Comparison of Organochlorine Pesticide Recovery from Limited Quantity Environmental Samples Using Solid Phase Extraction with All Carbon Microbeads and Graphitized Carbon Blacks

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Abstract

Introduction. We previously developed an optimized method for preparing samples using solid-phase extraction to detect 15 organochlorine pesticides (OCPs) in small amounts (100 mg of soil and 250 µL of river water) of environmental samples. In this white paper, we employ this method and compare the OCP recoveries between NanoPak-C All Carbon Microbeads and commercially available graphitized carbon black (GCB) media.

Methods- NanoPak-C All Carbon microbeads or graphitized carbon blacks (GCBs) were packed in SPE columns of 3 mL and 6 mL volumes at different bed weights (100 mg, 250 mg, and 500 mg). An optimized solid-phase extraction (SPE) procedure was applied to the analysis of trace amounts of OCPs in environmental samples using all-carbon beads and compared with commercial SPE columns containing graphitized carbon beads.

Results. All 15 pesticides could be detected in soil and river water samples using GC-MS, with various SPE columns packed with All Carbon microbeads. The average amount recovered was between 1-90 ppm (µg/kg). Very few, and no pesticides could be detected from soil and water samples, respectively, after sample preparation using graphitized carbon black SPE columns,

Conclusion. NanoPak-C All-Carbon reversed-phase packed SPE columns allow the extraction, elution, and subsequent detection by GC-MS of organochlorine pesticides from small quantities of environmental samples with complex matrix compositions across all volumes and bed weights. Under similar conditions, graphitized carbon black columns extract, but exhibit suboptimal elution, resulting in poor to no GC-MS detection of pesticides.

Introduction

Solid-phase extraction (SPE) is the most used method for analyzing pesticides in soil and river water. In comparison to traditional liquid-liquid extraction, SPE is a cost-effective, time-saving, and solvent-efficient process[1]. Organochlorine pesticides (OCPs) are synthetic chlorinated hydrocarbon derivatives widely used in global agriculture. Due to their toxicity, OCPs pose significant concerns for human health and the environment. Complex matrices hinder the detection and quantification of OCPs with liquid chromatography methods (column fouling and baseline noise). Thus, solid-phase Extraction (SPE) is a standard sample preparation method for cleaning up environmental samples and isolating OCPs before their introduction into chromatography systems. There is a dearth of SPE techniques to extract OCPs from small quantities of environmental samples.

NanoPak-C All Carbon Microbead is a reverse phase media with unique carbon chemistry comprising of a network of sp2 (natural graphite) & inter-graphite sp3 (covalent) carbon bonds (via crosslinker). We have developed an optimized method to prepare samples using solid phase extraction with NanoPak-C All Carbon Microbead (average microbead diameter

= 40 µm) to detect 15 OCPs (**Figure 1**) in small amounts (100 mg soil and 250 µL river water) of environmental samples [2].

Graphitized carbon blacks (GCBs) are commercially available adsorbents widely used in SPE workflows. Carbograph, Carbopack, Carboxen, and Carbotrap are some examples of GCBs. Their shape is spherical or irregular, and their size is typically greater than 40 µm. Herein, we employ this method and compare the OCP recoveries between All Carbon Microbeads and a commercially available graphitized carbon black (GCB) media.

