Environment Protection Engineering

Vol. 39 2013 No. 3

DOI: 10.5277/epe130310

ALY DERBALAH[1], AHMED ISMAIL[1]

REMEDIATION TECHNOLOGIES OF DIAZINON AND MALATHION RESIDUES IN AQUATIC SYSTEM

The study was carried out to evaluate the efficiencies of various remediation technologies (advanced oxidation processes and bioremediation) for removing diazinon and malathion residues from water. Nano photo-Fenton reagent (Fe^0(nano)/H_2O_2/UV) was the most effective treatment for diazinon and malathion removal while ultraviolet alone was the least effective one. Bioremediation of diazinon and malathion by effective microorganisms (EMs) removed about 100% of their initial concentration. There was no remaining toxicity in contaminated water after remediation except for ultraviolet alone on treated rats. Advanced oxidation processes with nanomaterials and bioremediation with effective microorganisms can be regarded as safe and effective remediation technologies.

1. INTRODUCTION

Organophosphorus pesticides (OPs) represent the most often applied group of insecticides for the last two decades. Their acute lethality is due to inhibition of acetyl cholinesterase (AChE), an enzyme vital to normal nerve function [1]. Because of their non-specific inhibition of AChE, which is present in insects as well as in humans, their dissipation in the environment and presence in spring waters [2] represent a serious threat to wildlife as well as to public health. Most commercial formulations containing OPs are thio-OPs with a P=S group. When applied in the field and transported into ground water, they are easily oxidized to oxons (P=O) by naturally present oxidants. The oxidation is even more efficient during disinfection of potable water when oxidants (like chlorine or ozone) are used [3]. The respective oxons are more polar and considerably more potent AChE inhibitors [1, 4]. For these reasons, there is a keen interest in research and development of purification technologies of OPs polluted wa-

[1]Department of Pesticides Chemistry, Faculty of Agriculture, Kafr El-Sheikh University 33516, Kafr El-Sheikh, Egypt, corresponding author A.S.H. Derbalah, e-mail: aliderbalah@yahoo.com

ter, their degradation products, and commercial co-products, i.e. isomers and/or O,O,S-trimethyl phosphorodithioate ([OOS(S)]) and O,O,S-trimethyl phosphorothioate ([OOS(O)]) [5].

Diazinon and malathion are thio-OPs with containing P=S groups and commonly used thionophosphorous organophosphate (OP) pesticides to control a variety of insects in agriculture and household environment [6]. Despite of their low persistence in the environment, they are considered nonspecific insecticides and highly toxic to animals and humans. Moreover, the toxicity of OPs is increased by their break-down products such as oxons which could be bio-activated within an organism or trough exposure to the sunlight.

Diazinon and malathion are common water pollutants in Egypt [7]. Therefore, due to great environmental and human risk of these organophosphorus insecticides in water resources, advanced methods are in demand for effective treatments of pesticides-polluted water to achieve complete mineralization of target pesticides and to avoid the formation of toxic end products [7]. Advanced oxidation processes (AOPs), which are constituted by the combination of several oxidants such as Fenton reagent and zinc oxide combined with hydrogen peroxide are characterized by the generation of very reactive and oxidizing free radicals in aqueous solution such as hydroxyl radicals which posses a great destruction power [8, 9].

An important feature of nanomaterials is that their surface properties can be very different from those characteristic of their macroscopic or bulk counterparts [10]. The application of nanoparticles as catalysts of the Fenton-like and photo-Fenton reactions has been described by several investigators [11]. In comparison with their microsize counterparts, nanoparticles show a higher catalytic activity because of their large specific surface where catalytically active sites are exposed [12]. The advantage of using nanoparticles as catalysts for Fenton reagent would be more than offset the disadvantage (associated with the use of iron(III) catalyst) of requiring ultraviolet radiation to accelerate the reaction.

Bioremediation of hazardous wastes is a relatively new technology that has undergone more intense investigation of recent decades. This process is focused on destroying or immobilizing toxic waste materials [13]. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site [14].

However after remediation of pesticide residues in water, toxicity assessment is needed to directly assess the potential hazard of original pollutants and its metabolites [8]. The use of AChE biosensor seems to be the most appropriate technique for unambiguous determination of toxicity of water samples and wastewater at different stages of degradation processes of organophosphorus compounds and carbamates. The main advantages of AChE bioassays results from their selective sensitivity to organophos-

phates toxic photoproducts and prompt response, which enables on line monitoring and control of photodegradation processes [15, 16].

