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Removal of chlorpyrifos using micelle clay complex and advanced treatment technology

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Received 12 June 2015; Accepted 21 August 2015

ABSTRACT

In this study, the stability of chlorpyrifos, a known pesticide, in fresh water and sludge was investigated. The results showed that chlorpyrifos underwent hydrolysis in both media to give two main metabolites: 3,5,6-trichloro-2-pyridinylol (TCP) and diethylthiophosphoric acid (DETP). The hydrolysis reaction in both media was found to follow first-order kinetics with a rate constant of 7.10×10^{-8} ($R^2 = 0.999$) in pure water and of 9.30×10^{-8} ($R^2 = 0.996$) in sludge. Chlorpyrifos degradation's half-life value in pure water was 112 d and in sludge was 85.9 d. The efficiency of Al-Quds University wastewater treatment plant demonstrated that the ultrafiltration-hollow fiber unit (UF-HF) was insufficient in removing chlorpyrifos from spiked secondary treated wastewater samples, whereas the combination of ultrafiltration-spiral wound unit (UF-SW) followed by activated carbon (AC) column was quite efficient and yielded a complete removal of the pesticide. Batch adsorption experiments using either AC or micelle-clay complex were performed and the experimental results were fitted to Langmuir and Freundlich isotherms. Adsorption analysis using Langmuir isotherm revealed that Q_{\max} for the AC was higher than that of the micelle-clay complex, while Freundlich isotherm showed almost similar values of n (3.413 vs. 3.745) for both adsorbents, despite the fact that the activated carbon filter showed higher k_F value. Filtration column of mixed micelle-clay complex and sand demonstrated a breakthrough point after the third fraction for chlorpyrifos removal and a complete removal for all fractions collected in the TCP experiment. This result reveals that mixed micelle-clay complex and sand column is much more efficient in removing TCP than removing its parent compound, chlorpyrifos.

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Presented at the 3rd International Conference on Water, Energy and Environment (ICWEE)
24–26 March 2015, Sharjah, United Arab Emirates

Keywords: Chlorpyrifos; Micelle clay complex; Pesticides; Pollutants removal; Activated sludge; Wastewater; Activated carbon

1. Introduction

Pesticides have been widely and increasingly used to control agricultural pests such as weeds, molds, rodents, and plant pathogens [1]. Pesticides are intensively used due to the high nutritional demand by the world's population which significantly increases year by year [2]. Pesticides are applied directly to soil, weeds, and plants. The fate of pesticides after application can follow several pathways: (i) a direct accumulation in the upper layer of soil, where they may undergo adsorption and deposition; (ii) degradation both on soil and plant surfaces; (iii) translocation into plant organs; and (iv) dissolution/transport from the plants and soil surfaces into water run-off with the consequent contamination of water resources [2–4]. There is a great public concern on the environmental fate of pesticides because the excessive application of pesticides has led to widespread contamination of soil, air, and water causing adverse effects on the environment and ecology [5–7]. Existing wastewater treatment plants (WWTPs) are often inefficient in removing these pollutants from wastewater. This leads to accumulation of toxic substances in the water body, thus causing human health risks and damage to the ecosystem [8]. Research in developed and developing countries are designed to find inexpensive and simple technologies for treating domestic and agricultural wastewater contaminated with pesticides to protect human health and the environment from adverse side effects caused by these substances and their derivatives [9,10].

Chlorpyrifos, (O,O-diethyl-O-3,5,6-trichloropyridin-2-phosphorothioate), 1, illustrated in Fig. 1, is an organophosphorous insecticide. It is widely used in domestic and agricultural practices, as insect control on both vegetable crops and ornamental plants [1,11,12]. The mode of action of chlorpyrifos involves the inhibition of acetyl cholinesterase (AChE) enzyme, thus causing to an accumulation of acetylcholine, a neurotoxic substance, which subsequently results in the death of the target organism [13,14]. The effectiveness and low cost of chlorpyrifos render it as one of the most commonly used pest control products in the world [11,12,15–18], and these factors have promoted the use of chlorpyrifos as a replacement for organochlorinated compounds [19]. Residues of many pesticides, among them chlorpyrifos, have been detected in wastewater, groundwater, surface water, and soil [20–26]. The detected concentrations of chlorpyrifos in both surface and ground water are in the range of 0.13–0.24 $\mu\text{g l}^{-1}$ [27].

