

A Bioinformatics-Driven Framework for Assessing Moringa Bioflocclulants Against Waterborne Microplastics

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ABSTRACT

India houses 42 of the 50 most microplastically polluted cities in the world, and conventional coagulation removes only 1.8-54%. Conventional coagulation has shown variable removal efficiency for microplastics, motivating the investigation of natural coagulants such as Moringa oleifera cationic protein (MOCP). However, the molecular interactions between MOCP and plastic-associated chemical structures remain incompletely characterized. In this exploratory bioinformatics study, an MOCP structure with a reported isoelectric point of approximately 10–10.5 was obtained from the RCSB Protein Data Bank. Four representative polymer fragments were evaluated: polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS). Ligand structures were obtained from PubChem, blind molecular docking was performed using AutoDock Vina, and the highest-scoring poses were examined in PyMOL. The predicted Vina docking scores were $-5.029 \text{ kcal mol}^{-1}$ for PET, $-4.613 \text{ kcal mol}^{-1}$ for PS, $-2.623 \text{ kcal mol}^{-1}$ for PE, and $-2.163 \text{ kcal mol}^{-1}$ for PP. PET and PS therefore produced more negative docking scores than PE and PP under the applied protocol. The shortest measured contacts ranged from 3.5 to 4.2 Å and indicated geometric proximity in the predicted poses, but did not establish a specific interaction mechanism. Because isolated polymer fragments do not reproduce the size, weathering, additives, surface charge, or biofilms of environmental microplastic particles, these findings should be interpreted as preliminary computational predictions. They identify MOCP as a promising candidate natural coagulant for future particle-level experimental validation.

INTRODUCTION

Microplastics are defined as plastic fragments between 0.1µm and 5mm in diameter derived from larger plastic debris or directly manufactured in the forms of microbeads or microfibers.[1] These plastics have been detected in various locations throughout the globe and found to be contaminating water supplies, oceans, rivers, and food. [2] This poses a major risk to the health and safety of individuals consuming this water or food. Approximately 11 million tons of plastic waste are deposited into oceans annually, 2.7 of which comes from rivers. Additionally, 385 trillion microplastic particles are currently floating on ocean

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surfaces.[3] India alone produces 14.16 million tons of plastic polymers annually, only 25% of which is recycled. These microplastics accumulate rapidly in living organisms; for example it has been estimated that the average individual ingests between 32,000 and 52,000 annually through the above sources of contaminated water supply and food.[4]

Several research studies have been carried out on microplastic removal from drinking water and wastewater treatment plants. Some of their findings were that initial coagulation and sedimentation in DWTPs (drinking water treatment plants), remove only 1.8% to 54.5% of microplastics. Advanced treatment plants push this percentage up to 86.6%, while larger-than-normal flocculant doses increase the percentage to 62%. [5] Additionally, the membrane technology and sludge used can themselves become a secondary source of microplastics in the water supply. This demonstrates that the current treatment is inadequate in the removal of microplastics. A study compared aluminum sulfate, the standard chemical coagulant, with moringa seed saline extract for removing aged PVC microplastics from low turbidity drinking water. With 8mg/L of aluminum sulfate, 98.7% aged PVC microplastic removal was achieved with a median diameter of 15 μ m. The effectiveness of aluminum sulfate as a microplastic-removing agent was established, although it was also determined that highly specific pH conditions are required. [6] A study conducted by Gao et al. synthesized cationic modified starch. This was done by reacting corn starch with CHPTAC. Its efficacy as a microplastic coagulant was determined. The average removal rate for polystyrene particles was determined to be 65.33%. Subsequently, higher removal rates were found for larger high-density aged microplastics; however, unlike aluminum sulfate, the efficiency remained constant over a range of pH values.[7]

Another bioinformatics-based study was conducted, where the dimerization behavior of a coagulant protein was found in *Moringa oleifera* seeds. Homology modeling was used to predict the structure of the M20.1 protein; docking was performed to investigate how the 2 M20.1 monomers interact. Additionally, binding free energy calculations and residue distance analysis were performed to identify energetic and structural factors driving the dimer formation. [8] They were able to successfully predict the 3d structure using homology modelling; they also found the residue responsible for stable dimer formation. Finally, they found that coagulation was similar for monomeric and dimeric versions of the protein, thus, it was deemed that dimerization is not essential for coagulation. Another study was conducted where 4 ML models were created to predict the efficiency of microplastic removal by coagulation under various conditions. [9] One of their models (RBF) attained an R^2 value of 0.96. They found water temperature to have the most significant negative effect, while coagulant ad showed the most positive correlation with net microplastic removal.

