Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

PRE-CONCENTRATION OF ORGANOCHLORINE PESTICIDES USING MICRO-SOLID PHASE EXTRACTION BASED ON BIO-SORBENT ALGINATE-SILICA BEADS DERIVED FROM OIL PALM FROND

(Pra-Kepekatan Racun Organoklorin Menggunakan Pengekstraksi Fasa Mikro-Pepejal Berasaskan Bio-sorben Alginat-Silika Komposit yang Diperoleh daripada Daun Pelepah Kelapa Sawit)

Azreen Asyikin Mhd Kamal[1], Siti Nur Afiqah Binti Mahazan[1], Nur Husna Zainal Abidin[1], Fariesha Farha Ramli[1,2], and Wan Nazihah Wan Ibrahim[1*]

[1]*Faculty of Applied Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2]*Faculty of Applied Science, Universiti Teknologi MARA, Perak Branch, Tapah Campus, 35400, Perak*

[*]*Corresponding author: wannazihah@uitm.edu.my*

Received: 27 September 2023; Accepted: 16 January 2024; Published: 29 April 2024

Abstract

This study focuses on the extraction of organochlorine pesticides (OCPs) contained in environmental water samples by using encapsulated alginate-silica (Alg-SiO_2) composite beads. The composite beads were made by ionic crosslinking interaction between polymeric alginate and silica particles derived from oil palm fronds and were characterized by ATR-FTIR, FESEM, and EDX to study their chemical and physical properties. The Alg-SiO_2 composite beads were then used as the sorbent material for the micro-solid phase extraction (μ-SPE) to extract OCPs from aqueous samples. Optimization of method development for extraction of targeted analytes (heptachlor, aldrin, and dieldrin) was done by varying the extraction time, extraction solvent, desorption time, and sorbent mass before the gas chromatography-mass spectrometry analysis. Analytical figures of merit for μ-SPE were calculated under the optimized extraction conditions. Under the optimum conditions of 50 mg of sorbent extracted for 5 mins and desorbed using n-hexane for 7 mins, good linearity was achieved based on the data from method validation, linear ranges of 1–10 mg/L for heptachlor, 0.1–1.0 mg/L for aldrin, and 0.2–1.4 mg/L for dieldrin, with a limit of detection of 0.09–0.85 mg/L. The proposed method was successfully applied to determine organochlorine pesticides in paddy water samples. The wide range of the calibration curves was linear, and the coefficients of regression were all around 0.979-0.993. The recoveries were obtained in a satisfactory range of 82-98%, and the precision RSD value was in the range of 0.19-4.52%.

Keywords: alginate, silica sorbent, organochlorine, oil palm, micro solid phase extraction

Abstrak

Kajian ini memfokuskan kepada pengekstrakan racun perosak organoklorin (OCP) yang terkandung dalam sampel air persekitaran dengan menggunakan kapsulan manik komposit alginat-silika (Alg-SiO_2). Manik komposit dibuat melalui interaksi pertautan silang ion antara polimer alginat dan zarah silika yang diperoleh daripada pelepah kelapa sawit, dan dicirikan oleh ATR-FTIR, FESEM, dan EDX untuk mengkaji sifat kimia dan fizikalnya. Manik komposit Alg-SiO_2 kemudiannya digunakan sebagai bahan

penjerap untuk pengekstrakan fasa pepejal mikro (μ-SPE) untuk mengekstrak OCP daripada sampel akueus. Pengoptimuman pembangunan kaedah untuk pengekstrakan analit yang disasarkan (heptaklor, aldrin, dan dieldrin) dilakukan dengan mempelbagaikan masa pengekstrakan, pelarut pengekstrakan, masa nyahjerapan dan jisim penjerap sebelum analisis spektrometer jisim gas kromatografi. Angka analisis merit untuk μ-SPE dikira di bawah keadaan pengekstrakan yang dioptimumkan. Di bawah keadaan optimum 50 mg penjerap yang diekstrak selama 5 min dan nyahjerap menggunakan n-heksana selama 7 min telah menghasilkan julat linear yang baik. Berdasarkan data yang diperoleh daripada pengesahan kaedah, julat linear 1–10 mg/L untuk heptaklor, 0.1–1.0 mg/L untuk aldrin, dan 0.2–1.4 mg/L untuk dieldrin, dengan had pengesanan 0.09–0.85 mg/L. Kaedah yang dicadangkan telah berjaya digunakan untuk menentukan racun perosak organoklorin dalam sampel air padi. Julat luas keluk penentukuran adalah linear, dan pekali regresi adalah sekitar 0.979-0.993. Pemulihan diperolehi dalam julat yang memuaskan iaitu 82-98% dan nilai ketepatan RSD dalam julat 0.19-4.52%.

Kata kunci: alginat, silika sorben, organoklorin, kelapa sawit, pengekstraksi fasa mikro-pepejal

Introduction

Oil palm plants have contributed to Malaysia's gross domestic product by around 37.9% compared to other agriculture plants [1]. As stated in the statistic report, 77% of all residues come from oil palm production, which includes its frond, empty fruit bunch, trunks, kernel, and shells. As the number of oil palm plants increases, the waste undeniably increases. Dry oil palm fronds (OPF) only generated 44-47 million tons, which were burned as one of the disposal methods. This causes air pollution to the environment. In contrast, agricultural wastes are renewable resources that can be utilized to create energy, bio-based products, and items with additional value [2]. Consequently, numerous research and reviews exist on how these agricultural wastes can be utilized as useful resources rather than discarded [3, 4]. Oil palm fronds have numerous underutilized natural polymers, including cellulose, hemicellulose, and lignin. According to Roslan et al., OPF may contain siliceous material, attracting the attention of many researchers to the potential of silica extracted from this biomass [5]. Silica is an inorganic substance with excellent mechanical strength, high chemical stability, and thermal stability. Its internal connection is composed of siloxane, while the surface is abundant in silanol. This provides silica with excellent hydrophilicity. Furthermore, this silica is environmentally tolerant, which makes it widely used as an adsorbent carrier [6]. Thus, this study utilized silica extracted from oil palm fronds, which follows the government's announcement on "Zero Waste Initiatives" by reusing the oil palm fronds' wastes into silica powders. A study by Onoja et al. showed that ash from acid-treated OPF could produce about 95.30% silica (SiO_2), which is employed in many applications such as in diverse array applications, enzyme immobilization carriers, as well as functional biomaterial [7]. The hydroxyl groups on the surface of silica particles make it easy to chemically modify the surface and introduce functional groups [8] by controlling the pore volume, pore size, and tortuosity, and surface chemistry on the particle allows for tunable release profiles in controlled release applications involving silica particles [9]. However, the light and fine particles of obtained silica properties make it complicated to collect and reuse after extraction. Hence, encapsulating the silica with tensile polymeric alginate proves to be a favorable option for improving their recyclability without the loss of any of the fine particles [10].

Alginate (Alg) is a biomaterial with several uses in biomedical science and engineering due to its advantageous features, such as biocompatibility and ease of gelation [11], with the presence of hydrophilic and reactive functional groups providing superior adsorption [12]. This biopolymer is rich in D-mannuronic acid (M block) and L-guluronic acid (G block), which can be easily cross-linked utilizing bivalent ions such as calcium ions to create hydrogels during the gelation process [13]. Hence, it is frequently used to create a gel-phase adsorbent material that is easier to manipulate than the raw precursor material [14]. Nevertheless, this biomaterial has high rigidity, fragility, and weak elasticity, as well as weak mechanical properties [15]. Organic and inorganic alginate-based composites have been produced to improve the mechanical and thermal stability, as well as the swelling

properties of pure alginate polymers, which have been intensively studied for the removal of pollutants from aqueous solution over the past decade [15]. One of the materials studied was inorganic silica matrices that provided several benefits regarding chemical and mechanical stability [16]. These combined characteristics of silica and alginate composites have drawn interest since they may be superior compared to their individual compounds [17], which enhances their high biocompatibility and reasonable physical and chemical durability [18]. Thus, alginate-silica composites were proposed in the study as a new class of materials with promising applications in a variety of disciplines, including biomedicine, bio-catalysis, bio-separation, and bio-sensing [19]. The proposed synthesis mechanism of alginate-silica adsorbent is shown in Figure 1 [20].