Chlorpyrifos is known for its ability to act as cytotoxic, genotoxic, re-productivity toxic and immune toxic to both human and animals [28–34]. It has been documented that exposure of birds and aquatic ecosystem to low concentrations of chlorpyrifos caused severe toxicity to these organisms [35,36].

Several techniques have been proposed for the removal of pesticides from water body. These include (i) physical methods such as nanofiltration [37] and adsorption on activated carbon (AC) [38,39]; (ii) chemical methods that involve oxidation by ozone [40]; (iii) aqueous chlorine [41] or Fenton, and photocatalytic destruction [44,42–45]; (iv) biological treatments [46]; (v) advanced oxidation processes [47]; (vi) aerobic degradation [48]; and (vii) adsorption by low cost adsorbents [49–53].

The objectives of this work were: (a) to investigate the kinetics and stability (degradation) of chlorpyrifos in pure water and sludge, (b) to study the removal efficiency of chlorpyrifos by adsorption onto micelle-clay complex, which was prepared from the reaction of octadecyltrimethylammonium (ODTMA) bromide with montmorillonite clay [54], and AC, (c) to investigate the efficiency of the advance membrane technology WWTP at Al-Quds University toward the removal of chlorpyrifos from spiked secondary treatment effluents, and (d) to compare the adsorption efficiency by the three different methods.

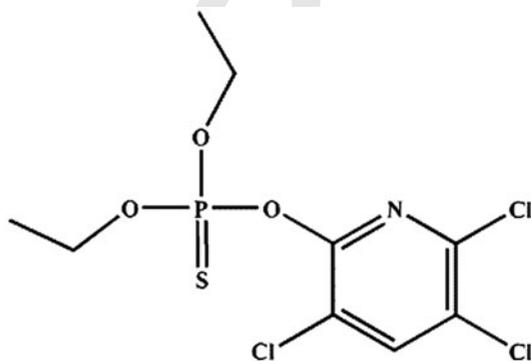


Fig. 1. Chemical structure of chlorpyrifos.



2. Experimental

2.1. Materials

All chemicals were of analytical grade. The clay used was Wyoming Na-montmorillonite SWY-2 obtained from the Source Clays Registry (Clay Mineral Society, Columbia, MO, USA). Quartz sand (grain size 0.8–1.2 mm) was obtained from Negev industrial minerals (Israel). Octadecyltrimethylammonium (ODTMA) bromide was obtained from Sigma Aldrich. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) were obtained in high purity from HPC company (Germany). AC (12–20 mesh) was obtained from Sigma (Sigma Chemical Company, USA). Deionized water was used to prepare all solutions. Methanol, acetonitrile, and water were HPLC grade and purchased from Sigma Aldrich (Israel). Magnesium sulfate was obtained from Sigma Aldrich (Israel).

2.2. Instrumentations

High-pressure liquid chromatography system consists of an alliance 2695 HPLC and a Micromass[®] Masslynx[™] detector tandem photodiode array from Waters (Milford, MA, USA). Data acquisition and control were carried out using Empower[™] software Waters (Milford, MA, USA). Analytes were separated on a 4.6 mm × 150 mm C18 XBridge[®] column (5 μm particle size) used in conjunction with a 4.6 mm × 20 μm XBridge[™] C18 column. Microfilter of 0.45 μm was used (Acrodisc[®] GHP, Waters).

pH meter model HM-30G; TOA electronics[™] was used to measure pH. C-18 (1 g) cartridges with 6 mL capacity and were purchased from Waters company (Milford, MA, USA).

The identification of all metabolites was performed using an LC system coupled to a hybrid linear quadrupole ion trap (LTQ)—Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The different components of WWTP at Al-Quds University as well as the characteristics of the membrane system were described previously [55,56].

