Stability and neurotoxic impact of organophosphate pesticides in aqueous environments

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Abstract:

Introduction/purpose: Organophosphates are widely used nowadays. They have applications as pesticides, drugs, plasticizers, flame retardants, or chemical warfare agents. Their acute toxicity is ascribed to inhibiting acetylcholinesterase (AChE), a key enzyme in the transmission of nerve impulses in animals. Their toxic effects manifest by acetylcholine accumulation in the nerve synapses and can lead to paralysis or death. Organo-thiophosphate pesticides (OPs) are used in large quantities. Their oxo-analogs can also be found in the environment due to oxidation. Once accumulated in the environment, they exhibit toxic effects on non-target organisms.

Methods: The hydrolysis of OPs in different pH was systematically analyzed, and their neurotoxic effects were evaluated. The concentration of the investigated pesticides during decomposition was monitored by ultra-performance liquid chromatography (UPLC). At the same time, a decrease in the toxicity of the treated samples was observed by measuring the activity of the enzyme AChE.

Results: OPs decompose rapidly in alkaline aqueous solutions but are highly stable in acidic solutions. Chlorpyrifos hydrolyzes the fastest and dimethoate the slowest. The toxicity of these OP solutions decreases over time, indicating that more toxic products were not formed.

Conclusion: The presented results can provide a sound basis for further efforts to find simple and efficient decomposition methods of OPs.

Keywords: organophosphate, pesticide, pH stability, toxicity.

Introduction

Water pollution is one of the biggest problems in modern society because it affects many different aspects; however, its most significant impact is on human health. Because of its vital importance, its quality must be within strictly defined limits. That is why it is necessary to continuously monitor the concentrations of toxic pollutants, including organophosphate pesticides (OPs), and reduce their presence as much as possible. Effective removal from water is also necessary to control the level of these compounds in food and the environment (Bootharaju & Pradeep, 2012; He et al, 2015; Rasmussen et al, 2015; Wang et al, 2013).

Extensive use of OPs leads to soil and water contamination (Peshin et al, 2020; Silva et al, 2019), OP residues in food (Baker et al, 2002), and public health impacts (Kunstadter et al, 2001; London et al, 2002). In addition to the inevitable acute and chronic impact on humans, overreliance on OPs for pest control has led to many problems for other species (Greaves & Letcher, 2017). Severe contamination of aquatic ecosystems by OPs can lead to mass mortality of aquatic organisms. That is why it is necessary to purify wastewater containing OPs before mixing it with other waters.

Depending on the persistence of particular OPs, they remain in the environment for a long time or are transformed into a more toxic form. Due to the high solubility of organophosphates (OPs) in water and their low persistence in soil, they have a strong potential to run off into surface water and leach into groundwater (Van Scoy et al, 2016). The most important degradation pathways of OPs in the environment are microbial degradation, hydrolysis, oxidation and photolysis (Van Scoy et al, 2016; Aćimović & Vasić Anićijević, 2022). These processes often produce oxo-forms as end products through photocatalytic oxidation and microbial metabolism, which is problematic due to the extreme toxicity of these compounds. On the other hand, hydrolytic degradation is the main inactivating route of OPs in the environment and usually does not yield oxo-forms as end products. The hydrolysis of OPs mainly depends on pH and temperature (Anićijević et al, 2022).

Dimethoate (DMT), malathion (MLT), and chlorpyrifos (CPF) are well-known contact and systemic OPs that have been in use for years. They are used against many insects in agriculture (Anićijević & Lazarević-Pašti, 2020). The acute toxicity of OPs is primarily due to their inhibition of acetylcholinesterase (AChE), an enzyme critical for proper nerve function (Anićijević & Karkalić, 2022). The oxidation of organo-thiophosphates results in the formation of oxo-analogs, which are even more toxic to AChE than the parent compounds. These oxo-analogs can also form in the environment due to various oxidizing agents in water and soil (Lazarević-Pašti et al, 2016; Vasić Anićijević, 2020).

