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A FIELD STUDY ON SOIL NUTRIENTS, ENZYME ACTIVITIES, AND INSECTICIDE RESIDUE DURING PHYTOREMEDIATION*

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Abstract

Phytoremediation is an eco-friendly treatment for reducing soil contamination. Cypermethrin is one of the most widely used pyrethroid insecticides against different pests, and its use causes soil contamination. A field study was conducted in cypermethrin contaminated soil to detect and monitor the changes in soil nutrients, enzyme activities, and 3-phenoxy benzoic acid (3-PBA, primary metabolite of cypermethrin insecticide). Phytoremediation was undertaken using two plant species: aster (*Callistephus chinensis* L. Nees) and Bermuda grass (*Cynodon dactylon* L. Pers.) in the absence and presence of iron oxide particles (0.01 g/kg of soil). Soil pH, soil organic carbon (SOC), total nitrogen (N), available phosphorus (P), and available potassium (K_2O) and relative metals content by EDXRF. The activities of soil urease (mg NH_4^+-N g^{-1} soil h^{-1}) and dehydrogenase (µg TPF g^{-1} soil h^{-1}) also increased in the treated soil samples as determined by the phenol-hypochlorite colourimetric method and the Triphenyl Tetrazolium Chloride (TTC) assay method. The urease enzyme activities of aster (5.363 ± 0.024) and Bermuda grass (4.816 ± 0.07) was found to be higher when compared with uncultivated soil (S_0) (3.74 ± 0.03), whereas the dehydrogenase activity of aster (0.00127 ± 0.0000) and Bermuda grass (0.00113 ± 0.0000) was also increased when compared with S_0 (0.0008 ± 0.0000) in the field experiment. Furthermore, the insecticide residue in the soil samples was determined by UV-Vis and GC-MS. The results showed the decrease in insecticide residue decrease in during phytoremediation in the presence of iron oxide particles. Phytoremediation demonstrated 96.33 % and 94.19 % PBA decrease using aster and Bermuda grass in 12 weeks period. The results demonstrated that aster and Bermuda grass showed promising potential for use as phytoremediating agents in insecticide contaminated soil.

Keywords: soil nutrients, enzyme activities, phytoremediation, aster, Bermuda grass, iron oxide particles, insecticide residue

Introduction

Cypermethrin is a highly active synthetic pyrethroid insecticide and is widely used to control insects and has been detected in organisms, including humans. Pyrethroids have been shown to pose neurotoxicity, hepatotoxicity, endocrine disruption, and reproductive risks in mammals (Wang *et al*., 2017). Cypermethrin and its metabolite, 3-phenoxybenzoic acid (PBA) have exerted adverse biological impacts on the environment; therefore, it is critically important to develop different methods to enhance their degradation (Xie *et al*., 2008). Phytoremediation is a versatile technology to treat polluted soils, pollutants, deposits, and groundwater in a profitable as well as environmentally friendly way through the usage of plants, and then thus be referred to as natural green biotechnology. The use of iron oxide particles has high potential in phytoremediation (Demangeat *et al.*, 2021).

Some species such as aster and Bermuda grass are better suited for phytoremediation due to their pre-adaption to environmental conditions of contaminated sites (e.g., weather and soil) (Swe Sint *et al*., 2021), their need for less care (e.g., frequent irrigation, and fertilizers) and their quality for restoring natural ecosystems. China aster (called “Maymyo-pan plant” in Myanmar) belongs to the family ‘Asteraceae’ and is native to China. China aster is one of the most popular annual flower crops cultivated widely due to its myriad colours ranging from violet, purple, magenta, pink, and white, and a comparatively longer vase life (Chaitra and Patil, 2007).

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Generally, asters grow best in moist, well-drained soils with plenty of sunlight. Bermuda grass (called “Myay Zar Myet” in Myanmar**)** belongs to the family ‘Poaceae’ and is native to Africa. Bermuda grass was evaluated for its ability to reduce oil sludge contamination in soil and the most efficient cultivar was chosen. Bermuda grass is an efficient species for phytoremediation of petroleum contaminated soil, and the selection of a more tolerant and efficient cultivar is possible. In general, Bermuda grasses are drought tolerant; that is, they survive dry soil conditions longer than most turfgrasses.

