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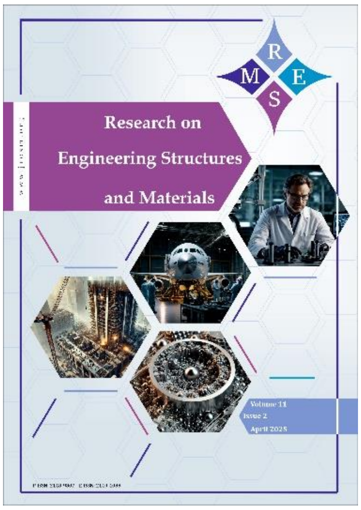

Treatability study of acclimatized industrial pharmaceutical wastewater using leaf-based natural coagulants with bioaugmentation

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Research Article

Treatability study of acclimatized industrial pharmaceutical wastewater using leaf-based natural coagulants with bioaugmentation

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Abstract

The recalcitrant nature of pharmaceutical wastewater, which contains high concentrations of active pharmaceutical ingredients (APIs), poses a significant challenge for conventional treatment systems. The present study examines a novel hybrid treatment process based on biological acclimatization after the use of secondary sewage and coagulation after the application of natural plant-based coagulants. Initially, a biological batch process was optimized in a period of 110 days using different ratios of wastewater to sewage, with the 1:3 ratio showing the best stability of degradation. Subsequently, a mixture of acclimatized and raw wastewater was treated using Azadirachta indica (Neem) and Moringa oleifera (Moringa) leaf powder. Results indicated that M. oleifera significantly performed better as compared to A. indica that is 94% reduction in Chemical Oxygen Demand (COD) as against 70% by Neem, at an optimal hydraulic retention time of 8 hours and a size of 150 µm. Mechanistic understandings based upon FTIR and NMR spectroscopy confirmed drug compound “partial degradation” of complicated aliphatic and aromatic molecules to less complicated by-products. Furthermore, FE-SEM and EDX analysis showed different morphological alterations and changes in the element composition, confirming the adsorption and bridging mechanisms of the natural coagulants. A techno-economic analysis indicates that this hybrid protocol is cost-effective with respect to the conventional chemical methods, with the sludge produced showing promise for valorization to serve as bio-fertilizer, this implies a circular economy approach to sustainable wastewater management.

1. Introduction

The Water scarcity and water quality deterioration have become global issues of crucial importance due to fast industrialization and the growth of the population [1]. Among effluents of industries, pharmaceutical wastewater is of special danger owing to the presence of Active Pharmaceutical Ingredients (APIs). These compounds frequently are recalcitrant, bio-accumulative and toxic (often even at trace concentrations of nano-grams to milligrams per liter). Conventional Wastewater Treatment Plants (WWTPs) are often inadequately equipped to break down these complex organic structures and consequently, "contaminants of emerging concern" are discharged into surface and ground water systems [2].

Current treatment methods have their own unique shortcomings. While biologically based treatment processes are economically feasible and environmentally friendly, they tend to fail when applied to pharmaceutical effluents. The toxicity and complicated chemical structure of APIs are very high, which can inhibit the action of microorganisms, resulting in the failure of the system or

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incomplete degradation [3]. On the other hand, physicochemical methods such as coagulation-flocculation by inorganic salts (e. g., Aluminum sulfate or Ferric Chloride) are effective and rapid. But they exhibit serious environmental disadvantages such as high operational cost, change of effluent pH, and production of huge amounts of toxic non-biodegradable sludge such that their disposal is complicated [4]. Consequently, there is a dire need for a hybrid therapy approach that employs an operation cost that is low for biological systems while maintaining the efficiency of coagulation with a lack of secondary pollution from the synthetic chemical systems.