This work aims to investigate the half-life of OP hydrolysis in aquatic environments across a pH range of 3.00 to 9.00 and to track the toxicity of OP solutions over time. Understanding these factors is essential for developing effective strategies to remove OPs from water. Toxicity is assessed by measuring AChE inhibition through an enzyme assay, as AChE inhibition is widely used as a biomarker in environmental monitoring (Grue et al, 1991; Legradi et al, 2018), indicating the eco-neurotoxicity of cholinesterase-inhibiting compounds.

Materials and methods

Stability of organophosphate in the aqueous buffer solution

The stability of OPs in media with different pH values was investigated in a phosphate buffer solution with a concentration of 50 mmol dm^{-3} (made with deionized water) with a pH value ranging from 3.00 to 9.00, in according with the pH range supported by the UPLC system. The 1×10^{-4} mol dm^{-3} OP solutions were incubated at 25 °C in an orbital shaker-incubator (Orbital Shaker-Incubator ES-20, Grant-bio) for 10 days. Aliquots were taken at relevant time points to measure the concentrations of the OPs, as described in the following section. Additionally, the decomposition of OPs at the same concentration was analyzed in tap water samples to simulate real-world conditions. All measurements were performed in triplicate, and uncertainties were calculated using the least significant differences (LSDs) test at a 95% significance level.

Ultra performance liquid chromatography analysis

A Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system was used to measure the concentration of DMT, MLT, and CPF, together with an adjustable UV photodiode array (PDA) detector controlled by the Empower software. Chromatographic separations were on 1.7 µm, 100 mm × 2.1 mm ACQUITY UPLC™ BEH C_{18} column (Waters).

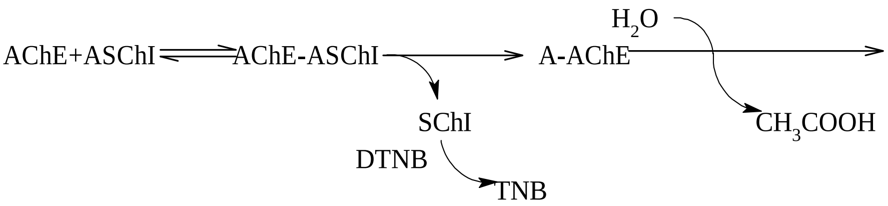
The solutions of OP concentrations of 1×10^{-5} mol dm^{-3} were analyzed under isocratic conditions with a mobile phase consisting of 10% acetonitrile and 90% water (v/v). The eluent flow rate was 0.25 cm^3 min^{-1}, and the injection volume was 5 mm^3. Under the described conditions, the retention times of DMT, MLT, and CPF were (2.37 ± 0.05) min, (2.48 ± 0.05) min, and (2.62 ± 0.05) min, respectively. The spectra for DMT, MLT, and CPF are shown in Figures 1 (c), 2 (c), and 3 (c) as well as their absorption maxima. Optical detection was performed at 205 and 200 nm for aliphatic MLT and DMT, respectively. For aromatic CPF, 230 nm was chosen from three characteristic absorption maxima at 200, 230, and 290 nm. The spectra for DMT, MLT, and CPF are shown in Figures 1 (c), 2 (c), and 3 (c).

The concentrations of OPs were determined using linear calibration curves which were constructed from standard pesticide solutions across a broad concentration range.

Neurotoxic measurements

AChE inhibition measurements were performed to track and quantify changes in the toxicity of OPs and to investigate whether hydrolysis under different pH conditions leads to the formation of more toxic compounds. These transformation products could have harmful effects at concentrations below the detection limits of Ultra Performance Liquid Chromatography (UPLC). AChE activity was measured using a modified version of Ellman's procedure (Ellman et al, 1961). The in vitro experiments were performed by the exposure of 0.5 IU commercially purified AChE from electric eel to the OP solutions obtained in adsorption experiments at 37 °C in 50 mmol dm^{-3} phosphate buffer (PB) pH 8.00 (final volume 0.650 cm^3). The enzymatic reaction was initiated by adding acetyltiocholine-iodide (ASChI) and 5.5'-dithiobis-2-nitrobenzoic acid (DTNB) as a chromogenic reagent. The reaction was allowed for 8 minutes before being stopped with 10% sodium dodecyl sulfate (SDS). The reaction product, thiocholine, reacts with DTNB to form 5-thio-2-nitrobenzoate, measured by its optical absorbance at 412 nm. It should be noted that in these measurements, the enzyme concentration was constant and set to give an optimal spectrophotometric signal.