Soil enzyme activities are greatly influenced by soil properties and could be significant indicators of insecticide contaminated soil for bioavailability assessment (Xian *et al*., 2015). A reliable assessment of quality of soil contaminated with organic products is possible by testing the activities of lipase, dehydrogenase, catalase, and ureases. The main role of urease is to allow the microorganisms to use urea as a source of nitrogen (Jingjing *et al.,* 2019). Dehydrogenase occurs in all living microbial cells. It is a quick and relatively simple method to determine the overall activity of microorganisms (Wolinska *et al*., 2016). This study also focuses on the determination of urease and dehydrogenase activities in soil microorganisms. Moreover, the changes of soil nutrients and insecticidal residue were assessed during phytoremediation using aster and Bermuda grass in the presence or absence of iron oxide particles.

Materials and Methods

Soil Sample Collection Site

Soil samples were collected from the surface layer (0-20 cm depth) of an agricultural field located in Myaungtagar Village (17º 11' 45'' N latitude and 95º 59' 09'' E longitude) Hmawbi Township, Yangon Region (Figure 1). This field is usually cropped with a rose and lady’s fingers rotation without treatment of any pyrethroids insecticide (including cypermethrin) for many years.

Figure 1. Sample collection site (Myaungtagar Village, Hmawbi Township, Yangon Region)

Study Design for Field Experiment

The field experiment was carried out on the farm at Myaungtagar Village, Hmawbi Township, Yangon Region from September (2021) to December (2021) to study the effect of magnetic iron oxide particles on the degradation of 3-phenoxy benzoic acid (3-PBA) in the insecticide contaminated soil. The field was prepared well by one deep ploughing, followed by three cross harrowing and planking. The field was constructed with four parallel rows of five-foot-long planting beds spaced 12 inches apart and treated with composted organic fertilizer (a mix of chicken dung and rice hull). The selected seeds were sown in another plot similarly treated with organic fertilizer.

The experiment was laid out with two treatments in a Randomized Complete Block (RCB) design by spraying with insecticide (cypermethrin) in each bed (control and treatment) and spraying with 1% iron oxide particles in the two treatment beds at the rate of 0.01 g/kg soil. Aster (Maymyo flower) plant (after the age of 20 days) and Bermuda grass (5 inches between two plants) were planted in this field. There were four rows in each field of work. The field experiments were arranged according to a random design that consisted of six treatments:

(a) **(b)** **(c)**

Figure 2. (a) Planting selected seeds (b) Preparing various treatments of the contaminated soil sample in the presence or absence of iron oxide particles (c) aster and Bermuda grass plants at 0 week

(a) **(b)** **(c)**

Figure 3. Aster and Bermuda grass plants after (a) 2 weeks (b) 8 weeks (c) 12 weeks

In the phytoremediation experiments, seedlings (2 weeks old) of the fresh plant species not previously exposed to cypermethrin were transplanted into a field containing soil contaminated with cypermethrin (100 µg/g). Plant maintenance that included watering, weeding, and pest control was done from the beginning of planting until harvest. The temperature was kept at 30 °C during the day and 27 °C at night. Water was added to the soil in each row every day to maintain the appropriate moisture content. The field study was performed with natural light. The study was continued after the screening period using two plant species that grew well in the cypermethrin-contaminated soil. Healthy plants with similar heights and biomasses of the selected species were transplanted into field plots for the main study. Samples of the soil were taken for analysis at 0, 2, and 12 weeks (with three replicates for each time) for the time of exposure to determine the amount of PBA formed, enzyme activities and the N, P, K contents over the course of 3 months.

Determination of Physicochemical Properties of Soil Sample

The moisture content of the soil sample was determined according to the oven drying method, pH value by pH meter, electrical conductivity (EC) value by electrical conductivity meter, organic matter content by Walkley and Black method based upon the oxidizable organic matter content, total nitrogen (N) content by Kjeldahl's method, cation exchange capacity by the method of Kappen (Jaremko and Kalembasa, 2014), total phosphorus (P) content by spectrophotometric method of Olsen for neutral and alkaline soil, potassium content by ammonium acetate extraction method using flame photometer, and the elemental analysis of the soil sample by EDXRF.

