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ASSESSING THE APPLICATION OF IRON OXIDE PARTICLES FOR REMEDIATION OF INSECTICIDE CONTAMINATED SOIL

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Abstract

In this study, iron oxide particles were synthesized and used to remediate insecticide contaminated soil. The influence of experimental variables such as reaction time, and particle dosage of iron oxide particles on the soil remediation were studied. The green synthesis of iron oxide particles was prepared by reducing iron (III) chloride solution with tea leaves extract for remediation of the soil. The optimized quantity of iron oxide particles was found to be 0.01 mL/kg of soil contaminated with 0.002 $\mu g\ g^{-1}$ of cypermethrin insecticide. The physicochemical properties of the soil sample were determined. Iron oxide particles were prepared by green method and characterized by modern techniques such as XRD, FT IR, TG-DTA and FESEM analyses. The degradation of insecticides residue in contaminated soil was studied. The residual insecticide in soil samples extracted from the experimental plot was examined by using UV-Vis spectrophotometer. Insecticide residue did not increase but firstly increased than decreased. The soil urease activity in the treated and contaminated soil was also determined. The stabilization of urease activity in the soils studied was due to iron oxide particles and these had light influence on soil organic matter. These results suggest that only proper amount of iron oxide particles plays a dominating role to control the urease activity of the soils.

Keywords: cypermethrin, remediation, iron oxide particles, contaminated soil, urease activity

Introduction

In recent years, the phasing out of organophosphate products such as diazinon and chlorpyrifos has prompted an increased use of pyrethroid insecticides for agricultural pest control (Bootharaju *et al*., 2012). Because of this concerns regarding the fate, toxicity, diffusion, and transformation of pyrethroids have significantly increased. Once the insecticide enters the soil it is partitioned between soil particles and soil solution. In soil particles, cypermethrin particularly binds with the soil organic matter. If organic matter is associated with soil particles, then it is favourable for binding insecticide.

Cypermethrin is classed as a type II pyrethroid and is commonly found in rivers, sediments, soils, and even foodstuffs (XIE *et al*., 2008). It possesses carcinogenic and cocarcinogenic potential and can produce compounds with endocrine activities, such as 3-phenoxybenzoic acid (PBA). Degradation of PBA is thus a vital step for remediation of cypermethrin pollution. One of the most important processes influencing the environmental behavior of an insecticide is its degradation in soil. Hydrolysis of the ester linkage producing PBA and cyclopropane carboxylic derivatives is the main degradation pathway of cypermethrin in soil. However, PBA is more mobile and persistent than cypermethrin in soils and it presents an environmental risk greater than its parent molecule. Degradation of PBA is a vital step for remediation of cypermethrin pollution.

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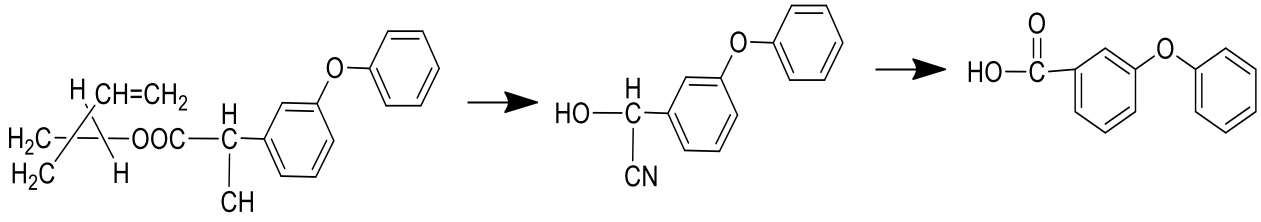

Figure 1 Degradation pathway of cypermethrin in soil

Iron oxides form naturally through the weathering of Fe-containing rocks both on land and in the oceans. They have attracted much attention due to their fine magnetic properties and applications in modern science (Fernández-Garcia *et al.*, 2007). Moreover, urea added to soils as fertilizer is rapidly hydrolyzed to ammonium carbonate in most soils through soil urease activity. Urease is a nickel containing enzyme which catalyzes the hydrolysis of urea to ammonia and carbon dioxide, it is produced by bacteria, algae, fungi and plants (Hameed *et al.*, 2018). The main role of urease is to allow the microorganisms to use urea as a source of nitrogen. Furthermore, urease plays an important role in nitrogen cycle in plants, urease produced by bacteria, also, acts as a virulence factor in many human infections. Ureases is the enzyme that degrades urea and is widely considered to be a good proxy of nitrogen (N) mineralisation. The aim of the present research work is to assess the application of iron oxide particles for remediation of insecticide contaminated soil by using green synthesized iron oxide particles and to determine the activities of urease enzyme of insecticide-contaminated soil.

