

Electrogenic Biodegradation Study of the Carbofuran Insecticide in Soil

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Abstract

In the development of this project the Carbofuran degradation was studied (2,3-dihydro-2,2-dimethyl-benzofuran-7-yl-methylcarbamate) in soil, during a 28 days period. The trials were made under natural conditions (control) and conditions using electrodes in a sediment microbial Fuel Cell (SMFC). The affinity between the pesticide and the graphite electrode directly conditioned the results obtained in this research. Thus, a percentage of pollutant recovery obtained from the soil was seven times bigger with the natural degradation treatment not electrogenically simulated than in the case of the electrogenic treatment. This difference disclosed a low efficiency in the pesticide degradation. Studies of recovering were complemented with ecotoxicity trials, using aquatic algae (*Pseudokirchneriella subcapitata*). The growth inhibition in all treatments decreased with the incubation time. For the control treatment, in the absence of electrodes, and despite not detecting Carbofuran at the end of the test, an inhibition of the algal growth of 37.6% was reached. This result suggested the presence of toxic metabolites in the soil extract, probably because of a partial degradation of the pollutant.

Keywords: Furadan, Ecotoxicity, Sedimentary Microbial Full Cell (SMFC).

INTRODUCTION

Despite the efforts made in order to find non-chemical methods for the management of pests, pesticides continue being an important tool in the agriculture development and their use has contributed to the food production and raw materials. Carbofuran (2,3-dihydro-2,2-dimethyl-benzofuran-7-yl-methylcarbamate) is a pesticide N-methylcarbamate (N-MC), a crystalline and colorless solid. Its solubility in water is relatively high with respect to other pesticides (330 mg/L), being soluble in other solvents such as acetone, ethyl acetate, acetonitrile and benzene. Carbofuran vapor pressure is 2.7 mPa at 33°C and the octanol/water partition coefficient is relatively low (1.7) (Evert, 2002) (Saxena, Ashok, & Musarrat, 1997). The chemical structure is as shown in figure 1. Although its commercialization is prohibited in Colombia, it is usually used in its granular presentation as Furadan® 3GR, with an active ingredient content of 3% Carbofuran.

In Colombia, this product is used to control pests in various crops, such as coffee, sugar cane, cereals, fruits, vegetables and tubers as potatoes, being this insecticide a basic necessity in the potato farming, a situation that has led farmers not only to a

permanent application, but also to increase progressively the dose and the number of applications (Chaparro García, Quijano Parra, Rodríguez Martínez, & Lisarazo Gutiérrez, 2017). Farmers are constantly in search of greater efficiency on the pesticide action, applying excessive doses which exceed up to five times the recommended doses by the manufacturer (Bonilla, Peinado, Urdaneta, & Carrascal, 2000) (Valencia, Valencia Ch, & Bravo, 2010).

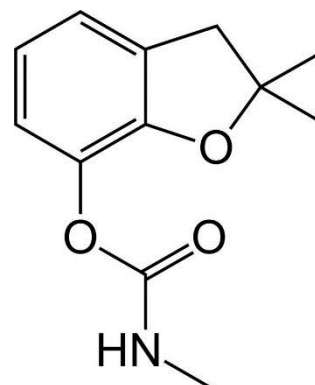


Figure 1. Carbofuran Structure. Adapted from: <https://scifinder.cas.org>

Carbofuran is classified in the toxicity range as moderate-high, therefore, it is considered highly toxic by inhalation ingestion and moderately toxic by dermal absorption (Hayes & Jr, 1982). Additionally, it was founded that the insecticide has a relatively weak mutagenic capacity (Saxena, Ashok, & Musarrat, 1997). According to the EPA, the use of Carbofuran carries considerable risks to the environment and human health (EPA, 2009).

Results of a research study conducted by the environmental hazard assessment groups of California and the Department of Food and Agriculture, revealed the presence of Carbofuran in the runoff water from the rice fields and in the Sacramento River (Nicosia, Carr, Gonzales, & Orr, 1991). Consequently, Carbofuran reaches the aquatic ecosystem and organisms face toxic exposure when it is adsorbed (Guptua, 2011) (Rodriguez, 2015) (Pinilla, 2017). Carbofuran is less common in groundwater than other pesticides. The main degradation route is hydrolysis (Figure 2), which generates several products, some of the most toxic are 3-hydroxycarbamate and 2,3-dihydro-2,2-dimethyl-benzofuran-7-ol, the rest of the derivatives are less toxic than Carbofuran (Castillo, Rojas, Solito, Nardelli, & Guasch, 2003).