In this study, the efficiency of advanced oxidation processes with various nanomaterials and bioremediation with effective microorganisms (EMs) was evaluated to achieve the total degradation of diazinon and malathion. The cholinesterase activity was measured to confirm complete detoxification of diazinon and malathion contaminated water after remediation.

2. EXPERIMENTAL

Chemicals. Diazinon and malathion with purity of 99.5% was purchased from Chem-service (USA). Zinc oxide (99.99%), zero valent iron (99.9%) and titanium dioxide were obtained from the Egypt Nanotech Company Limited, Giza, Egypt. Titanium dioxide was obtained from Egypt Nanotech Company Limited, Cairo, Egypt.

Photochemical remediation. The scope of the experiments included the following treatments: nano photo Fenton reagent (Fe^0(nano)/H_2O_2/UV), nano photo zinc oxide combined with hydrogen peroxide (ZnO(nano)/H_2O_2/UV), photo Fenton reagent (Fe^{2+}/H_2O_2/UV), photo zinc oxide combined with hydrogen peroxide (ZnO/H_2O_2/UV), titanium dioxide combined with hydrogen peroxide (TiO_2/H_2O_2/UV) and ultraviolet alone (UV). For photo Fenton reagent, a UV mercury lamp model VL-4.LC (80 W) at the wavelength range of 254–365 nm was employed for the irradiation of diazinon and malathion separately in water solutions. Ferrous sulphate and zero valent iron-nanoparticles were used as a source of iron catalyst. The solution was prepared by addition of desired amount of diazinon or malathion (1 mg·dm^{-3}) to distilled water and carefully agitated. Then, freshly prepared ferrous sulphate or zero valent iron-nanoparticles at the concentration of 50 mg·dm^{-3} as Fe was added followed by the addition of H_2O_2 at the concentration of 0.05%. After that the solution was completed with water up to 1 dm^3. The initial pH of the solution was adjusted to be 2.8 by using 1 M HCl at all experiments [9, 17]. The solution was transferred from the standard flask to a quartz cell (1 dm^3) and exposed to UV irradiation under constant temperature of 25 °C with steering (the distance between the lamp and the tested pesticides solution was 15 cm).The solutions from the irradiated samples were removed at regular intervals (10, 20, 40, 80, 160 and 300 min) for HPLC analysis.

For ZnO and TiO_2 catalysts, diazinon or malathion solutions at concentrations of 1 mg·dm^{-3} with appropriate amount of ZnO, TiO_2 or ZnO nanoparticles (300 mg·dm^{-3}) were shaken carefully before illumination followed by the addition of H_2O_2 (0.05%). Then the pH was adjusted to 7, the optimum pH for ZnO and TiO_2 catalysts [8, 17]. The suspension was kept for 30 min in the dark, prior to illumination to achieve the maximum adsorption of the tested insecticides onto the semiconductor surface. To

evaluate direct photolysis of the tested insecticides, an experiment using ultraviolet alone without catalyst was carried out for each insecticide to evaluate its photolysis. The solutions from the irradiated samples were collected at regular intervals (10, 20, 30, 60, 120, 240 and 300 min) for HPLC analysis. All photochemical experiments were repeated three times for accurate data.

Bioremediation technique. Effective microorganisms (EMs) formulation used for bioremediation of diazinon and malathion was obtained from the Egyptian Ministry of Agriculture, Cairo, Egypt. This formulation contains 60 species of beneficial microorganisms grown in special media and produced in Egypt under supervision of the Japanese EMRO Scientific Organization. The enrichment and propagation were carried out in 250 cm^3 sterilized Erlenmeyer flask using mineral salt medium (MSM) [18] and 5 $cm^3 \cdot dm^{-3}$ of EMs liquid concentrate. Then the culture was prepared in 250 cm^3 sterilized flasks containing 190 cm^3 of MSM, 10 cm^3 of EMs and supplemented with 1 $mg \cdot dm^{-3}$ of diazinon or malathion. The cultures were incubated at 30 °C, pH = 7 and 150 rpm as optimum conditions for the growth of the tested EMs [19]. Samples were collected after 0, 1, 2, 3, 4 and 5 weeks for monitoring the degradation of each tested insecticide. Control flasks of equal volume of MSM medium and the tested insecticides without the EMs were run in parallel at all intervals to asses abiotic loss of the tested insecticides. The collected water samples of each insecticide were filtered using syringe filter (0.2 μm) [19] followed by the HPLC analysis. Each experiment was repeated three times for accurate data.

