At the cross roads of environmental pollutants and phytoremediation: A promising bio remedial approach

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REVIEW ARTICLE

At the cross roads of environmental pollutants and phytoremediation: a promising bio remedial approach

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Abstract Environment damages by pollutants from various sources have become a very serious predicament posing grave threats to mankind, plant wealth and other life forms. It is a challenging and daunting task to overcome this problem for which various physical, chemical and biological remedies have been followed successfully in some cases. Among the biological sources, research focused on plants has generated promising results for establishing an inexpensive, safe and economically viable technology. Several plants of diverse origin have been exploited rewardingly for converting perilous compounds into harmless and less dangerous products. The present review describes mainly three pollutants from textile dyes, paint preservative and heavy metal contaminants which are having carcinogenic and other life threatening properties. Role of plants has been demonstrated convincingly in several examples for removal of toxic chemicals from the environment. Recently, plant biotechnology has opened up new avenues for phytoremediation research offering numerous advantages for determining precise and accurate process of remediation of pollutants under controlled experimental conditions. This overview is an attempt to present a concise description of research currently underway on phytoremediation.

Keywords Chromium · Paints · Phytoremediation · Textile dyes

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Abbreviations

BOD	Biological oxygen demand
COD	Chemical oxygen demands
CW	Constructed wetlands
GTN	Glycerol trinitrate
Cr (VI)	Hexavalent chromium
MIT	Methylisothiazolinone
MTBE	Methyl tertiarybutyl ether
OIT	Octhilinone
PAHs	Polyaromatic hydrocarbons
PCBs	Polychlorinated biphenyls
ROS	Reactive oxygen species
TCE	Trichloroethylene
TOC	Total organic carbon
TCE	Trichloroethylene
TNT	Trinitrotoluene
TPHs	Triphenyl phosphates
VOCs	Volatile organic compounds

Introduction

Environmental pollution is any discharge of material or energy into water, land, or air that causes or may cause acute (short-term) or chronic (long-term) damage to the Earth's ecological balance or that lowers the quality of life. In humanity's recent history, pollution has been primarily a local problem. The industrialization of society and the explosion of the human population, however, have caused an exponential growth in the production of industrial products and services. By-the-time, this growth led to a tremendous increase in waste by-products and the eminence of life on earth is strictly associated with the overall quality of the environment. More or less human carelessness and negligence in using natural

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resources have limited the abundance of land and wealth. Currently, millions of different chemicals are available on the Global market which have been either introduced from natural resources or synthesized artificially for the benefit of daily life, medicines, food production and industrial purposes and the majority of these compounds have a rather poor biodegradability. The indiscriminate discharge of untreated industrial and domestic wastes into water sources, the spewing of thousands of tons of particulates and airborne gases into the atmosphere, the "use-and-throw" attitude toward solid wastes, and the use of newly developed chemicals without considering potential consequences have resulted in major environmental disasters, including the formation of smog. Technology has begun to solve some pollution problems and public awareness of the extent of pollution will eventually force governments to undertake more effective environmental planning and adopt more effective pollution control measures.

Over the last century, the content of xenobiotic compounds in ecosystems has increased considerably. Nowadays, contaminated soils and waters pose a major environmental and human health problem that cannot be solved easily. Many synthetic substances, which include pesticides, solvents, dyes, and by-products of metal, chemical and petrochemical industries, are eventually released into natural vegetation, cultivated crop fields and environment, where they can either be harmful to human, animals or plants (Fig. 1). Among the hazardous synthetic-man-made substances, textile dyes, wall paints and heavy metals are the three major contaminants.

Hitherto, in attempting to preserve the environment, new and traditional methods of remediation using physical, chemical and biological principles are being studied (Agarwal et al. 2006). However, the current physico-chemical and engineering treatments are very costly and involve the digging up of contaminated soils and disposal of the wastes to a landfill, lead to contamination elsewhere and can create significant risks in excavation, handling and transport of hazardous materials (Vidali 2001). Alternately, bioremediation processes for the removal of hazardous pollutants have attracted a lot of focus from all over the world and this process involves microbes, plants and animals. Gifford et al. (2006) has accounted zooremediation as a tool for removal of pollutants from aquatic ecosystems, but animals are rarely considered for bioremediation initiatives owing to ethical or human health concerns (Fig. 2).

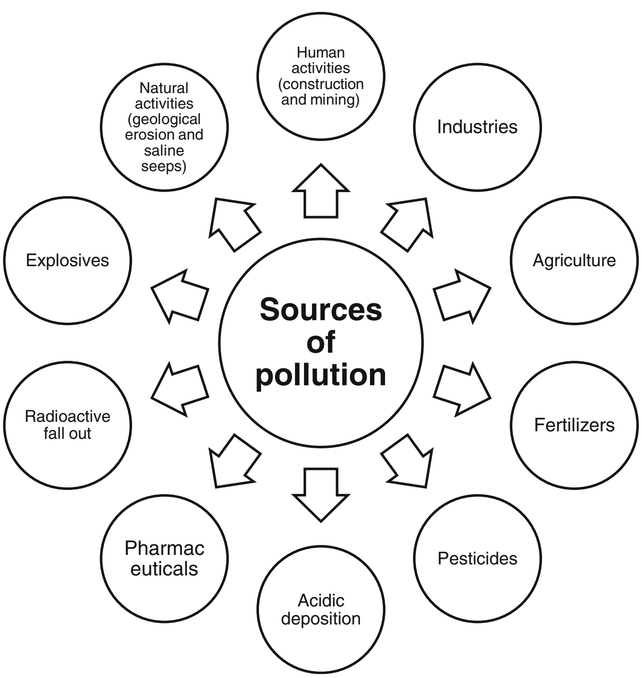
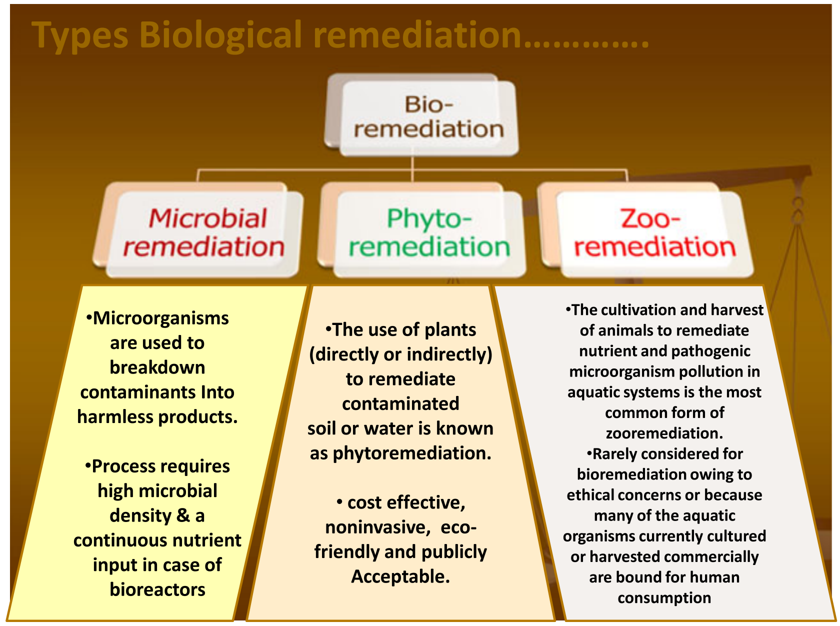


