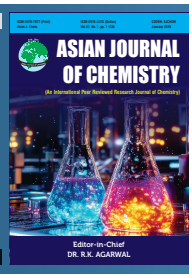
Asian Journal of Chemistry; Vol. 37, No. 5 (2025), 1118-1126

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33515



Efficacy of Protein Extracts of *Moringa oleifera* and *Benincasa hispida* Seeds for the Treatment of Microplastics

PRIYA AGARWAL[1,*], SATYA PRAKASH[1] and GAURAV SAINI[2]

[1]Department of Civil Engineering, Sharda University, Knowledge Park III, Greater Noida-201310, India
[2]Department of Civil Engineering, Netaji Subhas University of Technology, Sector-3, Dwarka, New Delhi-110078, India

*Corresponding author: E-mail: priyamittal1199@gmail.com

Received: 15 February 2025; *Accepted*: 1 April 2025; *Published online*: 30 April 2025; AJC-21978

Microplastics are emerging contaminants and have found significant interest due to their pervasive nature and potential health impacts on different life forms. A number of methods have been investigated so far, including the use of synthetic and recently natural coagulants, however, protein extracts derived from plant-based materials have not been extensively studied for such applications. Therefore, there is an urgent need to develop a sustainable treatment technology for the removal of microplastics. In this study, coagulation experiments were conducted under varying conditions such as pH, coagulant type and concentration using jar test apparatus for removal of microplastics from aqueous samples. The proteins extracted from two natural coagulants, *Moringa oleifera* and *Benincasa hispida*, have been studied for the removal of microplastics in aqueous samples. It was found that *M. oleifera* protein extracts achieved microplastics removal of 94.14 ± 11.0% at a dosage of 30 mL/L whereas *B. hispida* protein extracts achieved 88.29 ± 10.7% removal at 40 mL/L at pH 7. Therefore, the novelty of the study lies in evaluating the impact of *M. oleifera* and *B. hispida* seed protein extracts for the removal of microplastics. The findings suggest that the *M. oleifera* and *B. hispida* can be effective, sustainable, eco-friendly and affordable alternatives to chemical coagulants for treatment of microplastics in aqueous systems.

Keywords: Protein extracts, Natural coagulants, Sustainable, Coagulation, Microplastics.

INTRODUCTION

The production of plastic products has rapidly increased for diverse industrial uses including transportation, packaging, construction, agriculture, manufacturing, electronics, furniture, toy and leisure items, automobiles and medicine [1] as it is longlasting, resistant to degradation, inert and easy to shape, with very low production costs [2]. It has, therefore, become an important material for everyday use. The production of plastics has increased from 1.5 million metric tons in 1950 to 413.8 million metric tons in 2023 [3]. Plastic production is thus expected to increase at a significant rate hereafter [4]. However, improper handling and disposal of discarded plastic have led to their increasing accumulation in every compartment of the environment–air, water and soil. According to Geyer *et al.* [5], plastic wastes are recycled (9%), incinerated (12%), dumped in the environment, or landfilled (79%) after the end of their lifespan. The longevity of plastics is estimated to be hundreds or even thousands of years depending on their constitution and resultant properties [6]. However, plastics break down into smaller particles due to ultraviolet radiation from sunlight, wave action and wind abrasion [7]. This alarming trend highlights the urgent need for effective waste management and reduction strategies to address the growing plastic pollution crisis. Microplastics generally refer to plastic debris < 5 mm in diameter, including fibers, fragments and films [8]. According to their sources, two classifications of microplastics are currently recognized; primary and secondary microplastics, which are typically < 5 mm [9]. Primary microplastics are intentionally manufactured to this size to be added to personal care products, while secondary microplastics result from the fragmentation of larger plastic debris by physical, chemical and biological processes in the environment [10]. Major polymers encountered in microplastics include polyethylene terephthalate (PET), polyvinylchloride (PVC), polystyrene (PS), polyethylene (PE) and polypropylene (PP) [11]. Out of these, PE is one of the most widely used plastics globally, commonly used for manufacturing single-use plastic products, accounting

for approximately 50% of plastic wastes [12]. It has been estimated that the global discharge of microplastics would increase from 0.017 Mt in 2000 to 0.749 Mt in 2060 [13].

Consumption of water is a significant source of microplastics in humans [14] and these particles are also present in numerous food products typically packaged in plastic, thereby contributing substantially to environmental pollution and the accumulation of microplastics in ecosystems, including within various organisms.. Studies have detected microplastics in poultry meat [15], fish [16], shellfish [17], honey [18], sugar [19], salt [20], milk [21], tea [22], beer [23], fruits, vegetables [24], mother's milk [25] and human heart [26]. According to research, the effluent of wastewater treatment plants (WWTPs) is an important source of microplastics for the aquatic environment [27]. Since primary microplastics are smaller, they can spread throughout more aquatic systems and are more likely to be accidentally consumed, which disrupts the immune systems of different aquatic life [28]. The rising prevalence of microplastics in the environment is a significant global pollution concern. Microplastics can persist in the ecosystem for extended periods as insoluble in water and non-degradable, exacerbating environmental degradation and posing risks to wildlife and human health [29]. The impact of microplastics on human health varies based on the routes and sources of exposure, which can include ingestion, inhalation and dermal contact [30]. This widespread presence in our food and beverage supply raises concerns about the potential health implications of microplastics. It has been reported by the World Wildlife Fund (WWF) that humans consume up to 5 g of plastic (one credit card) every week (~ 700 mg/capita/day) [31]. The microplastics accumulate in the spleen, kidneys, brain, lungs, reproductive system, placenta and fetal tissues [32]. Moreover, microplastics have been detected in the human bloodstream too [33]. Such exposure to microplastics leads to oxidative stress and cytotoxicity and microplastics can be translocated to other tissues as well. Microplastics may also contribute to a higher incidence of immune or neurodegenerative diseases [34]. Microplastics have become emerging pollutants of growing concern and there is an urgent need to develop methods for the removal of microplastics. The large size plastic particles are removed *via* screening systems but the removal of small-sized microplastic particles is quite challenging and requires a series of treatment processes [35]. Microplastics can be removed (to various degrees) from aquatic systems and wastewater through physical, chemical and biological treatments, including coagulation, adsorption, magnetic separation, membrane filtration and photodegradation [36]. About 66% of microplastics in influent may be eliminated by current primary and secondary treatment methods [37]. Since wastewater treatment facilities discharge a significant portion of their plastics into the environment, it is imperative to reduce this pollution.