Experimental studies of the microplastic removal mechanisms of *Moringa oleifera*, computational studies on MOCP dimerization, and machine-learning coagulation efficiency studies were reviewed from the literature available to the authors. However, no study was found in the reviewed literature that directly tested the anticipated interactions between MOCP and typical fragments of plastics. This observation does not imply that there is no such study, as it was not conducted as a systematic literature review. The present

study is therefore an exploratory in silico study of interactions of MOCP with selected models of polymer fragments and the generation of hypotheses for particle validation experiments.

METHODOLOGY

Data collection

A list of keywords was defined to start the data collection process related to moringa and microplastic water pollution. The keywords defined were moringa, microplastic, water pollution, dataset, seed, composition, cationic starch. A marine microplastic dataset was identified from the database National Centers for Environmental Information, which had information on global microplastic concentrations in oceans. Another data source was the Journal of Contaminant Hydrology, which provided a dataset containing the different variants and structures of microplastics. Through this dataset, the common monomeric components of microplastics were identified as polyethylene, polypropylene, polystyrene, polycarbonate, polyurethane, polyamide, and polyethylene terephthalate. A paper from 3 Biotech, called Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance, identified the major protein of moringa as MOCP (Moringa Oleifera Cationic Protein), and determined its Isoelectric point as above PH 10.5, which is useful to determine its charge at common water pH values. Additionally, an article written by Amir Muhd, Arjulizan Rusli, and Muhammad Khalil Abdullah in 2024 titled “A review of microplastic surface interactions in water and potential capturing methods” spoke to the surface interactions of microplastics in water and explained their relative negative charges. It also detailed determinants of these charges, such as adsorption of natural organic matter, oxidation, and surface functionalization, and identified that weathering can cause microplastics to develop negatively charged functional groups and negative zeta potentials. Finally, through the following study conducted by Panigrahi et al. (2024) - “Removal of Pristine and UV-Weathered Microplastics from Water: *Moringa oleifera* Seed Protein as a Natural Coagulant.” MOCP, the coagulant protein in moringa, was used for microplastic removal.

Molecular Docking

The coagulant properties of *Moringa oleifera*'s MOCP are known from the aforementioned literature. The binding affinity between the MOCP protein and 4 common microplastic monomers was tested using AutoDock Vina, accessed from tamarind bio. A job was created and titled. The MOCP protein structure PDB file was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The ligand structures of the four microplastics were obtained as SDF files from PubChem. The four microplastic monomers tested were polyethylene (PE), Polyethylene Terephthalate (PET), Polypropylene (PP), and Polystyrene (PS). PE, PET, PP, and PS were selected as a focused test set because they were among the commonly reported polymer types identified in the reviewed data sources and provided contrasting chemical features. PE and PP represent predominantly nonpolar aliphatic structures, PS contains an aromatic ring, and PET contains both an aromatic ring and ester carbonyl groups. This comparison allowed the exploratory docking analysis to examine whether these structural differences were associated with variation in predicted docking scores. Polycarbonate, polyurethane, and

polyamide were excluded to maintain a defined initial scope and because these polymer classes can encompass chemically variable repeat units and formulations. The four selected models were not intended to represent all environmental microplastic types. A grid box was adjusted to boxX=101, boxY=13, boxZ=13, width=32, height=47, depth=37 to encompass the entire protein. Next, the ligand SDF file was uploaded, exhaustiveness was changed to 48, and the job was submitted. The result was taken from the binding site with the highest binding affinity.

