11TH INTERNATIONAL SCIENTIFIC CONFERENCE
ON DEFENSIVE TECHNOLOGIES
OTEH 2024
Serbia, Tara, 9-11 October 2024

ADSORPTION OF ORGANOPHOSPHATE PESTICIDES ON NITROGEN-DOPED CARBON CRYOGELS AND THE ASSESSMENT OF THEIR NEUROTOXIC EFFECTS

VLADAN J. ANIĆIJEVIĆ
University of Defence Belgrade, Military Academy, anicijevicj.v@gmail.com,

TAMARA TASIĆ
VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, tamara.tasic@vin.bg.ac.rs

VEDRAN MILANKOVIĆ
VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, vedran.milankovic@vin.bg.ac.rs

DALIBOR B. JOVANOVIĆ
Organization for the Prohibition of Chemical Eeapons, Hague, The Netherlands, jovcadach@hotmail.com

RADOVAN M. KARKALIĆ
University of Defence Belgrade, Military Academy, rkarkalic@yahoo.com

MILOŠ BALJOZOVIĆ
Surface Science and Coating Technologies, Empa, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland, milos.baljozovic@psi.ch

BILJANA M. BABIĆ
Institute of Physics, University of Belgrade, Belgrade, biljana.babic@ipb.bg.ac.rs;

IGOR A. PAŠTI
Faculty of Physical Chemistry, University of Belgrade, Belgrade, igor@ffh.bg.ac.rs

TAMARA D. LAZAREVIĆ-PAŠTI
VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, tamara@vin.bg.ac.rs

Abstract: *Efficient removal of different pollutants from the environment has become one of the most important challenges of modern society. The nitrogen-doped carbon cryogels were synthesized and characterized using XPS. All investigated materials have similar composition and structural disorder. By analyzing XPS spectra, the content of carbon, oxygen, and nitrogen can be obtained, and the existence of functional groups containing these elements can be detected. Dimethoate, malathion, and chlorpyrifos removal were investigated under stationary and dynamic conditions. Stationary conditions demonstrated successful removal of aliphatic dimethoate and malathion by all investigated materials. Conversely, the materials with the lowest and highest nitrogen content proved ineffective with aromatic chlorpyrifos. Under dynamic conditions, all materials effectively removed malathion and chlorpyrifos while exhibiting suboptimal performance for dimethoate adsorption. The demonstrated efficacy suggests the potential application of these materials in water treatment. The toxicity of these pesticide solutions decreases over time, indicating that more toxic products were not formed.*

Keywords: *carbon cryogel; organophosphate pesticides; water remediation; adsorption.*

1. INTRODUCTION

Unfortunately, organophosphate pesticides (OPs) pose a significant hazard to both humans and the majority of animals, primarily ascribed to their ability to inhibit the crucial enzyme acetylcholinesterase (AChE) [1].

An interesting class of carbonaceous materials nowadays is carbon cryogels (CC). They are especially attractive due to their developed and controllable mesoporosity [2]. Nitrogen-doped carbon cryogels (CCN) were synthesized by a sol-gel polycondensation process, employing

Corresponding author: V. Anićijević;
E-mail: anicijevicj.v@gmail.com
DOI: 10.5937/OTEH24085A

precursors such as resorcinol and formaldehyde. Subsequently, the hydrogels obtained are were dried and subjected to carbonization in an inert atmosphere [3, 4]. The procedure is described in detail in our paper [5]. The characterization and assessment of nitrogen-doped carbon cryogels as adsorbents was done using XPS to elucidate their structural properties and surface functionalities. Additionally, the investigation under both stationary and dynamic conditions provides valuable insights into the adsorption behavior of these materials, particularly their effectiveness towards different pesticide classes. Furthermore, the toxicity reduction assessment of treated samples offers a novel contribution, demonstrating the potential of nitrogen-doped carbon cryogels not only for efficient pesticide removal but also for mitigating the toxicity of contaminated water. Motivated by the significant threat posed by pesticides to non-targeted organisms and the challenges associated with their widespread use, this study addresses the pressing need for efficient pesticide remediation strategies.

