TOXICOLOGICAL EFFECTS OF ORGANOSPHORUS INSECTICIDES AND REMEDIATION TECHNOLOGIES OF ITS RESIDUES IN AQUATIC SYSTEM B. DIMETHOATE

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ABSTRACT

Studies on the currently used organophosphorus insecticides with respect their environmental levels, side effects and effective remediation technologies for it residues considered a source of major concern. This study was carried out to monitor the presence of organophosphorus in water resources in Kafr-El-Shiekh governorate, Egypt. Moreover, to evaluate toxicological effects of one of the most frequently detected compound (dimethoate), with respect to some biochemical parameters (acetylcholinesterase, alkaline phasphatase, Glutamic-Pyruvic Transaminase, Glutamic-Oxaloacetic Transaminase and Glutathion-S-transferase) in blood and histopathological changes of treated rats (kidney and liver). Furthermore, to evaluate the efficacy of different remediation techniques (advanced oxidation processes [AOPs] and bioremediation) for removing the most frequently detected compound (diemthoate) in water. Dimethoate was detected with high concentrations relative to other detected organophosphorus in water resources in Kafr-El-sheikh Governorate. The tested insecticide at dose level of 10 mg/kg induced significant toxicity against the treated rats relative to control with the respect to biochemical parameters and histopathological changes in selected rats organs. Photo-Fenton like reagent was the most effective treatment for dimethoate removal followed by Fe^{3+}/UV, H_2O_2/UV Fe^{3+}/H_2O_2 and UV systems respectively. Bioremediation of dimethoate by *Pseudomonas sp.* (EB20) removed around 70% of the dimethoate initial concentration. This study concluded that, dimethoate at the tested concentration level in water expected to induce side effects on human health. Bioremediation using *Pseudomonas sp.* (EB20) can be regarded as a safe remediation technology of dimethoate in drinking water and the photo-Fenton like reagent would be more preferable as effective treatment of dimethoate in wastewater.

Key words: water, dimethoate, toxicity, remediation, organophosphorus

INTRODUCTION

Wide spread use and disposal of organophosphorus compounds that have been used as an alternative to organochlorine compounds for pest control **(Mueller and Schwack, 2001)** resulted in the release of their residue into natural water, thus inducing an environmental problem **(Amoros *et al.*, 2000; Massoud *et al.*, 2007 a; Lasram *et al.*, 2009).** Pesticide pollution of surface waters and

wastewaters has increased sharply and it constitutes a major pollutant problem and health hazards due to an extensive use of these substances **(Evgenidou *et al.* 2007).**

Therefore, monitoring the presence of organophorphorus and carbamates insecticides in water and evaluation of its side effects on human health considered a source of major concern. Toxicity of organophosporus insecticides used compounds against human and animals were always evaluated by assessment of such biochemical parameters alterations and histopathological changes in tissues and organs (**Cronelius *et al.*, 1959; Ghanem *et al.*, 2006; Massoud *et al.*, 2010).** However, there is lack of evaluating the toxicity of organophosporus pesticides at low concentration levels near the environmental level. Since most previous studies were using high doses of the tested pesticides to expect significant effect of these insecticides, however, this is did not reflect real situation.

Among the different used organophosphorus insecticides, dimethoate [IUPAC Name- 0, 0 dimethyl S- (N methyl, carbamoylmethyl) phosphoro-dithioate], CAS No. 60-51-5 is an organophosphate available in the market by the trade name of Rogor. It is a systemic insecticide used for control of a wide variety of insect pests of fruits, and also vegetables and crop plants. In the WHO acute hazard ranking this is rated as moderately hazardous **(Singh *et al.*, 2009)**. USEPA has registered dimethoate as a systemic organophosphate insecticide but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements. Moreover, there is lack of evaluating the toxicity of organophosphorus pesticides such as dimethoate at low concentration levels near the environmental level (**Singh *et al.*, 2009**).

Due to the great environmental and human risk of pesticide residues in water resources Removal heavily used insecticides and their photoproducts from aquatic environment have become a very important task for human being. Thus, advanced methods are in demand for effective treatment of pesticides-polluted water to achieve complete mineralization of target pesticides and to avoid the formation of toxic end products (**Derbalah, 2009**). Advanced oxidation processes (AOPs), which are constituted by the combination of several oxidants, 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 (**Benitez *et al.*, 2002**).

The photo assisted-Fenton reaction process as advanced oxidation process proved to be very powerful in destroying persistent pesticides in the wastewater such as methyl parathion, methamidofos, fenotrothion and alachlor **(Penuela and Barcelo 1998; Failmann *et al.*, 1999; Derbalah *et al.*, 2004**). In this respect,

it is necessary to apply this method in water with low concentration level of pesticides (near to the environmental level) and by using natural river water to test the efficiency of this method under natural conditions, we would be able to generalize photo-Fenton reaction process for pesticides removal from water (**Derbalah *et al.*, 2004).** Bioremediation of chemo-pollutants becomes the method of choice because it is economically feasible and safer than chemical remediation technologies (**Massoud *et al.*, 2008**).*Pseudomonas* sp is known for their versatility in degradation of xenobiotic compounds such as pesticides in water **(Abd El-Razik, 2006; Massoud *et al.*, 2007 b; Derbalah *et al.*, 2008).**

Therefore, the present study aimed to monitor the presence of organophosphorus insecticides in different water resources in Kafr El-Sheikh Governorate Egypt, to evaluate the toxic effects of the most frequently detected compound in water resources (dimethoate), at low dose near the environmental levels with the respect to some biochemical's (AChE, ALP, GOT, GPT and GST) parameters in blood and histological changes in rats organs (liver and kidney) and finally to evaluate the efficacy of different remediation technologies (advanced oxidation processes and bioremediation) for the removal of the tested insecticide residue in the aquatic system.