Fig. 1 Sources of environmental pollutants

Against this backdrop, reports on plants growing in polluted areas without being seriously harmed indicate that it should be possible to detoxify contaminants using agricultural and biotechnological approaches. Higher plants possess a pronounced ability for the metabolism and degradation of many recalcitrant xenobiotics and are often considered as "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 and are shown to possess comparable metabolic pathways for degradation of organic pollutants. Thus, it appears that crops and cultivated plants can be used for the removal of hazardous persistent organic compounds and toxic metals from industrial wastewaters leading to the concept of phytoremediation.

Phytoremediation has been defined as the use of green plants and their associated microorganisms to remove, contain, or render harmless environmental pollutants. The use of plants for the removal of xenobiotics and heavy metals from spillage sites, sewage waters, sludge, and polluted areas is now an important experimental and practical approach over the past few decades. Knowledge of the physiological and molecular mechanisms of phytoremediation has begun to emerge together with biological and engineering strategies designed to optimize and improve the phytoremediation process. Phytoremediation also avoids the need for soil excavation and transport and causes less disruption to ecosystems than physical, chemical or microbial remediation. Numerous plant species have been explored and treatment systems have been set up. But the systems established overall seem to partially fulfill their task removing pollutants from various matrices with good efficiency and at comparatively low cost. Adki et al. (2012a, b, and 2011) have reported the general characteristics of the ideal candidate plant for phytoremediation (e.g. cactus): the plant should be so sturdy that it could decrease efficiently the load of toxic pollutants in the environment, the absorption and storage of toxic heavy metals and other organic pollutants in the plant systems should normally be safe that pose little or no any danger of entering into the food chain, the plant should also be an efficient scavenger of pollutants because of massive roots that are capable of

Fig. 2 Bioremediation methods



effectively mining a large part of any contaminated soil. Insight to the current state of affairs, environmental pollution remediation recommended a detailed study on the role of plant systems for bioremediation of toxicants and environmental pollutants. Hence, the present review is an attempt to study and understand the role of plant systems for bioremediation and biodegradation of environmental toxicants, mainly; textile dyes, a paint preservative (Troysan S89) and hexavalent chromium.

Aspects of environmental pollution

The World Health Organization (WHO) has estimated that about a quarter of the diseases facing humanity today occur due to the prolonged exposure to the environmental pollution. Most of these environment-related diseases are however not easily detected and may be acquired during childhood and manifested later in the adulthood. Improper management of solid and liquid waste is one of the main causes of environmental pollution in many cities and industrial sectors across the globe, especially in developing countries. Many of the developing countries lack waste regulations and proper disposal facilities, including the harmful waste that may be infectious, toxic or radioactive.

Municipal waste dumping sites and water reservoirs are designated places where the waste disposal is stored. Depending on a regional level of waste management, such waste may be dumped in an uncontrolled manner, segregated for recycling purposes, or simply burnt. Poor waste management poses a great challenge to the well-being of city residents, particularly those living adjacent to the dumpsites due to the potential of the waste to pollute water, food sources, land, air and vegetation. The poor disposal and handling of waste thus leads to environmental degradation, destruction of the ecosystem and poses great risks to public health.

Health effects of pollution on human

Effects of dyes on health

According to European Commission (Atkins 2000), some of the reactive dyes are recognized respiratory sensitizers. Breathing in respiratory sensitizers can cause occupational asthma. Once a person is sensitized, re-exposure to even very small amounts of the same dye may result in allergic symptoms such as a runny or stuffy nose, watery or prickly eyes, wheezing, chest tightness and breathlessness. Some dyes can cause similar allergic skin reactions. Certain reactive, vat and disperse dyes are recognized as skin sensitizers. A small number of dyes, based on the chemical benzidine, are thought to possibly cause cancer. Wastewaters and lands from an industrial area in India have been studied to assess the possible genotoxic health risk and environmental genotoxicity due to the textile industry effluents (Mathur et al. 2005). Allergic dermatoses and respiratory diseases are known to be caused by reactive dyes (Hatch and Maibach 1995; Manzini et al. 1996). Contact dermatitis, asthma (Thoren et al. 1980) and immunosensitivity (Park et al. 1991) have been reported in textile industry workers exposed to dyes. Previous studies have also suggested increased risks of colon and rectum cancers; however, these cancers relate mostly to dyes for synthetic fibres (De Roos et al. 2005).

Effects of paints and paint preservatives on health

The United States of America's Environmental Protection Agency (US EPA) classifies paint as one of its top five most hazardous substances. Prolonged or high exposure to paint and paint fumes can cause headaches, trigger allergies and asthmatic reactions, irritate skin, eyes and airways, and put increased stress on vital organs. As a result, paints are not environmentally friendly and can lead to the development of several health problems. The WHO has reported a 20–40 % increased risk of certain types of cancer (in particular lung cancer) for those who come into regular contact with, or work with paint while other researchers have pointed out to the added possibility of neurological damage.

Effects of chromium on health

Hexavalent chromium is recognized as a human carcinogen via inhalation [International Agency for Research on Cancer 1999]. Workers in many different occupations are exposed to hexavalent chromium. Problematic exposure is known to occur among workers who handle chromate-containing products as well as those who perform welding, grinding, or brazing on stainless steel. Within the European Union (EU), the use of hexavalent chromium in electronic equipment is largely prohibited by the Restriction of Hazardous Substances Directive. Hexavalent chromium compounds are genotoxic carcinogens. Chronic inhalation of hexavalent chromium compounds increases risk of lung cancer (lungs are especially vulnerable, followed by fine capillaries in kidneys and intestine). Soluble compounds, like chromic acid, are much weaker carcinogens. Chromate-dyed textiles or chromate-tanned leather shoes can cause or exacerbate contact dermatitis (Salnikow and Zhitkovich 2008). Ingestion of Cr(VI) can also cause irritation or ulcers in the stomach and intestines (http://www.epa.gov/region7/pdf/national_beef_leathersprime_tanning_chromiumVI_Fact_Sheet.pdf).