2.3. Methods

2.3.1. Micelle-clay complex preparation

The micelle-clay complex was prepared as described elsewhere [54]. Briefly the micelle-clay complex was prepared by stirring 12.0 mM of ODTMA with 10.0 g L⁻¹ clay for 72 h. Suspensions were centrifuged for 20 min at 15,000 rpm, supernatants were discarded and the complex was lyophilized.

2.3.2. Batch adsorption studies

Batch adsorption experiments were carried out for solutions of chlorpyrifos in the concentrations range of 10–700 mg L⁻¹. Experiments were performed in 100-mL Erlenmeyer flasks containing 0.125 g of either micelle-clay complex or AC. 25 mL of chlorpyrifos solutions having known initial concentration were then introduced to each flask. The flasks were shaken in an electric shaker for three hours at room temperature and then the content of each flask was centrifuged for 5 min and filtered through 0.45-μm cellulose nitrate microfilters. The equilibrium concentration of chlorpyrifos was measured by HPLC. The same procedure was repeated for chlorpyrifos metabolite, 3,5,6-trichloro-2-pyridinol (TCP), using 10–300 mg L⁻¹ with the same adsorbent dosage.

Kinetic studies on the extent of adsorption was determined by introducing 50 mL solution of 50 mg L⁻¹ chlorpyrifos or TCP in 100-mL Erlenmeyer flasks containing 0.50 g of micelle-clay complex and determining the concentration of the pesticide or its metabolite as a function of time.

2.3.3. Analysis of adsorption isotherms

Equilibrium relationships between adsorbent and adsorbate are described by adsorption isotherms. The most common models for adsorption processes are Langmuir and Freundlich adsorption isotherms. Langmuir isotherm is widely used for modeling equilibrium data and determination of the adsorption capacity. Its linear form is given by Eq. (1):

$$C_e/Q_e = 1/(KQ_{\max}) + C_e/Q_{\max} \quad (1)$$

where C_e is the equilibrium concentration of adsorbent (mg L⁻¹), Q_e is the equilibrium mass of adsorbent per gram of adsorbate (mg g⁻¹), K is the Langmuir constant (L mg⁻¹), and Q_{\max} is the maximum mass of adsorbent removed per gram of adsorbate (mg g⁻¹).

Freundlich isotherm describes equilibrium on heterogeneous surface and its linear form is given by Eq. (2):

$$\log q_e = \log k_f + (1/n) \log C_e \quad (2)$$

where C_e is the equilibrium concentration of solute (mg L⁻¹), q_e is the amount of solute adsorbed per unit weight of adsorbent (mg g⁻¹), k is the relative adsorption capacity of the adsorbent (mg g⁻¹), and n is the intensity of the adsorption (L mg⁻¹).

2.4. Efficiency of advanced membranes in Al-Quds University WWTP

Fig. 2 describes the layout of the advanced membrane technology WWTP; it includes two units of ultrafiltration (hollow fiber (HF-UF) and spiral wound (SW-UF)), AC, and reverse osmosis (RO), and this technology is installed in Al-Quds University WWTP which is located in Al-Quds University main campus (Abu-Dies, Jerusalem, Palestine). To study the removal efficiency of chlorpyrifos by this advanced WWTP, 50 mL of 480 g L⁻¹ chlorpyrifos solution were added to 500 L activated sludge effluent placed in the activated sludge reservoir (position number 1 in Fig. 2). Five samples (numbered from 1 to 5) were collected using pre-cleaned 500-mL amber glass bottles. The samples were then filtrated using 0.45-μm cellulose nitrate filters and stored at 4°C before being analyzed by HPLC.