HPLC analysis. The irradiated samples were analyzed directly by HPLC, 1100 series, Agilent Technologies, Palo Alto, CA, USA). The HPLC column used (i.d. 4.6 mm, length 250 mm) was filled with Wakosil-II 5 C18–100 (Wako). A mixture of acetonitrile (HPLC grade) and distilled water was used as a mobile phase for malathion (30:70) and diazinon (65:35) under the isocratic elution mode. The flow rate was maintained at 1 $cm^3 \cdot min^{-1}$ and the UV detector was employed at the wavelength of 202 nm for malathion [16] and 210 nm for diazinon [20]. First order rate constants were derived from plots by linear regression analysis for each experiment (chemical and bio) and the half-lives ($t_{1/2}$) were estimated [21].

Toxicity test. To confirm the complete detoxification of diazinon and malathion in treated water, a toxicity test was conducted on rats. Diazinon and malathion contaminated waters after treatment with $Fe^{2+}/H_2O_2/UV$, Fe^0(nano)/H_2O_2/UV, ZnO/H_2O_2/UV, ZnO(nano)/H_2O_2/UV, TiO_2/H_2O_2/UV, UV alone and EMs were orally administrated to the tested rats. The test was carried out to investigate the effect of possible remaining diazinon and malathion (parent or metabolites) in water samples after remediation on acetyl cholinesterase activity on treated rats relative to control.

Adult rats (*Sprague dauley*) with 100–120 g of weight, obtained from Faculty of Veterinary Medicine, Kafr-El-Shiekh University were used. Rats were housed in polypropylene cages under standard conditions with free access to drinking water and food. The animals were randomly divided into eight groups each comprising of five animals and the treated samples that possibly contain diazinon and malathion were given separately to rats as oral administration. Control group rats was fed with normal diet and given oral dose containing no diazinon and malathion. After 21 days, the rats were sacrificed under anesthesia and blood samples were taken by cardiac puncture in vials containing heparin. Plasma was separated and centrifuged at 4500 rpm for 15 min. The supernatant of blood sample was used to determine acetyl cholinesterase activity according to the method described by [22]. To determine the specific activity of cholinesterase, the total protein was determined according to the method described by [23]. The specific activity in μmole Ache/(min·mg) protein was calculated according to [22] from the equation:

$$\text{Activity} = \frac{\Delta OD_{\text{sample}} - \Delta OD_{\text{blank}}}{0.0124 PC \times Sv} \tag{1}$$

where: *OD* – optical density, *PC* – protein concentration (mg·cm^{-3}), *Sv* – sample volume (100 μL).

Enzyme activity data were statistically analyzed using one-way repeated measurement analysis of variance. Least significant difference (LSD) was used to separate means using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

3. RESULTS

3.1. DEGRADATION OF DIAZINON AND MALATHION BY ADVANCED OXIDATION PROCESSES

Losses in diazinon and malathion concentrations upon the irradiation time with respect to the rate and the complete degradation of the tested compounds have been determined. As shown in Figures 1, 2 as well as in Tables 1 and 2, the irradiation under Fe^0(nano)/H_2O_2/UV system gave the highest degradation rate and the lowest half-life times of diazinon and malathion followed by ZnO(nano)/H_2O_2/UV, TiO_2/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, ZnO/H_2O_2/UV and UV light alone, respectively. A complete degradation of diazinon and malathion was achieved under Fe^0(nano)/H_2O_2/UV followed by ZnO(nano)/H_2O_2/UV, TiO_2/H_2O_2/UV, Fe^{2+}/H_2O_2/UV and ZnO/H_2O_2/UV systems within 10, 20, 30, 60, 120, 240 and 300 min of irradiation, respectively (Figs. 1, 2). The complete degradation of the tested insecticides was not achieved under UV treatment.

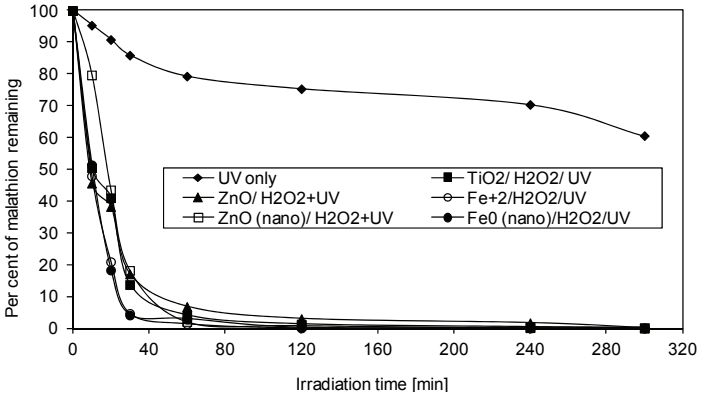

Fig. 1. Degradation of malathion at the initial concentration of 1 mg·dm^{-3} in water under Fe^0(nano)/H_2O_2/UV, ZnO(nano)/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, ZnO/H_2O_2/UV, TiO_2/H_2O_2/UV and UV alone systems