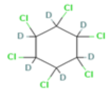
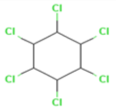
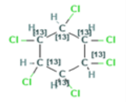
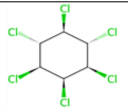
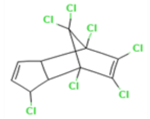
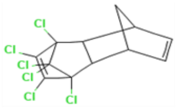
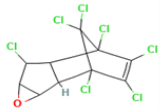
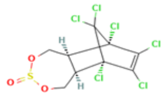
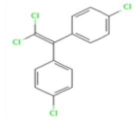
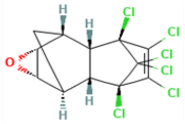
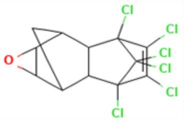
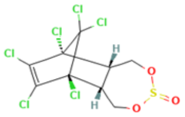
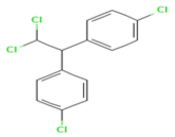
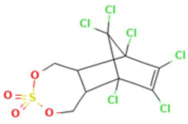
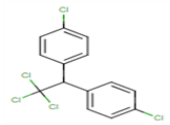
Sr. No.	Compound Name	Structure	Molecular Formula
1	Alpha HCH		$C_6H_6CL_6$
2	Beta HCH		$C_6H_6CL_6$
3	Gama HCH		$C_6H_6CL_6$
4	Delta HCH		$C_6H_6CL_6$
5	Heptachlor		$C_{10}H_5CL_7$
6	Aldrin		$C_{12}H_8CL_6$
7	Heptachlor Epoxide		$C_{10}H_5CL_7O$
8	Alpha Endosulfan		$C_9H_6CL_6O_3S$
9	p,p DDE		$C_{14}H_8CL_4$
10	Dieldrin		$C_{12}H_8CL_6O$
11	Endrin		$C_{12}H_8CL_6O$
12	Beta Endosulfan		$C_9H_6CL_6O_3S$
13	p,p DDD		$C_{14}H_{10}CL_4$
14	Endosulfan Sulpahte		$C_9H_6CL_6O_4S$
15	p,p DDT		$C_{14}H_9CL_5$

Figure 1. Name, structure depiction, and chemical formula of the 15 peptides.

Materials and Method

Chemicals

All the chemicals were of analytical reagent grade and used as received. Standard mixtures of 15 OCPs were purchased from Supelco (Merck, USA). This EPA pesticide was stored at 4°C and was used for the preparation of working standard solutions (1 PPM) in Deionized water obtained from a Milli-Q water purification system. A standard PTFE syringe filter, with a pore size of 0.45 µm and a diameter of 13 mm, was used for sample filtration. A commercial vendor donated graphitized black (GCB) media.

Soil Sample

Soil samples were directly collected in appropriate plastic bags (1 kg) between 0 and 20 cm deep in the ground from a pesticide-free local field. Before use, soil samples were carefully homogenized with a mortar and pestle, sieved (2mm mesh) to remove coarse particles and debris, and air-dried at room temperature. The pH of the soil in distilled water was determined using a 1:5 (w/v) soil-to-water suspension. A 4 g sample of soil was added to 20 mL of distilled water and stirred continuously for 5 minutes to ensure thorough mixing. The suspension's pH was then measured directly using a calibrated pH meter, and the recorded value was 8.0. Soil samples. were spiked at a working concentration (80 ppb) with a standard pesticide mix solution. Extractions of blank samples were done in parallel to the spiked ones.

Water Sample

Water samples were collected from a local river in a 100 mL sample collection bottle. Water samples were carefully centrifuged to remove larger contaminants, such as sand particles. Twenty milliliters of river water were collected and vortexed for 1 minute to ensure uniform mixing. The pH was then measured directly using a calibrated pH meter, yielding a recorded value of 7.56. Water samples were spiked with a working concentration (80 ppb) of a standard pesticide mix solution. Extractions of blank samples were done in parallel to the spiked ones.

Table 1.SPE column volumes and corresponding bed weights used in each column

No.	SPE Column Volume in milliliter (mL) bed weight of	Media Bed Weight in milligram (mg)
1	3 mL	100 mg
2	3 mL	250 mg
3	6 mL	500 mg

SPE columns

Table 1 lists the All Carbon Black and Graphitized Carbon Black media samples. Three different types of All Carbon bead SPE columns, with varying column lengths (3 mL and 6 mL) and bed weights (100 mg, 250 mg, and 500 mg), were used.

Instrumentation

Table 2 summarizes the compounds analyzed, detailing their retention times, precursor ions, identification ions, and associated collision energies.

