of aromatic rings increases, although most PAHs are probably not taken into plant tissue in significant amounts. The increased root growth and microbial levels, including PAH degrader numbers, appeared to be important factors influencing degradation rates (White et al., 2006). Microbial-contaminant interactions result in increased organic contaminant biodegradation in the soil. Additionally, the rhizosphere substantially increased the surface area where active microbial degradation can be stimulated. Microbial counts in rhizosphere soils can be one or two orders of magnitude greater than in non-rhizosphere soils (USEPA, 2000).

Metabolism is a possible mechanism within the plant for phytodegradation, which has been identified for a diverse group of organic compounds including the herbicide atrazine (Burken and Schnoor, 1997) and the chlorinated solvent TCE (Newman et al., 1997). Other metabolized compounds include the insecticide DDT, the fungicide hexachlorobenzene (HCB), PCP, the plasticizer diethylhexylphthalate (DEHP), and polychlorinated biphenyls (PCBs) in plant cell cultures (Sandermann et al., 1984; Harms and Langebartels, 1986; Wilken et al., 1995; Pivetz, 2001). Some plants may be able to take in toxic compounds and in the process of metabolizing available nutrients, detoxify them (USEPA, 2000). For instance, trichloroethylene (TCE) is possibly degraded in poplar trees and the carbon used for tissue growth, while the chloride is expelled through the roots. However, transpiration of TCE into the atmosphere has been measured (Newman et al., 1997).

For phytodegradation to occur within a plant, contaminants must be taken up by the plant prior to degradation. Early studies identified more than 70 organic chemicals representing many classes of compounds that were taken up and accumulated by 88 species of plants and trees (Paterson et al., 1990; Pivetz, 2001). Uptake is dependent on hydrophobicity, solubility, and polarity. Moderately hydrophobic organic compounds with log k_{ow} between 0.5 and 3.0 are most readily taken up by and translocated within plants. Very soluble compounds with low sorption will not be sorbed onto roots or translocated within the plant (Schnoor et al., 1995; Gao and Zhu, 2004). Hydrophobic (lipophilic) compounds can be bound to root surfaces or partitioned into roots but usually cannot be translocated further within the plant (Cunningham, 1997). Nonpolar molecules with molecular weights <500 will sorb to the root surfaces, whereas polar molecules will enter the root and be translocated (Bell, 1992). Plant uptake of organic compounds can also depend on type of plant, age of contaminant, and many other physical and chemical characteristics of the soil. Definitive conclusions can not always be made about a particular chemical. For example, when PCP was spiked into soil, 21% was found in roots and 15% in shoots after 155 days in the presence of grass (Qiu et al., 1994; Pivetz, 2001).

Organic contaminants could be transformed to less-toxic forms (USEPA, 2000). Thus, organic contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation. However, in some cases the contaminant or a hazardous metabolite, such as vinyl chloride formed from TCE, might be released into the atmosphere. Thus the contaminant or a hazardous metabolite might accumulate in vegetation and be passed on in later products such as fruit or lumber. Alfalfa has been studied by Kansas State University researchers for its role in the phytovolatilization of TCE of 100 and 200 μg/l (Narayanan et al., 1995; Pivetz, 2001) while black locust species were studied for use in remediating TCE in groundwater (Newman et al., 1997). Poplar trees were used with atrazine and volatile organic compounds in toxicity studies conducted in laboratory chambers and in the field when atrazine was mineralized, and deep-rooted poplars slowed migration of volatile organics (USEPA, 2000).

3.2. LIMITATIONS IN APPLICATIONS OF PHYTOREMEDIATION

Long-term field evaluation is critical to understand how well phytoremediation may work, the real cost of applications, and how to build methodologies to predict the interaction between plants and contaminants.

As a result of early information provided on phytoremediation, we know it is a relatively clean and inexpensive technology. However, disadvantages and limitations of phytoremediation need to be considered in the planning process.

- Root system limitations: Phytoremediation requires contaminants to be located within the area influenced by the plant root zone.
- Growth rate limitation: More time may be required to phytoremediate a site as compared with other cleanup technologies. Phytoremediation may not be the remediation technique of choice if a site poses acute risks for human and other ecological receptors.

- Contaminant limitation: Contaminated sites with widespread, low- to medium-level contamination within the root zone are the best candidates for phytoremediation processes. Areas with high contaminant concentrations often pose more acute risks. High concentrations also might inhibit plant growth, thus limiting effectiveness at some sites or parts of sites (Wang et al., 2007a; Peng and Zhou, 2008).
- Vegetation limitation: The remediation plan should identify and quantify, if possible, potential routes of ecological exposure and determine if accumulation of toxics in the selected plants will occur.

3.3. TECHNICAL CONSIDERATIONS

Several key factors need consideration when evaluating phytoremediation as a potential site remedy (USEPA, 2000; Qu et al., 2008):

- Assemble documented evidence for effectiveness of phytoremediation specific to the site matrix and contaminants.
- Consider protectiveness of the remedy during the time it takes plants to establish and to provide the needed phytoremediation processes.
- Determine if site cleanup is likely to occur within an acceptable amount of time.
- Develop an adequate backup or contingency plan in the event the phytoremediation plan does not succeed.
- Determine monitoring procedures needed to document efficacy of phytoremediation. Monitoring needs to address both the decrease in contaminant concentration and the fate of contaminants.

4. Application of Phytoremediation for Petroleum-Contaminated loess Soils in China

4.1. CHARACTERISTICS OF LOESS PLATEAU

Loess soil covers about 450,000 km^2 in Northern China. These yellow-colored soils vary in thickness from a few meters to hundreds of meters in depth. Climate in this region can be characterized as arid to semi-arid. Annual average temperature is 6.3°C with a frost-free period of 100–160 days. Maximum temperature is about 34°C and minimum about minus 27°C. Precipitation falls primarily from May to September and typically ranges between 80 and 450 mm annually, although annual potential evaporation is estimated at 2,000–3,200 mm.

In spring of 2004, the Changqing Oil Field Company of China announced that it had exploited a 400 million-ton oil field in the East District of Gansu Province for the next 2 decades (Asiainfo, 2004). In this region, fossil oil reserves are located in tertiary sandstone overlaid with thick loess soil sediments. During the exploitation process, drilling teams commonly disposed oil and deteriorated large areas of agricultural land causing severe contamination of soil, streams, and shallow groundwater aquifers. The provincial government urged the oil company to compensate local farmers for land damage and to take measures for reclamation of contaminated agricultural lands. Our research group took responsibility for studies of potential remediation methods and presented results to the government agencies. The Ministry of Environmental Protection of China paid attention to soil quality issues affected by heavy metal and petroleum contamination. Our project began in 2007 to study phytoremediation of oil-contaminated soil in the Loess Plateau by selection of effective plants based on field tests.

Soil texture and chemical constituents from samples of a typical loess soil in the Loess Plateau are shown in Table 1. Physical and dynamic properties are listed in Table 2.

TABLE 1. Characteristics of loess soil at the experimental site for phytoremediation.

Texture (%)			Bulk density (g/cm^3)	Organics (%)	Total N (%)	Total P (%)	Total K (%)	$CaCO_3$ (%)
Sand	Silt	Clay						
7.6	76.2	16.2	1.24	0.59	0.048	0.041	1.88	12.1

TABLE 2. Physical and dynamic properties of loess soils.

Number samples	Liquid limit (W_L) (%)	Plastic limit (W_P) (%)	Plasticity index (I_P), %	Relative collapsibility coefficient	Compressibility coefficient, cm^3/kg
76	28.6	17.5	11.1	0.074	0.105

Note:

Liquid limit: The volumetric water content of soil above which the soil behaves as a viscous liquid.

