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ORGANOPHOSPHORUS NEUROTOXINS REMEDIATION IN DYNAMIC CONDITIONS

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Abstract: *Organophosphorus neurotoxins (OPNs) are a class of chemical compounds widely known for their potent neurotoxic effects. OPNs work by inhibiting the enzyme acetylcholinesterase (AChE), which breaks down the neurotransmitter acetylcholine in the nervous system. This research investigates using spent coffee grounds carbonized at 900°C and subsequently activated with H_3PO_4 and KOH to remove chlorpyrifos (CHP) and malathion (MLT) from aqueous solutions. The activated carbon material is integrated into a filtration system designed to rapidly and efficiently remove these neurotoxins. In the initial cycle, the adsorption capacities for CHP and MLT were found to be 67% (11.7 mg g^{-1}) and 78% (12.8 mg g^{-1}), respectively, demonstrating high effectiveness in decontamination efforts. The filter was washed using 5 mL of 50% ethanol and reused for 10 cycles. It is shown that it exhibits durability, as it can be successfully regenerated and reused without significant loss in adsorption efficiency. As the material has high surface areas and shows no specific adsorption of the neurotoxins, it can be concluded that it can successfully be used to resolve chemical threats in water sources during military operations.*

Keywords: *filters, malathion, chlorpyrifos, spent coffee grounds, adsorption*

1. INTRODUCTION

Organophosphorus neurotoxins (OPNs) are a prominent class of chemical compounds recognized for their significant neurotoxic effects. Among these, malathion (MLT) and chlorpyrifos (CHP) are widely used pesticides that pose substantial risks to human health and the environment. Malathion and chlorpyrifos exert their toxic effects primarily by inhibiting acetylcholinesterase (AChE), a crucial enzyme responsible for the breakdown of the neurotransmitter acetylcholine in the nervous system. Inhibition of AChE results in the accumulation of acetylcholine at synaptic junctions, leading to continuous stimulation of muscles, glands, and central nervous system receptors. This can cause a range of symptoms from mild, such as headaches and dizziness, to severe, including respiratory paralysis and death [1, 2].

Though considered less toxic to humans than other organophosphates, MLT can cause serious health effects upon exposure. Acute exposure can result in symptoms like nausea, vomiting, abdominal cramps, diarrhea, excessive sweating, salivation, and muscle twitching. Chronic exposure has been linked to more severe consequences, including respiratory issues, cognitive impairments, and even cancer [3].

CHP is another potent organophosphorus compound extensively utilized in agricultural practices. Exposure to CHP has been associated with various adverse health effects. Acute exposure can lead to symptoms such as blurred vision, muscle weakness, convulsions, and, in extreme cases, death. Long-term exposure has been implicated in developmental and behavioral disorders, particularly in children, due to its profound impact on the developing nervous system [4].

Given the significant health risks posed by MLT and CHP, effective remediation techniques are essential to minimize their impact on public health and the environment. This research explores the use of spent coffee grounds (SCG) carbonized at 900°C and

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subsequently activated with H_3PO_4 and KOH for the remediation of these neurotoxins from aqueous solutions. Military operations demand the highest quality standards. These standards include material selection and performance requirements that would protect from air and water threats. Developing a filtration system incorporating this activated carbon material offers a promising solution for rapidly and efficiently removing MLT and CHP, thereby addressing chemical threats in water sources and ensuring safety during military operations and other critical scenarios.

