*Chapter 27*

# Potential Use of *Populus* for Phytoremediation of Environmental Pollution in Riparian Zones[1]

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## Introduction

Environmental pollution is a serious threat to human life and to our ecosystems. Riparian zones, the narrow band of land between terrestrial and aquatic systems, are especially vulnerable to environmental pollution because many pollutants are transported through these systems via surface or subsurface runoff. Pollutants include fertilizers (e.g., nitrates), pesticides, agrichemical by-products, heavy metals, trichloroethylene, halogenated phenolics, and other waste products (Schoeneberger 1994). Because agricultural and industrial pollutants are widespread, there is increasing interest on organisms that accumulate, detoxify, or degrade these substances. While it is known that plants and microorganisms modify their environments, their potential use as mitigative tools to clean pollutants has only recently gained acceptance (Brown 1995). Woody perennial plants are ideal for remedial purposes because they can be planted over large areas at low cost and can concentrate or degrade environmental pollutants over several years (Moffat 1995), while also providing other economic or ecological services. As metabolic pathways for pollutant detoxification, uptake, and/or degradation are described, woody plants can be selected or engineered to remediate specific environmental pollutants.

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## Why *Populus*?

*Populus* is well suited for use in phytoremediation (the use of specially selected and engineered plants for environmental remediation) plantings. *Populus* is easy to establish and grows quickly. Its high transpiration rate and wide-spreading root system make it ideal to intercept, absorb, degrade, and/or detoxify contaminants, while reducing soil erosion. Historically, this widely distributed genus has naturally grown in riparian areas, thus many genotypes are adapted for growth on potential remediation sites. *Populus* plantings are amenable to coppicing and short-rotation harvest, thereby helping to maintain sustained root vigor. Further, if a biofuels or fiber market is available, harvests can generate additional income that helps offset establishment costs (Strauss and Grado this volume). Although *Populus* is not part of the human food chain, many vertebrates and invertebrates use the trees for food, shelter, and reproductive sites. Such increases in biodiversity can contribute to sustained productivity of adjacent aquatic habitat and crop land (Dix et al. in press).

*Populus* is well studied, with established silvicultural, vegetative propagation, breeding, and harvesting protocols (Stettler et al. 1996). In addition, *Populus* is amenable to tissue culture manipulation, genetic engineering, and genetic mapping (various chapters this volume). Thus, *Populus* is an ideal candidate for genetic engineering and selection for absorption, detoxification, and/or degradation of environmental pollutants such as heavy metals, nitrates, pesticide residues, and other waste products.

## Pollutant-Neutralizing Trees

Plants have many mechanisms for neutralizing toxic pollutants including immobilization, absorption, and accumulation or sequestration of contaminants (except cytoplasmic toxins). Plants also support symbiotic, root-associated microorganisms that can contribute greatly to contaminant neutralization (Stomp et al. 1994). One example of phytoremediation is using metal accumulating plants to remove heavy metals from the soil. All plants can accumulate essential heavy metals from the environment and some accumulate nonessential metals such as cadmium (Cd), lead (Pb), and cobalt (Co) (Baker and Brooks 1989; Ernst et al. 1992; Ostry this volume; Salt et al. 1995). Tolerance to aluminum has been reported for several poplar and other plant species (Baker and Brooks 1989; Chung and Chun 1990; Ernst et al 1992). In addition, studies on landfills and municipal sludge recycling systems demonstrate the ability of *Populus* and other plants to take up and tolerate heavy metals and other potential pollutants (Salt et al. 1995; Schultz et al. 1995; Shrive et al. 1994). *Populus* designed for use in riparian remediation plantings also must be able to take up, translocate, and / or resist toxic pollutants. An ideal tree may have densely packed roots for inactivating toxins or removing them from the soil for subsequent translocation to the leaves and storage in roots or stems (Schoeneberger 1994; Stomp et al. 1994).