2. EXPERIMENTAL

2.1. XPS analysis

XPS analysis was done using SPECS Analyzer Phoibos 150. As an X-ray source, monochromatic Al Kalfa (400 W) was used. Data were acquired using SpecsLab2 software and processed using Unifit2013 with its internal database of sensitivity factors.

2.2. Adsorption studies

We conducted two sets of experiments to investigate the efficiency of adsorption of investigated OPs on CC. The first set was done in a batch system, while the second was done in a cryogel-modified nylon membrane filter.

Investigated carbon cryogels were dispersed in double-distilled water, and the desired amount of dimethoate (2-dimethoxyphosphinothioylsulfanyl-N-methylacetamide), malathion (diethyl 2-dimethoxyphosphinothioylsulfanyl-butanedioate), and chlorpyrifos (diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ^5-phosphane) stock solutions (Pestanal, Sigma Aldrich, Denmark) was added to provide the targeted concentration of adsorbent (10 mg cm^{-3}) and OP (5×10^{-4} mol dm^{-3}). The performance of each adsorbent was quantified as the OP uptake (the percentage of adsorbed OP from the solution), defined as:

$$AD\ /\ \% = \frac{C_0 - C}{C_0} \times 100 \qquad (1)$$

where C_0 and C stand for the OP concentration before and after the adsorption experiment, respectively [6].

2.3. UPLC analysis

The Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system, coupled with a tunable UV detector controlled by the Empower software, was employed for the study. Chromatographic separations were conducted using an ACQUITY UPLC™ BEH C18 column with dimensions of 1.7 µm, 100 mm × 2.1 mm (Waters). PDA spectra of dimethoate, malathion, and chlorpyrifos concentration 1×10^{-4} mol dm^{-3} are shown in Figure 1.

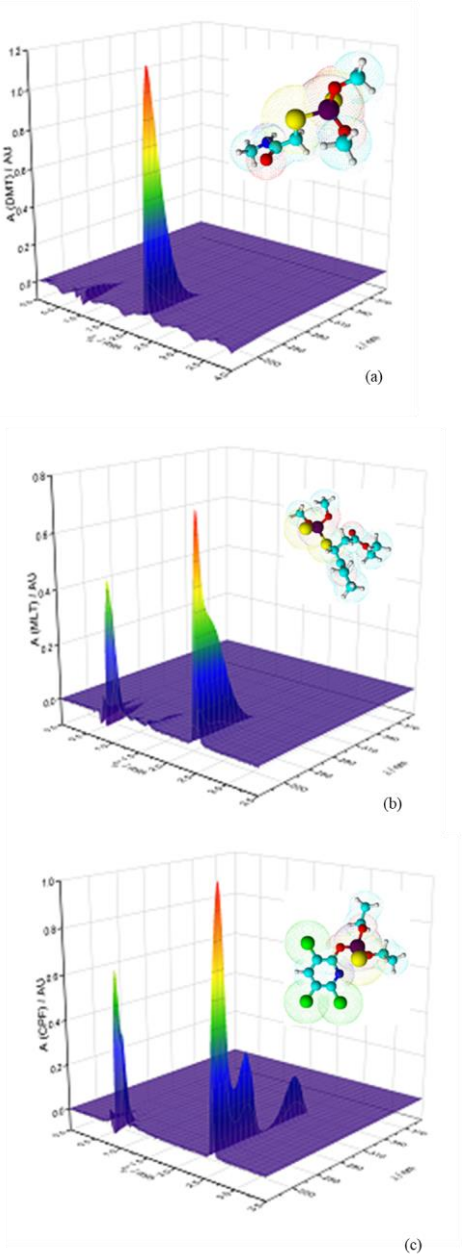

Figure 1. PDA spectra of dimethoate (a), malathion (b), and chlorpyrifos (c) concentration 1×10^{-4} mol dm^{-3}