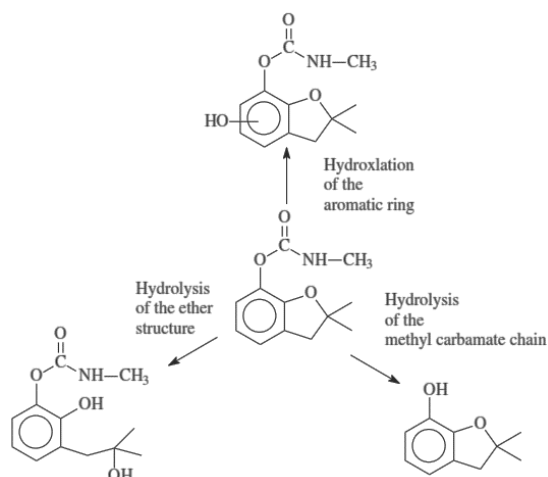


Figure 2. Carbofuran Biodegradation by the bacterium *Novosphingobium FND-3*. (Yan et al., 2007)

Carbofuran undergoes various physical, chemical and biological processes, when it is on the soil. Adsorption-desorption processes are the most important, since they influence on the pesticide degradation, transport, transformation, persistence and bioaccumulation in soil (Scow, 1993) (De Jonge, Breure, & Van Anandel, 1996). The pesticides adsorption in soil colloids prevents the accumulation of pesticides in surface and groundwater, as well as preventing their volatilization and biodegradation (Krishna & Philip, 2011). However, the pesticides desorption in soils facilitates their runoff and leaching into surface and groundwater, which makes the pesticide available to soil microorganisms, thus facilitating their biodegradation. The content of organic matter in the soil and the clay percentage are the most influential factors in the Carbofuran adsorption and desorption phenomena (Yazgan, Wilkins, Sykas, & Hoque, 2005) (Arias-Estevéz, López-Periágo, Martínez-Carballo, & Simal-Gándara, 2006) (Liyanage, Watawala, Aravinna, Smith, & Kookona, 2006). In addition, it has been observed that the Carbofuran adsorption is not influenced by the pH variation between 2 and 8 range. It was also observed that in soils enriched with compost the adsorption was maximum and the degradation was minimal, as well as a greater degradation was observed in submerged soils compared to unsaturated soils (Gupta, Ali, & Saini, 2006).

The biochemical (Figure 2), photochemical (Figure 3) and chemical (Figure 4) degradation control the environmental persistence of Carbofuran. One of the most effective ways to eliminate Carbofuran is microbial degradation. Previous studies reported the discovery of microorganisms capable of degrading Carbofuran and other pesticides from contaminated natural matrices (Yan, et al., 2007), (Bano & Musarrat, 2004). These degrading microorganisms might use the pesticide as their energy source (Plangklang, Reungsang, & Suphannafai, 2012).

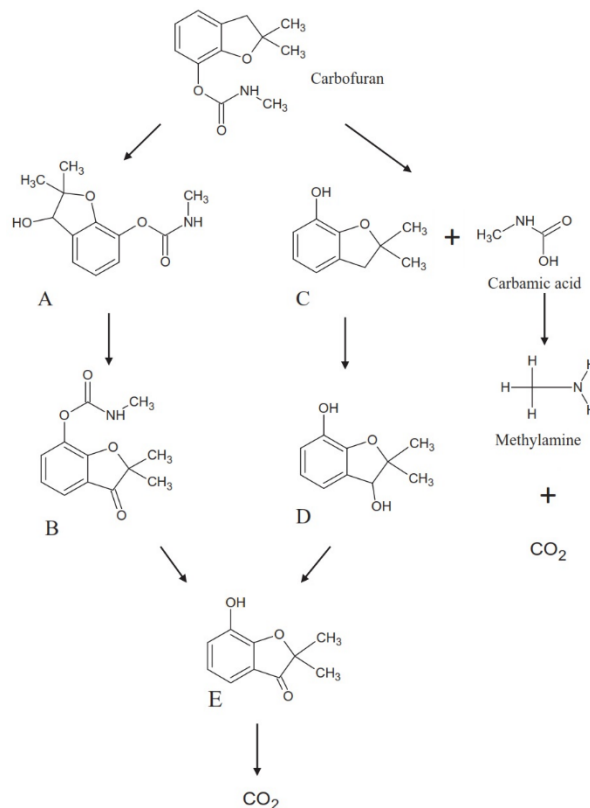


Figure 3. Principal ways of Carbofuran photochemical degradation. (Castillo, Rojas, Solito, Nardelli, & Guasch, 2003)

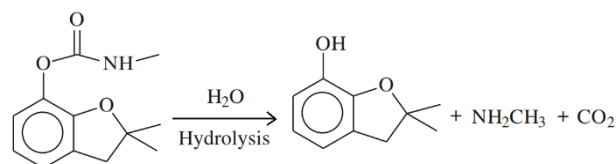


Figure 4. Hydrolysis of Carbofuran.

