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Rhizo-nano Remediation of Methylene Blue Dye in Soil

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ABSTRACT

Background: Discharge of textile effluents are major problems in most of the countries. These effluents contain xenobiotic compounds that affect both water and soil ecosystem.

Methods: The research has been performed at Parul Institute of Applied Sciences laboratory, Waghodia, Gujarat in the year 2022. Pots were filled with mycorrhizal soil spiked with methylene blue dye at various concentrations and pinch of iron oxide nanoparticle was spread over all pots except the control one. About 10 marigold seeds were sown per pot which were kept in green house for 3 months. After this, soil samples were collected and biodegradation of methylene blue dye was assessed by HPLC and GCMS technique in the lab.

Result: This research focuses on biodegradation of methylene blue dye on formation of mineral nano particle which is already available in soil as micronutrient *i.e* iron. Iron has been converted into nanoparticle *i.e* iron oxide which will act as nutrient supplement for the plants. Mycorrhizal association with iron oxide nano particle proved to be beneficial for plants by alleviating the adverse effects of methylene blue dye and ceasing soil pollution.

Key words: Dye degradation, Iron oxide nano particle, Methylene blue dye, Mycorrhiza.

INTRODUCTION

Untreated industrial wastewater discharged into ecosystems poses serious problems to the aquatic organisms, plants and humans. Environmentalists pay special attention to the textile sector since it uses a lot of water, dyes and chemicals for various textile processing (Ramchandran 2013). It is estimated that around 50% of the synthetic dyes used in the textile industries does not bind to the fabric and result in getting discharged into the environment (Rehman 2018). These coloured dye gets accumulated near the catchment area and allied agricultural field rendering them unfertile or crops growing there would be toxic and not fit for consumption. The compounds can leach into the groundwater and the use of those water sources for farming results in contamination of agricultural fields (Zhou 2001). A huge amount of colored wastewater is produced that is usually poisonous, resistant to biodegradation and sustainable in environment; thus, common conventional methods aren't effective (Ehrampoush 2010).

The use of bioremediation process or microbiological systems to ameliorate the negative effects of residual dyes on ecosystem is widespread accepted as a future key-technology which is sustainable and eco-friendly. Significant information on the structure and behaviour of isolated microbial communities' rhizospheric microbiota is now accessible. These microbial communities have beneficial effects along with dye degradation which includes increased plant growth, essential nutrient acquisition, pathogens tolerance and increased abiotic as well as biotic stress tolerance such as drought, temperature, salinity and antagonistic activities against the phyto-pathogens (Kumawat *et al*., 2022). Highly polluted sites are usually devoid of beneficial soil microorganisms. Naturally occurring allies like mycorrhizal association could be used to enhance plant survival on such difficult sites (Turnau *et al.*, 2002). Rhizosphere bioremediation has been shown to increase soil organic carbon, bacteria and mycorrhizal fungi which are all factors that promote the breakdown of soil organic matter (Schnoor, 1997). Many persistent organic chemicals like atrazine, hexachlorocyclohexane (HCH), aromatic hydrocarbons (BTEX) and polycyclic aromatic hydrocarbons (PAHs) are known to be degraded in the mycorhizosphere directly or indirectly (Korade and Fulekar 2009). Large-scale operations are very difficult and strict conditions must be followed in order to carry out the biodegradation process. Therefore, there is an urgent need to use new technologies such as nanotechnology for faster decomposition of dyes. (Sharma and Shirkot 2019).

Nanomaterials are atoms or molecules with a size range between 1 to 100 nm that can drastically modify their physiochemical properties which can be used to decontaminate the toxic organic and inorganic chemical of dye from the environment. Metal based nanoparticles are been used extensively due to their unique properties and

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novel characteristic in various applications (Verma *et al*., 2021). Iron deficiency is a widespread nutritional problem in plants growing mainly in high pH and calcareous soils (Pourjafar *et al*., 2016). Iron nanoparticles exhibit unique physical and chemical properties due to their ultrafine size and a high density of corner or edge surface sites (Sharma and Shirkot 2019). They will act as nano fertilizers which will provide nutrient supplement to the plants in dye contaminated soil.