Plastic limit: The volumetric water content of soil below which the soil no longer behaves as a plastic material.

Relative collapsibility coefficient: Calculated as $I_P = W_L - W_P$.

Relative collapsibility coefficient: Ratio between the change in height of natural soil after water saturation and the height of original natural soil.

Compressibility coefficient: The change in soil void ratio per unit increase in pressure.

4.2. CHARACTERIZATION OF PETROLEUM-CONTAMINATED LAND

Development of oil fields involves drilling wells on land or beneath the sea. Examples of possible environmental impacts associated with the recovery of hydrocarbons near production sites include

- Disruption of the land to construct pads for wells, pipelines, or storage tanks and to build a network of roads and other production facilities
- Pollution of surface water and groundwater from runoff and infiltration or leakage from broken pipes or tanks of contaminated surface water, wastewater, or fluids used in secondary recovery
- Oil seepage resulting from normal operations or large spills from accidents, such as pipe ruptures
- Release of drilling mud containing oil and toxic heavy metals

Petroleum hydrocarbons can exist in soil in four phases: vapor, solution, non-aqueous-phase liquids (NAPLs), and adsorbents to soil particles. Distribution of petroleum among these phases depends on three key processes (volatility, solubility, and sorptivity) and is influenced by chemical and physical conditions at a particular site.

Term remediation encompasses the concept of restoration, rehabilitation, cleanup of contaminants, or conversion of contaminants to less hazardous compounds. Remediation of petroleum-contaminated soils can be accomplished by two primary types of approaches: excavation and removal of the contaminated soil, or *in situ* treatment. *In situ* treatment offers advantages of minimum site disturbance, limited infrastructure requirements, and minimal exposure hazards. This may lead to lower expenses to complete the treatment process. Some of the conceptual possibilities of *in situ* remediation for petroleum-contaminated soils include soil venting with vacuum extraction to remove volatile hydrocarbons, bioremediation to promote breakdown of residual oil by microorganisms, hydraulic methods to collect and remove mobile liquid and dissolved oil, and soil flushing to wash out residual oil from soil pores (Alderman et al., 2007; Liu et al., 2008). Based on long-term field investigations of technical and economic issues associated with potential remedial methodologies, our evaluation determined phytoremediation, soil washing, and microbial remediation as most suitable for field application in petroleum-polluted loess soils.

4.3. POSSIBILITIES TO APPLY PHYTOREMEDIATION ON PETROLEUM-CONTAMINATED LAND

Phytoremediation uses plants as biological agents to remediate pollutants in soil and water. Phytoremediation of petroleum-impacted land using only single-process techniques has met with limited success due to slow rates of remediation (Liu et al., 2003). Alleviation of plant stress via plant growth promoting rhizobacteria can lead to more rapid rates of remediation and improved plant growth on contaminated land. A field study showed that 3 years after an oil spill, phytoremediation management of a contaminated site through vegetation establishment plus fertilizer addition led to a reduction of crude oil contaminants. In a separate study, vegetation with fertilizer was most effective in reducing concentrations of more recalcitrant hydrocarbon fractions (White et al., 2006).

According to Greenberg et al. (2007), to cleanup large areas of contaminated land, only integrated approaches can reach the highest efficiency expected. Based on our previous remedial experiments at a field contaminated by refinery-produced wastewater in Shandong Province (Liu and Zhu, 1995), optimal remediation processes combine phytoremediation, surfactant-washing, and micro-remediation as a following sequence:

Phytoremediation → Flushing with surfactant enhancement → Microbial degradation with fertilization

Some research results dealing with phytoremediation on petroleum-contaminated loess soils have been reported in China in recent years. A successful plot study showed that *Medicago sativa L.*, growing originally in the loess plateau, could remove 91–96% of petroleum contamination from soil with initial contaminant concentrations of 2,224–3,514 mg/kg (Zhang et al., 2008). Existence of peat in soil is helpful to growth of alfalfa roots in diesel fuel-contaminated soil, which is proposed for arid and semi-arid regions for geographically evaluating vegetative covers (Wang et al., 2007b; Lu and Shi, 2007).

Loess soils extensively cover much of Central Asia and Mongolia. Therefore plant species selection by the field experiments in Northwest China also can be applied in these areas due to many similarities in natural and topographic conditions. Plants that work best for remediation of particular petroleum contaminants may or may not be native to a particular area. Although native plants are most desirable, non-native species may be acceptable under the following circumstances (Hou et al., 2006):

- The introduced species do not create a new ecological risk.
- The introduced species are unable to propagate effectively in the wild (e.g., sterility, dependence on human cultivation, etc.).
- The introduced species are genetically altered and the plants must grow at the specific soil.

4.4. EXPERIMENTAL STUDY OF PHYTOREMEDIATION BY SELECTED PLANTS IN LOESS PLATEAU

On the basis of unfavorable natural conditions common in Northwest China, phytoremediation applications must select plant material that is resistant from drought, saline-alkali soil, strong wind, and cold temperatures. This is in addition to suitability for dissipation of mineral oils. Twelve herbaceous plant species commonly growing in the loess plateau and Gobi-desert of Northwest China were chosen for use in experimental field test plots (Table 3).

Loess soil collected for the experiment was artificially contaminated with petroleum hydrocarbons composed of diesel and lubricating oils mixed in a weight ratio of 1:1. The mixed oils with a density of 0.904 g/ml were sprayed on a pile of uncontaminated loess soil prior to mixing. The petroleum hydrocarbon concentration of the artificially contaminated soil was calculated as 549 mg/kg. The experimental soil was moved into a bamboo-framed greenhouse where the artificially contaminated soil was uniformly packed to a depth of 30 cm into 12, 1-m-squared plots.

Seedlings of the 12 plant species sprigged into each plot. The seedlings were 2–3 cm in height and spaced 15 cm apart, both horizontally and vertically. Plant growth was observed and recorded each day. Plants were irrigated every 5 days by using harvested rainwater collected from the roofs and yards of farmer's houses and stored in a number of cisterns. This guaranteed the irrigation water was without any organic contaminants.

The field experiments continued for 3 months from late March to the end of June in 2007. At the end of the experiment, soil samples were taken from each plot, and total petroleum hydrocarbon concentrations were determined by infrared spectrometry and gas chromatographic methods (Zeng and Lin, 2004).

This research showed oil concentrations were reduced by growing plants that originated from Northern China. Leguminous forage species, such as *Astragalus cicer L.* and *Medicago sativa L.*, showed higher hydrocarbon dissipation rates compared to gramineous forage species (Table 3). Some species, however, showed limited growth likely due to phytotoxicity of the contaminated soil.

In comparison of the 12 plant species, *Phalaris arundinacea* L. was given special consideration as it grows widely in Northwest Loess Plateau. Another field experiment was conducted to examine tolerance of *Phalaris arundinacea* L. to soil contaminated with different concentrations of petroleum hydrocarboins. *Phalaris Arundinacea* L. (reed canary grass) is a large, coarse grass having erect, hairless stems, usually from 60 to 140 cm tall. It occurs from a wide range of soil conditions at pH 4.9–8.9, with a temperature range from –30°C to 40°C in the Northern China. The Institute of Pasture and Ecology of Gansu recommended native reed canary grass for setting up artificial pastures under the adverse circumstances because of its hardiness, aggressive nature, and rapid growth (IPEG, 2006).

TABLE 3. Remedial effects of the different herbage at petroleum-contaminated loess fields.