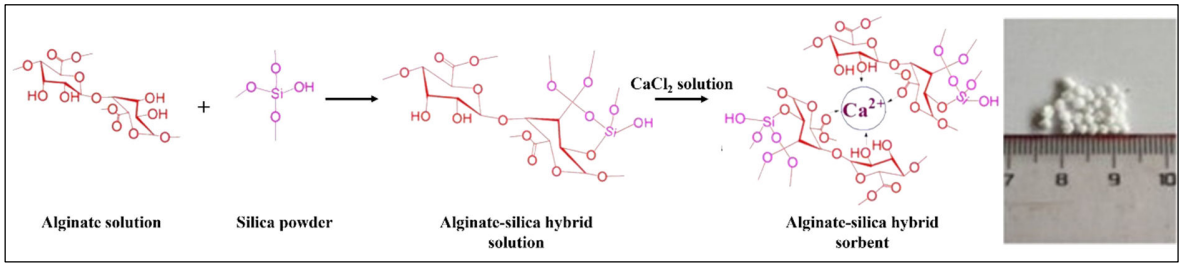

Figure 1. Synthesis mechanism of alginate-silica hybrid adsorbent

Amongst the aforementioned applications, very few studies of modification biowaste-silica for the pre-concentration of organochlorine pesticides have been investigated, as summarized in Table 1. Most of them applied different types of sorbents for OCPs or organophosphorus pesticides (OPPs). Organochlorine pesticides (OCPs) are one of the major pesticides that are used to manage insect pests on various types of crops. These OCPs are in the persistent organic pollutants (POPs) class, which are poisonous, bio-accumulative, and bio-magnified. The widespread adoption of these pesticides causes accumulation in the environment and food chains, and the incidence of OCP contamination in the human body can cause cancer, neurological system damage, generative diseases, and immune system disruption. The usage of OCPs is usually for pest and vector control in agricultural plantations such as oil palm, cocoa, and rubber in Malaysia [21]. However, the usage of OCPs has declined over the previous decade, as shown by various environmental monitoring studies [22, 23]. It is still possible to find OCPs and the products of their decomposition in the water, soil, sediment, and aquatic species in some regions of the world due to human activity. This is confirmed by several studies that have found the existence of OCPs in various samples in Malaysia. This includes the presence of OPCs (heptachlor and aldrin) in the surface water of Bertam and Terla River in Cameron Highland [24] and the detection of other types of OCPs such as endosulfan sulfate, 4-4'-DDT, and hexachlorocyclohexane isomers (α, β, γ, δ) in paddy soil situated in Machang, Kelantan [25] as well as detection of another group of OCPs, benzenehexachlorides (BHCs) in fish and sediment distributed in numerous countries including Malaysia [26].

These OCPs are present at the trace level, which needs sensitive, accurate, and efficient analytical procedures, which include sample preparation for their detection, identification, and quantification. Few sample preparations include liquid-liquid extraction (LLE) and solid phase extraction (SPE), which consume enormous volumes of toxic organic solvents and generate a substantial amount of waste that is unsafe for users and the environment [39]. Until today, SPE has mainly been used as sample preparation, especially on aqueous matrices, which mostly employ cylindrical cartridges packed with sorbent as SPE devices [40]. Countless researchers studied the miniaturization and solventless of SPE, such as dispersive SPE, or coined as μ-SPE. It is explained that this procedure implements loose

sorbent dispersed in the aqueous sample to allow the extraction of analytes and separated physically by magnetism, filtration, or centrifugation before the elution of analytes using solvent for the analysis [40]. This extraction method is favorable since it applies small amounts (microgram or milligram quantities) of sorbent, which allows the dispersion to occur and facilitates rapid and uniform interaction between sorbent and target analytes, resulting in faster equilibrium and enhancing the extraction efficiency [40]. Basheer et al. presented membrane-protected micro-solid-phase extraction (μ-SPE) as a replacement for classical multistep SPE, which allowed a reduction in the volumes of sorbent and desorption solvent as well as smaller sample volume and lower overall cost [41]. Sev eral studies implementing numerous types of μ-SPE techniques with results are summarized in Table 1. Therefore, the research aimed to investigate the use of the first-ever attempt of alginate-silica beads derived from oil palm frond as a promising bio-sorbent for the determination of OCPs in water samples with the aid of μ-SPE. Besides, this method works well for matrices that are moderately difficult to process by protecting the hydrophobic membrane and improving the sorbent as well as matrix influence to a greater extent.

Table 1. Summaries on analytical performance and extraction conditions of the developed procedure with other recently published works

Extraction Technique	**Sorbent Type**	**Sample Vol. (mL)**	**Desorption Solvent**	**Desorption Solvent Vol. (mL)**	**Total Extraction Time & Desorption Time (min)**	**LOQ (μg L^{-1})**	**RR (%)**	**Ref.**
d-μ-SPE	C18/Florisil/ Chromosorb G/AW-DCMS	100	EtAc	0.5	54	0.0017–0.0745	62.3-123	[27]
SBSE	SMNMa	60	ACN	1.50	57	0.23 – 2.94	82.6-104	[28]
d-μ-SPE	Zn-MOFb	20	ACN	0.20	~12	0.099 – 0.693	91.9-99.5	[29]
d-μ-SPE/ MSPE	Fe_3O_4@PDA-DES	100	ACN	1.50	~23	0.03 – 0.036	72.5-108	[30]
d-μ-SPE/ MSPE	Fe_3O_4@SiO_2 @KIT-6	100	ACN	2.00	~12	0.04 – 0.018	86.6-98.8	[31]
MIP-SPE	MIP	50	MeOH/HAc	1.50	-	0.23 – 0.41	79-104	[32]
d-μ-SPE	Fe_3O_4@TGA @TMU6	25	1-ButOH	0.10	35	1.65 – 3.30	88-107	[33]
SPE	PDMAT microbeads	100	DCM	10.0	-	0.006 – 1.99	69-139	[34]
SPE	PEG-CNT	70	ACN	0.80	-	0.033 – 0.099	95.2-99.8	[35]
d-μ-SPE/ MSPE	Fe_3O_4@APTES-GO /ZIF-8	30	MeOH	1.50	18	0.033 – 0.363	71.2-111	[36]
d-μ-SPE	MIL-101	10	EtAc	0.10	55	0.0083 – 0.053	87.6-98.6	[37]
d-μ-SPE/ MSPE	KHA/Fe_3O_4	30	MeOH	0.25	10	0.099 – 0.330	93.4-99.3	[38]
d-μ-SPE	Alginate – silica	10	n-hexane	1.00	22	0.27 – 2.55 (mg/L)	82 - 98	This work

Materials and Methods

Chemicals and materials

Three organochlorine pesticide standards: heptachlor ($C_5H_{10}Cl_7$, ≥ 98%), aldrin ($C_{12}H_8Cl_6$, ≥ 98%), dieldrin ($C_{12}H_{16}O$, ≥ 95%), Acetonitrile (C_2H_3N, 99.9%), and ethanol (C_2H_6, 99.9%, HPLC grade) were purchased from Sigma-Aldrich. Acetone (CH_3COCH_3, ≥ 99.5%) ACS reagent, ethyl acetate ($C_4H_8O_2$, 98%), n-hexane (C_6H_{12}, 99%), anhydrous calcium chloride ($CaCl_2$, ≥ 97%) and sodium alginate were all purchased from Sigma Aldrich (St. Louis, USA). Ultrapure water was collected from Barnstead Nanopure (Thermo Scientific).

Preparation of standard and sample solutions

A series of working solutions from stock solution, 1000 mg/L, were diluted in acetonitrile to prevent the decomposition of analytes and stored in amber glass at 4°C when not in use. Paddy water samples were collected from Bukit Gambir, Johor, and filtered through 0.45 µm nylon filter paper to remove any suspended particles. The samples were stored in a pre-cleaned bottle with acetone polyethylene at 4°C prior to the extraction. The sample was spiked with a standard mixture to give a final concentration of 5 mg/L of heptachlor, 0.5 mg/L of aldrin, and 1 mg/L of dieldrin common solution into a solution for a final volume of 10 mL for the precision and accuracy studies.

Preparation of silica derived from oil palm fronds

The method of synthesis was adapted from the literature [42]. Freshly harvested oil palm fronds (OPF) were then dried in the oven at 70 °C for 48 hours and ground into fine powder. The OPF powder was dispersed into 1 M HCl and constantly stirred for 2 hours at 400 rpm. The dispersion was allowed to cool at room temperature. The wet powder was washed with distilled water until the acid-treated OPF reached neutrality at pH 7. The neutralized OPF was filtered and dried in an oven at 80 °C for 24 hours. Then, the OPF powder was calcined in the furnace at 600 °C for 14 hours with a ramping time of 5 hours and a hold time of 9 hours at the rate of 2 °C/min. The white ash of silica was harvested and stored in capped vials at room temperature for further analysis.

Preparation of alginate-silica composite beads (Alg-Si)

Experimental methods for the preparation of alginate-silica were adapted and modified from the previous studies with minor modifications [43]. Alginate-silica (Alg-Si) composites were prepared by adding 2g of sodium alginate into 100 mL of distilled water and stirring using a magnetic stirrer at 60 °C to produce 2% (w/v) of sodium alginate solution until no longer coagulated. The heat was turned off, and the solution was left stirred. In the meantime, 10 mL of distilled water was added to 0.1g of silica powder and ultrasonicated with parafilm covered onto the beaker's mouth for approximately 10 mins. The silica solution was then added to the 2% (w/v) sodium alginate solution and stirred thoroughly. In the separated beaker, 4% (w/v) of $CaCl_2$ was prepared and diluted with distilled water. The Alg-Si mixture was then dropped wisely into the prepared $CaCl_2$ solution and left for 30 mins. The Alg-Si beads formed were filtered and rinsed with distilled water before being dried in an oven overnight at 80 °C to remove any excess cation solution. The Alg-Si was then stored and tested for the extraction procedure.

Characterization of alginate-silica composite beads

Several characterization methods were applied to study the physical and chemical properties of Alg-Si beads formedAttenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) with a resolution of 4 cm^{-1} and a total of 8 scans in the range of 500-4000 cm^{-1}. Scanning Electron Microscope (SEM) was used to determine the qualitative characteristics of the physical organization and morphology of pure alginate beads and alginate-silica beads as well as energy-dispersive X-ray spectroscopy (EDX) analyzer TEAM ™ EDS to measure the micro-area elements and the condition of element distribution in beads.

