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Exploring the Potential of Papaya (*Carica papaya*) Seeds as an Innovative Coagulant for Efficient Microplastic Removal

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Abstract: **Microplastics refer to particles less than 5 mm in diameter. They thus provide new, but not uniquely unprecedented, risks to ecosystems and human health through bioaccumulation and long-term environmental persistence. This paper reviews the appropriateness of papaya seeds as coagulants for the removal of microplastics from polluted water responsive to the alarming rise in the presence of microplastic pollution in waters in the Philippines. The research followed five stages: procuring, preparation of coagulant, preparation of microplastic samples, conducting coagulation experiments, and data analysis. There were experiments carried out with the use of ground papaya seeds for the treatment of artificial water samples with pre-set levels of microplastics. Coagulant extracted from papaya seeds was applied in varied amounts during the tests; this was followed by sedimentation to enable the aggregation and consequent removal of microplastics. Analysis was done quantitatively to check the efficiency of the coagulant in lowering the concentration of microplastics. Preliminary results were proof that this charge from papaya seed powder agglomerated the negatively charged microplastic particles, allowing them to be flocculated and removed from water. This study has demonstrated the potential for agricultural waste in the direction of sustainable methods of overcoming microplastic pollution while allowing research on natural coagulants as part of a potential wastewater treatment route.**

Keywords: **Microplastics, Coagulant, Carica papaya seeds, Microplastic pollution, Water treatment, Sustainable methods.**

I. INTRODUCTION

Microplastics (MPs) are smaller pieces of plastics that came about due to the degradation of larger plastics. Found in land, water, and even air, microplastics accumulate and persist due to its non-biodegradable nature (Rogers, 2024). Moreover, its small size allows it to be easily ingested by both humans and animals, resulting in various health complications.

Annually, 4 to 14 million tonnes of plastic enter the seas globally, contributing to microplastic pollution (Prakash, 2023). As a particle less than 5 mm in length, it has become a pervasive contaminant to both freshwater and saltwater ecosystems. In the Philippines alone, various bodies of water were polluted with a significant amount of microplastics.

Research in Laguna de Bay found a median of 15 microplastics per 1000 L of lake water in Brgy. Sampiruhan, indicating substantial pollution (Deocaris et al., 2023). In Lake Yambo and Lake Sampaloc, concentrations ranged from 344 to 989 n/m3 and 483 to 789 n/m3, respectively, with urbanized Lake Sampaloc showing higher levels (Natuel et al., 2023). In Mindanao, Cagayan de Oro River, results showed a mean concentration of 300 items/m3 of MPs dominated by blue-colored (59%), fiber (63%), 0.3–0.5 mm (44%), and polyacetylene (48%) particles, with the highest concentration of microplastics recorded near the mouth of the river, and the lowest in the middle area (Gabriel et al., 2023). Because microplastic continues to pollute bodies of water as result of human activities that generate waste water, various studies have been conducted to address this issue. Talvitie et al. (2017) conducted one study that used a membrane bioreactor to treat primary effluent and various tertiary treatment technologies (discfilter, fast sand filtration, and dissolved air flotation) to treat secondary effluent. During treatment, the MBR removed 99.9% of MPs (from 6.9 to 0.005 MP L^{-1}), the quick sand filter 97% (from 0.7 to 0.02 MP L^{-1}), the dissolved air flotation 95% (from 2.0 to 0.1 MP L^{-1}), and the discfilter 40-98.5% (from 0.5 - 2.0 to 0.03-0.3 MP L^{-1}). In the Philippines, Coagulation is an important step in the removal of microplastics, and natural coagulants surpass chemical alternatives due to their non-toxicity and cost-effectiveness (Reza et al., 2023).