The following reaction shows the principle of determining AChE activity using the Ellman test:



The physiological effects were quantified as AChE inhibition given as:

$$I\% = \frac{A_0 - A}{A_0} \times 100 \qquad (1)$$

where A_0 represents the AChE activity in the absence of OP, A is the AChE activity measured after exposure to a given OP. OP solutions with an initial concentration of 1×10^{-4} mol dm^{-3} were incubated in phosphate buffers (pH 3.00 to 9.00, 25 °C) and in tap water for 10 days to monitor the toxicity of the hydrolysis products formed spontaneously.

Results

Kinetics studies of organophosphate decomposition

The concentration of OPs was monitored in tap water and phosphate buffers with pH ranging from 3.00 to 9.00 for 10 days using UPLC analysis. Figures 1, 2, and 3 show the time dependence of the OP concentration. The spontaneous decay of DMT (Figure 1), MLT (Figure 2), and CPF (Figure 3) concentrations over time was relatively fast in neutral and alkaline buffers, as well as in tap water (pH 6.50). A decrease in OP concentrations was also observed in buffers with acidic pH, though it was slower. The decay followed exponential trends in all cases, consistent with pseudo-first-order kinetics. This outcome was expected, as the buffer solutions maintain constant H^+/OH^- concentrations over time, in alignment with previous literature (Wolfe et al, 1977). Consequently, the hydrolysis rate constants (k_h) were obtained by fitting the experimental data to the following equation:

$$C_{\mathrm{t}} = C_0 e^{-k_{\mathrm{h}} t} \qquad (2)$$

where C_t and C_0 are the remaining OP concentrations at a given time (t) and the initial OP concentration.

Dimethoate

The dependence of the degradation of DMT (Richendrfer & Creton, 2015, Elmorsy et al, 2022, Sparling & Fellers, 2007) with an initial concentration of 1×10^{-4} mol dm^{-3} on the pH value of the solution at 25 ºC and 35 ºC is shown in Figure 1 (a) and (b). The results show that an increase in the pH value increases degradation efficiency. The half-life of DMT at pH 9.00 and 25 °C is 8 days, and at 35 °C, it is 2 days.

The results also show that an increase in temperature of 10 °C accelerates the degradation about tenfold at pH 9.00. The less efficient degradation at pH 8.00 and 35 °C resulted in a half-life of 9 days. It practically means that a decrease in pH by 1 unit increases the length of degradation 4.5 times. This applies to the pH reduction from 9.00 to 8.00. For other changes, the pH degradation is several times longer.