Coagulation is a simple and cost-effective technology used in water and wastewater treatment plants. Coagulation is the process of destabilizing and aggregating particles into big flocs to remove contaminants from suspended particles and colloidal forms. After settling, the aggregates can be extracted from water by employing a solid-liquid separation technique [38]. Research on natural coagulants as alternatives to chemical coagulants has grown in the wake of the Sustainable Development Goal. Natural coagulants are more appealing than chemical coagulants since they are inexpensive, non-toxic, renewable and biodegradable [39]. Nevertheless, there is limited studies on using natural coagulants to remove microplastics; instead, most studies concentrate on removing turbidity and COD. According to recent study, *Moringa oleifera* and *Benincasa hispida* seed powder have proven efficient in removing microplastics successfully [40]. Charge neutralization and bridging are the most probable mechanisms of action of natural coagulants [41]. So far, the extracts of natural coagulants were used in water and wastewater treatment including the seed extracts of yard-long bean and snake gourd as efficient coagulants for removing turbidity [42], date seed extracts removed turbidity and colour in textile wastewater [43]. Recently, *M. oleifera* cationic protein (MOCP) and protein-coated sand (*f*-sand) were developed to remove pristine and photo-weathered polyethylene (PE) microplastics [44]. Utilizing these natural coagulants for coagulation is an appropriate and sustainable way to address the issue of microplastics in aqueous systems including wastewater effluent.

Coagulation-based removal of microplastics has been a focus of research for some time, although a significant portion of this work is based on the use of synthetic coagulants. Recently, there is a growing interest in a more sustainable approach, *i.e.* the use of plant-based products as coagulants for microplastics removal from aqueous systems. However, such work is very limited and lacks detailed investigation on plant-based proteins for microplastics removal. The present work is aimed at testing the potential effectiveness of a coagulant protein extracted from *M. oleifera* and *B. hispida* in removing microplastics. This study carried out the extraction of the protein responsible for promoting the coagulation/flocculation process according to a preliminary study and use of the proteins extracted from *M. oleifera* and *B. hispida* for microplastic treatment. This approach aims to utilize the natural coagulant properties of these proteins to enhance the removal process, offering a sustainable solution for addressing microplastic pollution in water systems. The study focuses on evaluating the efficiency of these extracts in binding and flocculating microplastics, contributing to environmental remediation efforts. Then the quality of water was evaluated pre and post-coagulation/flocculation treatment by determining the concentration of microplastics. This study aims to (i) assess the effectiveness of the coagulation technique for microplastic removal, (ii) thoroughly investigate the effect of coagulation factors such as pH, type and concentration of coagulant on the microplastics removal; and (iii) explore the potential of *M. oleifera* and *B. hispida* seed protein extracts for microplastics removal. The findings of this study could offer valuable insights for enhancing the removal process.

EXPERIMENTAL

Microplastic sample preparation: The synthetic microplastic samples were prepared using 100 mL Milli-Q water heated at 90 ºC and poured into disposable paper cups, lined with plastic on the inside and commonly used for serving beverages such as water, tea, coffee, *etc.* These cups were then prop-

erly covered with aluminum foil to prevent any dust particles from the surroundings entering and contaminating the prepared sample while left undisturbed for 15 min. The microplastics leach out from the inner lining of the cup into the solution, on exposure to hot water [40].

Characterization: The extracted microplastics were characterized using Fourier transform infrared (FTIR) spectroscopy (Agilent Cary 630) to identify their functional groups, bonds and chemical composition. The scanning electron microscopic (SEM) analysis (FEI Quanta 200) was used to determine the surface morphology of the particles.

Protein extraction of natural coagulants: Fig. 1 outlines the steps involved in protein extraction from natural coagulants. Protein extraction from natural coagulants involves isolating and purifying proteins from plants. The *M. oleifera* and *B. hispida* seeds were procured directly from the fruit. Then the process of sample preparation starts with washing, drying and grinding of seeds into a fine powder or paste. The sample was mixed with a solvent, such as water, saline solution or buffer, depen-ding on the protein's solubility. The pH can be adjusted to improve the solubility. The mixture was stirred or agitated for 1-2 h under controlled conditions to prevent denaturation. Filtration or centrifugation removes insoluble residues, yielding a protein-rich supernatant. Further purification can be achieved through precipitation methods such as salting out with ammonium sulfate, isoelectric precipitation or organic solvents. Then the extracts are stored at 4 to –18 ºC for further use.

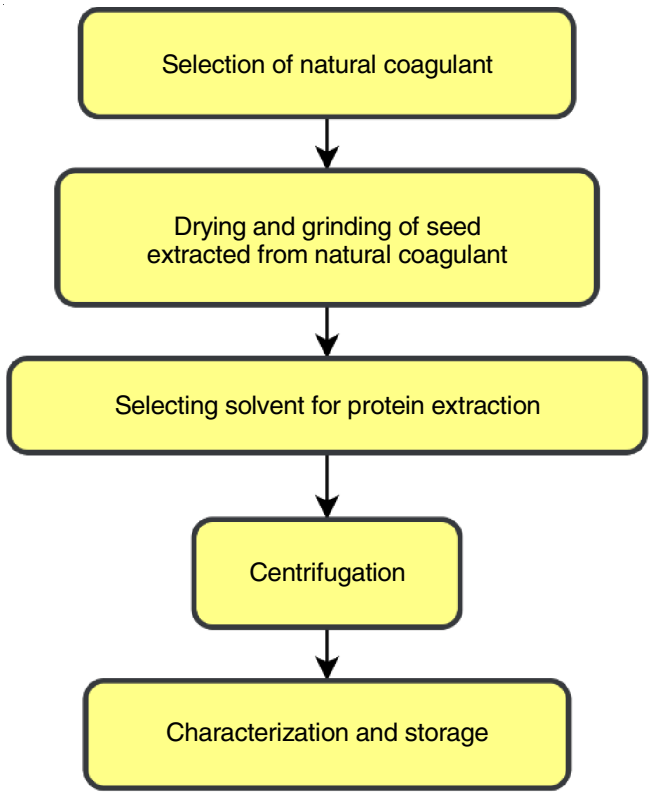


Fig. 1. Steps involved in protein extraction from natural coagulants

***M. oleifera* seed protein extraction:** In the extraction process of *M. oleifera* proteins, the defatting of seeds was carried out using three-position heating and stirring mantles. Here, 10 g of *M. oleifera* seed powder was placed inside the Soxhlet extraction chamber and 170 mL of *n*-hexane was added to the heating chamber. Then, the evaporation of hexane was carried out through three 30 min cycles to ensure the complete extraction of oils. After the hexane became colourless, the *M. oleifera* residue cake was dried for 24 h in desiccator [45,46]. The extraction process used a 1 M NaCl solution to enhance the protein solubility. The solution was added to the defatted *M. oleifera* seed cake and mixed using a magnetic stirrer for 30 min [47]. The resulting solution was centrifuged for 10 min at 6000 rpm. The solution was diluted at a factor of 10 and then the protein concentration was measured by the Bradford method (Thermo-Fisher, USA) [48], which is a colorimetric protein assay based on an absorbance shift from red to blue using a dye Coomassie Brilliant Blue G-250 (Bradford reagent). The assay measures absorbance at 595 nm (A_{595}) to be 0.45 and the protein concentration was calculated using a standard curve generated from known protein standards (usually bovine serum albumin (BSA)) as 2.25 mg/mL. Therefore, the protein concentration in the original sample was calculated by considering the dilution factor as 22.5 mg/mL.