Plant species	Plant growth	Hydrocarbon dissipation percentage, %	Note
Puccinellia tenuiflora (Griseb)	Fair	57.8	
Agropyron sibiricum (wild) Beauv.	Poor	–	Dead after 26 days
Hordeum bogdanii Wilensky	Good	74.1	
Bromus inermis Leyss.	Good	63.9	
Elymus sibiricus L.	Poor	–	Dead in 1 month
Festuca arundinacea Schreb	Good	70.6	
Dactylis glomerata L.	Good	77.0	
Phalaris arundinacea L.	Fair	83.2	
Astragalus cicer L.	Fair	91.9	
Medicago sativa L.	Fair	80.8	Growing slowly
Onobrychis viciaefoia Scop.	Fair	82.6	
Trifolium pratense L.	Poor	–	Dead in 2 months

The experiment to test petroleum hydrocarbon tolerance of *Phalaris arundinacea* L. was performed using the same method for artificial contaminated soils as described above. Six, 0.8 by 0.8-m plots were packed with the following oil concentrations: 0; 5,953; 9,018; 12,257; 15,426; and 19,630 mg/kg. *Phalaris arundinacea* L. seedlings, 3–5 cm in height, were transplanted to each plot on a 12-cm grid. Plant growth was observed each day and plants were irrigated with harvested rainwater every 3 days. The experiment lasted 4 weeks from September 17 to October 14. After finishing the experiment, soil samples were taken from each plot for analysis of petroleum hydrocarbon concentrations. The results are displayed in Table 4.

TABLE 4. Measurements of *Phalaris arundinacea* L. growth and oil dissipation in experimental soils.

Test No.	1	2	3	4	5	6
Petroleum conc. (mg/kg)	0	5,953	9,018	12,275	15,426	19,630
Height (cm)	42.6	58.4	60.8	62.1	51.5	32.3
Leaf length (cm)	16.7	20.2	22.8	23.4	22.1	15.5
Leaf width (cm)	10.8	11.6	11.9	13.8	11.3	9.1
Dissipation percentage (%)	0	24.7	27.1	28.8	22.5	13.6

Although a short-period field experiment could not greatly reduce oil concentrations, differences among the tested soils were apparent. *Phalaris arundinacea* L. grew well in loess soil with high petroleum concentrations. This shows tolerance of this perennial grass to petroleum-contaminated land. The highest dissipation percentage was observed with a petroleum concentration of 12,275 mg/kg, suggesting perhaps optimal conditions for plant growth and microbial activity. Higher oil concentrations might restrain plant growth and the resulting petroleum dissipation percentage. Analysis of plant stems and leaves showed no evidence of uptake of petroleum into the plants. This is consistent with results from other researchers. Based on the experimental results, petroleum concentration of 15,000 mg/kg might be considered as a limiting concentration for using *Phalaris arundinacea* L. in phytoremediation for cleaning up petroleum-contaminated loess soil in Northwest China. Results from test plots with artificially contaminated soil would need to be confirmed under field conditions.

The ecological advantages of using a perennial plant like *Phalaris Arundinacea* L. without tillage may include reduced risk of soil erosion and likely increased carbon content in soil. It also has tolerance to heavy metals that might also occur in this region. Moreover, *Phalaris Arundinacea* L. is also under consideration as a biofuel plant and has been proposed as a creation of new approaches for exploiting non-food crops on marginal agricultural lands in China (Xie et al., 2008). Hao and colleagues studied growth of *Phalaris arundinacea* L., and concluded that it could be adapted to transitional environmental conditions between wetlands to dry land. *Phalaris arundinacea* L. also showed high ability to reduce nitrogen and phosphorus in soil and to accumulate heavy metals in the rhizosphere (Hao et al., 2008). Application of fertilizers containing N and K might increase growth of *Phalaris arundinacea* L. and enhance its ability to promote reduction of petroleum hydrocarbons in the soil matrix (Qi and Zhou, 2001). Therefore, our research group has proposed that the local government advise farmers to plant *Phalaris arundinacea* L. in their fallow land for 2 years, if the land contains considerable oil residues from improper application of wastewater for irrigation.

5. Conclusions

Expansion of oil exploration in the loess plateau of Northwest China has increased concerns about petroleum pollution. For implementation of China's economic development strategy in the Northwest District, the environmental problems and risk assessment will mostly deal with oil field drilling, leakage of oil transport, and storage, particularly in rural areas where contaminated soils have become a potential threat to local people's health and crop safety. Our research seeks to investigate the current soil situation in Northwest China and to learn whether phytoremediation is a viable tool for reclamation of petroleum-contaminated loess soil. To evaluate field application of phytoremediation, 12 plant species were chosen for field experiments, and results showed that *Phalaris arundinacea* L., *Dactylis glomerata* L., *Festuca arundinacea* Schreb, *Hordeum bogdanii* Wilensky, and *Bromus inermis* Leyss. could grow well and effectively remediate oil-contaminated soils. Thus we have recommended phytoremediation applications for arid and semi-arid loess land. *Phalaris arundinacea* L. showed an obvious advantages for phytoremediation under adverse circumstances due to its hardiness, aggressive nature, and rapid growth.

Proposed alternative remediation measures should use simple and economical methods based a site-specific land evaluation process aimed at reducing potential risk. Organic compounds are the main category of contaminants subject to degradation in oil fields. High removal efficiency of organic pollutants from soil will usually be reached by integrated remedial methods instead of use of a single alternative.

An integrated approach for restoration of large fields polluted by petroleum consists of phytoremediation, surfactant flushing, and microbial bioremediation. This integrated strategy can be applied sufficiently and economically in the loess plateau. We are confident to suggest these procedures as feasible alternatives that would result in satisfactory environmental compliance. "Green" technologies without the potential risk to human health and crop quality are preferred for technical planning; however, recommendations must be made based on good evidence of safety and effectiveness. Scientific restoration and management of contaminated soil using phytoremediation can create a self-sustaining ecosystem that is resilient, largely self-maintaining, and provides a large ecological benefit. During the remediation design process, remediation plans should identify and, if possible, quantify potential avenues for ecological exposure to toxic compounds such as accumulation of toxic compounds in selected plants. In evaluating ecosystem restoration, it is important to compare the relative ecological risks posed by phytoremediation to those risks already occurring on site or those risks posed by alternative cleanup methods.

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PHYTOREMEDIATION OF CONTAMINATED GROUNDWATER

L. NEWMAN*
Brookhaven National Laboratory, Biology Department,
Upton, NY 11973 USA

Abstract Groundwater contamination is a problem facing many if not all nations. Most common engineering solutions are expensive and require dedicated personnel to set up and maintain. Phytoremediation offers a low cost, low technology approach to remediate groundwater. Phytoremediation has been shown to be affective for a variety of contaminants, with plants capable of degrading the chemicals to non-toxic metabolites. Ways are being developed to assist plants in reaching deep aquifer waters beyond the root zones of trees. Plant are being examined to determine the genes involved in the metabolism of the contaminants, as well as genetic engineering of plants for enhanced capabilities. Plant associated microbes are also being explored as a way to improve phytoremediation capabilities.

Keywords: phytoremediation, groundwater, solvents, hybrid poplar, combination technologies

1. Introduction and History

Environmental contamination can occur across all matrixes: air, water, and soil. Many types of contamination are readily apparent: mine spoils, petroleum spills, and air pollutants spewing from smoke stacks. However, some types remain hidden and are not readily apparent until people start to have health problems. Groundwater contamination falls into this category. For many people, groundwater is the source of water for drinking, cooking, and hygiene. And when the water becomes contaminated, exposure comes through several routes – dermal contact, ingestion, and inhalation; so even low levels of contamination in the water can have ready access to the human population that depends on the water.

