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Toxicological effects of methomyl and remediation technologies of its residues in an aquatic system

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This study was carried out to evaluate toxicological effects of methomyl at low concentration level with respect to some biochemical parameters (acetylcholinesterase [AChE], alkaline phosphates [ALP], glutamic-pyrovic transaminase [GPT], glutamic-oxaloacetic transaminase [GOT] and glutathion-S-transferase [GST]) and histopathological changes of treated rats organs (kidney and liver). Furthermore, to evaluate the efficacy of different remediation techniques (advanced oxidation processes [AOPs] and bioremediation) for removing the tested insecticide in aquatic system. The tested insecticide at dose level of 10 mg kg⁻¹ induced significant toxicity against the treated rats relative to control with respect to biochemical parameters and histopathological changes in treated rats. Photo-Fenton like reagent was the most effective chemical remediation treatment for methomyl removal in aquatic system followed by than Fe³⁺/UV, H₂O₂/UV, Fe³⁺/H₂O₂ and UV only systems, respectively. Bioremediation of methomyl using Pseudomonas sp. (EB20) isolate removed 77% of its initial concentration. This study concluded that, methomyl at the tested concentration level in water is expected to induce side effects on human health. Bioremediation using Pseudomonas sp. (EB20) can be regarded as a safe remediation technology of methomyl in drinking water. However the photo-Fenton like reagent would be more preferable as effective treatment of methomyl in wastewater.

Key words: Methomyl, residues, toxicity, remediation.

INTRODUCTION

Wide spread use and disposal of organophosphorus and carbamates compounds that have been used as an alternative to organochlorine compounds for pest control (Muller and Schwack, 2001) resulted in the release of their residue into natural water, thus inducing an environmental problem (Derbalah et al., 2004b). Dimethoate, malathion and methomyl considered to be priority pollutants in water due to the wide range use of these pesticides against different pests (Lasarm 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 (Derbalah et al., 2004b; Evgenidou et al., 2007). Therefore, evaluation of its side effects on human health considered a source of major concern.

Toxicity of organophosphorus and carbamates 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 these pesticides at low concentration levels near the environmental level. Since most previous studies were using high doses of the tested pesticides to expect significant toxicological effect of these insecticides, however, this is did not reflect real situation.

Due to the great environmental and human risk of pesticide residues in water resources, their removal becomes 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 possess a great destruction power to the organic pollutants (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 (Penuela and Barcelo, 1998; Fallmann 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), which would be able to generalize photo-Fenton reaction process for pesticides removal from water (Derbalah et al., 2004a). Bioremediation of chemo-pollutants becomes the method of choice because it is economically feasible and safer than chemical remediation technologies (Derbalah 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., 2007b; Derbalah et al., 2008).

Therefore, the present study aimed to evaluate the toxic effects of the most frequently detected compound in water resources (methomyl), 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 treated 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**

**Toxicity experiment**

**Animals' treatment**

For Toxicity assessments 8-week-old 80-100g Wistar male rats (*Rattus norvegicus*) obtained from Faculty of Medicine, Tanta University 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 h Light and 10 h dark cycles and given standard diet consist. 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 h and 21 days), group for control (without methomyl) and group for ethanol control.

Rats were treated with methomyl (99%) that 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⁻¹ (volume 1ml). Rats were scarified under anesthesia. Then tissue and blood samples were taken after 24 h (acute toxicity) and 21 days (sub-chronic toxicity). Blood samples were taken by cardiac puncture in vials containing heparin. Blood samples were centrifuged at 4500 rpm for 20 min and serum was collected 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 Ellman et al. (1961), Gornal et al. (1949), Beffield and Goldberg (1971), Reitman and Frankel (1957), and Rose and Wallbank (1986) were used for determining the activity of AChE (acetylcholinesterase), (ALP) alkaline phosphates, GPT (Glutamic-Pyrovic Transaminase), GOT (Glutamic-Oxaloacetic Transaminase) and GST (glutathion-S-transferase) in blood, respectively.

**Histopathological tests**

The histopathology test was conducted 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 the method described by Bancroft and Stevens (1996).