This study proposes a synergistic treatment strategy, which is Biological Acclimatization - followed by Natural Coagulation treatment. Acclimatization is the gradual adaptation of microbial communities to rising concentrations of pharmaceutical pollutants that will improve their ability to break down complex phenolic and aromatic compounds, which are toxic to non-acclimatized sludge [3]. To further polish the effluent and to remove the remaining suspended solids, plant-based natural coagulants are used in this study: Moringa oleifera and Azadirachta indica (Neem). Unlike synthetic polymers, these bio coagulants are biodegradable, non-toxic and sludge can be potentially valorized as bio-fertilizer agreeing with circular economy principles [5]. The treatment technologies, which are technically feasible, economically viable and environmentally sustainable [23].

While earlier studies have focused on either biological treatment or natural coagulation [6,7] separately, there are limited studies on the sequential coupling of acclimatized sludge treatment, *Azadirachta indica*, and *Moringa oleifera* for pharmaceutical wastewater. Furthermore, most existing types of literature are limited to the use of conventional parameters (COD, BOD) to measure the efficiency [4]. This study has an outstanding novelty in terms of the advanced spectroscopic techniques used, namely Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR), to give molecular evidence of the degradation. By examining structural changes in the functional groups and atomic environments, this study provides a mechanistic understanding of the structure breakdown of these hybrid processes over complex pharmaceutical matrices.

2. Objectives

- To optimize the biological acclimatization of raw pharmaceutical wastewater by different ratios of secondary sewage.
- To assess the effectiveness of natural coagulants (Moringa and Neem leaf powder) on the acclimatized effluent at various Hydraulic Retention Times (HRT).
- To validate the degradation mechanisms and structural morphological changes by using FTIR, NMR, FE-SEM and EDX analysis.
- To evaluate the economics and sludge valorization potential of this green protocol of treatment.

3. Materials and Methods

3.1. Waste Water Sampling and Characteristics

Raw pharmaceutical wastewater (representative sample) was collected from a pharmaceutical formulation industrial setup from the equalization tank located at Villianur, Puducherry. The industry is mainly producing antibiotics, analgesics (e.g., Aceclofenac, Paracetamol, Diclofenac, Albendazole, Aspirin etc.) and so on with a complex (organic) matrix. At the same time, secondary activated sewage was collected from the oxidation pond of a Common Effluent Treatment Plant (CETP) in Karuvadikuppam, located in Puducherry.

Upon collection, samples were sealed in high-density polyethylene (HDPE) storage containers at 4 °C to maintain their integrity without pre-degradation of samples. The initial physicochemical features for the raw wastewater and the secondary sewage (Table 1) were studied immediately upon their arrival at the lab, according to the norms prescribed by the Bureau of Indian Standards (IS 3025) and the APHA (American Public Health Association) protocols.

3.2. Preparation of Plant-Based Coagulants

Moringa oleifera is found all over the world, but it comes from India, Arabia, and the East Indies. It is a tree that loses its leaves in the winter and is common in tropical and subtropical areas. People have long valued it for its health benefits and use in cooking. In the last few years, it has also been found to have some medicinal uses. Researchers have found and grouped different organic compounds in the leaves and seeds of M. oleifera. These groups include organic acids, amino acids, carbohydrates, and other bioactive compounds. Glutamic acid is one of the most important parts of both leaves and seeds, and it is thought to be a key active coagulating agent. Because of these qualities, M. oleifera has become very popular as a natural coagulant for cleaning up wastewater. [18-20]

Azadirachta indica, commonly known as neem or Indian lilac, is widely distributed across tropical, subtropical, and moderately temperate regions. It is well recognized for its medicinal properties, particularly in the management of diabetes. Neem leaves are rich in bioactive compounds such as tannins, flavonoids, and fatty acids, which exhibit potential coagulation properties. Among these, tannins play a significant role in enhancing coagulation mechanisms. Therefore, tannins present in neem leaves can be considered as one of the key active coagulating agents, making *Azadirachta indica* a promising natural coagulant for wastewater treatment applications. [21]

Mature leaves of *Azadirachta indica* (Neem) and Moringa oleifera (Moringa) plants were collected from the farm and from local sources. To avoid the thermal deterioration of bioactive coagulating proteins, leaves were washed with distilled water and shade-dried at room temperature for 96 hours (Four days). The dried leaves were pulverized in a high-speed mechanical grinder and sieved to give a uniform particle size of 150 microns.

