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Removal of chlorpyrifos insecticide in constructed wetlands with different plant species

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A B S T R A C T

The objective of this study was to evaluate the remediation of water containing the insecticide chlorpyrifos by using constructed wetlands (CW) cultivated with *Polygonum punctatum*, *Cynodon* spp. and *Mentha aquatica*, operated under different hydraulic retention times: 24, 48, 96, 144 and 192 h. The system efficiency was based on reduction of the initial concentration of chlorpyrifos and toxicity of the contaminated water. The results showed that constructed wetlands are an excellent alternative for remediation of the insecticide chlorpyrifos in aqueous medium. It was observed that the average overall removal efficiency of the insecticide was 98.6%, and in the first hydraulic retention time, 24 h, chlorpyrifos was removed to levels below the detection limit in all CW. This result is mainly attributed to adsorption and microbial degradation. For the qualitative standard acute toxicity tests with *Daphnia similis*, for most samples there was a reduction in toxicity greater than 80%. It was reported that the ecotoxicological tests with the effluents of the constructed wetland are a good option as an indicator of the effectiveness of treatments and a promising alternative to complement the physical and chemical analyses.

Palavras-chave:
biorremediação
fitorremediação
organofosforado
pesticidas
toxicidade

Remoção do inseticida clorpirifós em sistemas alagados construídos cultivados com diferentes espécies vegetais

R E S U M O

Objetivou-se neste trabalho avaliar a remediação de água contendo o inseticida clorpirifós, por meio de sistemas alagados construídos (SAC) cultivados com as espécies *Polygonum punctatum*, *Cynodon* spp. e *Mentha aquatica* operados sob diferentes tempos de retenção hidráulica: 24, 48, 96, 144 e 192 h. A eficiência do sistema foi baseada na redução da concentração inicial do clorpirifós e toxicidade da água contaminada. Os resultados mostraram que os sistemas alagados construídos são uma excelente alternativa na biorremediação do inseticida clorpirifós no meio aquoso. Observou-se média geral de eficiência na remoção do inseticida de 98,6%, sendo que no primeiro tempo de retenção hidráulica, 24 h, houve remoção do clorpirifós para valores inferiores ao limite detecção para todos os SAC. Tal resultado é atribuído principalmente aos processos de adsorção e degradação microbiana. Para os ensaios padronizados qualitativos de toxicidade aguda com *Daphnia similis*, para a maioria das amostras observou-se a redução de toxicidade acima de 80%. Constatou-se que os testes ecotoxicológicos com os efluentes dos sistemas alagados construídos estudados são uma boa opção como indicativo da eficiência dos tratamentos e uma promissora alternativa na complementação de análises físicas e químicas.

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Introduction

Maintaining elevated levels of production linked with intensive land use makes the use of pesticides essential in agricultural areas. Among the organophosphate insecticides, chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is highlighted, which is globally produced and widely used both in agriculture and in urban areas. Chronic human exposure can cause long-term mutagenic, neurological, neuropathic, encephalopathic and visual disturbance effects (Agudelo et al., 2012).

In many studies, the successful use of constructed wetland (CW) has been verified as a low cost alternative for the treatment of wastewater (Matos et al., 2009), including runoff (Mustafa et al., 2009; Maillard et al., 2011).

Efficiency of the constructed wetland system can be influenced by several factors such as temperature, hydraulic retention time (HRT) and the plant species used. This one plays an important role on the biodegradation process, and its main functions include extracting substances, oxygen transferring to the substrate, supporting the growth of the bacteria biofilm, improved permeability of the substrate and aesthetics of the environment (Matos et al., 2009).

The high efficiency of constructed wetlands for removing organic matter and some nutrients such as N, P and K has been confirmed (Saeed & Sun, 2011; Ávila et al., 2013). However, studies are still incipient regarding the evaluation of CW for removal of organophosphate insecticides, such as chlorpyrifos.

The present study aimed evaluating the ability for remediation of water containing the insecticide chlorpyrifos by means of constructed wetlands cultivated with the species *Polygonum punctatum, Cynodon* spp. and *Mentha aquatica* for different hydraulic retention times (HRT). Efficiency of the system was evaluated by reduction of the concentration of chlorpyrifos and toxicity of the water previously contaminated with this active ingredient.

Material and Methods

The experiment was carried out in a greenhouse located at the Department of Agricultural Engineering at the Federal University of Viçosa. It was consisted of four pilot scale horizontal subsurface flow constructed wetland, which are referenced in this work as CW. The CW were manufactured of high-density polyethylene (HDPE) under concrete benches, with internal dimensions of 0.35 m high, 0.5 m wide and 2.0 m long, for a total surface area of 1.0 m^2 and total volume of 350 L. The troughs were filled with pea gravel (diameter = 7.0 mm D_{60}/D_{10} ratio = 1.6, void volume of 0.484 m^3 m^{-3}) to a height of 0.30 m, leaving an upper boarder of 0.05 m.

