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REMOVAL OF AZO DYE COMPOUNDS FROM PAPER INDUSTRIES WASTES USING PHYTOREMEDIATION METHODOLOGY

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ABSTRACT

Environment pollution is one of the human's unsolvable problems from the start of the industrial revolution and colonization. Phytoremediation is one of the measures in order to counter the problem of accumulation of the wastes in the soil in the form of inorganic and organic wastes. Treatment of soil for removal of these wastes is a tedious and uneconomical way. But, phytoremediation is one of the effective means in this regard. Certain species of plants like-grasses (rye, bermuda, sorghum, fescue); legumes (clover, alfalfa, cowpeas), sunflowers; Indian mustard; rape seed plants; barley, hops; crucifers; serpentine plants; nettles, dandelions have the inherent ability to accumulate these wastes within it, by absorption through root. They also have the ability to detoxify and metabolize them within the plant. This potential property is effectively used in the removal of the wastes like azo dyes, colorants, metal components, phenolic, and other organic and inorganic wastes. This paper documents, the phytoremediative activity of the plant *Eucalyptus spp*. in absorbing the azo dyes. The evidence for the effective role of *Eucalyptus spp*. in absorption of the azo dyes is experimentally shown by the gas chromatography- mass spectrometry analysis. Also, the planting of plants and trees for the phytoremediation in turn reduce the carbon dioxide in the atmosphere and thus, global warming and other residual effects of air pollution. Also, this method is cost effective and reliable.

Key words: Phytoremediation, *Eucalyptus spp*., Azo dyes, GC-MS

INTRODUCTION

Around the world, there is an increasing trend in areas of land, surface waters and

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groundwater affected by contamination from industrial, military and agricultural activities either due to ignorance, lack of vision, or carelessness. The build-up of toxic pollutants (metals, radionuclide and organic contaminants) in soil, surface water and ground water not only affects natural resources but also causes a major strain on ecosystems. Remediation of contaminated sites using conventional practices, such as 'pump-and-treat' and 'dig-and-dump' techniques, is often expensive, has limited potential, and is usually only applicable to small areas. Additionally, these conventional approaches to remediation often make the soil infertile and unsuitable for agriculture and other uses by destroying the microenvironment. Hence, there is need to develop and apply alternative, environmentally sound technologies (ESTs), taking into account the probable end use of the site; once it has been remediated.

Phytoremediation

Phytoremediation ('phyto' means plant) is a generic term for the group of technologies that use plants for remediating soils, sludges, sediments and water contaminated with organic and inorganic contaminants. Phytoremediation can be defined as "the efficient use of plants to remove, detoxify or immobilize environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants". Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. Phytoremediation involves growing plants in a contaminated matrix, for a required growth period, to remove contaminants from the matrix, or facilitate immobilization (binding/containment) or degradation (detoxification) of the pollutants. The plants can be subsequently harvested, processed and disposed. Plants have evolved a great diversity of genetic adaptations to handle the accumulated pollutants that occur in the environment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is a passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. Phytoremediation can be used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates. It can also be used for river basin management through the hydraulic control of contaminants. Phytoremediation has been studied extensively in research and small-scale demonstrations, but full-scale applications are currently limited to a small number of projects. Further research and development will lead to wider acceptance and use of phytoremediation.

There are several ways in which plants are used to clean up, or remediate, contaminated sites. To remove pollutants from soil, sediment and/or water, plants can break down, or degrade organic pollutants or contain and stabilize metal contaminants by acting as filters or traps. The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The root system provides an enormous surface area that absorbs and accumulates the water and nutrients essential for growth, as well as other non-essential contaminants. Researchers are finding that the use of trees (rather than smaller plants) is effective in treating deeper contamination because tree roots penetrate more deeply into the ground. In addition, pumping the water out of the ground and using plants to treat the contamination can treat deep-lying contaminated ground water.

Plant roots also cause changes at the soil-root interface as they release inorganic and organic compounds (root exudates) in the rhizosphere. These root exudates affect the number and activity of the microorganisms, the aggregation and stability of the soil particles around the root, and the availability of the contaminants. Root exudates, by themselves can increase (mobilize) or decrease (immobilize) directly or indirectly the availability of the contaminants in the root zone (rhizosphere) of the plant through changes in soil characteristics, release of organic substances, changes in chemical composition, and/or increase in plant-assisted microbial activity. Phytoremediation is an alternative or complimentary technology that can be used along with or, in some cases in place of mechanical conventional clean-up technologies that often require high capital inputs and are labor and energy intensive. Phytoremediation is an *in situ* remediation technology that utilizes the inherent abilities of living plants. It is also an ecologically friendly, solar-energy driven clean-up technology, based on the concept of using nature to cleanse nature.