PyMOL Visualisation

For the visualisation purpose, PyMOL software was used. First, the PDB file with the highest binding affinity was obtained from the AutoDock Vina analysis. Next, PyMOL was opened, and the PDB file was imported along with the MOCP PDB file. Under the Wizards tab in the toolbar, the measurement wizard was selected. For each docked complex, MOCP residues within 7.0 Å of the representative polymer fragment were inspected. For every retained measurement, the ligand atom, MOCP chain identifier, residue name and number, MOCP atom, and interatomic distance were recorded. Contacts were ranked according to distance and are reported in Table 2. No interaction type was assigned solely from the measured distance.

RESULTS

Molecular Docking Analysis

Molecular docking is a computational method used to predict binding affinity. It was used to determine the binding affinity between MOCP and the four different microplastic monomers. The receptor in each case was MOCP, and the ligand was the chosen microplastic monomer. Molecular docking utilizes the 3d structures of the protein and ligand and searches for the most energetically favourable orientation, position, and shape between the ligand and receptor. Blind docking was used, and the pose with the most negative binding affinity was selected for each monomer. The following results were obtained.

Protein name	Microplastic name	Predicted Vina docking score (kcal mol ⁻¹)
MOCP	Polyethylene (PE)	-2.623
MOCP	Polyethylene Terephthalate (PET)	-5.029
MOCP	Polypropylene (PP)	-2.163
MOCP	Polystyrene (PS)	-4.613

Table 1. Predicted AutoDock Vina scores for MOCP docked with representative fragments of polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS).

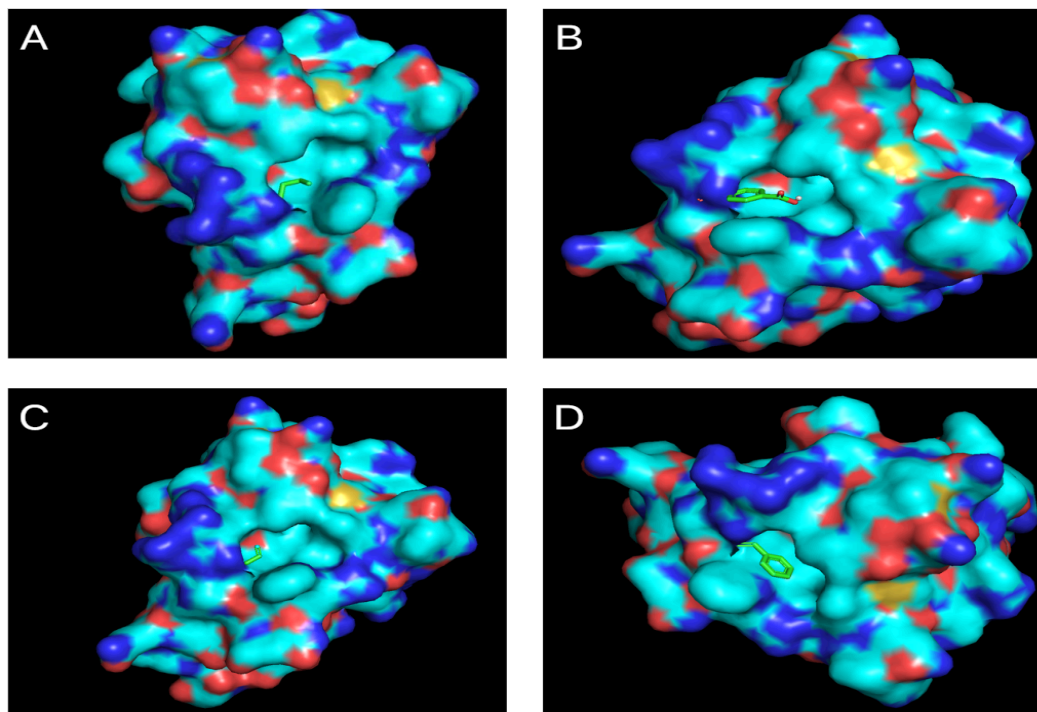


Figure 1. Molecular docking of representative polymer fragments with MOCP. Surface representations of MOCP generated in PyMOL. Polymer fragments correspond to (A) polyethylene (PE), (B) polyethylene terephthalate (PET), (C) polypropylene (PP), and (D) polystyrene (PS). Polymer fragments shown as green stick models at their predicted binding sites. Protein surfaces coloured by atom type (carbon, cyan; oxygen, red; nitrogen, blue; sulfur, yellow).