MATERIALS AND METHODS

Monitoring of the organophosphorus compounds in water resources

Sampling sites and description

Surface water samples were collected in summer 2008 for one time from three sites (Meet Yazid canal, No.7 drain, and Al-Gharbiya main drain) at River Nile, Rosetta branch in Kafrelsheikh Governorate in glass bottles. Water samples transferred directly to the laboratory for filtration, extraction and analysis of pesticides residues.Meet Yazid canal presents the main source for drinking water in Kafrelsheikh and Seedy Salem cities and adjacent village besides its use as a main source for irrigation water, serve about 150 fadden. Also, fishing activities are carried out. Al-Gharbiya main drain, one of many drainage canals present in the area of study, it receive drainage water from adjacent fields and from industrial area. It serve region about 156 fadden. No.7 drain, this canal is one of many drainage canals present in the area of study, it receive drainage water from adjacent fields (Drainage system of the irrigation water). It serve region about 64.282 Fadden ((**Maasoud *et al.*, 2007**a).

Extraction

In the laboratory, samples were extracted immediately after 24 hr of site collection using disposable solid phase extraction (SPE) with

octadecyl-bonded silica cartridges (C_{18}) (500 mg; Phenomenex Scientific) according by **Belden *et al.*, (2000)**.

Gas Chromatography Analysis

Analysis of final extract was conducted using a Hewlett Packed model 6890 equipped with a flame photometric detector (FPD) with phosphorus filter. The analytical column was a 30m×0.32mm HP-5, with a film thickness of 0.25_m. The 1_L SDME extract was injected into the GC-FPD in splitless mode at 250 ◦C. The column oven temperature was held at 125 ◦C for 2 min, then programmed at 75 ◦C/min to 200 ◦C for 1 min, 10 ◦C/min to 220 ◦C and held for 3 min (**Xiao *et al.*, 2006**). Detection limit and recovery of each detected compound were carried out according to the method described by **Xiao et al., (2006).**

Toxicity experiment

Animals' treatment

Adult Wistar male rats *Rattus norvegicus* 8 weeks of age and 80-100 gm in weight, obtained from Faculty of Medicine, Tanta University, Egypt were used. Wister rats were housed in wire cages under standard conditions with free access to drinking water and food. The rats were kept in temperature-controlled room with 14 hrs light and 10 hrs dark cycles and given standard diet. Before treatment, rats were made adaptation for two weeks during feeding. The animals were randomly divided into four groups each comprising of three animals. Two groups of tested insecticide (24 hrs and 21 days), group for control (without dimethoate) and group for ethanol control. Rats were treated by active ingredient of the tested insecticide (dimethoate) with a purity of 99% obtained from Kafr El Zyat for Chemicals and Pesticides Company Limited, Kafr-El-Zayat , Egypt. The tested insecticide was dissolved in ethanol and gave to rats by oral dose at level of 10 mg / kg. Rats were scarified under anesthesia. Then tissue and blood samples were taken after 24 hrs (acute toxicity) and 21 days (sub-chronic toxicity). Blood sampies were taken by cardiac puncture in vials containing heparin for enzymes activity determination. For histopathological test, rat organs (liver and kidney) were taken and kept in formalin 10 % for histopathological test (**Derbalah, 2009**).

Enzymes Assays

The colorimetric methods of **Elman *et al.*, 1961); Gornal *et al.*, 1949); Belfield and Goldberg, 1971); Reitman and Frankel, 1957); and Rose and Wallbank, 1986**) were used for determining the activity of AChE (acetylcholinesterase), (ALP) alkaline phasphates, GPT (Glutamic-Pyrovic Transaminase), GOT (Glutamic-Oxaloacetic Transaminase) and GST (glutathion-S-transferase) in blood , respectively.

Histopathological test

The histopathology test was carried out at Dep. of Histopathology, Fac. of Veterinary Medicine, Kafr El-Sheikh Univ. Egypt. This experiment was conducted to study the histopathological lesions of the organs (liver and kidney) in treated rats with the tested insecticide; these organs were removed and prepared for histopathological examination according to **Bancroft and Stevens (1996)**.