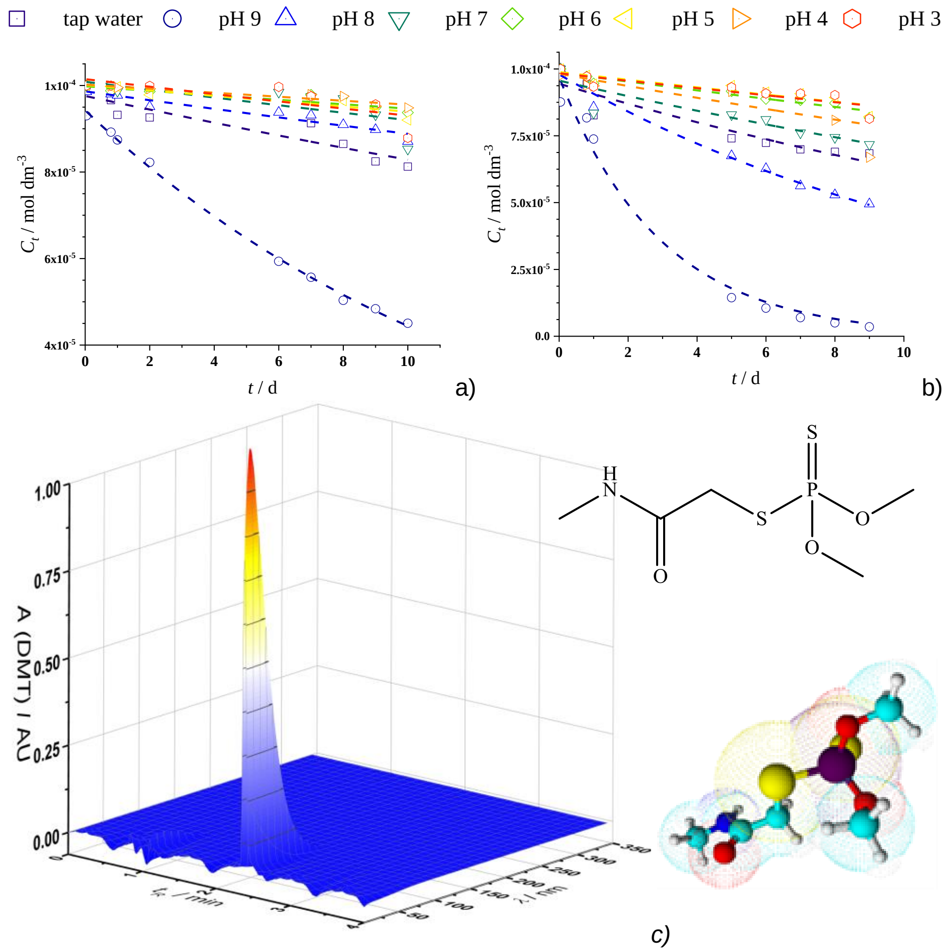
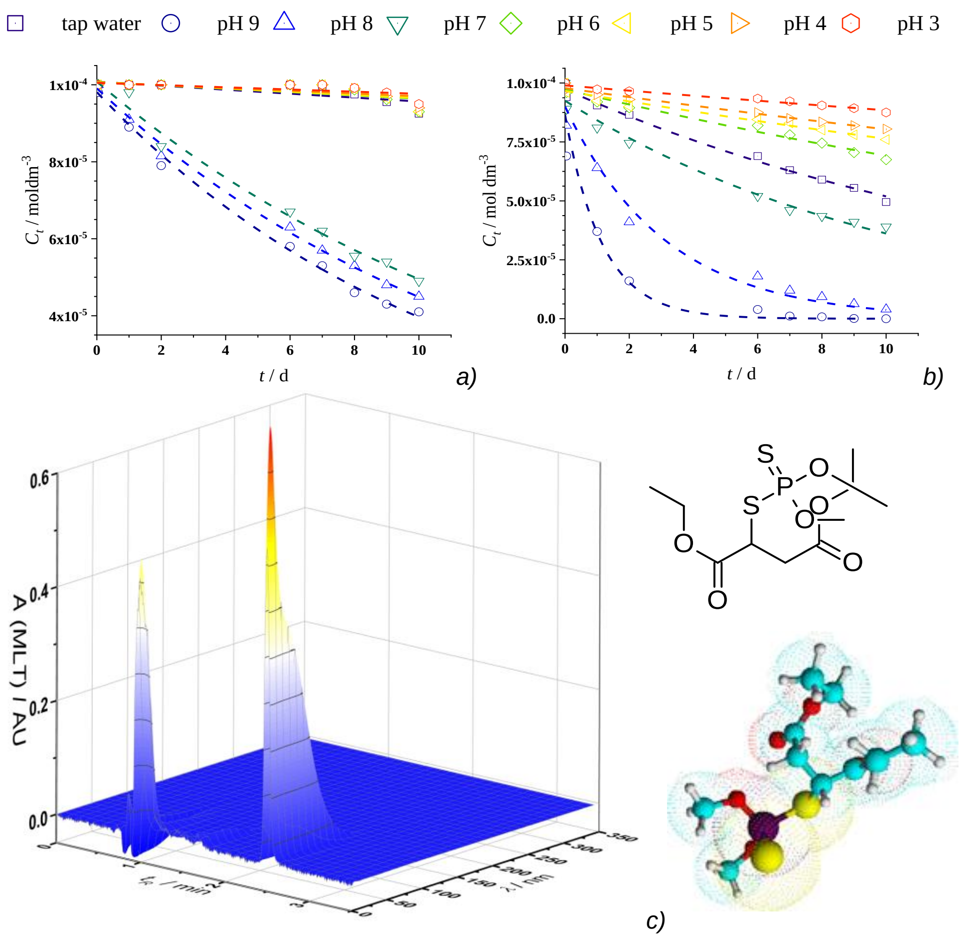