2.5. Filtration experiments

Column filter experiments were performed with 50/1 (w/w) mixtures of quartz sand and micelle-clay complex (13-cm layer) in a column of 18 cm in length and 4 cm in diameter. The mass of both micelle-clay complex and sand was 3 g and 147 g, respectively. The bottom of the column was covered by 3-cm layer of wool. Quartz sand was thoroughly washed by distilled water and dried at 105°C for 24 h. Either 50 mg L⁻¹ of chlorpyrifos or 75 mg L⁻¹ of 3,5,6-trichloro-2-pyridinol (TCP) were prepared by dilution of the stock solution

with distilled water and were passed individually through the columns. The flow rate was adjusted to 2.0 mL min⁻¹ at 25°C. Ten fractions of 100 mL each were collected and the concentration of the selected pesticide in each sample was determined by HPLC.

2.6. Stability study in pure water and activated sludge

Stability studies of chlorpyrifos were performed at 25°C by dissolving 100 mg L⁻¹ of chlorpyrifos either in pure water or in activated sludge, obtained from the WWTP located in the Al-Quds University. The kinetics in the activated sludge was performed under continuous aeration. Samples were collected at different time intervals, filtered using 0.45-μm cellulose nitrate filters and then analyzed by HPLC. The chemical structures of chlorpyrifos metabolites were identified by LC-MS.

2.7. Sample and standard preparations for chromatographic analysis

2.7.1. Stock solution

Stock solution was prepared by dissolving each pesticide (chlorpyrifos or TCP) standard in distilled water to a final concentration of 500 mg L⁻¹.

2.7.2. Calibration curves

The following diluted solutions were prepared from the stock solutions for chlorpyrifos and TCP: 0.10, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 mg L⁻¹.

The optimal HPLC conditions for the analysis of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) are summarized in Table 1.

3. Results and discussion

3.1. Effect of time

Fig. 3 summarizes the variation of chlorpyrifos's removal (percentage) vs. time as obtained in the batch

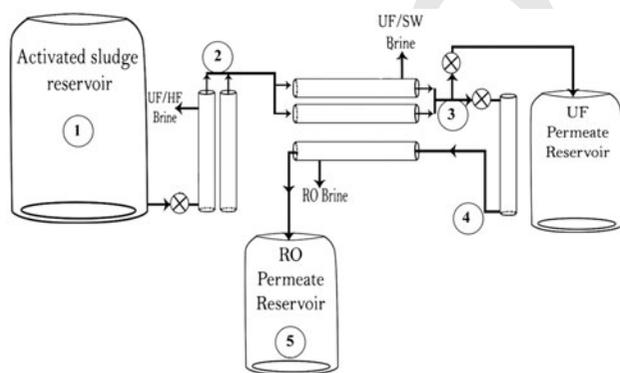


Fig. 2. Layout of WWTP showing all treatment processes including HF-UF filters (hollow fiber), SW-UF (spiral wound), AC, and RO filters. The numbers in the diagram indicate the sampling locations in the plant.

Notes: (1) the effluent of activated sludge sample, (2) the sample of HF-UF effluent, (3) the sample of UF-SW effluent, (4) the sample of AC effluent, and (5) the sample of RO effluent.

Table 1

Optimal chromatographic condition for HPLC analysis for chlorpyrifos or TCP; C-18 was selected as the separation column

Parameter	Chlorpyrifos	TPC
Wavelength	290 nm	230 nm
Flow rate	1 mL min ⁻¹	1 mL min ⁻¹
% ACN in mobile phase	85%	75%
% Water in mobile phase	15%	25%
Injection volume	10 μL	10 μL

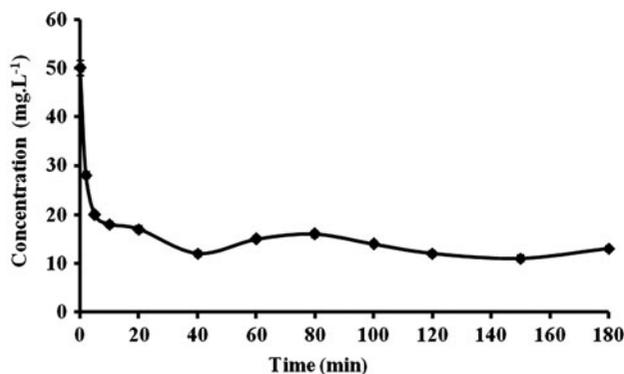


Fig. 3. Percentage removal (%) of chlorpyrifos using micelle-clay complex as a function of time. Initial concentration was 100 mg L⁻¹. Adsorbent dose was 5 g L⁻¹, pH 6.58, and T 25°C.

