Can Stress Enhance Phytoremediation of Polychlorinated Biphenyls?

Tomasz Kalinowski* and Rolf U. Halden

*The Swette Center for Environmental Biotechnology and The Center for Environmental Security,
The Biodesign Institute at Arizona State University, Tempe, Arizona.*

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Abstract

Phytoremediation—plant-facilitated remediation of polluted soil and groundwater—is a potentially effective treatment technology for the remediation of heavy metals and certain organic compounds. However, contaminant attenuation rates are often not rapid enough to make phytoremediation a viable option when compared with alternative treatment approaches. Different strategies are being employed to enhance the efficacy of phytoremediation, including modification to the plant genome, inoculation of the rhizosphere with specialized and/or engineered bacteria, and treatment of the soil with supplementary chemicals, such as surfactants, chelators, or fertilizers. Despite these efforts, greater breakthroughs are necessary to make phytoremediation a viable technology. Here, we introduce and discuss the concept of integrating controlled environmental stresses as a strategy for enhancing phytoremediation. Plants have a diverse suite of defense mechanisms that are only induced in response to stress. Here, we examine some stress-response mechanisms in plants, focusing on defenses involving physiological changes that alter the soil microenvironment (rhizosphere), and outline how these defense mechanisms can be co-opted to enhance the effectiveness of phytoremediation of polychlorinated biphenyls and other contaminants.

Key words: inducible defense mechanisms; PCB; phytoremediation; rhizodegradation; root exudates; stress response

Introduction

PHYTOREMEDIATION, THE REMEDIATION of contaminated soil and groundwater facilitated by vegetation, is a promising technology and has seen growing interest in the scientific community such that the last decade has seen an average 20% year-over-year increase in the number of publications in the field: in 2001, there were 150 phytoremediation articles indexed by ISI; in 2011, there were 588. Phytoremediation can enhance contaminant attenuation in a number of ways. Phytoextraction, the removal of contaminants via incorporation into plant root biomass that is subsequently harvested, has been a long-standing mode of phytoremediation, especially for metals (Sheoran *et al.*, 2009). Phyto-volatilization, the transpiration-mediated volatilization of contaminants (typically volatile organic pollutants [VOC], with trichloroethene [TCE] being the prime example), has also been successful (Gordon *et al.*, 1998).

Although promising results have been observed in the lab (Liu and Schnoor, 2008) and field (Mackova *et al.*, 2009), greater breakthroughs are necessary for the successful phytoremediation of polychlorinated biphenyls (PCBs) (van Aken *et al.*, 2010).

Attenuation rates are limited in part by the tendency of PCBs (and other compounds with a high octanol-water partitioning coefficients, K_{OW}) to sorb strongly to soils and sediments, thereby limiting their bioavailability. Remediation is also limited by slow transformation rates, due to the stability imparted on the structure by chlorine substituents and aromatic rings. These are some of the challenges and limitations that still should be overcome before full-scale implementations of phytoremediation can become practical more frequently.

Consequently, numerous efforts are being made to enhance the rate and effectiveness of phytoremediation. The initial and most sustained efforts have focused on genetic modifications of the plant (Dietz and Schnoor, 2001; Harms *et al.*, 2011). Typically, bacterial genes are expressed in plant cells, thereby conferring the ability to metabolize, accumulate, or simply tolerate the contaminant. For recalcitrant contaminants, such as PCBs, entire suites of genes must be transformed into the plant genome before contaminant attenuation is observed.

However, the creation of transgenic plants may be unnecessary. The rhizosphere, the soil area adjacent to plant roots, is one of the most diverse and rich microbial ecosystems known. Plants release an array of organic molecules from their root structure, thereby allowing the resident soil microbial community to flourish (Coleman *et al.*, 2004). Researchers have long been investigating how microbial communities residing on plant roots can be exploited for remediation purposes (Donnelly *et al.*, 1994). Much progress has been made, but it is

*Corresponding author: Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, 1001 S. McAllister Avenue, Tempe, AZ 85287. Phone: 480-727-0698; Fax: 480-727-0889; E-mail: tkalinow@asu.edu

clear that we are only beginning to understand the complex relationships between plants and the rhizosphere.