Hydrolysis is an alternative effective route of degradation, with average life times in waters between 2 days, at pH = 9.5, and 1700 days, at pH = 5.2 (CCME., 1999). Besides the pH, temperature has a direct influence on the degradation rate in hydrolysis.

Direct photolysis and photooxidation (Figure 3) by the free radicals mechanism, constitute an important degradation route of Carbofuran, since in laboratory were observed studies with a significant photo-decomposition over 96 hours (Lopez-Alvarez, Torres-Palma, & Peñuela, 2011)

Renewable energies in the future, will probably play a key role in global energy production. Current predictions for global energy have led to seek alternative energy sources, since nowadays non-renewable energy resources experience a consumption rhythm higher than the predictions raised. Therefore, this situation induce to a need for high efficiency energy transformation technologies and the use of alternative sources of renewable energy. (Tonia Tommasi & Giorgia

Lombardelli, 2017).

Microbial fuel cell technology represents a new alternative in water purification and renewable energy production. This technology mainly uses anaerobic bacteria which may already be present in wastewater. These microorganisms perform the purification and simultaneously they work as catalysts to generate electricity (Aziz, Memon, Shah, Soomro, & Parkash, 2013). The significant research aspects of microbial fuel cells are first to reduce the treatment cost and then simplify the conditions of process implementation (Min, Kim, Oh, Regan, & Logan, 2005).

The sedimentary microbial fuel cells (Figure 5), are a variant of the microbial fuel cells, where a first electrode (anode) is housed in the anaerobic sediments, and above this area is located a second electrode (cathode) on a water suspension, forming an aerobic environment (Hamdan Z, Darine A, Ananda Rao Hari, Lucy Semerjian, & Pascal Saikaly, 2017). In the sediment, the microorganisms oxidize the organic and inorganic matter, allowing the electrons produced during the oxidation be transferred from the microorganisms to a conductive surface (anode). The electrons that reach this conductive material are transferred to the cathode which is in the aerobic zone. In this area the oxygen is reduced by the acceptance of these electrons, combining with the protons that arrive through the solution in order to form water as a result of the whole process.

The difference in potential generated between both oxidation-reduction reactions allows this process to be used as electric current. (Donovan, Dewan, Heo, Lewandowski, & Beyenal, 2013) (Zhang, Tian, & He, 2001); Moreover, the SMFC may also be explored as a new technology for the organic pollutants elimination from sediments (Hong, Kim, & Chung, Alteration of sediment organic matter in sediment microbial fuel cells, 2010), (Yan, Song, Cai, Tay, & Jiang, 2012).

Therefore, this resistance generates a smaller potential difference. Several experiments have been carried out to improve the performance of the SMFC through the external resistance optimization (Song & Jiang, 2011), the improvement of the sediment conductivity (Babu & Mohan, 2012), and the materials modification in the electrodes and their configurations (An, Kim, Nam, & Chang, 2013); (Hong, Chage, Choi, & Ching, 2009); (Karra, et al., 2013) (Rodrigo, Boltes, & Esteve-Nuñez, 2013). It was also observed that the addition of electron donors such as glucose or cellulose to the sediments, allowed a better performance of the SMFC by increasing the substrate availability for the respiration of microorganisms at the anode (He, Shao, & Angenent, 2007); (Rezaei, Richard, & Logan, 2008). Voltages of up to 190 mV (2162 mW/m³) were achieved in a SMFC, whose anode is buried in sediments that contain approximate concentrations of 16,000 mg/Kg of TPH (Total Hydrocarbons derived from Petroleum). After 66 days, the degradation rates of TPH were 2% in the open control circuit and 24% in the active SMFC. Thus, it should be noted that SMFC technology in contaminated matrices accelerates (12 times) the natural biodegradation of soil compounds (Morri & Jin, 2012).

METHODOLOGY

The soil used during the experimental development, was obtained from the rice fields located in the town of Calasparra (Murcia-Spain). This soil was characterized in previous work developed in the Bioelectrogenesis Laboratory at the University of Alcalá. (Dominguez, 2012), (Rodrigo, Boltes, & Esteve-Nuñez, 2013).

Figure 6 shows the bioelectrogenic cells configuration, which are constituted by plastic with the following dimensions: 4.0 x 6.5 x 3.5 cm, and inside a total of 60 g of dry soil was housed. The materials used for the electrodes formation were: graphite sheet (Mersen) (3.0 x 3.0 x 0.5 cm) in the case of electrodes that function as an anode; carbon felt (Mersen) (4.0 x 6.5 x 3.5 cm) in the case of electrodes that function as a cathode, which contains a surface area of 0.7 m²/g.