Determination of Enzyme Activities

The activity of the urease enzyme (mg NH_4^+-N g^{-1} soil h^{-1}) was measured by colorimetric methods (McGarity and Myers, 1967) Detail were described in the reference. Dehydrogenase enzyme activity was assayed by modified 2, 3, 5-triphenyl tetrazolium chloride (TTC) reduction technique (Casida *et al.,* 1964). Five grams of soil was placed in a beaker and carefully mixed with 0.1 g of $CaCO_3$ and 1.5 mL of distilled water added into the mixture. Then, 1 mL of 1% TTC solution was added and the beakers were incubated at 30 ºC for 24 h after plugging with cotton. The resulting slurry was filtered and triphenyl formazan (TPF) was extracted with successive aliquots of methanol in a 50 mL volumetric flask. The absorption of the pink colour was read out with spectrophotometer at 485 nm.

Extraction and Characterization of Insecticide Residue

In brief, 3-PBA was extracted from 10 g of soil samples using methanol and dichloromethane (3:1, v/v) mixture and placed in a shaker at 150 rpm for 30 min. The supernatant liquid was centrifuged at 5000 rpm for 15 min three times. The residual insecticide (as its metabolite 3-PBA) in extracted soil samples from the experimental plot was examined by using UV-Vis spectrophotometer and GC-MS.

Figure 4. Extraction of insecticide residue in various treatments over weeks

Statistical Analysis

Values were expressed as means ± standard deviation (SD). All data were tested for normality and homogeneity using Leven's test. All results were conducted with three replicates. The experimental data were statistically analyzed by one-way analysis of variance (ANOVA) using Excel.

Results and Discussion

Physicochemical Properties of Soil Sample

The soil has a sandy loam texture. This research used a soil with low nitrogen, very low organic carbon, humus, electrical conductivity (EC), high K_2O, and very high phosphorus (P) contents in order to scientifically investigate the degradation of cypermethrin. The moisture content of the contaminated soil was found to be 2.83%. The pH value of the contaminated soil was found to be 6.76. The electrical conductivity value of the contaminated soil was found to be 0.08 mS/cm. The electrical conductivity of soil informs the ionic nature of the soluble compound to supply the needs of plants. The contaminated soil had an organic carbon content of 0.66 % and a humus content of 1.13%. Humus contains every element absorbed by growing plants, but not in the same proportions as in plants. The microbes become part of the soil humus, along with materials that have partially or entirely resisted the process of decomposition. Humus is a very important part of the ability of the soil to supply the needs of plants.

Table 1. Characteristics of the Soil Sample

Test Parameter	Content
Texture	Sandy loam
Moisture (%)	2.83
pH	6.76
Electrical Conductivity (mS/cm)	0.08
Organic carbon (%)	0.66
Humus (%)	1.13
Total Nitrogen (%)	0.14
CEC (meq/100 g)	11.75
Phosphorus (ppm)	81.85
K_2O (mg/100g)	24.71
Exchangeable Ca^{2+} (meq/100 g)	5.87
Exchangeable Mg^{2+} (meq/100 g)	4.56
Exchangeable K^+ (meq/100 g)	0.52
Exchangeable Na^+ (meq/100 g)	0.80

The total nitrogen content of the contaminated soil was found to be the lowest at 0.14%. Nitrogen helps plants make the proteins they need to produce new tissues. In nature, nitrogen is often in short supply, so plants have evolved to take up as much nitrogen as possible, even if it means not taking up other necessary elements. If too much nitrogen is available, the plant may grow abundant foliage but not produce fruit or flowers. The cation exchange capacity (CEC) of the soil was found to be 11.75 meq/100 g. The CEC is an essential measurement in agronomy and soil science to estimate the physicochemical state of a soil, which may be a good indicator of soil quality and productivity to supply the three important plant nutrients: calcium, magnesium, and potassium. The highest phosphorus content of the contaminated soil was found to be 81.85 ppm. Phosphorus stimulates root growth, helps the plant set buds and flowers, improves vitality, and increases seed size. It does this by helping transfer energy from one part of the plant to another. Organic matter and the activity of soil organisms also increase the availability of phosphorus.