Micro-solid phase extraction (µ-SPE)

Several parameters were optimized by changing one variable at a time (OVAT), including extraction time, desorption solvent, desorption time, and mass of sorbent. OVAT was chosen because of the simplicity of the system and the limited number of parameters under consideration. With only four parameters to be examined, OVAT provides a practical and

straightforward approach, allowing for a focused exploration of individual factors while ensuring resource efficiency. This OVAT method was adapted from Othman et al. with minor modification, where 50 mg of the beads were added to 10 mL of spiked aqueous sample and agitated at 900 rpm for 15 mins to allow the desorption process to occur in 12-Radley carousel system. The supernatant was discarded, and 1 mL of desorption solvent (acetonitrile) was added to the beads before sonicated for 7 mins. Under a gentle stream of nitrogen gas, the collected solvent was pre-concentrated to 500 µL and transferred to the vial for GC-MS analysis [44]. The schematic flow diagram of µ-SPE is illustrated in Figure 3.

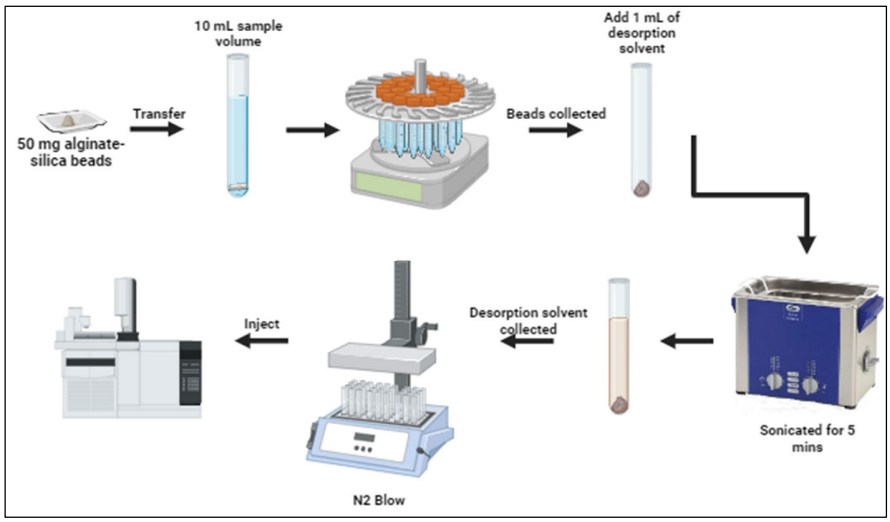

Figure 2. Micro-Solid Phase Extraction (µ-SPE)

Chromatographic conditions

The analysis was conducted using GC-MS, Shimadzu's (Kyoto, Japan) QP2010 GC-MS system, and a Shimadzu AOC-20i autosampler. The flow rate was set at 1.2 mL/min with pressure at 16 kPa at an average velocity of 41.633 cm/sec using helium as carrier gas. The injection was set without any intermediate splits. The separation was carried out on a silica capillary column (30 x 250 µm x 0.25 µm). The chromatographic conditions were optimized by varying the temperature. The temperature in the oven was increased from 150 °C (held for 1 min) to 200 °C at a rate of 30 °C per min (held for 2 mins), then to 250 °C at a rate of 10 °C per min, and finally to 280 °C at a rate of 20 °C per min. The data was collected in full scanning mode from 50 to 500 m/z to verify retention times. Selective ion monitoring (SIM) analysis was performed on all standards and sample extracts.

Method validation

Prior to sample analysis, the linearity (R^2), limit of detection (LOD), limit of quantification (LOQ), precision (RSD%), and accuracy (relative recovery%) of the method were evaluated.

Green analytical calculator (AGREE)

This analytical green calculator is a simple software applied in various research to evaluate the greenness of the analysis method. This calculator evaluated the greenness of the developed method when the AGREE metric values are near 1, correlated to a dark green. Meanwhile, a red color implies the least environmentally friendly, which is close to 0. This calculator has 12 scales, each of which corresponds to fundamental principles of green analytical chemistry, and the weight of each scale represents the importance of the principles of the analytical procedure [45]. The principle of the scale includes (1) the implementation of direct analytical techniques to avoid sample treatment, which can reduce the safety issues and health and environmental problems related to the methodology. However, direct analysis is usually inexecutable as the

appropriate form of the sample is required to increase its sensitivity; (2) the minimal usage of sample size and samples are recommended; (3) preferable *in situ* measurements whereby the location of analytical device should be close to the measurement location; (4) reduction usage of reagents and saves energy by the integration of analytical process and operations such as sampling with pre-concentration, simultaneous performance of derivatization with extraction or chromatographic determination; (5) automated and miniaturized methods; (6) no derivatization should be employed; (7) the proper management of analytical waste and a small volume of analytical waste should be implemented; (8) the multianalyte or multiparameter methods are preferred compared to methods using one analyte at a time; (9) the minimization of energy consumption in detection steps, sample preparation and analytical separation; (10) reagents obtained from renewable sources should be preferred; (11) toxic reagents should be eliminated or replaced by greener alternatives whenever possible; (12) the safety of the operator and environment whereby the several threats that are not avoidable should be selected [46].

Results and Discussion

Physical properties

Digital photographs were captured to examine the visual structure of the composite beads (Figure 4). Alg-Si beads, before drying, were obtained in asymmetrical white color and have a texture resembling hydrogel due to water content, as shown in (a). However, the size of Alg-Si shrunk to half after the drying process and turned to a yellowish color. In comparison, the pure alginate bead was prepared with a similar method and obtained as dark yellow beads.

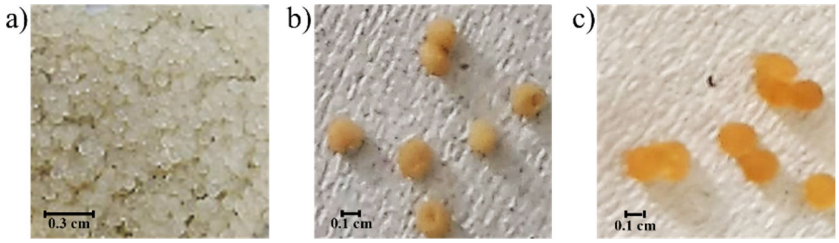

Figure 3. Digital photograph using phone camera with the scale of (a) Alg-Si beads before dried, (b) Alg-Si beads after dried overnight, (c) pure alginate beads after dried overnight

ATR-FTIR analysis

Figure 5 shows the spectra of OPF-silica powder, Alg-Si beads, and pure alginate beads. In the spectrum of OPF-silica powder, the existence of a broad band around 3407 cm^{-1} is attributed to the -OH stretching vibration of the silanol on the silica surface. The strong band at 805-1079 cm^{-1} corresponds to the asymmetric vibration of the Si-O-Si siloxane bond, which forms the silica matrix backbone [7]. The FTIR spectra of Alg-Si beads demonstrate that the absorption peaks at 3326 cm^{-1} correspond to the O-H absorption band, which is due to the stretching vibration of residual -OH groups and free hydrogen bonds [47]. The absorption peaks at 824 and 1029 cm^{-1} correspond to symmetric and asymmetrical stretching vibrations of Si-O-Si bonds, respectively. The bands at 1419 and 1598 cm^{-1} in the spectrum of the Alg-Si beads may be assigned to asymmetric and symmetric COO- deformation, respectively, confirming the interaction of carboxylate groups with silanol [48]. The FTIR spectra of pure alginate beads display characteristic bands at 3331 $cm^{-1,}$ which correspond to the OH stretching vibrations of hydrogen-bonded OH groups. Other distinctive peaks of pure alginate beads can be detected at 1424 cm^{-1} and 1598 cm^{-1}. They are caused by the asymmetric and symmetric stretching peaks of carboxylate (-COO), respectively. Table 2 summarizes the comparison of major absorption bands for OPF-silica powder, pure alginate beads, and alginate-silica beads with all the spectra in Figure 5.