2.4. Neurotoxicity assessment

Toxicity assessment of the samples before and after adsorption experiments was done *via* measurements of AChE inhibition. Besides performing AChE inhibition measurements to quantify the toxicity of the investigated OPs, these tests also provide information about potential transformations of OPs into more toxic forms due to the contact with adsorbents. It particularly relates to the formation of oxo-forms of OPs, which are much more toxic than corresponding thio-forms [7]. These oxidation

products can exert toxic effects at concentrations below the detection limits of UPLC. Hence, it is important to estimate the toxicity of the final samples *via* bioanalytical assay since their limit of detection is lower. AChE activity was assayed according to modified Ellman's procedure. It should be noted that in these measurements, the enzyme concentration was chosen to give an optimal spectrophotometric signal. Physiological effects were quantified as AChE inhibition given as AchE:

$$I\ /\ \% = \frac{A_0 - A}{A_0} \times 100 \qquad (2)$$

where A_0 is the AChE activity in the absence of OP and A is the AChE activity measured after exposure to a given OP [6].

3. RESULTS AND DISCUSSION

3.1. XPS analysis

The X-ray photoelectron spectra of the examined materials were recorded to determine their structure and chemical composition. The spectra of C1s, O1s, and N1s are shown in Figure 2.

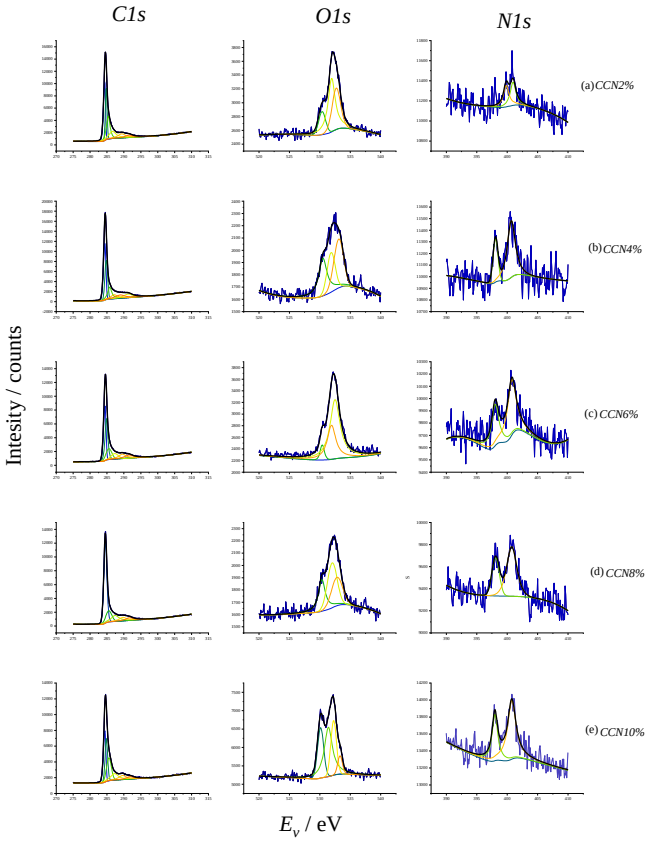

Figure 2. C1s (left), O1s (in middle), and N1s (right) XPS spectra of the CCN sample with (a) 2%, (b) 4%, (c) 6%, (d) 8% and (e) 10% N.

In the case of C1s spectra (Figure 2), deconvolution was performed for all the examined materials. Bands with different intensity maximum positions depending on the binding energy were obtained and can be assigned to different groups. The band with the highest intensity, which has a maximum at 284.5 eV, can be attributed to the C=C sp^2 bond, while other bands are also present: C–C at 285.1 eV, which is attributed to the sp^3 bond, C–O_x at 285.9 eV attributed to the epoxy and hydroxyl groups, C=O and N−C=O band at 287.5 eV and finally a weak intensity band at 290.0 eV attributed to π→π*. The component at 292.5 eV is ascribed to the same transition in N-containing carbons and carbons rich in carboxylic groups. O1s spectra (Figure 2) have a broad band in the region from about 530 to about 535 eV, which was deconvolved into several components. This band originates from various types of C–O bonds. Bands with maxima at 530.3 eV and 531.8 eV attributed to the C=O bond, at 532.5 eV attributed to the C–O–C bond, and at 533.7 eV belonging to C–OH and H–OH sp^3 bonds. N1s spectra (Figure 2) showed the presence of two main types of nitrogen moieties in the carbon structure: pyrrolic/pyridonic and quaternary nitrogen.