Table. 2 List of compounds along with their retention times, precursor ions,

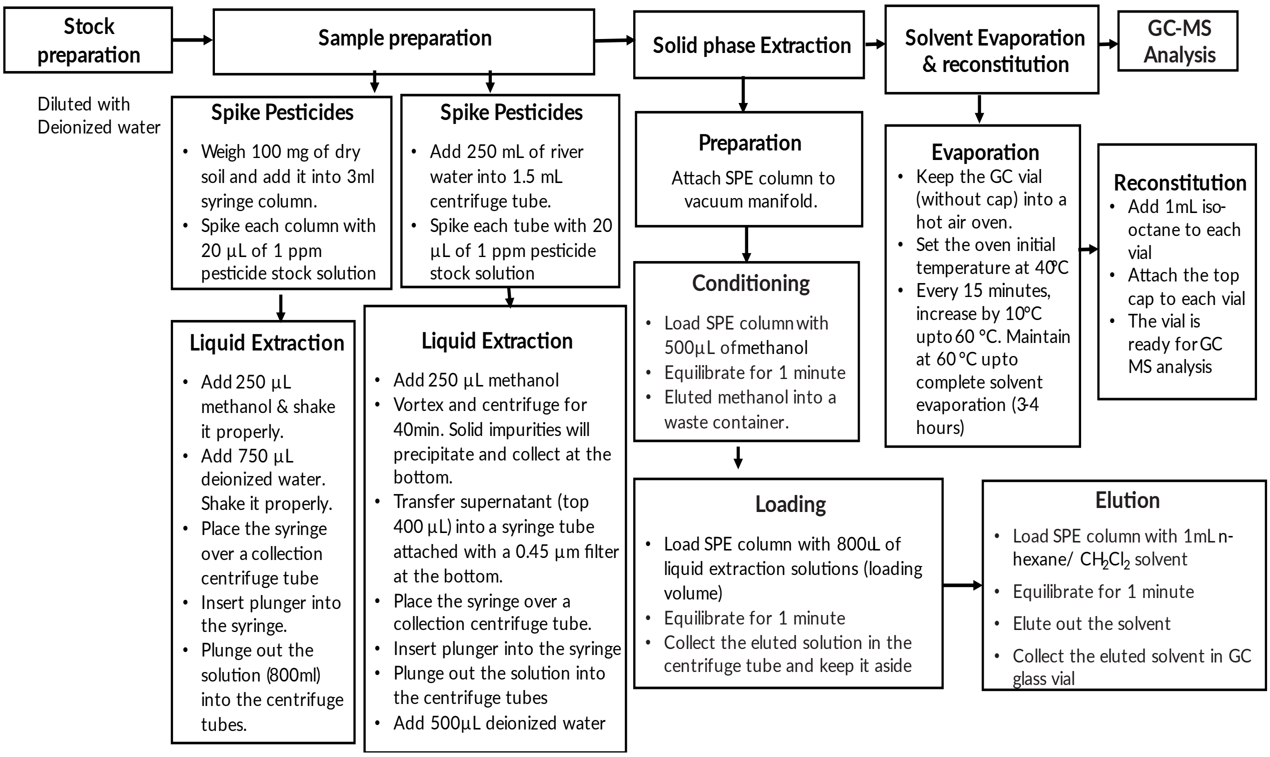
Sr.no.	Compound Name	Retention time	Precursor ion	Identification ion	Collision energy
1	Alpha HCH	7.74	218.8	182.9, 147, 109, 74	5, 20,40,45
2	Beta HCH	8.1	218.8	147, 74, 182.9, 109	20, 45,5,40
3	Gama HCH	8.27	218.8	182.9, 147, 109, 74	5, 20, 40,45
4	Delta HCH	8.72	218	182.9, 147, 109, 74	5, 20, 40,45
5	Heptachlor	9.5	271.8	236.8, 235.9, 116.9	10
6	Aldrin	10.14	262.9	227.9, 202.9, 193, 191	25, 30, 40,10
7	Heptachlor epoxide	10.82	352.8	316.8, 262.9	10
8	Alpha Endosulphan	11.5	240.9	205.9, 170, 136, 120.9	15, 25, 35
9	p,p DDE	11.83	317.8	282.9, 248, 210, 176.1	45, 30, 45, 15
10	Dieldrine	11.99	263	227.9, 202.9, 192.9	25, 30, 40
11	Endrin	12.38	262.8	228, 202.9, 193	30, 25, 40
12	Beta Endosulphan	12.59	236.8	142.9,118.9,106,	30, 45
13	p,p DDD	12.6	234.9	199, 165	20, 30
14	Endosulphan Sulphate	13.6	271.8	236.8, 142.9, 116.9	15, 45
15	p,p DDT	13.3	235	199, 165	20, 30