Textile dyes pollution

Approximately 10000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually throughout the world (Gomare et al. 2009). Due to the poor exhaustive properties of the dyes, unfixed dyes ultimately find its way into the environment. A very small amount of dye in water (10–50 mg/L) affects the aesthetic value, water transparency and gas solubility of water bodies (Banat et al. 1996). Textile wet processing (i.e. preparation, dyeing, printing and chemical finishing) has always been considered one of the worst industrial sectors in terms of water consumption and pollution. In treating 1 t of cotton fabric, the composite waste stream may have 200–600 ppm BOD (biological oxygen demand), 1,000–1,600 ppm of total solids and 30–50 ppm of suspended solids contained in a volume of 50–160 m^3 (Hirschler 1996). Nowadays, the increasing demand for non-fading colored textiles has led to a continuous growth in the use of azo-dyes. Azo dyes account for the majority of all textile dyestuffs produced and are the most commonly used synthetic dyes in the textile, food, paper making, color paper printing, leather and cosmetic industries (Chang et al. 2001). Sulfonated azo dyes are a class of synthetic organic colorants having great structural differences with a great variety of colors. Structurally azo dyes are characterized by their typical (N=N−) azo bonding. These dyes are extensively used for dyeing cotton fabrics and are consequently discharged into the effluents posing a serious environmental threat. The discharge of these sulfonated azo dyes not only have a negative aesthetic effects but also some azo dyes and their degradation products, sulfonated and unsulfonated aromatic amines are toxic or even carcinogenic (Myslak and Bolt 1998).

Pollution from paint preservatives

Paint, a generic term used for a wide range of surface coating products which include conventional solvent-borne formulations, varnishes, enamels, lacquers and water-based systems. The function of paints and coatings is to provide an aesthetically pleasing appearance, preservation, decoration as well as to help metal and other substrates to withstand exposure to both environment and every day wear and tear. Broadly, paint is composed of two general ingredients, the pigment and the fluid medium. The pigment usually consists of a mineral oxide or precipitated vegetable dye that determines its color, while the fluid medium is of oil, varnish or water in which the pigment is suspended. It contains binders, turpentine, cellulosic thickeners and other minor ingredients such as coalescing agent, defoamers and biocide preservatives. Several reports have shown that paints available in the market have harmful substances and containing volatile organic compounds (VOCs); which give off the characteristic smell of a fresh wet paint and are mostly found in the solvents and binders of the paint. These compounds have long lasting harmful effects and can give off damaging fumes, years after the paint has been applied. Research has shown that the air inside the homes is more harmful than the air outside. The preservatives help to protect the paints from microbial attack since waterborne paints are prone to contamination and spoilage by fungi, algae and many types of bacteria where the conditions of temperature and humidity are conducive for microbial growth. The microbial growth is very common in exterior emulsion paints but fungal growth also occurs in interior paints where the humidity level is generally high (Adki et al. 2011). To prevent this, the most important chemical classes of biocides used as film preservatives are derivatives of urea, isothiazoline-3-one, dithiocarbamates, benzimidazole, triazines, benzothiazole, carbamates, thiophthalimide, sulfenic acids, sulfones, triazoles and pyridine-N-oxide (VdL 2000). However, a range of anti-fouling paints does not contain biocides and exhibit a toxic effect (Karlsson and Eklund 2004; Jungnickel et al. 2008).

The preservative, Troysan S89, widely used in paint, as an algaecide as well as a fungicide, is a mixture of three different chemicals namely carbendazim, diuron and ochthilinone. Diuron is a substituted urea algaecide classified as phenyl urea herbicide, used to control a wide variety of broadleaf and grassy weeds of agricultural crops (Giacomazzi and Cochet 2004; Adki et al. 2011). Diuron inhibits photosynthesis hence used to control weeds on hard surfaces, such as, roads, railway tracks, and paths. It can be used for both pre-emergent and knockdown weed control. It is a white, crystalline, odorless solid and is stable towards oxidation and moisture under normal conditions and decomposes at 180–190°C. This chemical has a low acute toxicity on mammals but juveniles are more susceptible than adults (Hayes 1982). It is less toxic to birds but moderately toxic to fish and readily absorbed through the gut and lungs. It can be irritant to eyes and throat but less irritant to intact skin. Male rats showed changes in their spleen and bone marrow, when subjected to extremely high doses of diuron over a 2-week period. Changes in blood chemistry, increased mortality, growth retardation, abnormal blood pigment and anemia are a few of the other chronic effects exerted due to moderate to high doses of diuron over time (U. S. Environmental Protection Agency 1983, 1984, 1985, 1987; Food and Drug Administration 1986; Baltimore 1988; Kidd and James 1991; National Library of Medicine 1992). Diuron is known to be carcinogenic in rats and reported to cause bladder, kidney and breast cancers (Cox 2003). It is also having mutagenic and teratogenic effects such as delayed bone formation and reduced birth weight.

Another compound, carbendazim, is a systemic benzimidazole fungicide (Ministry of Agriculture, Fisheries and Food 1992) plays an important role in plant disease control of arable crops (cereals, oilseed rape), fruits, vegetables and ornamentals (Quian 1996). It is also used in post-harvest food storage and as a seed pre-planting treatment. It works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis. Carbendazim has extensive applications worldwide (WHO/FAO Joint Meeting 1994). Carbendazim is less toxic to rodent and non-rodent species via the oral, dermal, inhalational and intraperitoneal routes. In mice, it shows increased tumor formation (Du Pont 1991). It is believed to affect hormone functions (Friends of the Earth 2001; Commission of Europian Community 1999). Mantovani et al. (1998) showed the teratogenic toxic effects of carbendazim such as serious deformities including absence of eyes and hydrocephalus. It is also reported to disrupt the development of sperms and damage testicular development in adult rats. It is reported as a potent aneugen on cultured lymphocytes (Du Pont 1991; Mahmood and Parry 2001). Carbendazim is a stable compound with a long half life in the environment. It is decomposed in the environment with half-lives of 6–12 months on bare soil, 3–6 months on turf, and half-lives in water of 2 and 25 months under aerobic and anaerobic conditions, respectively (WHO 1993).

Octhilinone (OIT) is a mild weedicide, fungicide and bacteriocide, used as a preservative in various household products including paints. OIT is an isothiazolone chemical, which can react with cellular thiols, such as cysteine residues in proteins, because of its structure and electron density (Du et al. 2002; King et al. 2009). Methylisothiazolinone (MIT), a derivative of OIT, was found to be toxic to neurons in vitro involving in glutathion depletion (Du et al. 2002). OIT and MIT were found to increase significantly the amount of oxidized glutathion in the human liver cell line Hep G2 (Arning et al. 2008). OIT is responsible and associated with development of occupational asthma and several cases of occupational allergic contact dermatitis, mostly among the paint manufacturers (Thormann 1982; Mathias et al. 1983; Bourke et al. 1997; Korte-Aalto et al. 2007; Balaguer et al. 2008).