Chemical remediation of tested insecticide in aqueous system

A UV mercury lamb (model VL-4 LC (8W) was employed for the irradiation of the tested insecticide (dimethoate). Ferric chloride was used as a source of iron catalyst because it remains unchanged before and after oxidation and this made the study of the reaction and the future engineering scale-up simpler, because the system remained homogeneous (**Derbalah *et al.*, 2004**). The solution was prepared by addition of desired amounts of dimethoate technical grade (10 ppm) in distilled water. Then freshly prepared ferric chloride, $FeCl_3$, at concentration level of 1 mM as ferric ion was added followed by addition of H_2O_2 at 20 mg/l and the total volume was reached 100 ml by distilled water. The initial pH of the prepared solution was adjusted at 2.8 using hydrochloric acid 1 Molar for all experiments (**Derbalah *et al.*, 2004b; Derbalah, 2009**) using pH meter Jenway (Model 3510, PH/mV/Temperature Meter). All degradation experiments were carried out at room temperature. The solution was transferred from standard flask to glass cell and exposed to irradiation of UV lamp (the distance between the lamp and pesticides solution 15 cm)with a wave length of 265 nm (**Derbalah, 2009**). Illumination times were 10, 20, 40, 80, 160 and 320 min. Samples were removed at these regular intervals for HPLC analysis. Moreover, three experiments were carried out, the first in the absence of hydrogen peroxide (Fe^{3+}/UV) to account for the degradation of methomyl under iron, the second in the absence of iron to account for the degradation of methomyl under hydrogen peroxide (H_2O_2/UV) and finally the third in the absence of iron and hydrogen peroxide to account the degradation under UV light only. Moreover, to account the effect of light on Fenton degradation ability, one experiment was carried out in the presence of Fenton components under dark conditions. The irradiated samples were analyzed directly by HPLC system in the central laboratory of pesticides, Agriculture Research Center, El-Dokey, Egypt. A mixture of acetonitrile and distilled water (40:60) was used as mobile phase under the isocratic elution mode. The flow rate of mobile phase was maintained at 0.7 ml /min. The used detector was UV and the wavelength was 229 nm **(Nestorovska-Krsteska *et al.*, 2008).**

Bioremediation of tested insecticide in aqueous system

Pseudomonas sp. (EB20) was isolated from El-Hamoul water at Kafr-El-Sheikh Governorate, which polluted by persistent organic pollutants (POPs) (**Ashry *et al.*, 2006**) and identified according to its morphological and physiological parameters as described by **Holt (1984)**. The bioremediation test was carried out at Microbiology Laboratory, Dep. of Agric. Botany, and Fac. of Agric. Kafr El-Sheikh Univ. Egypt. The selected microbial isolate *Pseudomonas sp.* (EB20) was cultured onto Mineral Slat Medium (MSL) spiked with the tested insecticide (dimethoate) separately for 7 days and then the growing colonies was washed with three ml sterilized MSL medium. The cell suspension of 10^8cfu/ml (colony forming unit) was used to inoculate 100 ml MSL medium containing 10 ppm of the tested insecticide. The cultures were incubated at 30°C, pH (7) and 150 rpm as optimum conditions for the growth of the tested microbial isolate **(Derbalah et al. 2008)** for 14 days.). Samples were collected at 0, 3, 7, 10 and 14 days for monitoring the degradation of the tested insecticide. Control flasks of equal volume of MSL medium and the tested insecticide without the selected microbial isolate were run in parallel at all intervals to asses a biotic loss. The collected water samples of the tested insecticide were filtered using syringe filtered (**Derbalah *et al.*, 2008**) followed by HPLC analysis as mentioned before.

Statistical analysis Data were subjected to the analysis of variance test and Duncan's multiple range test using a computer program SAS (Version 6.12, SAS Institute Inc., Cary, USA).

RESULTS AND DISCUSSION

Monitoring of insecticide residues in water samples

The analysis of water samples from Kafr El-Sheikh Governorate at the three sampling sites (Meet Yazid canal, No.7 drain and Al-Gharbiya main drain) showed presence of numerous organophosphorus insecticides. The detected organophosphorus compounds were azinophos-methyl, Malathion, dimethoate, phenthoate and fenitrothion in water samples at all sampling sites as shown in Table (1). The concentrations of the detected organophosphorus insecticides ranged from 0.020 to 1.034 ppm. The detection limit and recovery of the detected compounds ranged from 25-56 ng/ml and 90-97%, respectively as shown in Table 1.

Both the detection frequency and concentration level of dimethoate and malathion were higher than the other detected organophosphorus insecticides at all sampling sites. With the concerning the sampling sites, the organophosphorus pesticides were abundant in the following order; Meet Yazid canal > Al-Gharbiya main drain > No.7 drain. These results are in agreement with the findings

of **Ashry *et al.*, (2006)** and **Massoud *et al.*, (2007a)**, they recorded that the occurrence of organophosphorus such as dimethoate, fenitrothion, malathion and phenthoate in irrigation and drainage water in Kafr El-Sheikh governorate.

Table (1): Organophosphorus compounds levels (ppm) in irrigation and drainage water of Kafrelsheikh Governorate as well as its detection limit and recovery.

Pesticides	Meet Yazid canal	No.7 drain	Al-Gharbiya main drain	Detection limits	Recovery percentage
Azinophos-methyl	N.D	N.D	0.02	0.27×10^{-3}	97
Malathion	0.59	1.034	0.3	0.23×10^{-3}	92
Dimethoate	0.42	0.83	0.73	0.56×10^{-3}	96
Phenthoate	N.D	N.D	0.017	0.25×10^{-3}	90
Fenitrothion	0.011	N.D	N.D	0.33×10^{-3}	91

*N.D: Not detected.