2. MATERIAL AND METHODS

2.1. Material synthesis and characterization

The adsorbent 900PC was prepared from SCG. After brewing a mixture of coffee obtained from the local market (80% Arabica and 20% Robusta) with boiling water, the mixture was allowed to cool to room temperature over 2 hours. SCG was then separated by filtration and left to dry at room temperature for 24 hours. The carbonization process was conducted in a tube furnace (Protherm Electrical Tube Furnace, Protherm Furnaces, Turkey) under a nitrogen flow of 100 L h^{-1}. The sample was heated to 900°C at a rate of 5°C per minute and maintained at this temperature for 60 minutes. Afterward, it was cooled to room temperature under a nitrogen atmosphere. The carbonized sample was then treated with H_3PO_4 (Centrochem, Stara Pazova, Srbija) at a mass ratio 1:2 (material: H_3PO_4) and returned to the furnace. It was heated again to 900°C at a rate of 5°C per minute under a nitrogen flow and maintained at this temperature for 60 minutes under a CO_2 flow of 100 L h^{-1}. Finally, the sample was cooled to room temperature under a nitrogen atmosphere. To prepare a stock suspension, 100 mg of the material was weighed and washed sequentially with 50 mL of 0.1 mol dm^{-3} NaOH, 50 mL of 0.1 mol dm^{-3} HCl, and 50 mL of deionized water, then suspended in 50 mL of 50% ethanol (J.T. Baker, Phillipsburg, NJ, USA), resulting in a final concentration of 2 mg mL^{-1}.

Synthesized material was characterized using SEM, EDX, FTIR, and Zeta potential measurements to determine the material's morphology, chemical composition, functional groups, and isoelectric point (IEP).

2.2. Adsorption experiments under dynamic conditions

To investigate adsorption under dynamic conditions, a commercial nylon membrane filter (pore size 0.22 µm, diameter 13 mm, KX Syringe Filter, Kinesis) was modified to contain a layer of adsorbent. This modification involved injecting 0.5 mL of the materials stock suspension into the commercial filter. The solvent was then removed from the filter using compressed air. The pesticide solution was injected through the modified filter at 1 mL per minute. The filtrate was subjected to UPLC analysis to determine the pesticide concentration. A control experiment was conducted using an unmodified filter.

To investigate the adsorbent regeneration and reuse, the filter was washed using 5 mL of 50% ethanol solution and reused as the adsorbent for the same pesticide.

The concentration of OPNs was determined using UPLC. A Waters ACQUITY UPLC with a PDA detector was used to determine the pesticide concentration. BEH C18 column was used under isocratic conditions: mobile phase A - 10% acetonitrile in water and B - pure acetonitrile. The injected volume was 5 µL, while the flow rate was 0.2 mL min^{-1} in all cases. The mobile phase in the case of the MLT consisted of 40%A and 60%B, while in the case of CHP, it consisted of 20%A and 80%B for individual detection. The retention time for MLT under these conditions was 2.5 min, while it was 2.7 min for chlorpyrifos. Both pesticides were detected at a wavelength of 200 nm. Control experiments were performed identically but without the presence of the material.

2.3. Neurotoxicity assessment

The physiological effects of the treated solutions were analyzed through AChE inhibition measurements using a modified Ellman's procedure. 2.5 IU of commercially purified AChE from an electric eel was exposed to the organophosphate solutions in a 50 mmol dm^{-3} phosphate buffer at pH 8.0 and 37 °C in a final volume of 0.650 mL. The enzymatic reaction was initiated by combining AChI with DTNB as the chromogenic reagent. The reaction was allowed to proceed for 8 minutes before being stopped with 10% sodium dodecyl sulfate (SDS). Thiocholine, the reaction product, reacts with DTNB to form 5-thio-2-nitrobenzoate, whose optical absorption was measured at 412 nm using UV-VIS Perkin Elmer, Lambda 35 spectrophotometer. The enzyme concentration was maintained at a level that produced an optimal spectrophotometric signal. The physiological effects were quantified as AChE inhibition, calculated as follows:

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

where A_0 and A stand for the AChE activity in the absence of OP and the one measured after exposure to a given OP.

3. RESULTS

3.1. Physicochemical properties of the material

SEM micrography and EDX analysis were performed to examine the materials' morphology and elemental composition. The SEM micrograph and EDX spectrum are given in **Figure 1**. The SEM micrograph shows that the material has a granular, heterogeneous, and highly porous structure, resembling the previously reported morphology of the precursor, SCG [5]. EDX analysis revealed a high percentage of C, O, and P, 46.27, 36.76, and 6.97 at.%, respectively. Besides mentioned, N, K, Ca, Mg, Na, and Si are also present in the sample as the chemical footprint of the precursor.