Figure 1 – Dependence of DMT degradation at pH from 3.00 to 9.00 and in tap water (pH 6.5) at 25 °C (a), 35 °C (b), and PDA spectra of concentration 1×10^{-4} mol dm^{-3} (c)

Malathion

The dependence of the degradation of MLT (Richendrfer & Creton, 2015, Elmorsy et al, 2022, Sparling & Fellers, 2007) with an initial concentration of 1×10^{-4} mol dm^{-3} on the pH value of the solution at 25 ºC and 35 ºC is shown in Figure 2 (a) and (b).



a)

b)

c)

Figure 2 – Dependence of MLT degradation at pH from 3.00 to 9.00 and in tap water (pH 6.5) at 25 °C (a) and 35 °C (b), *and PDA spectra of concentration* 1×10^{-4} *mol dm*$^{-3}$ *(c)*

As for DMT, the results show that an increase in the pH value leads to an increase in degradation efficiency. The half-life of MLT at a concentration of 1×10^{-4} mol dm^{-3} at pH 9.00 at 25 °C is 7.5 days, and at 35 °C it is 1 day, while it completely decomposes in 10 days at 35 °C. The presented results also show that an increase in temperature of 10 °C accelerates the degradation of MLT several times under the given experimental conditions (pH 9.00). This means that a decrease in pH by 1 increases the length of degradation by 2 times. As in the case of DMT, this applies to the reduction of pH from 9.00 to 8.00; for other changes in pH, the degradation is several times longer.

Chlorpyrifos

The dependence of the degradation of CPF (Ubaid ur Rahman et al, 2021) with an initial concentration of 1×10^{-4} mol dm^{-3} on the pH value of the solution at 25 ºC and 35 ºC is shown in Figure 3 (a) and (b).