# Nitrates

One of the largest fertilizer pollutants from both agricultural and urban sources is nitrates ($NO_3$). Large amounts of nitrates have already entered ground and surface waters, adversely affecting the health of aquatic organisms, humans, and other components of the ecosystem (Duda 1982; Komor and Magner 1996; Lowrance 1992). Nitrates are also indirect by-products of livestock operations (Welsch 1991). Large root systems of certain *Populus* genotypes have an affinity for nitrates. For example, in simulated groundwater depletion studies *Populus* x *canadensis* roots substantially reduced the concentration of nitrates continuously over a 2-month period (O'Neill and Gordon 1994). Furthermore, wide-spread root systems of *Populus* are more effective than confined root systems in uptake of these nutrients (Licht 1992). Clonal and species differences in root growth and root physiology are common in *Populus* (Pregitzer et al. 1990). Nitrate uptake, although it varies with environmental conditions, is apparently heritable in selected *Populus* spp. (Nguyen et al. 1990; Pregitzer et al. 1990; Pregitzer and Friend 1996). Thus, planting *Populus* clones selected for their large root masses on sites with potential for nitrate runoff could increase absorption of nitrates. Nitrate uptake in these poplars could perhaps be further enhanced by genetic engineering or selective breeding of poplar for larger root mass or increased protein storage (Coleman this volume). For example, root system growth was stimulated in *P. alba* x *P. glandulosa* (Chung et al. 1989), *P. davidiana* Dode (Lee et al. 1989), *P. deltoides* x *P. nigra* (Charest et al. 1992) and *P. nigra* x *P. maximowczii* (Charest et al. 1992) by transformation with *Agrobacterium rhizogenes*.

## Chemical Tolerance and Detoxification

Herbicides are commonly sprayed on crops to eliminate competing weeds. However, herbicides and their by-products that reach streams by direct runoff, leaching, erosion, and other processes can be toxic to aquatic plants and animals. For planting between the crops and streams, herbicide-tolerant poplars could be produced or selected to remove, detoxify, degrade, or tolerate selected pollutants including herbicides. *In vitro* selection, genetic engineering, genetic screening (e.g., marker- assisted selection), and other molecular techniques have potential for producing or selecting genotypes with improved remediation efficiency.

### Chemical tolerance

*In vitro* techniques were developed for detecting somaclonal variation in the tolerance of *Populus* to herbicides (Michler and Haissig 1988). Using such *in vitro* techniques, 4 hybrid *Populus* lines were selected for increased tolerance to glyphosate and sulfometuron methyl (Michler and Haissig 1988). In addition, several *Populus* variants selected for tolerance to sulfometuron methyl were found to have increased acetolactate synthase (ALS) activity (Michler 1993; Riemenschneider and Haissig 1991; Riemenschneider et al. 1988).

Genetic engineering techniques are available for transferring tolerance mechanisms into *Populus*. Mutant acetolactate synthase genes, *crs*1-1 and *als*, that confer resistance to sulfonylurea or the herbicide chlorsulfuron were individually used to transform *P. tremula* x *P. alba* (Brasileiro et al. 1992; Chupeau et al. 1994). Transgenic *Populus* cells containing the mutant *als* gene grew on a selective media containing 200 nM chlorsulfuron (Chupeau et al. 1994), and transgenic plants expressing the mutant *crs*1-1 gene were completely resistant to high doses of chlorsulfuron (Brasileiro et al. 1992). *P. alba* x *P. grandidentata* was genetically transformed with the mutant *aro*A gene for 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that confers tolerance to the herbicide glyphosate (N-(phosphonomethyl)-glycine) (Donahue et al. 1994; Fillatti et al. 1987; Karnosky et al. this volume). Transgenic *Populus* plants expressing the mutant *aro*A gene demonstrated more herbicide tolerance than the control *Populus* (Donahue et al. 1994).

In work to increase tolerance to air pollution, hybrid aspen (*P. sieboldii x P. grandidentata*) was transformed with an *E. coli* glutathione reductase (*GR*) gene (Endo et al. this volume). *GR*-expressing, transgenic aspen displayed resistance to oxidative stress caused by the herbicide paraquat (methyl viologen: 1,1-dimethyl-4,4-bipyridium dichloride) or sulfur dioxide ($SO_2$). This approach could potentially confer tolerance to oxidative stresses caused by other environmental pollutants. Pollutant-tolerant *Populus* could be further selected or engineered with remediatory functions, or used to support growth of remediatory microorganisms.