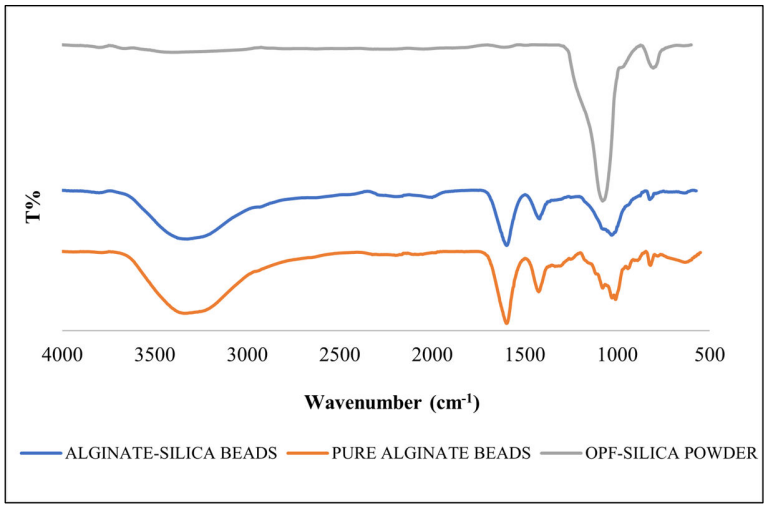

Figure 4. ATR-FTIR spectra of OPF-silica powder, Alg-Si beads, and pure alginate beads

Table 2. Comparison of major absorption bands for OPF-silica powder, Alg-Si beads, and pure alginate beads

Major Functional Groups	OPF-Si powder	Alg-Si beads	Pure alginate beads
	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
Stretching of O-H	3407	3326	3331
Antisymmetric stretching of COO-groups	-	1598	1598
Symmetric stretching of COO-groups	-	1419	1424
Asymmetric stretching of Si-O-Si	1079	1029	-
Symmetric stretching of Si-O-Si	805	824	-

Field emission scanning electron microscopy

The morphology of Alg-Si beads and pure alginate beads was investigated by field emission scanning electron microscopy (FESEM). Under magnification 45X, 1000X, 3000X, and 5000X, micrographs of pure alginate and Alg-Si beads were viewed. Alginate and calcium chloride can form a rigid structure due to the sequential placement of G units on alginate, which generates a space for calcium ions to bind, with each ion able to link to four G units [49]. In accordance with Figure 4 (a-b), the surface of pure alginate beads was rough, irregular in shape, and possessed a deep and extensive fissure span. At the same time, the morphology study of Alg-Si beads in Figure 6 (c-d) found that the gully and folds on the surface of the hybrid material diminished after interaction with SiO2; the surface of the "wrinkles" ring shrunk and progressively became more regular, with no discernible fold ring appearing. Briefly, the surface structure of Alg-Si beads was more homogeneous and regular than that of pure alginate composite beads. In addition to having a rough surface, the Alg-Si beads have cavities created during the cross-linking process. The formation of cavities during sonication, in which alginate and silica combine to produce a homogenous web [50]. Moreover, these cavities facilitate pore diffusion during adsorption due to the significance of the inner specific surface with a low diffusion resistance in the composite beads (involving increased adsorption potential and rate). In conclusion, the composite particles of alginate and silica exhibited a porous network structure. A large number of hydroxyl and carboxyl groups in sodium alginate provided numerous chemical and physical cross-linking and reactive sites, which enhanced the deformation of resistance of cross-linked structures [51].

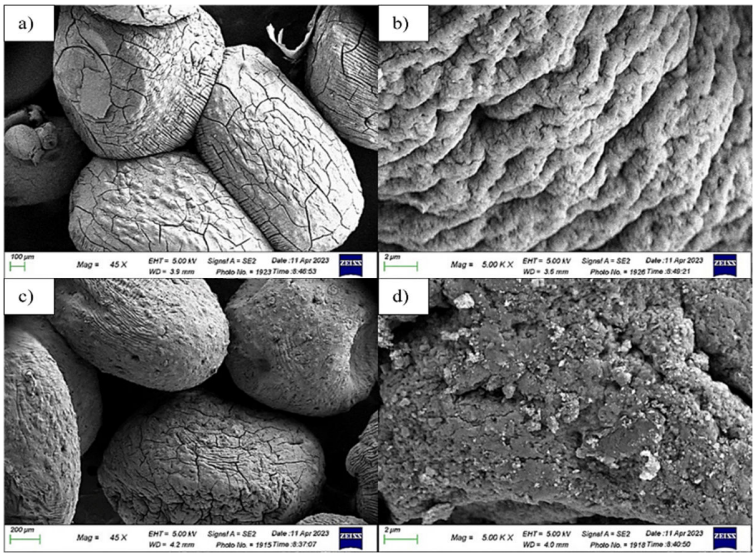

Figure 5. FESEM images for pure alginate beads at a) 45X, b) 1KX and Alg-Si beads at c) 45X, d) 5KX

Energy dispersive X-ray (EDX) analysis

In conjunction with energy dispersive X-ray (EDX), energy spectra of Alg-Si beads and pure alginate beads were measured to characterize the beads. It permits the quantification of the active molecules retained within the beads. Figures 7 a) and b) depict the EDX analysis of pure alginate and Alg-Si beads, respectively. EDX analysis revealed that pure alginate beads consisted primarily of oxygen (68.83%), calcium (28.55%), and a minor amount of sodium (2.62%) by weight. There were no additional impurity peaks in the EDX spectra, and this was consistent with the results obtained in the FTIR. Details of the chemical composition of pure alginate and Alg-Si beads were tabulated in Table 3.

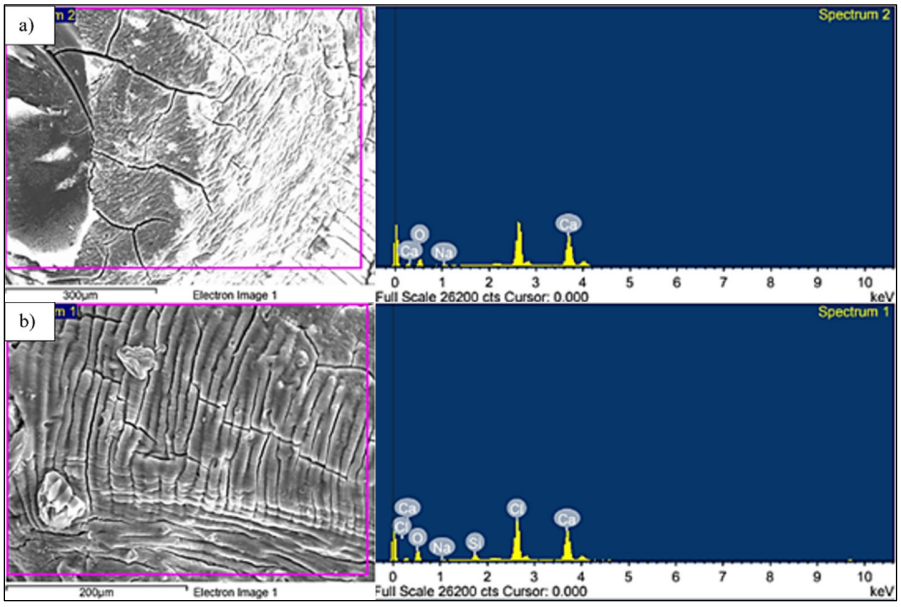

Figure 6. a) The EDX analysis of pure alginate beads and b) the EDX analysis of Alg-Si beads

Table 3. Chemical composition of pure alginate beads and alginate-silica beads based on EDX analysis

Element	Pure Alginate Beads		Alginate-Silica Beads	
	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)
O	68.83	83.89	59.05	76.64
Na	2.62	2.23	0.71	0.64
Ca	28.55	13.89	18.16	9.41
Cl	-	-	19.55	11.45
Si	-	-	2.53	1.87

Optimization parameters of µ-SPE

Heptachlor, aldrin, and dieldrin were chosen as the target analytes for this analysis since they are the most frequently used OCPs in the agriculture industry due to their excellent efficacy in pest control and cost-effectiveness [52]. In order to find the optimal conditions for the extraction of OCPs performance from aqueous samples, several significant variables that can affect the extraction performance, including extraction time, desorption solvent, desorption time, and sorbent mass, were studied. Optimization was conducted by using OVAT (one variable at a time) in which the distilled water samples were spiked with each OPC at a concentration of 1 mg/L in a 10 mL solution. Triplicate extractions were performed for every parameter optimized in this analysis using gas chromatography-mass spectroscopy (GC-MS).

Effect of extraction time

In the µ-SPE principle, the sorbent is encapsulated in a polysaccharide alginate matrix; as a result, its sorption behavior is based on the equilibrium of mass transfer between the sample matrix and the sorbent, as opposed to the exhaustive extraction as in cartridge- or disc-based SPE. The sorption time was one of the most important parameters for enhancing the precision and sensitivity of the extraction procedure, as it is vital to allow enough time to ensure the equilibrium between the aqueous phase and sorbent [53]. Thus, various extraction times were evaluated in the range of 2.5 to 15 minutes to study the optimum time for the sorbent-analyte to achieve an equilibrium state. The impact of contact time between the sorbent and target analytes is depicted in Figure 6. It was discovered that the adsorption efficiency of OCPs was significantly increased up to 15 minutes, especially for heptachlor and aldrin. However, the adsorption of dieldrin was practically constant after 10 minutes. This could be due to the saturation of the surface area of the sorbent as time progressed and the competitive adsorption rate of the analytes [54]. Further extraction time after 15 minutes was not advisable as a few studies show that it will cause back extraction of analytes from the acceptor into the sample solution [55]. Thus, the optimal extraction time was determined to be 15 mins and was employed in subsequent experiments. The average was reported with associated error bars representing the standard deviation (n=3) in Figure 8.

Effect of desorption solvent

According to the principle of “like dissolves like”, compounds dissolve in a solvent when their chemical properties, particularly their polarities, are similar [56]. Polar and non-polar solvents are able to dissolve polar and non-polar compounds, respectively [57]. In this analysis, the desorption solvents n-hexane, ethyl acetate (EA), acetone, acetonitrile (ACN), and ethanol were assessed, with increasing polarity from n-hexane to ethanol to determine which one provided the maximum analyte recovery using µ-SPE technique. Figure 9 demonstrates that n-hexane performed better than the other solvents regarding desorption. N-hexane, which is non-polar with a polarity index of 0.1, provides a more favorable performance in desorbing the target analytes due to the hydrophobicity properties of the OCPs [58]. Meanwhile, due to its reduced compatibility with the hydrophobic OCPs in terms of polarity, ethanol exhibited the lowest desorption efficiency. Hence, the principle “like dissolves like” was applicable and produced the greatest results among various solvents used, and n-hexane was regarded as the optimal desorption solvent for further analysis.