Toxicity of dimethoate on some biochemical parameters in rats:
The obtained data in Table (2) and (3) showed that the activity of GST and ALP enzymes were decreased either after 24 hr or 21 days of treatment with dimethoate at dose level of 10 mg/kg relative to control. The results also showed a significant increase in GPT and GOT enzymes activity after 24 hrs treatment, while the activity of the same enzymes were decreased after 21 days of treatment with dimethoate relative to control. The significant differences in liver functions enzymes in rats treated with dimethoate and control due to the damage of hepatic tissue such as cell necrosis which disrupt the normal liver functions. The histopathological changes found in liver tissue elsewhere in this study confirmed this approach. The decrease of hepatic enzymes in rats treated with dimethoate after 21 days may be due to the presence of ethanol as a solvent which acts as free radical producer, increasing the enzyme inhibition (**Sivapiriya *et al.*, 2006**).The increase of hepatic enzymes (GPT and GOT) after 24hrs of treatment with dimethoate due to that the liver is often primary target organ for the toxic effect of xenbiotics and the elevation of these defense enzymes is expected due to the early damage in the hepatic cells (**Massoud *et al.*, 2010).** However, the decrease of hepatic enzymes in rats treated with dimethoate after 21 days may be due to the presence of ethanol as a solvent which acts as free radical producer, increasing the enzyme inhibition (**Sivapiriya *et al.*, 2006**). Moreover, the detoxification of the tested insecticide with the time after treatment increased by these defense enzymes and subsequently their activity with time gradually decreased.

The obtained data showed significant increases in the activity of GPT and GOT enzymes after 24 hrs of treatment with ethanol only while the activities of the same enzymes were decreased after 21days

of treatment. The decrease of hepatic enzymes in rats treated with ethanol only may be due to ethanol acts as free radical producer, increasing the enzyme inhibition caused by the free radicals (**Sivapiriya *et al.*, 2006**).

The results of acetylcholinesterase activity revealed that the activity decreased after treatment with dimethoate at dose level of 10 mg/kg relative to the control either after 24 hrs or after 21days of treatment. These results are in agreement with findings of many researchers they reported that the most prominent clinical effects of poisoning with OPs were related to their inhibition of the activity of blood acetylcholinesterase (AChE) (**Timur *et al.*, 2003; Hazarika *et al.*, 2003**). The obtained results indicated that, the activity of AChE enzyme was increased either after 24 hrs or after 21 days of treatment ethanol only relative control as shown in Tables (2 and 3). The increase in AChE activity in rat treated with ethanol relative to dimethoate may be due to that ethanol known to significantly reduce the inhibition of the AChE inhibitors (**Sivapiriya *et al.*, 2006**).

Table(2): Effect of dimethoate at dose level of 10 mg/kg on activity of some biochemical's parameters in rats' blood after 24 hrs of treatment.

Treatment	AChE (U/L)	ALP (IU/L)	GPT (Units/ml)	GOT (Units/ml)	GST (Units/ml)
Control	5.51×10^{-1} ± 0.035ab	1.28×10^{-1} ± 0.0002a	8.49×10^{-1} ± 0.007^{d}	10.58×10^{-1} ±0.007d	1.15×10^{-1} ± 0.033c
Ethanol	5.86×10^{-1} ± 0.023a	4.67×10^{-2} ± 0.000d	9.58×10^{-1} ± 0.009^{c}	14.47×10^{-1} ± 0.003c	2×10^{-3} ± 0.0005b
Dimethoate	4.8×10^{-1} ± 0.0115c	8.21×10^{-2} ± 0.000b	25.64×10^{-1} ± 0.0051a	23.87×10^{-1} ± 0.003a	1.2×10^{-2} ± 0.0005a

*a, b and c letters shows the significance and non-significance between the means at p value of 0.05 using Duncan's multiple range test.

Table (3): Effect of dimethoate at dose level of 10 mg/kg on activity of some biochemical's parameters in rats blood after 21 days of treatment.

Treatment	AChE (U/L)	ALP (IU/L)	GPT (Units/ml)	GOT (Units/ml)	GST (Units/ml)
Control	9.73×10^{-1} ± 0.012b	1.128×10^{-1} ± 0.0007b	26.07×10^{-1} ± 0.009a	21.93×10^{-1} ± 0.005b	17.3×10^{-3} ± 0.0017b
Ethanol	14.65×10^{-1} ± 0.0115a	4.77×10^{-2} ± 0.0003d	10.27×10^{-1} ± 0.00d	4.19×10^{-1} ± 0.002c	1.3×10^{-3} ± 0.0002c
Dimethoate	9.26×10^{-1} ± 0.0585b	1.52×10^{-1} ± 0.0025a	11.46×10^{-1} ± 0.004c	22×10^{-1} ± 0.063b	4.6×10^{-3} ± 0.0003c

*a, b and c letters shows the significance and non-significance between the means at p value of 0.05 using Duncan's multiple range test.

Histopathological changes

The histopathological changes in the kidney

In normal histological structure of the kidney, the cortex contains glomerular tufts scattered in between proximal and distal convoluted tubules (Figs. 1A). Slight congestion was observed after 24 hrs of with

dimethoate (Fig. 1B). However, after 21 days of treatment with dimethoate, tubular vacuolar degeneration was observed (Fig.2B). In general, results of histopathology experiments showed that, there were no change in both of control and I ethanol in all organs of rats that treated with the tested insecticide after 24 hrs and 21 days of treatment. The results in this study agree with the findings of **Kerem *et al.*, (2007)** and **Afshar *et al.*, (2008)** they reported kidney damage such marked tubular dilation, hydropic degeneration in tubular lining epithelium, moderate congestion and hemorrhage in the cortical male Wistar rats exposed some organophosphate pesticides.

