Uses of *Trichoderma* spp. to Alleviate or Remediate Soil and Water Pollution

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I. Introduction

Trichoderma spp. are very common in soil and on plant roots; they are among the most abundant culturable fungi in many soils. They also colonize plant debris, and in some cases the sexual stage (genus *Hypocrea*) is found on twigs or other similar materials (Harman *et al.*, 2004a; Klein and Eveleigh, 1998). The fungi in this genus are genetically quite diverse, with a number of different capabilities between different strains.

It is the purpose of this chapter to consider how these prolific fungi may be used in remediation or amelioration of pollutants in the environment, with foci on mechanisms and capabilities of the fungi and their emergence as novel and useful tools to improve agriculture and environmental quality. Other reviews (Harman, 2000; Harman *et al.*, 2004a; Lynch, 2003) examined the agricultural and biocontrol applications of these fungi including their abilities to control plant diseases and to enhance plant growth and the uses and nature of their economically important enzymes (Harman and Kubicek, 1998), and so these uses will be considered only in passing here.

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II. *Trichoderma* spp. Are Opportunistic Plant Symbionts

Recently these fungi have been demonstrated to be opportunistic avirulent plant symbionts (Harman *et al.*, 2004a). They clearly are opportunistic, since they can proliferate, compete, and survive in soil and other complex ecosystems. They are capable of invading roots but are typically restricted to the outer layers of the cortex (Yedidia *et al.*, 1999), probably because of production by the fungi of several classes of compounds that act as signals for the plant to activate resistance responses based on chemical and structural mechanisms (Harman *et al.*, 2004a). This root infection, followed by limitation of fungal proliferation within the root, allows the fungi to grow and develop by using the energy sources of the plant. Not only do the fungi grow based on resources provided by the plant, but they also are carried through soil and occupy new soil niches as a consequence of root colonization. Thus root-associated *Trichoderma* spp. derive numerous benefits from plants.

Plants also derive numerous advantages from root colonization by these opportunistic root symbionts. These include the following:

- Protection of plants against diseases by direct action of the *Trichoderma* strains on pathogenic microbes (Chet, 1987) or other deleterious soil microflora (Bakker and Schippers, 1987).
- Protection against plant pathogens because of systemic induction of resistance; this permits plants to be protected widely separated temporally or spatially from application of *Trichoderma* (Bigirimana *et al.*, 1997; Harman *et al.*, 2004a; Yedidia *et al.*, 1999, 2000, 2003). For example, through induced resistance, *Trichoderma* spp. can control foliar pathogens even when it is present only on the roots.
- Enhancement of plant growth and development, especially of roots. The activity of *Trichoderma* spp. added to soil increases plant growth and development. This fact seems counterintuitive, since no doubt the root colonization and induction of resistance is energetically expensive to the plants, but it is a phenomenon that is commonly observed on a variety of plants (Chang *et al.*, 1986; Harman, 2000; Lindsey and Baker, 1967). Some of this improved plant growth no doubt occurs as a consequence of control of pathogenic or other deleterious microbes, but it also has been demonstrated in axenic systems (Lindsey and Baker, 1967; Yedidia *et al.*, 2001), so it is no doubt a consequence of direct effects on plants as well as a biological control phenomenon (Harman *et al.*, 2004b).

These facts directly demonstrate that *Trichoderma* spp. have a strong beneficial effect upon plants. Thus, at least some strains function as plant symbionts. This is a strain-specific ability, however, since some strains in some conditions may produce toxic metabolites, and so the balance between toxicants and growth-promoting effects determines their net effect (Ousley *et al.*, 1993). However, with other strains, negative effects usually are not seen regardless of inoculum level or environmental conditions (Harman, 2000).

It should be noted that root-colonizing *Trichoderma* strains are not the only organisms that provide similar benefits. For example, the PGPR (plant growth promoting rhizobacteria), including strains of *Pseudomonas* and *Bacillus* spp. (Kloepper *et al.*, 1993; Ryu *et al.*, 2003), both induce systemic resistance and enhance plant growth. Several other fungi—including nonpathogenic strains of *Fusarium* and *Rhizoctonia* spp., mycorrhizal fungi, and *Penicillium* spp.—may colonize superficial layers of roots and induce systemic resistance (Fravel *et al.*, 2003; Hwang and Benson, 2003; Pozo *et al.*, 2002). This suggests that the ability to (1) infect plant roots, (2) induce the plants to limit the level of infection and induce generalized resistance mechanisms in the plant, and (3) enhance plant growth and development evolved independently numerous times within different fungal genera and is a useful survival strategy (Harman *et al.*, 2004a).