The four experimental units that made up the system were: CW_C without vegetation, CW_T cultivated with tifton 85 grass (*Cynodon* spp.), CW_M cultivated with mint (*M. aquatica*), and CW_K cultivated with knotgrass (*P. punctatum*). Because one of the essential components of the constructed wetland system is the biofilm, before adding the water containing the insecticide chlorpyrifos, domestic sewage was added to provide organic matter to the system as well as nutrients for the crops. Therefore, after construction of the treatment units and implementation of the plant species, all systems were provided with diluted domestic sewage (ratio of 25% sewage and 75% water) for 30 days, followed by the specific treatments. The pesticide-contaminated solution was formulated by adding the commercial insecticide Lorsban 480 BR (48% w/v chlorpyrifos) to treated water, in order to obtain a final concentration of 1.0 mg L^{-1} of the active ingredient.

For treatment of water containing chlorpyrifos, the following hydraulic retention times (HRT) were established: 24, 48, 96, 144 and 192 h. The HRT over 24 h were obtained by recirculating the contaminated water, for example, the 48 h HRT was achieved by recirculating the contaminated water in two-HRT of 24 h, and so on. Three cycles of analysis were performed, where each configuration was an experimental repetition.

Separation of the organophosphorus pesticide chlorpyrifos from water was performed according to the method proposed by Vieira et al. (2007), namely Liquid-Liquid Extraction with Low Temperature Partitioning (LLE-LTP), with adaptations for specific extraction of chlorpyrifos such as proportion between sample and solvent volumes 1:1 (v/v). Analysis of the organic extracts for quantification of chlorpyrifos was performed by gas chromatograph using a Shimadzu model GC-2014 with Ni electron capture detection (GC/ECD), auto injector AOC-20i.

The specie *Daphnia similis* was used to perform the acute ecotoxicological test. The methodology used for the test follow the proposed by the Brazilian Association of Technical Standards (ABNT) (ABNT, 2009).

The experiment was conducted using a split-plot design, where the plots were the treatments (vegetation type or absence of vegetation) and the subplots are the periods, in a randomized block design (RBD), in which each cycle represents a block. The data obtained were subjected to two-way analysis of variance.

Results and Discussion

The method used for quantification by GC presented a detection limit of 10 μg L^{-1}. In samples it was not possible to identify the peak referring to the insecticide chlorpyrifos, assuming its concentration was equal to the detection limit for statistical analysis of the data, i.e., equal to 10 μg L^{-1}, as in the study of Matamoros et al. (2007).

As can be seen in Table 1, it was not necessary to apply the mean comparison test, since interaction of the F-test was not significant.

Table 1. Analysis of variance for the efficiency data in the removal of the insecticide chlorpyrifos in the studied constructed wetland (plot) during the hydraulic retention times (subplots)

SV	DF	SS	MS	F	
Blocks	2	15.1508	7.5754	1.4296	ns
CW	3	15.8969	5.2990	1.0000	ns
Residual (a)	6	31.7938	5.2990		
Plots	11	62.8415			
HRT	4	17.3386	4.3346	0.9544	ns
CW x HRT	12	55.3266	4.6106	1.0152	ns
Residual (b)	32	145.3303	4.5416		
Total	59	280.8370			

ns - Non-significant ($p > 0.05$); CW - Constructed wetlands; HRT - Hydraulic retention time; SV - Source of variation; DF - Degrees of freedom; SS - Sum of squares; MS - Mean square

Table 2 presents the average values of insecticide removal efficiency in the four constructed wetlands as a function of the hydraulic retention times.

Although no statistical differences among treatments were observed, there is high efficiency of pesticide removal, with overall average of 98.6% and individual averages ranging from 93 to greater than 99% for removal of chlorpyrifos by the constructed wetlands.

Agudelo et al. (2010), when studying the removal of chlorpyrifos in subsurface horizontal flow constructed wetland cultivated found similar results with reed (*Phragmites australis*). The authors found an average removal efficiency of 96.2% with values ranging from 92.2 to 97.4%, not addressing the influence of time on the results. The high efficiency of the four constructed wetlands in this study may be attributed to the junction of construction factors, such as water depth, horizontal subsurface flow, particle size of the support medium, and the functions performed by each constituent system (support medium, plants, biofilm, radiation) (Agudelo et al., 2010), as well as the characteristics of the studied insecticide.