***B. hispida* protein extracts:** In the extraction process of *B. hispida* proteins, the seeds were cleaned and ground in cold phosphate-buffered saline (PBS), which creates an isotonic environment and keeps the pH steady. PBS and powdered seeds were combined in a 1:4 (w/v) ratio to help release the proteins into the mixture. Overnight, the mixture was stirred at around 4 ºC. The mixture was filtered to get rid of the solid seed material after homogenization. For 25 min, the supernatant was centrifuged at 10,000 g. Saturated ammonium sulfate (80%) was added to precipitate and concentrate the proteins. After that, the precipitated proteins were dissolved in a minimum amount of buffer and dialyzed using a cellophane membrane against a 5 mM of sodium phosphate buffer (pH 7). The solution was centrifuged once again after dialysis to eliminate any insoluble elements that might have developed during the precipitation and dialysis processes. After the elimination of water from the dialysate by lyophilization, a concentrated protein was produced that can be used further [49]. The solution was diluted at a factor of 10 as the protein concentration was high. The absorbance of sample was determined as 0.28 at 595 nm and the standard curve (created from BSA standards) shows that an absorbance of 0.28 corresponds to a protein concentration of 1.4 mg/mL. Therefore, the protein concentration in original solution of *B. hispida* seed extract was determined as 14 mg/mL using Bradford method. The *M. oleifera* and *B. hispida* extract were stored at 4 to –18 ºC to maintain the coagulation protein in the extract. It generally loses coagulation activity at room temperature because the coagulation protein present in the solution decreases.

Coagulation-flocculation process: The jar test is the most extensively used experimental apparatus for investigating and optimizing coagulation-flocculation with 1 L jars equipped with six-paddle stirrers. The procedure was followed to test the effectiveness of the coagulants using 500 mL leached synthetic samples placed in glass jars. The pH values of 5, 7 and 9 were commonly selected for experiments. The pH of the samples

was adjusted by adding 0.1 M HCl and 0.1 M NaOH to analyze the change in the release of microplastics. The protein extracts of *M. oleifera* and *B. hispida* in different dosages (10-60 mL) were added to the prepared sample. This allowed testing of the coagulant concentrations to determine the optimal dosage for effective treatment. The coagulant caused microplastics to destabilize and aggregate. Then, the stirrers were activated to mix the coagulant evenly throughout the sample at a high speed for 2 min and then slow speed for 20 min. After mixing, the coagulated particles began to clump together to form larger flocs, allowing the water to undergo a flocculation process. Then the jars were left undisturbed for 90 min to allow the flocs to settle to the bottom of the jar in the sedimentation process. The supernatant was separated and further characterized to determine the optimal dosages of coagulants.

Quantification of microplastics: The number of microplastics was determined using a naeubers counting chamber. After treatment, the supernatant using different *M. oleifera* and *B. hispida* dosages at pH 5, 7 and 9 was collected using a pipette and diluted at 1:3 as it is highly microplastic concentrated. The diluted sample of 10 µL was added to the counting chamber and a coverslip was placed on it so that dust particles would not interfere. The counting chamber was placed under a trinocular microscope and the grid lines of the chamber were observed. Then, the number of microplastics was counted at 400X in the selected 5 squares out of large 9 squares using the logical count method.

Depending on the number of microplastics (MPs) in the selected 5 number of squares, the concentration of microplastics in the samples can be calculated using the formula mentioned below:

$$\text{Concentration (MPs/mL)} = \frac{\text{No. of MPs} \times \text{Dilution factor} \times 10^4}{\text{Number of squares}} \quad (1)$$

The concentration for each pH and dosage was calculated by taking the average of 5 samples.

Removal efficiency: The difference in microplastics after treatment was calculated by subtracting the concentration of the sample after treatment from the concentration of the initially prepared sample. The removal efficiency was calculated using the formula:

$$\text{Removal efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad (2)$$

where C_i represents the concentration of the initially prepared sample and C_f represents the concentration of sample after treatment.

RESULTS AND DISCUSSION

Identification of polymer type: The FTIR analyses of microplastics extracted from the prepared sample to identify the polymer type. The bands at 2918 cm^{-1} and 2848 cm^{-1} can be attributed to the CH stretch of all the hydrocarbon constituents present in the polymers. The peaks positioned at 1464 and 1471 cm^{-1} correspond to CH_2 bend and the peaks located at 732 cm^{-1} represent CH_2 rock (Fig. 2). Therefore, it is concluded that these peaks in samples closely match HDPE [50].

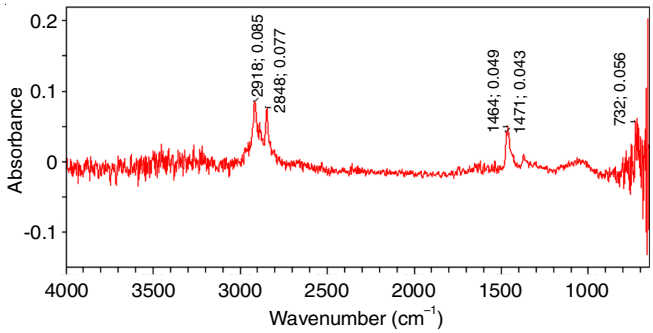


Fig. 2. FTIR spectra of microplastics extracted from the disposable cup

Thus, FTIR results of the different samples show that the plastic films extracted from the samples were HDPE grade plastics and that any microplastics leached through exposure to hot water/beverage were made of HDPE.

Morphology of extracted microplastics: The extracted microplastics were viewed under ESEM at 1000 to 25,000X magnifications as shown in Fig. 3. The microplastics were seen to be either irregularly shaped or to have a distinct shape (such as spherical or rod-like). Moreover, the microplastics of smaller sizes were dispersed throughout the field of vision and formed tiny, agglomerated clusters. For each sample, a range of particle sizes was indicated by the presence of particles of different sizes throughout the field of vision.

Concentration of microplastics in the prepared sample: The average number of microplastics in the prepared sample was determined as 13.6 ± 0.54 at a dilution of 1:3. Then the microplastics concentration in the samples was calculated (eqn. 1) as 82,000 ± 3240 MPs/mL.