III. Rhizosphere Competence and Co-Metabolism

A few strains of *Trichoderma* are highly rhizosphere competent, but most strains are not (Chao *et al.*, 1986). The most effective strains can colonize roots of virtually all plant species, as exemplified by *T. harzianum* strain T22, and this has been tested thousands of times in academic and commercial trials (Harman, 2000). The spores of the fungus, or other forms of biomass, can be added in any way that the fungi come into contact with roots. Once this occurs, T22 proliferates over the entire root system and grows with roots as they penetrate into soil (Harman, 2000). Thus the fungus is carried deeply within the soil profile even when applied only to the upper soil layer. Moreover, the addition of T22 or other strains results in denser root growth and may increase deep rooting by as much as twofold. In this way, the fungus enhances root exploitation of the soil volume both laterally and vertically, which also increases the volume of soil exposed to the fungus (Harman, 2000). Finally, root colonization occurs across a wide range of soil types (Harman, 2000; Harman and Björkman, 1998), perhaps because the fungus exists within the outer layers of roots and therefore is less exposed to adverse soil conditions. An important part of the ecological success of *Trichoderma* spp. relies upon their biocontrol abilities. As the levels of T22 in soil are increased, the fungus becomes more and more dominant on roots, thus excluding other microbes (Harman, 2000); the reliability of this ability of T22 has been proven in more than a decade of use in commercial horticulture and agronomy (Harman, 2000).

Thus T22 and other strains of *Trichoderma* form robust and stable, self-organizing symbiotic root-microbial systems. While many other microbes are known to colonize roots, we are unaware of any that are as reliable in their performance.

Trichoderma spp. can grow on and in plant roots and derive nutrients from plants. At the same time, the fungus provides benefits to the plant (Harman, 2004a). This creates a co-metabolic system that probably has substantial potential for alleviation of soil pollution problems (Fig. 1).

IV. Root Enhancement by *Trichoderma* spp.

T. harzianum strain T22 now is widely used in plant agriculture, both for its abilities to control plant diseases and to increase plant root growth (Harman, 2000). The organism applied even as a seed treatment can provide long-term benefits to plants. An example of increased root growth caused by a seed treatment on maize and on soybeans from grower fields is shown in Fig. 2. Root colonization from seed treatments typically results in about one order of magnitude greater colonization (colony forming units per g dry weight of roots) than occurs natually from the native strains in soils (Harman and Björkman, 1998). This level of colonization can be increased by additions of conidia or other inoculum to the developing root system.

V. Enhanced Extraction and Biodegradation of Toxicants

Microbes with capabilities to degrade or remove toxicants are known in many genera. However, a common failing in the use of some of these is that the microbes used frequently are overtaken or outgrown by other microbes, especially when glucose or other nutrients are added. This may necessitate their use in highly controlled conditions, such as a bioreactor. This may increase costs associated with removal of polluted materials, sterilization, and media components. The *Trichoderma* root

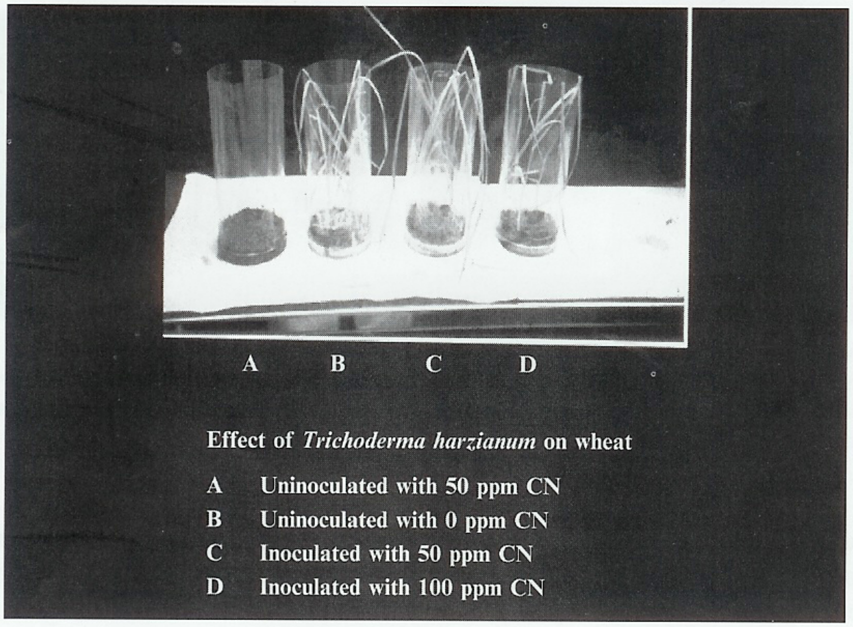


FIG. 1. Detoxification of cyanide by *Trichoderma* spp. growing on plant seeds and roots; an example of the utility of rhizosphere competence and co-metabolism. Soils were amended or not amended with various levels of KCN, as noted, and planted to wheat seeds. Even at 50 μg/g of cyanide, wheat seeds were killed in the absence of *Trichoderma*, but in its presence, the seedling survived even at 100 μg/g. *Trichoderma* in this case grew on the surfaces of germinating seeds and roots and degraded cyanide as indicated by its disappearance from soil. The fungus produces at least two separate cyanide-degrading enzymes, rhodanese and formamide hydrolase (Ezzi, 2002, 2003; see text also). The image is from M. Ezzi and J. M. Lynch, unpublished.

system, especially in the case of strains such as T22, is sufficiently stable that such issues can largely be overcome.