The pollution of soil and water with heavy metals is an environmental concern today. Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, and their remediation in soils and sediments are among the most technically difficult. The projected cost for remediation of areas containing mixtures of heavy metals and organic pollutants by conventional means is $ 35.4 billion over the next five years**.** The high cost of existing cleanup technologies led to the search for new cleanup technologies that has the potential to be low-cost, low-impact, visually benign, and environmentally sound. Phytoremediation is a new cleanup concept that involves the use of plants to clean or stabilize contaminated environments. The most studied phytoremediation technology is phytoextraction, a plant-based cleanup method involving the use of metal accumulating plants to extract metal contaminants from soil. Once metals have been sequestered in the tissues, the above-ground portions of the plant can be harvested resulting in the permanent removal of metals from the site. Phytoextraction is not the answer to all environmental problems, but rather it is another tool to be used in conjunction with existing remediation technologies. Over the past decade, researchers have sought to perfect this remediation technology. The majority of phytoextraction research has focused on finding the ideal metal-accumulating plant and the means by which metals can be liberated from the soil for root uptake. At present, Indian mustard (*B. juncea*) is among the most viable candidates for the phytoextraction of a number of metals including Cd, Cr (IV), ^{137}Cs, Cu, Ni, Pb, U, and Zn. Only a fraction of the phytoremediation research addresses the use of *B. juncea* for the phytoextraction of Zn even though this element is one of the most prevalent heavy metals at contaminated sites. Zinc is among the least toxic of all metal contaminants to humans, and this may be the reason, why other metals have been given priority in phytoremediation research efforts in the past. Few studies have focused on the development of specialized agricultural practices for phytoextraction, although most researchers agree that this is an area that warrants further attention. Mineral nutrition profoundly influences the growth of plants and the absorption of nutrients by plant root, two areas with which phytoextraction is greatly concerned. With the exception of Zaurov et al.[1], all plant nutrition information for *B. juncea* with regards to phytoremediation has been anecdotal e.g. Ebbs et al.[2]; Ebbs and Kochian[3,4]; Kumar et al.[5]). Proper plant nutrition has the potential to be an effective, low cost agronomic practice for enhancing the phytoextraction of heavy metals by plants, but more research is required before fertilizers can be used effectively for this purpose.

Phytoremediation process

Depending on the underlying processes, applicability, and type of contaminant, phytoremediation can be broadly categorized as:

(i) ***Phytodegradation***: Use of plants to uptake, store and degrade contaminants within its tissue,

(ii) ***Phytostimulation or rhizodegradation***: Use of rhizospheric associations between plants and symbiotic soil microbes to degrade contaminants,

(iii) ***Phytovolatilisatio****n*: Use of a plant's ability to uptake contaminants from the growth matrix and subsequently transform and volatilize contaminants into the atmosphere,

(iv) **Phytoextraction**: Use of plants to absorb, translocate and store toxic contaminants from a soil matrix into their root and shoot tissue,

(v) ***Rhizofiltration***: Use of roots to uptake also stored contaminants from an aqueous growth matrix and

(vi) ***Phytostabilisation***: Plant-mediated immobilization or binding of contaminants into the soil matrix; thereby, reducing their bioavailability.

The following list gives the media, contaminants and typical plants for the types of phytoremediation listed above.

Application	Media	Contaminants	Typical plants
Phytovolatization	Soil, groundwater, landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); Aromatics (BTEX); Chlorinated aliphatics (TCE); Nutrients; Ammunition wastes (TNT,RDX)	Phreatophyte trees (poplar, willow, cottonwood, aspen); Grasses (rye, bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)
Rhizodegradat-ion	Soil, sediments, land application of waste water	Organic contaminants (pesticides aromatic, and polynuclear aromatic hydrocarbons	Phenolics releasers (mulberry, apple, osage orange); Grasses with fribous roots (rye, fescue, bermuda); Aquatic plants for sediments
Phytostabilization	Soil, sediments	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U), Hydrophobic organics (PAH,PCB,DDT, dieldrin)	Phreatophyte trees to transpire large amounts of water (hydraulic control); Grasses to stabilize soil erosion; Dense root systems are needed to sorb/bind contaminants
Phytoaccumula-tion/extraction	Soil, Brownfields, sediments	Metals(Pb, Cd, Zn, As, Cu, Cr, Se, U) with EDTA addition for Pb, Selenium	Sunflowers; Indian mustard; rape seed plants; barle, hops; crucifers; Serpentine plants; nettles, dandelions
Degradation	Soil, groundwater, landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); Aromatics (BTEX); Chlorinated aliphatics (TCE); Nutrients; Ammunition wastes (TNT,RDX)	Phreatophyte trees (poplar, willow, cottonwood, aspen); Grasses (rye, bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)