Materials and Methods

Preparation of iron oxide particles

Iron oxide particles were synthesized by green method. Briefly, by adding 0.01 M $FeCl_3.6H_2O$ solution to the tea extract in a 1:1 volume ratio. Fe_3O_4 particles were immediately obtained with the reduction process (Kanagasubbulahshmi *et al*, 2017). The mixture was stirred for 60 min and then allowed to stand at room temperature for another 30 min to obtain colloidal suspension. Mixture was centrifuged and washed several times with ethanol and then dried at 60 ˚C under vacuum. Finally, the black iron oxide particles were obtained. Tea leaves have the best reduction capability against ferric chloride when compared to other parts of the plants that is observed by the external color change (Bharadwaz *et al.*, 2015).

Characterization of iron oxide particles

The prepared black iron oxide particles were characterized by XRD, FT IR and TG-DTA techniques at the Universities Research Center, University of Yangon and by FESEM technique at the Science and Industrial Research, Osaka University. The prepared iron oxide particles were characterized by means of XRD method and estimated the crystallite size of these particles according to Debye-Scherrer's formula. The prepared iron oxide particles were characterized by means of FT IR method and estimated the functional group of the iron oxide particles. The TG-DTA was used to investigate the decomposition and phase formation that occurs during heat treatment of the prepared iron oxide particles. The FESEM signals that derived from electron sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.

Soil Sample Collection

Soil samples was collected from the surface layer (0-20 cm depth) of an agricultural field located in Phya Thee Village (at 21° 48' latitude north and 94° 57' longitude east), Myaing Township, Pakokku District, Magway Region, cropped with a chilli and tomato rotation without treatment of any pyrethroids for several years.

Figure 2 Sampling site of soil in Phya Thee village

Physicochemical Properties of Soil Sample

The moisture content of the soil samples was determined according to the reported method. The pH content of the soil sample was determined according to the standard method by using pH meter. Electrical conductivity of the soil sample was determined by using electrical conductivity meter. Organic matter content of the soil sample was determined by using walkley and black method based upon the oxidizable organic matter content. Total N content was determined by using Kjeldahl's method. Cation exchange capacity content of the soil samples were determined according to the method of Kappen (Jaremko *et al.,* 2014*)*. Total P of the soil samples was determined by using Olsen method for neutral and alkaline soil (measured by spectrophotometer). Potassium content of the soil samples was determined by using ammonium acetate extraction method (measured by Flame photometer).

Experimental Design for Determining Insecticide Degradation in Soil

Dried soil samples (10 g), H_2O (1mL) and 0.0001% cypermethrin (1mL) were placed in 150 mL aliquots. Fe_3O_4 particles was dissolved in water at doses of 0 % Fe_3O_4 (P 0), 1% Fe_3O_4 (P 1), 2% Fe_3O_4 (P 2), 3% Fe_3O_4 (P 3), respectively. A control soil sample, without adding iron oxide particles, was prepared in a similar manner. Each treatment in triplicate was amended with cypermethrin dissolved in water to obtain insecticide concentration of 0.002 µg/g, which is the common residual field concentration after application in contaminated soils. All the containers were covered with perforated aluminum foil to ensure gas exchange and then incubated at 25 ℃. The samples were aged for several weeks. After 1, 2, 3, 4 and 5 weeks, soil samples were collected to determine the amount of secondary metabolite of cypermethrin (3-phenoxy benzoic acid, 3- PBA) formed.