**Chemical detoxification**

Other remediatory approaches are aimed toward direct degradation or detoxification of toxic pollutants. *P. alba* x *P. tremula*, *P. tremula* x *P. alba*, and *P. trichocarpa* x *P. deltoides* hybrids were transformed with a *bar* gene that codes for the enzyme phosphinotricin acetyl transferase (PAT) (Chupeau et al. 1994; De Block 1990; Devillard 1992). PAT inactivates the commercial herbicide phosphinotricin (glufosinate, Basta) by acetylation (De Block 1990).

Ongoing work at the University of Washington and Washington State University demonstrated that *Populus* hybrids (*P. trichocarpa* x *P. deltoides*) can oxidize trichloroethylene (TCE) to produce carbon dioxide and other metabolites. Further experiments are underway to determine the capacity of *Populus* to remove and degrade TCE from groundwater (Strand et al. 1995). Pioneering work was initiated to enhance environmental detoxification by genetically engineering trees with genes that encode remediatory functions (Stomp et al. 1994). Two genes from *Alcaligenes eutrophus*, *tfd*B and *tfd*C, were isolated and cloned in an attempt to detoxify halogenated phenolics. One gene, *tfdB*, encodes a chlorophenol hydroxylase, and the other gene, *tfd*C, encodes a chlorocatecol 1,2-dioxygenase. Chlorophenols are sequentially hydroxylated by these 2 enzymes to form chlorocatechol. Subsequently, the ring is cleaved to create chloro-*cis-cis*-muconate. Initial tests are underway using 2,4-dichlorophenol, a breakdown product of 2,4-dichlorophenoxyacetic acid (2,4-D), and trichloroethylene. Gene constructs have been made with *tfd*B and *tfd*C under the control of a cauliflower mosaic virus (CaMV) *35S* constitutive promoter for transformation of *Populus*, black locust (*Robinia pseudoacacia*), and sweet gum (*Liquidambar styraciflua*). Subsequent studies are assessing active enzyme levels, uptake, and fate of TCE in these trees (Stomp et al. 1994). Such studies in direct detoxification further demonstrate the potential of *Populus* for phytoremediation.

## Soil Conditions and Microorganisms

Success of poplar plantings in remediating a riparian site is dependant on soil conditions and microorganisms. Soil chemistry plays a pivotal role in this process with soil pH and chelating agents affecting uptake of metals. For example, many metals in soils are bound to oxides. Plants can dissolve these oxides and enhance their solubility by releasing reductants from the roots. However, soil pH can influence metal bioavailablity and uptake. Plants growing in soils with low pH typically display higher metal toxicity because of decreased metal adsorption to soil particles. This, in turn, can increase concentrations of metals in the soil solution and subsequent leaching (Salt et al. 1995).

Trees support a diverse population of soil microorganisms, including bacteria, ecto- and endomycorrhizal fungi, actinomycetes, and blue green algae. In turn, many of these microorganisms help tree establishment and growth by greatly increasing the uploading capacity of roots. These soil microorganisms are instrumental in the processes of remediation, stabilization, and filtration of water and soil. Like trees, soil microorganisms participate directly or indirectly in these processes. Direct remediation occurs when organisms take up, store, detoxify, or degrade toxic compounds and their derivatives. Indirect remediation occurs through beneficial effects on associated organisms directly involved in remediation. Thus, overall effectiveness of remediation processes is based on interactions among the plant species, the type(s) of pollutants, and the soil microflora (Stomp et al. 1994).

Mycorrhizae, symbiotic associations between soil fungi and roots, can greatly increase the root surface area and provide a low-resistance pathway for water transport (Koide 1990). These symbioses can influence the plant's ability to take-up metals, and possibly influence plant tolerance to heavy metals. However, site conditions can influence the development of mycorrhizal associations. In natural conditions and with advanced stand age, *Populus* roots generally form ectomycorrhizae. When these roots are flooded, in the early stages of stand establishment, or in very fertile soils, they may form vesicular arbuscular mycorrhizae or no mycorrhizal associations (Heilman et. al. 1996).