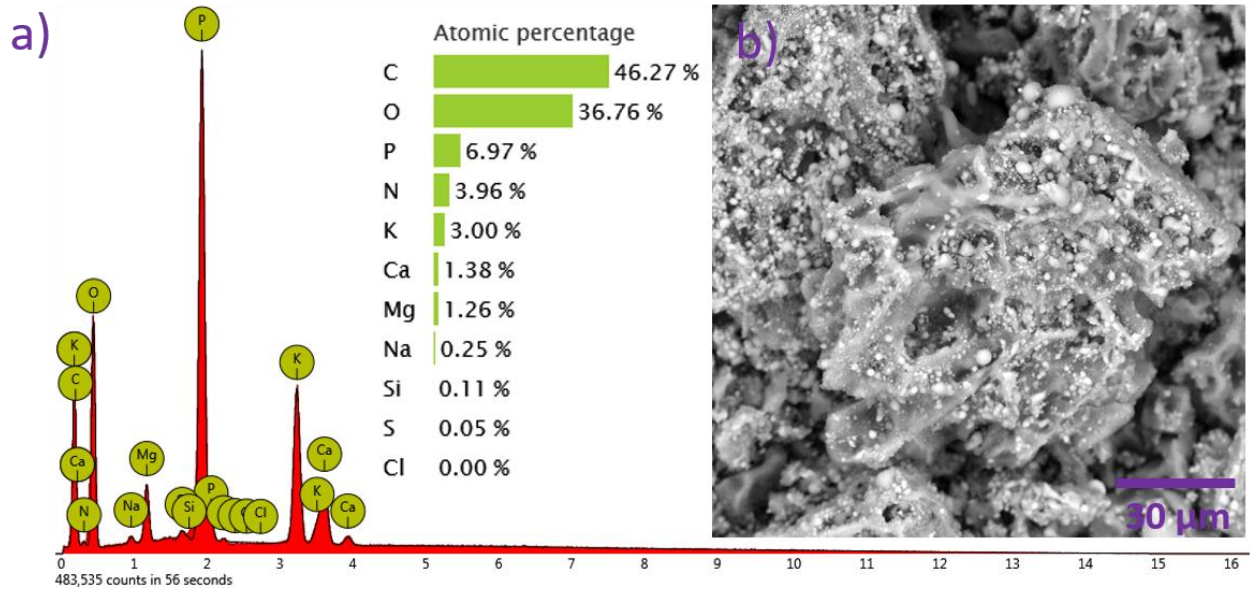

Figure 1. a) EDX spectrum and elemental composition in at.% of 900PC; b) SEM micrograph of 900PC (magnification 2000×)

The FTIR spectrum of 900PC is given in **Figure 2.** The observed bands in the spectrum correspond to specific vibrations. The band at 1554 cm^{-1} indicates the presence of condensed aromatic rings, signifying aromatic carbon-carbon double bonds (C=C) within the carbon structure. These aromatic rings enhance the material's π-electron system, improving its adsorption properties through π-π interactions with adsorbates. The band at 1227 cm^{-1} is assigned to the stretching vibration of phosphorus-oxygen double bonds (P=O), suggesting the presence of phosphorus-containing functional groups. These groups may stem from phosphorus compounds in the precursor material or be introduced during the carbonization and activation processes, potentially enhancing the material's adsorption affinity for certain contaminants. The band at 1079 cm^{-1} is linked to the coupling of aromatic carbon-hydrogen (C-H) in-plane deformation vibrations and carbon-oxygen (C-O) stretch vibrations in primary alcohols, indicating the presence of aromatic C-H bonds and hydroxyl (OH) functional groups, likely derived from phenolic compounds in the precursor material. These functional groups contribute to the material's hydrophilicity and surface reactivity, affecting its adsorption behavior towards polar contaminants [6]. The band at 877 cm^{-1} corresponds to vibrations from 3-4 condensed aromatic rings, pointing to carbonaceous structures with multiple fused aromatic rings. These condensed aromatic structures enhance the material's graphitic nature and provide additional adsorption sites for contaminants through π-π interactions [7].