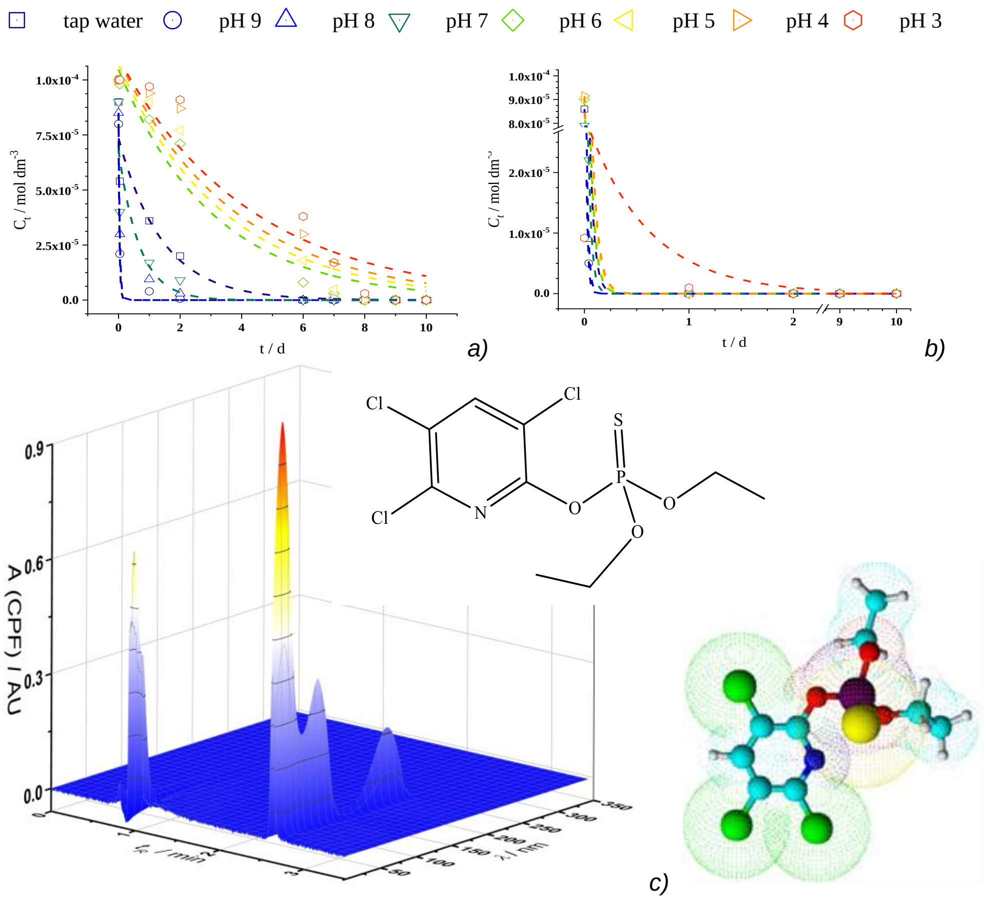


Figure 3 – Dependence of CPF degradation at pH from 3.00 to 9.00 and in tap water (pH 6.5) at 25 °C (a) and 35 °C (b), *and PDA spectra of concentration 1×10^{-4} mol dm^{-3} (c)*

As in the previous cases, the results show that, with an increase in the pH value, the degradation efficiency increases. The half-life of CPF at a concentration of 1×10^{-4} mol dm^{-3} at pH 9.00 and 25 °C is 29 min, while at 35 °C, the value is only 4 min. The degradation of aromatic CPF at pH 9.00 is much faster than in the case of both tested aliphatic OPs. At 25 °C, the time required for a complete degradation of CPF is significantly shorter than 1 d (measured in minutes) for all pH values higher than 6.00 and in tap water.

On the other hand, at 35 °C, complete degradation is very fast for all tested pH values and almost immediate for the basic ones. The presented results also show that an increase in temperature of 10 °C accelerates the degradation of CPF by 7.5 times at pH 9.00, 8.00, and 7.00. At pH values lower than 7.00, the degradation of CPF is slowed down from 1 000 to 2 000 times when the temperature increases by 10 °C.

Toxicity assessment of organophosphate solutions

OP solutions of the initial concentration of 1×10^{-4} mol dm^{-3} were left in phosphate buffers (pH ranging from 3.00 to 9.00, 25 °C) and in tap water for 10 days to monitor the toxicity of the spontaneous hydrolysis products. The toxicity of the OP solution was evaluated using the AChE inhibition assay, as already described above. Aliquots for AChE inhibition assays were taken at the beginning of the experiment and then after 1, 2, 6, and 10 days.

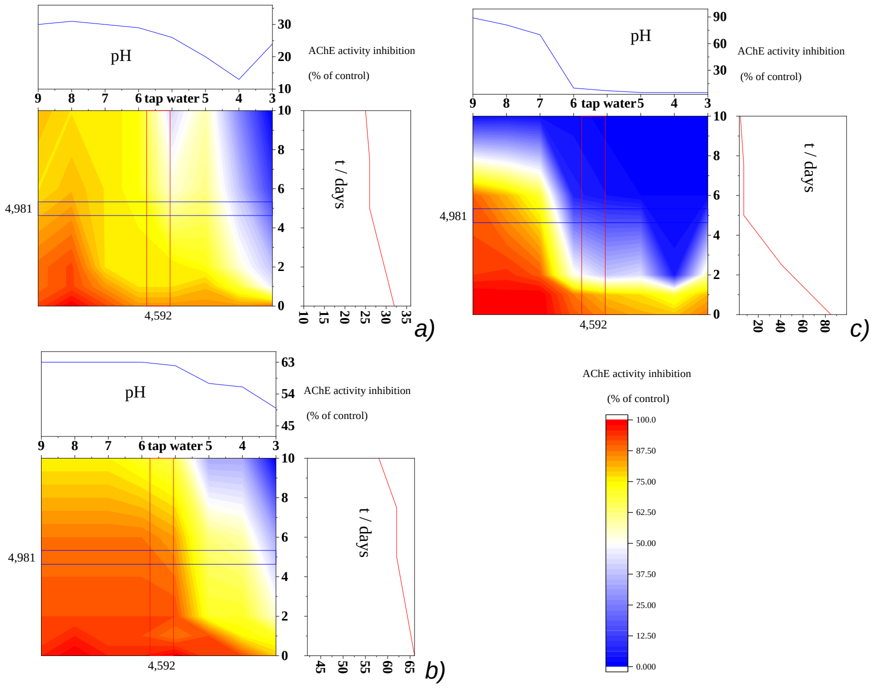


Figure 4 – Toxicity of the solutions of DMT (a), MLT (b), and CPF (c) measured during 10 days at pH from 3.00 to 9.00 for the initial concentration of Ops 1×10^{-4} mol dm^{-3}