Genetic selection and manipulation of rhizosphere microorganisms can potentially improve biological remediation of soil and water. Several ectomycorrhizal fungi immobilized the herbicide chlorpropham, while other ectomycorrhizal fungi degraded chlopropham to 3-chloroaniline (Rouillon et al. 1989). *Paxillus involutus*, an ectomycorrhizal colonizer of conifer and hardwood species, was transformed by particle bombardment with the hygromycin phosphotransferase gene (*HPT*) as a selectable marker and the β-glucuronidase (*GUS*) gene as a reporter gene. The transgenes were actively expressed after stable integration into the fungal genome, and the ability to form ectomycorrhizal roots was unaffected (Bills et al. 1995). Thus, the potential to genetically engineer mycorrhizal fungi with remediatory functions is demonstrated. Populations of rhizosphere microflora could be increased indirectly by genetically increasing *Populus* root mass through transformations such as with *Agrobacterium rhizogenes*. Increased root mass could support larger populations of rhizosphere microorganisms that could also be genetically engineered with improved remediatory functions (Stomp et. al. 1994). However, ethical concerns must be thoroughly addressed before such strategies can be implemented in the field (Yang et al. this volume).

## Strategies and Considerations for Plantings

For efficient and sustained remediation, *Populus* planted at remediation sites must tolerate prevailing site conditions such as excess nitrates and herbicides, as well as damage by insect pests and diseases. Accumulation of pollutants is toxic to many plants; thus, these plants must tolerate existing pollution levels and higher concentrations than normally exist within the plant. However, many native poplars traditionally recommended for riparian zones are relatively slow growers and may be intolerant of pollutants. Genetic engineering and selection can potentially improve the tolerance of *Populus* trees to various pollutants. Such pollution tolerance could amplify potential biomass benefits of *Populus* plantings.

Pest outbreaks are common in riparian *Populus* plantings (Ostry et al. 1988), and can threaten remediation activity of the planting. Genetic engineering, genetic selection, and *in vitro* selection can facilitate the development of *Populus* clones with enhanced pest resistance (Cervera et al. this volume; Ebinuma et al. this volume; Ellis and Raffa this volume; Heuchelin et al. this volume; Ostry this volume; Powell and Maynard this volume). Eventually, techniques developed by this research will be used to develop a variety of *Populus* cultivars and clones with improved resistance/tolerance to insects and disease. In addition, planting establishment and maintenance guidelines should include strategies for integration with other pest management techniques (e.g., enhancing natural controls). Such approaches could minimize the need for additional pesticide application to *Populus* plantings.

Because *Populus* biomass plantings usually require intensive management, they should be established at least 1 planting zone away from the stream. These plantings could serve as an effective intermediate buffer zone for absorption and degradation of environmental pollutants. The plant zones adjacent to the stream could be designed to delay or absorb excess chemicals and soil from *Populus* plantings. As mentioned earlier, soil microorganisms found in poplar plantings have a primary role in site remediation. These microorganisms also must tolerate the pollutants and other conditions at the site.

Finally, potential applications of this technology beyond riparian forest buffer are numerous. Similarly designed *Populus* plantings could be used to remediate industrial waste sites, agricultural waste water, sewage, and mine land. For example, in the Pacific Northwest, bioengineers are designing systems that use *Populus* biomass plantings for recovering nitrates and other fertilizers from irrigated waste water, or removing urea and heavy metals from dairy waste, human sewage, and landfill leachate. Most of these practices are exploiting the nitrogen affinity and high water consumption of hybrid *Populus* (Gary Kuhn, USDA Natural Resource Conservation Service, personal communication).

## Conclusion

Planting poplar near riparian zones and toxic waste sites has generated considerable interest as an economical method to remediate toxic sites while providing income and environmental benefits. Planting *Populus* in riparian zones may provide unique opportunities for remediation of multiple toxins. *Populus* has high potential for environmental remediation because its biology is well studied, and its management, production, genetic engineering, genetic selection, and *in vitro* manipulation techniques are well developed and readily available. Demonstration plantings have been established in several communities to limit movement of potential ground-water contaminants. Such plantings are used to remediate leachate from contaminated landfill and waste water systems while producing biomass and providing wildlife habitat. However, phytoremediation of pollution in urban and rural landscapes is a long process and is primarily effective only on pollutants near the surface. It is a relatively environmentally safe process that can be used for large areas. Removal of pollutants by this method does not necessarily require much energy (Stomp et al. 1995). Additional research, development and field trials are needed before the specific biochemical processes involved in pollutant uptake, transport, and accumulation are fully understood. Environmental impacts of using *Populus* remediation plantings must also be thoroughly evaluated before such plantings can be fully utilized.

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