The relationship between roots and the resident microbial community of the rhizosphere can simultaneously be described as symbiotic, commensal, mutualistic, and parasitic. The most studied of the symbiotic relationship-forming microorganisms have been nodule-forming *Rhizobium* spp., capable of fixing nitrogen in legumes, and arbuscular mycorrhizae—strains which provide an extension of the root network and aid in the harvest of nutrients, primarily phosphorus (P). More recently, it has come to light that the rhizosphere imparts many additional benefits to the plant, including protection from bacterial parasites (Bais *et al.*, 2001; Salem, 2003; Badri *et al.*, 2009), from toxic organics in the soil, and from macro parasites (i.e., grazing caterpillars) (Bezemer and van Dam, 2005).

In exchange for these services that the rhizosphere provides, the plant exudes photosynthetically fixed carbon at the roots, primarily in the form of organic acids, as well as free amino acids. In addition, it is known that plants exude many other, more complex organic molecules (e.g., flavonoids, terpenoids) whose function is still debated. Microbial PCB degradation has been shown to be stimulated by many of these compounds, including citric acid (White *et al.*, 2006), linalool, terpenoids (Luo *et al.*, 2007), phenolics, and flavonoids (Narasimhan *et al.*, 2003; Leigh *et al.*, 2006).

Most relevant to remediation efforts is the fact that plants can modulate the composition and amount of exudates released in response to environmental stresses they experience. It has long been thought that plant exudates are intended to directly mitigate environmental stress, for example, excretion by plant roots of citric acid for chelation of free metal ions in the presence of excess aluminum ions (Al^{3+}) (Ma *et al.*, 1997a). However, recent evidence suggests that root exudates may also function by enhancing the rhizosphere's microbial community's ability to mitigate chemical and environmental stress.

The manipulation of innate plant stress responses may represent a novel and ecologically sustainable approach that stimulates the rhizosphere, thereby increasing contaminant attenuation rates. Here, we examine a number of innate plant stress responses, with a focus on how these might influence phytoremediation. To this end, we have included only stresses that could feasibly be integrated into existing or future phytoremediation applications.

In addition, we are focusing primarily on the remediation of PCBs as a proxy for hydrophobic organic pollutants in general. This selection was guided by both the challenges posed by this group of widespread pollutants and recent advances in the use of phytoremediation as a remediation tool for PCBs. Polychlorinated biphenyls have been identified as carcinogens and endocrine disruptors that reduce the primary productivity of aquatic and terrestrial ecosystems and bioaccumulate up the food chain (van den Berg *et al.*, 2006). They are U.S. Environmental Protection Agency (EPA) priority pollutants and are ranked number five on the list of priority pollutants in the 2007 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). An estimated 1.5 million tons of PCBs have been produced worldwide (van Aken *et al.*, 2010). The majority of this chemical mass is believed to be still in use and, thus, poses a risk of further release into the environment (Diamond *et al.*, 2010),

despite the 1970s ban of these compounds in the United States (Pieper, 2005). Nowadays, more than 400 soil and sludge sites in the United States are known to be contaminated with PCBs (Varanasi *et al.*, 2007).

Environmental Stresses

In this section, environmental stresses with the potential to positively or negatively affect phytoremediation are discussed. These environmental stresses and their potential impacts are summarized in Table 1.

Aluminum

The phytotoxicity of aluminum (Al) depends on its speciation. The ionic form, Al^{3+} , is much more toxic than the chelated form, presumably because the chelated form is bound and unable to participate in reactions with sensitive plant proteins. The primary physical manifestation of Al stress in plants is the suppression of root elongation. However, to counter the toxicity effect, some plant species exude Al-chelating substances, most often citric, malic, or other organic acids. The ability of an organic acid to alleviate these toxicity effects is dependent on its chelating ability; citric acid, when present in concentrations equimolar to Al, has been shown to completely negate the ability of Al to inhibit root elongation (Hue *et al.*, 1986; Ma *et al.*, 1997b).