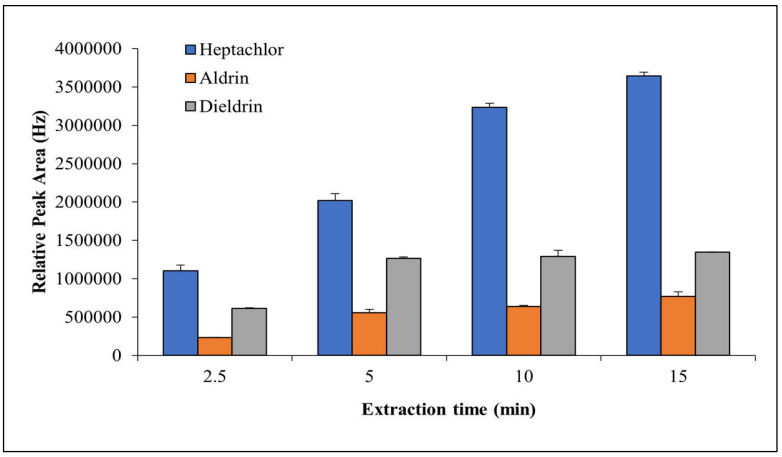

Figure 7. Graph of average relative peak height (Hz) against extraction time (min)

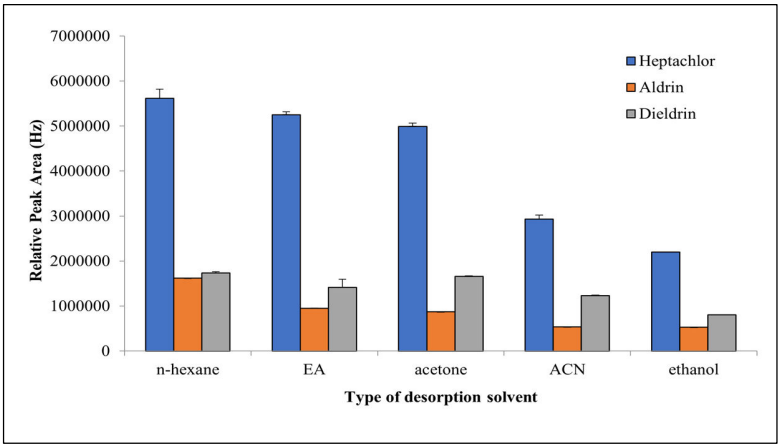

Figure 8. Graph of average relative peak height (Hz) against desorption solvent

Effect of desorption time

Desorption time was an additional significant element that could influence the peak area of the analytes. It has been determined that ultrasonication is an effective method for enhancing the desorption procedure [59]. The effectiveness of ultrasonic-assisted desorption is dependent on the parameters that influence the acoustic cavitation created during the process [60]. In addition to the ultrasonic power, the total amount of analytes eluted is also influenced by the time consumed. As depicted in Figure 10, there were only minor changes in desorption efficiency between 1,3,5,7 and 10 mins of ultrasonication. As the ultrasonic time increased from 3 to 7 minutes, a modest increase in the desorption of heptachlor, aldrin, and dieldrin was detected. Regardless of the result, at 1 and 10 mins, the trend of the graph appears to increase, although the data appears to be consistent at 3,5 and 7 mins, which are the span of time in the middle. Adding to this, extended ultrasonication decreased the efficacy of the OCP desorption. This result can be attributed to its comparatively high hydrophobicity among these analytes, indicating that the analyte prefers to diffuse into the hydrophobic pores of alginate-silica. It has been shown that ultrasonication can facilitate the desorption of adsorbed molecules and boost pore diffusion in the sorption process [60]. As a result of the re-adsorption of the target analytes, the peak area of each target OCP steadily decreased after a 7-minute desorption time [61]. Thus, the optimal desorption time for further analysis was determined to be 7 minutes.

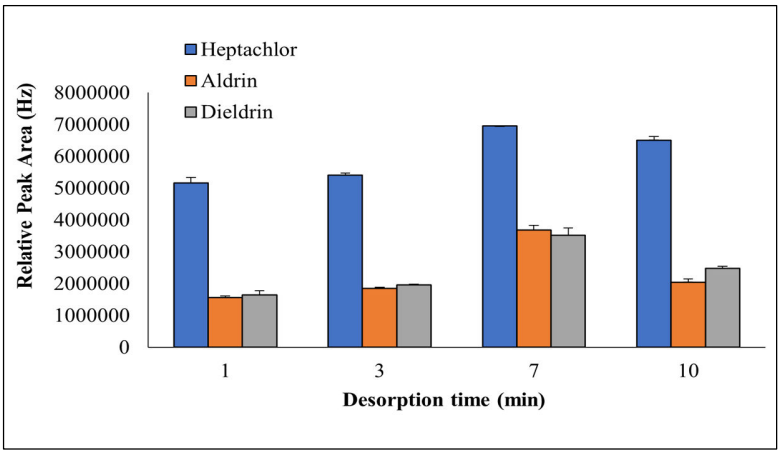

Figure 9. Graph of average relative peak height (Hz) against desorption time (min)

Effect of sorbent mass

In this study, 20, 30, 40, 50, and 60 mg of Alg-Si beads were used to optimize the sorbent mass. The results in Figure 11 demonstrate that the maximum peak area of analytes is attained with 50 mg of sorbents. With increasing sorbent mass, no discernible increase in peak area was seen; instead, the peak areas continued to diminish. This may be owing to the saturation of Alg-Si beads as the primary sorption site; hence, the use of an excessive amount of sorbent (more than 50 mg) made desorption more difficult and demanded a larger volume of desorption solvent [62]. Since the peak area for the analytes was at its maximum at 50 mg, 50 mg of sorbent was used for the following analysis.

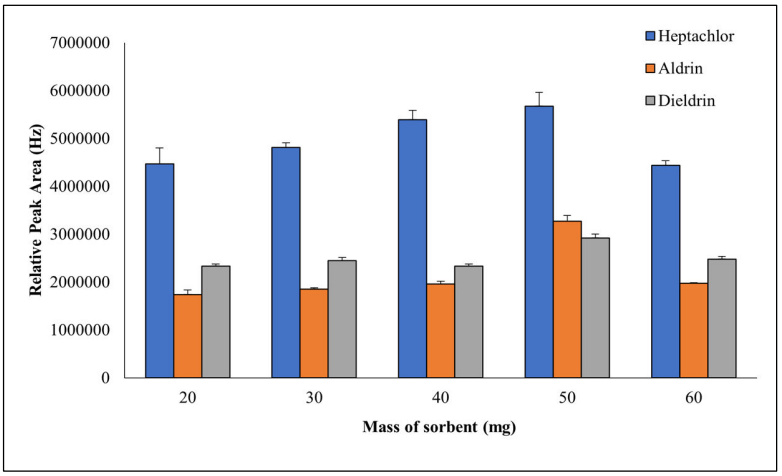

Figure 10. Graph of average relative peak height (Hz) against mas of sorbent (mg)

Optimum µ-SPE conditions.

The final adopted Alg-Si-µ-SPE conditions were: 15 min extraction time; n-hexane as desorption solvent; 7 min of desorption time; sorbent mass, 50 mg. Hence, these optimum extraction conditions were applied for subsequent studies.

Matrix-matched calibration

In analytical procedures, matrix effect (ME) can manifest as an undesired enhancement or suppression of the analytical response due to the eco-elution of interfering matrix components. Therefore, ME is a source of error in quantitative chromatography analyses of complex matrices such as pesticides, and it may be

compound or matrix-dependent. Commonly, ME is determined by comparing the slope of the calibration curve of analytes spiked into a blank paddy water sample to the slope of the calibration curve derived by spiking analytes into the solvent. In this study, matrix-matched calibration was performed on blank pesticides of varying concentrations of the OCPs.

Method validation

The proposed µ-SPE-GC-MS analytical method for the determination of three pesticides in paddy water samples under optimized experimental conditions was deemed valid. The analytical figures of merit, including the limit of detection (LOD), the limit of quantification (LOQ), the linear range, the relative recoveries (RR), the determination coefficient (R^2), and the precision (as relative standard deviation, RSD), were therefore determined. A calibration curve for each pesticide was constructed by adding six concentration levels of each analyte to a blank sample of paddy water. Heptachlor at 1, 4, 7, 10, and 13 ppm; aldrin at 0.3, 0.5, 0.7, 1, and 1.3 ppm; and dieldrin at 0.2, 0.6, 1, 1.4, 1.8 mg/L. Using least-squares linear analysis, the contours and corresponding regression equations were determined and subsequently validated by R^2 values. The spiked concentration differs because of the varying detection limits and anticipated ambient concentrations of the analytes [63], in which the calculated values indicate the expected levels of a particular substance or analyte in the surrounding environment. The optimized, developed µ-SPE procedure was validated to determine the adsorption capacity of Alg-Si particles as a functional bio-sorbent using paddy water as a sample, as shown in Table 4.