Concentration of microplastics after using protein extracts: The concentration of microplastics in the sample was determined after using *M. oleifera* and *B. hispida* protein extracts.

***M. oleifera* protein extracts:** The number of microplastics calculated in the counting chamber is mentioned in Table-1. It is the average of 5 samples with their standard deviation. The average number of microplastics seen at a 1:3 dilution following treatment with *M. oleifera*-extracted proteins at different pH and doses is shown in Table-1. The findings at pH 5 demonstrate a distinct pattern: the microplastic count stays comparatively high at lower protein concentrations (10 mL/L), with an average of 11.6 ± 0.54 microplastics. The microplastic count drops further to 4.2 ± 0.44 microplastics as the *M. oleifera* protein concentration rises to 60 mL/L, resulting in the most significant reduction as there are more active coagulants available to interact with microplastics. This suggests that *M. oleifera* proteins at higher concentrations have a greater effect on lowering the quantity of microplastics. At pH 7, the dosage of 10 mL/L consistently produced 9 ± 0 microplastics throughout the five samples, but at 30 mL/L decreased to an average of 0.8 ± 0.44 microplastics, indicating good efficacy. The microplastic count did, however, marginally increase to 2.6 ± 0.54 as the dosage was increased to 40 mL/L. It then climbed further at 60 mL/L, with an average of 8.4 ± 0.54 microplastics. According to this, coagulation efficiency peaks at 30 mL/L and decreases with increasing dosage. At pH 9, for a dosage

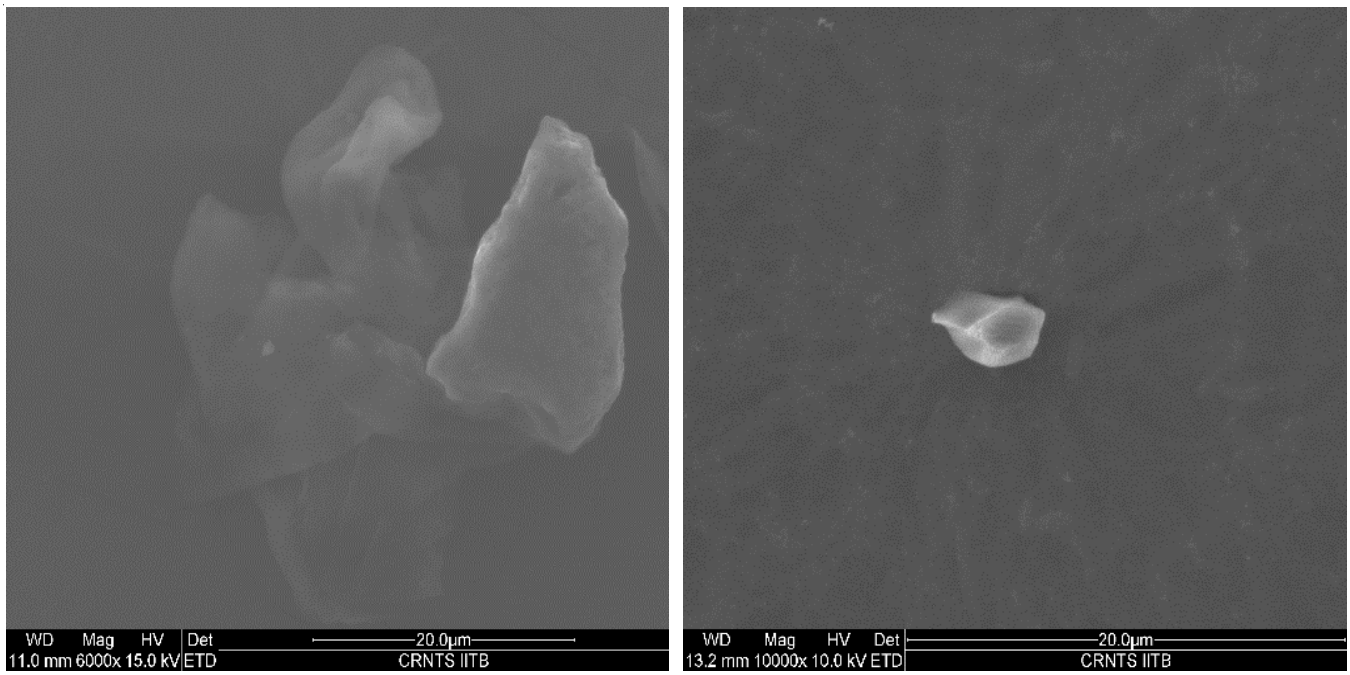


Fig. 3. SEM image representing different sizes and shapes of microplastics

TABLE-1
AVERAGE NUMBER OF MICROPLASTICS ON DILUTION 1:3 AFTER USING EXTRACTED PROTEINS OF *Moringa oleifera* AT pH 5, 7 AND 9

Sample pH	10 mL/L	20 mL/L	30 mL/L	40 mL/L	50 mL/L	60 mL/L
5	11.6 ± 0.54	11 ± 0	7.2 ± 0.44	6.8 ± 0.83	4.4 ± 0.54	4.2 ± 0.44
7	9 ± 0	4.8 ± 0.44	0.8 ± 0.44	2.6 ± 0.54	5.4 ± 0.54	8.4 ± 0.54
9	10.6 ± 0.54	7 ± 0	3 ± 0	2.2 ± 0.44	4 ± 0	7.8 ± 0.44

of 10 mL/L, the average number of microplastics was 10.6 ± 0.54, which decreased to 2.2 ± 0.44 MPs at 40 mL/L. However, the microplastic count increased again to 7.8 ± 0.44 microplastics at a higher dosage of 60 mL/L. This suggests that the optimal dosage for microplastic removal at pH 9 is around 40 mL/L, with effectiveness decreasing beyond this point. After finding the number of microplastics in 5 squares of the counting chamber at dilution 1:3, the concentration of microplastics in samples was determined using eqn. 1.

The concentration of microplastics that remained after treatment with *M. oleifera*-extracted proteins at various dosages (10 to 60 mL/L) and pH values (5, 7 and 9) is summarized in the Table-2. Effective microplastic removal at higher dosages was demonstrated by the gradual decrease in the microplastic concentration at pH 5 as the dosage increases, from 69,600 ± 3240 microplastics at 10 mL/L to 25,200 ± 2640 microplastics at 60 mL/L. The microplastic concentration first sharply drops at pH 7, hitting a low of 4,800 microplastics at 30 mL/L from 54,000 ± 0 microplastics at 10 mL/L, then rises again to 50,400 ± 3240 microplastics at a higher dosage of 60 mL/L. Likewise, the microplastic concentration decreases at pH 9 from 63,600 ± 3240 microplastics at 10 mL/L to 13,200 ± 2640 microplastics at 40 mL/L, increasing at higher dosages. *M. oleifera* seeds contain cationic proteins that can act as effective coagulants. This shows that the effectiveness of removing microplastics is greatly influenced by pH and dosage, with the best outcomes occurring under particular circumstances.