- Stock Solution Preparation: A stock solution of the same was prepared by dissolving 3.0 g of the respective leaf powder in 100 mL of distilled water. From the mother solution 3ml of optimum dosage is added to the 1000 ml of sample taken in the beaker. The optimum dosage is determined by using the turbidity test. The suspension was agitated for 30 min using a magnetic stirrer to solubilize the active coagulation.

3.3. Biological Acclimatization Strategy (The Batch Mode)

A stepwise biological acclimatization procedure was used to activate the activated sewage microorganisms to the recalcitrant pharmaceutical effluent. The experiments were carried out using laboratory-scale aerobic bioreactors in batch mode with a capacity of 10 liters.

- Experimental Design: Six different volumetric ratios of Raw Pharmaceutical Wastewater (RPW) to Secondary Sewage (SS) were studied (1:1, 1:2, 1:3, 1.5:2.5, 2:1, 3:1).
- Operational parameters aerators were used to provide continuous aeration to maintain a Dissolved Oxygen (DO) level (4-7mg/ L). The reactors were run at ambient room temperature for a duration of 110 days.
- Maintenance: To maintain the microbial viability, fresh secondary sewage was added at 15 days interval. Samples were withdrawn for monitoring the changes in pH, Turbidity, Total Dissolved solids, COD, and BOD at every 5-day intervals.
- Steady State Determination: The optimal ratio was determined as per the "Steady State" condition i.e., the condition where the COD varied within ± 5% over three successive sampling intervals.
- The above research work is carried out in the ambient conditions prevailing in the laboratory of the study area, that is warm and humid climatic condition, where the relative humidity will reach saturation levels in winter (NOV, DEC and JAN) and the temperature ranges from 25-35°C

3.4. Hybrid Treatment Coagulation-Flocculation Studies

In order to simulate a "shock loading" situation and test the robustness of the natural coagulants, the last phase of experiments involved the use of a mixture of 50% Acclimatized Effluent (optimized ratio 1:3) and 50% Raw Pharmaceutical Wastewater. Standard Jar Test experiments were carried out for the optimization of the coagulation process. The protocol was based on the following:

- Rapid Mixing: 120 rpm for 2 minutes (for destabilizing of colloids).
- Slow mixing: 45 rpm for 15 minutes (to encourage growth of a floc).
- Sedimentation: Samples were allowed to settle for different Hydraulic Retention Times (HRT) of 2, 4, 6 and 8 hours.

Supernatant was extracted from a depth of 2 cm below the surface for analysis of residual pH, Turbidity, Alkalinity, TDS, COD, and Phosphate.

3.5. Analytical and Instrumentation Technique

For testing the validity of the degradation mechanisms and structural changes, advanced analyses (spectroscopic and microscopic analysis) were done:

- Physicochemical Analysis: pH, Turbidity, TDS, Alkalinity, COD, and Phosphate (IS 3025 Part 58) were monitored.
- Surface Morphology (FE-SEM/EDX): The surface texture as well as its elemental composition of the sludge before and after treatment was analyzed with the Field Emission Scanning Electron Microscope coupled with an Energy Dispersive X-ray spectroscopy. Samples were sputter-coated with a thin layer of gold prior to imaging to effectively increase surface conductivity and prevent electron charging.
- Molecular Characterization: (FTIR & NMR):
  - FTIR: Transformations of functional groups were done by Fourier Transform Infrared Spectrometer (TA instruments Q600 SDT) in a range of (4000-400 cm^{-1}) using the KBr pellet technique.
  - NMR: Nuclear Magnetic Resonance (1 H-NMR) spectra were recorded on a (400 MHz) spectrometer (Bruker Avance II) (0 to 220 ppm) to identify the degradation of certain of the organic protons. Samples were dissolved in deuterated water (D2O).