By examining the dependence of the zeta potential of the materials' suspension on pH (**Figure 3**), it can be seen that the isoelectric point of 900PC is at pH=4.87. As the suspension's initial pH value used in adsorption experiments was 6, it can be concluded that 900PC has a slightly negatively charged surface.

The specific surface area of 900PC, determined by N_2 adsorption, is 846 $m^2\ g^{-1}$, while the total pore volume is 0.557 $cm^3\ g^{-1}$.

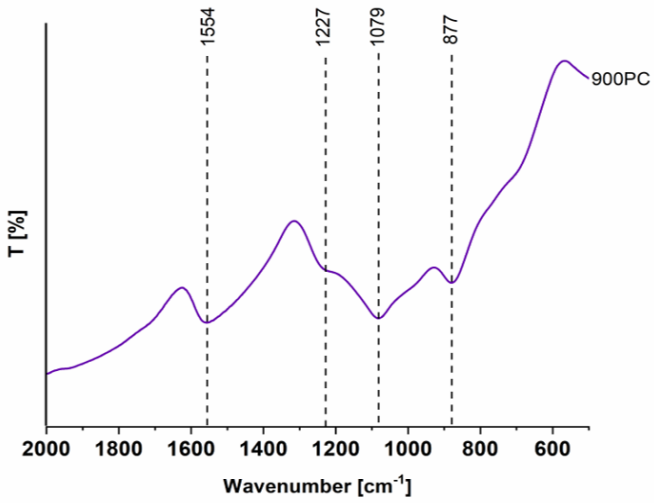

Figure 2. FTIR spectrum of 900PC

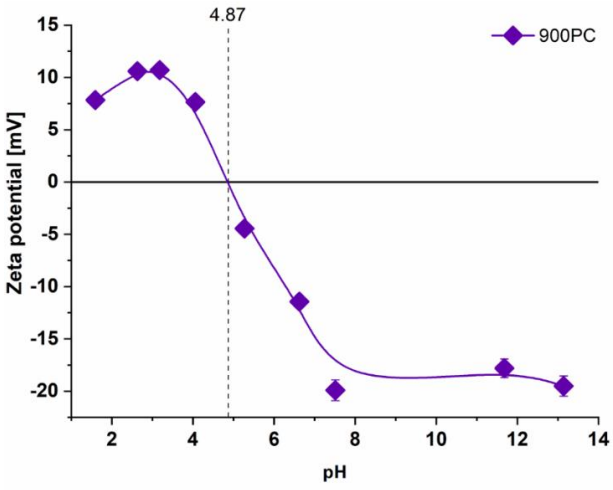

Figure 3. Dependence of zeta potential of pH with

indicated IEP for 900PC

3.2. Adsorption of OPNs onto 900PC under dynamic conditions. Regeneration and reuse.

The obtained results are shown in **Figure 4**. By observing the presented results, it can be concluded that the material can successfully remediate 98% of MLT and 97% of CHP under dynamic conditions. It can be regenerated using 5 mL of 50% ethanol solution and reused for at least 10 cycles without significantly impacting the adsorption capacity, as it loses around 10% of its efficiency after the 10th cycle. These results indicate that 900PC is not a selective adsorbent, making it suitable for application in the remediation of various chemical threats from water sources.