For each of the analytical curves, good R^2 values for each analyte were obtained in the range of 0.9796 to 0.9935. The LOD varied between 0.85 mg/L for heptachlor, 0.09 mg/L for aldrin, and 0.19 mg/L for dieldrin, as presented in Table 4, while the LOQ varied between 2.55 mg/L for heptachlor, 0.27 mg/L for aldrin, and 0.57 mg/L for dieldrin. Concerning precision, the examined OCPs exhibited intraday precision values of 10% or less. The intraday precision RSD% ranged from 2.52 – 2.78% for heptachlor, 2.34 – 3.29% for aldrin, and 3.52 – 4.52% for dieldrin. Meanwhile, the interday precision ranged from 1.82 – 2.31%, 0.30 – 1.91%, and 0.19 – 2.11% for heptachlor, aldrin, and dieldrin, respectively. Enrichment factor (EF) and extraction recovery (ER%) were also determined to evaluate the method's effectiveness. All the target pesticides provide RR (%) values above 80%, ranging from 82 – 84%, 90 – 91%, and 95 – 98%, for heptachlor, aldrin, and dieldrin, respectively. This result is comparable with another study conducted by Huang et al., which obtained a range of 87.6 – 98.6% for the same method with the application of different sorbents [64]. The EF values for the pesticides ranged between 6.74 – 16.87 for heptachlor, 9.64 – 34.73 for aldrin, and 4.38 – 20.25 for dieldrin. The results are summarized in Table 5. Meanwhile, Figure 12 shows the chromatogram of blank paddy water sample in three different analyses (blank, spiked at 1 mg/L and 10 mg/L, 0.1 mg/L and one mg/L, and 0.2 and 1.4 mg/L for heptachlor, aldrin, and dieldrin, respectively). All targeted analytes were detected in less than 10 minutes.

Table 4. Performance of the proposed method under optimized conditions

Sample	Analytes	Linearity (mg/L)	Correlation Coefficient	Regression Coefficient, R^2	LOD (mg/L)	LOQ (mg/L)
Paddy water	Heptachlor	1-10	y = 1246479x + 102580	0.9796	0.85	2.55
	Aldrin	0.1-1	y = 710263x + 166656	0.9908	0.09	0.27
	Dieldrin	0.2-1.4	y = 469676x + 62885	0.9935	0.19	0.57

Table 5. Method precision for intra- and inter-day (RSD), relative recovery (RR%), and enrichment factor (EF)

Analytes	Concentration (mg/L)	Intra-day RSD (%, n=3)	Inter-day RSD (%, n=9)	%RR	EF
Heptachlor	1	2.52	1.82	84	6.74
	10	2.78	2.31	82	16.87
Aldrin	0.1	2.34	0.30	90	9.64
	1	3.29	1.91	91	34.73
Dieldrin	0.2	3.52	0.19	95	4.38
	1.4	4.52	2.11	98	20.25

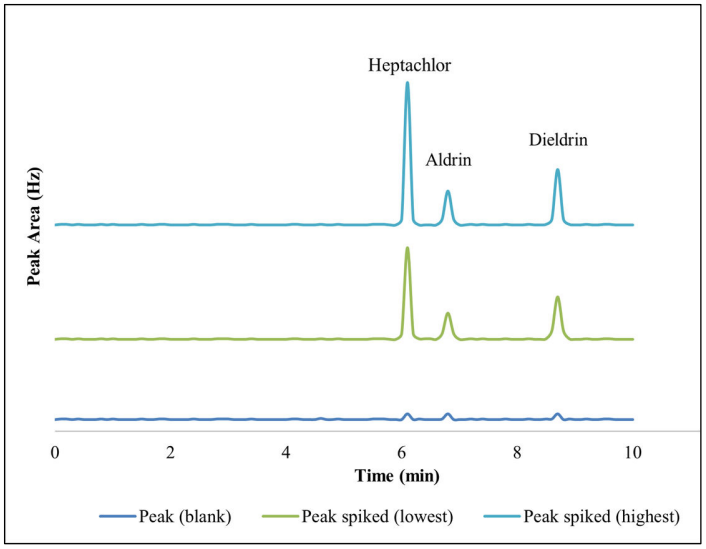

Figure 11. Chromatogram of paddy water sample at unspiked (blank), spiked (lowest concentration), and spiked (higher concentration)

Figure 12 shows the proposed interaction mechanism between the targeted analytes' OCPs and the obtained alginate-silica sorbent in this study. The extraction of OCP on alginate-silica sorbent is based on adsorption, whereby it possesses a porous structure comprising a substantial number of active sites accessible for binding with analytes [65]. It is explained that the binding type is contingent upon the nature of interactions between the adsorbent's surface and the molecular species of the analyte. For this interaction between OCP and alginate-silica composite, the interaction is physical, primarily governed by weak van der Waals forces, which is known as physisorption [65].

Green analytical calculator (AGREE)

The greenness of the developed method was evaluated using the AGREE metric calculator, which falls under a score (Figure 13) of 0.56. According to Rajendran et al., the determination of green classification lacks a predefined scale or metric; it hinges on the number of segments exhibiting a green color, with the value on the central pictogram approaching 1.0 [45]. The assessment implied an adequate green analytical methodology as it meets specific criteria, including (4) the integration of analytical processes for simultaneous steps, such as extraction and sonication (6), no derivatization agent was used during analysis and extraction, (10) no reagent used in the procedure, (12) the threats to the operator and environment were minimum due to minimum organic solvent consumption which approximately less than 1mL. The other two criteria represented by red are (3) the offline position of the analytical device and (9) the high consumption of energy-intensive techniques as the analysis was performed using GC-MS. The red colors of

the assessment will be improved for future work. Nevertheless, by concentrating solely on the advancement of sample preparation techniques, this study can be regarded as an environmentally friendly method in comparison to conventional SPE and LLE techniques.

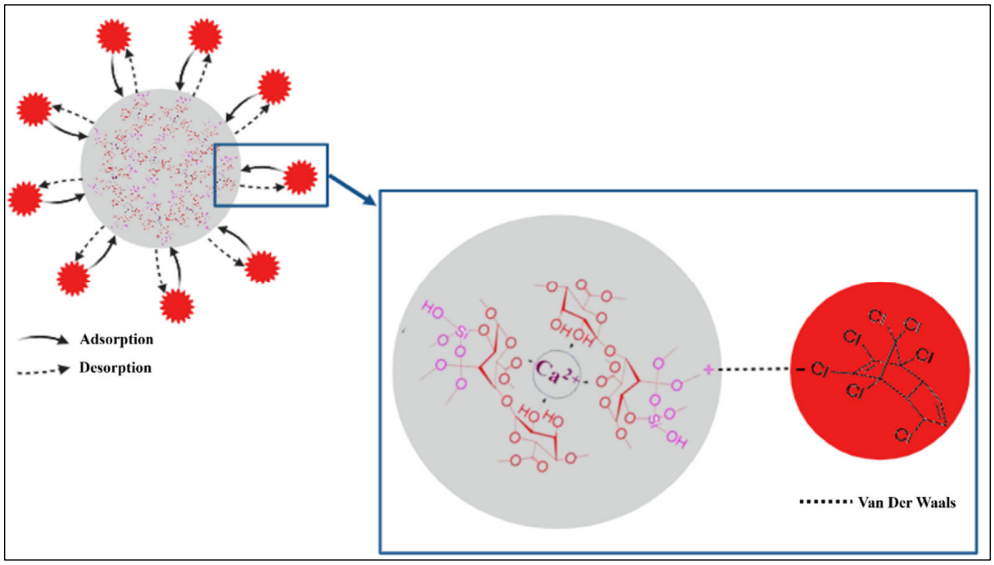

Figure 12. Interaction mechanism of extraction between alginate-silica sorbent with OCP (heptachlor) by van der Waals interaction

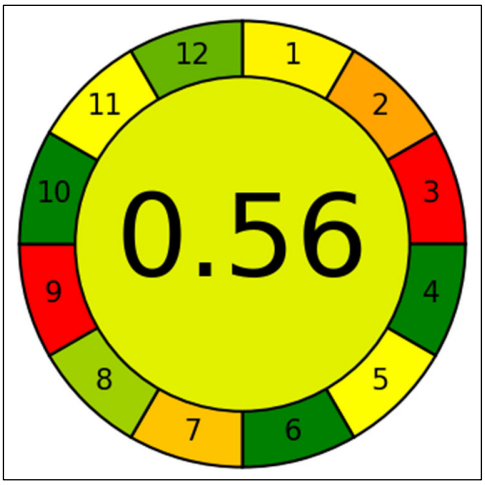

Figure 13. AGREE metric for greenness assessment of method developed

Conclusion

In conclusion, the chemical and physical properties of Alg-Si beads prepared by the encapsulation method were examined by characterization with ATR-FTIR and FESEM and showed that the functional groups of O-H, Si-O-Si, and COO^- groups contribute to the van der Waals, the interaction between prepared Alg-Si beads sorbent with targeted analytes. An uneven, rough surface area from the FESEM analysis of the sorbents could be attributed to the porosity of the beads obtained. The performance of Alg-Si-µ-SPE to extract OCPs from paddy water samples using alginate-silica beads was optimized, which included extraction time, desorption solvent, desorption time, and mass of sorbent.

