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Research Article
Toxicity Assessment of Chlorpyrifos, Malachite Green and Tetracyclines by Microtox® Assay: Detoxification by Ultrasonic

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Abstract

Background and Objective: Organophosphorus insecticides (OPIs), biocides and bactericides (antimicrobial) residues can accumulate in plant, fish, water, milk, animals and cause adverse health effects. Therefore, new and fast tools for detection and removal OPIs such as chlorpyrifos (CPF) and other xenobiotics are very important. This study was carried out for the first time to evaluate the toxicity of CPF, Malachite Green (MG), leucomalachite green (LMG) and tetracyclines (TC) using Microtox® assay and to remove CPF residues from apple juice by ultrasonic. **Methodology:** Acute toxicity of standard CPF, CPF residues and removal of residues from apple juice by ultrasonic were studied. Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), doxycycline (DC), Malachite Green (MG) and leucomalachite green (LMG) were evaluated by Microtox®. **Results:** CPF and CPF residues are very toxic with $EC_{50,\ 5\ min,\ 15\ min}$ = 30.39 and 54.08 μg L^{-1} of CPF and 22.35 and 49.855 μg L^{-1} of CPF residues, respectively. CPF can be effectively and rapidly degraded by ultrasonic and the half-life time account 11.44 min with strongly correlated by sonication times and power. MG is very toxic with $EC_{50,\ 5\ min,\ 15\ min}$ = 365.8 and 192.56 μg L^{-1}. CTC and DC are toxic with $EC_{50,\ 5\ min,\ 15\ min}$ = 9.664 and 4.628 mg L^{-1} of CTC and 19.888 and 5.208 mg L^{-1} of DC, respectively, while, OTC and LMG are harmful based on the toxicity categories established in the EU legislation. **Conclusion:** It is concluded that toxicity of CPF residues and their intermediate products can determined by Microtox® assay. CPF was effectively and rapidly degraded by ultrasonic. CPF, MG and TCTs act as toxic compounds in the environment at low concentration levels since they were detected in the environment at these low concentrations by Microtox® assay.

Key words: Detoxification, Microtox®, chlorpyrifos, ultrasonic, malachite green, tetracyclines

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Toxicity assessment of pesticides on human can be measured based on the epidemiological studies or estimated from the results of experimental animals. In addition, the toxicity of synthetic pesticides to aquatic organisms is an important marker for evaluating the potential adverse effects on environmental and ecosystem[1]. Organophosphorus insecticides such as chlorpyrifos (CPF), O, O-diethylO-3, 5, 6-trichloro-2-pyridyl phosphorothioate, became one of the largest selling OPIs in the world and had both agricultural and urban uses[2]. CPF is non-polar natural; therefore, it can accumulate in the organic phase in environmental[3]. It persists approximately 120 days in soil and degrades to 3, 5, 6-trichloro-2-pyridinol which broken down to organochlorine compounds and carbon dioxide[4]. However, worldwide the accumulation of xenobiotics such as CPF in an ecosystem has become a serious public health concern. Moreover, CPF and other pesticides can induce hepatic, renal, reproductive and genotoxicity to experimental animals[5-9] and adverse effects to agriculture workers[10].

Currently, some techniques for determination and removal of OPIs residues were used and development. These techniques such as physical methods e.g., filtration, activated carbon adsorption and chemical methods e.g., ozonation, aqueous chlorine and Fenton treatment are common for removing OPIs residues from water, vegetables and foods. It has been reported that ultrasonic treatment was active for OPIs elimination from aqueous solution[11]. Moreover, previous studies generally paid attention to removal parent OPIs compounds by ultrasonic, while few studies talked the intermediate products and the assessment of toxicity after ultrasonic treatment.

Malachite Green (MG) is a synthetic compound commonly used as a dyestuff to color fabric and paper. It has antimicrobial activity and used as biocide to control protozoal and fungal in aquaculture worldwide. It works as an ectoparasiticide and has high activity against fungal and protozoal infections of fish and fish eggs[12]. It has been reported that MG residues were found in fish and transfer to human consumption. Therefore, it has become a greatly restricts compound due to the toxic effect and their potential risk effects of consumers untreated fish[13]. Previous studies reported that MG has toxic effects on mammals and other microorganisms. It caused disruption in immune and reproductive systems and has genotoxic and carcinogenic properties[14]. It was stopped for using as a dye in numerous countries. US Food and Drug Administration[15] stopped it for using in food as an additive. In contrast, due to the effectiveness, cheap cost and availability of MG, it is still widely used as biocide in different parts worldwide, especially in developing countries. It has been reported that MG has selected as a significant chemical for carcinogenicity testing. There are interesting and concern about the residues and the fate of MG and its reduced form (leucomalachite green, LMG) in aquatic and ecosystems, due to the toxic effect and potential risk hazards to human health and ecosystems.