Moreover, since the fungus-root association is stable and the fungi actually colonize root surfaces, there is an exchange of bioactive molecules between the fungus and the plant. The fungi gain their nutrients from the plant and produce a series of disease resistance signaling molecules that have a strong effect upon plant metabolism (Harman *et al.*, 2004a) and provide the basis for this symbiotic co-metabolic plant-fungus association. Other molecules—for example, enzymes that degrade toxicants in soils or water—also can be produced from this synergistic platform (Fig. 1). This co-metabolic system, composed of both plant and fungal metabolites, including perhaps some factors uniquely synthesized only during the interaction, may represent an

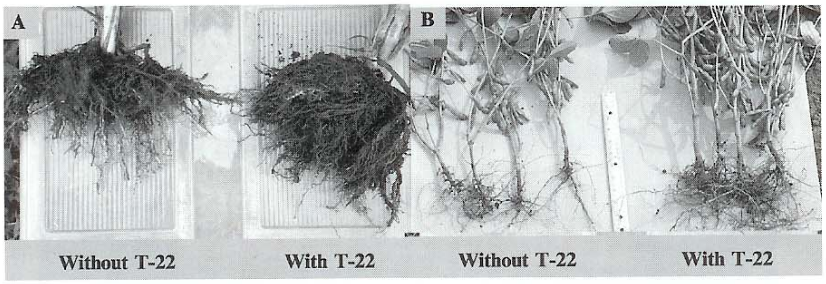


FIG. 2. Enhancement of root growth of maize and soybeans by a seed treatment with *T. harzianum* strain T22 in grower fields. Enhancement of root growth occurs over at least the life of annual crops and is a major component that leads to yield increases and its commercial use. Figure from Harman (2000), with permission.

advantage for applications in bioremediation systems for cleanup for strategies either on accumulation of toxicants in plant tissues or for breakdown of toxic compounds.