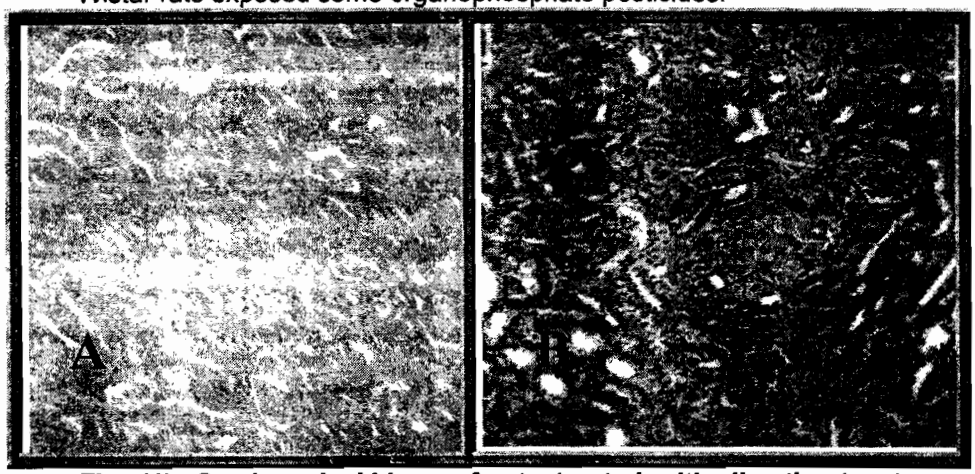

Fig. (1): Sections in kidney of rats treated with dimethoate at dose level of 10 mg/kg after 24 hrs of treatment (B) relative to control (A).

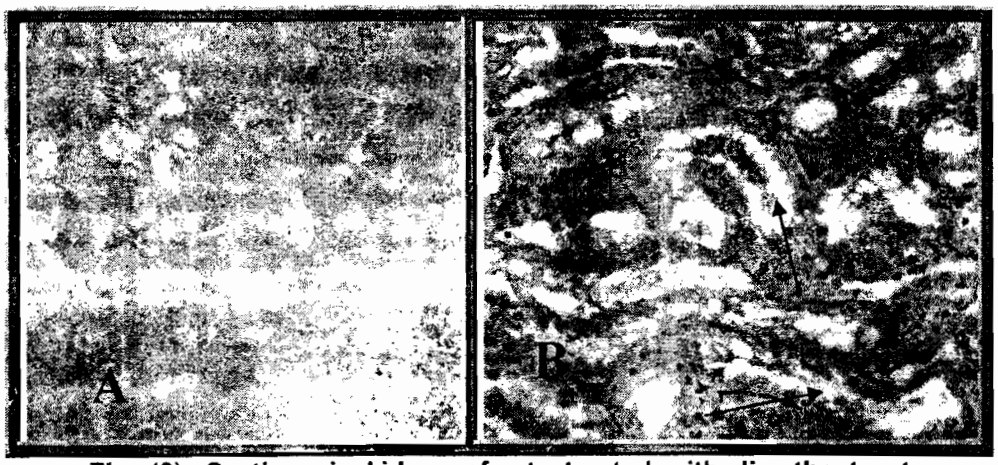

Fig. (2): Sections in kidney of rats treated with dimethoate at dose level of 10 mg/kg after 21 days of treatment (B) relative to control (A).

The histopathological changes in the liver

Normal liver structure appeared in the form of hepatic lobules in which there were centrally located central veins, which were surrounded by hepatocytes arranged in the form of hepatic cords separated from each other by hepatic sinusoids (Figs.3A). In the rats treated with dimethoate after 24 hrs, cell necrosis of hepatocytes was observed (Fig.3B). However, after 21 days of treatment perivascular mononuclear cell infiltration (Fig. 4B), moderate hepatocellular necrosis with mononuclear cell infiltration (Fig. 4C) and focal hemorrhage were observed (Fig. 4D).

The liver is well known target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotics (**Roganovic and Jordanova, 1998**). Therefore, the liver tissue damage in rats treated with dimethoate such as inflammatory ,cell infiltration, hemorrhage, calcification, vacuolar degeneration, dilation of sinusoids, vascular congestion and necrosis was expected (**Sayım, 2007; Yehia *et al.*, 2007**). These changes are entirely consistent with the changes in various biochemical parameters that were also observed in this study.