MATERIALS AND METHODS

Test plant selection

Tagetes patula L. commonly called as Marigold was selected for development of mycorrhizal soil at laboratory scale in Parul Institute of Applied Sciences laboratory, Waghodia, Gujarat in the year 2022. Marigold seeds were procured from local market. Before sowing, the seeds were surface-sterilized for 2-3 minutes with 0.1% mercuric chloride and rinsed several times with distilled water to avoid fungal contamination. These seeds were further used for the development of mycorrhizal soil.

Soil Sampling and characterization

Soil used for the development of mycorrhizal soil, was collected from a depth of 0-15 cm along the banks of Sindhrot dam, Vadodara, Gujarat. Stones and plant remains were removed from the soil, air dried and screened through 2 mm stainless steel sieve and stored in plastic bag at room temperature. The soil was then characterized for physico-chemical parameters *i.e*. pH, electrical conductivity, moisture content, organic carbon, kjeldahl nitrogen, phosphorus and potassium were analyzed using Standard methods described in APHA.

Greenhouse experimental design

For the development of mycorrhizal soil, a greenhouse experiment was set up using pot culture technique under controlled environmental conditions. The experiment was carried out in 3 pots, each pot containing 3 kg sand-soil mixture. For better aeration and drainage of water, perforations were made at the base of the pots. A mixture of soil and sand (3:1) was used for growing host plants to provide porosity in the soil. About 3 gm VAMOZ mycorrhizal inoculum was mixed with 3 kg of sand-soil mixture per pot in 1:1 ratio. Ten sterilized marigold seeds were then sown in each pot and their growth was monitored for 3 months. The plants were watered regularly and provided with Hogland solution to provide essential nutrients. Approximately 10 ml of Hogland solution was provided per pot, every 15 days. All pots were placed in a greenhouse with natural sunlight at temperatures of 27-28°C. After 3 months, dense mass of roots were formed colonized with AMF and mycorrhizal spores. Further, roots of the plants were chopped and mixed in the same soil which will be our 'soil based mycorrhizal inoculum' for further experiment.

Preparation of iron oxide nanoparticle

For the synthesis of iron oxide nano-particles in solid phase, powders of $FeCl_3.6H_2O$ (1.35 g), $FeCl_2.4H_2O$ (0.50 g) and KCl (3.9 g) were mixed and grounded in a mortar at room temperature for 30 min. Yellow coloured paste was obtained. KOH powder (1.22 g) was added in mortar followed by grinding for another 30 min at room temperature. Dark brown paste was formed. During grinding and KOH addition significant amount of heat and some vapour was given off in the first few minutes. The product was then washed with distilled water. Product was kept in sonicator for 15 minutes and centrifuged for 15 minutes at 3500 RPM. Several times this process was repeated until no Cl^- ion could be detected in the centrifugate. The product was then dried in vacuum at 50°C, maghemite was formed. By increasing the temperature, hematite (Fe_2O_3) was formed *i.e.* iron oxide. Flowchart of preparation process is shown in Fig 1.

Spiking of dye and nanoparticle in the soil

Mycorrhizal inoculum obtained from green house experiment was used. About 4 pots were filled with mycorrhizal soil spiked with methylene blue dye at various concentrations *viz*. 0 (control), 10, 25 and 50 mg/kg. Pinch of iron oxide nanoparticle was spread over all pots except the control one. About 10 marigold seeds were sown per pot amended with methylene blue dye and iron oxide nanoparticle. This was carried out in triplicate set (total 12 pots) and the pots were kept in green house at a temperature of 27-28°C with the natural light for 3 months as shown in Fig 2(a) and (b). After this soil samples were collected at an interval of 1 month to evaluate degradation of dye. Biodegradation of methylene blue dye was assessed by HPLC and GCMS technique in the lab.