The K_2O content of the contaminated soil was found to be 24.71 mg/100 g. Potassium is one of the three major fertilizer elements. In fertilizer and soil analyses, however, potash signifies the hypothetical potassium oxide, K_2O. In reality, there is no K_2O in fertilizers. Furthermore, K_2O is not absorbed by plants. Plant roots absorb most of their potassium as potassium ions K^+. The exchangeable calcium content of the contaminated soil was found to be 5.87 meq/100 g. Calcium is used by plants in cell membranes, at their growing points, and to neutralize toxic materials. In addition, calcium improves soil structure and helps to bind organic and inorganic particles together. The exchangeable magnesium content of the contaminated soil was found to be 4.56 meq/100 g. Magnesium is the only metallic component of chlorophyll. Without it, chlorophyll cannot capture the sun's energy, which is needed for photosynthesis.

The exchangeable potassium content of the contaminated soil was found to be 0.52 meq/100 g. Potassium improves the overall vigor of the plant. It helps the plants make carbohydrates and provides disease resistance. It also helps regulate metabolic activity. The exchangeable sodium content of the contaminated soil was found to be 0.80 meq/100 g. Sodium cations (Na^+) are not plant nutrients, so they are not wanted by the plants. When exchangeable sodium is present in quantities greater than or equal to 5 % of (CEC), it makes the clay particles unstable in rainwater. This shows up as dispersion or cloudiness in water. Dispersive soils have poor water entry and drainage and are hard to dry. This study provides information about the nature of soil and the nutrients present in it, allowing a farmer to plan the amount of fertilizers and nutrients required to increase crop yield (Table 1).

EDXRF Analysis

The relative abundance of some elements: Si, Fe, K, Ca, Ti, Mn, Zr, Ba, Cr, Sr, Zn, Y, Cu, and Rb in insecticide-contaminated soil is determined by EDXRF (Figure 2 and Table 2). According to EDXRF, the insecticide-contaminated soil contained silicon (Si) as the major constituent and iron (Fe) as the second major constituent, followed in decreasing order by potassium (K), calcium (Ca), titanium (Ti), manganese (Mn), zirconium (Zr), barium (Ba), chromium (Cr), strontium (Sr), zinc (Zn), yttrium (Y), copper (Cu), and rubidium (Rb).

Table 2. Elemental Analysis of the Soil Sample

Element	Relative Abundance (%)
Silicon (Si)	54.729
Iron (Fe)	25.643
Potassium (K)	8.398
Calcium (Ca)	7.198
Titanium (Ti)	2.451
Manganese (Mn)	0.627
Zirconium (Zr)	0.765
Barium (Ba)	0.716
Chromium (Cr)	0.372
Strontium (Sr)	0.169
Zinc (Zn)	0.074
Yttrium (Y)	0.039
Copper (Cu)	0.078
Rubidium (Rb)	0.034

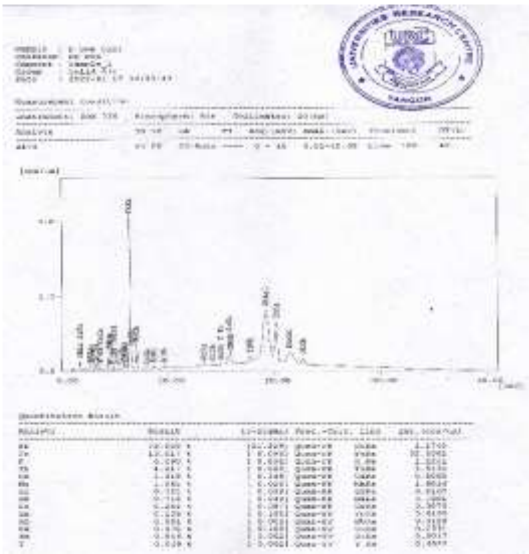


Figure 5. EDXRF spectrum of contaminated soil

The high content of silicon resists the damage to crops caused by pathogenic microorganisms and that of other elements provided as plant nutrients. Iron (Fe) is one of the major elements present in the soil, mostly in the form of oxide. Generally, the most dominant oxidation state is Fe^{3+}. Iron oxides are very important components in most soils, as they have a major influence on the chemical, physical, and microbial properties of soils. Because of their size (usually 5-200 nm), iron oxides possess a large specific surface area and highly reactive surfaces. The average particle's diameter was determined to be approximately 33.423 nm. Larger iron oxide particles degraded slowly, indicating that the reaction is surface area dependent. Potassium (K) is commonly supplied to the soil as farm manure and as commercial fertilizers.