***B. hispida* protein extracts:** The number of microplastics observed on the counting chamber after treatment in the selected 5 squares of counting chamber are mentioned in Table-3.

Table-3 summarizes the number of microplastics observed at various pH (5, 7 and 9) and dosages (10 to 60 mL/L) of *B. hispida* extracted proteins. at pH 5, using a 1:3 dilution. At 10 mL/L dosage, the number of microplastics remained consistently high, averaging 12.4 ± 0.54. The microplastic count decreased to 5.2 ± 0.44 microplastics at 50 mL/L but rose again to 7 ± 0 microplastics at 60 mL/L. This indicates that the most effective dosage for microplastic removal at pH 5 is 40

TABLE-2
CONCENTRATION OF MICROPLASTICS AFTER REMOVAL USING EXTRACTED PROTEINS OF *Moringa oleifera*

pH/Dosage	10 mL/L	20 mL/L	30 mL/L	40 mL/L	50 mL/L	60 mL/L
5	69600 ± 3240	66000 ± 0	43200 ± 2640	40800 ± 4980	26400 ± 3240	25200 ± 2640
7	54000 ± 0	28800 ± 2640	4800 ± 2640	15600 ± 3240	32400 ± 3240	50400 ± 3240
9	63600 ± 3240	44400 ± 0	18000 ± 0	13200 ± 2640	24000 ± 0	46800 ± 2640

TABLE-3
AVERAGE NUMBER OF MICROPLASTICS ON DILUTION 1:3 AFTER USING EXTRACTED PROTEINS OF *Benincasa hispida* AT pH 5, 7 AND 9

Sample pH	10 mL/L	20 mL/L	30 mL/L	40 mL/L	50 mL/L	60 mL/L
5	12.4 ± 0.54	12 ± 0	7.6 ± 0.89	5.6 ± 0.89	5.2 ± 0.44	7 ± 0
7	11.2 ± 0.44	6.6 ± 0.54	5 ± 0	1.6 ± 0.89	3.4 ± 0.89	2.8 ± 0.44
9	11.4 ± 0.89	8.2 ± 0.44	5.8 ± 0.83	3.8 ± 1.09	2.4 ± 0.89	3.2 ± 0.44

mL/L. While at pH 7, the average microplastic count at 10 mL/L was 11.2 ± 0.44 which dropped to 5 microplastics at 30 mL/L. But at 40 mL/L, the microplastic count dramatically dropped to 1.6 ± 0.89 microplastics, indicating peak efficacy. At higher dosage, however, the microplastic count increased somewhat, averaging 2.8 ± 0.44 microplastics at 60 mL/L. This suggests that at pH 7, 40 mL/L is the ideal dosage for microplastic elimination; above this, the effectiveness begins to decrease. The average microplastic count at pH 9 was 11.4 ± 0.89 at 10 mL/L. With increased efficacy, the count further decreased to 3.8 ± 1.09 microplastics at 40 mL/L. At 50 mL/L, the microplastic count was at its lowest at 2.4 ± 0.89 and at 60 mL/L, it slightly increased to 3.2 ± 0.44 microplastics. This indicates 50 mL/L as the most effective dosage for removing microplastic at pH 9, with efficacy marginally declining at higher concentrations.

The concentration of microplastics that remained after the treatment with proteins isolated from *B. hispida* at various pH levels (5, 7 and 9) and doses (10 to 60 mL/L) is summarized in Table-4. At pH 5, the microplastic concentration dropped from 74,400 ± 3240 at 10 mL/L to 31,200 ± 2640 at 50 mL/L and then slightly rising to 42,000 ± 0 at 60 mL/L. The concentration dramatically decreased at pH 7, going from 67,200 ± 2640 at 10 mL/L to a low of 9,600 ± 2640 at 40 mL/L before increasing once more to 16,800 ± 2640 at 60 mL/L. The microplastic concentration increased to 19,200 ± 2640 at 60 mL/L after falling from 68,400 ± 2640 at 10 mL/L to 14,400 ± 2640 at 50 mL/L at pH 9. This suggests that the best conditions for removing microplastics are 40 mL/L and pH 7 indicating the greatest reduction in microplastic concentration. The high concentration of active proteins in extracted *M. oleifera* and *B. hispida* allowed rapid and effective microplastics removal even at very low dosages. However, the extraction process for these proteins is expensive than preparing the other forms of natural coagulants.

A comparitive study towards the efficiency of removal of microplastics: Fig. 4a shows the percentage of microplastics removed using protein extracts from *M. oleifera* at varying dosages (10 to 60 mL/L) and pH values (5, 7 and 9). Elimination efficiency rose from 15.12 ± 8.5% at 10 mL/L to 69.26 ± 9.9% at 60 mL/L at pH 5. After reaching a peak of 94.14 ± 11.0% at 30 mL/L at pH 7, the removal dropped to 38.53 ± 9.4% at 60 mL/L. From 22.43 ± 8.8% at 10 mL/L to 83.90% at 40 mL/L, efficiency increased at pH 9 before declining to 42.92% at 60 mL/L. Overall, the most successful removal was at 30 mL/L, with pH 7 exhibiting the highest peak efficiency. Fig. 4b displays the percentage removal of microplastics using protein extracts from *B. hispida*. The removal efficiency rose from 9.26 ± 8.3% at 10 mL/L to a high of 61.95 ± 9.6% at 50 mL/L at pH 5, after which it fell to 48.78 ± 5.9% at 60 mL/L. With modest drops at higher dosages, removal was more successful at pH 7, peaking at 88.29 ± 10.7% at 40 mL/L after beginning at 18.04 ± 7.9% at 10 mL/L. The elimination efficiency started at 16.58 ± 7.8% at 10 mL/L at pH 9 and increased to 82.43 ± 10.4% at 50 mL/L before slightly declining to 76.58 ± 10.2% at 60 mL/L. In general, 40 mL/L showed the best

TABLE-4
CONCENTRATION OF MICROPLASTICS AFTER REMOVAL USING EXTRACTED PROTEINS OF *Benincasa hispida*

pH/Dosage	10 mL/L	20 mL/L	30 mL/L	40 mL/L	50 mL/L	60 mL/L
5	74400 ± 3240	72000 ± 0	45600 ± 5340	33600 ± 2640	31200 ± 2640	42000 ± 0
7	67200 ± 2640	40800 ± 3240	54400 ± 0	9600 ± 2640	20400 ± 2640	16800 ± 2640
9	68400 ± 2640	49200 ± 2640	34800 ± 4980	22800 ± 6540	14400 ± 2640	19200 ± 2640