Extraction of 3-phenoxy benzoic acid in soil and characterization

Three samples from each treatment was used to determine the residue of PBA in the soil. In brief, PBA was extracted from 10 g soil samples using methanol and dichloromethane (3:1, v/v) and placed in shaker at 150 rpm for 30 min. The supernatant liquid was centrifuged at 5000 rpm for 15 min in three times. The residual insecticide (as its metabolite 3-phenoxybenzoic acid, 3-PBA) in contaminated soil samples extracted from the experimental plot was examined by using UV-Vis spectrophotometer.

Determination of the urease enzyme activity in soil and characterization

The activity of the urease enzyme (mg NH_4^+ -N g^{-1} soil h^{-1}) was measured by the determination of ammonia released after soil sample incubation with a urea solution by using colorimetric methods (Jingjing *et al*., 2015). A 10 g of fresh soil was placed in a 100 mL volumetric flask and treated with 1 mL of toluene, 10 mL buffer (pH-7) and 5 mL of 10 % urea solution (freshly prepared). After a throughout mixing the flask was incubated for 3 h at 37 ˚C in dark. After incubation the volume of the flask was made to 100 mL distilled water and shaken thoroughly and transferred the filtrate through Whatman No.5 filter paper. The ammonia released as a result of urease activity was measured by indophenol blue method. 0.5 mL of the filtrate was taken into a 25 mL volumetric flask and 5 mL of distilled water was added. Then 2 mL of phenolate solution (mixture of 20 mL of stock A (62.5 g phenol crystals dissolved in a minimum volume of methanol and make up the volume up to 100 mL with ethyl alcohol after adding 18.5 mL acetone and 20 mL of stock B (27 g NaOH dissolved in 100 mL distilled water and kept in freezer)) was added. Therefore, 1.5 mL of sodium hypochlorite solution was added. The final volume of the flask was increased up to 25 mL with distilled water and the blue colour was read out with the spectrophotometer at 630 nm. Urease activity was determined by a calibration curve.

Results and Discussion

Characteristics of Prepared Iron Oxide Particles

The green synthesis of iron oxide particles using extract of green tea which is a cheap and local resource. Synthesized iron oxide particles utilizing green tea (*Camellia sinensis*) extract containing a range of polyphenols. Polyphenols in plants act as both a reducing agent and a capping agent to provide a robust coating on metal particles in a single step and leads to colour change yellowish brown to brownish black. This colour change gave the confirmation of the synthesis of iron oxide particles. This denotes the tea leaves have premier competence to synthesize of iron oxide particles than other parts of the plants such as seeds, fruit.

The XRD analysis results of iron oxide powders prepared at 60 ˚C are shown in Figure 3. The results indicate that, at 200 ˚C or 1 h, no other obvious diffraction peaks are found, except the peaks belonging to α-Fe_2O_3 phase. The XRD pattern points out that the product reduced at 300 ˚C is a mixture of Fe_3O_4 and α-Fe_2O_3 phases with Fe_3O_4 as the main phase. The crystalline natures of the prepared iron oxides were identified by XRD analysis. Four miller indices [(111), (311), (422), and (531)] of magnetite sample matched with the standard data. This revealed that the resultant iron oxide are cubic structure. After that, the particle sizes of iron oxide samples was calculated by using Debye-Scherrer's equation and the particle size of iron oxide particles was found to be 33.518 nm.

The FT IR spectrum of iron oxide particles is shown in Figure 4 (Nakamoto, 1997). The absorption peaks at 893.07, 792.77, 572.88, and 451.36 cm^{-1} correspond to the vibration of Fe-O group. FT IR analysis gave the stretching vibrations at 3448, 1622, 572 and 451 $cm^{-1.}$ These peaks represent the reducing agent role in the formation of Fe_3O_4 particles. The peak at 3448 cm^{-1} corresponds to the –OH bond stretching denotes the aqueous phase as well as the reduction of ferric chloride. Remaining unclear peaks represents small amount of organic acids which is responsible for the low pH of the sample which helps to the synthesis of the Fe_3O_4 particles. The peaks at 572 and 451 cm^{-1} correspond to the inorganic stretching indicates the Fe_3O_4 particles.