Calcium (Ca) plays a vital role in plant growth, specifically cell wall formation, cell division, and pollination. Calcium also promotes healthy soil structure by loosening soils and stabilizing organic matter, which increases the soil's water- and nutrient-holding capacity. The more calcium is in the soil, the higher the pH of the soil can become. Titanium (Ti) is considered a beneficial element for plant growth. When plants experience Fe deficiency, Ti helps induce the expression of genes related to Fe acquisition, thereby enhancing Fe uptake and utilization and subsequently improving plant growth. The other nutrient elements, manganese (Mn), chromium

(Cr), zirconium (Zr), barium (Ba), strontium (Sr), zinc (Zn), yttrium (Y), copper (Cu), and rubidium (Rb), are used in very small amounts by higher plants, thereby justifying the name "micronutrients" or trace elements. This is due to the relatively small quantities of micronutrients in sands and organic soils and the low availability of most of these elements under very alkaline conditions.

Table 3. Characteristics of the Farm Soil Samples

Samples	Moisture %	pH	Total N %	CEC	Available Nutrients	
				K^+ (meq/100g)	P (ppm)	K_2O (mg/100g)
S_0 (0 week)	0.84	7.26	0.18	0.48	79.11	22.40
S_{Fe} (0 week)	0.77	7.39	0.20	0.73	89.07	34.47
S_{Fea} (2 weeks)	0.82	7.19	0.16	0.97	106.96	45.40
S_{Feb} (2 weeks)	0.70	7.17	0.14	0.73	74.60	34.47
S_{Fea} (4 weeks)	1.80	7.07	0.18	1.24	142.57	58.04
S_{Feb} (4 weeks)	0.84	7.06	0.16	0.97	82.74	45.40
S_{Fea} (8 weeks)	0.85	6.73	0.19	2.29	343.08	103.53
S_{Feb} (8 weeks)	0.73	7.13	0.14	1.16	151.20	54.43
S_{0a} (12 weeks)	1.016	7.05	0.142	1.28	169.86	60.66
S_{Fea} (12 weeks)	1.21	6.90	0.16	1.26	113.48	59.57
S_{0b} (12 weeks)	1.442	6.56	0.142	0.28	114.72	13.4
S_{Feb} (12 weeks)	2.68	7.68	0.13	0.43	53.442	20.34

pH and Major Nutrients of Farm Soil Samples in Different Treatments

The pH values of the contaminated farm soil at different times (0, 2, 4, 8, and 12 weeks), determined by the pH meter, are shown in Table 3. The pH values of the control soil samples at 0 week for S_0 and S_{Fe} were higher than those of the other soil samples (S_{0a}, S_{Fea}, S_{0b}, and S_{Feb}) for 0, 2, 4, 8, and 12 weeks, except for S_{Feb} at 12 weeks (Table 3). From these observations, the contaminated farm soil samples were found within the range of 6.5-7.68, and this range of pH is generally very compatible with plant root growth. It also affects the microbial population in soils. The optimum range for most plants is between 5.5 and 7.5. However, many plants have adapted to thrive at pH values outside this range. Therefore, the pH values measured in this agricultural soil are consistent with the determined optimum values. If the pH value is less than 5.5, it also affects the activity of soil microorganisms, thus affecting nutrient cycling and disease risk. From the study of total nitrogen percent in the contaminated farm soil samples by Kjeldahl's method, the control samples for week 0 (S_0 and S_{Fe}) were higher than those of the other soil samples (S_{0a}, S_{Fea}, S_{0b}, and S_{Feb}) for weeks 0, 2, 4, 8, and 12 (Table 3). Thus, the decrease in nitrogen levels reduces the leaf area, chlorophyll content, photosynthesis, and biomass production. This is because more nitrogen is consumed by the plants, which results in more nitrogen depletion in the soil.