In addition to measuring AChE activity inhibition over 10 days, we also tracked changes in inhibition using the measured concentrations of DMT, MLT, and CPF over time (C_t) and the OP inhibition curves. The results in Figure 4 show a rapid decrease in the acute toxicity of water contaminated with OPs at pH levels above 7.00.

Discussion

A fundamental understanding of the OP hydrolysis mechanisms is paramount for properly planning OP disposal and removal (Tomasi et al, 2005; Anićijević et al, 2022). For example, understanding OP hydrolysis could help optimize chemical treatments of contaminated samples to maximize OP decomposition into non-toxic products without the need for aggressive chemicals or excessive use of alkalis. The results in Figures 1, 2, and 3 show that the estimated half-lives of OPs in aqueous solution decrease with increasing pH, though the relationship is not strictly linear. Additionally, the stability of these molecules shows varied pH dependence. Further research is needed to fully resolve the link between the OP structure and stability at different pH levels in aqueous media.

Importantly, matrix effects in spiked tap water appear to have only a minor impact on the rate of hydrolysis (Figure 4). Therefore, these results can be reliably used to estimate OP half-lives in aqueous media based primarily on pH values. However, other factors, such as microbial activity or oxygen saturation levels, could also affect OP decomposition. (Lockridge et al, 2019).

Finally, the data presented in Figure 4 suggest that the inhibition of AChE activity decreased monotonically over time in all samples tested. Therefore, by combining the results of the pH stability of OPs with their AChE inhibition curve, the inhibition of AChE activity in the contaminated water samples can be reliably predicted for up to 10 days (Figure 4).

Conclusion

The investigated OPs have varying half-lives, and since their half-lives decrease rapidly with increasing pH in alkaline media, this suggests that alkaline hydrolysis is an effective method for removing DMT, MLT, and CPF from water. Unlike microbial degradation or photocatalytic oxidation, alkaline hydrolysis does not result in the accumulation of more toxic byproducts during the degradation process. Therefore, if alkaline hydrolysis removes OPs, no particular care should be taken to monitor the degradation process because the risk of toxic product formation is negligible. Matrix effects in tap water were found to have a negligible impact on the rate of OP hydrolysis, making the presented data reliable for estimating OP half-lives in contaminated water. Additionally, toxicity data, measured as AChE inhibition, can be used to assess acute toxicity following water contamination. Estimates for periods longer than ten days can be derived by combining stability data with AChE inhibition curves for OPs. However, these results should be interpreted cautiously, and further systematic studies are recommended to evaluate the hydrolytic stability of OPs fully.

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Estabilidad e impacto neurotóxico de pesticidas organofosforados en ambientes acuosos

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CAMPO: ciencias ambientales, química física
TIPO DE ARTÍCULO: artículo científico original

Resumen:

Introducción/objetivo: Los organofosforados se utilizan ampliamente en la actualidad. Tienen aplicaciones como pesticidas, fármacos, plastificantes, retardantes de llama o agentes de guerra química. Su toxicidad aguda se atribuye a la inhibición de la acetilcolinesterasa (AChE), una enzima clave en la transmisión de impulsos nerviosos en animales. Sus efectos tóxicos se manifiestan por la acumulación de acetilcolina en las sinapsis nerviosas y pueden provocar parálisis o muerte. Los pesticidas organotiofosforados (OP) se utilizan en grandes cantidades. Sus oxoanálogos también se pueden encontrar en el medio ambiente debido a la oxidación. Una vez acumulados en el medio ambiente, presentan efectos tóxicos en organismos no objetivo.