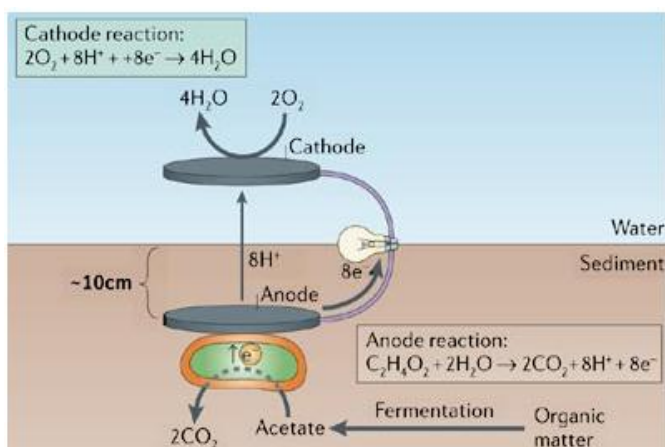


Figure 5. Sediment microbial fuel cell (Lovley, 2006)

Due to several limiting factors such as the mass-substrate transfer in the sediments and the low electrical conductivity, especially in fresh water, the internal resistance in a SMFC is generally higher than in a conventional MFC (He, Shao, & Angenent, 2007) (Song & Jiang, 2011).

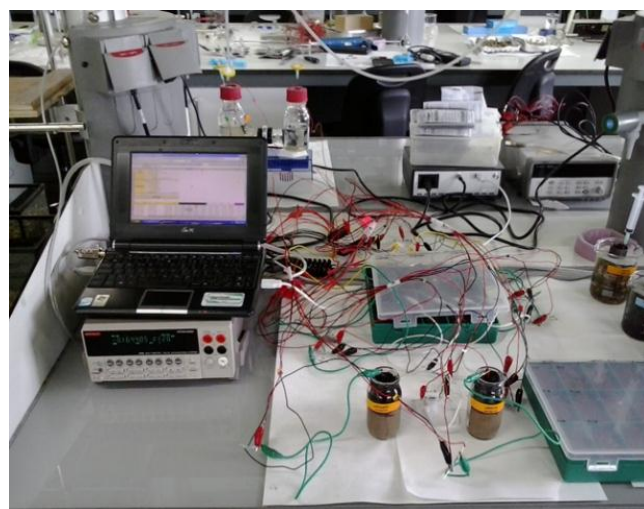


Figure 5. Experimental setup.

The connections between electrodes and the copper conductor cables were made by applying an Epoxy conductor resin from Circuit Works, followed by an insulating resin. The anode was buried 1cm from the surface and the floor was left covered with a water sheet, anode and cathode were finally connected through a 220 KΩ resistor and monitored by a multimeter (Keithley 7700).

The contaminated soil was subjected to five types of treatment over three different incubation times (2,16 and 28 days), the procedure was duplicated. The different treatments are described below.

Treatment 1: Soil under conditions of natural sterilized degradation (Es), without electrodes, with application of a 500 ppm solution of HgCl₂.

The 10 mL dose of HgCl₂ solution was applied on days 1 and 18 counted from the beginning of the experiment. Treatment 2: Soil under conditions of natural degradation (N) and without electrodes. Treatment 3: Soil under conditions of natural degradation with not connected electrodes (NCE). Treatment 4: Soil under conditions of electrogenic degradation (EI), with anode and cathode connected to each other through a conductor cable. Treatment 5: Soil under conditions of sterilized electrogenic degradation (SEs) with a solution of HgCl₂, at the same times as treatment 1.

The pollutant application was carried out 26 days after the cell installation, 10 mL of a 15 ppm solution of Carbofuran (Standard 98%, SIGMA-ALDRICH).

The Carbofuran extraction in soils was performed by mechanical agitation for 24 hours, using 120 mL of ethyl acetate as solvent. The obtained extracts were passed through filters (Prat Dumas 20 μm) with anhydrous sodium sulfate, then these extracts were taken to dryness and dissolved in 10 mL of methanol, successively with methanol they were used for the quantification of the Carbofuran concentration with HPLC.

The methanoic extracts were analyzed by high precision liquid chromatography (HPLC) coupled to a diode beam detector (Varian), and controlled by the Galaxie software. Table 1 summarizes the working conditions in the HPLC analysis.