It has already been demonstrated that the addition of citric acid to PCB-contaminated soil increases the bioavailability of PCBs (White *et al.*, 2006). The additional input of organic acids also drastically changes the microenvironment immediately adjacent to the root: pH decreases, total biological oxygen demand (BOD) increases, and many other ionic compounds in addition to Al, such as phosphorus, are chelated, and often made more bioavailable (Cesco *et al.*, 2010; Jones, 1998). Furthermore, some of these stresses may have synergistic effects; Ma *et al.* observed that Al presence coupled with P deficiency

TABLE 1. POTENTIAL IMPACTS OF ENVIRONMENTAL STRESSES ON PHYTOREMEDIATION

Stress	Plant responses that may enhance contaminant attenuation	Plant responses that may retard contaminant attenuation
Ionic aluminum	Secretion of citric, malic, and other organic acids	Suppression of root elongation
Nutrient deficiency	Exudates of organic acids, additional symbiotic relationships with AM, release of flavonoids, architectural changes in root development (more density); increased fine root hair turnover	Decreased plant primary productivity
Grazing	Production of monoterpenes, alkaloids	Decreased photosynthesis
Anoxia	Oxygenation of the roots, oxidative burst (ROS)	Decreased primary productivity
Gamma-irradiation	Increased growth rate	Plant death

ROS, reactive oxygen species; AM, arbuscular mycorrhizae.

enhanced the excretion of citric acid more than P deficiency alone.

Additional research is necessary to determine what the relative magnitude of these phenomena is on these processes. It is difficult to directly compare studies measuring citric acid secretion with studies measuring the effects that citric acid have on PCB bioavailability and soil microflora, because studies examining the former are often conducted under hydroponic (soil-free) conditions, while the latter are necessarily conducted in soil. Nevertheless, it is possible to get a rough estimate; under 50 μM Al^+ concentrations, *Cassia tora* L. roots secreted approximately 6–8 μM citric acid per hour per g of root dry weight (Ma *et al.*, 1997a). Meanwhile, plant-soil microcosms amended with a 1 mM citric acid solution showed approximately 600% increased leaf content of PCBs, and a 65% increase in total removal of PCBs, compared with unamended controls (White *et al.*, 2006). In the soil microenvironment immediately adjacent to the root, where mobility of exudates is relatively low, it is conceivable that concentrations of exudates can reach 1 mM or higher at secretion rates of 6 μM per hour per g of root dry weight. However, a study directly examining the effect of this phenomenon on PCB removal in soil is necessary before any conclusions can be drawn.

In light of these known effects, amending PCB contaminated soil with low concentrations of Al may be an alternative to amending with a low-molecular-weight organic acid. The direct application of such chelating agents to the soil introduces the risk of mobilizing contaminants; whereas inducing plants to secrete their own chelating agent directly at the roots minimizes these risks. Furthermore, a degradable amendment such as citric acid would need to be applied repeatedly, thus increasing cost; an elemental amendment such as Al, however, would only need to be applied once and would function on a continuing basis. Replacing a degradable amendment with an elemental amendment could significantly reduce the recurring cost of long-term phytoremediation projects.

Phosphorus/nitrogen deficiency

Currently, plants in phytoremediation projects are routinely fertilized with nitrogen (N) and phosphorus (P) (Sheoran *et al.*, 2009). However, there have been no studies conducted that determine whether this is a sound practice for the remediation of organic pollutants.

N and P are the two principal limiting nutrients in terrestrial ecosystems. This is not due to lack of presence, but lack of availability. Both N and P are often present in abundant amounts, though in a form that is inaccessible to plants. As a result, plants have evolved elaborate mechanisms for increasing the availability of these nutrients.

Phosphorus is often unavailable for root uptake. At low pH (<6), P forms insoluble compounds by reacting with Al, iron (Fe), and organic matter. Meanwhile, in alkaline environments, it binds with calcium (Ca) and magnesium (Mg) to form only slightly more soluble phosphates (Jones, 1998; López-Bucio *et al.*, 2002). Nitrogen, on the other hand, is often abundant but unavailable to plants in the form of di-nitrogen.