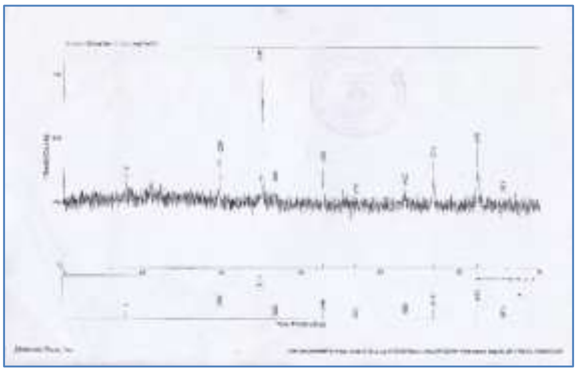

Figure 3 XRD diffractogram of prepared iron oxide particles

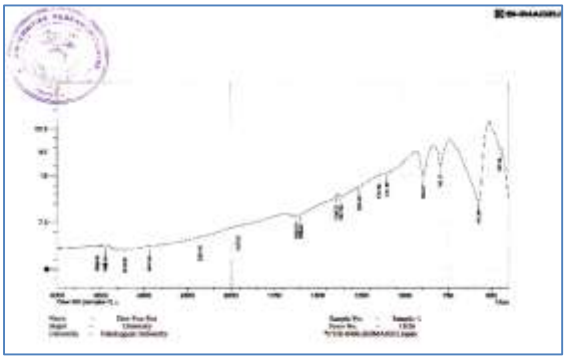

Figure 4 FT IR spectrum of prepared iron oxide particles

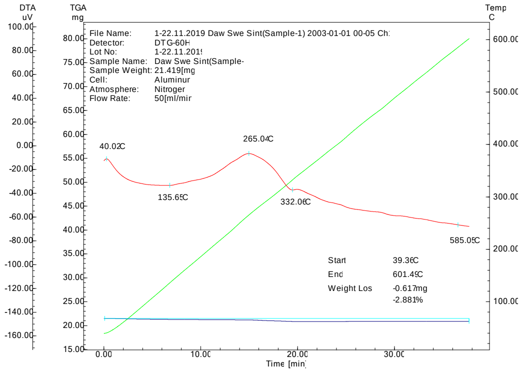

Figure 5 TG-DTA thermograph of prepared iron oxide particles

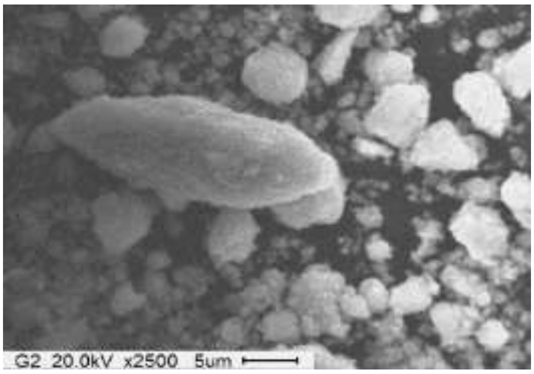

Figure 6 FESEM microphotograph of prepared iron oxide particles

TG and DTA curve of iron oxide particles is shown in Figure 5. The temperature range is 40 ˚C to 600 ˚C. TG-DTA analysis, it was found that total weight loss is 2.881 %. One exothermic peak and one endothermic peak were corresponding to phase transition and oxidation reaction takes place in the sample. The endothermic peak was observed at about 332.06 ˚C due to the removal of organic volatile materials and the exothermic peak was observed at about 265.04 ˚C. From DTA data, above 200 ˚C, exothermic peak and endothermic peak were observed due to the phase transformation. In this research, surface morphology of the prepared iron oxide samples were studied by using FESEM as shown in Figure 6. From the FESEM result of iron oxide particles by green method indicated that the particles are spherical in shape with a narrow size distribution and it can be seen that the particles agglomeration, indicating a good connectively between the grains together and the pore size of which is 0.7 cm. The FESEM micrographs indicated the porous nature of the surface.