Several studies have been conducted in regards to developing natural coagulants for the purpose of removing microplastics from the water. Liemin et al. (2023) discussed Cationic-modified starch (CS) effectively removing microplastics by utilizing its positive charge to attract and trap the plastic particles in water. The positively charged CS interacts with MPs, promoting coagulation where the particles clump together, facilitating their removal from the water. Moreover, they add that CS's efficiency in removing MPs is attributed to its ability to capture a wide range of MP sizes, types, and ages, with higher removal rates observed for larger, high-density, and aged microplastics. In a similar vein, the study of Avazpour and Noshadi (2024) explored the use of *Moringa oleifera* along with anionic polyacrylamide in enhancing the coagulation process for the removal of microplastics from water. Findings suggest that the natural-based *Moringa oleifera* aids in the aggregation of microplastics by acting as a coagulant in the treatment process, due to being rich in various bioactive compounds, including proteins, polysaccharides, and phenolic compounds, which contribute to its coagulation properties. The proteins in *Moringa oleifera*, particularly the water-soluble proteins, play a significant role in destabilizing microplastic particles, which facilitates the aggregation of the particles.

Among other natural coagulants, papaya seeds can be a promising alternative solution for microplastic pollution. *Carica papaya*, or papaya, is a popular fruit around the world. It grows in tropical or subtropical climates, with an international trade value of approximately $200 million in 2009. Consumption of papaya fruits generates a large amount of food waste, mainly discarded papaya peels and seeds, which account for 15-20% of the weight. As a result, it is critical to reduce the amount of papaya waste by using it in various applications, including as a bio-coagulant in wastewater treatment (Amran et al., 2021). Studies on papaya (*Carica papaya*) seeds show a promising advantage in its transformation into a natural coagulant. According to the study conducted by Yimer and Dame (2021), because papaya seed contains positively charged proteins, it acts as a coagulant by binding with negatively charged particles (silt, clay, bacteria, and toxins, for example), allowing the ensuing flocs to settle and yield pure water. Also, papaya seed powder can combine with particles in water and settle to the bottom. Papain (Papaya proteinase) is the most important protein present, with 345 amino acid residues and a single sequence of properly bound and mature peptides. Papaya seeds were also utilized to cure water-containing fecal germs. The following studies were conducted on papaya seed as a water purifier.

II. MATERIALS AND METHOD

The research was conducted through five (5) phases: (A) collection of materials, (B) preparation of the coagulant, (C) MPs sample preparation, (D) coagulation experimentation, and (E) data collection and analysis. Creating step-by-step procedures is essential to have a proper flow when conducting research. The study was conducted in the Laboratory of Davao City National High School (DCNHS). The FTIR spectrometer analysis was performed at the laboratory of Davao Medical School Foundation (DMSF), Davao City.

A. Collection of Materials

The study will use papaya seeds as coagulants as they are proven to have coagulant properties (Widiyanti et al., 2023). The researchers purchased the papaya in a local market in Davao City. The fruit was discarded and its seeds for coagulant testing. The materials, apparatus, equipment, and location of the experiment were also prepared and sterilized by the researchers to ensure a clean, safe, and efficient working environment.

B. Creating Coagulants

The seeds of Papaya fruit were washed to remove other particles and then dried in the sunlight for 3 days to ensure the water contents of the seeds were absorbed. After drying, the seeds of Carica Papaya were then ground into a fine powder using mortar and pestle. The powdered seeds were then stored in a dark and air-tight container to avoid any type of aerial contamination (Agarwal et al., 2024). The seeds and their powdered form of Papaya (*Carica papaya*) are shown in Figure 01.

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Figure 01: Papaya (*Carica papaya*) seeds and its powdered form.

C. Water Sample Preparation and Characterization

In conducting the coagulation experiment, the researchers created synthetic water samples in order to ensure controlled conditions which eliminates the variability and unknown contaminants present in natural bodies of water. This ensures that the experiment focuses solely on the effect of the papaya seeds on microplastic removal. Additionally, the researchers tested the water samples for its pH and temperature to allow replicability of the exact experimental conditions.

Table 01: Average turbidity, pH, and temperature of the water samples collected

Parameters	Average
pH	7
Temperature	32.2°C

Sample Preparation

The researchers created the synthetic samples by using plastic-coated paper cups, which are commonly used. This object can create microplastics through the leaching process. For the water, the researchers used distilled water for the synthetic samples as it only focuses on the effectiveness of *Carica papaya* seeds as novel coagulants (Amran et al., 2014). The researchers used thirteen (190 mL) plastic-coated paper cups. The cups were rinsed using distilled water at room temperature and then applied with 100 mL hot distilled water at 90 °C. The researchers then covered the cups and left undisturbed for 15 minutes to avoid aerial contamination (Ranjan et al., 2021).