A. ENHANCED PLANT REMOVAL OF TOXICANTS

1. *Arsenic*

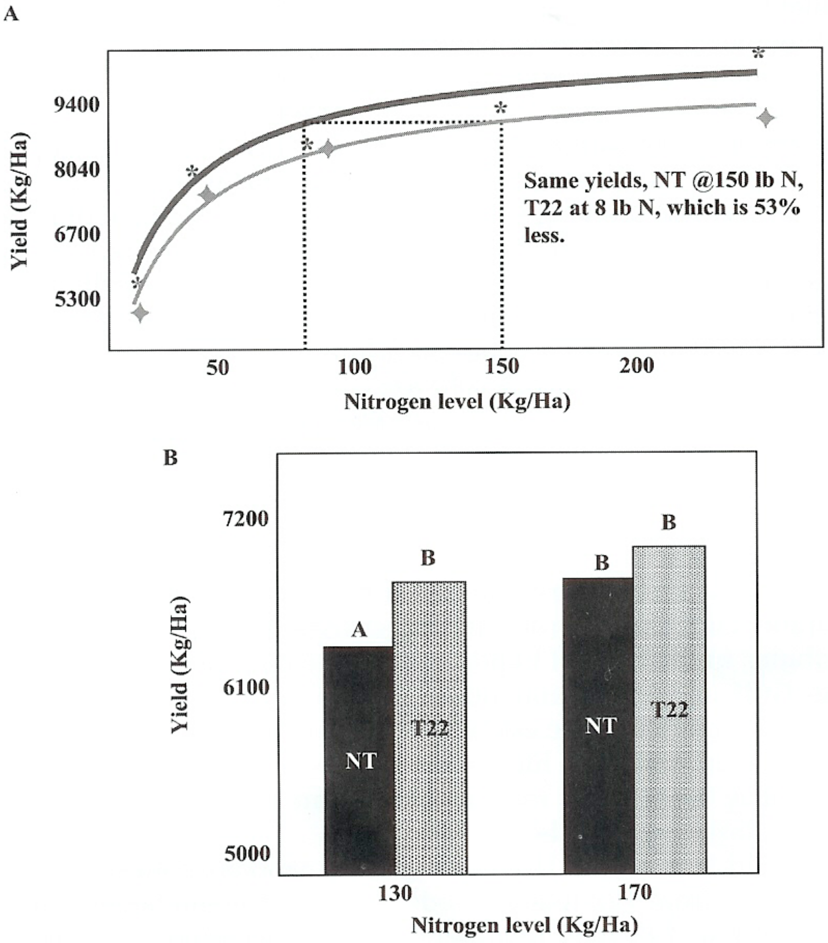
Arsenic was applied to orchards and other farm lands as a pesticide and herbicide between approximately 1930 and 1960 in various forms, most commonly lead arsenate (Woolson, 1975). Lead concentrations in at least some sites do not rise to actionable levels, but arsenic more frequently is of concern. Typical values in old orchard soils range between 30 and 150 μg/g of soil, while eastern U.S. background levels are 7.5 to 12 μg/g. Assessments of risks to human health posed by arsenic-polluted soils suggest that levels above about 20 ppm of bioavailable arsenic may be a health hazard. This is an emerging problem; many hundreds of thousands of hectares are affected, and so this is likely to become a serious problem for farmers and developers. Other sites also are polluted with this metalloid as a result of smelting operations and wood-treating sites and leaching from treated lumber used for landscaping, playgrounds, and other purposes. There are few low-cost and effective solutions for the cleanup of relatively low but toxic levels (e.g., 20–150 μg/g) of arsenic-polluted soils. However, ferns have been identified that hyperaccumulate arsenic in their fronds from soils at levels between 2 and 200 times the level in soils (Ma *et al.*, 2001a,b). Commercial fern growers routinely treat very small fern plantlets with T22 because it enhances growth and survival. Such ferns are being commercialized by Edenspace Systems Corporation (Edenferns™). In the summer of 2003, we purchased ferns for field trials in an arsenic-polluted site that were, of course, already treated with T22. Soon after we transplanted the ferns to the field, we applied T22 as a drench at the rate of 9 kg/ha (the product contained about 10^9 cfu/g of T22). About 6 weeks later we sampled the roots. The level of *Trichoderma* spp. on roots of ferns that did not receive the drench treatment but that were treated at the nursery had a mean value of log 1.9 cfu/g roots, while ones that received the drench had 3.3 cfu/g roots, which is an increase of more than one order of magnitude. The level of arsenic taken up into ferns was about tenfold higher than the level in soil regardless of treatment. Unfortunately, this level of uptake times the total plant biomass still resulted in very small reductions in the course of a single growing season. Use of these tropical-adapted ferns may be more practical in southern regions, where they can grow as a perennial than in upstate New York, where they are poorly adapted. However, there are other plants that are better adapted to northern climates and that under proper conditions can hyperaccumulate arsenic. Testing of these plant-microbe associations is currently underway. In addition, the abilities of T22 and other root-associated microbes to enhance uptake of heavy metals in hyperaccumulating plants is being evaluated concurrently.

2. *Nitrates*

Agricultural systems and industrial processes place large quantities of nitrates and phosphorus into waterways. This pollution problem contributes to the zone of hypoxia along the coast of the United States in the Gulf of Mexico and other regions worldwide and may also encourage growth of toxic estuarine microbes such as *Pfiesteria*. These environmental costs are high—the EPA estimates that harmful algal blooms may have been responsible for an estimated $1 billion in economic losses during the past decade.

In the mid-1990s, G. E. Harman and his coworkers noticed that maize plants grown from T22-treated seeds were greener and larger than ones grown without the seed treatment, which suggested a capability of these fungi to enhance nitrogen uptake. A series of field experiments were undertaken in which we grew maize under different nitrogen fertilizer levels with or without a seed treatment with the beneficial fungus. Maize usually responds to nitrogen fertilizer by increases in yields up to a level, defined as the yield plateau, beyond which yields usually do not increase further. This yield plateau is considered the maximum yield for the particular cultivar and environment. In the presence of T22, (1) plants responded (increased growth) more rapidly to N fertilizer than in its absence (Harman, 2000), and (2) at tasselling, plants were greener and had a greater stalk diameter (Harman, 2000).

The nitrogen response curves consistently showed an improvement in nitrogen utilization with T22 in our trials (Harman, 2000) (Fig. 3).



Same yields, NT @150 lb N, T22 at 8 lb N, which is 53% less.

FIG. 3. (A) Nitrogen fertilizer yield relationships in a trial at Cornell University in 2000 in the presence or absence of T22 and (B) yields at two different nitrogen levels in a large (23 replications) trial at Cornell the same year. The lines in (A) are significantly different at P = 0.05, and in (B), bars with different letters are significantly different at the same probability level. (A) is from Harman (2001); (B) is from Harman and Donzelli (2001). Both are used with permission.

These experiments suggested that nitrogen fertility levels could be reduced by 40–50% (Harman, 2000, 2001; Harman and Donzelli, 2001, see also Fig. 3), with no reduction in yield. It is not clear whether this response was due simply to more efficient use of fertilizer or to mining of organic nitrogen pools in the soil. Such responses were associated with more abundant deep roots (Harman, 2000), which suggests that one key factor is greater density and depth of rooting, since deeper and denser root systems would be expected to both better intercept applied nitrogen and also utilize the nutrient at deeper depths of roots.