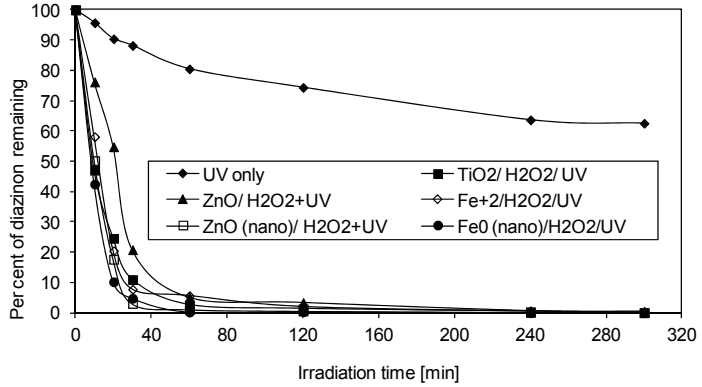

Fig. 2. Degradation of diazinon at the initial concentration of 1 mg·dm^{-3} in water under Fe^0(nano)/H_2O_2/UV, ZnO(nano)/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, ZnO/H_2O_2/UV, TiO_2/H_2O_2/UV and UV alone systems

Table 1

Half-lives ($t_{1/2}$) and correlation coefficients (R^2) for malathion in aqueous system under various advanced oxidation processes

Treatment	R^{2*}	$t_{1/2}$ [h]
UV only	0.91	8.25±0.1
TiO_2/ H_2O_2/ UV	0.93	0.54±0.05
ZnO/ H_2O_2+UV	0.91	0.62±0.03
Fe^{+2}/H_2O_2/UV	0.89	0.58±0.04
ZnO(nano)/ H_2O_2+UV	0.92	0.46±0.03
Fe^0(nano)/H_2O_2/UV	0.88	0.41±0.04

Data scatter form those from the experiments by the standard deviation.

Table 2

Half-life values ($t_{1/2}$) and its correlation coefficient (R^2) for diazinon in aqueous system under advanced oxidation processes

Treatment	R^2	$t_{1/2}$ [h]
UV only	0.94	7.70±0.05
TiO_2/ H_2O_2/ UV	0.92	0.49±0.03
ZnO/ H_2O_2+UV	0.89	0.67±0.05
Fe^{+2}/H_2O_2/UV	0.91	0.61±0.06
ZnO(nano)/ H_2O_2+UV	0.89	0.44±0.041
Fe^0(nano)/H_2O_2/UV	0.83	0.42±0.031

Data scatter form those from the experiments by the standard deviation.

3.2. BIODEGRADATION OF DIAZINON AND MALATHION USING EMS

The degradation ability of the EMs against malathion and diazinon is shown in Fig. 3 and in Table 3.

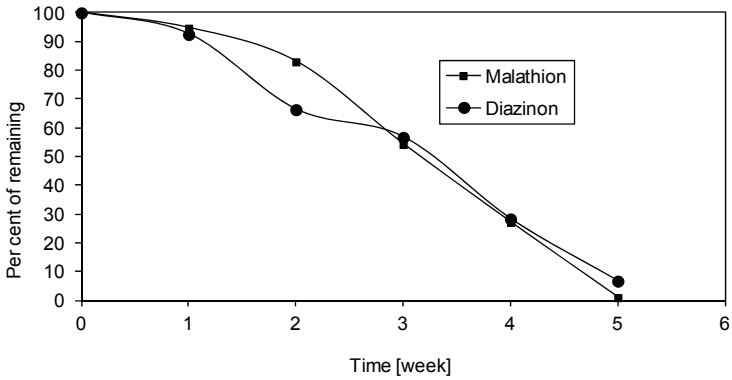

Fig. 3. Biodegradation of diazinon and malathion at the initial concentration of 1 mg·dm^{-3} in water by effective microorganisms (EMs)

Table 3

Half-life values ($t_{1/2}$) and its correlation coefficient (R^2) for malathion and diazinon in aqueous system using EMs

Treatment	R^2	$t_{1/2}$ [h]
Diazinon	0.98	16.61±0.61
Malathion	0.96	17.44±1.10

Data scatter form those from the experiments by the standard deviation.