In fact, bactericide and other antibiotics are playing an important role in controlling many diseases in agriculture, veterinary and aquaculture sectors. The tetracyclines (TCs) group, such as chlortetracycline (CTC), oxytetracycline (OTC) and doxicycline (DOC), is the most important antibacterial used. They are the efficiency, available and cheapest class of antibiotic worldwide[16]. Both oxytetracycline (OTC) and doxicycline (DOC) are widely used due to the highest activity as antibacterial to control diseases in fish. It can be used at a dose of 75 mg kg^{-1} b.wt., daily for 4-10 days. OTC/DOC is widely used for control diseases in fish by adding to the diet of fish[17]. In addition, treatment of fish through injection, dipping and eating by some antibacterial e.g., the fluorescent OTC for marking are considered as an essential method in fisheries investigation and management. Previous studies reported that these treatments lead to accumulate the antibacterial OTC residues of OTC in farmed fish at a level more than the accepted tolerance (2.0 μg g^{-1}) of OTC[18-20]. Also, OTC was found in the sediments of the treated fish farm due to the highly persistent in sediments[20]. However, Bjorklund *et al.*[21] stated that antibacterial TCs residues in animal tissues have thermal stability. It has been reported that TCs residues and other antibiotics were destroyed by heat treatment[22,23].

Regarding the present of CPF, MG and antibiotics (e.g., TCs, OTC, DOC and CTC) residues in milk, juice, food, fish, sediment and aquaculture, the research for new methods and fast techniques for detection and evaluate the toxic effect of these compounds are important issues. Currently, the new technique for study acute toxicity (Microtox acute toxicity test) by using the marine luminescent *Vibrio fischeri* bacteria has been reported and widely used to determent the toxic effect of several compounds[24]. In this method, the decreases in light emission of luminescent bacteria after exposure to toxic chemicals can measure by Microtox® Analyzer.

The method and technique used by the Microtox® Toxicity Analyzer had been previously described and standardized (ISO, 2009)[25]. The Microtox® test is considered relatively inexpensive, offers a fast testing procedure and the results obtained with the Microtox® test correspond well with others standard toxicity tests[26,27].

Pengphol *et al.*[11] generally paid attention to removal parent OPIs compounds by ultrasonic, while assessment of toxicity of the intermediate products by Microtox has not yet been studied. The current study was designed to evaluate the toxicity of chlorpyrifos, malachite green, leucomalachite green and tetracyclines by Microtox® assay. Effect of ultrasonic on detoxification of chlorpyrifos in apple juice was also evaluated.

MATERIALS AND METHODS

Chemicals: The following chemicals, analytical standard grade, were selected for this study: chlorpyrifos (97%) was obtained from TaeGeuk Cop., South Korea and Pestban® 48% EC from Agrochem, Alwatneia Co., Alex., Egypt. Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), doxycycline (DC), Malachite Green (MG) and leucomalachite green (LMG) were purchased from Sigma-Aldrich (Louis Mo, USA).

Microtox® reagents and supplies: Microtox® supplies, including cuvettes, freeze-dried luminescent bacteria, *Vibrio fischeri* (14G4106A), diluent solution (2% NaCl, 14C4039), Osmotic Adjusting Solution (OAS, 20% NaCl, 14F4091) and reconstitution solution (13F4065), were supplied by Modern Water Inc., New Castle, DE 19720, USA. The acute toxicity tests were done by the Microtox® Model 500 Toxicity Analyzer from Modern Water Inc (Pesticide Chemistry Department, National Research Centre, Egypt). Microtox testing was performed according to the standard procedure recommended by the manufacturer.

Luminescent bacteria assay: Toxicity of testing compounds was evaluated using the bioassay based on the inhibition of the luminescence emitted by the bacteria *V. fischeri*. The mechanism of toxicity and inhibition in light emitted from bacteria is a result of the interaction of the enzyme luciferase, reduced flavin and a long-chain aldehyde in the presence of oxygen. The metabolic energy produced in this pathway changes to chemical energy, through the electron transport system, into visible light. This metabolic pathway is intrinsically linked to cellular respiration, so disruption of normal cellular metabolism causes a decrease in light production[28].

The acute toxicity endpoint was determined for 5 and 15 min as the effective concentration (EC_{50}) of a chemical that causes a 50% of the reduction in the luminescence of the bacteria. The EC_{50} values were obtained by following the Microtox® basic test protocol. In addition, EC_{50} values were expressed as mg L^{-1} or µg L^{-1} and µM. The acute toxicity studies have been carried out in Pesticide Chemistry Department, Chemical Industries Research Division, National Research Centre, Egypt, at 2016 and 2017 according to standard method[29] and good practice.

Preliminary experiments were carried out to find the suitable concentration to determine the EC_{50} values for each compound. Then, the concentrations of testing compounds were 0.1 mg L^{-1} of CPF, 40 mg L^{-1} of TC, CTC and DC, 100 mg L^{-1} of OTC, 0.4 mg L^{-1} of MG and 160 mg L^{-1} of LMG, respectively. The solutions were freshly prepared and used immediately. According to Commission of the European Communities[30], the toxicity categories based on the EC_{50} values were classified into "very toxic to aquatic organisms" ($EC_{50} \leq 1$ mg L^{-1}), "toxic" (EC_{50} in the range of 1-10 mg L^{-1}) and "harmful" (EC_{50} in the range of 10-100 mg L^{-1}), which are established in legislation (Directive 93/67/EEC). These categories are applied in this study to classify the target compounds[30]. However, methanol used as the solvent for test solution at percentage 2%.

Determination of CPF residues: Chlorpyrifos (Pestban® 48% EC) was added to apple juice samples at concentration 0.10 mg L^{-1}. Then, juice samples were mixed and divided into two sub-samples. The first sub-samples were used for determination CPF residues by Microtox. The second sub-samples were used for studding the degradation effect by ultrasonic. In the same time, blank sample was used as a control.