RESULTS AND DISCUSSION

Mycorrhizal and normal soil comparison

Physico-chemical properties of the developed mycorrhizal soil had better pH, moisture holding capacity, OC, N and K than in the original soil. Comparison between normal soil and mycorrhizal soil has been depicted in Table 1.

The pH of the mycorrhizal soil was found to be 7.42 which is considered as neutral. Electrical conductivity was

Table 1: Comparison between normal soil and mycorrhizal soil after 3 months.

Parameters	Normal soil	Mycorrhizal soil
pH	6.88	7.42
Electrical conductivity (µmhos/cm)	8.0	8.4
Moisture content (%)	25.92	26.10
Total organic carbon (%)	0.36	0.50
Kjeldahl nitrogen (%)	0.20	0.31
Phosphorus (%)	0.028	0.011
Potassium (%)	0.33	0.39
Spore count (per 100 gm soil)	-	320

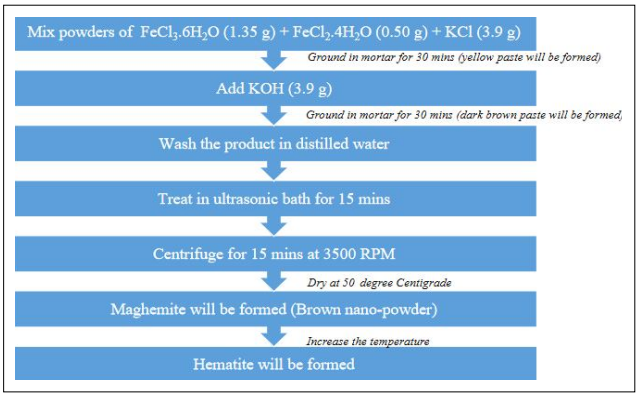

Fig 1: Preparation process of iron oxide nanoparticle.

8.4 in mycorrhizal soil which indicates effective movement of ions. Moisture content of mycorrhizal soil was 0.18% more than normal soil which depicts it can retain more water as compared to normal soil. The organic carbon of the developed mycorrhizal soil was found to be higher (0.50%) than in the collected soil (0.36) which indicates higher organic matter for the survival of various microbial population. Nitrogen content was found to be 0.11% more in mycorrhizal soil as compared to normal soil which is essential for chlorophyll molecule for generating food for the plant. The phosphorous content did not vary much during development of mycorrhizal soil and was found to be lower. Soil potassium levels recorded 0.06% increased in mycorrhizal soil in comparison with normal

Fig 2: (a) When marigold seeds were sown in mycorrhizal soil (b) Growth of marigold after three months.

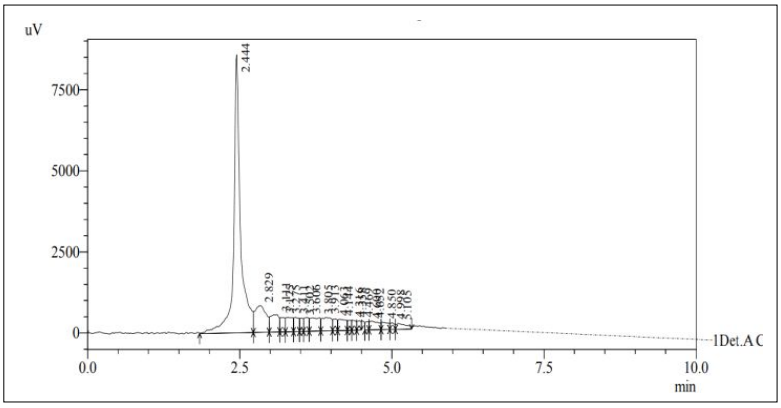

Fig 3(a): Pot 1 with no MB dye and nanoparticle.