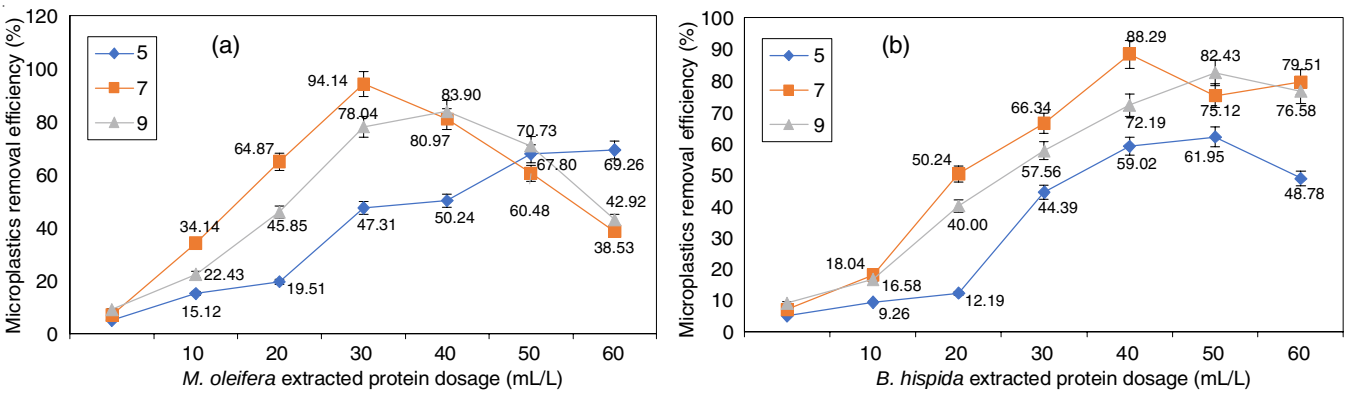


Fig. 4. Percentage removal of microplastics using extracted proteins of (a) *M. oleifera* and (b) *B. hispida*

removal of microplastics, at pH 7 for protein extracts from *B. hispida*. The comparison between *M. oleifera* and *B. hispida* protein extracts for the removal of microplastics revealed that *M. oleifera* extracts generally achieved higher peak efficiencies but exhibited significant declines at higher dosages. The adsorption and charge neutralization of particle surfaces are considered the main mechanisms for coagulation. These proteins adsorb onto the surface of microplastics and neutralize the charge. Then the particles begin to aggregate to form larger particles called flocs which can be further removed [44,51].

A recent study used chitosan for removing polyethylene microplastics in synthetic wastewater and achieved 81.5/% removal at a pH of 6 with 100/mg/L dosage [52]. Another study investigated the use of *B. hispida* and *M. oleifera* seeds powder that resulted in 83.73 ± 1.41% at 100 mg/L and 86.99 ± 1.41% at 150 mg/L, respectively, over synthetic coagulants: alum resulted in 86.58 ± 1.22% removal at 50 mg/L [40]. While the majority of studies have focused on chemical coagulants, Yao *et al.* [53] applied polyaluminum sulfate (PAS) at a concentration of 7.5 g/L, resulting in polystyrene (PS) microplastics removal efficiency of 90.4% [53]. Among the different coagulants tested, Polyaluminium chloride (PAC) alone removed 97% PS microbeads while $Al_2(SO4)_3$ and $FeCl_3$ removed 67 % and 48 % PS microbeads, respectively [54]. It was demonstrated by Yu *et al.* [55] that the highest PS microplastics removal efficiency of 94.28% was achieved when active silicic acid (10 mg/L) was used as a coagulant aid in conjunction with a polyaluminum ferric chloride (60 mg/L). Additionally, a study conducted in Poland assessed the effectiveness of $AlCl_3$·6HO and $FeCl_3$·6HO at a concentration of 0.05 g/L, achieving in PVC microplastics removal of 89.2 ± 6.2% and 66.8 ± 14%, respectively [56]. Furthermore, an investigation on the use of $Al_2(SO_4)_3$ and anionic polyacrylamide (APAM) resulted in 93.47%, 81.25% and 29.48% removal for PA, PS and PE microplastics, respectively. The combination of chemical coagulant with natural coagulant ($Al_2(SO_4)_3$+MO) resulted in a 50% reduction of $Al_2(SO_4)_3$. The microplastics removal efficiencies of 92.99%, 80.48% and 28.94% for PA, PS and PE microplastics, respectively were obtained with 40 mg/L of $Al_2(SO_4)_3$ and 60 mg/L of *M. oleifera* [51].

These chemical coagulants used in water treatment can be costly and harmful to the environment. They can produce toxic waste and cause contamination of water with metals, which can be a threat to human health. Such as, the use of aluminum salts has been linked to Alzheimer's disease [39]. Therefore, natural coagulants are found to be more effective and can be used as an alternative to chemical coagulants as they are sustainable and environmentally friendly. Natural coagulants have functional groups that neutralize negatively charged microplastics. Overall, *M. oleifera* extracts are highly effective at optimal dosages, while *B. hispida* extracts provide more consistent removal across varying conditions. Although their efficiency in eliminating microplastic is beneficial, their increased expense might prevent them from being widely used in water treatment. It is essential to balance the advantages of effective microplastic removal and the practicality of using these materials. To maximize the usage of *M. oleifera* and *B. hispida* seed powders in water purification procedures, it is essential to address the cost concerns and explore more economical extraction methods. This approach would help harness their effective microplastic removal capabilities while ensuring practicality and sustainability in water treatment processes.