Métodos: Se analizó sistemáticamente la hidrólisis de los OP a diferentes pH y se evaluaron sus efectos neurotóxicos. La concentración de los pesticidas investigados durante la descomposición se controló mediante cromatografía líquida de ultra alta resolución (UPLC). Al mismo tiempo, se observó una disminución de la toxicidad de las muestras tratadas midiendo la actividad de la enzima AChE.

Resultados: Los OP se descomponen rápidamente en soluciones alcalinas acuosas, pero son muy estables en soluciones ácidas. El clorpirifos se hidroliza más rápido y el dimetoato, más lentamente. La toxicidad de estas soluciones de OP disminuye con el tiempo, lo que indica que no se formaron productos más tóxicos.

Conclusión: . Los resultados presentados pueden proporcionar una base sólida para futuros esfuerzos encaminados a encontrar métodos de descomposición de OP simples y eficientes.

Palabras claves: organofosforado, pesticida, estabilidad del pH, toxicidad.

Стабильность и нейротоксическое действие фосфорорганических пестицидов в водной среде

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Резюме:

Введение/цель: Органофосфаты широко используются в современном мире. Они используются в качестве пестицидов, лекарств, пластификаторов, антипиренов или боевых отравляющих веществ. Их острая токсичность объясняется ингибированием ацетилхолинэстеразы (АХЭ), ключевого фермента передачи нервных импульсов у животных. Их токсическое действие проявляется накоплением ацетилхолина в нервных синапсах и может привести к параличу или смерти. Тиофосфаторганические (ОФ) пестициды используются в больших количествах. Их оксоаналоги могут попасть в окружающую среду вследствие окисления. Накапливаясь в окружающей среде, они оказывают токсическое воздействие на нецелевые организмы.

Методы: В ходе исследования систематически проводились анализ гидролиза пестицидов ОФ в различных условиях pH и оценка их нейротоксического воздействия. Концентрацию исследуемых пестицидов ОФ в процессе разложения контролировались методом сверхэффективной жидкостной хроматографии (СЭЖХ). Одновременно при измерении активности фермента АХЭ наблюдалось снижение токсичности обработанных образцов.

Результаты: Пестициды ОФ быстро разлагаются в щелочных водных растворах, но они чрезвычайно стабильны в кислых растворах. Хлорпирифос гидролизуется быстрее, а диметоат − медленнее. Токсичность этих растворов со временем снижается, что указывает на то, что более токсичные продукты не образуются.

Выводы: Помимо того, представленные результаты могут стать хорошей основой в дальнейшем поиске простых и эффективных методов разложения ОФ пестицидов.

Ключевые слова: органофосфат, пестицид, ph стабильность, токсичность.

Стабилност и неуротоксични утицај органофосфатних пестицида у воденим срединама

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Сажетак:

Увод/циљ: Органофосфати данас имају широку примену. Користе се као пестициди, лекови, пластификатори, успоривачи пламена или хемијски бојни агенси. Њихова акутна токсичност се приписује инхибицији ацетилхолинестеразе (AChE), кључног ензима у преносу нервних импулса код животиња. Токсични ефекти се манифестују акумулацијом ацетилхолина у нервним синапсама и могу довести до парализе или смрти. Органотиофосфатни (ОФ) пестициди се користе у великим количинама. Њихови оксоаналози се такође могу наћи у животној средини услед оксидације. Када се акумулирају у животној средини, испољавају токсичне ефекте на нециљане организме.

Методе: Систематски је анализирана хидролиза ОФ пестицида у различитим pH условима, при чему су процењена њихова неуротоксична дејства. Концентрација испитиваних ОФ пестицида током разлагања праћена је течном хроматографијом ултраперформансе (UPLC). Истовремено, примећено је смањење токсичности третираних узорака мерењем активности ензима AChE.

Резултати: Органотиофосфатни пестициди се брзо разлажу у алкалним воденим растворима, али су веома стабилни у киселим растворима. Хлорпирифос се хидролизује најбрже, а диметоат најспорије. Токсичност раствора ових ОФ пестицида се временом *смањује, што указује на то да се токсичнији производи нису формирали.*

Закључак: Приказани резултати могу пружити добру основу за даље напоре у проналажењу једноставних и ефикасних метода за разградњу ОФ пестицида.

Кључне речи: органофосфати, пестициди, pH стабилност, токсичност.

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