**Chemical remediation of tested insecticide in aqueous system**

A UV mercury lamb (model VL-4 LC (80W) was employed for the irradiation of the tested insecticide (methomyl). 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., 2004a). The solution was prepared by addition of desired amounts of methomyl technical grade (10 ppm) in distilled water. Then freshly prepared ferric chloride, FeCl₃, at concentration level of 1 mM as ferric ion was added followed by addition of H₂O₂ 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., 2004a; 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 (Fe³⁺/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 (H₂O₂/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-Dokej, Egypt. A mixture of acetonitrile and distilled water (20:80) 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 231 nm (Tamimi et al., 2008).

**Biorremediaion 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
The increase of hepatic enzymes after 24 h or after 21 days of treatment with methomyl was due to the fact that the liver is often primary target organ for the toxic effect of xenobiotics and the elevation of these defense enzymes is expected due to the early damage in the hepatic cells (Massoud et al., 2010). Moreover, the histopathological changes found in liver tissue elsewhere in this study confirmed this approach. However, the decrease of hepatic enzymes in rats treated methomyl after 21 days with ethanol 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). Furthermore, the detoxification of the tested insecticide with the time after treatment increased by these defense enzymes and subsequently their activity with time gradually decreased.

Data in Tables 1 and 2 showed that, the activity of GST and ALP enzymes were decreased either after 24 h or 21 days of treatment with ethanol relative to control treatment. On the other hand, the activity of GPT and GOT enzymes were increased after 24 h of treatment with ethanol while after 21 days of treatment the same enzymes activity were decreased comparing with control as shown in Tables 1 and 2. The decrease of hepatic enzymes in rats treated with ethanol may be due to the reason mentioned elsewhere in this study (Sivapiriya et al., 2006).

Referring to acetylcholinesterase activity, the obtained results revealed that, the activity decreased after 24 h or after 21 days of treatment with methomyl at dose level of 10 mg/kg relative to control treatment as shown in Tables 1 and 2. The inhibition of acetylcholinesterase activity in treated rats relative to control treatment due to that methomyl and other carbamates known as acetylcholinesterase inhibitors (Derbalah 2009). Moreover, the obtained results indicated that, the activity of AChE enzyme was increased either after 24 h or after 21 days of treatment by oral administration with ethanol comparing with control treatment (Tables 1 and 2). The increase in AChE activity in rat treated with ethanol

### Table 1. Effect of methomyl at dose level of 10 mg/kg on activity of some biochemical parameters in rats after 24 h of treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AChE (U/L)</th>
<th>ALP (U/L)</th>
<th>GPT (Units/ml)</th>
<th>GOT (Units/ml)</th>
<th>GST (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$5.11 \times 10^3 \pm 0.035bc$</td>
<td>$1.28 \times 10^3 \pm 0.0002b$</td>
<td>$8.493 \times 10^1 \pm 0.007d$</td>
<td>$10.58 \times 10^1 \pm 0.007c$</td>
<td>$1.15 \times 10^2 \pm 0.003b$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$5.86 \times 10^3 \pm 0.023b$</td>
<td>$4.67 \times 10^2 \pm 0.0006d$</td>
<td>$9.58 \times 10^1 \pm 0.009c$</td>
<td>$14.47 \times 10^1 \pm 0.002a$</td>
<td>$2 \times 10^3 \pm 0.0005c$</td>
</tr>
<tr>
<td>Methomyl</td>
<td>$6.92 \times 10^3 \pm 0.012a$</td>
<td>$1.455 \times 10^2 \pm 0.0002a$</td>
<td>$13.12 \times 10^1 \pm 0.006a$</td>
<td>$14.02 \times 10^1 \pm 0.002b$</td>
<td>$2.9 \times 10^2 \pm 0.0043a$</td>
</tr>
</tbody>
</table>