Conclusion

In today's world, plastic is one of the materials that is utilized the most frequently. Despite its harmful effects on all organisms, it is favoured due to its affordable, strong and adaptable. It becomes microplastic once disposed into the environment, which increases the risk. Therefore, coagulation has emerged as a promising method for removing microplastics. In this study, a synthetic solution was prepared using microplastics extracted from common-use disposable cups. These microplastics were removed by extracted proteins of two natural coagulants *viz. M. oleifera* and *B. hispida*, which have been successfully applied to water and wastewater treatment earlier. *M. oleifera* protein extracts achieved optimal microplastic removal of 94.14 ± 11.0% at a dosage of 30 mL/L and pH 7, demonstrating their high effectiveness in this specific condition. However, their removal efficiency decreased significantly at higher dosages, indicating a peak performance at this optimal dosage. In contrast, *B. hispida* protein extracts were more consistent, peaking at 88.29 ± 10.7% removal at 40 mL/L and maintaining stable efficiency across various dosages and pH levels. Further, the sludge obtained after treatment using *M. oleifera* and *B. hispida* is generally considered non-toxic and biodegradable. It is generated in smaller amounts than alum sludge and can be potentially used in agricultural applications. Unfortunately, protein extracts have several limitations; they degrade over time when exposed to UV light and temperature change. The large-scale extraction of these proteins could be economically unfeasible compared to synthetic coagulants. It may be challenging to scale up the use of extracted proteins of *M. oleifera* and *B. hispida* in terms of sourcing enough plant materials and maintaining effectiveness in larger systems. There are several research gaps and issues that need to be further examined and explored in future research related to *M. oleifera* and *B. hispida*. It is recommended to use real water and wastewater samples, instead of synthetic samples, in future studies to validate the effectiveness of *M. oleifera* and *B. hispida* in removing *M. oleifera* and *B. hispida*.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Sharda University for providing the facilities to conduct the FTIR analysis and to the Indian Institute of Technology (IIT) Bombay Sophisticated Analytical Instruments Facilities (SAIF) for allowing access to their ESEM (Environmental Scanning Electron Microscopy).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D. Pan, F. Su, C. Liu and Z. Guo, *Adv. Compos. Hybrid Mater.*, **3**, 443 (2020); https://doi.org/10.1007/s42114-020-00190-0
2. O. Bajt, *FEBS Open Bio*, **11**, 954 (2021); https://doi.org/10.1002/2211-5463.13120
3. Statista 2023, Annual Production of Plastics Worldwide from 1950 to 2023. Statista Research Department, Accessed on November 21, 2024; https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/
4. A. Rafey and F.Z. Siddiqui, *Int. J. Environ. Anal. Chem.*, **103**, 3971 (2023); https://doi.org/10.1080/03067319.2021.1917560
5. R. Geyer, J.R. Jambeck and K.L. Law, *Sci. Adv.*, **3**, e1700782 (2017); https://doi.org/10.1126/sciadv.1700782
6. D.K.A. Barnes, F. Galgani, R.C. Thompson and M. Barlaz, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **364**, 1985 (2009); https://doi.org/10.1098/rstb.2008.0205
7. S. D'Angelo and R. Meccariello, *Int. J. Environ. Res. Public Health*, **18**, 2392 (2021); https://doi.org/10.3390/ijerph18052392
8. Z. Gao, K. Wontor, J.V. Cizdziel and H. Lu, *Microplast. Nanoplast.*, **2**, 10 (2022); https://doi.org/10.1186/s43591-022-00029-z
9. M. Kushwaha, S. Shankar, D. Goel, S. Singh, J. Rahul, K. Rachna and J. Singh, *Mar. Pollut. Bull.*, **209**, 117109 (2024); https://doi.org/10.1016/j.marpolbul.2024.117109
10. N. Bikiaris, N.F. Nikolaidis and P. Barmpalexis, *Cosmetics*, **11**, 145 (2024); https://doi.org/10.3390/cosmetics11050145
11. B. Udovicki, M. Andjelkovic, T. Cirkovic-Velickovic and A. Rajkovic, *Int. J. Food Contam.*, **9**, 7 (2022); https://doi.org/10.1186/s40550-022-00093-6
12. R.U. Duru, E.E. Ikpeama and J.A. Ibekwe, *Waste Dispos. Sustain. Energy*, **1**, 117 (2019); https://doi.org/10.1007/s42768-019-00010-2
13. G. Suzuki, N. Uchida, K. Tanaka, O. Higashi, Y. Takahashi, H. Kuramochi, N. Yamaguchi and M. Osako, *Environ. Pollut.*, **348**, 123855 (2024); https://doi.org/10.1016/j.envpol.2024.123855
14. A. Vega-Herrera, X. Borrell-Diaz, P.E. Redondo-Hasselerharm, E. Abad, M. Llorca, C.M. Villanueva and M. Farré, *Water Res.*, **220**, 118645 (2022); https://doi.org/10.1016/j.watres.2022.118645
15. M. Kedzierski, B. Lechat, O. Sire, G. Le Maguer, V. Le Tilly and S. Bruzaud, *Food Packag. Shelf Life*, **24**, 100489 (2020); https://doi.org/10.1016/j.fpsl.2020.100489
16. L. Alberghini, A. Truant, S. Santonicola, G. Colavita and V. Giaccone, *Int. J. Environ. Res. Public Health*, **20**, 789 (2022); https://doi.org/10.3390/ijerph20010789
17. D.B. Daniel, P.M. Ashraf, S.N. Thomas and K.T. Thomson, *Chemosphere*, **264**, 128554 (2021); https://doi.org/10.1016/j.chemosphere.2020.128554
18. B. Basaran, Z. Özçifçi, E.D. Kanbur, H.T. Akçay, S. Gül, Y. Bektas and Ü. Aytan, *J. Food Compos. Anal.*, **135**, 106646 (2024); https://doi.org/10.1016/j.jfca.2024.106646
19. P. Makhdoumi, M. Pirsaheb, A.A. Amin, S. Kianpour and H. Hossini, *J. Food Compos. Anal.*, **119**, 105261 (2023); https://doi.org/10.1016/j.jfca.2023.105261
20. N. Manimozhi, V. Rani, C. Sudhan, D. Manimekalai, R. Shalini and K.M. Abarna, *Reg. Stud. Mar. Sci.*, **53**, 102350 (2022); https://doi.org/10.1016/j.rsma.2022.102350
21. G. Kutralam-Muniasamy, F. Pérez-Guevara, I. Elizalde-Martínez and V.C. Shruti, *Sci. Total Environ.*, **714**, 136823 (2020); https://doi.org/10.1016/j.scitotenv.2020.136823
22. D. Xing, T. Zhao, X. Tan, J. Liu, S. Wu, J. Xu, M. Yan, B. Sun, S. Liu and P. Zheng, *Food Chem.*, **455**, 139636 (2024); https://doi.org/10.1016/j.foodchem.2024.139636
23. Y. Li, L. Peng, J. Fu, X. Dai and G. Wang, *Analyst*, **147**, 1099 (2022); https://doi.org/10.1039/D2AN00083K
24. N.-N. Lazăr, M. Călmuc, S.-A. Milea, P.-L. Georgescu and C. Iticescu, *Heliyon*, **10**, e28291 (2024); https://doi.org/10.1016/j.heliyon.2024.e28291
25. A. Saraluck, T. Techarang, P. Bunyapipat, K. Boonchuwong, Y. Pullaput and A. Mordmuang, *J. Clin. Med.*, **13**, 4029 (2024); https://doi.org/10.3390/jcm13144029
26. E. Persiani, A. Cecchettini, E. Ceccherini, I. Gisone, M.A. Morales and F. Vozzi, *Biomedicines*, **11**, 264 (2023); https://doi.org/10.3390/biomedicines11020264
27. S.A. Arat, *Water Pollut. Res.*, **89**, 1771 (2024); https://doi.org/10.2166/wst.2024.082
28. M.N. Issac and B. Kandasubramanian, *Environ. Sci. Pollut. Res. Int.*, **28**, 19544 (2021); https://doi.org/10.1007/s11356-021-13184-2
29. K. Ziani, C.-B. Ionita-Mîndrican, M. Mititelu, S.M. Neacsu, C. Negrei, E. Morosan, D. Drăgănescu and O.-T. Preda, *Nutrients*, **15**, 617 (2023); https://doi.org/10.3390/nu15030617
30. A. Nawab, M. Ahmad, M.T. Khan, M. Nafees, I. Khan and I. Ihsanullah, *J. Hazard. Mater. Adv.*, **16**, 100487 (2024); https://doi.org/10.1016/j.hazadv.2024.100487
31. Y. Chen, Y. Meng, G. Liu, X. Huang, G. Chai and Y. Xie, *Environ. Pollut.*, **355**, 124192 (2024); https://doi.org/10.1016/j.envpol.2024.124192
32. Y.S. Ibrahim, S. Tuan Anuar, A.A. Azmi, W.M.A. Wan Mohd Khalik, S. Lehata, S.R. Hamzah, D. Ismail, Z.F. Ma, A. Dzulkarnaen, Z. Zakaria, N. Mustaffa, S.E. Tuan Sharif and Y.Y. Lee, *JGH Open*, **5**, 116 (2021); https://doi.org/10.1002/jgh3.12457
33. M. Liu, J. Liu, F. Xiong, K. Xu, Y. Pu, J. Huang, J. Zhang, Y. Pu, R. Sun and K. Cheng, *Environ. Geochem. Health*, **45**, 2803 (2023); https://doi.org/10.1007/s10653-022-01458-8
34. K. Kadac-Czapska, J. Oœko, E. Knez and M. Grembecka, *Antioxidants*, **13**, 579 (2024); https://doi.org/10.3390/antiox13050579
35. W. Gao, Y. Zhang, A. Mo, J. Jiang, Y. Liang, X. Cao and D. He, *Green Anal. Chem.*, **3**, 100042 (2022); https://doi.org/10.1016/j.greeac.2022.100042
36. L. Dayal, K. Yadav, U. Dey, K. Das, P. Kumari, D. Raj and R.R. Mandal, *JJ. Hazard. Mater. Adv.*, **16**, 100460 (2024); https://doi.org/10.1016/j.hazadv.2024.100460
37. S. Ziajahromi, P.A. Neale, L. Rintoul and F.D.L. Leusch, *Water Res.*, **112**, 93 (2017); https://doi.org/10.1016/j.watres.2017.01.042
38. N. Girish, N. Parashar and S. Hait, *Sci. Total Environ.*, **899**, 165723 (2023); https://doi.org/10.1016/j.scitotenv.2023.165723
39. B. Koul, N. Bhat, M. Abubakar, M. Mishra, A.P. Arukha and D. Yadav, *Water*, **14**, 3751 (2022); https://doi.org/10.3390/w14223751
40. P. Agarwal, S. Prakash and G. Saini, *Cleaner Water*, **1**, 100010 (2024); https://doi.org/10.1016/j.clwat.2024.100010
41. T. Reza, Z.H. Mohamad Riza, S.R. Sheikh Abdullah, H. Abu Hasan, N. Ismail and A.R. Othman, *Toxics*, **12**, 12 (2023); https://doi.org/10.3390/toxics12010012
42. S. Mathupreetha, W.M.A.P. Wijekoon, W.K.C.N. Dayanthi and L.F.U. Nusrath, *Total Environ. Eng.*, **2**, 100009 (2025); https://doi.org/10.1016/j.teengi.2025.100009
43. M.H. Abdul-Rahman-Adrin, N. Ngadiman, N.H. Abdullah, R. Hamdan and M.S. Abdullah, *IOP Conf. Ser. Earth Environ. Sci.*, **1453**, 012052 (2025); https://doi.org/10.1088/1755-1315/1453/1/012052
44. C. Panigrahi, S. Kamal, J. Qin, S. Ziemann, E. Rahman, M. House, C. Dutcher and B. Xiong, *Environ. Eng. Sci.*, **41**, 477 (2024); https://doi.org/10.1089/ees.2024.0135
45. E. Ali, S. Muyibi, H. Salleh, M. Alam and M. Salleh, *J. Water Resour. Prot.*, **2**, 259 (2010); https://doi.org/10.4236/jwarp.2010.23030
46. S.A. Muyibi, S.A. Abbas, M.J.M.M. Noor and F.R. Ahmadon, *IIUM Eng. J.*, **4**, 1 (2003).
47. S.B. Kurniawan, A. Ahmad, N.S.M. Said, K. Gustinasari, S.R.S. Abdullah and M.F. Imron, *Environ. Adv.*, **14**, 100441 (2023); https://doi.org/10.1016/j.envadv.2023.100441