However, there is a complication to this positive story. Persons who conducted commercial trials (more than 500 have been done so far) reported that occasionally there were negative growth responses to T22 in maize. We now have verified that this is true by examination of inbred lines. Maize line Mo17 responds very positively (Harman *et al.*, 2004b), other lines respond less strongly, and a few—such as inbred line A661—are negatively affected by T22 (Harman *et al.*, 2004b, unpublished). Fortunately such responses can be quantified when plants are very young; 2-week-old plants already show changes in growth that are predictive of responses and plant performance throughout the season. Thus the increased growth capabilities and associated nitrogen responses are rapidly predictable. It is likely that reactions of other plants may also be measurable at an early stage, thereby permitting rapid assessment of the genetic responses of different varieties to T22.

B. Degradation of Pollutants

The largest commercial use of *Trichoderma* spp. is in the production of enzymes. These fungi are prolific producers and secreters of enzymes and are widely used, as both native (mutated) and transgenic strains for production of cellulases and other carbohydrate lyases of plant cell wall components; they also are efficient producers of enzymes such as chitinases. *Trichoderma* chitinases probably will be used for commercial applications soon (Donzelli *et al.*, 2003). In all cases, multiple forms of the enzymes exist with different modes of action; these enzymes and their genes were thoroughly reviewed in 1998 (Harman and Kubicek, 1998).

1. *Cyanide*

Cyanide and metallocyanides have been released into the environment from the metal plating and mining industries, and large quantities are also produced as wastes and emissions from the manufacture of paints, synthetic fibers, pesticides, and formerly, in the production of fuel gas from carbonization of oil and coal (Dubey and Holmes, 1995; Shirifin *et al.*, 1966). There are an estimated 50,000 former coal gasification sites in the United States left from the coal gas era of 1840–1950 (http://www.heritageresearch.com/manufactured_gas_E.htm). In the water gas process, retorted coal was blasted with super-heated steam to produce a line fuel composed of hydrogen, methane, and carbon monoxide plus HCN, NH_4, and H_2S impurities. The ammonia was captured with sulfuric acid and sold as fertilizer; the H_2S was scrubbed with ferric oxide and went to the manufacture of sulfuric acid; and HCN also reacted with ferric oxide to form ferric ferrocyanide, a.k.a. Prussian blue. This valuable pigment was used in paints, textiles, and inks. None of these operations were quantitative; thus, former gas sites may contain high levels of cyanide, metallocyanides, and metal sulfides and organic components of the coal tar residues that constituted approximately 7–8% of the coal gasified.

A second, more recent source of environmental concern is the practice of leaching trace amounts of gold and silver from abandoned mine tailings with CN solutions. Thousands of tons of cyanide are applied to open piles of rubble; after infiltrating the pulverized rock, the solutions run into impoundments where the metals are extracted and the cyanide is recycled to the tailings. Large volumes of dilute cyanide solutions may be lost to ground and surface waters through leaks and spills, plus catastrophic spills have poisoned whole river systems (http://www.grida.no/inf/news/news00/news17.htm). Several states and countries (e.g., Wisconsin, Montana, Turkey) are enacting laws to forbid further open cyanide leaching operations, but millions of acres of mine lands and water sheds are already contaminated from some of these ventures.

Trichoderma spp. have recently been reported to produce two separate enzymes that degrade cyanide (Ezzi and Lynch, 2002; Ezzi *et al.*, 2003). The enzymes and the reactions they catalyze are provided below:

Formamide hydrolase

$$HCN + H_2O \rightarrow HCONH_2 \rightarrow\rightarrow CO_2 \text{ and } NH_3$$

Rhodanese

$$S_2O_3^- + CN^- \rightarrow SO_3^{2-} + CNS^-$$

These enzymes are constitutively produced and secreted into the medium by all strains that have been tested (Ezzi and Lynch, 2002; Ezzi *et al.*, 2003), and the rhodanese has been characterized (Ezzi *et al.*, 2003). This discovery provides a useful biological method for remediation of cyanide. In addition to cyanide, most polluted sites contain metallocyanides (e.g., Prussian blue). These compounds are insoluble and are not particularly toxic themselves, but they release soluble cyanide over time. However, *Trichoderma* spp. take up Prussian blue—$Fe_4[Fe(CN)_6]$—into the thallus of the fungi, where it is then degraded (J. M. Lynch, D. Redman, and N. Isla, unpublished).

Importantly, *Trichoderma* spp. are resistant to a wide range of toxic compounds (Harman *et al.*, 2004a), and some strains will grow even at levels of up to 2,000 μg of cyanide per g of soil, a level that is 10,000 times the EPA allowable limit. This rather remarkable tolerance for cyanide and other toxicants is associated with a very active cellular detoxification system based on permeases such as ABC transporters recently discovered in these fungi (Harman *et al.*, 2004a).