Furthermore, the high adsorption capacity of the sorbent is demonstrated by the data obtained from method validation, which reveals linear ranges of 1-10 mg/L, 0.1-1.0 mg/L, 0.2-1.4 mg/L, for heptachlor, aldrin, and dieldrin, respectively with LOD and LOQ in between of 0.09-0.85 mg/L and 0.27-2.55 mg/L, respectively. The relative recoveries (RR) have been found in the range of 82-98% for all targeted analytes. OCPs were extracted from genuine paddy water samples, demonstrating the effectiveness of the developed sorbent. Most of the calibration curves were linear, and all regression coefficients were in the range of 0.97-0.99. The intra-day precision varied from 2.34-4.52% while the inter-day ranged from 0.30-2.31% by spiking the three analytes into a sample solution. The miniaturized solvents volume (μL) and sorbents mass (mg) utilized in this method could be considered a green method and safe for the environment. Furthermore, the silica produced from oil palm fronds (OPF) is likely to evolve considerably in various applications and not limited to analytical and material chemistry only.

Acknowledgment

The authors would like to convey their sincere appreciation to the Kurita Oversea Research Grant, Reference number: 22pmy058-K1 [100-TNCPI/INT 16/6/2 (065/2022)], for their financial support for this research. The authors also acknowledge the Faculty of Applied Science of Universiti Teknologi MARA for the facilities provided to carry out this project.

References

1. Department of Statistics Malaysia (2019). Gross domestic product. https://www.dosm.gov.my/portal-main/release-content/e37cf376-8b79-11ed-96a6-1866daa77ef9. [Access online 02 July 2023].
2. Marsin, F. M., Wan Ibrahim, W. A., Nodeh, H. R., and Sanagi, M. M. (2020). New magnetic oil palm fiber activated carbon-reinforced polypyrrole solid phase extraction combined with gas chromatography-electron capture detection for determination of organochlorine pesticides in water samples. *Journal of Chromatography* A, 1612: 460638.
3. Sulaiman, F., Abdullah, N., Gerhauser, H., and Shariff, A. (2011). An outlook of Malaysian energy, oil palm industry and its utilization of wastes as useful resources. *Biomass and Bioenergy,* 35(9): 3775-3786.
4. Ozturk, M., Saba, N., Altay, V., Iqbal, R., Hakeem, K.R., Jawaid, M. and Ibrahim, F. H. (2017). Biomass Bioenergy: An overview of the development potential in Turkey and Malaysia. *Renewable Sustainability Energy Reviews,* 79: 1285-1302.
5. Roslan, A. M., Zahari, M. A. K. M., Hassan, M. A., and Shirai, Y. (2014). Investigation of oil palm frond properties for use as biomaterials and biofuels. *Tropical Agriculture and Development,* 58(1): 26-29.
6. Zhong, L. B., Yin, J., Liu, S. G., Liu, Q., Yang, Y. S., and Zheng, Y. M. (2016). Facile one-pot synthesis of urchin-like Fe–Mn binary oxide nanoparticles for effective adsorption of Cd(II) from water. *RSC Advances,* 6(105): 103438-103445.
7. Onoja, E., Chandren, S., Razak, F. I. A., and Wahab, R. A. (2018). Extraction of nanosilica from oil palm leaves and its application as support for lipase immobilization. *Journal of Biotechnology*, 283: 81-96.
8. McCarthy, S. A., Davies, G. L., and Gun'ko, Y. K. (2012). Preparation of multifunctional nanoparticles and their assemblies. *Nature Protocols*, 7(9): 1677-1693.
9. Barbé, C. J., Kong, L., Finnie, K. S., Calleja, S., Hanna, J. V., Drabarek, E., … and Blackford, M. G. (2008). Sol–gel matrices for controlled release: from macro to nano using emulsion polymerization. *Journal of Sol-Gel Science and Technology,* 46: 393-409.
10. Chiew, C. S. C., Yeoh, H. K., Pasbakhsh, P., Poh, P. E., Tey, B. T., and Chan, E. S. (2016). Stability and reusability of alginate-based adsorbents for repetitive lead (II) removal. *Polymer Degradation and Stability,* 123: 146-154.
11. Lee, K. Y. and Mooney, D. J. (2012). Alginate: properties and biomedical applications. *Progress in Polymer Science,* 37(1): 106-126.
12. Crini, G. (2005). Recent developments in polysaccharide-based materials used as adsorbents

in wastewater treatment. *Progress in Polymer Science*, 30(1): 38-70.
13. Vijayalakshmi, K., Devi, B. M., Sudha, P. N., Venkatesan, J., and Anil, S. J. J. N. N. (2016). Synthesis, characterization and applications of nanochitosan/sodium alginate/microcrystalline cellulose film. *Journal Nanomedicine Nanotechnology,* 7(6): 419.
14. Cataldo, S., Gianguzza, A., Milea, D., Muratore, N., and Pettignano, A. (2016). Pb (II) adsorption by a novel activated carbon alginate composite material. A kinetic and equilibrium study. *International Journal of Biological Macromolecules,* 92: 769-778.
15. Thakur, S., Pandey, S., and Arotiba, O. A. (2016). Development of a sodium alginate-based organic/inorganic superabsorbent composite hydrogel for adsorption of methylene blue. *Carbohydrate Polymers*, 153: 34-46.
16. Pannier, A., Soltmann, U., Soltmann, B., Altenburger, R., and Schmitt-Jansen, M. (2014). Alginate/silica hybrid materials for immobilization of green microalgae *Chlorella vulgaris* for cell-based sensor arrays. *Journal of Materials Chemistry B*, 2(45): 7896-7909.
17. Singh, V., Srivastava, P., Singh, A., Singh, D., and Malviya, T. (2016). Polysaccharide-silica hybrids: design and applications. *Polymer Reviews*, 56(1): 113-136.
18. Jaganathan, H., and Godin, B. (2012). Biocompatibility assessment of Si-based nano-and micro-particles. *Advanced Drug Delivery Reviews*, 64(15): 1800-1819.
19. Choudhari, S. K., Premakshi, H. G., and Kariduraganavar, M. Y. (2016). Development of novel alginate–silica hybrid membranes for pervaporation dehydration of isopropanol. *Polymer Bulletin,* 73: 743-762.
20. Shi, J., Zhang, H., Yu, Y., Zou, X., Zhou, W., Guo, J., ... and Zhao, Y. (2020). Adsorption properties of calcium alginate-silica dioxide hybrid adsorbent to methylene blue. *Journal of Inorganic and Organometallic Polymers and Materials,* 30: 2114-2125.
21. Santhi, V. A., and Mustafa, A. M. (2013). Assessment of organochlorine pesticides and plasticisers in the Selangor River basin and possible pollution sources. *Environmental Monitoring and Assessment,* 185: 1541-1554.
22. Borrell, A., and Aguilar, A. (2007). Organochlorine concentrations declined during 1987–2002 in western Mediterranean bottlenose dolphins, a coastal top predator. *Chemosphere,* 66(2): 347-352.
23. Hernandez, F., Bakker, J., Bijlsma, L., De Boer, J., Botero-Coy, A. M., de Bruin, Y. B., and Hogendoorn, E. A. (2019). The role of analytical chemistry in exposure science: Focus on the aquatic environment. *Chemosphere,* 222: 564-583.
24. Abdullah, M. P., Abdul Aziz, Y. F., Rozali Othman, M., and Wan Mohd Khalik, W. M. A. (2015). Organochlorine pesticides residue level in surface water of Cameron Highlands, Malaysia. *Iranica Journal of Energy & Environment,* 6(2): 141-146.
25. Osman, B. E., and Khalik, W. M. A. W. M. (2018). Data on organochlorine concentration levels in soil of lowland paddy field, Kelantan, Malaysia. *Data in Brief*, 20: 999-1003.
26. Mat Saad, A., and Hassan Asari, F. F. A. (2019). Distributions of organochlorine pesticides in sediment and aquatic biota: Benzenehexachlorides (BHCS). *Proceedings of the International Conference on Design Industries & Creative Culture:* pp. 28-39.
27. Nascimento, M. M., da Rocha, G. O., and de Andrade, J. B. (2021). Customized dispersive micro-solid-phase extraction device combined with micro-desorption for the simultaneous determination of 39 multiclass pesticides in environmental water samples. *Journal of Chromatography A,* 1639: 461781.
28. Gorji, S., Bahram, M., and Biparva, P. (2019). Optimized stir bar sorptive extraction based on self-magnetic nanocomposite monolithic kit for determining bisphenol A in bottled mineral water and bottled milk samples. *Analytical and Bioanalytical Chemistry Research*, 6(1): 137-156.
29. Amiri, A., Tayebee, R., Abdar, A., and Sani, F. N. (2019). Synthesis of a zinc-based metal-organic framework with histamine as an organic linker for the dispersive solid-phase extraction of organophosphorus pesticides in water and fruit juice samples. *Journal of Chromatography*

A, 1597: 39-45.