The EMs showed high potential in the degradation of the tested insecticides with half-life values of 17.44 and 16.6 days for malathion and diazinon, respectively. 99% of malathion initial contents was degraded within five weeks while 93% of diazinon was degraded within the same time. On contrary, the degradation percentages of malathion and diazinon were negligible at the end of incubation time in control samples (data not shown). This indicates that abiotic losses of diazinon and malathion are negligible.

3.3. TOXICITY ASSESSMENT

The complete detoxification of diazinon and malathion in water samples treated with Fe^0(nano)/H_2O_2/UV, ZnO(nano)/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, TiO_2/H_2O_2/UV, ZnO/H_2O_2/UV, UV alone and EMs was confirmed by measuring the effect of these treated samples on the activity of acetylcholinesterase relative to control in treated rats. There were no significant differences in cholinesterase activity in the blood of rats treated with water samples after remediation relative to control treatment except for UV treatment only (Table 4).

4. DISCUSSION

Chemical remediation of malathion and diazinon by different advanced oxidation processes have been reported before [15, 17, 24]. However the degradation half-life times of these insecticides were much lower (few minutes) than that obtained in the present study. The slow degradation under different advanced oxidation processes in this study may be due to the low concentration used (1 $mg \cdot dm^{-3}$) of the tested insecticides. Hence it increases the ability of the advanced oxidation processes components to compete with diazinon and malathion in reacting with generated hydroxyl radicals as hydroxyl radicals scavenger [8,9]. Also, chloride and carbonate ions naturally present in water with high concentration react as hydroxyl radical scavenger [25].

The irradiation under Fe^0(nano)/H_2O_2/UV gave the highest degradation rates of diazinon and malathion followed by ZnO(nano)/H_2O_2/UV, TiO_2/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, ZnO/H_2O_2/UV and UV light alone, respectively. The higher degradation rate of diazinon and malathion by irradiation under Fe^0(nano)/H_2O_2/UV and ZnO(nano)/H_2O_2/UV relative to the degradation under other photochemical remediation systems. This is due to the facts that the stabilized nanoparticles offer much greater surface area and reactivity which leads to higher generation rate of hydroxyl radicals relative to the normal particles [26] and this subsequently induces faster degradation of the tested insecticides. For example, the effect of ZnO particle size on the photodegradation efficiency can be ascribed to two reasons. When the size of ZnO crystals decreases, the amount of the dispersion particles per volume in the solution may increase and, as

a result, photon absorbance may be enhanced. The surface area of ZnO photocatalyst will also increase as the size of ZnO crystals decreases, which promotes adsorption of more insecticide molecules on the surface [27].

The degradation rates of diazinon and malathion under photo Fenton reagent (nano or normal) were higher than those under $ZnO/H_2O_2/UV$ (nano or normal). This may be due to high generation rates of hydroxyl radicals under photo Fenton reagent through many resources (Eqs. (2)–(6)) relative to $ZnO/H_2O_2/UV$ (Eqs. (7)–(10)) [8].

$$H_2O_2 + h\nu \rightarrow 2\ {}^{\bullet}OH \tag{2}$$

$$Fe^0 + H_2O_2 \rightarrow Fe^{2+} + 2HO^- \tag{3}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \tag{4}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H \tag{5}$$

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \tag{6}$$

$$ZnO + h\upsilon \rightarrow ZnO\ (e_{-cb}/h_{+vb}) \rightarrow e_{cb}^- + h_{vb}^+ \tag{7}$$

$$e_{cb}^- + h_{vb}^+ \rightarrow \text{heat} \tag{8}$$

$$h_{vb}^+ + OH^-\ (\text{or}\ H_2O)_{ads} \rightarrow {}^{\bullet}OH\ (+ H^+) \tag{9}$$

$$e_{cb}^- + O_2 \rightarrow {}^{\bullet}O_2^- \tag{10}$$

where e_{cb}^- are conduction band electrons, h_{vb}^+ – valence band holes.