Degradation of CPF by ultrasonic: Ultrasonic was carried out using Ultrasonic (Sonics and Materials, INC. 53 Church Hill RD. Newtown, CT USA) with a probe diameter of 13 mm at a high frequency of 20 kHz and power output of 750 W with different sonication time 1, 5, 10, 15, 20 and 25 min. To reduce energy, ice was used for cooling during the sonication process and energy was given through Sonicator probe.

Sample extraction and preparation: Apple juice samples (100 mL) were extracted three times by using dichloromethane (1:1 v/v). The combined extracts were dried by anhydrous sodium sulphate (10 g) and evaporated under vacuum at 35°C in a rotary evaporator to dryness. Then, the residue was re-dissolved in methanol and made up to 2 mL with deionized water. Methanol used in all measurement not exceeded 2% of solution.

Toxicity of CPF before and after sonication: The reduction in CPF toxicity after sonication was determined by compared the toxicity before and after sonication time. The reduction in toxicity of CPF was calculated by the Eq.1[31]:

$$\text{Toxicity reduction (TR \%)} = \frac{EC_{50A} - EC_{50B}}{100 - EC_{50B}} \quad (1)$$

where, EC_{50A} and EC_{50B} are the EC_{50} value of toxicity after and before sonication.

Half-life time of chlorpyrifos ($T_{1/2}$): The half-life time of chlorpyrifos in apple juice after different sonication time could be calculated from degradation curve by the Eq. 2[32]:

$$\text{Half life time } (T_{1/2}) = \frac{\text{Ln } 2}{k} = \frac{0.693}{k} \quad (2)$$

The reaction constant rat (K) was obtained from semilogarithmic linear regression line by the Eq. 3 and 4[32]:

$$R(t) = R(0) \exp(k.t) \quad (3)$$

$$\ln R(t) = \ln R(O) + k.t \quad (4)$$

where, R(t) is the CPF residue (μg L^{-1}) at time t, R(0) is the initial amount of CPF (t = 0), k is the reaction rate constant (decay constant) and *t* is the time (min) and $t_{1/2}$ is the half-life.

Quality control: The effect of storage time on the quality and activity of bacteria *Vibrio fischeri* was tested. Phenol was used as standard and tested in the Microtox assay to confirm the sensitivity of bacteria. The obtained data were compared with the data in Microtox quality assurance guidelines[25].

RESULTS AND DISCUSSION

Results of acute toxicity of testing compounds by using *Vibrio fischeri* are shown in the Table 1-6 and Fig. 1-4. The EC_{50} values in mg L^{-1}, μg L^{-1} or μM were calculated and the categories of toxicity classification according to the CEU legislation (Directive 93/67/EEC) are shown[30].

Quality control: The activity of bacteria *Vibrio fischeri* was determined using phenol at a concentration of 100 mg L^{-1} as standard. These results showed that phenol EC_{50} at 5 min was 20.31 mg L^{-1}. According to Azur Environmental[29], a good bacterium *V. fischeri* has EC_{50} value between 13-26 mg L^{-1} at 5 min of phenol. Therefore, the bacteria *V. fischeri* used in this study was active and the EC_{50} at 5 min of phenol was 20.31 mg L^{-1}.

Table 1: EC_{50} values and toxicity classification of chlorpyrifos and their residues in apple juice by Microtox

	EC_{50}						
	Light Intensity (%) and (95% confidence limits)		Equivalent values (μg L^{-1})		Slope		#Toxicity classification (% bioluminescence
Compounds	5 min	15 min	5 min	15 min	5 min	15 min	inhibition)
CPF (Standard)	0.3039 (0.1331-0.6941)	0.5408 (0.2077-1.408)	30.39	54.08	0.7581	0.7376	Very toxic
CPF (Residues)	0.1490% (0.0697- 0.3184)	0.3657 (0.0483-2.764)	22.35	49.855	0.6634	0.6443	Very toxic

#Toxicity categories based on the EC_{50} toxicity endpoint: ≤1 mg L^{-1} Very toxic to aquatic organisms, 1-10 mg L^{-1} Toxic, 10-100 mg L^{-1} Harmful

Table 2: Chemical structure and toxicity of chlorpyrifos ($EC_{50, 15\ min}$, μM)

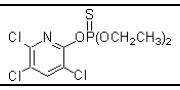
Compound	Chemical structure	$EC_{50, 15\ min}$ (μg L^{-1})	Molecular weight (g)	$EC_{50, 15\ min}$ (μM)
Chlorpyrifos		54.08	350.6	0.154

Table 3: EC_{50} values and toxicity classification of malachite green and leucomalachite green by Microtox

	EC_{50}						
	Light Intensity (%) and (95% confidence limits)		Equivalent values (mg L^{-1})		Slope		#Toxicity classification (% bioluminescence
Compounds	5 min	15 min	5 min	15 min	5 min	15 min	inhibition)
MG	9.145 (4.822-17.34)	4.814 (0.2738-84.65)	0.3658	0.19256	1.773	2.565	Very toxic
LMG	19.43 (nd)	14.43 (6.107-34.09)	31.088	23.088	4.595	2.384	Harmful

MG: Malachite green, LMG: Leucomalachite green, #Toxicity categories based on the EC_{50} toxicity endpoint: ≤1 mg L^{-1} Very toxic to aquatic organisms, 1-10 mg L^{-1} Toxic, 10-100 mg L^{-1} Harmful, nd: No date