The CEC is an essential measurement in agronomy and soil science to estimate the physicochemical state of soil, the three important plant nutrients: calcium, magnesium, and potassium. Plants use calcium in their cell membranes at their growth points and to neutralize toxic materials. In addition, calcium improves soil structure and helps to bind organic and inorganic particles together. Magnesium is the only metallic component of chlorophyll. Potassium occurs in the soil in three forms: as exchangeable (available) potassium (K^+) adsorbed onto the soil CEC; fixed by certain minerals, from which it is released very slowly into an available form; and in unavailable mineral forms. The CEC values of potassium K^+, available nutrients K_2O, and phosphorous (P) values of the control soil samples (0 week) for S_0 and S_{Fe} were found to be lower than those of the other soil samples (S_{0a}, S_{Fea}, S_{0b}, and S_{Feb}) for (0, 2, 4, 8, and 12 weeks), except S_{Feb} for 12 weeks (Table 3). Phosphorous (P) is a major nutrient required for energy storage and transfer, cell division, and tissue development in plants like aster and Bermuda grass. Among N, P, and K, the iron oxide-treated soil samples (S_{Fea} and S_{Feb}) for aster and Bermuda grass have the highest values of available P and K_2O.

Moisture Variation in Different Treatments

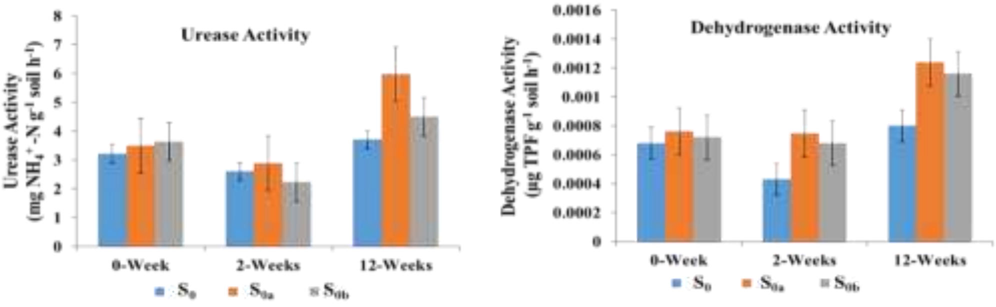
The moisture contents of the contaminated farm soil Table 3 at different times (0, 2, 4, 8, and 12 weeks) are determined by the oven drying method. The moisture contents of the control soil samples (0 week) for S_0 and S_{Fe} were lower than those of the other soil samples (S_{0a}, S_{Fea}, S_{0b}, and S_{Feb}) for (0, 2, 4, 8, and 12 weeks). Soil moisture is the water stored in the soil and is affected by precipitation, temperature, soil characteristics, and more. The size of the soil particles and pores affects how much water the soil can hold and how that water moves through the soil.

Enzyme Activities Variation in Different Treatments

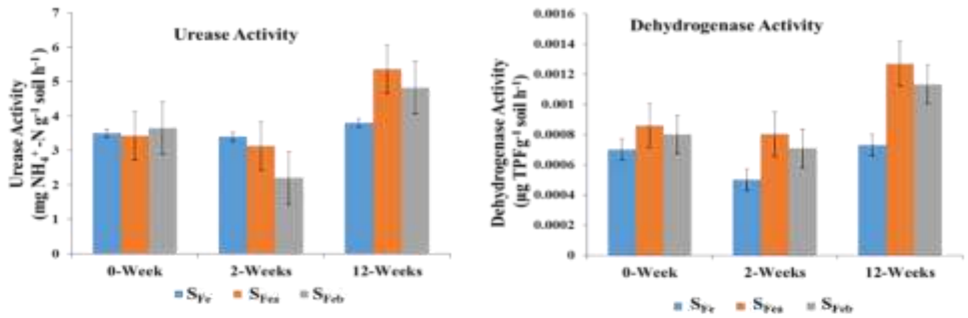
During the incubation periods, the effects of iron oxide particles on urease and dehydrogenase activities were found to increase, as shown in Figure 6-a and b. According to this figure, the changes in urease and dehydrogenase activities depend on the dosage of iron oxide particles. The degradation of cypermethrin in soil is mostly attributed to microorganisms. Urease and dehydrogenase activities are appropriate substitute biomarkers of general microbial activities in soils. In addition to iron oxide particles, urease and dehydrogenase activities significantly increased over the course of 12 weeks.