Docking results showed that, according to the applied docking protocol, the PET and PS had more negative Vina scores than PE and PP, and were ranked as PET ($-5.029 \text{ kcal mol}^{-1}$) > PS ($-4.613 \text{ kcal mol}^{-1}$) > PE ($-2.623 \text{ kcal mol}^{-1}$) > PP ($-2.163 \text{ kcal mol}^{-1}$). These values only refer to the individual isolated fragments and their best-scoring rigid-receptor poses. These should therefore be considered to be comparisons to the preliminary rather than evidence for binding to or removal of intact environmental microplastic particles by MOCP. The net positive charge anticipated for MOCP at a reported pI and the negative zeta potentials reported for some weathered microplastics justify experimental testing in the future, but electrostatic interactions at the particle scale were not directly modelled here.

Interaction Analysis Using PyMOL

The interaction analysis was carried out in PyMOL. PyMOL is an open-source molecular visualisation program for viewing and analysing 3D structures of proteins and small molecules from coordinate files such as .pdb. It is used here to measure the closeness of ligand-protein contacts as inter-atomic distances. Residue-resolved contacts for the four predicted complexes are summarized in Table 2. In this hypothetical example, the shortest contact for PE occurred between ligand atom C2 and Leu42-CD1 in MOCP chain A (3.9 Å). The shortest PET contact occurred between O2 and Arg54-NH1 (3.5 Å), while

PP showed a contact between C3 and Val46-CG1 (4.1 Å). For PS, the shortest contact occurred between aromatic atom C6 and Phe68-CZ (4.2 Å). These distances describe geometric proximity within the predicted poses and do not, by themselves, establish a particular bonding or energetic mechanism.

Docked Complex	Ligand atom	MOCP chain	MOCP residue and number	MOCP atom	Distance (Å)
MOCP–PE	C2	A	Leu42	CD1	3.9
MOCP–PET	O2	A	Arg54	NH1	3.5
MOCP–PP	C3	A	Val46	CG1	4.1
MOCP–PS	O6	A	Phe68	CZ	4.2

Table 2. Interaction analysis distances in ångströms (Å) calculated using PyMOL that show residue-resolved contacts between MOCP and the four representative polymer fragments.