Table 1. Assignation of chemical groups in studied cryogels, as determined using XPS analysis

Element	E_b / eV	Assignation
C	284.5	C=C (sp^2)
	285.1	C–C (sp^3)
	285.9	C–O_x
	287.5	C=O
	290.0	π→π*
	292.5	π→π* (N-containing C)
O	530.3	C=O
	531.8	C=O
	532.5	C–O–C
	533.7	C–OH, H–OH
N	398.8	Pyrrolic/Pyridonic N
	400.8	Quaternary N

All tested cryogels have bands with the same maximum position, but their intensities differ, as shown in Table 1. Band intensities are directly proportional to the content of certain groups in the material, and these data can be used for quantitative composition determination.

Table 2. Chemical composition of investigated carbon cryogels

Sample	C at.%	N at.%	O at.%
CCN2%	97.06	0.24	2.70
CCN4%	98.19	0.39	1.42
CCN6%	97.55	0.59	1.85
CCN8%	96.74	0.73	2.53
CCN10%	93.90	1.00	5.08

The chemical composition of the tested materials is

shown in Table 2. From Table 2, it is evident that the amount of nitrogen incorporated into the material during the synthesis is about 10% of the added amount. By analyzing the displayed spectra, the content of carbon, oxygen, and nitrogen can be obtained, and the existence of functional groups containing these elements can be detected.

3.2. OP adsorption on carbon cryogels

From the data presented in Figure 3, it can be seen that aliphatic organophosphates, dimethoate, and malathion are very successfully adsorbed on all tested cryogels under static conditions in batch studies. Dimethoate could not be detected in the supernatant decanted after adsorption. It means that the dimethoate concentration dropped below 1×10^{-8} mol dm^{-3}, which is the detection limit of the instrument used for this analysis (UPLC) under the given experimental conditions. These results suggested that all dimethoate from the solution is adsorbed on all tested materials. In the case of malathion, it was possible to detect low concentrations of pesticides in the decanted supernatants, in concentrations ranging from 10^{-7} to 10^{-8} mol dm^{-3}, which is practically negligible considering that the initial malathion concentration was 5×10^{-4} mol dm^{-3}.

On the other hand, with aromatic chlorpyrifos, the situation is different. The material with the highest nitrogen content, CCN10, turned out to be completely inefficient in this case. In contrast, the CCN8 proved to be the most suitable adsorbent, which adsorbed 43% of the available chlorpyrifos. Materials CCN2, CCN4, and CCN6 adsorbed about 20% of the available pesticide.

The adsorption of 5×10^{-4} mol dm^{-3} malathion, chlorpyrifos, and dimethoate on 10 mg cm^{-3} carbon cryogels dopped with nitrogen was also examined under the dynamic conditions in filter studies. 10 mg of each of the tested adsorbents was injected into nylon filters. Next, 1 cm^{-3} of 5×10^{-4} mol dm^{-3} solution of each tested organophosphate is filtered through the modified nylon filter within 1 min. In the filtrate, the concentration of the remaining pesticides was determined chromatographically. The results are presented in Figure 3.

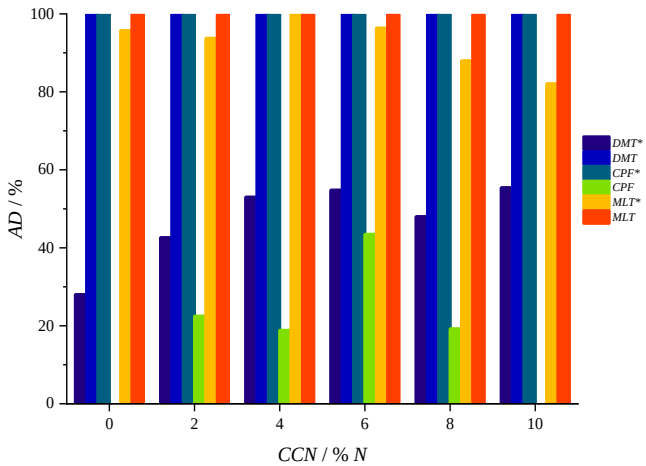

Figure 3. OPs removal using N-doped carbon cryogels under stationary and dynamic conditions (marked by *).