After 12 weeks, the urease and dehydrogenase activities in the controls (or treated soil in the absence of iron oxide particles, S0) were found to reach 3.74 mg NH_4^+-N g^{-1} soil h^{-1} and 0.0008 µg TPF g^{-1} soil h^{-1}, whereas 5.975 mg NH_4^+-N g^{-1} soil h^{-1} and 0.00124 µg TPF g^{-1} soil h^{-1} were found in aster (S_{0a}) and 4.494 mg NH_4^+-N g^{-1} soil h^{-1} and 0.00124 µg TPF g^{-1} soil h^{-1} in Bermuda grass (S_{0b}). The percentages of urease and dehydrogenase activities were found to increase by 161.49 and 155 % in aster (S_{0a}) and 121.45 and 155 % in Bermuda grass (S_{0b}) compared with controls (assumed to be 100 %) through 12 weeks.

After 12 weeks, the urease and dehydrogenase activities in the presence of iron oxide particles (S_{Fe}) were found to be reached 3.8 mg NH_4^+-N g^{-1} soil h^{-1} and 0.00073 µg TPF g^{-1} soil h^{-1}, 5.363 mg NH_4^+-N g^{-1} soil h^{-1} and 0.00127 µg TPF g^{-1} soil h^{-1} in aster (S_{Fea}) and 4.816 mg NH_4^+-N g^{-1} soil h^{-1} and 0.00113 µg TPF g^{-1} soil h^{-1} in Bermuda grass (S_{Feb}). The percentage of urease and dehydrogenase activities in the presence of iron oxide particles was found to increase by 141.13 and 174 % in aster (S_{Fea}) and by 126.74 and 154.79 % in Bermuda grass (S_{Feb}) in comparison with S_{Fe} (assumed to be 100 %) for 12 weeks.



(a)



(b)

Figure 6. Soil urease and dehydrogenase activities in insecticide contaminated soil treated with aster and Bermuda grass (a) in the absence and, (b) in the presence of iron oxide particles

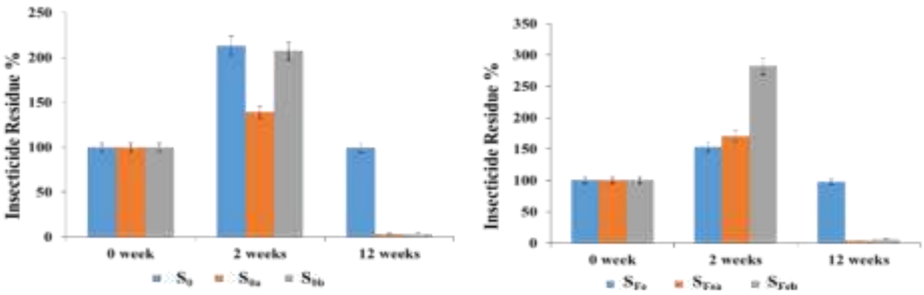
Insecticide Residue Percent in Contaminated Soil Samples

The effect of iron oxide particles prepared with total phenol extract from tea leaf waste as a reducing agent on phytoremediation was studied using UV-Vis and GC-MS for insecticide residue from contaminated soil.

In comparison with $S_{0\ in\ a}$ 0-week treatment, the percentages of insecticide residue in the S_0, S_{0a}, and S_{0b} treatments were found to be 213.25, 139.29, and 207.4 % PBA formed through 2 weeks of experiments and 99.2, 3.53, and 3.67 % PBA formed through 12 weeks of experiments (Figure 7-a). In comparison with S_{Fe} in the 0-week treatment, the percentages of insecticide residue in the S_{Fe}, S_{Fea}, and S_{Feb} treatments were observed to be 153.28, 170.37, and 282.35 % PBA formed after 2 weeks of experiments, and 97.89, 3.67, and 5.82 % PBA formed (no cypermethrin residue) through 12 weeks of experiments (Figure 7-b). All treatments showed phytoremediation efficiency for insecticide-contaminated soil.