Plants have numerous adaptive traits for coping with limiting N and P conditions: alteration of pH through secretion exudates (Dakora and Phillips, 2002; Bertin *et al.*, 2003), cultivation of symbiotic relationships with arbuscular mycorrhizae (AM) or *Rhizobia* spp. (Parniske, 2008), release of

flavonoids (Cesco *et al.*, 2010), and architectural changes in root development (Fig. 1). Under nutrient limitation, plants drastically increase their branching and lateral root density, thereby increasing the total root surface area as well as total volume of soil canvassed (López-Bucio *et al.*, 2003).

All these stress responses to nutrient limitation are known or hypothesized to enhance PCB attenuation. It has already been demonstrated that AM and *Rhizobia* spp. enhance phytoremediation of PCBs (Teng *et al.*, 2010). Flavonoids and other plant phenolic compounds, which can be structurally similar to PCB, are known to support the growth of PCB-degrading microorganisms (Donnelly *et al.*, 1994; Fletcher and Hegde, 1995). Greater canvassing of the soil by the root structure should also enhance PCB removal.

The turnover rate of fine root hairs also increases with nutrient deficiency. It is known that in perennial plants, as much as 70% of the fine root hairs (defined as less than 2 mm in diameter) produced in a single growing season die and constitute a significant source of energy for the rhizosphere. This input of dead root hairs is believed to be a source of biomass for the rhizosphere throughout the growing season, not just at the end. Further, the phenolic content of these fine root hairs doubles immediately before root death (Leigh *et al.*, 2002). We suggest that the large input of phenolic-laden biomass from fine root hair turnover could stimulate PCB degradation by creating additional selective pressure for microbes adapted to phenolic and aromatic compounds.

The increased input of organic matter into the rhizosphere, the increase in the total soil in contact with roots and consequently the size of the rhizosphere, and the increased phenolic content of roots due to N/P deficiency lead us to suggest that such environmental stress may positively impact net PCB transformation rates in the rhizosphere, or perhaps at a minimum, offset the loss in phytoremediation efficacy due to reduced overall plant growth resulting from not applying fertilizers.

Grazing

Throughout their evolutionary history, plants have developed a variety of adaptations for coping with grazing: some have increased production of lignin and other compounds that make leaf matter unpalatable; others have increased total biomass production to offset losses due to grazing (Oosterheld and McNaughton, 1991). Generally, defense mechanisms against herbivores can be separated into two categories: inducible and constitutive. Inducible defenses typically come at a great cost to the plant, often decreasing reproductive ability in exchange for robustness (Baldwin *et al.*, 2001). This presents an intriguing opportunity where by maintaining a constant moderate grazing stress, a plant can be coaxed toward diverting resources from growth and reproduction toward robustness and defense. In practice, this often results in the growth of elaborate root systems that produce a suite of complex organic defense molecules—compounds which may potentiate the rhizodegradation of halogenated aromatics (Kessler and Baldwin, 2002; Singer *et al.*, 2003; Luo *et al.*, 2007).

For example, *Nicotiana tabacum*, when experiencing grazing-induced stress, increases production of nicotine and monoterpenes, despite the increased metabolic cost. Nicotine alone can account for as much as 8% of the plant's nitrogen sink, and nicotine is only one alkaloid in a suite of inducible

FIG. 1. Schematic drawing illustrating changes in plant physiology in response to environmental stress. Nitrogen deficiency (N ↓): increased lateral root elongation. Phosphorous deficiency (P ↓): increased fine root hair production, which may promote growth of polychlorinated biphenyls-degrading microorganisms. Aluminum stress: citric and other organic acids exuded, stimulating growth of the rhizosphere. Grazing/parasite stress: phenolic compounds synthesized both in roots and leaves. (A) Fine root hair with background concentrations of phenolic compounds. (B) Phenolic content of root hairs increases before senescence. (C) After senescence of fine-root hair, phenol-metabolizing bacteria have increased presence in the rhizosphere due to the phenolic loading from fine root hair turnover. Adapted from Lopez-Bucio *et al.* (2003).

defense mechanisms (Baldwin, 2001; Bezemer and van Dam, 2005). Interestingly, nicotine is primarily produced in the roots of plants, and then transported to leaves; it is unknown how much of the alkaloids produced at the roots are released, through fine root hair turnover or other means, into the rhizosphere.