Pollution from heavy metal (chromium)

Industries often produce infinite toxic substances, organic and inorganic as well, which are many of the times discharged directly in to the sea, rivers, lagoons and lands. Due to the strong corrosion resistance, hexavalent chromium [Cr (VI)] has been widely applied in a wide range of industries, including electroplating, wood preservation, leather-tanning and alloy production (Yu et al. 2008). Disposal of these industrial wastes containing high levels of chromates results in anthropogenic contamination of pristine environments, in spite the industrial effluent standards which must not exceed 0.75 and 0.25 mg/L of Cr(III) and Cr(VI) in water discharge, respectively (Mongkhonsina et al. 2011). Chromium can exist in a number of states in natural environment and Cr (VI) draws serious public health and legislative concerns because of its extremely high toxicity, mutagenicity and teratogenecity and listed as class A human carcinogens by the US EPA (Desai et al. 2008). Chromium oxyanoins can readily permeate through biological membranes and their intracellular reduction results in the dire consequences of the chromate induced toxicity by generation of Cr(V), Cr(III) valence states and reactive oxygen species (ROS) which damage cellular components including DNA, proteins and lipids (Rodriguez et al. 2011).

Physical and chemical methods for remediation of environmental pollutants

The numerous physico-chemical methods for subtraction of pollutants from environment have been reported (Lear et al. 2007; Shen et al. 2007; Hu et al. 2011) and are still underway to discover a sustainable remediation technology. Dozens of remediation technologies developed internationally could be divided in two general categories of incineration and non-incineration. These methods were applied depending on the source of contamination. The contamination of groundwater by toxicants is a matter of utmost concern to the public health. Remediation of contaminated groundwater is of highest priority since billions of people all over the world use it for drinking purposes. Hashim et al. (2011) has reviewed 35 approaches for groundwater treatment including chemical and physico-chemical treatment processes for removal of heavy metals. Remediation of metal-contamination countenances a particular challenge, because unlike organic contaminants, metals cannot be degraded in their native toxic form to simpler, non/less toxic components hence must be removed.

Dye wastewater is usually treated by physico-chemical treatment processes which include flocculation combined with flotation, electroflocculation, membrane filtration, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation, and katox treatment methods. However, these technologies are generally ineffective in color removal, expensive and less adaptable to a wide range of dye wastewaters (Banat et al. 1996). Adsorption has been observed to be an effective process for color removal from dye wastewater. Use of activated carbon has been found to be effective, but it is too expensive. Many studies have been undertaken to investigate the use of low-cost adsorbents for color removal (Ramakrishna and Viraraghavan 1997; Crini 2006; Gupta and Suhas 2009). However, these low-cost adsorbents have generally low adsorption capacities and require large amounts of adsorbents (Srinivasan and Viraraghavan 2010). Therefore, there is a need to find new, economical, easily available and highly effective forward-looking technologies for these methods.

The electrokinetic methods have been put for soil remediation that demonstrated effectiveness of the technology in removal of pollutants either singly (Shen et al. 2007) or in multiples (Alcántara et al. 2012). Diuron has been previously shown to be degraded by using electro-Fenton process (Edelahi et al. 2004). Degradation of carbendazim by physicochemical method by using UV/H_2O_2 was reported earlier (Mazellier et al. 2003). The coupling of two or more methods for soil remediation suggested efficacy of the system together (Davezza et al. 2011). Nanoremediation is an advanced technology that uses nanoparticles (NPs) for the environmental remediation. The recent developments on the use of inorganic NPs for environmental remediation in polluted soil, water and gas have been reviewed by Sánchez et al. (2011). So far, in attempting to preserve environment, new and traditional methods of remediation using physical, chemical and biological principles are being studied (Agarwal et al. 2006). However, the physico-chemical treatments have numerous disadvantages, including high cost, low efficiency, and inapplicability, to a wide variety of metals, as well as formation of huge quantities of toxic by-products, further creating disposal problems of contaminated wastes (Wani et al. 2007).

Cleaning up contaminated soil by the chemical and physico-chemical methods is a costly enterprise-the overall cost to remediate affected sites in the EU is estimated to be between €59 and €109 billion (Commission of the European Communities, 2002) that cannot be affordable to developing countries. Furthermore, current methods of soil remediation do not really solve the problem. In Germany, for instance, only 30 % of soils from contaminated sites are cleaned up in soil remediation facilities (SRU 2004); the remaining soil must be stored in waste disposal facilities. This does not solve the problem; it merely transfers it to future generations.

Microbial remediation of environmental pollution

Considering the hazards and disadvantages of physico-chemical remediation processes, alternative approaches are shifting towards the use of conventional biological methods to treat wastes (Jadhav et al. 2010). These methods are gaining more importance nowadays because of their lesser cost, effectiveness and eco-friendly nature. The metabolites produced after biodegradation are mostly non toxic or comparatively less toxic. Microbial remediation is the process whereby wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities.

Several reports have been cited here for microbial bioremediation of pesticides from Troysan S89. The white rot fungus *Phanerochaete chrysosporium* BKM-F-1767 was shown to degrade 10 and 14 mg/L diuron, in nitrogen limited synthetic medium and on ash wood chips, respectively. Co-immobilized bacterial cultures of *Arthrobacter* sp. and *Delftia acidovorans* were found to be effective for diuron removal when compared to their free cell cultures. Previous studies have showed that various microorganisms were able to degrade diuron partially in to 3,4-dichloroaniline (3,4-DCA) which was found to be accumulated in the medium (Tixier et al. 2000, 2001, 2002). *Arthrobacter* sp. N2 was shown to degrade diuron completely (Widehem et al. 2002). Several researchers have detected the degradation of herbicide carbendazim by using microbial sources either by pure cultures or in consortia. A microbial consortium capable of degrading the herbicide carbendazim was developed by enrichment of soil samples obtained from paddy fields and the degradation has been studied in glass column reactors using carbendazim as a sole source of carbon (Pattanasupong et al. 2004), while Zhang et al. (2005) had demonstrated carbendazim degradation by the new bacterial species.

The use of biological agents (plants and microbes) for decolorization of hazardous textile dyes is becoming a promising option. Microbial degradation of sulfonated azo dyes with possible degradation pathways have been reported earlier

(Lu and Hardin 2006; Kalme et al. 2007; Dawkar et al. 2010). Recalcitrant nature of azo dyes led failure in their degradation by typical biological (e.g. activated sludge) and physico-chemical (e.g. flocculation, coagulation) treatments, which largely promoted the transfer of the azo-dyes from the waste-water to the sludge, causing additional disposal problems. Furthermore, the incomplete degradation of azo-dyes may lead to the production of toxic by-products, such as aromatic amines (Heinfling et al. 1998; Kim and Shoda 1999; Manu and Chaudhari 2002) that can be transformed into highly reactive electrophiles, which have been described as mutagenic and carcinogenic (Gottlieb et al. 2003; Yoo et al. 2001), thus posing a significant health risk.