At all levels of contamination of the soil, aster and Bermuda grass soil respiration was found to be significantly higher than that soil respiration. The root system and soil moisture provide a suitable environment in the soil for microorganisms, contaminants, and organic molecules nutrient interactions. (Germida *et al.*, 2002). Therefore, it seems that higher root biomass produced by aster and Bermuda grass in contaminated soils, is responsible for more microbial activity in their rhizosphere.

Aster and Bermuda grass with iron oxide particles were found to possess the greater degradation efficiency in phytoremediation. During cultivation, the plants were watered regularly. This may be because soil saturation with water decreases the oxygen levels and thus prevents the oxidation of iron oxide particles. Insecticides, which are persistent in aerobic environments, are more readily degraded under reducing conditions. The results showed that the iron oxide particles played the most important role in the degradation of insecticide residue in the soil, compared with natural degradation in soil without iron oxide particles.



(a) **(b)**

Figure 7. Insecticide residue (PBA) percent in contaminated soil treated with aster and Bermuda grass (a) in the absence of iron oxide particles and (b) in the presence of iron oxide particles through 12 weeks experiments

Cypermethrin is relatively stable under sunlight, and, though it is probable that photodegradation plays a significant role in the degradation of the product, its effects in soils are limited. Degradation in the soil occurs primarily through cleavage of the ester linkage to give PBA and carbon dioxide. Some carbon dioxide is formed through the cleavage of both the cyclopropyl and phenyl rings under oxidative conditions. The half-life of cypermethrin in typical fertile soil is between 2 and 4 weeks. Cypermethrin is adsorbed very strongly on soil particles, especially in soils containing large amounts of clay or organic matter.

The role of functionalized iron oxide particles in nanomaterial and biomedical applications often relies on achieving the attachment of ligands to the iron oxide surface in sufficient numbers and with proper orientation (Korpany *et al*., 2017). The results of this study could be due to relationships between the ligand chemical structure and surface binding on magnetic iron oxide particles (~30 nm) for a series of related benzoic acid derivatives. The structure of the resultant ligand-surface complex was primarily influenced by the relative positioning of hydroxyl and carboxylic acid groups within the ligand. The chemical structure of benzoic acid derivatives enables fast and stable covalent binding on the surface of magnetite (Fe_3O_4) particles, which act as catchers and carriers for magnetic removal. The results of studies have shown that, with iron oxide particles applied, the levels of cypermethrin and its secondary metabolite, PBA, can be lowered.

Conclusion

The 12-week long experiment revealed an increase of available phosphorous and K_2O and enzyme activities in soil and a decrease of nitrogen content and insecticide residue. The percentage of total nitrogen content decreased from 0.18% (S_0) in 0 week to 0.16% in asters and 0.13% in Bermuda grass. The available phosphorous content increased from 79.11 ppm (S_0) in 0 weeks to 113.48 ppm in aster and decreased to 53.442 ppm in Bermuda grass through 12 weeks. The available K_2O content increased from 22.40 mg/100 g (S_0) in 0 week to 59.57 mg/100 g in asters and decreased to 20.34 mg/100 g in Bermuda grass through 12 weeks. Through a 12-week experiment, the percentage of urease and dehydrogenase activities increased 41.13 and 74.0 % in aster (S_{Fea}) and 26.74 and 54.79% (S_{Feb}) in Bermuda grass, while the percentage of insecticide residue in contaminated soil decreased from 100 % (S_0) in 0 week to 3.67 % in aster and 5.82% in Bermuda grass. The phytoremediation process increases soil urea and dehydrogenase activity.

The growth of plants with iron oxide particles in insecticide contaminated soils has favorable effects on the changes in soil nutrients, the enzyme activities, and the degradation of PBA in the contaminated soil. The findings of this study showed that aster and Bermuda grass can be recommended as native alternative options for phytoremediation in some area of the country.

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