Terpenoids represent another class of secondary plant metabolites that have been shown to facilitate PCB degradation in microcosm studies (Hernandez *et al.*, 1997; Kim *et al.*, 2003). Their production is only induced in response to stress: Terpenoids are metabolically expensive due to their need for extensive reduction, and they can build up in leaf matter and cause toxicity in the plant itself. Inducing the production of secondary plant metabolites through controlled, low-level grazing stress may be one way to potentiate the rhizosphere's remedial power by providing selective pressure for microorganisms with the ability to transform complex aromatic structures.

Anoxia

Anoxia, and its lesser cousin hypoxia, could be considered the most interesting stress treatment. An anaerobic environ-

ment is necessary for the metabolic reductive dehalogenation of higher chlorinated PCBs and other haloorganics by microbes, while an aerobic environment is needed for the complete mineralization of lower chlorinated PCBs. Consequently, sequential anaerobic-aerobic treatments have the most potential for removing PCBs (Vasilyeva and Strijakova, 2007).

However, anoxia poses a challenge for phytoremediation, because anoxia is detrimental to the health of almost all macroflora. Nevertheless, plants often have to deal with anoxic environments, and have developed elaborate coping mechanisms that could be co-opted for remediation purposes. These defense strategies are most developed in plants adapted to growing in marshes, bogs, and other environments that routinely are flooded and become anaerobic, but they are present in a limited form in other flora as well, including *N. tabacum* (Drew, 1997; Vartapetian and Jackson, 1997).

One strategy for coping with anoxia is oxygenation of the roots. For example, in the seagrass *Zostera marina* [another promising candidate for phytoremediation of PCBs (Huesemann *et al.*, 2009)], oxygen is supplied to the roots during times of photosynthesis. However, roots still experience anoxia every night (Smith *et al.*, 1988). This daily supply

of oxygen to a normally anaerobic environment may create precisely the series of oxidative states necessary for the complete metabolic mineralization of PCBs.

γ -irradiation

More than 50 years ago, it was discovered that small doses of gamma (γ) irradiation can stimulate plant growth, and lead to more robust plants in general (Sax, 1955, 1963). Increased presence of oxidases within plant cells, faster growth rates, and additional branching in both shoots and roots are all gamma-ray induced changes in plant physiology (Wi *et al.*, 2007) that, technically, may positively impact PCB rhizodegradation. Further, γ -irradiation would be relatively inexpensive to implement in the field with a handheld device.

Conclusions and Future Work

Plants have stress-induced responses that are known to influence the microbial community of the rhizosphere. Some of these innate stress responses may enhance the attenuation rate for PCBs and other contaminants of concern through a variety of mechanisms, (i.e., stimulation of the rhizosphere through root exudates, change in root growth patterns, etc.) and, as such, provide a potential mechanism by which implementations of phytoremediation may be improved. We have reviewed here what is known about plant responses to certain stresses, and what is known about how these responses may impact the transformation of certain contaminants. However, the majority of our knowledge in these areas comes from research in which the two phenomena are studied separately. In addition, many publications of phytoremediation applications omit detailed information on the stresses that the plants experienced, making a literature meta-analysis of field results not possible. Further research directly aimed at studying the relationship between environmental stress and contaminant attenuation rates is necessary.

Intentionally exposing plants to any environmental stress in phytoremediation applications will involve balancing a series of trade offs; stress will impose a burden on the plant and can lead to less plant growth. However, it is clear that some of the stress responses may also enhance contaminant attenuation. Directed studies will be necessary to determine exactly to what extent these stress responses will off-set the overall reduced growth of the plant in phytoremediation applications. Some strategies for mitigating the negative impacts of stress may include only introducing them for brief periods, or in succession. Included in these investigations should also be the relative costs for implementing environmental stress mitigation measures (e.g., cost of applying fertilizer).

If future research demonstrates a positive association with stress-induced plant defense mechanisms and phytoremediation effectiveness, then the controlled use of environmental stresses could be used in concert with existing phytoremediation-enhancement strategies to address the challenges that should be overcome to establish phytoremediation as an effective environmental restoration technology.

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