Microbial bioremediation for the removal of hazardous compounds has received quite a lot of attention from researchers all over the world with the high potentiality of prokaryotic systems to perform a variety of functions, but has some limitations. The use of microbes might lead to infections to humans that is why the method could not readily be used and required special restrictions. Obviously, there is an urgent need for alternative, cheap and efficient methods to clean up heavily contaminated industrial areas. This could be achieved by a relatively new technology known as phytoremediation, which uses plants to remove pollutants from the environment. Plants also possess some inherent metabolic pathways that can breakdown a wide range of toxicants but the fact was much less realized, hence the use of phytoremediation processes for the removal of toxicants is comparatively an unexplored methodology.

Phytoremediation: a brief overview- from a concept to the application

Phytoremediation is the use of plants and/or their associated microorganisms for the environmental cleanup. This is an emerging biotechnological application which operates on the principles of biogeochemical cycling (Raskin and Ensley 2000; Raju et al. 2008). This technology makes the use of the naturally occurring processes by which plants and their associated rhizospheric microflora degrade and sequester organic and inorganic pollutants (Pilon-Smits 2005). Plants are autotrophic organisms capable of using sunlight and carbon dioxide as sources of energy and carbon. However, plants rely on the root system to take up water and other nutrients, such as nitrogen and minerals, from soil and groundwater. As a side effect, plants also absorb a diversity of natural and man-made toxic compounds for which they have developed diverse detoxification mechanisms (Eapen et al. 2007). Pollutant-degrading enzymes in plants probably originate from natural defense systems against the variety of allelochemicals released by competing organisms, including microbes, insects and other plants (Singer 2006). From this viewpoint, plants can be seen as a natural, solar-powered pump-and-treat systems for cleaning up contaminated environments, leading to the concept of phytoremediation (Pilon-Smits 2005).

The term phytoremediation, from the Greek phyto, meaning "plant", and the Latin suffix remedium, "able to cure" or "restore", was coined by Ilya Raskin in 1994 and is used to refer to plants which can remediate a contaminated medium. Phytoremediation takes advantage of the plant's ability to remove pollutants from the environment or to make them harmless or less dangerous (Raskin 1996). It can be applied to a wide range of organic and inorganic contaminants. During the 1980s, the US Government initiated a large program for the development of environmental cleanup technologies (The Comprehensive Environmental Response, Compensation, and Liability Act or Superfund), which has accelerated the growth of a new productive research field worldwide (Krammer 2005). Though, this technology has been used for hundreds of years to treat human wastes, reduce soil erosion and protect water quality, research focusing specially on the phytoremediation of contaminated soils has only grown significantly in the last 25 years.

Mechanism of phytoremediation

Understanding the basic physiology and biochemistry that underlie various phytoremediation processes is very important to improve the applicability of this plant based method. In the following section, basic processes for phytoremediation are briefly summarized (Table 1) (Morikawa and Erkin 2003).

Table 1 Mechanism of phytoremediation

Phytostabilization	Immobilization of pollutants in rhizosphere by the process of precipitation
Rhizodegradation	Co-metabolic degradation of contaminants in the rhizosphere region by soil microbes
Phytoaccumalation/ phytoextraction	The extraction of metals or organics by plant roots from contaminated soil and water to translocate them to above ground shoots
Phytodegradation	Transformation or degradation of organics and/ or inorganics taken up by plants
Phytovolatilization	The volatilization through stomata of volatile chemicals taken up by plants from the media
Evapotranspiration	The combined effects of plants both to evaporate water on their leaf surfaces and to vaporize water at the stomata
Rhizofiltration	Use of hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants

Recent reports on phytoremediation

Phytoremediation has been implemented for environmental remediation since 1980s and its applicability is still underway of progress for sustainable remediation. A lot of advancement has been progressed in the utilization of plants for cleaning up environment. The Supplementary Table summarizes the recent research carried out worldwide. In the present review, out of various pollutants mentioned so far, phytoremediation of textile dyes, pesticides from Troysan S89 and heavy metal (chromium) has been discussed comprehensively.

Plants are natural attenuators to stress in the environment usually possessing properties to detoxify their surroundings, and may be suitable for use in phytoremediation. Under normal circumstances, plants have to metabolize endogenous chemicals (e.g. natural pesticides and growth hormones) and exogenous xenobiotics (e.g. herbicides, synthetic pesticides and textile dyes) (Scott-Craig et al. 1998; McCutcheon and Schnoor 2003; Ghodake et al. 2009). Plants have also shown to possess metabolic pathways for degradation of textile dyes (Kagalkar et al. 2009; Patil et al. 2009). Phytoremediation dominates over microbial and other physico-chemical methods because of cost effectiveness, safety, easiness to manage due to the autotrophic system of larger biomass requiring little nutrient inputs (Cunningham and Berti 2000). Depending on these facts, some of the plants have been tested on field for phytoremediation studies (Cunningham and Ow 1996) and on constructed wetlands have been used to treat dye wastewater domestic and industrial effluents (Carias et al. 2007; Nilratnisakorn et al. 2008). *Salsola vermiculata*, a desert plant, has been proved to be a low cost option for the removal of large organic molecules (Bestani et al. 2008).

Phytoextraction is an aspect of phytoremediation that involves the removal of toxins, especially heavy metals and metalloids, by the roots of hyperaccumulator plants with subsequent transport to aerial plant organs which are able to accumulate concentrations up to 100-fold more than those normally found in non-accumulators species (Brunetti et al. 2011). A number of plants have been studied for Cr uptake that included, *Prosopis sp*., *Typha angustifolia*, and *Convolvulus arvensis* (Haque et al. 2009; Dong and Wu 2007; Gardea-Torresdey et al. 2004). In addition, *Leersia hexandra* Swartz and *Salsola kali* have been reported as Cr hyperaccumulator (Zhang et al. 2005; De la Rosa et al. 2007). Moreover, *Prosopis* and *C. arvensis* have been accounted to tolerate, uptake, and reduce Cr(VI) to the less toxic Cr(III) (Aldrich et al. 2003; Montes-Holguin et al. 2006).

Though, extensive research has been focused to develop effective and efficient phytoremediation techniques for hyperaccumulation of metals (Padmavathiamma and Loretta 2007) and other organic molecules such as herbicides, pesticides (Kawahigashi 2009; Benekos et al. 2010), phytoremediation is still at its initial stages of research and development. Available data on phytoremediation of environmental pollutants is limited. Many of the plants should be checked for their phytoremediation potential and the knowledge should be explored for environmental welfare.