The initial process of chlorpyrifos removal in the CW is its adsorption on plant roots and the support medium (Budd et al., 2011). In this experiment, sewage was added before initiating the treatments, creating a biofilm and certainly favored the removal of chlorpyrifos from the aqueous medium. The biofilm plays an essential role, since the surface of the organic matter allows for greater adsorption of the pesticide. Chlorpyrifos distribution among water and biofilm is a complex process affected by pesticide physico-chemical characteristics. The water solubility, soil organic carbon-water partitioning coefficient (K_{oc}) and octanol/water partition coefficient (K_{ow}) of chlorpyrifos are 1.4 mg L^{-1}, 4.7 (log) and 4981 cm^3 g^{-1} respectively (Dores & De-Lamonica-Freire, 2001). Such characteristics make the organophosphate chlorpyrifos a pesticide with high retention in the constructed wetlands, as suggested by Vymazal & Březinová (2015), that pesticides with high K_{oc} value (log K_{oc} > 3.0) has high removal in CW.

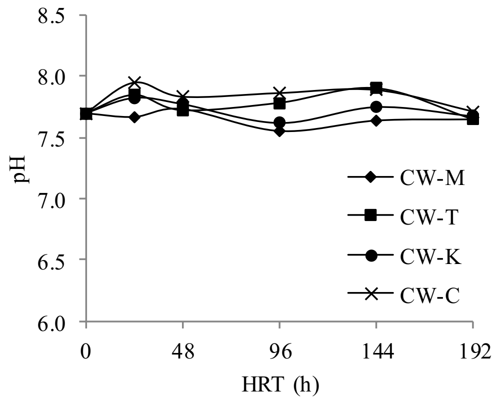
In addition to adsorption, the supply of domestic sewage at the start of the three experimental blocks was crucial for the high removal efficiency, allowing the insertion of an appropriate biota for biodegradation. Biotic and abiotic processes act to promote degradation of the pesticide. In this stage microbial degradation acts intensely, being is the main route of chlorpyrifos degradation. According to Budd et al. (2011), the higher the organic material content in the system, greater is microbial stability.

The pH was measured in samples collected during the experiment and the average values obtained are shown in Figure 1, where the pH of the contaminated water affluent to

Table 2. Chlorpyrifos removal efficiency (%) for the hydraulic retention times

CW	HRT (h)				
	24	48	96	144	192
CW_C	>99	>99	>99	>99	98
CW_K	>99	>99	93	>99	98
CW_M	>99	>99	>99	>99	>99
CW_T	>99	>99	>99	>99	>99

CW_M - Constructed wetland cultivated with mint; CW_T - Cultivated with tifton 85 grass; CW_K - Cultivated with knotgrass; CW_C - Without vegetation; HRT - Hydraulic retention times



CW_M constructed wetland cultivated with mint, CW_T cultivated with tifton 85 grass, CW_K cultivated with knotgrass and CW_C without vegetation

Figure 1. Average pH in the CW effluents in function of the hydraulic retention time (HRTs)

the system was 7.7. It was observed that there were no large variations in pH values of all CW for the retention times, which were maintained between 7.6 and 8.0. This fact benefits microbial activity, where the ideal pH range is from 6.0 to 9.5 for the bacteria activity responsible for organic degradation (Metcalf & Eddy, 2014).

The local temperature also favored the microbial degradation process, since it remained above 20 °C during most of the period, and therefore it was not a limiting factor for bacterial activity. Additionally, the fact that the experiment was conducted in a greenhouse, the internal temperature was higher than the external temperature.

Chlorpyrifos and its main degradation product, TCP (3,5,6-tricloro-2-piridinol) are often considered toxic to microorganisms. However, experiments with constructed wetlands have shown good adaptation of the microbial community to these compounds, since it increases with increasing concentration of the pesticide (Anwar et al., 2009; Karpuzcu et al., 2013). It was found by Agudelo et al. (2011) that the microorganisms continue to grow after application of the insecticide and possibly use it as a carbon source along with TCP, which accelerates the removal of both.

The contaminated water was continuously fed in at the inlet and flowed slowly through the porous medium under the surface of the bed in a horizontal path, with a flow applied of the order of 2.5 L h^{-1} for the HRT of 24 h. This type of flow resulted in elevated contact of chlorpyrifos with the biofilm formed on the roots and support medium and high contact with a network of aerobic, anoxic and anaerobic zones. In general, when compared to other constructed wetland configurations, such as surface flow or vertical flow system, the horizontal subsurface flow system promotes greater efficiency (Vymazal & Březinová, 2015).