Table 1. HPLC conditions

Conditions in the HPLC analysis	
Column	C18 Phenomenex Kromasil 150x4.6mm, 5um.
Injected volume	50 μL
Flow	0,7 mL/min
Mobile Phase	60% Acetonitrile- 40% Water
Wavelength	(200-300) 210 λ
Retention Time	5 min

In order to quantify the toxic effect of the Carbofuran degradation derivatives, the algae growth (*Pseudokirchneriella subcapitata*) was figured, which were exposed to the substances in the different extracts, calculated during a 96 hours period.

Additionally, to have cells in exponential growth phase, a culture was prepared 72 hours before the ecotoxicity test start. For this, the algae were reconstituted following the protocol established by the manufacturer, and they were taken to 25mL of culture media, composed of deionized water and specific nutrients for its growth. It was kept in the incubator at 23°C and in a soft agitation for three days until its use in the test.

Volumes of contaminated soil extracts with Carbofuran were taken to dryness and resuspended with phosphate buffer at pH 7, and afterwards they were used in the toxicity tests. A 1:10 dilution of mentioned extracts was carried out in order to observe the algae growth, this dissolution ratio was made taking into account the LC50, calculated in previous studies 2.5 ppm (Iesce, and others, 2006).

After three days of algae incubation and before starting the test, the culture optical density was estimated through a spectrophotometer (Shimadzu UV-VIS 1800) at a wavelength of 670 nm, with the aim of adjust the number of cells per milliliter needed (20,000-50,000 cc/mL) for the ecotoxicity test start. The analysis was performed following the indications of the OECD 201 Guide Test, in 96-well plates. All the samples and controls established in the protocol were tested in four replicates.

The algae growth was followed by a Luminometer-Fluorimeter FLUOROSKAN FL, (Thermo Fisher), measuring the chlorophyll emission at a wavelength of 680nm and an excitation of 440nm. The measurements were made immediately after the plate preparation, and in a 24 hours interval time over four days.

The inhibition that an organism can suffer in the presence of a certain substance is given as:

$$\% \text{ Extract inhibition} = \frac{\text{GRC} - \text{SGR}}{\text{SGR}} * 100$$

GRC= Growth rate of the positive control containing uncontaminated medium.

SGR = Sample growth rate with contaminated extract

RESULTS

The results obtained from the soil characterization used are shown in Table 2.

Table 2. Soil Physicochemical characteristics

Parameter	Results
Sand (%)	37,09
Silt (%)	23,21
Clay (%)	39,63
Texture	Clay Loam
Organic Matter (%)	0,17
Calcium Carbonate (%)	52,03
Humidity (%)	39,00
pH	8,01
Electric Conductivity (mS/cm, 25°C)	241,50

From the data in Table 2, the CLAY LOAM texture is highlighted, this characteristic indicates a high water retention capacity. The slightly alkaline pH is associated with the high carbonate content. Microorganisms are not significantly affected at this pH, the activity is reduced to pH values lower than 5.5 (Navarro Blaya & Navarro García, 2003). Organic matter is in a low range, this figure probably influenced the electrogenic response, because of current and degradation are associated with the microorganisms activity.

The extraction carried out 48 hours after the soil exposure to the Carbofuran (Table 3), showed that at least the soil has the capacity to irreversibly adsorb 20% of the Carbofuran. This contaminant retention is directly related to the percentage of clays. There was no a significant difference between treatments (<2%), ruling out a possible influence of microbiological activity on extraction capacity.

Table 3. Percentage of Carbofuran extraction, 48 hours after application.

Treatments	Percentage of Extraction (%)
Sterile	53,9
Natural	51,7
Natural with Electrodes	68,3
Electrogenic	77,6
Sterile Electrogenic	80,9

The high percentage of extraction for the sterile treatment (Table 3), indicated the efficiency of the extraction technique to recover the Carbofuran applied in the soil. The extraction values in all those treatments in the presence of electrodes,

(Natural with Electrode, Electrogenic and Sterile Electrogenic) indicated very high recovery percentages.

These results led to perceive a greater Carbofuran affinity with the graphitic material of the electrode than for the soil particles, which would suppose a lower bioavailability of the pesticide. However, the results showed that such contaminant adsorbed on the electrode, is obtained in a quite efficient way through the extraction technique employed.

The pesticide affinity for graphite electrodes is due to its porosity and high contact surface, which adsorbs carbofuran through electrostatic interactions with its surface functional groups (carbonyl, hydroxyl, carboxyl and ether). A recent study about the water treatment contaminated with Carbofuran, by adsorption with activated carbon, revealed a great pollutant adsorption capacity (296.5 mg of Carbofuran/g of activated carbon). This study confirmed the pesticide affinity for a carbonaceous material similar to graphite (Chang et al., 2014) from the extractions carried out on days 16 and 28, after the Carbofuran application, the percentage of extraction was calculated and the Carbofuran recovery percentage was determined for each treatments (Table 6). The values observed after 28 incubation days revealed that the highest recovery percentages were for sterile and electrogenic sterile treatments. These high recovery values are associated with a low Carbofuran degradation as a consequence of biological inactivity in the soil. The reduction of recovery values with respect to time in sterile treatments may be related to chemical and photochemical degradations explained above.