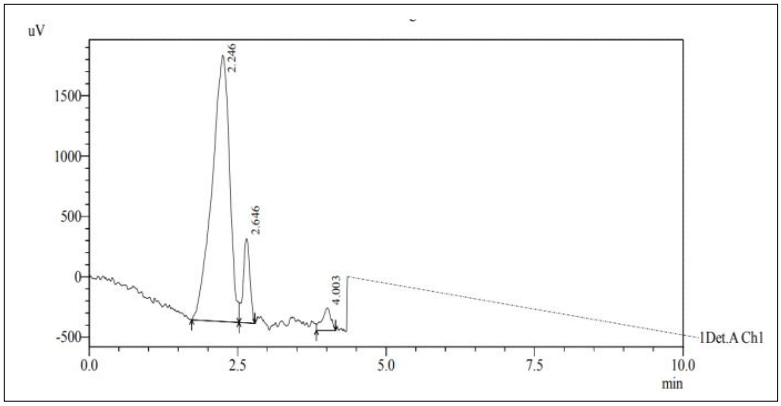

Fig 3(b): Pot 2 with iron oxide nanoparticle and 10mg/kg of MB dye.

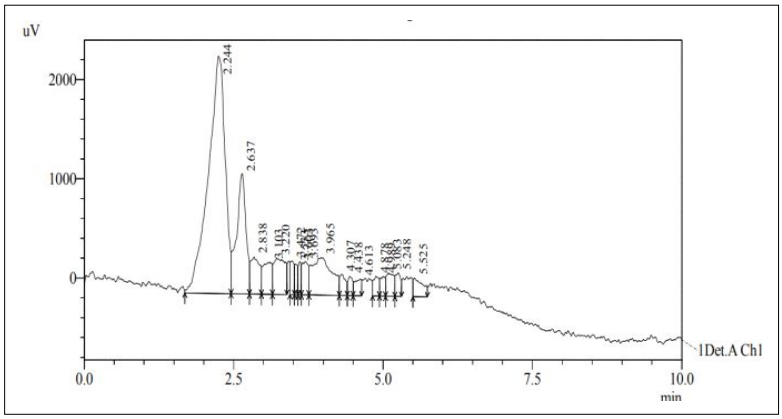

Fig 3(c): Pot 3 with iron oxide nanoparticle and 25mg/kg of MB dye.

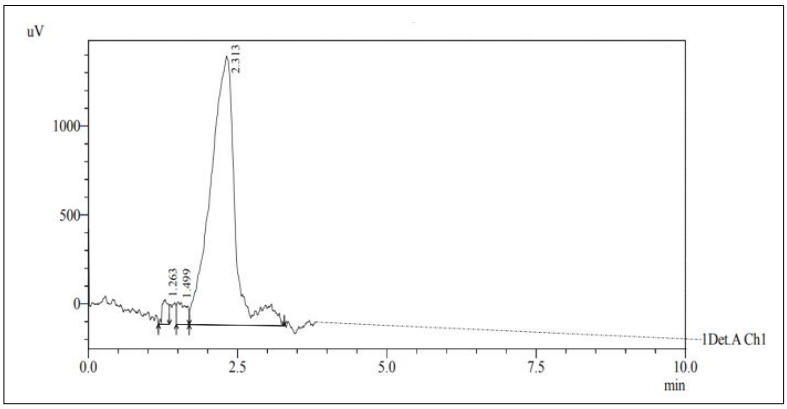

Fig 3(d): Pot 4 with iron oxide nanoparticle and 50mg/kg of MB dye.