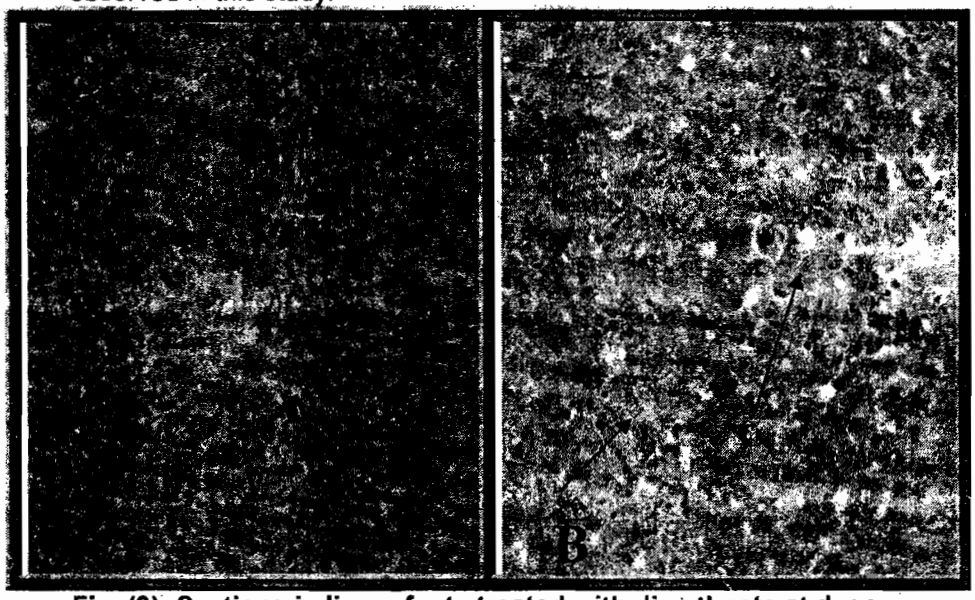

Fig. (3): Sections in liver of rats treated with dimethoate at dose level 10 mg/kg after 24 hrs of treatment (B) relative to control (A).

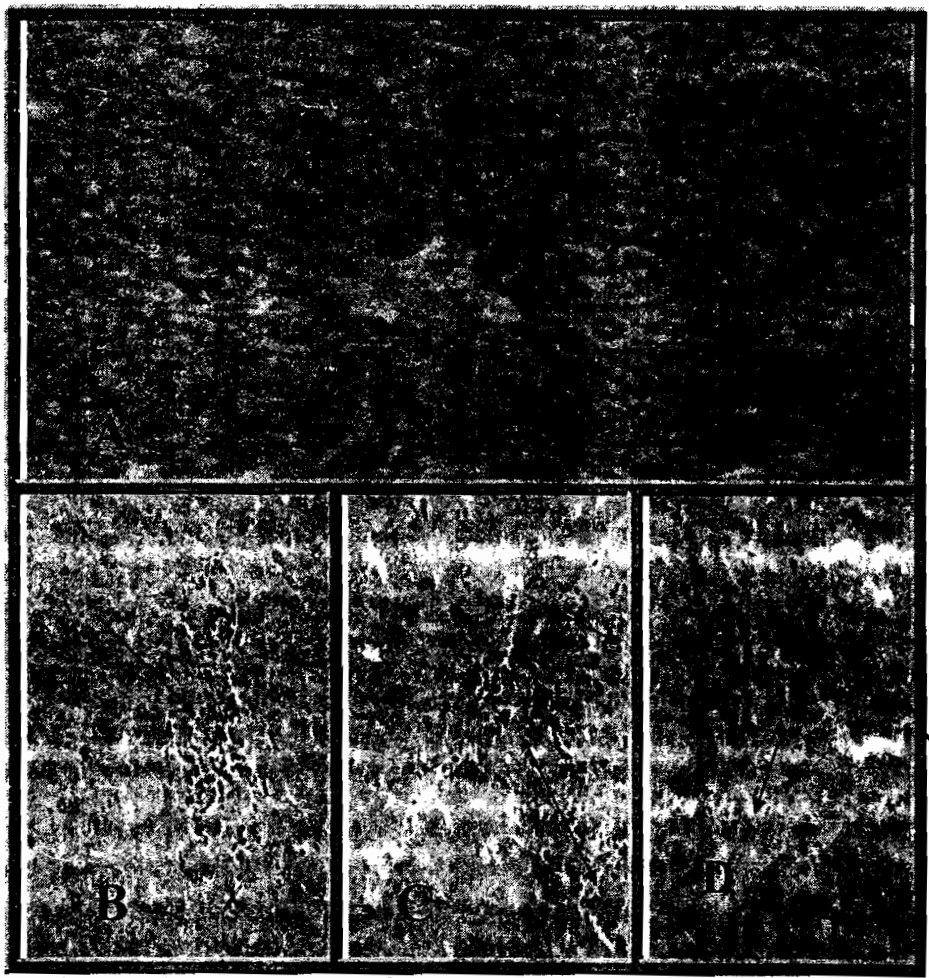

Fig. (4): Sections in liver of rats treated with dimethoate at dose level 10 mg/kg after 21 days of treatment (B), (C) and (D) relative to control (A).

Chemical remediation of the tested insecticide in aqueous solution

The loss in dimethoate initial concentration with the irradiation time under UV, H_2O_2/UV, Fe^{3+}/UV, Fe^{3+}/H_2O_2 and Fe^{3+}/H_2O_2/UV systems was evaluated. The results in Fig.5 showed that, the degradation rate of the tested insecticide were greatly enhanced by irradiation under Fe^{3+}/H_2O_2/UV relative to H_2O_2/UV, Fe^{3+}/UV, Fe^{3+}/H_2O_2 and UV systems. More than 86% of dimethoate initial concentration was degraded under Fe^{3+}/H_2O_2/UV system within 320 min of irradiation time compared with 52.8, 51.4, 50 and 36% in the presence of,

Fe^{3+}/UV, Fe^{3+}/H_2O_2, H_2O_2/UV, and UV systems, respectively within the same irradiation time (Fig.5).