*To whom correspondence should be addressed.

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Because shallow aquifers can easily become polluted from improper disposal of waste, virtually all industrial societies have groundwater pollution problems. However, because it is not readily visible with massive contamination sites or clouds of pollutants rolling across the landscape, people are not always aware of the problem; thus, monitoring and detection rates are lower than for other polluted sites. In societies where there are limited resources or will to deal with environmental problems, groundwater problems are easily ignored.

Most groundwater pollution remediation and monitoring is costly and requires equipment that takes a level of sophistication to install and maintain. Monitoring requires drilling wells and specialized equipment for sampling, and often special handling of samples prior to analysis.

Remediation is most often done through expensive and complicated equipment. Pump-and-treat systems using activated carbon filtration are the least expensive or complicated, but even these can include U.V. oxidation, stripping towers, chemical oxidation, air stripping, soil vapor extraction, or the introduction of reactive chemicals into the aquifer to catalyze oxidation. In the 1960s and 1970s, researchers started looking at using biological systems, namely microbes, to degrade pollutants, including those found in groundwater. However, keeping the microbes at biologically active concentrations when released in the environment proved challenging.

In the late 1980s and early 1990s, a handful of researchers started looking in another direction; namely, using plants rather than microbes to remediate contaminated groundwater. These pioneers included Dr. Milton Gordon at the University of Washington, along with colleagues Drs. Stuart Strand, Paul Heilman, and Lee Newman; Dr. Jerald Schnoor at the University of Iowa along with student Dr. Louis Licht; and Dr. Raymond Hinchman at Argonne National Laboratory with colleagues Drs. Christina Negri and Ed Gatliff. Others also contributed to the field, but these three were some of the first to do so.

In practice, the idea is that the plants take up water, and when in the presence of water-soluble compounds, will take up the compounds as well. The goal would be to select plants that are also capable of degrading the compound, rather than releasing it to the atmosphere along with the water vapor. The plant of choice for all groups was the hybrid poplar.

Poplars have been planted as windbreaks in Europe for centuries, and in the early 1900s people began to hybridize the European, American, and Asian varieties to induce disease resistance and increase growth. Much of the early work on the hybridization program was funded by the forestry industry. In the 1970s when the United States suffered its first energy crisis, the U.S. Department of Energy started funding poplar breeding programs to use the plant as a bioenergy feedstock. Major breeding centers for poplars in the U.S. developed in the upper Midwest and the Pacific Northwest sections of the country. As the energy crisis waned, there was less interest in growing poplars for biofuels and more interest from the paper industry to grow poplar for pulp production.

With this large amount of data about family lines, disease resistance, and most critically, growth and water uptake availability, poplar was a natural choice for early studies and deployment of phytoremediation. Hybrid poplars grow exceedingly rapidly, with varieties that can average 3–5 m per year for the first 5–7 years of growth. Along with this growth is a comparable water uptake rate to supply the rapidly growing plant.

In recent years, the variety of plants used to phytoremediate ground water has increased. Willow, which is closely related to poplar, is another plant used for phytoremediation due to its rapid growth and high water uptake capabilities, as is the eucalyptus tree. Other rapidly growing, high-water-using plants are also being studied. In many cases, the plants being considered are native to a given area, in that the plantings can be used not only for remediation but also for site reclamation purposes. Many studies have been done in recent years looking at other species of plants to determine their ability to take up and degrade groundwater contaminants.

2. Plant Selection

More than for any other form of phytoremediation, the plants used to treat groundwater contamination need to be healthy and actively transpiring; rapid growth is also a big plus. Therefore, the first selection criteria needs to be, will this plant thrive on this site? The decision has to take into consideration not only will the plant thrive in a particular geographic region, but also, will it thrive in the ecological niche of the site to be remediated? If the site has other contaminants, such as heavy metals, does the plant have a high degree of tolerance to heavy metal stress? If the site is in a low-lying area, is the plant capable of handling periodic flooding? If the area has very sandy soil, can the plant handle low water conditions for its upper roots and while it is getting established? Some of these conditions can be altered or managed with engineering solutions, but each engineered measure increases cost.

The second consideration is the goal of the remedial action. If the goal is only to remediate the site, then any workable plant species can be considered. However, if there is a secondary goal, such as restoring the site to ‘natural’ conditions, like remaking the site into a park or public-use facility, then plants have to be selected that will meet those requirements.

And finally, the plant has to be able to interact with the contaminant in a viable way. This means that the plant roots should be able to reach the contaminant; the plant should be able to take up the contaminant; the plant should have at least a moderate resistance to the toxicity of the contaminant; and the plant should ideally be able to degrade the contaminant. Again, some of these criteria are not absolutely critical, as will be addressed later, but if most or all of these criteria can be met, the result will be lower costs for implementation.

As previously mentioned, hybrid poplar is often the plant of choice for groundwater remediation. Its rapid growth, high water intake, and broad range of growing conditions make it an excellent choice for this type of remediation. Additionally, poplars in general do not take up a significant level of heavy metals (although there are varieties that can take up bio-available metals), thus rendering them less susceptible to metal toxicity on sites with multiple types of contamination.

In recent years many other plants have been used depending on the site location and plans. Willows are another common plant selection, as are Eucalyptus plants in Australia, Koa trees in Hawaii, as well as a variety of ‘native’ plants to other locations. Common features for plants chosen for groundwater remediation are rapid growth and high water uptake rates.

3. Plant Contaminant Interactions

For optimal results, plants should not only take up the contaminant in the groundwater but should also degrade the contaminant within the plant tissue to non-toxic metabolites or to carbon dioxide, which is then released to the atmosphere or utilized within the plant’s photosynthetic pathway. In 1994, Sandermann proposed the ‘green liver’ theory, which states that plants have many of the same metabolic enzymes as mammals, and that the entire plant has the potential to detoxify contaminants the same way as a mammalian liver with its associated enzymes.

In 1997, the Gordon group (Newman et al., 1997) demonstrated that plant cultures that were free of bacteria were able to degrade trichloroethylene (TCE) to the same metabolites seen in human metabolism of the compound, trichloroethanol and di- and tri-chloro acetic acid. These same plants cells were also able to degrade the TCE to carbon dioxide, albeit at low levels. Whole plant systems were also able to degrade the TCE, but interestingly, the ratio of metabolites was different in the different plant tissues, with the trichloroacetic acid being most prevalent in the leaves. Later work by the same group (Newman et al., 1999) showed the production of the same metabolites in plants grown in the field and exposed to controlled levels of TCE.

Later studies looking at carbon tetrachloride (Wang et al., 2004) and perchloroethylene (James et al., 2009) have shown that not all compounds are degraded as rapidly or completely. Methyl-tertiary-butyl ether (MTBE), a common gasoline additive and common groundwater contaminant, is degraded little in trees (Ma et al., 2004), and in the case pine and poplar, a significant fractions volatilized to the atmosphere (Arnold et al., 2007). It should be noted there are many other compounds in contaminanted groundwater that have been successfully phytoremediated, including benzene, 1, 4-dioxane, arsenic, perchlorate, and nitrates, to name a few.

4. Designing a Site and Other Factors to Consider

The application of phytoremediation is different than with other remediation technologies in that there is a need to think like a farmer, rather than an engineer. Installing electrical equipment or a concrete pad does not have the same needs as installing a living biological system. Thus, for many engineers, collaborating with local farmers or agriculture or forestry personnel can be invaluable.