Applications of plant biotechnology for phytoremediation studies

Several plant-based experimental systems can be employed for the phytoremediation research viz. cell extracts, differentiated organ cultures such as roots and shoots, dedifferentiated plant cell cultures such as callus and cell suspensions, explants such as leaf disks and excised roots, plants in hydroponic culture or in potted soil under greenhouse cultivation or in the field. Plant tissue cultures share several common features with intact plants grown either hydroponically or in the field; however, these different culture systems also possess important unique properties (Doran 2009).

Plant tissue culture is a convenient tool for phytoremediation studies. As indicated in Fig. 3, in vitro plant cell and organ cultures have been applied in numerous studies intended to identify the capacity of plant cells' tolerance to, assimilation, detoxification, metabolism, and storage of a wide variety of organic and heavy metal pollutants. The complex network of interactions of various factors in stress tolerance could be studied at the initial stages of plant development under controlled conditions (Kumar et al. 2008). Dedifferentiated plant cells are cultured in the form of a callus or cell suspensions; differentiated organs such as roots and shoots can also be propagated in vitro. Because they grow, relatively quickly and do not require exogenous hormones in the medium, genetically transformed hairy roots and shooty teratomas are often used in tissue culture instead of untransformed roots and shoots. The forms of tissue culture most frequently employed are cell suspensions and hairy roots (Doran 2009).

Plant biotechnology using tissue culture, somaclonal variation and in vitro selection, offers the opportunity to develop new germplasm, better adapted to the changing demands (Skirvin et al. 1993; Alibert et al. 1994). Somaclonal variation and in vitro selection seem to be an appropriate technology for the development of new plant variants with enhanced metal accumulation and extraction properties (Herzig et al. 1997; Guadagnini et al. 1999). Further, improvement of a plant variety with better phytoremediation capacity by transgenic technology primarily requires plant tissue cultures.

Differentiated organ cultures for phytoremediation

Differentiated organ cultures include multiple shoots and root cultures that have been established by using suitable growth

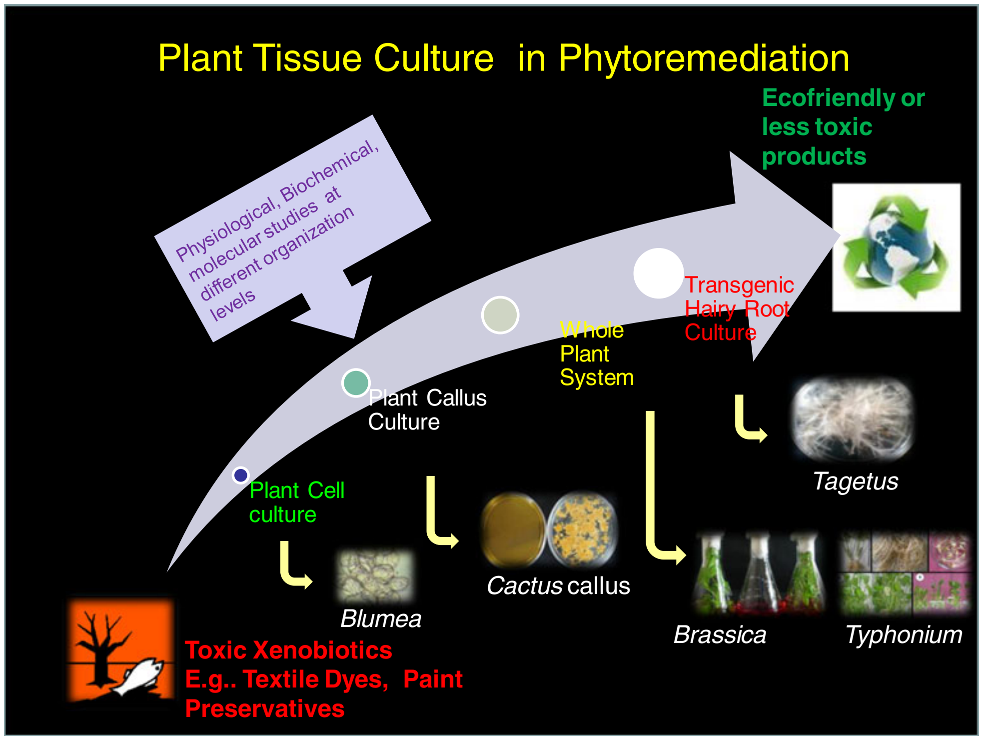


Fig. 3 Applications of plant tissue culture in phytoremediation research (Source: Acknowledgements to Dr. Umesh B. Jagtap, Department of Biotechnology, Shivaji University, Kolhapur for the inclusion of the figure)

regulators, either auxins, or cytokinins or combinations of both. Once established, these in vitro cultures can be propagated indefinitely and are available on demand. For the plants with slower growth rate (like cacti) micropropagation could enhance their rapidity and availability. Therefore, the time required to carry out experimental investigations may be substantially reduced using plant tissue cultures rather than whole plants. In this regards, Smykalova et al. (2010) has reported large scale and rapid screening of heavy metal tolerance in flax/linseed (*Linum usitatissimum* L.) tested in vitro. Because in vitro plant cultures are grown and maintained free from microbial contamination, they can be used to distinguish the responses and metabolic capabilities of plants from those of microorganisms normally present in the rhizosphere or in plant tissues (Chaudhry et al. 2005; Lebeau et al. 2008). Plant cultures can help to carry out studies under more but easily controlled conditions than with soil-growing plants, particularly with regard to medium composition, nutritional parameters, growth regulator levels, and medium additives. Although effecter substances can be added to soil, they may be rendered unavailable to plants due to the adsorption or binding with soil components. Extracellular complexation of substrates is minimized in tissue cultures, thus facilitating substance transport and uptake directly by the cells.

Callus and cell suspension cultures for phytoremediation

Main advantage in use of callus and suspended plant cells is lacking many of the barriers used by ex vitro plants to regulate penetration of chemicals from the environment, such as leaf wax, bark, cuticles, epidermis, and endodermis, and do not depend on translocation processes for tissue-specific metabolic activity. Hence, better and more uniform uptake of external components is generally expected in plant cell cultures (Camper and McDonald 1989; Lucero et al. 1999). The ability to feed in vitro cultures relatively large amounts of contaminants that would be unavailable from the soil at similar levels (Lucero et al. 1999) allows the recovery of metabolites and intermediates in quantities suitable for analysis (Laurent et al. 2007), providing a significant advantage for biochemical and metabolic research. This benefit is amplified when plant cells are cultivated in bioreactors in vitro in multiliter volumes (Knops et al. 1995). Because of the reduced amounts of starch, chlorophyll, and other pigments in cultured plant cells, compared with whole plants, isolation of reaction products from plant tissue cultures may be easier, require fewer purification steps, and yield samples of higher purity than from intact plants (Schmidt 2001).