A major advantage, that is listed above, is the low cost. For example, the cost of cleaning up one acre of sandy loam soil at a depth of 50 cm with plants is estimated at $ 60,000-$ 100,000 compared to $ 400,000 for the conventional excavation and disposal method. One reason for this low cost is phytoremediation may not require expensive equipment or highly specialized personnel, and can be relatively easy to implement. One major concern with phytoremediation is the possible affects on the food chain. For example, vegetation is used that absorbs toxic or heavy metals and moles or voles eat the metal contaminated plants. The predators of the moles or voles then become victims of intoxication. All though the possibilities of such scenarios are being looked at, more fieldwork and analysis is necessary to understand the possible effects, phytoremediation can have.

Azo dyes

Three different mechanisms for azo dye carcinogenicity were identified; all involving metabolic activation to reactive electrophilic intermediates that covalently bind DNA. These mechanisms are: (i). Azo dyes that are toxic only after reduction and cleavage of the azo linkage to give aromatic amines, mostly via intestinal anaerobic bacteria. The aromatic amines are metabolically oxidized to reactive electrophilic species that covalently bind DNA. (ii). Azo dyes with structures containing free aromatic amine groups that can be metabolically oxidized without azo reduction and (iii) Azo dyes that may be activated via direct oxidation of the azo linkage to highly reactive electrophilic diazonium salts. Each mechanism may be compound specific; thus, azo toxicity is probably caused by more than one mechanism. Although, it is not possible to predict azo dye carcinogenicity with absolute certainty, it is possible to establish certain guidelines. Because some species of intestinal anaerobic bacteria (and in some cases, hepatic azo reductases) may reduce any azo compound to aromatic amines. Those containing aromatic amine subgroups are known to be carcinogenic, such as benzidines, must be suspect carcinogen. Information about human carcinogenicity of other specific aromatic amines is scanty, and various short-term mutagenicity tests may provide some guidance. Other *in vitro* tests can directly assay new azo dyes. Although, it is unlikely that azo dyes can be developed that can be guaranteed not to generate constituent aromatic amines, it may be possible to select aromatic amines that are not toxic.

Measured data concerning the emissions of azo dyes to the environment in Denmark are not available. This applies both for the production (processing) and the use phases. The major route of release during the production phase is through wastewater effluent from the processing industries, mainly from textile and to a smaller extent from leather. In the present survey, it is assumed that releases from the remaining trades: paper mills, printing, plastics and paint industries are negligible and approximately zero. In addition, it shall be noted that no manufacture of dyes takes place in Denmark. There is a potential release of dyes to the wastewater during the consumption (use phase) of the end-products (paints, varnishes, textiles etc.) from industries as well as private households. However, the predominant potential release route from end-use is from waste deposited in landfills. The potential atmospheric release route may be through particulate matter from soils, which are treated with sludge, from waste deposits (land-fills), from incineration of waste and from emissions of the processing industry. It is estimated that the atmospheric release route is insignificant and approximately zero. Agricultural soil fertilized with sludge may give rise to releases of dyes to soil/groundwater. In addition, landfill deposit of dyes contained in products may cause release of dyes to soil/groundwater, too. The estimated Danish releases are shown in Table 1. The preconditions for the estimates are also given. It should be noted, that the release to landfills is assumed to be associated, exclusively with the consumption of end-products (use phase).