All analyses were performed on an Agilent (USA) GC-7890B, MS-7000D, gas chromatography (GC)–mass spectrometry (MS) system. The GC was fitted with a Column1- DB-5ms Ultra Inert (60-325 degree Celsius) column (15m * 250um * 0.25um) and flow-1ml/min, and Column2- DB-5ms Ultra Inert (60-325 degree Celsius) column (15m * 250um * 0.25um) and flow-1.2 ml/min. Helium was used as the carrier gas at a flow rate of 2.250

ml/min, and Nitrogen was used as CID gas at a flow rate of 1.50 ml/min. The sample injection volume was 2 µL at an injection temperature of 280 °C. All injections were made in splitless mode. The mass spectrometer was run in electron ionization (EI) mode with an electron energy of 70 eV, scanning a range of 10–500 amu. The MS parameters were set as source temperature - 280 °C, MS1 and MS2 Quad temp – 150 °C, and transfer line temperature – 280C. The data was processed by Mass hunter Quantitative Analysis software. The All OCPs were identified by retention time and specific ions (given in table 2). They were quantified by comparison to the internal standard.

Pesticide SPE and GC-MS detection Method

The SPE method was optimized for several parameters, such as: elution solvent, target analyte concentration, SPE column bed weight, and SPE column length. This method development is published elsewhere [2]. Figure 2 presents the flowchart of the various steps. 1 mL N-hexane: DCM (70:30) was used to elute the analytes retained on the cartridge. The eluent was collected and dried in the oven at 59°C. 1 mL of iso-octane was used to reconstitute the residue, and used GCMS system for analysis.



Spike Pesticides

- Weigh 100 mg of dry soil and add it into 3ml syringe column.
- Spike each column with 20 µL of 1 ppm pesticide stock solution

Spike Pesticides

- Add 250 mL of river water into 1.5 mL centrifuge tube.
- Spike each tube with 20 µL of 1 ppm pesticide stock solution

Liquid Extraction

- Add 250 µL methanol & shake it properly.
- Add 750 µL deionized water. Shake it properly.
- Place the syringe over a collection centrifuge tube
- Insert plunger into the syringe.
- Plunge out the solution (800ml) into the centrifuge tubes.

Liquid Extraction

- Add 250 µL methanol
- Vortex and centrifuge for 40min. Solid impurities will precipitate and collect at the bottom.
- Transfer supernatant (top 400 µL) into a syringe tube attached with a 0.45 µm filter at the bottom.
- Place the syringe over a collection centrifuge tube.
- Insert plunger into the syringe
- Plunge out the solution into the centrifuge tubes
- Add 500µL deionized water

Preparation

Attach SPE column to vacuum manifold.

Conditioning

- Load SPE column with 500µL of methanol
- Equilibrate for 1 minute
- Eluted methanol into a waste container.

Loading

- Load SPE column with 800uL of liquid extraction solutions (loading volume)
- Equilibrate for 1 minute
- Collect the eluted solution in the centrifuge tube and keep it aside

Elution

- Load SPE column with 1mL n-hexane/ CH_2Cl_2 solvent
- Equilibrate for 1 minute
- Elute out the solvent
- Collect the eluted solvent in GC glass vial

Evaporation

- Keep the GC vial (without cap) into a hot air oven.
- Set the oven initial temperature at 40°C
- Every 15 minutes, increase by 10°C upto 60 °C. Maintain at 60 °C upto complete solvent evaporation (3-4 hours)

Reconstitution

- Add 1mL iso-octane to each vial
- Attach the top cap to each vial
- The vial is ready for GC MS analysis

Figure 2. Flowchart of the analytical method.