Adsorption using filters under dynamic conditions showed a different trend of results compared to the experiment in batch. From the results given in Figure 3, it can be seen that aromatic chlorpyrifos is the one that is completely adsorbed to all tested materials. The remaining traces of pesticides in the filtrates could not be detected since their concentration was below the instrument's detection limit (1×10^{-8} mol dm^{-3}). It is also obvious that under dynamic conditions, aliphatic malathion is adsorbed in a high percentage on all investigated cryogels. Material CCN4 showed the best result and almost completely adsorbed the initial amount of pesticides (99%). Material CCN8 adsorbed 96% malathion and material CCN4 94% under the given experimental conditions. CCN6 and CCN10, with 88% and 82% of adsorbed malathion, respectively, showed slightly lower performance. Even though the percentage of adsorbed pesticides varies from 82 to 99, we can say that all investigated materials under dynamic conditions are effective adsorbents for the remediation of malathion due to the fact that amounts that could be found in real samples are significantly lower than investigated.
Unlike chlorpyrifos and malathion, the investigated materials yielded significantly inferior results in terms of dimethoate adsorption under dynamic conditions. The adsorption percentages range from 43% to 56% of the initial concentration. The uptake increased with the increase of nitrogen content in the adsorbent structure. It is evident that nitrogen addition has a favorable effect on dimethoate adsorption efficiency under dynamic conditions, but nitrogen content does not play a significant role in the given framework. Although the adsorption efficiency under these conditions is about 50%, this result, although significantly poorer than the adsorption of the other two pesticides, is by no means negligible, considering the already discussed situation in the environment. Moreover, there are cases when the adsorption rate is critical (e.g., adsorption of chemical warfare with the structure of organophosphate) and plays a more significant role than adsorption efficiency. Under those circumstances, materials that adsorb many potential pollutants under dynamic conditions have the advantage, even if adsorption efficiency is not 100%.

3.3. Physiological effects

As shown in Figure 4, AChE inhibition is induced for the initial concentration of OPs used in the adsorption experiments, which is connected to the neurotoxic effects of OPs. Also, it is noticeable that AChE inhibition for the samples without the adsorbent for batch studies decreases in comparison to those in filter studies. This discrepancy is attributed to the previously discussed instability of OPs at room temperature, where batch samples are exposed for 24 hours, whereas filter studies involve only a brief exposure of a few minutes. Chlorpyrifos showed complete inhibition of AChE activity under the given experimental conditions for both initial and apparent initial concentrations since it is highly toxic in both amounts.

However, after the adsorption of OPs on carbon cryogels, purified water samples in the cases of malathion and

dimethoate in batch studies and chlorpyrifos and dimethoate in filter studies induced no AChE inhibition. Other samples induced lower AChE inhibition compared to non-purified samples, suggesting a decrease in solution toxicity after incubation with all investigated carbon cryogels. This observation also confirms the absence of oxidation and the formation of more toxic byproducts during the interaction between OPs and cryogels.

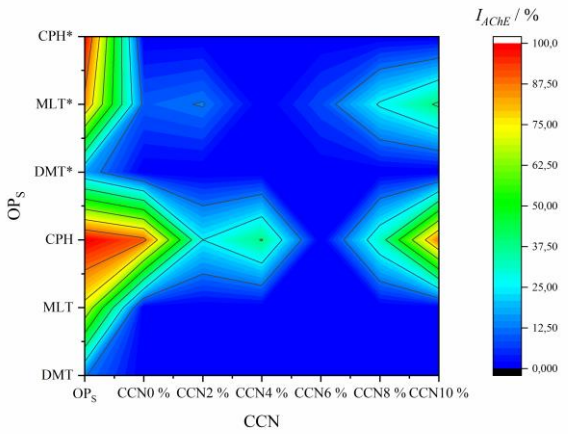

Figure 5. Inhibition of AChE activity in the presence of chlorpyrifos, malathion, and dimethoate before (without adsorbent added) and after the adsorption on investigated carbon cryogels in batch and filter*