When thinking like a farmer planning a site, it is important to consider plant requirements for healthy growth. Soil with sufficient nutrients, access to water until the plants get established, and sufficient sunlight for growth are all important. Timing is important as well when preparing the soil, as you do not want to be plowing during the rainy season or when the soil is frozen.

Security can be more of an issue, as it is more difficult to secure a field than a building with doors and locks. And it is not just people that the site needs to be secured against; planting trees can be an irresistible lure to the local wildlife, and a herd of deer can kill off an entire plantation in a very short time. Other, smaller, wildlife such as insects that feed on trees must be managed, and a plan needs to be in place to handle all these issues.

Timing of the planting is important as well, as you want to make sure that the trees have sufficient time to become established before it becomes either too hot or too cold for them to survive. This means that the site has to be prepped and ready for the trees to be installed as soon as the weather is warm enough to support them.

4.1. MOST COMMON PROBLEM AND SOLUTION

The most common problem that prevents the deployment of phytoremediation to treat groundwater contamination is concern about the ability of plants to actually reach and interact with the groundwater contamination. There can be many reasons why this might not happen, but three of the major ones are depth to groundwater, interfering geological formations, or a thick aquifer that would prevent roots from reaching the contamination. Even relatively shallow depths to groundwater can also be a problem if there is a high precipitation level in the area, and the soil has a high water-holding capacity that eliminates the need for the roots to reach out to the groundwater. Intervening geological formations can be a shale layer, heavy clay, or even an intervening clean aquifer. Thickness of the aquifer can have an impact on the ability of the plants to reach the contamination. Compounds that are heavier than water, such as TCE, may be most concentrated at the bottom of the aquifer, and even if dispersed evenly throughout the aquifer, the volume of water moving through a thick aquifer may make it difficult for the overlaying plants to have a strong impact it.

4.1.1. Deep Rooting Methods

Most hybrid poplars are planted with short stem cuttings, with the cutting being between 9 and 18 in. in length. However, much longer cuttings are available, and this has led to the development of deep rooting of the cuttings for more rapid phytoremediation. This method works well for aquifers that are up to 25 ft in depth.

Many levels of complexity are involved in the deep-rooting of the cuttings. At its most basic, holes or trenches are dug that reach deep into the ground, sometimes down to the water table, and the extended cutting is planted in the hole or trench. If the depth to groundwater is relatively shallow, and the major problem is either intervening geological formations or high precipitation levels, this is a fast way to get tree roots directly in contact with the contaminated aquifer. This method has been used by companies such as Ecolotree, which was founded by Dr. Louis Licht (http://www.ecolotree.com/).

However, much more complex methods have been developed. Dr. Ed Gattliff of Applied Natural Sciences, who developed and patented a technology called TreeMediation that will reach more complex deep-water aquifers (http://www.treemediation.com/). In this technique, holes are drilled in the soil down to the aquifer. The hole is lined to prevent lateral root growth along the length of the stem, and a long cutting, either rooted or unrooted, is placed in the hole. The hole is backfilled with a variety of materials, depending on the site. Because the cutting cannot send out lateral roots, all its rooting energy is diverted to the bottom of the cutting that is in the aquifer. Depending on the depth to the aquifer, it may be necessary to supply nutrients and oxygen through tubing along the length of the cutting. This method allows for direct root contact of the tree with the contaminated aquifer as soon as the cutting starts to develop roots.

This technology has been used on many sites for a variety of contaminants, including solvents, nitrogen, and tritium. Drawbacks of this technology are the increased cost for planting, and the decrease in plant stability within the soil due to the inhibition of lateral root formation that the plant depends on to seek out nutrients.

4.1.2. Pump and Irrigate

Another method for getting plant roots into contact with contaminated groundwater relies on the traditional agronomic practice of irrigation. With a pump-and-irrigate technology, problems of deep or thick aquifers are eliminated, and trees are in contact with the contaminated groundwater from the time the irrigation system is turned on. Additionally, the well or wells can be sunk such that they draw from the most contaminated part of the aquifer, releiving the worry about having the plants draw water from portions of the aquifer that have lower levels of contamination. The situation of the plantation can also be adjusted so that it does not have to sit directly above the contamination, but can be located where there is sufficient land for the trees.

This type of system does need additional monitoring. Suction lysimeters are typically deployed to ensure that the contamination does not migrate beyond the root zone of the trees. This is especially important if the plantation is situated above a clean area, or if there is an intervening clean aquifer between the soil surface and the contaminated aquifer. Our group has deployed this type of system for the remediation of 1,1,1-trichloroethane, trichloroethylene, nitrates, and mixed organic waste from a landfill. Other groups have used this as well for treatment of landfill leachate to prevent it from entering groundwater systems.

The advantage of this type of system is there are no depth limitations, nor is there a problem when geological formations are between the soil surface and the contaminated groundwater. The plants start remediation as soon as the system is turned on, and the plantation can be managed as a more typical tree plantation to optimize growth. Drought years will not tax the plants; in fact, the system will be more efficient if the trees are getting water only through the irrigation system. Additionally, the amount of water remediated can be monitored by installing a flow meter on the system. Drawbacks of the system are increased maintenance of the irrigation system, the need to monitor the soil pore water through the lysimeters, and the reliance of the trees on the irrigation, making it essential that the system stays on through the summer months.

4.2. MONITORING

Monitoring a phytoremediation groundwater site has many of the same components as any other remedial action site: monitoring the flow rate of the water under the site, the concentration of the contaminant at check points, and looking for metabolites that would indicate that degradation of the contaminant is occurring.

However, with phytoremediation there are many other factors to consider. First and most obvious is monitoring the health of the trees. Phytoremediation of groundwater is an active process, with the removal of groundwater taking place through the innate transpiration of water and the co-uptake of the contaminant. If the plants are stressed, not actively growing or transpiring, or worse, dead, then there will be no phytoremediation of groundwater. The next parameter to check is to determine if the tree roots are in contact with the groundwater. This can be done by direct observation, either through excavation of areas around the base of the tree to see if the roots are into the groundwater, or if the soil allows, the installation of minirhizotrons will allow for the in situ observation of root development without the need for excavation.

A physical parameter that differs from standard measurements is the groundwater elevation. There are many discussion as to the impact of the trees on the groundwater and whether the trees cause a cone of depression in the center of the plantation due to water uptake, or if they will raise water levels as they remove more water from the aquifer. Thus, although this is one of the measurements asked for, it is still not clear what result would best indicate that the trees are impacting the aquifer.

The most critical observation to take place on the phytoremediation site is the analysis of the plant tissue. Tree core observations have been done my many groups, including Vroblesky (Vroblesky et al., 1999) and Burken (Ma and Burken, 2003). This method of monitoring trees for uptake of the parent compound looks at the level of the contaminant in the tree trunk before it is metabolized in the leaf tissue. The second tree tissue routinely examined is the leaves. Leaves are collected and stored (−80°C is best) and then analyzed for the presence of either the parent compound or metabolites. This work was first done for TCE by Gordon (Newman et al., 1997), but since then many others have looked for a variety of compounds in the leaf. Finally, transpiration of the compound or metabolites from leave tissue is examined. This can be done with Teflon bags wrapped around individual leaf clusters, or using more complex instrumentation such as open path FT-IR (Newman et al., 1999).

Presence of either parent compound or metabolite in the plant tissue is the best indication that the plant is taking up the contaminant. Little or no transpiration and higher levels of metabolites than parent compound in the leaves are all positive signs that the plants are taking up the contaminant, but are metabolizing it before it is released to the atmosphere.