Thus *Trichoderma* spp. (1) degrade cyanide by activity of characterized enzymes, (2) take up and degrade metallocyanides, (3) are resistant to high levels of a variety of toxicants, and (4) some strains are highly rhizosphere competent and form stable, long-lasting plant microbe communities. This self-organization provides a highly robust platform for degradation of cyanide and other pollutants. This system does, in fact, work rather well in remediation of cyanide in microcosm studies, as shown in Fig. 1, and this has also been reproduced by using other plants, including dicots. The wheat plants shown in the figure are sensitive to cyanide, but they are protected from its toxicity by release of enzymes from the plant-colonizing *Trichoderma* strains. Thus the *Trichoderma*-plant associations provide a useful point from which to develop novel remediation strategies for this serious pollutant.

However, this system would be even more effective if the plants used were themselves resistant to cyanide. Recently such plants have been identified. A variety of willow (*Salix eriocephala* var. Michaux) was found to take up both potassium cyanide and potassium ferrocyanide and to translocate them throughout the plant. Most importantly, the cyanide and ferrocyanide are degraded within the plant so that they do not accumulate within the aerial portions of the plant (Ebbs *et al.*, 2003). The combination of willows plus rhizosphere competent *Trichoderma* strains are expected to provide very effective methods of removal of cyanide and metallocyanides from polluted sites; research and development on this combination is underway.

2. *Polyphenols*

When olives are pressed, about 11 L of water are released for every liter of oil. This "black water" contains very high levels of phenolic and polyphenolic compounds; the biological oxygen demand may be

400-fold higher than that of usual municipal waste waters. The polyphenolics are toxic, so the water is essentially sterile. Moreover, at least a portion of the polyphenolics are resistant to anaerobic microbial degradation, making processing of such waste waters difficult.

However, *Trichoderma* spp. are highly tolerant to the toxicity of olive oil waste water, just as they are to many other compounds (M. Lorito, unpublished). Thus, if the waste water is diluted 1:3 and aerated, the fungi can grow and proliferate. While so doing, they produce polyphenol-degrading enzymes that destroy the polyphenolics (Fig. 4A, see color insert) and so remove them from solution (Fig. 4B). Similar procedures have been used to treat waste water with *Phanerochaete chrysosporium* or *Pleurotus ostreatus*, which are basidomycetes known to produce copious levels of polyphenol oxidases, laccases, and related enzymes expected to degrade polyphenolics (Kissi *et al.*, 2001). *P. chrysosporium* after 9 days of incubation removed about 50% of the phenolics and color, while *P. ostreatus* required 12 days to reach this same level (Kissi *et al.*, 2001). By contrast, *Trichoderma* strain TC3 removed about 90% of the materials, which resulted in a high level of color removal, after only 6 days of incubation. Thus these fungi appear to provide useful methods to cleanse olive oil waste waters.

3. *Polycyclic Aromatic Hydrocarbons*

Along with cyanides, gas works sites—as well as other polluted soils, including those polluted with petroleum, coal tar, and shale oil—contain complex polycyclic aromatic hydrocarbons (PAHs). These compounds are frequently toxic, mutagenic, and/or carcinogenic (Keith and Telliard, 1979). Numerous fungi, especially wood-rotting fungi (Andersson *et al.*, 2003; Pointing, 2001) and bacteria, sometimes in combination (Boonchan *et al.*, 2000), have been demonstrated to degrade these compounds to one degree or another.

More recently, other fungi including *Mucor* spp., *Penicillium* spp., *T. harzianum*, and *T. virens* have been shown to effectively degrade pristane when the compound was used as the sole carbon source (Ravelet *et al.*, 2000; Saraswathy and Hallberg, 2002). In our research, *T. harzianum* TH1 has been shown to degrade hexadecane and pristane in flask culture (J. M. L. and P. J. Phillips, unpublished). The addition of glucose as a co-metabolic substrate tripled the degradation rate compared with flasks containing only the hydrocarbons as substrates (J. M. L. and P. J. Phillips, unpublished). These capacities are usually strain dependent. Moreover, there exists great variation in the resistance of different strains to the toxic effects of PAHs. For example, strain T22 was only slightly inhibited in the application of 20 g/L to the