The photodegradation of dimethoate under UV light is due to the direct absorbance of UV light (photolysis) **(Derbalah *et al.*, 2004)**. While the degradation of dimethoate under H_2O_2/UV system due to firstly, the direct photolysis and secondly due the generation of hydroxyl radicals from hydrogen peroxide eq. (1) **(Benitez *et al.*, 2002; Derbalah *et al.*, 2004; Derbalah, 2009)**

$$H_2O_2 + h\nu \rightarrow 2\ ^{\bullet}OH \qquad (1)$$

However, the photodegradation of dimethoate under Fe^{3+}/UV system is due to the direct photolysis of the tested compounds by absorbance of UV light. Moreover, due to the indirect photolysis of this compound by the hydroxyl radicals generated from in the presence of UV light as shown in eq. (2)

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

The degradation of dimethoate under Fe^{3+}/H_2O_2 system is due to the generation of hydroxyl radicals under this system in the absence of the light as shown in eq. 3

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \qquad (3)$$

The degradation rate of dimethoate under H_2O_2/UV system was slightly slower than its degradation under Fe^{3+}/UV system which may be due to the lower formation rate of hydroxyl radicals under H_2O_2/UV system relative to Fe^{3+}/UV system (**Derbalah *et al.*, 2004**). This low generation rate of hydroxyl radicals under H_2O_2/UV system compared to Fe^{3+}/UV system can attributed to the fact that hydrogen peroxide absorbs weakly above 300 nm and that hydroxyl radicals are only generated through direct photolysis of hydrogen peroxide [**Benitez et al., 2002**]. On the other hand, under Fe^{3+}/UV system the $Fe(OH)^{2+}$ complex is the predominant species for generating hydroxyl radicals absorbed light at wavelengths up to 410 which may lead to the generation of hydroxyl radicals under this system more than H_2O_2/UV system (**Derbalah *et al.*, 2004**).

The great enhancement in tested insecticide degradation rate under photo-Fenton's like reagent system (Fe^{3+}/H_2O_2/UV) relative to the other advanced oxidation processes is due to the higher generation rate of hydroxyl radicals under this system (Fe^{3+}/H_2O_2/UV) than the other systems (**Derbalah *et al.*, 2004; Derbalah, 2009**). This high generation rate of hydroxyl radicals under photo-Fenton like reagent due to many reasons. Firstly, the photolysis of hydrogen peroxide itself, which leads to the formation of hydroxyl radicals eq. (1) (**Benitez *et al.*, 2002**). Secondly, the photolysis of Fe $(OH)^{2+}$ complex the predominant specie of Fe^{3+} for generating hydroxyl radicals eq.(2) (**Larson *et al.*, 1991**). Thirdly, the photodecarboxylation of ferric carboxylate complexes generate ferrous ion (eq.3), which can in turn react with hydrogen peroxide to generate additional hydroxyl radicals (eq.4) (**Pignatello and Sun, 1995**).

$H_2O_2 + h\nu \rightarrow 2\ ^{\cdot}OH$ (1)

$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\cdot}OH$ (2)

$Fe^{3+}(RCO_2)^{2+} + h\nu \rightarrow Fe^{2+} + CO_2 + R^{\cdot}$ (3)

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

Results of present study about dimethoate agree with finding of many researchers (**Benitez *et al.*, 2002; Oller *et al.*, 2007; Tamimi *et al.*, 2008**) who reported that, total disappearance of dimethoate and other organosphosphorus insecticides.

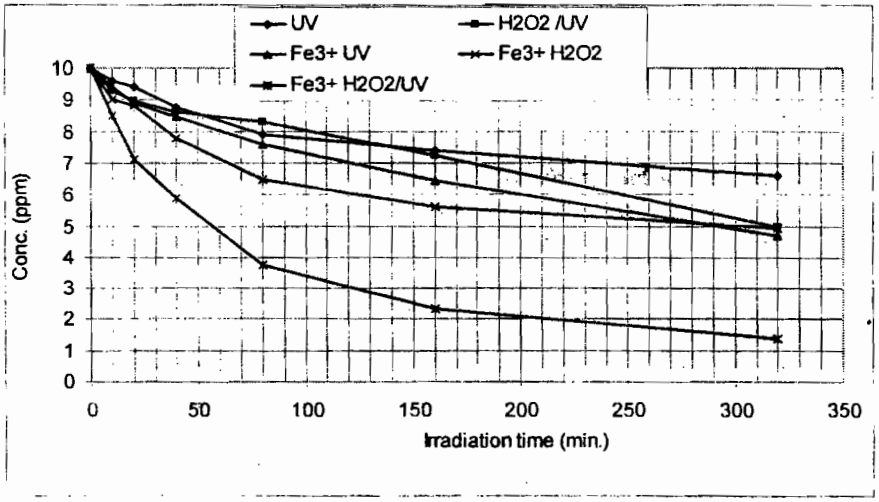

Fig. (5): Degradation of dimethoate at initial concentration of 10 mg/l under $Fe^{3+}/H_2O_2/UV$, H_2O_2/UV Fe^{3+}/UV system and Fe^{3+}/H_2O_2 in distilled water.