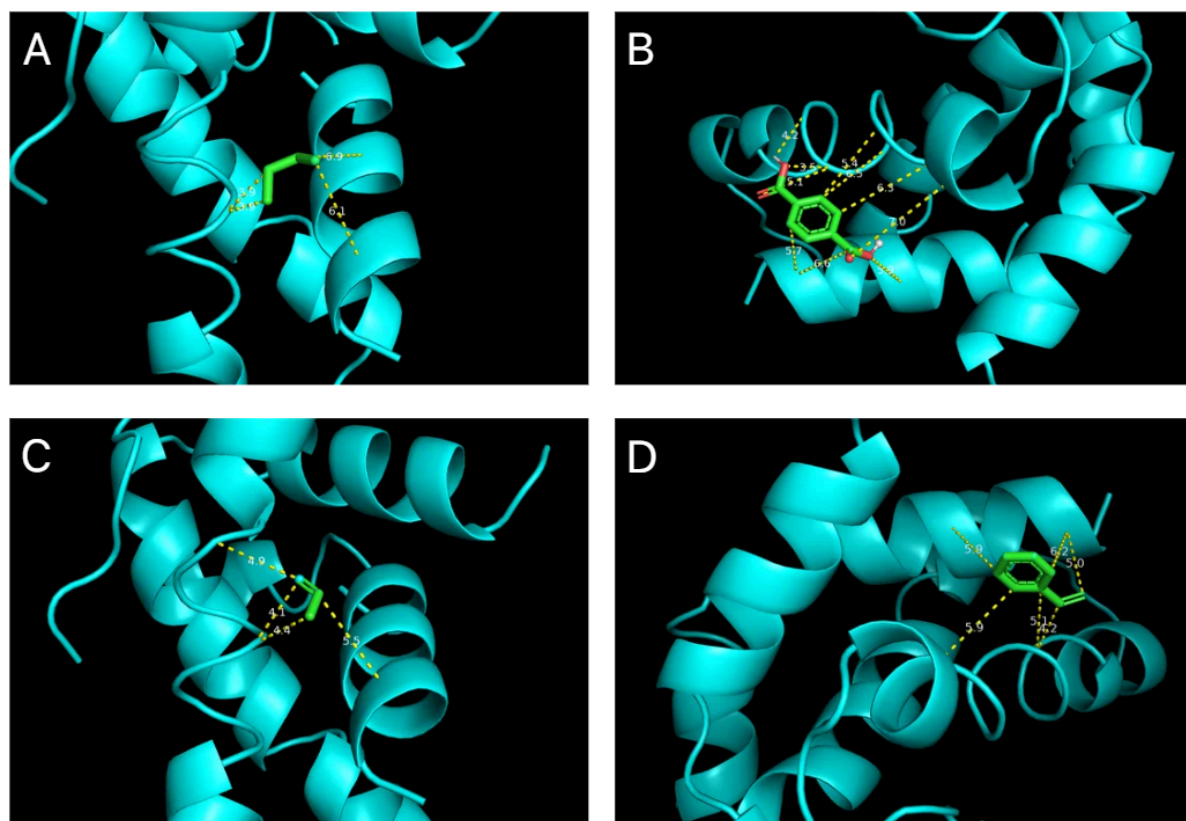


Figure 2. Distances between docked microplastic polymer atoms and nearby atoms on residues of MOCP. Representations of MOCP generated in PyMOL showing the docked binding poses of representative monomers corresponding to (A) polyethylene (PE), (B) polyethylene terephthalate (PET), (C)

polypropylene (PP), and (D) polystyrene (PS). The representative monomers are displayed as green stick models within the predicted binding pocket. Yellow dashed lines are distances measured using the PyMOL Measure tool between atoms of the docked representative monomers and the nearest atoms of MOCP. Distances were labelled in ångströms (Å).

DISCUSSION

Conventional coagulation removes only 1.8-54% of MPs[5], while moringa is a demonstrated experimental coagulant. However, the molecular basis of Moringa-microplastic binding had not been characterised. Through literature screening, MOCP was identified as the coagulant protein and its PI was found to be 10.5. The four selected representative polymer fragments to be tested were identified as PE, PET, PP, PS. Blind docking using AutoDock Vina was used, as MOCP has no known plastic-binding pocket, resulting in a search of the entire surface to identify the microplastic with the most negative binding affinity. Next, PyMOL inter-atomic distance was used to characterise the nature of the contact. From the blind docking, the following binding affinities were found for each mp monomer at its best PET (−5.029) > PS (−4.613) >> PE (−2.623) > PP (−2.163).

PET and PS produced more negative predicted docking scores than PE and PP under the applied protocol. One possible explanation is that PET and PS contain aromatic rings, which are absent from the PE and PP fragments. Aromatic groups may support stronger dispersion interactions and, when appropriately positioned relative to aromatic or cationic amino-acid side chains, could permit π – π or cation– π interactions. The ester carbonyl groups of PET could also contribute dipolar contacts. However, the present analysis did not establish the identities and orientations of all interacting residues or quantify individual energetic components. Therefore, these interactions represent plausible explanations for the observed score differences rather than confirmed binding mechanisms. Similarly, the shortest measured contacts of 3.5–4.2 Å indicate close noncovalent proximity in the predicted poses, but distance alone cannot distinguish among dispersion, electrostatic, hydrophobic, π -related, or other energetic contributions. Residue-level interaction mapping, molecular-dynamics simulations, and energy-decomposition analyses would be required to evaluate these hypotheses. Docking supplies a molecular explanation for the removal efficiencies found experimentally through various studies cited. It also predicts that binding is polymer-dependent, a prediction that studies using a single polymer cannot test. MOCP is cationic at environmental pH below 10-10.5. Weathered microplastics have negative zeta potentials, which bring them into proximity and allow the shorter-range LDFs to stabilise the contact and coagulate the microplastics.