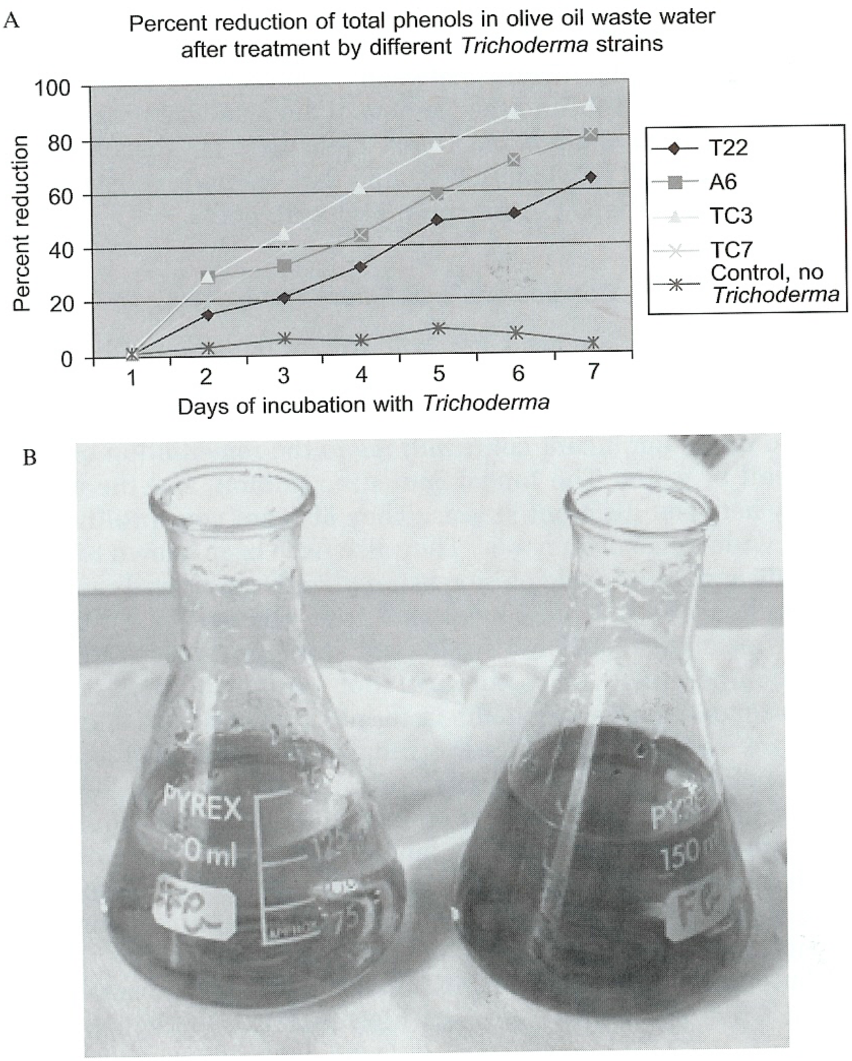


FIG. 4. Degradation of polyphenolics from olive oil waste waters by the activities of *Trichoderma* spp. The fungi were added to diluted (1:3 waste:water dilution) waste water and the content of polyphenolics in the mixture was followed over time. Phenolic compound content was determined by using the method of Folin-Denis based on the commercial substrate (Folin-Ciocalteu reagent; Sigma Scientific). A 5 ml total reaction mixture consisted of 250 μl of the reagent, 500 μl of sodium carbonate 20%, 100 μl of the sample to be tested, and distilled water. The phenolic concentration was determined based on a standard curve with differing concentrations of tannic acid. (A) The percentage degradation of the polyphenols; (B) the change in water color by the process. Analysis of variance of arcsine transformed percent values of all *Trichoderma* treatments were significantly different ($P < 0.05$) from controls without the strains.

surface of nutrient medium, but strain T12 was completely inhibited at this same concentration. This is particularly intriguing, since T22 was prepared by protoplast fusion between *T. harzianum* strain T12 and *T. atroviride* strain T95, and the genome of the resulting progeny is that of T12 (Stasz, 1990; Stasz *et al.*, 1988). Thus there is substantial potential for selection of *Trichoderma* strains that are both resistant to and degrade PAHs, perhaps as root colonizing symbionts.