Thus, it is clear that microbial degradation was strongly favored in this experiment, with regard to the supply of organic material and biota, pH, temperature and retention time. Biodegradation and adsorption were probably the processes that most contributed to the efficiencies greater than 99% in the first analysis time (24 h) for constructed wetlands planted with tifton 85 grass, knotgrass, mint and without vegetation.

Besides adsorption on the roots and support medium and biodegradation, chemical degradation resultant from hydrolysis also acts to remove chlorpyrifos from the aqueous medium, which is favored in alkaline media (Selvi et al., 2005). The main product of this process is the TCP, which, as already mentioned, can be biodegraded. Karpuzcu et al. (2013) affirmed that the same microorganisms capable of degrading chlorpyrifos act in the degradation of TCP.

The plant species used in this experiment, tifton 85 grass, knotgrass and mint, presented good growth performance during the experiment. However, the constructed wetland system without vegetation (CW_C) did not present statistical difference when compared to the cultivated systems (Table 2). This fact indicates that the retention time of 24 h is sufficient for removal of chlorpyrifos with 99% of efficiency, regardless of whether or not the CW is vegetated.

Despite the higher variation amplitude according to the environmental conditions, the half-life of chlorpyrifos on average is 50 day in water, according with the literature (Dores & De-Lamonica-Freire, 2001). As mentioned previously, in less than 24 h the constructed wetland resulted in 99% removal of chlorpyrifos from the aqueous medium, indicating the potential of these systems their benefits when used, for example, to treat runoff water from agricultural areas.

In Table 2, it can be observed a decrease in average efficiency, from 99 to 93, 98 and 98% in the CW cultivated with knotgrass at retention times of 96 and 192 h, and in the CW without vegetation at the HRT of 192 h, respectively. Possibly, it is due to the desorption process. Thus, chlorpyrifos molecules that were adsorbed on the support medium, roots and biofilm or even sediment were liberated in the passage of recirculated water and were carried away, which resulted in increased concentration of chlorpyrifos in these samples. However, this phenomenon did not significantly compromise the efficiency of the systems.

Therefore, according to the presented data, it can be inferred that the constructed wetland for this study are capable of removing the insecticide chlorpyrifos from the aqueous medium, with efficiency exceeding 99%, at a retention time of 24 h. These systems can be a potential alternative to treat runoff water from agricultural areas contaminated with chlorpyrifos.

Toxicity assays

For qualitative standardized acute toxicity tests of chlorpyrifos in the affluent contaminated water, whose concentration was 1 mg L^{-1} of the insecticide, high toxicity was observed, with death of 100% of the organisms immediately after exposure. This toxicity is linked to inhibition of the enzyme acetylcholinesterase caused by the action of chlorpyrifos (Cáceres et al., 2007). For the effluent samples from the system, in line with the results of removal efficiency for chlorpyrifos presented earlier, high reduction of toxicity to organisms was evidenced, as it can be observed in Figure 2, where the symbol * represents a significant difference with the control ($p \leq 0.05$), characterizing these samples as toxic and the bars are standard deviation.

Note that for the systems cultivated with mint (CW_M), tifton 85 grass (CW_T) and the non-vegetated (CW_C), at the retention time of 24 h, the samples remained toxic (Figure 2). In addition, for the first two systems, CW_M and CW_T, they maintained 100% toxicity. Concentration of chlorpyrifos in these samples (Table 2) is less than 10 μg L^{-1}. However, given the sensitivity of these organisms, many other factors possibly led to this result, such as concentration of toxic substances to *Daphnia*, including chlorpyrifos degradation products, organic acids and even pathogenic microorganisms.

The CW cultivated with knotgrass presented toxicity in the effluent corresponding to the time of 48 h. In the case that domestic sewage causes toxicity in the first HRTs, the CW_K attenuated this effect at the beginning, which was not observed in the HRT of 48 h. Another possibility is the release of a root exudate, such as organic acids, in this HRT.

For the CW_K, which presented 100% toxicity for the retention time of 192 h, the active factor was most likely the