*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 2. Effect of methomyl at dose level of 10 mg/kg on activity of some biochemical’s parameters in rats after 21 days of treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AChE (U/L)</th>
<th>ALP (U/L)</th>
<th>GPT (Units/ml)</th>
<th>GOT (Units/ml)</th>
<th>GST (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$9.73 \times 10^3 \pm 0.012b$</td>
<td>$1.128 \times 10^3 \pm 0.0007a$</td>
<td>$26.07 \times 10^1 \pm 0.009a$</td>
<td>$21.93 \times 10^1 \pm 0.005a$</td>
<td>$1.73 \times 10^2 \pm 0.002a$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$14.56 \times 10^3 \pm 0.012a$</td>
<td>$4.77 \times 10^2 \pm 0.0003c$</td>
<td>$10.27 \times 10^1 \pm 0.002d$</td>
<td>$4.19 \times 10^1 \pm 0.002d$</td>
<td>$1.3 \times 10^3 \pm 0.0002d$</td>
</tr>
<tr>
<td>Methomyl</td>
<td>$8.44 \times 10^3 \pm 0.0 c$</td>
<td>$4.95 \times 10^2 \pm 0.0004b$</td>
<td>$16.39 \times 10^1 \pm 0.004c$</td>
<td>$14.70 \times 10^1 \pm 0.006c$</td>
<td>$6 \times 10^2 \pm 0.0005c$</td>
</tr>
</tbody>
</table>

*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.
relative to methomyl may be due to that ethanol known to significantly reduce the inhibition of the AChE inhibitors (Sivapiriya et al., 2006).

Histological changes in different rat organs after treatment with methomyl

**Histopathological changes in kidney**

In normal histologic structure of the kidney, the cortex contains glomerular tufts scattered in between proximal and distal convoluted tubules (Figures 1A and 2A). Kidney of rats treated with methomyl at dose level 10 mg/kg after 24 h of treatment showed no changes (Figure 1B). However, for rats treated with methomyl at the same dose after 21 days of treatment showed cystic tubular degeneration (Figure 2B) and advanced tubular degeneration with cystic dilatation of the lumens (Figure 2C).

**Histopathological changes in 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 (Figures 3A and 4A). The liver of rats treated with methomyl at dose level 10 mg/kg after 24 h of treatment was normal as control (Figure 3B). However, the liver of rats treated with methomyl at dose level 10 mg/kg after 21 days of treatment showed advanced scarring of the hepatocytes cytoplasm (Figure 4B) and advanced sinusoidal congestion (Figure 4C). The increase of hepatic enzymes (GPT and GOT) after 24 h of treatment with methomyl mentioned before support the histopathological changes recorded in liver. Since the liver is often primary target organ for the toxic effect of xenobiotics and the elevation of these defense enzymes is expected due to the early damage in the hepatic cells (Roganovic, 1998; Massoud et al., 2010).

**Chemical remediation of the tested insecticide in aqueous solution**

The loss in methomyl initial concentration with the irradiation time under UV, H\textsubscript{2}O\textsubscript{2}/UV, Fe\textsuperscript{3+}/UV, Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2} and Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2}/UV systems was evaluated. The results in Figure 5 showed that, the degradation rate of the tested insecticide were greatly enhanced by irradiation under Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2}/UV (Fenton like reaction) relative to UV, H\textsubscript{2}O\textsubscript{2}/UV, Fe\textsuperscript{3+}/UV, Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2} systems. More than 90% of methomyl initial concentration was degraded under Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2}/UV system within 320 min of irradiation time compared with 52, 46, 45 and 38% of the same insecticide in the presence of, UV, H\textsubscript{2}O\textsubscript{2}/UV, Fe\textsuperscript{3+}/UV, Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2} and Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2}/UV systems, respectively within the same irradiation time (Figure 5).

The photodegradation of methomyl under UV light is due to the direct absorbance of UV light (photolysis). (Derbalah et al., 2004a). While the degradation of methomyl under H\textsubscript{2}O\textsubscript{2}/UV system due to firstly, the direct photolysis and secondly due to the generation of hydroxyl radicals from hydrogen peroxide equation (1) (Benitez et al., 2002; Derbalah et al., 2004a; Derbalah, 2009).

\[
\text{H}_{2}\text{O}_{2} + \text{hv} \rightarrow 2 \cdot \text{OH} 
\]

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

\[
\text{Fe(OH)}^{2+} + \text{hv} \rightarrow \text{Fe}^{3+} + \cdot \text{OH} 
\]

The degradation of methomyl under Fe\textsuperscript{3+}/H\textsubscript{2}O\textsubscript{2} system is due to the generation of hydroxyl radicals under this system in the absence of the light as shown in equation 3

\[
\text{Fe}^{2+} + \text{H}_{2}\text{O}_{2} \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \cdot \text{OH} 
\]