Figure 02: Heating 500 mL of distilled water to be applied and plastic-coated paper cups

After exposure to hot water, the thirteen (13) leached samples were then poured into a large container with the remaining 4.7 L distilled water to create synthetic water samples. Mechanical Homogenization was conducted in order to assume and have equal concentration of microplastic particles per 500 mL which will be later used for coagulation experiments (Stuparu et al., 2021).

MPs Characterization:

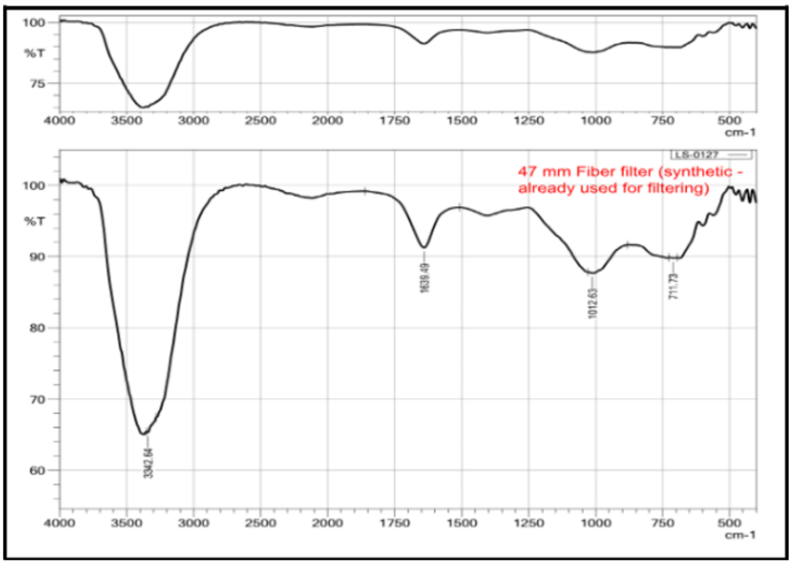

Figure 03: FTIR Analysis Results for the filtered leached Sample.

A leached sample was filtered using a 0.47 mm fiber filter to remove the supposedly generated particles to be used for conducting FTIR spectrometer analysis. FTIR spectrometer analyses were conducted on the fiber filter used by the researchers to determine the type of microplastics existing in the water sample, its chemical compounds, appearance, and bonds (Käppler et al., 2016). The FTIR spectrometer analysis was conducted at Davao Medical School Foundation (DMSF), Davao City.

Table 02: Characteristic IR absorption peaks of functional groups present in the fiber filtered sample.

Functional Group	Type of Vibration	Frequency (cm^{-1})
Hydroxyl group	O-H	3342.64
Aromatic	C=C	1639.49
Ester group	C-O	1012.63
Aromatic	=C-H	711.73

The results of the FTIR spectrometer show different chemical bonds and functional groups within the sample absorb specific wavelengths of infrared radiation corresponding to their vibrational energy levels (Nandiyanto 2019). This absorption causes the molecules to vibrate at characteristic frequencies, which allows their presence to be identified in the filter. The presence of the ester and aromatic functional groups strongly indicates that the used fiber filter contains microplastics. The presence of hydroxyl groups indicates the presence of water.

The presence of aromatics (=C-H) and esters (C-O) in the results strongly indicates the existence of Polyethylene Terephthalate (PET) (Alidadykhah,2022). The aromatic (C=C) group indicates the existence of Polystyrene (PS) (Ashby,2013). Polyethylene Terephthalate (PET) is microplastic with defining features under the functional groups being ester and aromatic groups which enhances the plastic's thermal stability and rigidity (being used in paper cups) (Sintac, 2024). Polystyrene (PS) plastic has an aromatic group as its defining structure on its functional group content. (Lin & Liu, 2021). This type of plastic is commonly used in beverages, and packaging (Ruitai, 2023). The microplastics can be seen as fragments, pellets, or fiber-shaped particles under a microscope (Yusuf et al., 2023).