The natural treatment with a 0% recovery, showed the greatest transformation of the entire trial, even greater than the electrogenic and natural treatments with the presence of electrodes. This result is explained by the Carbofuran affinity of towards the graphite electrode, therefore this behavior could decrease the microorganisms interaction with the contaminant, reducing its bioavailability and, hence, resulting in a lower biodegradation rate.

Table 4. Carbofuran Percentage recovered after 16 and 28 days of the application.

Treatments	% Recovery (16 days)	% Recovery (28 days)
Sterile	95,9	92,4
Natural	0,00	0,00
Natural with Electrodes	60,2	31,7
Electrogenic	71,8	69,5
Sterile Electrogenic	89,1	82,2

The results obtained in the ecotoxicity tests (Table 5), revealed that although Carbofuran was not detected in the natural treatment, an inhibition of 37% persisted after 28 days of the test. This value is explained by considering the presence of toxic metabolites of Carbofuran still present in the sample. The decrease of the inhibition percentage with respect to time in all

the treatments indicated that the inhibition is directly related to the decrease in the contaminant concentration. The above statement is corroborated with the high inhibition values presented by sterile, electrogenic and sterile electrogenic treatments, which correspond to the highest percentages of Carbofuran recovery.

Table 5. Percentage of inhibition in the Alga growth (*P. subcapitata*) in several soil extracts.

Time (Days)	S(%)	N(%)	NWE(%)	EI(%)	SEI(%)
2	100	100	100	100	100
16	100	100	100	45,1	43,2
28	49,1	37,6	25,9	54,9	45,9

Although the electrogenic biodegradation efficiency in soil has been demonstrated with other contaminants such as atrazine, and DBT with graphite plates (Dominguez, 2012), (Rodrigo, Boltes, & Esteve-Nuñez, 2013); the chemical nature of the pollutant seems to be decisive when choosing the material of the anode and avoiding undesirable interactions. In future investigations, for the Carbofuran degradation in soils with electrogenic methods, it is recommended to explore new materials in the electrodes, which allow a improved interaction of the contaminant with the microorganisms.

CONCLUSIONS

Carbofuran shows an affinity for the graphite electrode (anode) in both connected and disconnected systems at a cathode. Thereby, soil microorganisms reduce the contaminant bioavailability and the Carbofuran biodegradation is hindered.

The insecticide transformation is not favored under electrogenic conditions after 28 days of testing. The residual levels at the end of the test turned out to be seven times higher compared to the natural degradation.

The ecotoxicity at the end of the treatments, determined by the inhibition in the alga growth (*Pseudokirchneriella subcapitata*), showed a decrease tendency with respect to the time, for all the treatments evaluated. Presenting the greatest reduction in toxicity in the Natural Treatment With Electrodes (not connected), which was reduced 4 times compared to the initial inhibition.

The treatment subjected to natural degradation presented a complete transformation of Carbofuran after 28 days, but the soil toxicity was only reduced by 70%, this suggests the presence of intermediate metabolites of Carbofuran degradation capable of conferring toxicity to the soil. The elimination of these intermediate compounds must be evaluated to achieve an efficient treatment of contaminated soil.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