5. Genetic Manipulation of Plants

Although many plants appear to have the ability to degrade chlorinated solvents and other groundwater contaminants, there are still volatile emissions from plants as not all of the contaminant is degraded during its residence time in the plant. This has led some researchers to try to understand the molecular basis for degradation of contaminant, and also to insert genes into the plant that will increase the degradation rate. There has been considerable work done to engineer plants for increased metal tolerance or herbicide degradation, and recently to degrade energetic compounds such as TNT or RDX (reviewed in Doty, 2008; Van Aken, 2008; and Dowling and Doty, 2009). However, much less work has been done to engineer plants for enhanced remediation of groundwater.

Strycharz et al. (2009) has done work to identify the P450 genes in plants that may be involved in the degradation of contaminants such as TCE. Gordon (Doty et al., 2000; Banerjee, 2002) and then Doty et al. (2007) have led the research in placing exogenous P450 genes from mammalian systems into plants to increase degradation of chlorinated solvents. These engineered plants have much higher degradation rates for groundwater solvents, but use of mammalian genes in plants for these types of uses still generates much discussion.

6. Plants and Bacteria

A new area being explored is the role of bacteria that colonize the vascular tissue of plants, the endophytes. Dr. Jerald Schnoor recently discovered an endophytic bacteria-colonizing poplar that has the ability to degrade energetic compounds. And Drs. Daniel van der Lelie, Safiyh Taghavi, and Jaco Vangronsveld have identified dozens of endophytic bacteria from hybrid poplars and other plants and have engineered some of these to assist plants in degradation of groundwater contaminants (Barac et al., 2004; Van Aken et al., 2004). Some of these bacteria, particularly Enterobacter sp. 638, seem to have a significant impact on plant growth (Taghavi et al., 2009). There have been several recent articles about these bacteria, including analysis of genomes that have been sequenced by the Department of Energy's Joint Genome Institute.

There exists the potential for these bacteria to be genetically manipulated to include genes encoding degradative pathways for common organic groundwater contaminants. Also, as plant growth is a factor in plant water, and therefore contaminant uptake, simply having faster growing plants will enhance the phytoremediation effect. There is also the potential for plants being used for phytoremediation to also be harvested for bio-energy production. The idea that plants can be grown on waste sites and nonagricultural land, and clean the site as well as produce biomass needed for energy production, is exceedingly attractive.

7. Acceptance by the Public and Regulators

In general, phytoremediation to treat groundwater contamination is a technology that is well received by the general public. Most people are able to understand the need for long-term treatment options for the remediation of aquifers that have been contaminated during the course of years or decades. The more expensive and complex to install and operate systems are important for when there is an immediate potential impact on human and environmental health or when contamination levels would be toxic to the plants, or when the spill has just occurred and immediate physical removal would be most protective of the environment.

However, for long-term application where there is a desire to get away from heavily instrumented and engineered sites, phytoremediation is an excellent option. Communities can support and assist in the maintenance of the trees, and often take pride in caring for living systems that are cleansing their environment.

Regulators, depending on their comfort level and knowledge about the technology, may have more concerns. Their job is to protect the environment, and for many there is the feeling that phytoremediation is not yet a 100% proven technology. Depending on the contaminant of concern, the plant may not be able to fully degrade the chemical within the plant tissue, which can lead to a release of the chemical to the atmosphere. In some areas where there is either a zero emission policy for remedial technologies, or in areas where there is a large human population very close to or immediately down-wind of the remediation site, volatilization of the contaminant may be a concern.

There can also be concerns about animals feeding on the plants or the general hardiness of the plants. Seasonal variations will also have a large impact on efficiency of the site; areas with long winters, where plants will not be actively transpiring and thus removing contaminant from the aquifer, may need either a backup technology for that time or may not be suitable for phytoremediation.

However, most regulators have been interested in the technology and are willing to learn more about it. And the more regulators and the effected public learn about new technologies, the more they will turn to them when faced with problems such as contaminated groundwater.

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EVAPOTRANSPIRATION COVERS FOR LANDFILLS

S. A. ROCK*
United States Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, 26 W. Martin Luther King Blvd., Cincinnati, Ohio 45268, rock.steven@epa.gov

Abstract Safe disposal or containment of waste continues to be one of the world's largest environmental challenges. If not properly handled, wastes from municipal, commercial, industrial, and mining sources can pollute surface and groundwater, and release damaging gases. One potentially useful technology is the evapotranspiration (ET) cover for landfills and waste sites. Designed to use engineered soil and vegetation layers, an ET cover absorbs, holds, and releases precipitation in order to minimize percolation into the waste mass. This chapter describes some of the physical, legal, and economic considerations of ET covers.

Keywords: evapotranspiration covers, landfill covers, solid waste, phytotechnologies

Waste generation in the United States, from all residential, commercial, and industrial sources is about 2 kg per capita per day. Despite efforts to encourage recycling, waste reduction, and other alternatives, landfill disposal is the primary method used in this country to handle this waste stream. Most of the waste generated is contained in lined facilities. There is some diversion or separation of waste streams mandated by local law. Many areas do not allow disposal of "green waste," tree trimmings and yard waste, in landfills. These materials tend to be composted. Certain materials, such as batteries, solvents, and paints, are not allowed to be landfilled with municipal waste. Automobile and truck tires are also restricted from landfill disposal. Modern American landfills tend to be well engineered, highly controlled, and very large compared to landfills only a few decades ago.

*To whom correspondence should be addressed.

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In addition to the highly controlled sector of landfills, there are also uncounted former landfills, abandoned landfills, and open dumps that were started, filled, and closed in a non-regulated or pre-regulation environment, leaving a legacy of potential environmental problems. Many of those sites contain mixed municipal and industrial waste, and none of them are likely to have excluded any materials from the waste mass.

1. Background

There are three main areas of regulatory concern regarding landfilled waste, and the same broad concerns apply to working, closed, or abandoned sites. The three areas threatened by landfills are water pollution, air pollution, and physical contact. Both groundwater and surface water may be contaminated if it comes in contact with waste by groundwater or precipitation. Direct physical or dust-borne contact with waste can spread contaminants and disease. Rodents and other vectors who contact waste can also spread disease. Release to the air of toxics and climate-change gases may have local and global impact.

The most immediate environmental hazard from landfills is the threat to groundwater. Precipitation as either rain or snow can infiltrate the surface of a waste mass, percolate through the mass, pick up dissolved or suspended contaminants, and carry those contaminants as leachate to groundwater. This contaminated groundwater, or the leachate, can travel to drinking water wells or emerge as surface water, sometimes carrying the pollutants for long distances.

The second regulatory concern is the generation of gases through biological activity in a landfill. Organic waste such as food, yard waste, and paper can be consumed by microbes in the presence of water and in the absence of oxygen to produce methane and carbon dioxide, two primary greenhouse gases. Methane from landfills has also been known to cause explosions and fires when unintentionally concentrated and accidentally ignited.

The third regulatory concern is physical contact with the waste, either through direct exposure to humans, through support of rodents and other disease vectors, or through litter scattered by wind. Landfill design and regulatory strategies have been designed to prevent or mitigate these three concerns.

In 1976, the U.S. Resource Conservation and Recovery Act (RCRA) established regulations for landfill establishment, operation, and closure. The protective strategy adopted by RCRA was to surround the waste with an impermeable wrapping that prevented aqueous and gaseous transport pathways, effectively isolating the waste from water which triggers both gas production and leachate formation. This was to be accomplished through a system of bottom liners, careful layering of waste, engineered slopes, and an impermeable cover. Any precipitation that landed on the site would be diverted away from the waste, leaving it dry and isolated from transport mechanisms, and biologically inactive. Many variations of materials and techniques can be employed to accomplish this isolation, all of which may be grouped as conventional covers.