experiments. In this study, 100 mL of 100 mg L⁻¹ chlorpyrifos solutions were brought into contact with 0.5 g micelle-clay complex at 25°C. Samples were collected and analyzed for chlorpyrifos concentration by HPLC. Examination of Fig. 3 demonstrates that the optimum contact time for the removal of chlorpyrifos is 30 min ± 5 min. The adsorption kinetics can be divided into two steps: a fast step revealing at 5 min contact time to reach the equilibrium, while the second step is a slower one with 30 min contact time to reach the equilibrium. The overall removal efficiency of chlorpyrifos by micelles-clay complex at these conditions reaches 90% after 180 min contact time.

3.2. Adsorption isotherms

The adsorption isotherms of chlorpyrifos at different concentrations using both micelle-clay complex and AC were determined at 25°C. The data were fitted to both Langmuir and Freundlich isotherms. The adsorption parameters were calculated from Eqs. (1) and (2) and are summarized in Table 2. Comparison of the correlation coefficient (R^2) values for chlorpyrifos obtained from both adsorption isotherms for micelles-clay complex and AC reveals that Langmuir isotherms fits the data better than Freundlich isotherm.

Table 2 reveals that the Langmuir adsorption parameters for AC ($Q_{max} = 76.9 \text{ mg g}^{-1}$ and $K = 0.098 \text{ L mg}^{-1}$) are higher than those obtained with micelles-clay complex ($Q_{max} = 11.49 \text{ mg g}^{-1}$ and $K = 0.017 \text{ L mg}^{-1}$). On the other hand, both adsorptions gave similar values for n but the adsorption by micelles-clay complex yielded larger value of k_F .

Table 3 lists the adsorption parameters for the removal of the major metabolite of chlorpyrifos, TCP,

by micelles-clay complex and AC using both Langmuir and Freundlich isotherms. Inspection of Table 3 demonstrates that Langmuir isotherms is better fit the data for AC, while Freundlich isotherm is better fit for micelles-clay complex as judged by the R^2 values. The micelle-clay complex yielded higher value of Q_{max} (26.18 mg g^{-1}) than AC (23.5 mg g^{-1}) with similar K value for both adsorbents (0.302 and 0.357 L mg^{-1}). The n and k_F values for AC were found to be higher than those obtained by micelle-clay complex.

3.3. Removal of chlorpyrifos by the advance membrane WWTP

Fig. 4 summarizes the percentage removal efficiency of chlorpyrifos by HF-UF, SW-UF, AC, and RO filters (see Section 2). The results indicate that the removal efficiency of HF-UF was 68%. On the other hand, 100% removal was reached by SW-UF and the AC filters, thus eliminating any need for the RO unit.

3.4. Filtration experiments

Fig. 5 displays the percentage removal of chlorpyrifos and TCP by continuous flow method using bench top columns. The initial concentration of chlorpyrifos was 50.0 mg L⁻¹ and that of TCP was 75.0 mg L⁻¹. For both experiments, the column consisted of a mixture of sand and micelle-clay complex in a ratio of 50/1 (w/w). The flow rate was adjusted to 2 mL per min at a temperature of 25°C; the total volume eluted through the columns was 1,000 mL. Fig. 5 indicates that the breakthrough point was reached after the third fraction which indicates that this column is inefficient in removing chlorpyrifos at these conditions. To enhance the removal efficiency of these types of columns, several parameters are needed to be optimized such as weight percentage of the micelle-clay complex, the column length, and flow rate.

It is planned that such changes to these parameters will be addressed during the design of large-scale columns which will be integrated within the existing WWTPs in the near future.