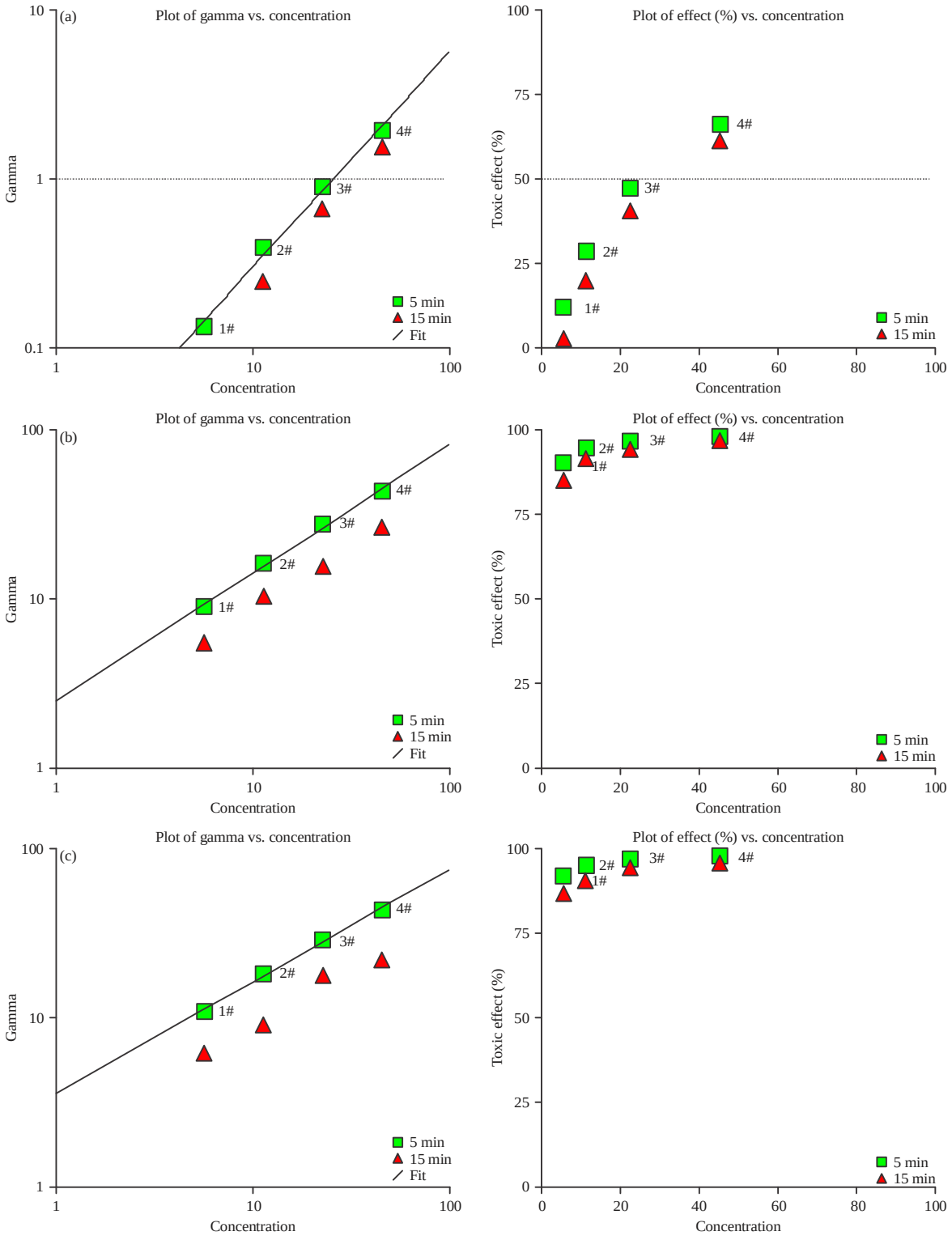


Fig. 1(a-c): Chart of toxicity of (a) Phenol (mg L^{-1}), (b) Chlorpyrifos standard (µg L^{-1}) and (c) Chlorpyrifos (CPF)-residues (µg L^{-1}) by Microtox at 5 and 15 min

Toxicity of CPF: As shown in Table 1 and 2, the $EC_{50,\ 5\ and\ 15\ min}$ values for CPF were 30.39 and 54.08 µg L^{-1} while at 15 min; the EC_{50} was 0.154 µM based on CPF molecular weight. These results confirmed that CPF is very toxic to *V. fischeri*, according