$TiO_2/H_2O_2/UV$ showed high ability for the degradation of the tested insecticides in this study and this agrees with the findings of [15]. However, it possesses lower degradation efficacy of the tested insecticides than (Fe^0(nano)/H_2O_2/UV) and (ZnO(nano)/H_2O_2/UV) systems in this study. This may be due to the presence of nanoparticles in these two systems which offer much greater surface area and reactivity which leads to higher generation rate of hydroxyl radicals relative to $TiO_2/H_2O_2/UV$. The degradation of the tested insecticides under $TiO_2/H_2O_2/UV$ system due to that the semiconductor TiO_2 in the presence of light, valence band holes (h_{vb}^+) and conduction band electrons (e_{cb}^-) are photogenerated (Eq (11)). Hydroxyl radicals generated through water oxidation by photo-generated valence band holes according to Eqs. (12)–(14) are known to be the most oxidizing species. Hydroxyl radicals react rapidly and non-selectively with organic molecules leading to the production of numerous oxidation intermediates and final mineralization products. In the

presence of air, other species might contribute to the oxidation of the organic molecules such as H_2O_2 or even superoxide radicals which are produced by oxygen reduction through photo generated conduction band electrons.

$$TiO_2 + h\nu \rightarrow e^-_{cb} + h^+_{vb} \quad (11)$$

$$TiO_2(h^+) + H_2O_{ad} \rightarrow TiO_2 + {}^\bullet OH + H \quad (12)$$

$$TiO_2(h^+) + HO_{ad} \rightarrow TiO_2 + {}^\bullet OH \quad (13)$$

$$TiO_2(h^+) + RX_{ad} \rightarrow TiO_2 + {}^\bullet RX^+ \quad (14)$$

After 120 min of irradiation, the degradation rates of the remaining diazinon and malathion were much slower than during the first 120 min under all photochemical remediation systems. This might be due to low concentration of the remaining diazinon and malathion (lower than 5% of the initial concentration) which led to high delivery rate of Fe^{2+}/H_2O_2 and ZnO/H_2O_2 corresponding to higher concentrations of these reagents. Subsequently their ability to react with hydroxyl radicals as hydroxyl radicals scavenger is increased to compete with diazinon and malathion (Eqs. (15), (16)) [8, 9, 25]. Also, chloride and carbonate ions naturally present in water react as hydroxyl radical scavengers [39] as shown in Eqs. (17) and (18).

$$Fe^{2+} + {}^\bullet OH \rightarrow Fe^{3+} + + OH \quad (15)$$

$${}^\bullet OH + H_2O_2 \rightarrow {}^\bullet HO_2 + H_2O \quad (16)$$

$$Cl^- + {}^\bullet OH \rightarrow {}^\bullet Cl + + OH^- \quad (17)$$

$$CO_3^{2-} + {}^\bullet OH \rightarrow {}^\bullet CO_3^- + OH^- \quad (18)$$

The degradation rates of malathion and diazinon were the lowest under UV system alone comparing to other systems and this is due to the degradation under this system induced through the direct photolysis only and this agrees with the findings of [8].

Concerning the bioremediation of diazinon and malathion, EMs showed high degradation ability against those compounds in water. This may be due to that the effective microorganisms are a mixture of various microorganisms [28]. It has also been described as a multi-culture of coexisting anaerobic and aerobic beneficial microorganisms [29]. Therefore, their degradation ability must be faster and more effective than using one microorganism. The main species involved in EMs include: lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, and *Streptoccus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustrus* and *Rhodobacter spaeroides*) yeasts

(*Saccharomyces cerevisiae* and *Candida utilis*), actinomycetes (*Streptomyces albus* and *Streptomyces griseus*) and fermenting fungi (*Aspergillus oryzae* and *Mucor hiemali*) [29]. Bioremediation or biodegradation of malathion and diazinon by several microbial isolates have been reported elsewher[30]. However the degradation half-life times of these insecticides were much lower (few hours or days) than their half-life times in the present study by using EMs. The slower degradation under this study may be due to low concentration of the tested insecticides in this study.

As a conclusion, EMs could be used in aerobic system for treating agricultural wastes which represent the first point for discharge of many chemicals into environment. The effective and stable degradation capacity of the EMs technology in utilizing and degrading these compounds reflects their efficacy in biotechnological application for the bioremediation of such contaminated water. Since EMs live in symbiotic relationships and their influence on the environment are sum of all activities of these microorganisms, they are more stable in retaining their ability to completely degrade diazinon and malathion. The metabolites formed by one type of microorganism may be utilized by other group of organisms. Therefore, this study suggests that microorganisms endowed with this property of degradation of toxic pollutants are a boon to mankind. Future studies on the genes responsible for enhanced biodegradation may enable one to elucidate the exact degradation pathway involved in its microbial biodegradation.