Furthermore, Fig. 5 demonstrates that micelle-clay complex is very efficient for the removal of TCP. 1,000 mL of 75 mg L⁻¹ of TCP was eluted through the column filter. At the end of the experiment, a complete removal was observed for all fractions. 3.0 g of micelle clay complex completely removed 75.0 mg of TCP; it implies that 1.0 g of ODTMA complex (clay micelles complex) has the potential to remove 81.3 mg of TCP. The negatively charged and polar TCP may

40

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80

85

Table 2

Adsorption isotherms parameters for chlorpyrifos on both clay-micelles complex and AC, using Langmuir and Freundlich equations. Contact time = 3 h and $T = 25^\circ\text{C}$

Type of adsorbents	Langmuir equation (1)			Freundlich equation (2)		
	R^2	Q_{\max} (mg g^{-1})	K (L mg^{-1})	R^2	n (± 0.05)	k_F
Micelle-clay complex	0.910	11.49 ± 0.02	0.017 ± 0.01	0.890	3.413 ± 0.01	1.683 ± 0.01
Activated carbon	0.997	76.9 ± 0.02	0.098 ± 0.01	0.890	3.745 ± 0.01	18.75 ± 0.01

Table 3

Adsorption isotherms parameters for TCP on both clay-micelles complex and activated carbon, using Langmuir and Freundlich equations. Contact time = 3 h, and $T = 25^\circ\text{C}$

Type of adsorbents	Langmuir equation (1)			Freundlich equation (2)		
	R^2	Q_{\max} (mg g^{-1})	K (l mg^{-1})	R^2	n (± 0.05)	k_F
Micelle-clay complex	0.943	26.18 ± 0.02	0.302 ± 0.01	0.993	1.29 ± 0.01	2.10 ± 0.01
Activated carbon	0.967	23.5 ± 0.02	0.357 ± 0.01	0.887	1.68 ± 0.01	3.20 ± 0.01

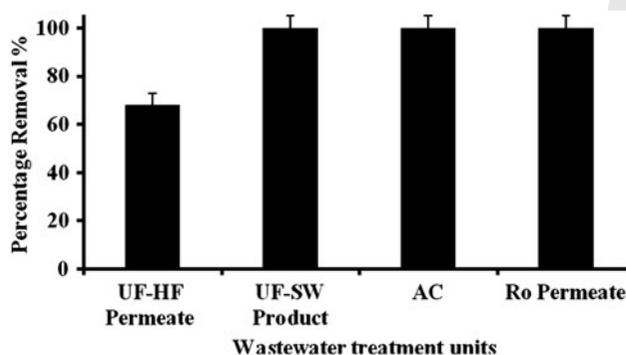


Fig. 4. Percentage removal of chlorpyrifos from Al-Quds University WWTP which includes ultrafiltration-hollow fiber unit (UF-HF), ultrafiltration-spiral wound unit (UF-SW), activated carbon column (AC), and RO filters.

be the cause for its high adsorption capacity and significant removal.

3.5. Stability of chlorpyrifos in sludge and characterization of its metabolites

Biodegradation analysis was performed in batch cultures using chlorpyrifos solutions (100 mg L^{-1}) to determine its degradation rate and verify the formation of various metabolites formed in sludge. The biodegradation study of chlorpyrifos was monitored by HPLC. The various chlorpyrifos's metabolites formed during the study period were identified by LC-MS. Accurate analysis by MS and MS/MS was made to identify chlorpyrifos degradation products and an ion chromatogram (XIC) of chlorpyrifos

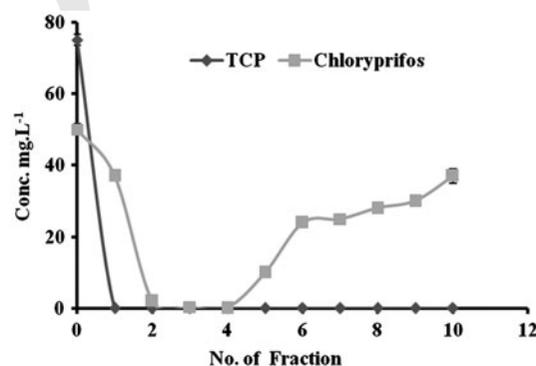


Fig. 5. Filtration of chlorpyrifos and trichloro-2-pyridinol (TCP) by a laboratory filter, which includes a micelle-clay complex mixed with excess sand at 1:50 (w/w). The flow rate was 2.0 mL min^{-1} . Initial concentrations of chlorpyrifos and TCP were 50.0 and 75.0 mg L^{-1} , respectively, pH 6.58, and $T = 25^\circ\text{C}$.