In a conventional cover system, the water balance for a site is very simple: input of water in the form of rain or snow (precipitation) equals outflow of water in the form of runoff. An alternative cover paradigm may be as effective in protecting groundwater, isolating waste from contact, and preventing gas generation. In fact, there may be several effective alternate paradigms, although this chapter focuses only on evapotranspiration (ET) covers. Also known as vegetative covers, ET covers work by turning the landfill cover into a site-specific vegetated ecosystem that fuctions as an engineered water handling system.

2. ET Cover Design Considerations

Using plant technologies to affect environmental improvement is not startlingly new. People have been growing plants to modify their environment for millennia. In contrast to a conventional cover, the ET cover system does not aim for total exclusion of water from the site. The system, which is sometimes called a 'sponge-and-pump' in contrast to the conventional 'raincoat' cover, allows a certain amount of water to be stored in the soil-root layer or rhizosphere, where it is held until the plants can use and transpire it. The water balance for an ET cover is somewhat more complicated than the conventional cover: the input of water equals the interception by plants, runoff, plus storage followed by evapotranspiration. Evapotranspiration is the combination of evaporation that would occur in a particular spot in the absence of plants, and the transpiration that occurs as plants process water for nutrient transport, cooling, and structure. The rate of ET depends on plants species and placement, as well as climatological characteristics of temperature, wind speed, humidity, and growing season. Evapotranspiration can be estimated using the Penman-Monteith equations.

Because ET covers work differently in different areas, all installations need site-specific designs. Climate-based applications can be broadly understood on a regional basis by separating a region (country or continent) by annual precipitation or by native climax ecosystems. The best candidate sites are those where native climax vegetation evapotranspiration exceeds precipitation. Generally speaking, those parts of the region that receive between 20 and 50 cm of precipitation each year are considered semi-arid with a native climax ecosystem of prairie grassland. Areas with more than 50 cm per year are classified as humid with a native climax woodlands or forest. Less than 20 cm per year is possibly too arid to sustain dense vegetative cover, although evaporation in very arid areas tends to far exceed precipitation.

Design of an ET cover in any climate depends on the water-holding capacity of the soil. Water must be stored in the layer of soil within the range of plant influence, either in direct contact with the roots or within range of the effect of plant capillary suction. Plant root influence also depends on soil characteristics. For example, a sandy soil will allow easier penetration by roots, while a more silty soil will hold more water but restrict root penetration. Climate determines types of plants available, while soil type determines water-holding capacity and hence soil cap depth needed for water storage. Therefore, depth of the soil layer influences plant selection by dictating the necessary root architecture.

In arid and semi-arid prairie grasslands, a tremendous diversity of plants can be utilized for ET covers. Some thrive during the cool and wet spring months but then yield to hot weather species during the summer. Some spread quickly into disturbed areas while others wait for the shade provided by the early species. Some have spreading, shallow root systems and some extend long roots that give them the capacity to withstand droughts. These plant characteristics help determine which species or combination of species will tap into and use the water that will be stored in the cover system. Prairie species, like most plants, have most of their roots in the top meter of soil, although some grasses send roots 8 m deep or deeper. In semi-arid areas, most ET covers are designed with a 1-m water-holding layer that is planted with a variety of plant species. In wetter climates, a thicker soil layer is needed to capture the greater precipitation. Since trees have a greater root structure than grasses to support their larger biomass, designers of ET covers can use more depth for water holding. It is possible to design a water-storage layer more than 2 m thick that may be within the root zone of some trees.

A storage component of an ET system that relies on a very thick soil layer for water-holding capacity has an additional consideration, however – it may be uneconomical to install such a layer. One type of design uses an installed soil layer that is about 1 m, and in addition, this design relies on the existing cover and waste mass itself to provide additional water-storage capacity. In some older landfills that were closed with a soil cap before RCRA laws were enacted or simply abandoned without proper closure, the boundary between surface soil and waste can be indistinct. On several sites that have clean soil containing increasing percentages of waste with depth, the U.S. EPA has noted trees growing quite well, presumably sending roots into and amongst the waste. Among these sites are the Center Hill landfill in Cincinnati, Ohio that received municipal waste and incinerator ash; the Ohio EPA lead (Green II) site, near Logan Ohio, that received municipal and hazardous wastes; and the U.S. federal Superfund sites known as the Industrial Excess Landfill and the Woodlawn Landfill, both having received mixed industrial and municipal waste. Each of these sites did not discourage incidental vegetation, and supported levels of grasses and trees that apparently rooted into the waste layer without detrimental effect to the plants.

The most common trees proposed for use on ET covers are hybrid poplars or hybrid willows. These trees are members of the Salicaceae family. They are hydrophilic and phreatophytic, which means they tend to thrive in water-rich areas, are undamaged by overwatering or inundation, and withstand drought with a deep and extensive root architecture. Despite popular misconceptions, tree roots do not seek water nor do they sense water behind barriers. Trees can and will follow water through a soil column, and where possible, roots will follow and extend as deep as necessary to obtain sufficient moisture. Some groundwater fluctuates annually or seasonally. Therefore, a rising water table may inundate tree roots. Many tree species will shed or slough off roots that are under water and deprived of oxygen. Some non-Salicaceae trees may even die under these circumstances. Phreatophytes maintain their roots even when saturated and are still in place when the water table descends. Therefore, they are immediately ready to draw water from the deeper seasonal water level.

Methods for planting a vegetation cover rely on site-specific designs based on local microclimate, time of year, soil characteristics, and topography. Planting techniques depend on the site and species selected. For trees of the Salicaceae family, there are several successful tree establishment methods and at least an equal number of ways to be unsuccessful. Trees may be purchased from commercial or public nurseries in a variety of sizes from one-half to 3 m tall. These species may be planted 1-m deep depending on the soil excavation method. For many years, the pulp, paper, and lumber industries, as well as the U.S. Forest Service, have developed cultivation techniques for short-rotation woody crops. It has been estimated that thousands of hybrid poplar varieties have been groomed through natural genetic selection in order to thrive in a wide range of conditions. Because these trees root from cuttings and grow quickly (2 m per year is not uncommon), they are useful for many applications such as biofuels, wind breaks, and wood products.

Design of the cover system must also take into account the seasonal nature of planted systems. Depending on climatological location, the 'pumping' or active transpiration phase of the growing season may be between 5 and 7 months when ET greatly exceeds precipitation. During the remainder of the year, any precipitation must either runoff, evaporate directly from the soil, or be stored in the soil. This seasonal storage must have the capacity to last throughout the dormant season until the vegetation leafs out and resumes active growth during the spring. Some areas have precipitation patterns that allow for this to easily occur, such as in the Midwestern U.S. where fall and winter weather is typically dry followed by rains and thunderstorms during the spring and summer. Other regions receive the bulk of their precipitation during the time when plants have shut down. These climate locations may need too much storage capacity to make the system feasible.

3. Regulation

Having plant roots in direct contact with the waste may raise regulatory and public concerns regarding safety of the ET cover, since the purpose of these systems is generally to isolate waste from contact. The general concern is that plant roots may take hazardous waste into their roots, transfer the chemicals to the aboveground portions of the plant, and release a hazard into the environment. This concern can generally be addressed by carefully considering characteristics of the plants, the subsurface environment, and the chemical of concern. For example, root zones or rhizospheres are conducive to microbial activity. One result of a biologically active root zone is that methane, which can be generated by anaerobic decomposition in a landfill situation, can be consumed in the aerobic portion of the soil. Rates for methane generation and degradation are dependent on waste composition, soil moisture, gas transfer mechanisms through soil, and extent of methane-consuming microbes available.