EXPERIMENTAL

Materials and methods

Procedure

In this experiment to demonstrate phytoremediation, two plants were grown in the soil around the work place, into which the waste water from the industry was released. Plant of the same variety grown in normal soil was considered. It is taken as the control. Three test plants are numbered as 219, 413 and 419 grown in different areas of the soil around the industry, where the waste water is released. All these plant components are tested by collecting their leaves. These leaves were ground and samples were prepared by using microwave digestion method. In this method, the leaves were ground well and are then dried in a microwave oven. These samples were then tested using VOC method on gas-chromatography to analyze the components present in the plants. This work was done to prove that phytoremediation is an effective method in removing contaminants from the soil, which make the soil unfit for agriculture. The contaminants here are from the paper industry, which mainly consists of compounds like dyes (azo dyes in particular). The main method used to evaluate the results in this process is gas-chromatography (VOC method), which is done using GC-MS equipment.

Calculations

For headspace gas calculations, concentration of each compound in ppm by volume:

$$\text{Concentration, ppm (vol)} = [24.5(A) \times (10^3)] / [(B) \times (MW)]$$

Where, A = Quantified amount of a compound, (μg); B = Injection volume (mL) and MW = Molecular weight of the compound (g/mole).

Note: 24.5 L/mole is the molar volume of an ideal gas at $25^{o}C$

For liquid sample calculations, concentration of each compound in percent by volume is:

Concentration, % by vol = [(Y) x (0.1)]/[(Z) x (D)]

Where, Y = Quantified amount of a compound for one injection, (μg),

Z = Injection volume, (μL), and

D = density of the compound, (g/mL).

For liquid samples, concentration of each compound in g/L:

Concentration, g/L = Y/Z

where: Y = Quantified amount of a compound for one injection, (μg), and

Z = Injection volume, (μL)

Total VOC, g/L is the sum of the concentrations of each compound.

RESULTS AND DISCUSSION

The standard methods are used to analyze the samples. The characteristics of paper and pulp industrial waste water was estimated (Table 1). After the treatment and before the treatment of the pulp and paper mill effluents results are shown, These are characteristics of treated pulp and paper mill effluents, ground water and surface waters

Fig.1: The farm used for the cultivation of the *Eucalyptus Spp*.

Fig. 2: Numbering of the sample along with the control

Fig. 3: Preparation of sample for the GC-MS analysis

Table 1: Physico-chemical and chemical composition of treated paper mil effluents collected from ITC, Bhadrachalam

Parameters	Tolerance limits (industrial) prescribed by ISI (IS: 2490-1981) for their discharge into		Treated paper mill effluent mean
	Inland surface water	Land for irrigation	
pH	5.5-9.0	5.5-9.0	7.75
E.C. (ds/m)	-	-	1.81
TS (mg/L)	-	-	1320

Cont…

Parameters	Tolerance limits (industrial) prescribed by ISI (IS: 2490-1981) for their discharge into		Treated paper mill effluent mean
	Inland surface water	Land for irrigation	
TDS (mg/L)	2100	2100	1260
TSS (mg/L)	100	200	60
BOD (mg/L)	30	100	21
COD (mg/L)	250	-	65
Acidity (mg/L)	-	-	52
Ca^{2+} (mg/L)	-	-	162
Mg^{2+} (mg/L)	-	-	19
Na^{+} (mg/L)	-	-	169
K^{+} (mg/L)	-	-	40
CO_3^{2-} (mg/L)	-	-	-
HCO_3^{-} (mg/L)	-	-	244
Cl^{-} (mg/L)	1000	600	198
SO_4^{2-} (mg/L)	-	-	408
SAR	-	-	3.34
Water class	-	-	C3SI

For the estimation, GC-MS was used. The method is VOC analyzing method and the sample preparation was done by the extraction process by using microwave digestion. From the above, it can be observed that azo dye is the only compound that is found in the test plants and which is not present in the control, which implies that the plants in the contaminated soil are absorbing the azo dyes from soil.

Analysis of *Eucalyptus spp* (Control) in GC-MS

The phytoremediation is an effective method to treat the soil and wastewater from pulp industry. The control waste was analysed by GC-MS and the compounds present in the leaf extract of *Eucalyptus* were found out. In the control, 1,8-cineole (eucalyptol), (E)-4, 8-

dimethyl-1, 3, 7-nonatriene (DMNT), (E,E)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMNT), (E,E)-Alpha-farnesene, (E,E,E)-3, 7, 11, 15-tetramethyl-1, 3, 6, 10, 14-hexadecapentaene (TMHP), beta-caryophyllene, alpha-humulene, germacrene D, beta-cubebene, menthol, thymol, methyl salicylate and 1,8-cineole (eucalyptol) were present.