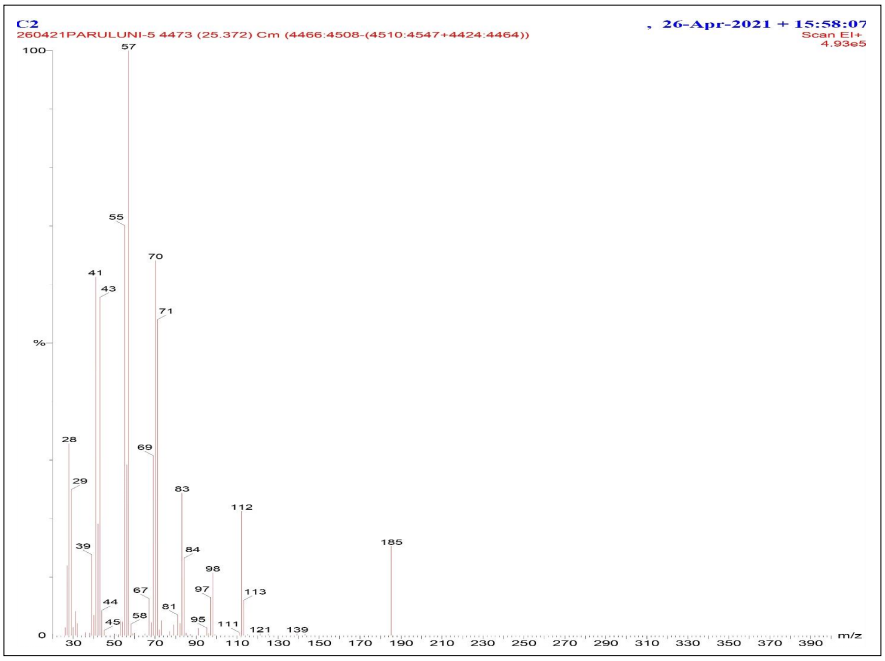

Fig 4: GC-MS analysis of compounds formed after dye degradation.

soil which will trigger activation of more ATP molecules and beneficial enzymes.

HPLC analysis

HPLC chromatogram (Fig 3 (a), (b), (c), (d)) confirmed the degradation of methylene blue dye in all the three samples of pot (except the control one). Pot-1 consisted of only mycorrhizal soil (control) with no MB dye in it which produced major peak at retention time of 2.444. Pot-2 consisted of mycorrhizal soil + pinch of iron oxide nanoparticle + 10 mg/kg of MB dye which produced major peak at retention time of 2.246 indicating 60% dye degradation. Pot-3 consisted of mycorrhizal soil + pinch of iron oxide nanoparticle + 25 mg/kg of MB dye which produced major peak at retention time of 2.244 indicating 62% dye degradation. Pot-4 consisted of mycorrhizal soil + pinch of iron oxide nanoparticle + 50 mg/kg of MB dye which produced major peak at retention time of 2.313 indicating 64% dye degradation.

GC-MS analysis

Gas chromatography and mass spectra (GC–MS) analysis was done to investigate the metabolites formed during the biodegradation process. GC–MS analysis showed formation of these compounds, *viz.* oxalic acid (molecular weight = 314, m/z = 29), formic acid (molecular weight = 289, m/z = 41), carbonic acid (molecular weight = 212, m/z = 43), acetic acid (molecular weight = 197, m/z = 69), octanol (molecular weight = 158, m/z = 70) after degradation as shown in Fig 4.

CONCLUSION

Analytical techniques like HPLC and GCMS demonstrated that degradation of dye resulted with significant reduction of phyto toxic metabolites with the formation of many beneficial metabolites like oxalic acid, confirming its safety to discharge and harmless to the environment. Nano particle acts as nano fertilizer and minimize the potential adverse effect of dye and its residue in soil. Optimum use of mineral nano particle along with association of mycorrhizal soil will increase the nutrient content in plants along with degradation and decolourization of textile dye. Rhizo Nanotechnology could provide eco-friendly alternatives for environmental management without harming the natural environment. Furthermore, before these nanomaterials are widely used, their health effects and environmental fate must be addressed.

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Conflict of interest: None.

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