Furthermore, few environmental contaminants are taken up in their parent form by plants, and even fewer are released intact. Some plants stimulate the biodegradation of chemicals in situ, while others transform contaminants within the plant tissues. Collectively, these processes are known as phytoremediation. Some volatile solvents have been measured in the transpiration stream from a plant, but the quantities have been extremely low, and the concentrations in the groundwater have been much higher than would be expected at most landfills. Some plants do accumulate metals in leaves and shoots which may be consumed by insects and other herbivores, but again, in a landfill situation, this is a very unlikely scenario as the rate and extent of accumulation is generally very small.

In a small number of cases, it may be the intention of the cover designer to include a treatment component as part of the plan. Recently, a cover system was proposed that would remediate waste as a "moving front"

(Fletcher et al., 1997). In this system, an initial treatment phase would clean the soil. Then, the cleaned soil layer would be used for water-storage capacity in a containment phase. Such a plan may be technically feasible, though awkward to regulate since the environmental rules typically tend to rigidly separate treatment from containment. One strategy that was successfully proposed and accepted by the Ohio EPA for a former landfarm was to demonstrate containment in part due to ET vegetation and allow phytoremediation to continue during the post-closure period. The expectation on the part of the land owner was that the planted "treatment cover" would not only contain the waste, but at some point would remediate the contaminants to the target cleanup levels determined by the state. The site owner could then close the site under the clean closure rules, thereby discontinuing maintenance of the cover and runoff collection and treatment system, as well as eliminating periodic sampling events.

State regulations generally do not have provisions for these treatment covers. Similarly, there are no specific federal guidelines for phytoremediation or for ET covers. Therefore, these technologies are typically only accepted and considered truly protective if they can show performance that achieves the regulatory goals (ITRC, 2003, 2009). The U.S. federal RCRA requirements, which are often the basis for state regulations, include a provision that landfill covers should follow a specific set of guidelines, "or equivalent" RCRA. There is no guidance as to how to demonstrate equivalence, or even what the standard is to which an alternative should be equivalent. The situation becomes more complicated when an alternative involves a completely different way to accomplish the same goal, as when an ET cover is compared to a conventional cover.

In an effort to gather data for eventual guidance on alternative covers, the U.S. EPA Office of Research and Development (ORD), in conjunction with the Remediation Technology Development Forum (RTDF), launched the Alternative Cover Assessment Program (ACAP) in 1997. Phase one of this multi-year study involved a survey of existing field sites in conjunction with an analytical comparison of existing computer models for predicting and evaluating performance of various landfill cover systems. This survey discovered that despite 28 projects that measured alternative cover performance, none of the results were nationally applicable and few had any direct comparison to conventional covers. Similarly, while there were many computer models that have been used for cover systems, none was consistently accurate for the unique situation of the ET cover. Most of the codes tested did not account well for unsaturated episodic water flow such as follows rain events. Most did not incorporate the effect of vegetation that changes seasonally or more often. Phase two of the project was installation of 12 field sites across the country during 1999 and 2000. These sites are located in eight U.S. states that span the range of climates from semi-arid to very humid, from southern Georgia to Utah to Oregon. Many of the sites have installed drainage lysimeters that can directly compare two cover systems, such as an ET cover constructed immediately adjacent to a conventional cover. Phase three, underway currently, involves collecting and analyzing the data from the test facilities (Albright et al., 2004; Benson et al., 2001). More information on ACAP can be found on the Internet at www.DRI.edu. Each site involved in the ACAP has a site-specific design, usually paired with an appropriate conventional cover for the site. Since the climatic range of the different test sites is broad, there is an equally great variety in ET cover configurations.

Although percolation from a prescriptive cover is site specific, some estimates on typical expected percolation rates can be derived from the literature. An alternative cover can be said to be equivalent to a soil cover (e.g., an impermeable cover design) if the percolation rate is less than 10 mm/year in semi-arid and drier climates, or less than 30 mm/year in humid climates (Bolen et al., 1999, 2001). In order to estimate percolation rates, a water-balance evaluation can be conducted which consists of measuring and/or estimating all variables in the water balance including precipitation (P), runoff (R), evapotranspiration (ET), and percolation or infiltration (Pr).

$$P = Pr + ET + R$$

Accuracy of each variable depends on installation, location, quality of instruments, and time for monitoring and analysis.

Methods of estimating evapotranspiration (ET) vary in precision and accuracy as well. Potential evapotranspiration (PET) can be estimated with reasonable accuracy given accurate measurements of wind, solar incidence, humidity, and precipitation. However, this is not entirely applicable since at most sites ET will be less than PET during at least some portion of the year. There are commercially available devices and methods for estimating water use from crop plants, grasses, and trees, which may be employed to derive a reasonable site-specific estimate of ET at a given time. Extrapolation from point data to a season or year is best done conservatively. For example, sap-flow measurements from a stand of cottonwood trees at a project in Texas indicated that water usage by those trees varied considerably from day to day and month to month depending on weather and water availability, from 15 l per tree per day in mid summer, to 1 l in October in the first full growing season (USEPA, 2003). Only after measuring for several entire days each month during two growing seasons was there any confidence in predicting the water usage of those trees over time.

Surface runoff (R) can be measured using a catchment system such as diversion structures that prevent runon from adjacent areas and capture runoff for measurement. There are standard engineering techniques for diverting and measuring runoff with a high degree of accuracy. Finally, percolation (Pr) can only be measured by catchment in the subsurface, which requires construction of a test facility or lysimeter designed for that purpose.

4. Economics

ET cover applications have a fairly recent history with the first tree covers planted in the 1980s and the first prairie covers tested around the same time. Since that time there have been nearly 100 full-scale installations in the U.S. ranging from a few hectares to more than 600 ha. Sites that have been closed with ET covers include Superfund, industrial, mine waste, and municipal solid waste sites.

Although precise cost estimates are hard to find and difficult to verify, ET covers seem to cost half or less to design, install, and maintain than conventional covers. Some sites report savings more than $100,000 per ha, leading to total installed savings of tens of millions of dollars per installation.

There may also be long-term maintenance savings as ET covers are somewhat self-repairing in that a thick vegetative cover discourages erosion, and an unconsolidated monolayer of soil tends to fill in gaps that would otherwise be the result of waste settling and earth shifting.

It is also conceivable that an ET cover with trees or other crops could be cultivated to produce commercial products. Wood could be harvested on a rotation that allows continuous landfill coverage, for example every year every fifth row of trees could be harvested. Cutting 20% of a tree cover would not significantly decrease the system efficacy. Since poplar and willow trees can be harvested to encourage re-growth from their stumps, the vegetation would regenerate, thus ensuring a continuous supply of wood while maintaining the ET cover. Such a system could produce an additional income stream for landfill operators. Trees and woody shrubs also sequester carbon from atmospheric carbon dioxide. At some point there may be commercial value in carbon credits for stored biomass. Another by-product of the establishment of an ET cover that mirrors a native ecosystem is habitat creation for birds, insects, and mammals. Diversity in plants translates into increased food and shelter opportunities for wildlife. Sometimes public sentiment favors a wooded vista over the traditional closed-landfill grassy knoll.

Any planted system will require time to develop. Grasses or trees planted in 1 year will not reach full capacity for several years. In some cases, time is not a critical factor; while in other cases, a multi-year development time would be unacceptable. Some abandoned sites have developed sustainable ecosystems in the absence of human intervention. In a few cases it might be acceptable to understand that an ET cover has established itself on an untended site and could be protective of human health and the environment.

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