Table 4

Effect of various treatments of malathion and diazinon contaminated water on the activity of cholinesterase in rat relative to control treatment

Treatments	Specific activity of cholinesterase	
	Malathion	Diazinon
Control	$5.78\times10^{-1}\pm0.05$a	$5.38\times10^{-1}\pm0.051$a
(EMs)	$5.43\times10^{-1}\pm0.04$a	$4.62\times10^{-1}\pm0.038$a
UV only	$2.03\times10^{-1}\pm0.02$b	$2.87\times10^{-1}\pm0.011$b
TiO_2/ H_2O_2/ UV	$4.99\times10^{-1}\pm0.03$a	$4.77\times10^{-1}\pm0.033$a
ZnO/ H_2O_2+UV	$5.37\times10^{-1}\pm0.031$a	$5.04\times10^{-1}\pm0.045$a
Fe^{+2}/H_2O_2/UV	$5.52\times10^{-1}\pm0.041$a	$5.25\times10^{-1}\pm0.047$a
ZnO(nano)/ H_2O_2+UV	$5.59\times10^{-1}\pm0.050$a	$5.35\times10^{-1}\pm0.051$a
Fe^0(nano)/H_2O_2/UV	$5.18\times10^{-1}\pm0.042$a	$4.91\times10^{-1}\pm0.043$a

Data scatter form those from triplicate experiments by standard deviation.

Letters a and b show the significance and non-significance between the means using Duncan multiple range test.

To evaluate the efficacy of various tested remediation techniques in removing diazinon and malathion from drinking water, toxicity assessment was carried out with

respect to biochemical test. The results showed that the treated water samples either after chemical or bioremediation had no significant effect on the activity of AChE relative to control except for UV alone (Table 4). This implies that diazinon and malathion which polluted water samples before remediation were completely removed in all remediation techniques except for UV alone. Also this means that the aqueous solutions polluted with diazinon and malathion were completely detoxified to AChE non-inhibiting products when a bioassay was conducted with AChE as a sensitive target of diazinon and malathion [16].

5. CONCLUSIONS

The photo-Fenton reagent and photo zinc oxide combined with hydrogen peroxide showed much promise in the complete degradation and detoxification of diazinon and malathion in contaminated water, especially by using zero valent iron and zinc oxide nanoparticles. EMs formulation is promising as effective and safe bioremediation technique for diazinon and malathion removal from water.

REFERENCES

[1] CHAMBERS W.H., *Organophosphorus compounds: an overview*, [in:] *Organophosphates, Chemistry, Fate and Effects*, J.E. Chambers, P.E. Levi (Eds.), Academic Press, San Diego, CA, 1992, 3–17.
[2] COUPE R.H., BLOMQUIST J.D., *Water-soluble pesticides in finished water of community water supplies*, J. AWWA, 2004, 96 (10), 56.
[3] MAGARA Y., AIZAWA T., MATUMOTO N., SOUNA F., *Degradation of pesticides by chlorination during water purification*, Water Sci. Technol., 200, 30 (7), 119.
[4] JOKANOVIC M., *Biotransformation of organophosphorus compounds*, Toxicol., 1994, 166 (3), 139.
[5] RICE D.W., WISNIEWSKI J.A., JOWA L., HOWD R.A., DIBARTOLOMEIS M.J., *Health Risk Assessment of Malathion Coproducts in Malathion-Bait used for Agricultural Pest Eradication in Urban Areas*, Pesticide and Environmental Toxicological Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, California, 1997.
[6] COX C., *Diazinon*, J. Pestic. Reform., 1992, 12 (3), 30.
[7] MASOUD A.H., EL-FAKHRANY I.I., ABD EL-RAZIK M.A.S., *Monitoring of some agrochemical pollutants in surface water in Kafr El-Sheikh Governorate*, J. Pest. Cont. Environ. Sci., 2007, 15, 21.
[8] DERBALAH A.S., *Chemical remediation of carbofuran insecticide in aquatic system by advanced oxidation processes*, J. Agric. Res. Kafrelsheikh Univ., 2009, 35 (1), 308.
[9] DERBALAH A.S., NAKATANI N., SAKUGAWA H., *Photocatalytic removal of fenitrothion in pure and natural waters by photo-Fenton reaction*, Chemosphere, 2004, 57, 635.
[10] FENG J., HU X., YUE P.L., *Novel bentonite clay-based Fe-nanocomposite as a heterogeneous catalyst for photo-Fenton discoloration and mineralization of Orange II*, Environ. Sci. Technol., 2004, 38, 269.
[11] FENG J., HU X., YUE P.L., *Discoloration and mineralization of Orange II using different heterogeneous catalysts containing Fe: a comparative study*, Environ. Sci. Technol., 2004, 38, 5773.
[12] SHANNON M.J., UNTERMAN R., *Evaluating bioremediation: distinguishing fact from fiction*, Annual Rev. Microbiol., 1993, 47 (24), 715.