Microplastics, defined as particles smaller than 5 mm, are easily identifiable under a microscope due to their small size and distinct physical features. They come in various shapes, including fibers, fragments, pellets, films, foams, and microbeads. Their surface texture can be smooth or rough depending on degradation, and some, like polyethylene and polypropylene, may appear translucent or transparent under magnification (Rushdi et al. 2023). The crude powder of *C. papaya* ripe seed was dark black in color, fine in texture, odorless, and had a salty flavor (Nayak & Shee, 2020).

Figure 04: Appearance of microplastic particles under a microscope (Fiber-Shaped Particle).

Figure 05: Appearance of microplastic particles under a microscope (Irregular-Shaped Particles).

Hot Needle Testing

To distinguish microplastics while observing them under the microscope, researchers conducted hot needle testing on the remaining synthetic water sample before the coagulation process. The research conducted by Perez et al. (2022), stated that the hot needle test can be used to distinguish between natural and plastic particles based on their thermoplastic properties. Plastic particles melt, and non-plastic materials are burned into ashes. This helps them identify the microplastics visually from other impurities. The hot needle test, also known as the hot point test, helps researchers identify suspected microplastics under optical microscopy by probing their physical melt or deformation behavior. It is a low-cost and practical method that can be used widely (Beckingham et al., 2023).

MPs Concentration

For the determining the concentration, three (3) - 10 µL were collected in each 500 mL water sample to quantify the amount of microplastics. To improve the accuracy and reliability of the results, the samples were taken from different areas of the 500 mL water sample, ensuring that the data represents the entire sample homogeneously. Manual Counting was applied as it is one of the alternative ways for quantification (Osorio, 2021). The data collected from manual counting were then averaged to calculate the microplastic concentration in particles per milliliter (MPs/mL). Based on the quantification conducted by the researchers, there is approximately 2530 MPs/mL.

D. Coagulation Experimentation

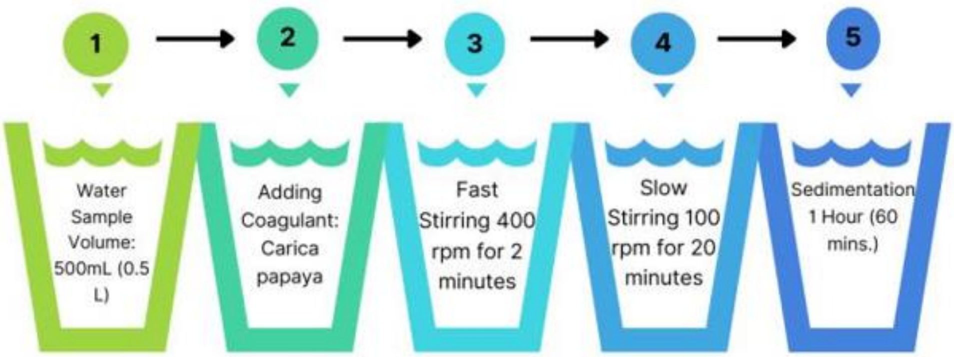

Figure 06: Schematics of the coagulation experiment.

In conducting a coagulation experiment, the researchers used the test jar/magnetic stirrer as it is commonly used in this type of experimentation such as the study of Li et al. (2015), and Agarwal (2024). The researchers have created three different amounts of doses of each of the coagulants. (100 mg L^{-1}, 150 mg L^{-1}, and 200 mg L^{-1}) to be used in this study. It is then followed by preparing the water sample with MPs, each containing 0.5L (500mL).

The samples were then rapidly stirred (at 400 rpm) for 2 minutes, followed by 100 rpm for 20 minutes (Wiśniowska et al., 2024). After mixing, it is then followed by an hour (60 mins.) of sedimentation. The initial rapid stirring at 400 rpm for 2 minutes ensures thorough distribution of the coagulant in the synthetic water sample, followed by a controlled mixing speed of 100 rpm for 20 minutes to promote the formation of larger, more stable flocs without disrupting them, thereby enhancing their settling capacity The subsequent 60-minute sedimentation phase allows gravity to act on the aggregates which allows for efficient removal of microplastics from the water sample (El-taweel et al., 2023). The samples used in the experiment were performed triplicate to have more accurate findings.

Figure 07: The Coagulation process.

Figure 08: The different samples after the Coagulation Experiment.