Results & Discussion

Soil analysis

Figure 3 presents the percentage pesticide recovery using the All Carbon microbeads and Graphitized Carbon black packed SPE columns. Significantly higher recovery of OCPs was obtained using All Carbon microbeads across all the SPE columns. All 15 pesticides could be recovered using the various SPE columns packed All Carbon microbeads. The average pesticide amount recovered was between 1-80 ppm (µg/kg) with the highest recovery of the pesticide p,p,-DDT. However, for the SPE columns of graphitized carbon black media, only two pesticides, endosulphan sulphate and p,p-DDT could be recovered using the 3 mL 100 mg bed weight and 3 mL, 250 mg bed weight SPE columns, respectively. No pesticides could be recovered using 6 mL, 500 mg bed weight.

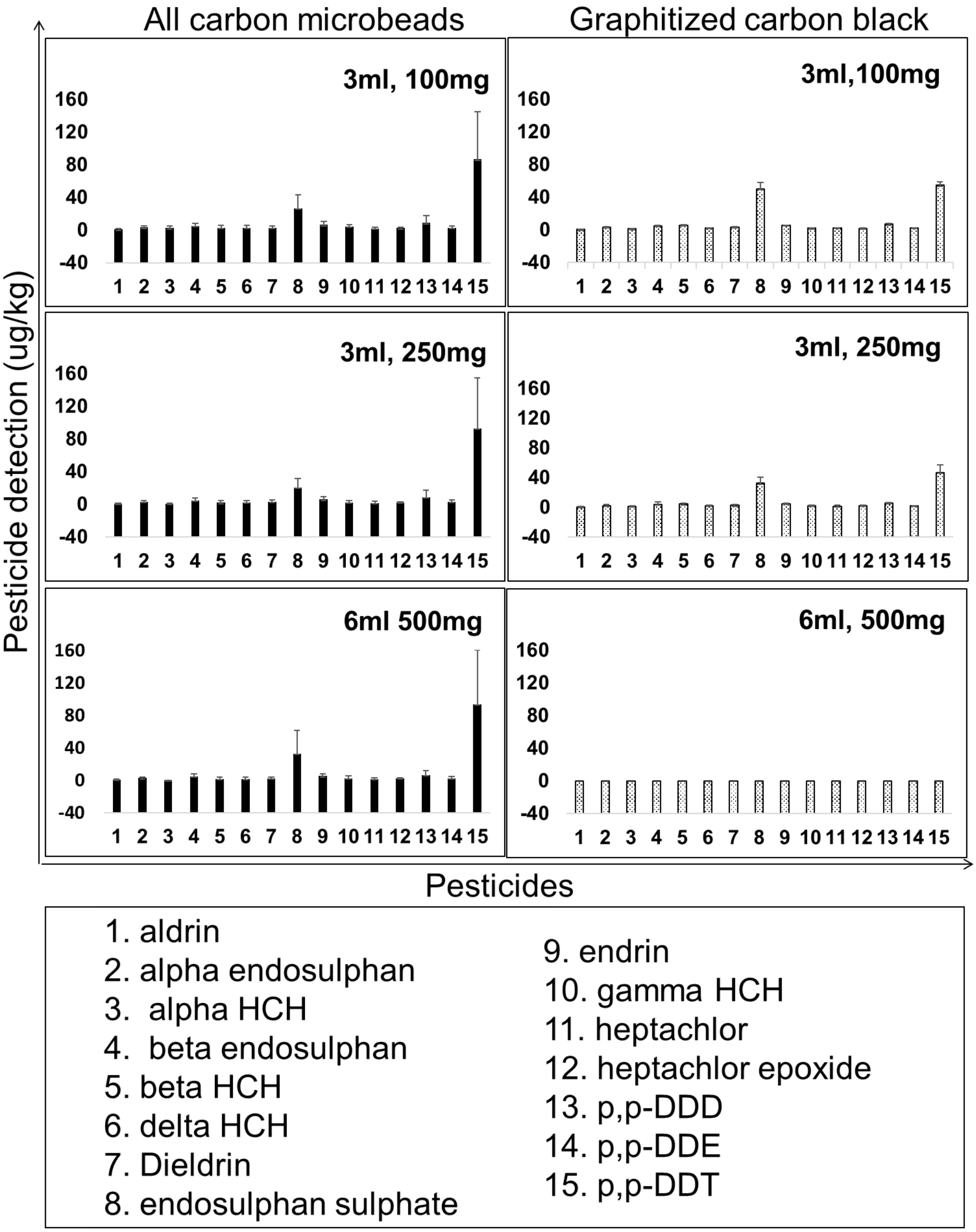


Figure 3. *Pesticide recovery from soil samples using All carbon microbead and graphitized carbon black packed SPE columns.*

Water analysis

Figure 4 presents the percentage pesticide recovery using the All Carbon microbeads and Graphitized Carbon black packed SPE columns. Significantly higher recovery of OCPs was obtained using All Carbon microbeads across all the SPE columns. All 15 pesticides could be recovered using the various SPE columns packed with All Carbon microbeads. The average pesticide amount recovered was between 1 and 90 ppm (µg/kg), with the highest recovery of the pesticide endosulphan sulphate. Conversely, no pesticides were detected after extraction with various graphitized carbon black SPE columns.