In Patil et al. 2013 study on homology modelling and protein-protein docking of M02.1, they identified residues driving dimerization and found coagulation comparable for monomer and dimer. This study, however, docked the protein to itself rather than to a pollutant. Therefore, there was no ligand-level characterisation of the protein-plastic interface. This study docks MP representative monomers directly to MOCP. In 2026, Batista et al. performed an experimental study where they used MOS-SE at 30 mg L^{−1}

pH 6.0 and found that it was able to remove 98.5% of aged PVC. They identified zeta potential and charge neutralisation as the primary mechanisms. This study was purely macroscopic and inferred charge neutralisation from bulk zeta potential. The present docking and distance measurements suggest that short-range noncovalent contacts could accompany electrostatic attraction; however, they do not quantify the relative contributions of electrostatic, dispersion, hydrophobic, or other forces. Panigrahi et al. (2024) also performed a study on MOCP coagulation of pristine and UV-weathered MPs. However, this study was also experimental and did not provide a microscopic rationale regarding why weathering changes MOCP performance. The molecular docking study of polyurethane resin against microbial cutinase enzymes serves as precedent for treating a plastic monomer as a ligand against a protein receptor. [10] Additionally, a combined GCMC/DFT/QSAR study of pollutant adsorption on nanoplastics indicated that π - π interactions, hydrophobic forces, dispersion, electrostatic interactions, and hydrogen bonding all act together on nano/microplastic monomers. These previous findings provide a basis for considering several possible interaction types, but they do not confirm that any particular mechanism produced the score differences observed in the present study. [11]

A key limitation of this study was that it docked microplastic monomers, while real microplastics are high-molecular-weight polymers that present long and weathered surfaces. Therefore, the affinities found by this study describe intrinsic chemical binding affinity of a repeat unit instead of the total interaction energy of the microplastic. This would scale with contact surface area and therefore be far larger. Docking reliably produces binding geometry (within 2 Å of experimental RMSD structures). Additionally, Vina's function is based on drug-like ligands binding to enzyme pockets rather than polymer fragments on a protein surface. This also results in an underestimation of the absolute value of the binding affinity, as it would be greater for the larger weathered polymers. Next, molecular docking inherently treats the protein as rigid and approximates the effects of water rather than computing water molecules individually. Since all the microplastics are hydrophobic, their coagulation increases entropy and is energetically favourable. Docking estimates this interaction rather than computing its exact effect. Therefore, due to this, the binding affinity is underestimated, especially for PE and PP, as they have almost no polar groups. As touched upon previously, docking also does not model the oxidation of functional groups. This oxidation generates the surface charge and promotes coagulation, resulting in another underestimation of the absolute value of the binding affinity. Further extensions of this study could include a molecular dynamics study. Metrics like MM-PBSA/MM-GBSA would help us calculate a more accurate binding affinity that would address the lack of explicitly calculated effects of water and the assumption of a rigid protein. It is also more accurate than molecular docking. Another extension would be to use repeating weathered monomers for docking instead of the single unweathered unit. This would help model an actual polymer as well as better account for the effects of weathering. Per-residue contact mapping could be performed to identify which amino acids from the protein the microplastic binds to. Finally, an experimental study could be performed to verify theoretical binding affinities. This would address mixing, particle collisions, pH, salts, polymer size, and weathering.

CONCLUSION

In conclusion, this study assesses the capability of moringa components as a microplastics removal agent from the water to address the problem of microplastics water pollution. This exploratory computational study predicted energetically favorable docking poses between MOCP and the four tested representative polymer fragments, with PET and PS producing more negative Vina scores than PE and PP under the applied protocol. These results do not establish the binding or removal effectiveness of MOCP against intact environmental microplastic particles. Instead, they identify MOCP as a promising candidate natural coagulant for future experimental validation using particles that vary in polymer type, size, weathering state, additive content, surface charge, and biofilm coverage. Such validation is necessary before conclusions can be drawn regarding practical microplastic-removal performance. It utilizes a bioinformatics-driven approach identifying defatted moringa as effective organic entity. MOCP was predicted to interact with all major MP types, supporting its use as a broad-spectrum natural coagulant. It can be tested experimentally to validate the assessment of this bioinformatics analysis.

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