The new plant variants with enhanced phytoremediation properties could be developed with somaclonal variation and in vitro selection technology. The isolation of highly metal-tolerant cell lines from the callus or suspension cultures of non-metallophytes like *Datura innoxia* L. has been reported by Jackson et al. (1984), suggesting that non-metallophytes should possess the ability for high level metal tolerance. Huang et al. (1987) have reported that tomato callus cell lines, selected on 5 mM Cd, showed a higher tolerance of Cd, a slightly higher tolerance of Cu, but not improved Hg, Zn, Pb or Ag tolerance. Nickel-tolerant callus lines of *Setaria italica* L. were developed by Rout et al. (1998). Callus cultures of *Ginkgo biloba* L. have been selected on a medium containing 10 or 100 μM Cd (Nehnevajova et al. 2002). Aluminium tolerance has been induced by in vitro selection in rice, maize and wheat (Jan et al. 1997; Ramgareeb et al. 1999; Sibov et al. 1999).

Protoplasts culture and somatic hybridization in phytoremediation research

Plant breeding could help bringing together the characteristics of two or more plants with counter-properties of phytoremediation to get a hybrid, ideal phytoremediation plant. But owing to the drawbacks such as time consumption, more manpower requirement, the plant breeding could not be used. Though, the possibility of using molecular techniques to engineer a larger plant for phytoremediation is attractive; unfortunately, the number or types of genes responsible for tolerance to and removal of pollutant have not been elucidated. Ingrouille and Smirnoff (1986) observed that tolerance and hyperaccumulation were genetically independent in *Thlaspi caerulescens*, and most genetic studies of metal tolerance in other species have concluded that tolerance is a polygenic trait. As an alternative to the isolation and characterization of all genes involved in metal tolerance, Brewer et al. (1999) has attempted to produce somatic hybrids between the zinc hyperaccumulator hybridize *T. caerulescens* with a related species of higher biomass *Brassica napus* by electrofusion of protoplasts isolated from each species and the growth of the plant in tissue cultures on high-zinc media as well as on high-Zn soil conclusively demonstrated the utility of somatic hybridization as a technique for the production of metal tolerant plants with greater phytoremediation capacity.

Hairy roots

Hairy roots are produced by genetic transformation using *Agrobacterium rhizogenes*. An overview of hairy root biology and applications, including detailed procedures for inducing hairy root cultures, is provided by Hamill and Lidgett (1997) and Bapat and Ganapati (2005). Suza et al. (2008) have highlighted in their review the advances in the use of hairy roots to assess plants for their potential in removing important water and soil pollutants such as metals, explosives, radionuclides, insecticides, and antibiotics. Hairy roots offer the important advantages of greater genotypic and phenotypic stability compared with dedifferentiated plant cells (Doran 2009), thus providing a more reliable and reproducible experimental system over time. They also have simpler culture requirements as exogenous plant growth regulators are not needed. Because hairy roots are themselves the products of genetic transformation of plant cells with bacterial DNA, further genetic modification to introduce genes for improved phytoremediation traits via the Ri (root-inducing) plasmid of *A. rhizogenes* is relatively straightforward. Alternatively, transgenic hairy roots can be initiated from already transformed plant material. Transgenic hairy root cultures are a useful tool in metabolic studies and for screening genetic transformants prior to regeneration of whole plants with enhanced phytoremediation potential.

Transgenic plants

Typically, transgenic plants exhibiting new or improved phenotypes are engineered by the over expression and/or introduction of genes from other organisms, ranging from bacteria to mammals. Historically, transgenic plants for phytoremediation were first developed in an effort to improve heavy metal tolerance; for example, tobacco plants (*Nicotiana tabacum*) expressing a yeast metallothionein gene for higher tolerance to cadmium, or *Arabidopsis thaliana* over expressing a mercuric ion reductase gene for higher tolerance to mercury (Misra and Gedamu 1989; Rugh et al. 1996). Transgenic narrow-leaved cattails (*Typha angustifolia* Linn.) created by transforming a hyperaccumulation gene into this plant, grown in contaminated sites have been reported to remediate heavy metals (Lincoln and Eduardo 2002). The first attempts to transform plants for phytoremediation of organic compounds targeted explosives and halogenated organic compounds in tobacco plants (French et al. 1999; Doty et al. 2000). These initial efforts in developing transgenic plants for phytoremediation lead to their applications to remediate contaminated sites. Transgenic poplars with improved phytoremediation abilities plants have been applied in the field to remove metal contaminants (Peuke and Rennenberg 2005).

Although the motivation to exploit plant cultures in phytoremediation research are convincing, this approach also has its limitations and drawbacks (Doran 2009). Plant tissue cultures cannot represent or simulate many aspects of whole plant cultivation, hence, the applicability of in vitro cultures depends in many ways on the original purpose of the research and is neither a practical nor a commercially feasible technology for direct application in large-scale phytoremediation operations; require sterile culture conditions throughout the process. However, a rapid progress in plant biotechnology is very promising to remediate hazardous chemicals using tissue culture technology.

Phytoremediation applicability

Phytoremediation technologies could be subdivided into two broad fields (Schröder 2003): the first dealing with the removal of compounds from the environment by phytoextraction, phytodegradation, phytoaccumulation, and pump-and-treat. The second field deals with the stabilization of compounds within the site of interest (e.g. phytostabilization and hydraulic control). Many phytotechnologies are at the demonstration level, but relatively a few have been applied in practice on large sites. Those options that may prove successful at a higher scale are (a) phytoextraction of metals, As and Se from marginally contaminated agricultural soils, (b) phytoexclusion and phytostabilisation of metal- and As-contaminated soils, (c) rhizodegradation of organic pollutants and (d) rhizofiltration/ rhizodegradation and phytodegradation of organics in constructed wetlands. Each incidence of pollution in an environmental compartment is different and successful sustainable management requires the careful integration of all relevant factors, within the limits set by policy, social acceptance and available finances. Many plant stress factors that are not evident in the short-term laboratory experiments can limit the effective deployment of phytotechnologies at field level. The current lack of knowledge on physicochemical and biological mechanisms that underpin phytoremediation, the transfer of contaminants to bioavailable fractions within the matrices, the long-term sustainability and decision support mechanisms should be understood to identify future R&D priorities that will enable potential end-users to identify particular technologies to meet both statutory and financial requirements.