E. Data Collection and Analysis

After the coagulant process and sedimentation, a 10 µL portion of the supernatant of the samples were collected by using a micropipette for quantification of the microplastics. The quantification of microplastics was done manually as it is one of the alternative ways of quantification (Perez, et al.,2022). Collecting and analyzing multiple samples from the supernatant improves the reliability of results, and averaging these replicates reduces the impact of outliers or human counting errors, providing a more accurate representation of microplastic concentrations. The researchers used a compound microscope at 60x magnification for quantification and collected three (3) supernatant samples for each leached sample. The three results per sample were then computed into the average data which was converted to MPs/mL (Agarwal et al., 2024). In order to analyze the data from conducting the coagulant experiment, the researchers will use analysis of variance (ANOVA) , considering the researchers have made three (3) treatments (different doses) of *Carica papaya* seeds as coagulants for microplastic removal (Kaufmann & Schering, 2014). The researchers used this statistical analysis as it allows for the comparison and analysis between the means of the different dosages of *Carica papaya* seeds for MPs removal efficiency.

For computing the efficiency of the Papaya (*Carica Papaya*) seeds as natural coagulant, certain formulas and computations were conducted. The formula for the computation of coagulant efficiency is:

$$\text{Removal Efficiency (\%)} = \frac{C_o - C_f}{C_o} \quad 100\%$$

Which C_o represents the initial concentration of the microplastics in a 500 mL sample and C_f represents the final microplastic concentration after applying the natural coagulant. The units for the microplastic sample concentration are count-based therefore, the researchers used the unit particles per milliliter (particles/mL). The variable T represents the removal efficiency of each sample. The constant 100% serves as the conversion of the solution from decimal to percentage. Then the researchers computed for the mean of the different treatments in order to find the average effectiveness of the natural coagulant. The N represents the amount of replicate per treatment which for this case, is three (3).

$$\text{Mean } (\bar{X}) = \frac{T1 + T2 + T3}{N}$$

Research Design

The researchers of this study will employ and practice true experimental design, as this design allows the researchers to control and manipulate the variables which are the doses of *Carica papaya* seeds in order to determine its effectiveness on microplastic removal. This research design and analysis chosen by the researchers will ensure strong and reliable results.

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III. RESULTS AND DISCUSSION

Table 03: Removal Efficiency in % of Papaya (*Carica papaya*) seeds on Microplastics

Treatment (mg/L⁻¹)	Trial 1	Trial 2	Trial 3	Mean ($\bar{X}$)
100	69.72%	74.31%	68.85%	**70.96%**
150	76.28%	78.50%	81.42%	**78.73%**
200	84.98%	84.58%	83.00%	**84.19%**

Table 02 presents the removal efficiency of papaya (Carica papaya) seeds on microplastics across three treatment concentrations: A (100 mg L^{-1}), B (150 mg L^{-1}), and C (200 mg L^{-1}). The mean removal efficiencies are 70.96% for treatment A, 78.73% for treatment B, and 84.19% for treatment C, indicating a clear trend of increased effectiveness with higher concentrations.

Table 04: Descriptive Statistical Analysis for the Removal Efficiency.

Descriptive Statistics

	MPs Removed (%) T1	T2	T3
Valid	3	3	3
Missing	0	0	0
Mean	70.960	78.733	84.187
Std. Deviation	2.934	2.578	1.047
Minimum	68.850	76.280	83.00
Maximum	74.310	81.420	84.980

The removal rates for treatments T1, T2, and T3 were 70.96%, 78.73%, and 84.19%, respectively. The reduction in standard deviation from 2.93% in T1 to 1.05% in T3 indicates a narrowing of variability and improved consistency in the process. The significant increase in removal efficiency from T1 to T3 demonstrates the effectiveness of the intervention, suggesting that the refinement of the procedure leads to more reliable outcomes.