biodegradation was conducted as well. The benefit of using very selective extracted ion chromatograms by FTICR-MS generated with a tight mass-to-charge ratio window of ± 0.0010 units around each selected protonated molecule (i.e. $[\text{M} + \text{H}]^+ \pm 1.0 \text{ mDa}$) is to reduce the signal complexity of the total ion current trace (data not shown).

Fig. 6 summarizes the degradation rate of chlorpyrifos in fresh water and sludge. The results reveal that chlorpyrifos underwent degradation in both, sludge, and fresh water. The hydrolysis reaction in both media was found to follow first-order kinetics with a rate constant of 7.10×10^{-8} ($R^2 = 0.999$) in pure water and of 9.30×10^{-8} ($R^2 = 0.996$) in sludge.

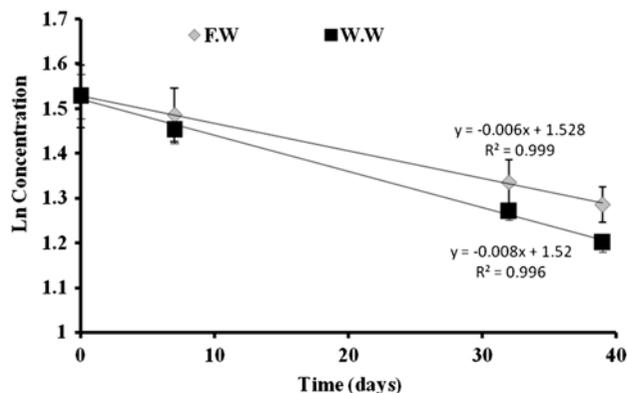


Fig. 6. Kinetics of chlorpyrifos degradations in the activated sludge and fresh water at 25°C; initial concentration of chlorpyrifos was 100 mg l⁻¹, pH 6.58, and T = 25°C.

Chlorpyrifos degradation's half-life in pure water was 112 d and in sludge was 85.9 d. In similar studies on the biodegradation of chlorpyrifos by different specific strains of bacteria and fungus, the half-life values reported were between 12 and 17 d as compared to 128 d in mineral salt liquid as a control [57]. Our biodegradation results in pure water are very close to the results obtained by the control; however, the half-life in sludge is sevenfold larger than that obtained in specific bacteria. This result is not surprising since the concentrations of these strains in the sludge are expected to be much lower than in pure strain. Fig. 7 illustrates the extracted ion chromatogram for chlorpyrifos solution in sludge after 40 d of biodegradation. The chromatogram shows two major biodegradations products at retention times of 3.30 and 7.36 min based on the accurate m/z ratio. Table 6 lists the various proposed metabolites according to their molecular anions, chemical formula, retention time, chemical structure, and molecular weights.

Chlorpyrifos was found to undergo degradation in natural environmental waters. It was reported that the hydrolysis of chlorpyrifos ester group is the most labile function in the pesticide and the sole degradation pathway proceeds by its cleavage [58–60]. The cleavage of the phosphate ester bond of chlorpyrifos results in the formation of two metabolites: 3,5,6-trichloro-2-pyridinol (TCP) and diethylthiophosphoric acid (DETP) [61,62]. In most cases, it was found that such ester cleavage occurs as a result of microbial activity via the phosphatase enzymes found in such bacteria [63–67].