Bioremediation of the tested insecticide in aqueous solution

The degradation ability of the selected microbial isolate *Pseudomonas* sp.(EB20) to dimenthoate was illustrated in Fig (6). *Pseudomonas* sp (EB20) showed high potential in the degradation of the tested insecticide. Since around 70% of dimethoate of initial concentration level (10 ppm) was degraded within two weeks of incubation with *Pseudomonas* sp (EB20). On the other hand, the degradation percentage of dimethoate reached to 4% at the end of incubation time in control or non-inoculated samples. This is implied that the quote of tested insecticide decay due to temperature effect and photodecomposition or volatilization is very slight or negligible. The degradation of dimethoate may be attributed to the secretion of enzymes from either tested bacterial or fungal strains which are capable of degrading pesticides (**Bollag and Liu, 1990**). The genus *Pseudomonas* showed the highest rate of dimethoate degradation, and has considerable potential for the biotransformation and biodegradation of pesticides with widely differing chemical structures (**Massoud *et al.*, 2007b; Derbalah *et al.*, 2008**).

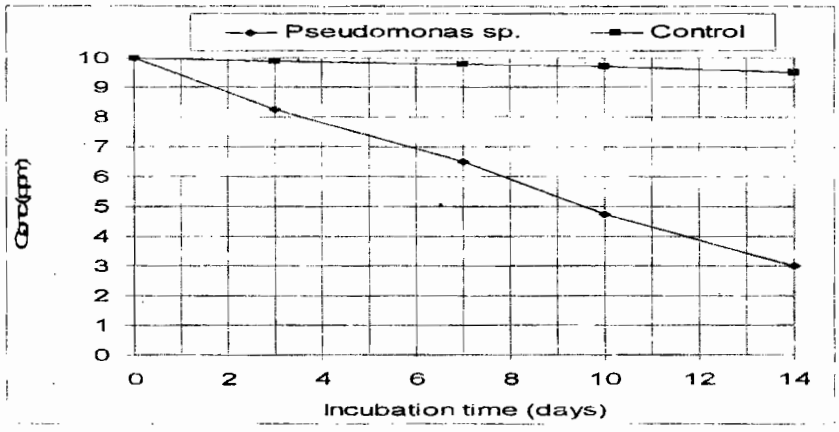

Fig. (6): Biodegradation of dimethoate at concentration level of 10 ppm by *Pseudomonas* sp. (EB20) isolate in aquatic medium.

CONCLUSIONS

Dimethoate was detected with high level in selected water resources in Kafr-El-Sheikh Governorate. Dimethoate induced toxicological effects in treated rats relative to control treatment with the respect to enzymes activity and histological changes in treated organs. More extensive studies are needed to evaluate the toxicity of dimethoate at concentration level near the environmental level which in return helps to evaluate its real toxicity. Photo-Fenton like reagent was the most effective treatment of the removal of dimethoate residues in aquatic system and may be preferable in wastewater treatment. *Pseudomonas* sp (EB20) could be regarded as a safe removal treatment of dimethoate in drinking water.

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الملخص العربى

التأثيرات السامة لمبيد الدايمثويت ومعالجة متبقياته في البيئة المائية

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أجريت هذه الدراسة لعمل حصر سريع واستكشاف وجود المبيدات الفوسفورية في مصادر المياه بمحافظة كفرالشيخ . كذلك لدراسة التأثيرات السامة لأحد اكثر المبيدات الفوسفورية اكتشافا في مصادر المياه و هو الدايمثويت من خلال تأثيره على بعض الإنزيمات ((AChE و GOTوGPT وGST). والتغيرات الهستولوجية التي يحدثها في الكبد و الكلى للفئران المعاملة بتركيز ١٠مجم/ كيلوجرام. أجريت هذه الدراسة أيضا لتقييم كفاءة بعض طرق المعالجة (الأكسدة المتقدمة – المعالجة البيولوجية) للتخلص من متبقيات الدايمثويت في المياه. أوضحت النتائج أن الدايمثويت كان من أكثر المركبات من حيث عدد مرات الاكتشاف و مستوى التركيز في مصادر المياه المختلفة. الملاثيون عند مستوى ١٠مجم/كجم وزن أحدث تأثيرات سامة للفئران المعاملة سواء على مستوى التأثيرات البيوكيميائية أو الهستولوجية مقارنة بالكنترول.طريقة مشابه الفينتون الضوئي (Fe^{+3}/ H_2O_2/UV) كانت اكفأ طرق المعالجة في التخلص من مبيد الدايمثويت في المياه. المعالجة البيولوجية لمبيد الدايمثويت باستخدام عزلة ال *Pesudomonus sp* أدى الى التخلص من حوالي ٧٥ من متبقيات الدايمثويت في المياه. هذه الدراسة توضح ان مبيد الملاثيون بالتركيزات المنخفضة يمكن أن يحدث تأثيرات سامه على صحة الإنسان. كما توضح الدراسة ايضا ان المعالجة البيولوجية باستخدام عزلة ال *Pesudomonus sp* يمكن استخدامها كطريقة معالجة فعالة وآمنة للتخلص من متبقيات الدايمثويت في مياه الشرب بينما طزيقة مشابه الفينتون تعتبر طريقة مفضله للتخلص من متبقيات الدايمثويت في مياه الصرف.