- [1] An, J., Kim, B., Nam, J., & Chang, I. S. (2013). Comparison in performance of sediment microbial fuel cells according to depth of embedded anode. *Bioresour. Technol*, 127, 138–142.
- [2] Arias- Estevez, M., López-Periago, E., Martínez-Carballo, E., & Simal-Gándara, J. (2006). Carbofuran sorption kinetics by corn crop soils. *Bulletin of environmental contamination and toxicology*, 77(2), 267-273.
- [3] Aziz, S., Memon, A. R., Shah, S. F., Soomro, S. A., & Parkash, A. (2013). Prototype designing and operational aspects of microbial fuel cell-review paper. *Science International*, 25(1).
- [4] Babu, M. L., & Mohan, S. V. (2012). Influence of graphite flake addition to sediment on electrogenesis in a sediment-type fuel cell. *Bioresour. Technol*, 110, 206–213.
- [5] Bano, N., & Musarrat, J. (2004). Characterization of a novel carbofuran degrading *Pseudomonas sp.* with collateral biocontrol and plant growth promoting potential. *FEMS microbiology letters*, 231(1), 13-17.
- [6] Bonilla, J. P., Peinado, J. E., Urdaneta, M. A., & Carrascal, E. (2000). *Informe Nacional sobre el uso y manejo de plaguicidas en Colombia, tendiente a identificar y proponer alternativas para reducir el escurrimiento de plaguicidas al Mar Caribe*. Ministerio del Medio Ambiente, Bogotá.
- [7] Castillo, A. E., Rojas, J., Solito, R. M., Nardelli, I., & Guasch, G. (2003). Métodos para determinar carbofuran (2,3 -dihidro-2, 2-dimetilbenzofuran-7-il metilcarbamato). *Agrotecnia*, 10, 15-20.
- [8] Chang, K., Chen, C., Lin, J., Hsien, J., Wang, Y., Zhao, F., . . . Chen, S. (2014). Rice straw-derived activated carbons for the removal of carbofuran from an aqueous solution. 29(1), 47-54.
- [9] Chaparro García, A. L., Quijano Parra, A., Rodríguez Martínez, R., & Lisarazo Gutiérrez, L. F. (2017). Desarrollo y validación de un método ambientalmente amigable para la determinación de carbofurano en suelos. *Corpoica Ciencia y Tecnología Agropecuaria*.
- [10] De Jonge, R. J., Breure, A. M., & Van Andel, J. G. (1996). SDe Jonge, R. J., Breur Reversibility of adsorption of aromatic compounds onto powdered activated carbon (PAC). 30(4), 883-892.

- [11] Dominguez, A. G. (2012). *Biodegradación electrogénica del herbicida Atrazina en suelos*. Universidad de Alcalá, Universidad Rey Juan Carlos.
- [12] Donovan, C., Dewan, A., Heo, D., Lewandowski, Z., & Beyenal, H. (2013). Sediment microbial fuel cell powering a submersible ultrasonic receiver: New approach to remote monitoring. *Journal of Power Sources*, 233, 79-85.
- [13] EPA. (2009). *Rules and Regulations*. EPA.
- [14] Evert, S. (2002). Environmental fate of carbofuran. Environmental Monitoring Branch. *Department of Pesticide Regulation*.
- [15] Gupta, V. K., Ali, I., & Saini, V. K. (2006). Absorption of 2, 4-D and carbofuran pesticides using fertilizer and steel industry wastes. *Journal of colloid and interface science*, 299(2), 556-563.
- [16] Gupta, R. C. (2011). *Toxicology of organophosphate & carbamate compounds*. Academy press.
- [17] Hamdan Z, H., Darine A, S., Ananda Rao Hari, Lucy Semerjian, & Pascal Saikaly. (2017). Assessment of the performance of SMFCs in the bioremediation of PAHs in contaminated marine sediments under different redox conditions and analysis of the associated microbial communities. *Science of the Total Environment*, 1453-1461.
- [18] Hayes, W. J., & Jr. (1982). *Pesticides study in Man*. Baltimore: Williams & Wilkins.
- [19] He, Z., Shao, H., & Angenent, L. T. (2007). Increased power production from a sediment microbial fuel cell with a rotating cathode. *Biosensors and Bioelectronics*, 22(12), 3252-3255.
- [20] Hong, S. W., Chage, I. S., Choi, Y. S., & Ching, T. H. (2009). Experimental evaluation of influential factors for electricity harvesting from sediment using microbial fuel cell. *Bioresour. Technol*, 100, 3029-3035.
- [21] Hong, S. W., Kim, H. S., & Chung, T. H. (2010). Alteration of sediment organic matter in sediment microbial fuel cells. *Environmental pollution*, 158(1), 185-191.
- [22] Iesce, M. R., Della-Greca, M., Cermola, F., Rubino, M., Isidori, M., & Pascarella, L. (2006). Transformation and Ecotoxicity of Carbamic Pesticides in Water (5 pp). *Environmental Science and Pollution Research*, 13(2), 105-109.
- [23] Karra, U., Huang, G. X., Umaz, R., Tenaglier, C., Wang, L., & Li, B. K. (2013). Stability characterization and modeling of robust distributed benthic microbial fuel cell. *Bioresour Technol*, 477-484.
- [24] Krishna, K. R., & Philip, L. (2011). Bioremediation of single and mixture of pesticide-contaminated soils by mixed pesticide-enriched cultures. *164*(8), 1257-1277.
- [25] Liyanage, J. A., Watawala, R. C., Aravinna, A. P., Smith, L., & Kookona, R. S. (2006). Sorption of carbofuran and diuron pesticides in 43 tropical soils of Sri Lanka. *Journal of agricultural and food chemistry*, 54(5), 1784-1791.
- [26] Lopez-Alvarez, B., Torres-Palma, R. A., & Peñuela, G. (2011). Solar photocatalytic treatment of carbofuran at lab and pilot scale: Effect of classical parameters, evaluation of the toxicity and analysis of organic by-products. *Journal of hazardous materials*, 191(1), 196-203.
- [27] Lovley, D. R. (2006). Bug juice: harvesting electricity with microorganisms. *Nature Reviews Microbiology*, 4(7), 497-508.
- [28] Min, B., Kim, J., Oh, S., Regan, J. M., & Logan, B. E. (2005). Electricity generation from swine wastewater using microbial fuel cells. *Water research*, 39(20), 4961-4968.
- [29] Morri, J. M., & Jin, S. (2012). Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. *Journal of hazardous materials*, 213, 474-477.
- [30] Navarro Blaya, S., & Navarro García, G. (2003). Química agrícola: el suelo y los elementos químicos esenciales para la vida vegetal. *Mundi-prensa*.
- [31] Nicosia, S., Carr, N., Gonzales, D. A., & Orr, M. K. (1991). Off-field movement and dissipation of soil-incorporated Carbofuran from three commercial rice fields. *Journal of environmental quality*, 532-539.
- [32] Pinilla, L. A. C., Serrezuela, R. R., David, J., Díaz, S., Martínez, M. F., & Benavides, L. C. L. (2017). Natural Reserves of Civil Society as Strategic Ecosystems: Case Study Meremberg. *International Journal of Applied Environmental Sciences*, 12(6), 1203-1213.
- [33] Plangklang, P., Reungsang, A., & Suphannafai, W. (2012). Bioremediation of carbofuran contaminated soil under saturated condition: soil column study. *Biodegradation*, 23, 473-485.
- [34] Reimers, C. E., Tender, L. M., Ferting, S., & Wang, W. (2001). Harvesting energy from the marine sediment-water interface. *Environmental science & technology*, 35(1), 192-195.
- [35] Rezaei, F., Richard, T. L., & Logan, B. E. (2008). Enzymatic hydrolysis of cellulose coupled with electricity generation in a microbial fuel cell. *Biotechnol. Bioeng.*, 101, 1163-1169.
- [36] Rodrigo, Q. J., Boltos, K., & Esteve-Núñez, A. (2013). Microbial-electrochemical bioremediation and detoxification of dibenzothiophene-polluted soil. *Chemosphere*, 61-65.
- [37] Rodríguez Serrezuela, R., & Carvajal Pinilla, L. A. (2015). Ecological determinants of forest to the