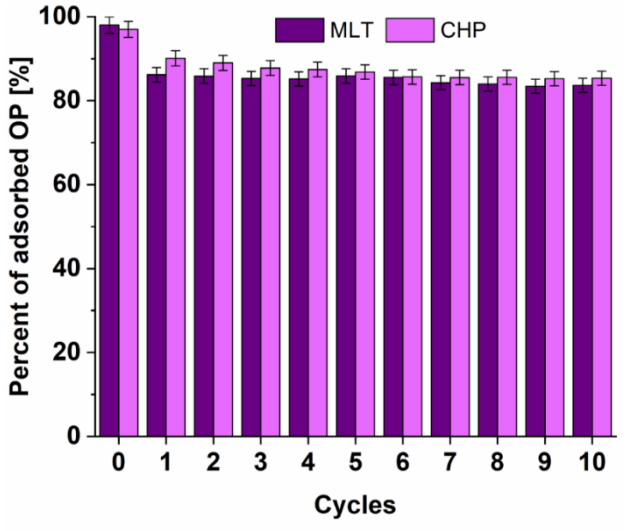

Figure 4. Histogram representing the percent of adsorbed OP through 10 cycles of regeneration and reuse

3.3. Assessment of AChE activity inhibition after MLT and CHP remediation using 900PC

The neurotoxicological assessment was performed as described in Section 2.3, and the histogram showing the difference in the activity inhibition of AChE before and after the adsorption of MLT and CHP is shown in **Figure 5**. It can be seen that the AChE inhibition due to the contact with samples was highly reduced after the adsorption in all cases, indicating that no more toxic products, such as OPNs' oxo-forms, were formed during this process. The reduction in AChE activity inhibition is crucial for the intended application, as if more toxic products are forming during the process, the interaction of OPNs with the material could lead to more severe neurotoxic effects.

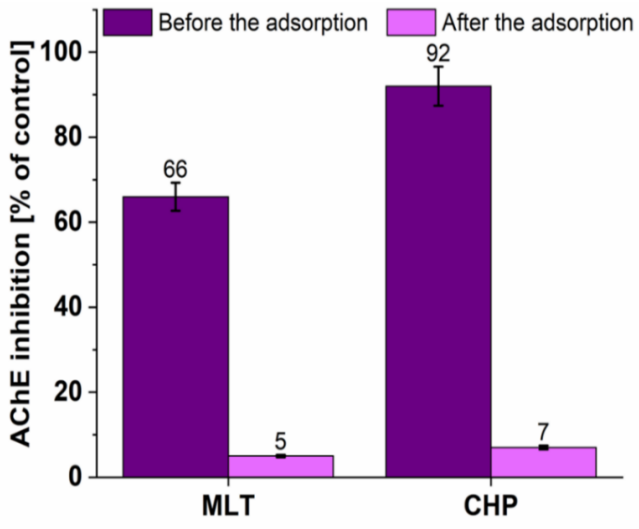

Figure 5. Histogram of AChE activity inhibition before and after the adsorption of MLT and CHP using AC-SCG

4. CONCLUSION

In conclusion, this research demonstrates that the 900PC material, produced from SCG carbonized at 900°C and activated with H_3PO_4 and KOH, is highly effective in remediating OPNs, MLT, and CHP from aqueous solutions. The material exhibits excellent adsorption capacities, successfully removing 98% of MLT and 97% of CHP. This high level of performance is attributed to the material's highly porous structure, which enables efficient adsorption of these toxic compounds. As this material did not exhibit significant differences in the percent of adsorbed OPN, it is concluded that it is non-selective and that its application is not limited just to OPNs.

Furthermore, the 900PC material shows impressive durability and reusability. It retains its high adsorption efficiency over at least 10 regeneration cycles, losing only about 10% of its capacity. This reusability is achieved by regenerating the material with a 50% ethanol solution, demonstrating a practical and cost-effective approach for maintaining its performance.

Neurotoxicological assessments indicate that the 900PC material significantly reduces AChE inhibition after the adsorption of MLT and CHP, suggesting that the adsorption process does not produce harmful by-products. This finding underscores the safety and reliability of using 900PC in filtration systems designed to remove chemical threats in water sources.

The study highlights the potential application of 900PC material in developing filtration systems for military operations and other critical scenarios where stringent quality standards for material selection and performance are required. By providing protection against neurotoxins in water, the 900PC material contributes to enhanced safety and protection during operations.

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