48. M. Bradford, *Anal. Biochem.*, **72**, 248 (1976); https://doi.org/10.1016/0003-2697(76)90527-3
49. D. Churiyah and L.K. Darusman, *Hayati J. Biosci.*, **16**, 161 (2009); https://doi.org/10.4308/hjb.16.4.161
50. R. Chércoles Asensio, M. San Andrés Moya, J.M. de la Roja and M. Gómez, *Anal. Bioanal. Chem.*, **395**, 2081 (2009); https://doi.org/10.1007/s00216-009-3201-2
51. S. Avazpour and M. Noshadi, *Chemosphere*, **358**, 142215 (2024); https://doi.org/10.1016/j.chemosphere.2024.142215
52. S. Prasetyo, C.A. Santos, A.K. Sugih and H. Kristianto, *Sustain. Chem. Environ.*, **9**, 100225 (2025); https://doi.org/10.1016/j.scenv.2025.100225
53. J. Yao, Z. Peng, W. Chen, Q. Lin, M. Cheng, H. Li, Y. Yang and H.Y. Yang, *Mar. Pollut. Bull.*, **186**, 114347 (2023); https://doi.org/10.1016/j.marpolbul.2022.114347
54. C. Li, R. Busquets and L.C. Campos, *Chemosphere*, **364**, 143145 (2024); https://doi.org/10.1016/j.chemosphere.2024.143145
55. K. Yu, Y. Zhang, S. Yang and L. Chen, *Environ. Eng. Sci.*, **41**, 459 (2024); https://doi.org/10.1089/ees.2023.0332
56. S. Ziembowicz, M. Kida and P. Koszelnik, *Materials*, **16**, 4070 (2023); https://doi.org/10.3390/ma16114070