Physicochemical Properties of Soil Sample

The soil has the sandy loam texture. This research used a soil with low nitrogen, very low organic carbon and electrical conductivity (EC), medium cation exchange capacity (CEC), high K_2O and very high phosphorous (P) content in order to scientifically investigate on the degradation of cypermethrin. The pH value of the contaminated soil was found to be 8.079. The electrical conductivity value of the contaminated soil was found to be 0.09 µS/cm. The organic carbon content of the contaminated soil was found to be 0.36 %. Humus content of the contaminated soil was found to be 0.62 %. The nitrogen value of the contaminated soil was found to be the lowest value 0.13 %. CEC is important for maintaining adequate quantities of plant available calcium Ca^{++}

(19.10) meq/100 g , Mg^{++} (0.68) meq/100 g, K^{+} (0.43) meq/100 g, and Na^{+} (0.85) meq/100 g, respectively. The highest phosphorous content of the contaminated soil was found to be 45.03 ppm.

Table 1 Characteristics of the Soil Sample

Test Parameter	Content
Texture	Sandy loam
Sand (%)	67.56
Silt (%)	15.00
Clay (%)	17.44
Organic carbon (%)	0.36
Humus (%)	0.62
Total Nitrogen (%)	0.13
CEC (meq/100 g)	21.06
Phosphorus (ppm)	45.03
K_2O (mg/100 g)	20.27
Exchangeable Ca^{++} (meq/100 g)	19.10
Exchangeable Mg^{++} (meq/100 g)	0.68
Exchangeable K^{+} (meq/100 g)	0.43
Exchangeable Na^{+}(meq/100 g)	0.85

The iron oxide particles efficiency on soil remediation

After 4 weeks' incubation, the amounts of PBA degradation were found to be slightly higher in the 2nd week in all treatments with respect to Control (P0). These findings indicated that adequate addition of iron oxide particles could increase the degradation of cypermethrin. However, the degradation levels were similar after a long enough incubation period. Apparently, there is no movement of cypermethrin from soil to air and its degradation in soil is mostly attributed to microorganisms.

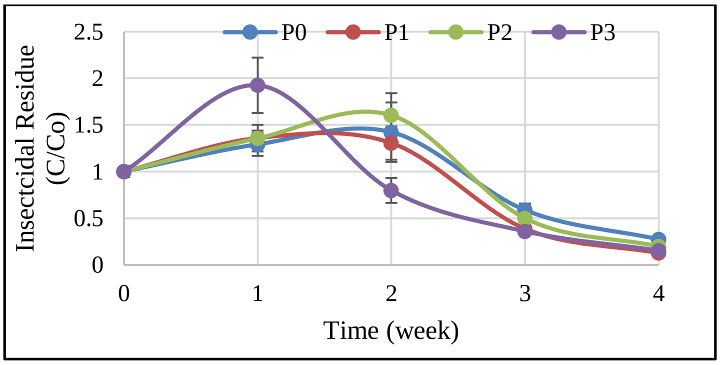

Figure 7 Insecticide residue in soil by different dosage of iron oxide particles treatments

Enhancement of Urease Enzyme Activity

The effect of iron oxide particles on urease activity was found to be increased during the incubation periods. The results of determination of urease enzyme activity in contaminated in soil are shown in Figure 8.