There are many remediation techniques available, but due to cost, time, and logistical concerns, relatively a few are applicable to contaminated soils and waters. Whichever may be the technique, in general, remediation technologies are concerned with the two facts: they either remove the contaminants from the substratum ("site decontamination or clean-up techniques") or reduce the risk posed by the contaminants by reducing exposure ("site stabilization techniques"). Numerous plants have been attempted for phytoremediation of a variety of pollutants on the field; a few of them have been summarized in the Supplementary Table. More than 400 plant species have been identified to have potential for soil and water remediation. Among them, *Thlaspi, Brassica, Sedum, and Arabidopsis species* have been mostly studied for metal remediation (Lone et al. 2008). A significant uptake and transport of chromium in all the three tree species viz. *Azadirachta indica* A. Juss. (Neem), *Melia azedarach* Linn. (Wild Neem) and *Leucaena leucocephala* (Lam) de Wit (Subabool) raised over the tannery sludge suggesting that these plants could be employed in phytoremediation of soils contaminated with heavy metals (Sakthivel and Vivekanandan 2009).

Direct in situ appliances whether to contaminated sites or to industrial effluent treatment plants could result in the considerable removal of the pollutants, but the positive modifications in the technology might increase the potentiality of the phytoremediation process. A few of the advancements in situ phytoremediation applications include hydroponics and constructed wetlands for phytoremediation.

Hydroponic phytoremediation

The cultivation of plants and their phytoremediation applicability by hydroponic cultures has been reported for many of the pollutants (January et al. 2008; James and Strand 2009; Liu and Schnoor 2008). Hydroponic solutions are enriched with various macro and micro nutrients, providing a nutrient status which is closer to that of the soil in which the plant usually grows and can be used for the cultivation and/or maintenance of plants for phytoremediation. As a phytoremediation application, the use of hydroponics provides a cost effective method. Aubert and Schwitzguébel (2004) carried out the screening of four different plant species (*Rheum rabarbarum, Rumex acetosa, Rumex hydrolapatum* and *Apium graveolens*), in hydroponic solutions for the removal of sulfonated anthraquinones. Many plant species have the capacity to absorb large quantities of water from hydroponic solutions. The water absorption capacity of a plant is a factor that should be considered while performing studies in hydroponic solutions because it reflects the overall health of the plant. Lower water absorption capacity for the plant *R. acetosa* in hydroponic solution indicated that the plant might not be in optimum health under hydroponic conditions and thus the metabolism and transpiration could probably be reduced as compared to native growth conditions. Owing to the difficulties in using adult terrestrial plants such as *Rhubarb* and common sorrel, plants could be cultured under hydroponic conditions (Aubert and Schwitzguébel 2004). But, the cultivation and experimentation with plants in hydroponic systems showed a few major disadvantages that included the leaves of the same age and same stage of growth and development could not be collected in case of plants grown in hydroponics (Page and Schwitzguébel 2009), the level of enzymes like cytochrome P450 and peroxidases changed with the growth of the plants; which made difficulty in exactly confirming the role of these enzymes in the detoxification of dyes (Page and Schwitzguébel 2009). To overcome these problems with wild plants grown in hydroponics or in wetlands for phytoremediation, the importance of tissue culture based technologies has been stressed.

Constructed wetlands

CW are basically treatment systems that mimic the functions of natural wetlands by the use of processes involving wetland vegetation, soils and their associated microbial populations to improve water quality, with the benefit that the specific design of CW allows higher treatment efficiencies (Davies et al. 2005). The main role of wetland vegetation is attributed to the modification of soil texture, hydraulic conductivity and soil chemistry by the growth of plant roots and rhizomes. Phytoremediation with CW uses the storage of inorganics and the degradation of organics to clean contaminated waters. A broad range of effluents can be managed, e.g. municipal, domestic and industrial wastewaters, landfill leachates or products of sludge dewatering, containing organic pollutants, trace elements or radionuclides (Schröder et al. 2007).

Different models of CW could be engineered to achieve conditions closer to those prevalent at the contaminated sites or in order of achieving sustainable remediation management. A vertical flow constructed wetland was designed so as to work in intermittent feeding mode (8 feeding cycles per day) which enhanced the characteristics like constant hydrolic permeability and maximized the oxygen transfer rate and was tested for the removal efficiency of the dye Acid Orange 7 (Davies et al. 2005). CW with a continuous re-circulating system has been employed for phytoremediation of chromium by two tropical plants *Penisetum purpureum* and *Brachiaria decumbens* individually from tannery waste waters (Mant et al. 2006). Environmental parameters such as BOD, COD, TOC content, hardness and alkalinity etc. of the industrial effluents were evaluated to assess the applicability of CW as a phytoremediation technology.

Conclusion

Most scientific and commercial interest in phytoremediation now focuses on phytoextraction and phytodegradation that use selected plant species grown on contaminated sites. The plants are then harvested to remove the pollutants that have been accumulated in their tissues and also to get biomass together. Depending on the type of contamination, the plants can either be disposed off or used in alternative processes, such as burning for energy production. In essence, phytoextraction removes pollutants from the contaminated soils, concentrates them in biomass and further concentrates the pollutants by combustion. And such mixed-benefit strategies could be considered 'para-phytoremediation'. This etymological construction recognizes concurrent or post-remediation uses for phytoremediation plants in addition to the environmental detoxification (Rugh 2004).

Some of the metals can be recovered from plant tissue (phytomining) [e.g. humans have restored potassium (potash) for centuries] that has economic importance (Meagher 2000). In addition to accumulating toxic minerals in their tissues, plants are also able to take up a range of harmful organic compounds, including some of the most abundant environmental pollutants such as polychlorinated biphenyl (PCB), halogenated hydrocarbons (trichloroethylene, TCE) and ammunition wastes [nitroaromatics such as trinitrotoluene (TNT) and glycerol trinitrate (GTN)]. Subsequent metabolism in plant tissues then mineralizes or degrades such pollutants to non- or less-toxic (Peuke and Rennenberg 2005). Compared with conventional methods of soil remediation, the use of plants provides several striking advantages. It is cheap: after planting, only marginal costs apply for harvesting and field management, such as weed control. It is a carbon-dioxide neutral technology: if the harvested biomass is burned, no additional carbon dioxide is released into the atmosphere beyond what was originally assimilated by the plants during growth. Phytoremediation is also a potentially profitable technology as the resulting biomass can be used for heat and energy production in specialized facilities.

Spectacular advances in recent times pertaining to genomics, proteomics, metabolic engineering and in other genetic engineering technologies have opened new avenues for understanding the mechanisms involved in phytoremediation of hazardous chemicals. Additionally, availability of high-tech instruments has considerably helped to learn how the various cellular mechanisms involved converting toxins to nontoxic products. The selection of hyper accumulator plants could be an easier task for formulating efficient strategies for phytoremediation. In this connection, achievements and progress in plant biotechnology research will help to remove environmental pollutants more easily in the near future.

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