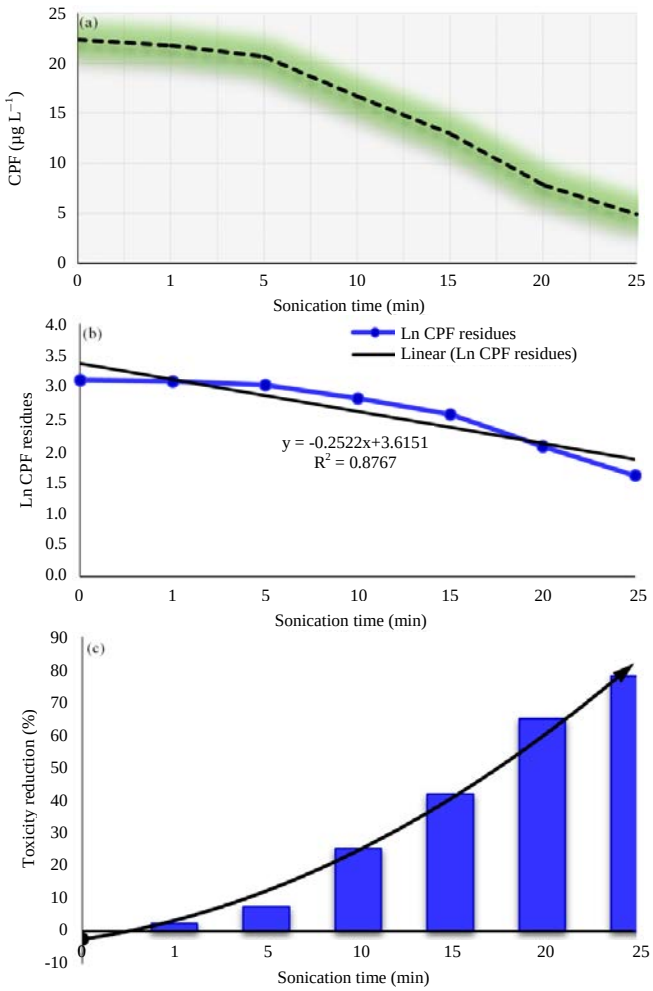
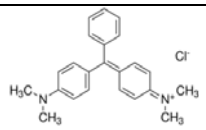
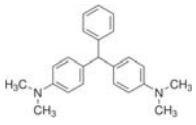


Fig. 2(a-c): (a) Degradation curve of chlorpyrifos residues in apple juice under different sonication time, (b) Linear regression line and (c) Toxicity reduction to marine luminescent *Vibrio fischeri* bacteria after 15 min

Table 4: Relationship between chemical structure and toxicity ($EC_{50, 15\ min}$, µM) of MG and LMG agents

Compounds	Chemical structures	$EC_{50, 15\ min}$ (mg L^{-1})	#Relative toxicity to MG	Molecular weight (g)	$EC_{50, 15\ min}$ (µM)	#Relative toxicity to MG
MG		0.19256	1.00	364.911	0.528	1.00
LMG		23.088	119.90	330.466	69.865	132.32

MG: Malachite green, LMG: Leucomalachite green, #Relative toxicity to MG: $EC_{50, 15\ min}$ of LMG/$EC_{50, 15\ min}$ of MG, values <1 have high toxicity and >1 have low toxicity

to the EU legislation (Directive 93/67/EEC) classification of toxicity[30]. In contrast, toxicity of CPF residue "commercial formulation" was increased and the $EC_{50, 5\ and\ 15\ min}$ values were 22.35 µg L^{-1} and 49.855 µg L^{-1} at 5 and 15 min. The high toxicity of CPF commercial formulation compared to CPF "active ingredient" could be due to the effect of solvent and emulsifying agents, which used in formulation.

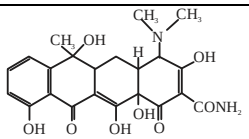
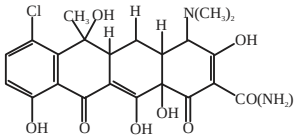
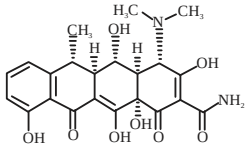
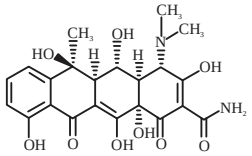
Previous studies generally paid attention to removal parent OPIs compounds by ultrasonic[12]. While assessment of toxicity of the intermediate products by Microtox not studies

Table 5: EC_{50} values and toxicity classification of some antibiotic (tetracycline, chlortetracycline, oxytetracycline and doxycycline) by Microtox

	EC_{50}						
	Light Intensity (%) and (95% confidence limits)		Equivalent values (mg L^{-1})		Slope		#Toxicity classification (% bioluminescence
Compounds	5 min	15 min	5 min	15 min	5 min	15 min	inhibition)
TC	104.7 (13.77-795.9)	126.0 (47.00 -337.7)	41.880	50.4	1.002	0.8932	Harmful
CTC	24.16 (15.20 -38.41)	11.57 (6.191- 21.60)	9.664	4.628	1.472	2.421	Toxic
DC	49.72 (9.115- 271.2)	13.02 (10.74-15.78)	19.888	5.208	1.551	2.227	Toxic
OTC	79.37 (2.805- 2245)	nd	79.370	nd	0.8482	nd	Harmful

TC: Tetracycline, CTC: Chlortetracycline, DC: Doxycycline, OTC: Oxytetracycline, #Toxicity categories based on the EC_{50} toxicity endpoint: $\leq$1 mg L^{-1} Very toxic to aquatic organisms, 1-10 mg L^{-1} Toxic, 10-100 mg L^{-1} Harmful, nd: Not detected

Table 6: Relationship between chemical structure and toxicity ($EC_{50, 15 min}$, µM) of the tested antibiotic agents

Compounds	Chemical structures	$EC_{50, 15min}$ (mg L^{-1})	#Relative toxicity to TC	Molecular weight (g)	$EC_{50, 15min}$ (µM)	#Relative toxicity to TC
TC		50.4	1.000	444.43	113.403	1
CTC		4.628	0.0918	478.88	9.664	0.085
DC		5.208	0.1033	444.43	11.718	0.1033
OTC		nd	nd	460.43	nd	nd