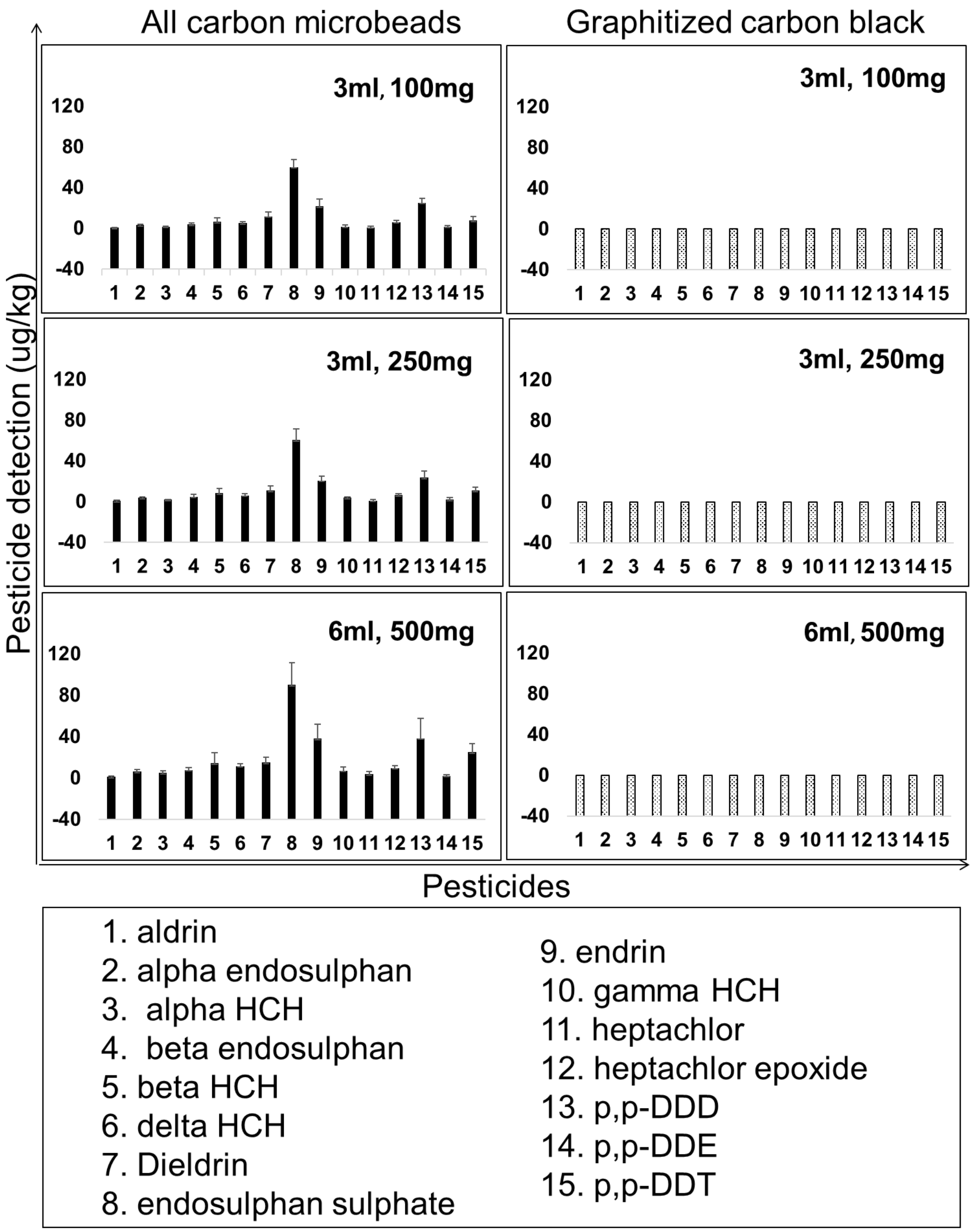


Figure 4. Pesticide recovery from water samples using All carbon microbead and graphitized carbon black packed SPE columns.

The recovery differences between the All Carbon microbeads and graphitized carbon microbeads are due to their selectivity differences. The All Carbon microbeads exhibit a reverse-phase chromatography mechanism. Thus, it absorbs and elutes the pesticides, allowing downstream detection. Its composition comprises a network of carbon-carbon double bonds known as aromatic sp2-hybridized carbon with delocalized π orbital electrons. π-π interactions occur between an aromatic or unsaturated analyte and graphitic stationary phases. The differences in these interactions contribute to analyte selectivity.

Conversely, graphitized carbon blacks are adsorbents similar to activated carbon. Their mechanism of adsorption is commonly attributed to the presence of micropores in their structure. It is driven by several parameters, including pore size and distribution, as well as surface area. They vary from very weak to medium/strong sorbents. Thus, depending on this strength, they will capture an analyte very efficiently, but the release or elution of these adsorbed analytes is poor to suboptimal. Thus, their primary use is during SPE sample clean-up to remove impurities or nuisance compounds.

The results taken together indicate that the All Carbon microbeads reverse phase media, in conjunction with the analytical method, allow the SPE of all the OCPs employed in this study. If the final goal of the sample preparation is only the extraction of OCPs from soil and river water samples, both All Carbon microbead and graphitized carbon black media are suitable. However, if the goal is to extract and elute OCPs for further downstream analysis, the All Carbon microbeads reverse-phase media, in conjunction with the analytical method, are better suited. This capability would enable the application of methods for assessing pesticides in soil and potential risks of soil contamination and surface water contamination in adjacent streams and reservoirs, using All carbon black SPE columns with desirable bed weights and column types.

Conclusion

NanoPak-C All-Carbon reversed-phase packed SPE columns allow the extraction, elution, and subsequent detection by GC-MS of organochlorine pesticides from small quantities of environmental samples with complex matrix compositions across all volumes and bed weights. Under similar conditions, Graphitized carbon black columns extract, but exhibit suboptimal elution, resulting in poor to no GC-MS detection of pesticides. The results indicate that the streamlined SPE procedure using the NanoPak-C All Carbon microbead media is suitable for extracting and detecting organochlorine pesticides from soil or water samples, regardless of whether they are available in limited or large quantities.

References

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