abundance of *Lutzomyia longiflocosa* in Tello, Colombia. *International Journal of Ecology*, 2015.

- [38] Saxena , S., Ashok, B. T., & Musarrat , J. (1997). Mutagenic and genotoxic activities of four pesticides: captan, foltaf, phosphamidosn and furadan. *IUBM Life*, 1125-1136.
- [39] Scow, K. M. (1993). Effect of sorption-desorption and diffusion processes on the kinetics of biodegradation of organic chemicals in soil. 73-114.
- [40] Song, T. S., & Jiang, H. (2011). Effects of sediment pretreatment on the performance of sediment microbial fuel cells. *Bioresour. Technol*, 102, 10465–10470.
- [41] Tonia Tommasi, & Giorgia Lombardelli. (2017). Energy sustainability of Microbial Fuel Cell (MFC): A case study. *Journal of Power Sources*, 438-447.
- [42] Valencia , Y. A., Valencia Ch, E. M., & Bravo, I. (2010). *Validation of an analytical method for carbofuran determination on soils by high performance liquid chromatography with uv detection (hplc-uv)*. *Revista Colombiana de Química*.
- [43] Yan, Q. X., Hong, Q., Han, P., Dong, X. P., Shen, Y. J., & Li, S. P. (2007). Isolation and characterization of a carbofuran-degrading strain *Novosphingobium* sp. FND 3. *FEMS microbiology letters* , 271(2), 207-213.
- [44] Yan, Z., Song, N., Cai, H., Tay, J. H., & Jiang, H. (2012). Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide. *Journal of hazardous materials*, 199, 217-225.
- [45] Yazgan , M. S., Wilkins, R. M., Sykas, C., & Hoque, E. (2005). Comparison of two methods for estimation of soil sorption for imidacloprid and carbofuran. *Chemosphere*, 60(9), 1325-1331.
- [46] Zhang, F., Tian, L., & He, Z. (2001). Powering a wireless temperature sensor using sediment microbial fuel cells with vertical arrangement of electrodes. *Journal of Power Source*, 196(22), 9568-9573.