TC: Tetracycline, CTC: Chlortetracycline, DC: Doxycycline, OTC: Oxytetracycline, #Relative toxicity to TC = $EC_{50, 15 min}$ of each compound/$EC_{50, 15 min}$ of TC, values less than "1" have high toxicity and more than "1" have low toxicity, nd: Not detected

until now. CPF residues were determined in apple juice and subjected to sonication to remove these residues. Toxicity reduction (TR %) of CPF were account 2.4, 7.5, 25.3, 42, 65 and 78% after sonication time 5, 10, 15, 20 and 25 min. Results showed that CPF can be effectively and rapidly degraded by ultrasonic and the half-life time account 11.44 min with strongly correlated by sonication times and power (Fig. 2a-c). This finding suggested that CPF residues and their intermediate products could be an assessment by Microtox® assay.

Previous studies showed that CPF has high toxicity to Daphnia with LC_{50} 1.7 µg L^{-1} [33]. However, it has been reported that methanol is the most common solvent used for test solution[34]. It has EC_{50} value approximately 25 mg mL^{-1} [35]. The percentage of solvent used in stock solution was studied by several authors and accounted to 4, 5, 8 and 10%[27,26,36,37], respectively. Therefore, this personage must not be more than 10% of chemical solutions.

These results revealed that CPF induced complete toxic effect after 5 min exposure and the $EC_{50, 5 min}$ value was 30.39, while low toxic effect with high EC_{50} = 54.08 µg L^{-1} after 15 min exposure time was recorded. However, other pesticides such as malathion and bentazon recorded the complete toxic effects after 5 min exposure[38]. In contrast, the toxicity of some pesticides to the bacteria is increased with increasing exposure time for example, diazinon, carbofuran, diquat, chlormequat, paraquat, difenzoquat, tetrachlorvinphos and propanil. These pesticides have higher an EC_{50} value at

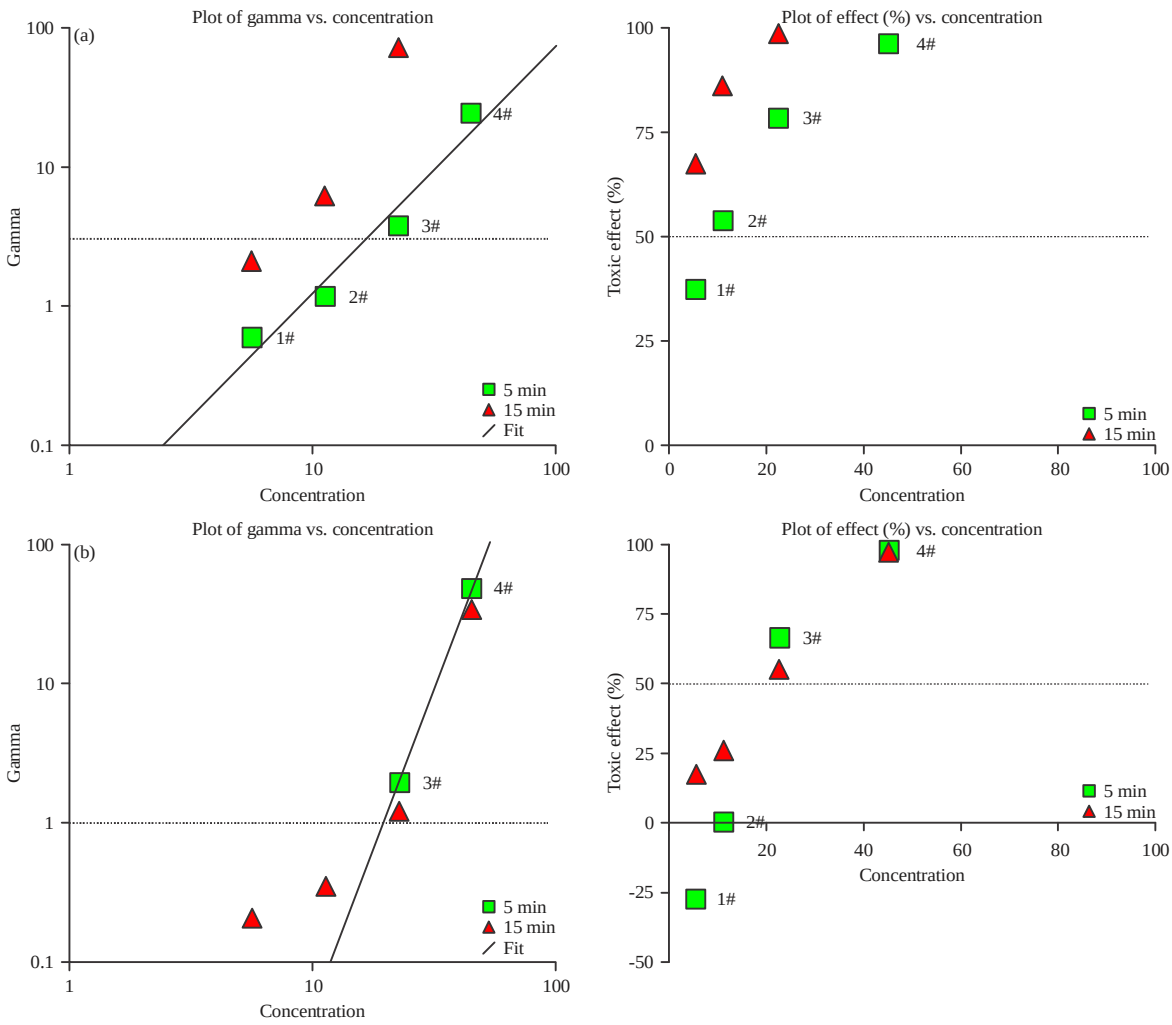


Fig. 3(a-b): Chart of toxicity for (a) Malachite green and (b) Leucomalachite green (mg L^{-1}) determined by Microtox at 5 and 15 min