[13] VIDALI M., *Bioremediation. An overview*, Pure Appl. Chem., 2001, 73, 1163.
[14] SIMONIAN A.L., EFREMENKO E.N., WILD J.R., *Discriminative detection of neurotoxins in multicomponent samples*, Anal. Chimica Acta, 2001, 444, 179–186.
[15] KRALJ M.B, FRANKO M., TREBSE P., *Photodegradation of organophosphorus insecticides. Investigations of products and their toxicity using gas chromatography–mass spectrometry and AChE-thermal spectrometric bioassay*, Chemosphere, 2007, 67, 99.
[16] KALLEL M., BELAIDA C., BOUSSAHEL R., KSIBIA M., MONTIELA A., ELLEUCHA B., *Olive mill wastewater degradation by Fenton oxidation with zero-valent iron and hydrogen peroxide*, J. Hazard. Mat., 2009, 163, 550.
[17] KOULOUMBOS V.N., TSIPI D.F., HISKIA A.E., NIKOLIC D., VAN BREEMEN R.B., *Identification of photocatalytic degradationproducts of diazinon in TiO_2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight massspectrometry*, J. Am. Mass Spectry, 2003, 14, 803.
[18] ABDEL-MEGEED A., *Psychrophilic degradation of long chain alkanes*, Dissertation, Hamburg –Harburg, Technical University Hamburg–Harburg, Germany, 2004, 158.
[19] DERBALAH A.S., MASSOUD A.H., BELAL E.B., *Biodegrability of famoxadone by various microbial isolates in aquatic system*, Land Contam. Reclama., 2008,16 (1), 13.
[20] SHEMER H., LINDEN K.G., *Degradation and by-product formation of diazinon in water during UV and UV/H_2O_2 treatment*, J. Hazard. Mater., 2006, B136, 553.
[21] MONKIEDJE A., SPITELLER M., *Degradation of Metalaxyl and Mefenoxam and Effects on the Microbiological Properties of Tropical and Temperate Soils*, Int. J. Environ. Res. Public Health, 2005, 2, 272.
[22] ELLMAN G.L., COURTNEY K.D., ANDRES V., FEATHERSTONE R., *A new and rapid calorimetric determination of acetyl cholinesterase activity*, Biochem. Pharmacol., 1961, 7, 88.
[23] GORNALL A.G., BARDAWILL C.J., DAVID M.M., *Determination of serum protein by means of the biuret reaction*, J. Biol. Chem., 1949, 177, 751.
[24] ZHANG Y., PAGILLA K., *Treatment of malathion pesticide wastewater with nanofiltration and photo-Fenton oxidation*, Desalination, 2010, 263, 36.
[25] PARE B., SINGH P., JONNALAGADDA S.B., *Visible light induced heterogeneous advanced oxidation process to degrade pararosanilin dye in aqueous suspension of ZnO*, Indian J. Chem., 2008, 47, 830.
[26] HE F., ZHAO D., LIU J., ROBERTS C.B., *Stabilization of Fe–Pd nanoparticles with sodium carboxymethyl cellulose for enhanced transport and dechlorination of trichloroethylene in soil and groundwater*, Ind. Eng. Chem. Res., 2007, 46, 29.
[27] WANG H., XIE C., ZHANG W., CAI S., CAI Z., YANG Z., GUI Y., *Comparison of dye degradation efficiency using ZnO powders with various size scales*, J. Hazard. Mater., 2007, 141, 645.
[28] HIGA T., *What is EM Technology*, College of Agriculture, University of Ryukyus, Okinawa, Japan, 1995.
[29] *EM Trading Effective Microorganisms for a Sustainable Agriculture and Environment*, Effective Microorganisms@emtrading.com, http://www.emtrading.com.html 2000.
[30] SHAN X., JUNXIN L., LIN, L., CHUANLING Q., *Biodegradation of malathion by Acinetobacter johnsonii MA19 and optimization of cometabolism substrates*, J. Environ. Sci., 2009, 21, 76.