Analysis of *Eucalyptus spp* (Sample 219) in GC-MS

After treatment of waste water, the *Eucalyptus* leaves were collected and analysed with the GC-MS. In this, sample 219 is having 1,8-cineole (eucalyptol), (E)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT), (E,E)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMNT), (E,E)-alpha-farnesene, (E,E,E)-3, 7, 11, 15-tetramethyl-1, 3, 6, 10, 14-hexadecapentaene (TMHP), beta-caryophyllene, alpha-humulene, germacrene D, beta-cubebene menthol, thymol, methyl salicylate,1,8-cineole (eucalyptol), N,N-disubstituted 1, 4-diaminobenzene and amines 2-bromo-4, 6 dinitroaniline. So that one can arrive at the conclusion that the Eucalyptus leaf using the phytoremediation removes these dyes. 2-Bromo-4, 6 dinitroaniline and N, N-disubstituted 1, 4-diaminobenzene azo dye compounds came into the leaves.

Analysis of *Eucalyptus spp* (sample 419) in GC-MS

After treatment of wastewater, the *Eucalyptus* leaves were collected and analysed with the GC-MS. In this, sample 419 is having 1,8-cineole (eucalyptol), (E)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT), (E,E)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMNT), (E,E)-alpha-farnesene, (E,E,E)-3, 7, 11, 15-tetramethyl-1, 3, 6, 10, 14-hexadecapentaene (TMHP), beta-caryophyllene, alpha-humulene, germacrene D, beta-cubebene menthol, thymol, methyl salicylate,1,8-cineole (eucalyptol) and amines 2-bromo-4,6 dinitroaniline. The only two azo dye compounds came into the leaves. So this implies that these dyes are removed by the *Eucalyptus* leaf using the phytoremediation. 2-Bromo-4,6 dinitroaniline and N,N-disubstituted 1,4-diaminobenzene azo dye compounds came into the leaves.

Analysis of *Eucalyptus spp* (sample 413) in GC-MS

After treatment of wastewater, the *Eucalyptus* leaves were collected and analysed with the GC-MS. In this, sample 413 having 1,8-cineole (eucalyptol), (E)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT), (E,E)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMNT), (E,E)-alpha-farnesene, (E,E,E)-3, 7, 11, 15-tetramethyl-1, 3, 6, 10, 14-hexadecapentaene (TMHP), beta-caryophyllene, alpha-humulene, germacrene D, beta-cubebene menthol, thymol, methyl salicylate,1,8-cineole (eucalyptol), N,N- disubstituted 1,4-diaminobenzene and amines 2-bromo-4,6 dinitroaniline. The only two azo dye compounds came into the leaves. So that one can assume that the *Eucalyptus* leaf removes these dyes using the phytoremediation. This is the most advanced method and low cost method to remove the metals and azo dye compounds. Here 219, 413 and 419 are the different *eucalyptus spp*.

Components	219	419	413	Control
1,8-Cineole (eucalyptol),	P	P	P	P
(E)-4, 8-Dimethyl-1, 3, 7-nonatriene (DMNT)	P	P	P	P
(E,E)-4, 8, 12-Trimethyl-1, 3, 7,11-tridecatetraene (TMNT)	P	P	P	P
(E,E)-Alpha-farnesene	P	P	P	P
(E,E,E)-3, 7, 11, 15-Tetramethyl-1, 3, 6, 10, 14-hexadecapentaene (TMHP)	P	P	P	P
Beta-Caryophyllene	P	P	P	P
Alpha-Humulene	P	P	P	P
Germacrene D	P	P	P	P
Beta-Cubebene	P	P	P	P
Menthol	P	P	P	P
Thymol	P	P	P	P
Methyl salicylate	P	P	P	P
1,8-Cineole (eucalyptol)	P	P	P	P
2-Bromo-4,6-dinitroaniline	P	P	P	N.P
N,N- Disubstituted 1,4-diaminobenzene	P	P	P	N.P

Where,

P. - Present

N.P. - Not present

CONCLUSION

The present work makes it clear that the plants have effective bioremediative role in absorbing harmful contaminants from the soil and waste water, which is coming out from the paper and pulp industry. The results are expressing the fact that azo dye compounds are present in the paper and pulp industrial effluent, which can be treated by these *Eucalyptus* leaves using the method as an effective and low cost treatment method to treat the pulp and paper industrial effluents. Also, planting trees for phytoremediation will add values in environment. With this information, one can conclude that phytoremediation is an effective method in treating contaminated waters and lands.

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