Table 05: Results of Statistical Analysis: ANOVA

ANOVA - MPs Removed (%)

Cases	Sum of Squares	df	Mean Square	F	p
Treatmen	265.108	2	132.554	24.325	0.001
Residual	32.696	6	5.449		

Note. Type III Sum of Squares

The statistical analysis of the removal efficiency presents a compelling case for the effectiveness of the treatment. The ANOVA results indicate a significant impact of the intervention, with an F-value of 24.325 reflecting strong differences among the test groups. A p-value of 0.001 further substantiates that the observed variations in means between T1, T2, and T3 are not attributable to random chance, thereby highlighting the meaningful enhancements in MPs removed across the experimental phases. This statistical evidence reveals the efficacy of the treatment in improving removal efficiency.

Table 06: Post Hoc Test Results

Post Hoc Test
Standard

Post Hoc Comparisons - Treatments

		Mean Difference	SE	t	P
T1	T2	-7.773	1.906	-4.078	0.015
	T3	-13.227	1.906	-6.939	0.001
T2	T3	-5.453	1.906	-2.861	0.065

The post hoc analysis indicates significant differences in removal efficiency among the treatment phases. T1 (70.96%) significantly differs from T2 (78.73%), highlighting a marked improvement. T2 also shows a significant increase compared to T3 (84.19%), albeit less pronounced. The comparison between T1 and T3 further emphasizes the substantial enhancement in removal efficiency across the phases, affirming the treatment's effectiveness. The results of the study shows the potential of Carica papaya seeds as green coagulants for microplastic removal, with 84.19% being the maximum removal efficiency at 200 mg/L. This shows that powderized papaya seeds can be used as a cost-effective solution for microplastic removal, as 30% to 50% of the seed is often discarded as a waste (Davila et al., 2014). This is also to address the pollution of microplastic on a global scale. The statistical validation using ANOVA, with a p-value of 0.001, which further confirms the reliability of the results. Because of the results, Papaya seeds can be served as a non-toxic alternative for the commonly-used coagulants such as $Al2(SO4)3$ (Aluminum Sulfate) which had a removal efficiency rate of approxinately 80% and $Fe2(SO_4)3$ (Ferric Sulfate) with approximately removal efficiency rate of 79% from the study conducted by Prokopova et al. (2021). These conventional coagulants also leave harmful residues in treated waters that pose risks to human health and aquatic ecosystems if not adequately removed during subsequent treatment processes (Ziembowicz et al., 2023). These synthetic coagulants can affect the pH, and can create sludge that are non-biodegradable which poses a threat in aquatic ecosystems (Kurniawan et al., 2020). Papaya seeds showed comparable efficacy to other green coagulants such as Cationic-modified starch and Moringa oleifera with removal efficiency of 89% and 90% - 96% respectively. This is due to their protein content, which promotes microplastic aggregation. This makes them a viable and affordable choice for wider water treatment applications (Amran et al., 2021).

IV. CONCLUSIONS AND RECOMMENDATIONS

The use of Papaya (Carica papaya) seeds as a natural coagulant demonstrated effective removal of microplastics due to the coagulating properties of the positively charged proteins in the seeds. Based on the statistical tests conducted, the results showed a significant difference between the treatments (100 mg L^{-1}, 150 mg L^{-1}, and 200 mg L^{-1}) in their ability to remove microplastics, with increasing concentrations leading to higher removal efficiencies, making it effective in removing microplastics from synthetic water samples.

For further research:

1. **Proper Characterization and Identification Techniques:** More accurate characterization of microplastic particles must be practiced. Aside from FTIR, Raman spectroscopy and fluorescence microscopy for more accurate characterization. These techniques should be also applied for analyzing the chemical and physical properties during the coagulation process of Carica papaya seeds and the microplastics.

2. **Accurate Quantification Methods:** Despite manual counting being practiced for easier alternatives, it is still encouraged to have more reliable, precise and accurate quantification methods for the concentration of microplastic in the samples. It is recommended that the study of Agarwal et al. (2024), which uses the Neubauer Counting Chamber for quantifying microplastics in their samples, should be followed.

3. **Application and Optimization of varying Conditions (Parameters):** While the study focuses on the given parameters, It is recommended to focus on testing the coagulant's performance across a broader range of factors, such as the levels of turbidity, pH, and temperature. The variables such as the concentration of the microplastics, amount of doses,

and the stirring duration and the intensity of the coagulation process should also be optimized. This will ensure the solution will be effective under real-world conditions and can be used for larger-scale operations.

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