It should be emphasized that the presence of TCP in wastewaters are more common than the presence of its parent compound, chlorpyrifos, due to the fact

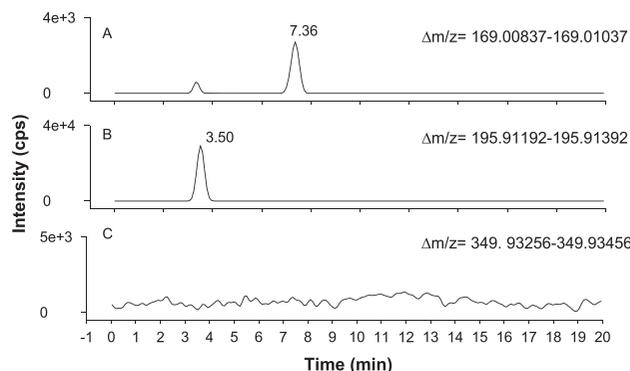


Fig. 7. The extracted ion chromatograms (XICs) by LC/ESI-FTICRMS acquired in positive ion mode (C) and in negative ion mode (A and B) of a chlorpyrifos solutions after 40 d of biodegradation. The ions monitored are displayed in each trace and correspond to the most abundant protonated or deprotonated molecules, [M + H]⁺ or [M - H]⁻, using a restricted window of ±0.0010 m/z unit centered around each selected ion (right side).

that TCP is much more water soluble and thus it is considered a risky and dangerous soil and aquatic pollutant. Further, it is worth noting that TCP is listed in Environmental Protection Agency (EPA) as potential endocrine-disrupting chemical [63,68–72].

Our proposed diagram for chlorpyrifos degradation and biodegradation to TCP and DETP is shown in Fig. 8.

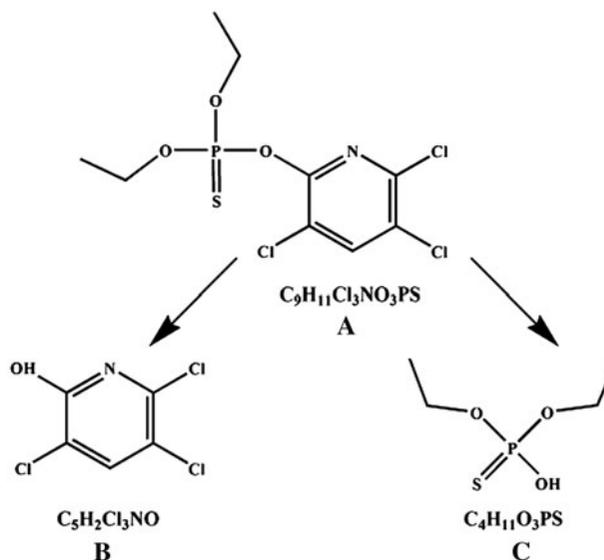


Fig. 8. Proposed transformation pathway for the biodegradation of chlorpyrifos (A), to its metabolites, TCP (B), and DETP (C).

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4. Conclusions

The advanced WWTP utilizing UF (HF and SW), AC, and RO demonstrated that UF-HF unit is not efficient in removing chlorpyrifos from spiked wastewater, whereas UF-SW followed by AC filters were sufficient for a complete removal of chlorpyrifos.

The chlorpyrifos stability study revealed that chlorpyrifos was unstable in both water and sludge and it underwent degradation to two metabolites, TCP and DETP.

In adsorption kinetics, it can be concluded that the optimum contact time for chlorpyrifos removal is 30 ± 5 min. The overall removal of 0.5 g of micelle clay complex in 100 mg L^{-1} reaches 90% after 180 min.

Adsorption isotherms studies on ODTMA micelle-clay complex and AC revealed that under steady-state conditions both adsorbents can be efficient in chlorpyrifos removal. The micelle-clay complex demonstrated an efficient removal for chlorpyrifos and its metabolite (TCP) in filtration experiment with fast removal for TCP, which implies that 1 g of ODTMA, can remove 81.26 mg of 75 mg L^{-1} TCP and 30.65 mg of 50 mg L^{-1} chlorpyrifos after elution of 1,000 mL solutions.

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