30. Wang, D. D., Lu, Z. H., Guan, X. Y., Yang, M. N. O., Guo, H. M., and Yang, Z. H. (2021). Magnetic polydopamine modified with choline-based deep eutectic solvent for the magnetic solid-phase extraction of sulfonylurea herbicides in water samples. *Journal of Chromatographic Science,* 59(1): 95-102.
31. Zhang, M., Mei, J., Lv, S., Lai, J., Zheng, X., Yang, J., and Cui, S. (2020). Simultaneous extraction of permethrin diastereomers and deltamethrin in environmental water samples based on aperture regulated magnetic mesoporous silica. *New Journal of Chemistry,* 44(37): 16152-16162.
32. Arias, P. G., Martínez-Pérez-Cejuela, H., Combès, A., Pichon, V., Pereira, E., Herrero-Martínez, J. M., and Bravo, M. (2020). Selective solid-phase extraction of organophosphorus pesticides and their oxon-derivatives from water samples using molecularly imprinted polymer followed by high-performance liquid chromatography with UV detection. *Journal of Chromatography A,* 1626: 461346.
33. Shakourian, M., Yamini, Y., and Safari, M. (2020). Facile magnetization of metal–organic framework TMU-6 for magnetic solid-phase extraction of organophosphorus pesticides in water and rice samples. *Talanta,* 218: 121139.
34. Özer, E. T., Osman, B., and Parlak, B. (2020). An experimental design approach for the solid phase extraction of some organophosphorus pesticides from water samples with polymeric microbeads. *Microchemical Journal,* 154: 104537.
35. Amiri, A., Baghayeri, M., and Vahdati-Nasab, N. (2020). Effective extraction of organophosphorus pesticides using sol–gel based coated stainless-steel mesh as novel solid-phase extraction sorbent. *Journal of Chromatography A,* 1620: 461020.
36. Senosy, I. A., Guo, H. M., Ouyang, M. N., Lu, Z. H., Yang, Z. H., and Li, J. H. (2020). Magnetic solid-phase extraction based on nano-zeolite imidazolate framework-8-functionalized magnetic graphene oxide for the quantification of residual fungicides in water, honey and fruit juices. *Food Chemistry*, 325: 126944.
37. Huang, Z., and Lee, H. K. (2015). Micro-solid-phase extraction of organochlorine pesticides using porous metal-organic framework MIL-101 as sorbent. *Journal of Chromatography A,* 1401: 9-16.
38. Chahkandi, M., and Amiri, A. (2019). Hydroxyapatite/Fe_3O_4 nanocomposite as efficient sorbent for the extraction of phthalate esters from water samples. *Inorganic Chemistry Research,* 3(1): 50-64.
39. Sajid, M., Khaled Nazal, M., Rutkowska, M., Szczepańska, N., Namieśnik, J., and Płotka-Wasylka, J. (2019). Solid phase microextraction: apparatus, sorbent materials, and application. *Critical Reviews in Analytical Chemistry,* 49(3): 271-288.
40. Naing, N. N., Tan, S. C., and Lee, H. K. (2019). Micro-solid-phase extraction 16. Solid-Phase Extraction, 443.
41. Basheer, C., Alnedhary, A. A., Rao, B. M., Valliyaveettil, S., and Lee, H. K. (2006). Development and application of porous membrane-protected carbon nanotube micro-solid-phase extraction combined with gas chromatography/mass spectrometry. *Analytical Chemistry*, 78(8): 2853-2858.
42. Abidin, N. H. Z., Ibrahim, W. N. W., Raharjo, Y., Nodeh, H. R., Wahab, R. A., Yaakob, M. K., and Hanapi, N. S. M. (2024). Microextraction experimental and forcefield theoretical modelling study on exploring a silica-enriched oil palm frond biomass for the determination of polycyclic aromatic hydrocarbons in *Psidium guajava*. *Microchemical Journal*, 197: 109885.
43. Zaini, N., Hanapi, N. S. M., Ibrahim, W. N. W., and Anis, A. L. (2020). Selective determination of acidic drugs in water samples using online solid phase extraction liquid chromatography with alginate incorporated multi-walled carbon nanotubes as extraction sorbent. *Indonesian Journal of Chemistry,* 20(5): 987-999.
44. Othman, N. Z., Hanapif, N. S. M., Ibrahim, W. N. W., and Saleh, S. H. (2020). Alginate incorporated multi-walled carbon nanotubes as dispersive micro solid phase extraction sorbent for selective and efficient separation of acidic drugs in water samples. *Nature Environment and Pollution*

Technology, 19(3): 1155-1162.
45. Rajendran, S. (2022). Formulating and evaluation of magnetic effervescent tablets containing ionic liquid used as microextraction tool for multi-analytes pharmaceutical active compounds analysis. Thesis of Master of Science, Universiti Malaysia Terengganu.
46. Pena-Pereira, F., Wojnowski, W., and Tobiszewski, M. (2020). AGREE—Analytical GREEnness metric approach and software. *Analytical Chemistry,* 92(14): 10076-10082.
47. Yang, M., Xia, Y., Wang, Y., Zhao, X., Xue, Z., Quan, F. and Zhao, Z. (2016). Preparation and property investigation of crosslinked alginate/silicon dioxide nanocomposite films. *Journal of Applied Polymer Science*, 133(22).
48. Xu, S. W., Jiang, Z. Y., Lu, Y., Wu, H., and Yuan, W. K. (2006). Preparation and catalytic properties of novel alginate− silica− dehydrogenase hybrid biocomposite beads. *Industrial & Engineering Chemistry Research,* 45(2): 511-517.
49. Draget, K. I., Stokke, B. T., Yuguchi, Y., Urakawa, H., and Kajiwara, K. (2003). Small-angle X-ray scattering and rheological characterization of alginate gels. 3. Alginic acid gels. *Biomacromolecules,* 4(6): 1661-1668.
50. Amouzgar, P., Chan, E. S., and Salamatinia, B. (2017). Effects of ultrasound on development of Cs/NAC nano composite beads through extrusion dripping for acetaminophen removal from aqueous solution. *Journal of Cleaner Production,* 165: 537-551.
51. Pawar, S. N., and Edgar, K. J. (2012). Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials,* 33(11): 3279-3305.
52. Fosu-Mensah, B. Y., Okoffo, E. D., Darko, G., and Gordon, C. (2016). Assessment of organochlorine pesticide residues in soils and drinking water sources from cocoa farms in Ghana. SpringerPlus, 5: 1-13.
53. Asgharinezhad, A. A., Ebrahimzadeh, H., Mirbabaei, F., Mollazadeh, N., and Shekari, N. (2014). Dispersive micro-solid-phase extraction of benzodiazepines from biological fluids based on polyaniline/magnetic nanoparticles composite. *Analytica Chimica Acta*, 844: 80-89.
54. Zaini, N., Hanapi, N. S. M., Ibrahim, W. N. W., Osman, R., Kamaruzaman, S., Yahaya, N., and Anis, A. L. (2022). Dispersive micro-solid-phase extraction (D-μ-SPE) with polypyrrole-graphene oxide (PPy-GO) nanocomposite sorbent for the determination of tetracycline antibiotics in water samples. *Malaysian Journal of Analytical Sciences*, 26(5): 953-964.
55. Hernando, M. D., Mezcua, M., Fernández-Alba, A. R., and Barceló, D. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta,* 69(2): 334-342.
56. Abidin, N. N. Z., Sanagi, M. M., Ibrahim, W. A. W., Endud, S., and Shukri, D. S. M. (2014). Portable micro-solid phase extraction for the determination of polycyclic aromatic hydrocarbons in water samples. *Analytical Methods,* 6(15): 5512-5518.
57. Deshmukh, S., Kumar, R., and Bala, K. (2019). Microalgae biodiesel: A review on oil extraction, fatty acid composition, properties and effect on engine performance and emissions. *Fuel Processing Technology*, 191: 232-247.
58. Zarrinmehr, M. J., Daneshvar, E., Nigam, S., Gopinath, K. P., Biswas, J. K., Kwon, E. E., ... and Bhatnagar, A. (2022). The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. *Bioresource Technology*, 344: 126303.
59. Hamdaoui, O., Naffrechoux, E., Tifouti, L., and Pétrier, C. (2003). Effects of ultrasound on adsorption–desorption of p-chlorophenol on granular activated carbon. *Ultrasonics Sonochemistry*, 10(2): 109-114.
60. Breitbach, M., and Bathen, D. (2001). Influence of ultrasound on adsorption processes. *Ultrasonics Sonochemistry*, 8(3): 277-283.
61. Rozaini, M. N. H., Yahaya, N., Saad, B., Kamaruzaman, S., and Hanapi, N. S. M. (2017). Rapid ultrasound assisted emulsification micro-solid phase extraction based on molecularly imprinted polymer for HPLC-DAD determination of bisphenol A in aqueous matrices. *Talanta,* 171: 242-249.
62. Dahane, S., García, M. G., Bueno, M. M., Moreno,

A. U., Galera, M. M., and Derdour, A. (2013). Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography–quadrupole-linear ion trap-mass spectrometry. *Journal of Chromatography A,* 1297: 17-28.

63. Batt, A. L., Furlong, E. T., Mash, H. E., Glassmeyer, S. T., and Kolpin, D. W. (2017). The importance of quality control in validating concentrations of contaminants of emerging concern in source and treated drinking water samples. *Science of the Total Environment*, 579: 1618-1628.
64. Huang, Z., and Lee, H. K. (2015). Micro-solid-phase extraction of organochlorine pesticides using porous metal-organic framework MIL-101 as sorbent. *Journal of Chromatography A*, 1401: 9-16.
65. Ponnuchamy, M., Kapoor, A., Senthil Kumar, P., Vo, D. V. N., Balakrishnan, A., Mariam Jacob, M., and Sivaraman, P. (2021). Sustainable adsorbents for the removal of pesticides from water: a review. *Environmental Chemistry Letters,* 19: 2425-2463.