The degradation rate of methomyl under H\textsubscript{2}O\textsubscript{2}/UV system was slightly slower than its degradation under Fe\textsuperscript{3+}/UV system which may be due to the lower formation rate of hydroxyl radicals under H\textsubscript{2}O\textsubscript{2}/UV system relative to Fe\textsuperscript{3+}/UV system (Derbalah et al., 2004a). This low generation rate of hydroxyl radicals under H\textsubscript{2}O\textsubscript{2}/UV system compared to Fe\textsuperscript{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\textsuperscript{3+}/UV system the Fe(OH)\textsuperscript{2+} complex is the predominant species for generating hydroxyl radicals absorbed light at wavelengths up to 410 which may lead to the generation...
Figure 4. Sections from liver of rats after 21 days of treatment with methomyl at dose level 10 mg/kg (B and C) relative to control (A).

Figure 5. Degradation of methomyl at initial concentration of 10 mg/L under Fe$^{3+}$/H$_2$O$_2$/UV, H$_2$O$_2$/UV, Fe$^{3+}$/UV system and Fe$^{3+}$/H$_2$O$_2$ in distilled water. H$_2$O$_2$=20mg/l, FeCl$_3$=1mmol, pH=2.8.
of hydroxyl radicals under this system more than 
\( \text{H}_2\text{O}_2/\text{UV} \) system (Derbalah et al., 2004a).

The great enhancement in tested pesticide degradation rate under photo-Fenton’s like reagent system (\( \text{Fe}^{3+}/\text{H}_2\text{O}_2/\text{UV} \)) relative to the other advanced oxidation processes is due to the higher generation rate of hydroxyl radicals under this system (\( \text{Fe}^{3+}/\text{H}_2\text{O}_2/\text{UV} \)) than the other systems (Derbalah et al., 2004a; 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 radical’s Equation (1) (Benitez et al., 2002). Secondly, the photolysis of \( \text{Fe} (\text{OH})^{2+} \) complex the predominant specie of \( \text{Fe}^{3+} \) for generating hydroxyl radicals Equation (2) (Larson et al., 1991).

Thirdly, the photodecarboxylation of ferric carboxylate complexes generate ferrous ion (Equation 3), which can in turn react with hydrogen peroxide to generate additional hydroxyl radicals (Equation 4) (Pignatello and Sun, 1995).

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{hv} & \rightarrow 2 \cdot \text{OH} \\
\text{Fe(OH)}^{2+} + \text{hv} & \rightarrow \text{Fe}^{2+} + \cdot \text{OH} \\
\text{Fe}^{3+} (\text{RCO}_2)^{2+} + \text{hv} & \rightarrow \text{Fe}^{2+} + \text{CO}_2 + \text{R} \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^{-}
\end{align*}
\]

The degradation of methomyl and other carbamate insecticides have been reported before (Benitez et al., 2002; Oller et al., 2007; Tamimi et al., 2008; Derbalah, 2009).

**Bioremediation of the tested insecticide in aqueous solution**

The ability of the selected microbial isolate *Pseudomonas* sp (EB20) for the biodegradation of the tested pesticide was illustrated in Figure 6. The results in Figure 6 indicated that, *Pseudomonas* sp (EB20) showed high potential in the degradation of the tested insecticide. Since around 77% of methomyl of initial concentration level (10 ppm) was degraded within two weeks of incubation with *Pseudomonas* sp (EB20). On the other hand, the tested pesticide degradation percentages reached to 6.6% 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, photodecomposition and volatilization is very slight or negligible. The degradation of methomyl may be attributed to the secretion of enzymes which are capable of degrading pesticides (Bollag and Liu, 1990). The genus *Pseudomonas* showed the highest rate of methomyl degradation and has considerable potential for the biotransformation and biodegradation of acetylcholinesterase inhibitors insecticides with widely differing chemical structures (Massoud et al., 2008; Derbalah et al., 2008).

**Conclusions**

Methomyl induced toxicological effects in treated rats relative to control with the respect to enzymes activity and histological changes in treated rats’ organs. More extensive studies are needed to evaluate the toxicity of methomyl at concentration level more close the
environmental level which in return helps to evaluate its toxicity under real environmental conditions. Photo-Fenton like reagent was the most effective treatment for the removal of methomyl residues in aquatic system and may be preferable in wastewater treatment. *Pseudomonas* sp. (EB20) could be regarded as a safe removal treatment of methomyl in drinking water.

**REFERENCES**