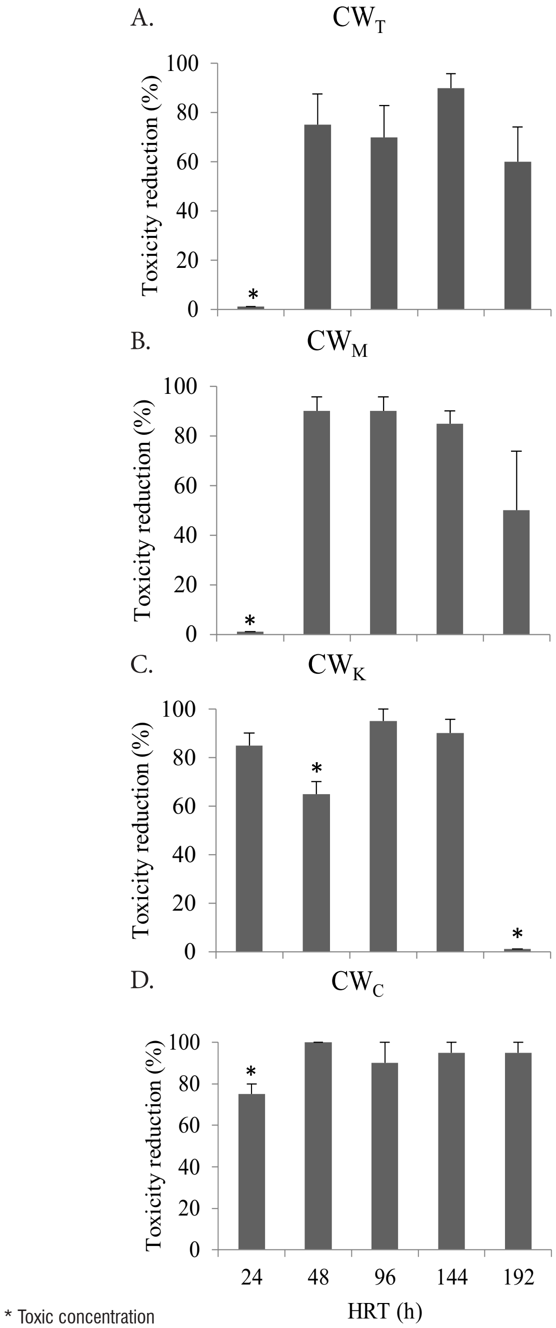


Figure 2. Average results of the toxicity test for the samples after treatment in the constructed wetlands (CW). (A) CW_T cultivated with tifton 85 grass; (B) CW_M cultivated with mint; (C) CW_K cultivated with knotgrass and (D) CW_C without vegetation

presence of the main compound resulting from chlorpyrifos degradation, TCP. In a study performed by Karpuzcu et al. (2013), evaluating the degradation of chlorpyrifos in constructed wetland, they found that TCP reaches its peak concentration after 7 days of chlorpyrifos application. This fact is similar to the toxicity presented in the HRT corresponding to the 8 days after application (192 h). Cáceres et al. (2007), when analysing the toxicity of chlorpyrifos and TCP to *Daphnia carinata*, found that TCP is significantly more toxic to *Daphnia* spp. that chlorpyrifos, indicating that this may have influenced toxicity at the time of 192 h.

It can also be observed (Figure 2) that in the CW_M and CW_T, although they remained non-toxic by the statistical analysis, a reduction in toxicity for the HRT of 192 h was confirmed. This reduction was not observed in the non-vegetated CW, which corroborates with the hypothesis that in this CW biodegradation is less, even though removal of chlorpyrifos from the aqueous medium was the same as in the other CW. This assumption also suggests that the degradation of chlorpyrifos occurs at different rates in vegetated CW, being more pronounced in the CW cultivated with knotgrass. This fact confirms that the aerenchyma present in the knotgrass can positively influence the degradation process of chlorpyrifos, transferring oxygen to the root zone.

The high sensitivity of *Daphnia similis* and the fact that this organism is abundant in natural aquatic ecosystems make its application in tests with chlorpyrifos an excellent indicator of water contamination and efficiency of treatment systems. Thus, ecotoxicological tests with effluents of constructed wetland are presented as a promising alternative to complement the physical and chemical analyses. For bioremediation of pesticides in particular, these assays are extremely useful, since their degradation compounds may exhibit greater toxicity than the parent compound, as is the case chlorpyrifos, indicating that quantitative analysis of the pesticide in question alone is not sufficient to conclude about its environmental impact.

Conclusions

1. In the present study, it was demonstrated that the constructed wetlands are a promising alternative for bioremediation of the insecticide chlorpyrifos in aqueous medium and can be applied from treatment of runoff water from agricultural areas.

2. For the hydraulic retention time of 24 h, the removal of chlorpyrifos was below to the detection limit (10 μg L^{-1}), implying an efficiency greater than 99% for the CW_C, CW_M, CW_K and CW_T.

3. Ecotoxicological assays with the effluent of the constructed wetlands showed to be an alternative to complement the physical and chemical analyses, indicating efficiency of the treatments. For CW_M, CW_T and CW_C, no toxicity was observed in HRT higher than 48 h.

Acknowledgments

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