5 min than the EC_{50} value at 15 min. This effect may be due to the toxic effect of these pesticides on bacteria by different ways with increasing the exposure time. Other pesticides are very toxic to *V. fischeri* such as thiobencarb, which have EC_{50} value of 30 µg L^{-1}. However, other studies reported different results of CPF than that obtained in our study. In addition, other pesticides showed different toxicity using the Microtox. This difference may be due to the purity of testing pesticide, the type of pesticide formulations, a solvent used for preparation stock solution and variations in the cell suspension[38-40].

Toxicity of MG and LMG: The EC_{50} values (mg L^{-1}) and the categories of toxicity classification according to the EU legislation (Directive 93/67/EEC) of MG and LMG are shown in Table 3. Malachite Green (MG) showed the highest toxic (Very toxic) effect and it increased throughout the time of exposition, with EC_{50} values of 0.3658 mg L^{-1} (5 min) to 0.19256 mg L^{-1} (15 min). In the contrast, leucomalachite green (LMG) showed a low toxic effect (harmful) than MG with EC_{50} values of 31.088 mg L^{-1} (5 min) to 23.088 mg L^{-1} (15 min). The EC_{50} values found in the current study for MG and LMG showed that MG compound has a highly toxic effect on *Vibrio fischeri*. Based on molecular weight, the $EC_{50,\ 15\ min}$ of MG is 0.528 µM compared to 69.865 of LMG (Table 4, Fig. 3). However, the toxicity of MG and LMG has been confirmed to *V. fischeri*[41]. They reported that MG and LMG have $EC_{50,\ 30\ min}$ = 0.031 mg L L^{-1} and $\geq$39.9 mg L L^{-1}, respectively. Malachite green was classified as "very toxic to aquatic organisms". These results showed the relation between chemical structures of MG and LMG and their toxic effect on *V. fischeri*. The MG can convert by reduction possess to obtain