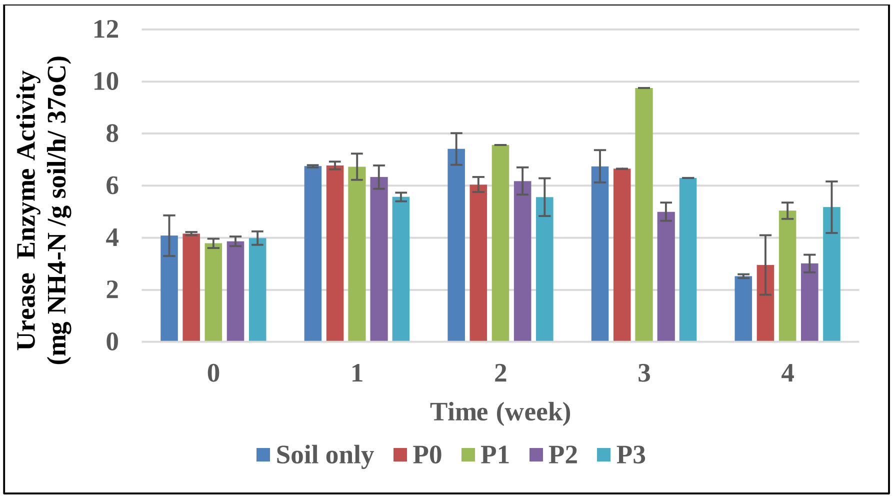

Figure 8 Urease Enzyme Activity of insecticide contaminated soil

The change of urease activity depended on iron oxide particles dosage. In blank stage, urease activity had a slight increase. Under the addition of different iron oxide particles, all treatments showed a little positive impact on urease activity. The present study showed that urease enzyme quality was improved by addition of proper iron oxide particles. In 1 week, urease activity content of five treatments had little difference. But in the 3rd week period, it had the highest value of urease activity due to the high 1 % iron oxide particles. At the 4 weeks, urease activity in soil was found to decrease. Moreover, the first two had very little change in all treatments, this means that proper dose of iron oxide particle is conducive to the preservation of urease activity. However, urease activity picked up quickly in jointing stage and reached the top of growth. These results clearly indicate that urease activity in iron oxide particles treatment increased compared to dose and time. P1 treatment of urease activity was higher than other treatments, moreover, 3 week in four stages had the highest urease activity. Thus, the different dose of iron oxide particles and soil urease activity related to the time.

Conclusion

In this research, synthesis of iron oxide particles by green method were carried out. The results of this study suggest that addition of iron oxide particles could enhance degradation of cypermethrin and its most persistent metabolite, PBA. Thus, in agricultural practice, adequate application of iron oxide particles was an efficient method to reduce the accumulation of cypermethrin and PBA in soil and significantly decreased environmental risks. The present study showed that high soil urease activity due to iron oxide particles. The variance analysis results indicated iron oxide particles can influence the soil urease activity. It is concluded that proper iron oxide particles is important for the beneficial to the remediation of insecticide contaminated soil.

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References

Bharadwaz, A. and Battacharjee, C. (2012). “Extraction of Polyphenol from Dried Tea Leaves”. *International Journal of Scientific and Engineering Research*, vol. 3(5), pp. 1-5

Bootharaju, M.S, and Pradeep, T. (2012). “Understanding the Degradation Pathway of the Pesticide, Chlorpyrifos by Noble Metal Nanoparticles”. *Langmuir*, vol. 28, pp. 2671-2679

Fernández-Garcia, M., and José A. R. (2007). *Metal Oxide Nanoparticles.* Upton, USA: Brookhaven National Laboratory, pp. 1-60

Hameed, H.D. and Entesar, H. E. (2018). “Evaluation of purified urease activity from Proteaus mirabilis using iron oxide nanoparticles and measurement of urea concentration in blood”. *Engineering and Technology Journal*, vol. 36, pp. 152-155

Jaremko, D. and Kalembasa, D. (2014). “A comparison of methods for the determination of cation exchange capacity of soils”. *Ecological Chemistry* and *Engineering S. Journal*, vol. 21(3), pp. 487-498

Jingjing, S., Mijia, Z., Xiaoqia, Y., Chi, Z. and Jun, Y. (2015). “Microbial, Urease Activities and Organic Matter Responses to Nitrogen Rate in Cultivated Soil”. *The Open Biotechnology Journal*, vol. 9, pp. 14-20

Kanagasubbulahshmi, K. and Kadirvelu, K. (2017). “Green Synthesis of Iron Oxide Nanoparticles using *Lagenaria siceraria* and Evaluation of its Antimicrobial Activity”. *Journal of Life Science*, vol. 2, pp.422-427

Nakamoto, K. (1997). *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, New York: Parts A and B, John Wiley& Sons, pp. 52-68

XIE, W.-J., Jian-Min, Z., Huo-Yan W. and Xiao-Qin, C. (2008). “Effect of Nitrogen on the Degradation of Cypermethrin and Its Metabolite 3-Phenoxybenzoic Acid in Soil”, Pedosphere, vol. 18(5), pp. 638-644