VI. Conclusions and Future Prospects

Trichoderma spp. are used substantially for enzyme production and in agriculture for both plant protection against diseases and for plant growth promotion. Products and services based on these fungi are poised to make significant contributions to the remediation or alleviation of soil or water. The fungal genus is common, and there is great diversity between different strains. They also are very prolific producers of a wide range of enzymes. They also have been shown to be plant symbionts and are capable of increasing plant biomass and root growth and simultaneously protecting plants from disease by a number of different mechanisms. Some strains are strongly rhizosphere competent, which permits them to colonize roots, grow, and persist on roots and to provide long-term benefits in terms of plant health and productivity. This capability permits the fungi to form durable and robust plant associations in a wide variety of soil conditions. The symbiotic nature of the interaction permits co-metabolic processes in which the fungus gains nutrients from the plants and produces bioactive molecules that stimulate plant growth and resistance to stresses and stimulate the production of metabolites that are of value to the plants. Some of these compounds, including enzymes, may be highly useful in degradation of toxic soil pollutants; this capability is enhanced by the fact that *Trichoderma* spp. possess high intrinsic resistance to toxic compounds, probably by virtue of a strong detoxification system. The abilities of rhizosphere-competent *Trichoderma* spp. to enhance root growth is expected to enhance the capability of hyperaccumulating plants to remove toxic metals and metalloids. Research just conducted suggests that the presence of the fungi increased removal of arsenic from soils by hyperaccumulating ferns in the genus *Pteris*. Other data demonstrate that root colonization by the fungi increases nitrogen fertilizer use efficiency in maize but that there is a strong maize genotype response. Positive responding lines can be identified by growth after only 2 weeks. The use of positive responding maize lines and reduced levels of nitrogen fertilizer are expected to reduce nitrate pollution of waterways and, incidentally, to increase farm profits. Further, the same fungi produce enzymes that degrade cyanide, and they accumulate, and then degrade, metallocyanides such as Prussian blue. They do so in co-metabolic combinations with plants. We anticipate that combinations of rhizosphere competent *Trichoderma* strains with willows that take up and themselves degrade ferrocyanides and cyanide will provide novel and effective solutions to problems of soil pollution with cyanide. Further, these fungi also produce enzymes that, in aerated reactors, degrade polyphenolics. Finally, they possess capabilities to degrade at least some PAHs. No doubt additional uses of the fungi, and their associations with plants, will be found in the pollution remediation field. They appear to provide highly promising new tools for the treatment or amelioration of soil and water pollution.

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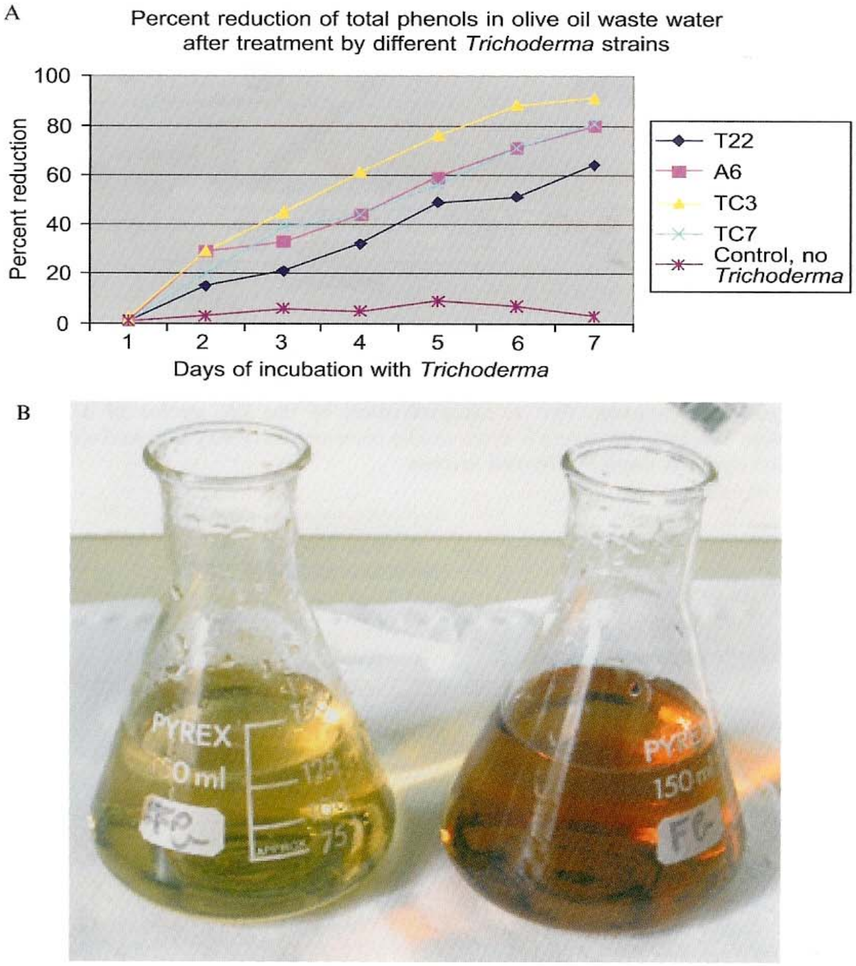

HARMAN *ET AL.*, FIG. 4. Degradation of polyphenolics from olive oil wastewaters by the activities of *Trichoderma* spp. The fungi were added to diluted (1:3 waste:water dilution) wastewater, the fungi were added and the content of polyphenolics in the mixture was followed over time. Phenolic compound content was determined by using the method of Folin-Denis based on the commercial substrate (Folin-Ciocalteu reagent; Sigma Scientific). A 5 ml total reaction mixture consisted of 250 μl of the reagent, 500 μl of sodium carbonate 20%, 100 μl of the sample to be tested, and distilled water. The phenolic concentration was determined based on a standard curve with differing concentrations of tannic acid. (A) The percentage degradation of the polyphenols; (B) the change in water color by the process. Analysis of variance of arcsine transformed percent values of all *Trichoderma* treatments were significantly different ($P < 0.05$) from controls without the strains.