4. CONCLUSION

In conclusion, our study elucidates the complex relationship between the properties of nitrogen-doped carbon cryogels and their adsorption performance towards organophosphate pesticides. Despite minimal alterations in carbon morphology, nitrogen incorporation induces significant changes in textural properties and subtle variations in chemical composition, shaping adsorption behavior. Overall, our findings highlight the complexity of OP adsorption onto nitrogen-doped carbon cryogels, emphasizing the need for a comprehensive understanding of material properties and OP characteristics for effective pesticide remediation strategies. By shedding light on the complicated interplay between chemical structure, surface properties, and adsorption mechanisms, our study contributes to the advancement of adsorbent materials for environmental applications, making way for innovative and sustainable water treatment technologies.

Acknowledgment

This work was partially supported by the Ministry for Science, Technological Development and Innovation of the Republic of Serbia (451-03-66/2024-03/200017 and 451-03-65/2024-03/200146), and Ministry of defence Republic of Serbia (VA-TT/1/22-24).

References

[1] ANIĆIJEVIĆ, V., TASIĆ, T., MILANKOVIĆ, V., BREITENBACH, S., UNTERWEGER, C., FÜRST, C., BAJUK-BOGDANOVIĆ, D., PAŠTI, I. A. and LAZAREVIĆ-PAŠTI, T., *How Well Do Our Adsorbents Actually Perform? - The Case of Dimethoate Removal Using Viscose Fiber-Derived Carbons*, International Journal of Environmental Research and Public Health, 2023.

[2] MINOVIĆ, T. Z., GULICOVSKI, J. J., STOILJKOVIĆ, M. M., JOKIĆ, B. M., ŽIVKOVIĆ, L. S., MATOVIĆ, B. Z. and BABIĆ, B. M., *Surface characterization of mesoporous carbon cryogel and its application in arsenic (III) adsorption from aqueous solutions*, Microporous and Mesoporous Materials 201 (2015) 271-276.

[3] BABIĆ, B., KALUĐEROVIĆ, B., VRAČAR, L. and KRSTAJIĆ, N., *Characterization of carbon cryogel synthesized by sol–gel polycondensation and freeze-drying*, Carbon 42 (2004) 2617-2624.

[4] BABIĆ, B. M., VRAČAR, L. M., RADMILOVIĆ, V. and KRSTAJIĆ, N. V., *Carbon cryogel as support of platinum nano-sized electrocatalyst for the hydrogen oxidation reaction*, Electrochimica Acta 51 (2006) 3820-3826.

[5] LAZAREVIĆ-PAŠTI, T., ANIĆIJEVIĆ, V., KARKALIĆ, R., BALJOZOVIĆ, M., BABIĆ, B. and PAŠTI, I. A., *Nitrogen-Doped Carbon Cryogels as Adsorbents: Efficient Removal of Organophosphate Pesticides from Water and Assessment of Toxicity Reduction*, C 10 (2024) 56.

[6] TASIĆ, T., MILANKOVIĆ, V., BATALOVIĆ, K., BREITENBACH, S., UNTERWEGER, C., FÜRST, C., PAŠTI, I. A. and LAZAREVIĆ-PAŠTI, T., *Application of Viscose-Based Porous Carbon Fibers in Food Processing—Malathion and Chlorpyrifos Removal*, Foods 12 (2023) 2362.

[7] LAZAREVIĆ-PAŠTI, T., ANIĆIJEVIĆ, V., BALJOZOVIĆ, M., ANIĆIJEVIĆ, D. V., GUTIĆ, S., VASIĆ, V., SKORODUMOVA, N. V. and PAŠTI, I. A., *The impact of the structure of graphene-based materials on the removal of organophosphorus pesticides from water*, Environmental Science: Nano 5 (2018) 1482-1494.