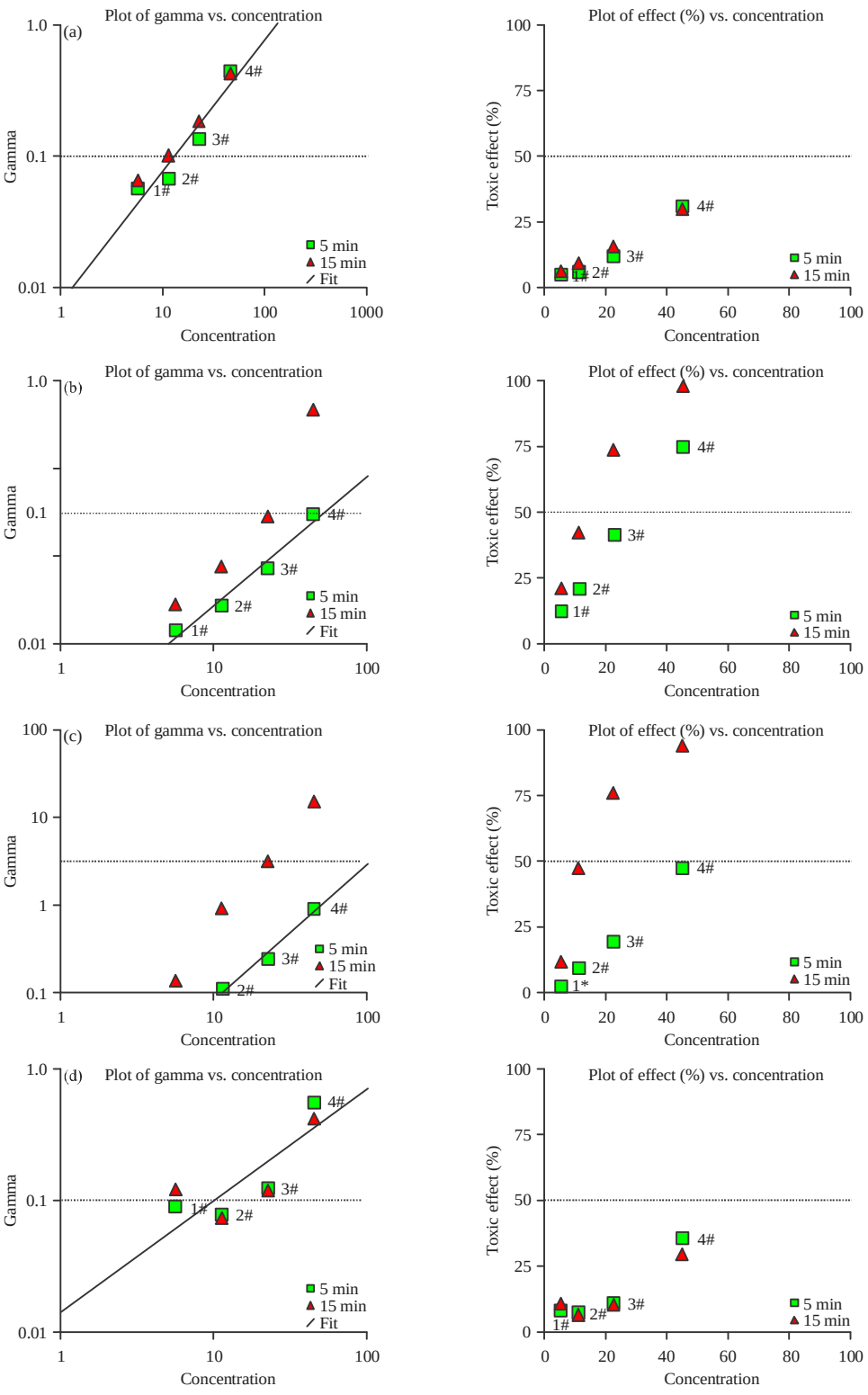


Fig. 4(a-d): Chart of toxicity for some antibiotic, (a) Tetracycline, (b) Chlortetracycline, (c) Doxycycline and (d) Oxytetracycline determined by Microtox at 5 and 15 min

leucomalachite green (LMG) which more persistence and low toxic (119 and 132 fold)than MG on *V. fischeri*. In contrast, LMG is structurally similar to the leucoforms of other carcinogenic triphenylmethane dyes[42].

Toxicity of antimicrobial compounds: Toxic effects were observed in the case of chlortetracycline (CTC) and doxycycline (DC) (Table 5, Fig. 4). Both CTC and DC have toxic effects and they increased throughout the time of exposition, with EC_{50} values of 9.664 and 19.888 mg L^{-1} (5 min) to 4.628 and 5.208 mg L^{-1} (15 min), respectively. In contrast, tetracycline (TC) showed a low toxic effect (harmful) compared to CTC and DC, with EC_{50} values of 41.88 mg L^{-1} (5 min) which increased throughout the time of exposition to 50.4 mg L^{-1} (15 min). Based on molecular weight, the $EC_{50, 15\,min}$ of TC, CTC, DC accounted 113.403, 9.664 and 11.718 µM, respectively. The high toxic effect of CTC may be due to the chemical structure, which contains halogen atom "Cl" compared to TC and DC.

Bactericides or antimicrobial compounds are executively used in farms to control bacteria diseases in both agriculture and veterinary sectors. For example, Boatman[43] reported that approximately 2500 t of TCs were used annually as veterinary drugs in Europe and TC, CTC and OTC as the greatest practical ones. The wastes or residues of these compounds will find their way to the agricultural field, water, milk and the environment. TCs has toxic effects on both aerobic sludge bacteria and microalgae sludge compared to other antimicrobial compounds[44,45]. Pesticides, antimicrobials and other veterinary drugs administered by oral route are lower absorbed and emitted with animal faeces. About 70-80% of antibacterial drugs used in feed pellets of animals are accumulated in the environment[46]. Animal wastes (faeces) consider as a good source of plant nutrients (fertilizer) in agriculture farmers. This organic matter (fertilizer) is suitable for enhanced soil building and source of fiber, minerals and non-protein nitrogen[47]. Currently, aquafarming e.g., fish farming is used animals, pottery wastes and fish processing wastes as a source of proteins and amino acids[48]. Therefore, new methods are very important for detection pesticides, MG and TCs residues in agriculture, aquaculture and eco-system. Our results revealed that the method of toxicity determination by using Microtox® is a new, standardized, fast, relatively inexpensive and a good tool for toxicity evaluation of environmental pollutants such as pesticides, biocide and antimicrobials in agriculture, aquaculture and veterinary sectors.

Available findings have been confirmed the results of the present study[31,41,43,49-52]. For example, Jones and Huang[31], reported that CPF induced complete effect after 5 min exposure and the $EC_{50, 5\,min}$ value was 25.06% (210 µg L^{-1}) of initial concentration 0.84 mg L^{-1}, while low toxic effect with high EC_{50} = 31.57% (265.18 µg L^{-1}) after 15 min exposure time was recorded. In contrast, other studies reported low toxic effect of CPF with EC_{50} = 2.84 mg L^{-1} after 30 min[53]. In addition, results of malachite green and antibiotics are confirmed by the results of other studies[41,54].

CONCLUSION

It can be concluded that Microtox® assay can be used to determine CPF and their intermediate products residues in apple juice. CPF can be effectively and rapidly degraded by ultrasonic. Microtox® assay can be used to determine CPF, MG and TCTs in the environment at low concentration levels. It assay can be used to determine the overall toxicity of pesticides residues and their metabolites in fruits, vegetables, fish, water, juice, milk and food products.

SIGNIFICANCE STATEMENTS

In the present study, ultrasonic was used as a new method for detoxification of insecticides. It can be beneficial for removal pesticides residues from food and water. As well, to evaluate the overall toxicity of chlorpyrifos, malachite green, leucomalachite green and tetracyclines using Microtox® assay. This study will help the researcher to uncover the critical areas of pesticides residues and detoxification that many researchers were not able to explore. Ultrasonic and Microtox can be used for detoxification and determination the overall toxicity of xenobiotic such as pesticides residues and their metabolites in fruits, vegetables, fish, water, juice, milk and food products. Thus, a new method on toxicity evaluation and detoxification may enhance the removal of pesticides and evaluate the overall toxicity in foods and water.

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