3.6. Statistical Analysis

All the experiments were performed in triplicate to ensure the reliability, reproducibility of the results. The physicochemical characteristics of raw pharmaceutical wastewater, secondary sewage, acclimatized wastewater, and treated effluents were analyzed for each replicate separately, and the results are presented as mean ± standard deviation (SD). Parameters such as pH, turbidity, total dissolved solids (TDS), total alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and phosphate at various hydraulic retention times (2, 4, 6, and 8 h) were assessed during the acclimatization period and after the treatment of natural coagulants.

Statistical analysis was performed using SPSS version 2.5. One-way analysis of variance (ANOVA) was applied to assess the significance of difference between treatment groups (Neem and Moringa coagulants) and across different retention times. Where appropriate, a post hoc multiple comparison test was used to identify significant differences between individual groups. A p-value of < 0.05 was considered statistically significant.

Removal efficiencies were determined from the comparison of initial and final concentrations, and values of observed standard deviations were used to measure experimental variability and consistency. The fact that low variation of SD values is observed during replicate measurements indicates good analytical precision and stable experimental conditions during the acclimatization and coagulation-flocculation processes, and thus the robustness and reproducibility of the experimental findings.

4. Results and Discussion

4.1 Initial Characteristics of Raw Pharmaceutical Wastewater and Secondary Sewage

Table 1 summarizes the initial physicochemical properties of the raw pharmaceutical wastewater (RPW) and secondary sewage (SS) used in the current study. The RPW had a significant pollution load with its very high turbidity, total dissolved solids (TDS), alkalinity, and chemical oxygen demand (COD) and phosphate levels, revealing the complex and chemically intensive nature of pharmaceutical effluent. BOD/COD ratio (0.29) of RPW is very low, less than 0.3, which indicates that the organic compounds are poorly biodegradable and that the effluent comprises recalcitrant and inhibitory organic substances, which are known to negatively influence the normal operation of the biological treatment process. These properties of the pharmaceutical wastewater are not new to the previous literature [8, 9].

Table 1: Physicochemical characteristics of Raw Pharmaceutical Wastewater (RPW) and Secondary Sewage (SS) used in the study

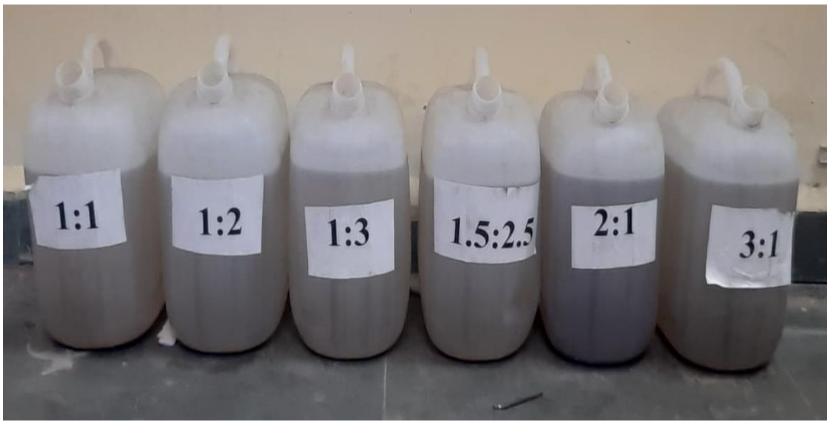
Parameter	RPW (Mean ± SD)	SS (Mean ± SD)	Test Method	F-value	p-value	Post Hoc (Tukey)
pH	6.46 ± 0.2	6.96 ± 0.1	IS 3025 (Part 11)	18.75	0.012*	RPW ≠ SS
Turbidity (NTU)	490 ± 16	58.8 ± 5.4	IS 3025 (Part 10)	1520.3	<0.001**	RPW ≠ SS
TDS (mg/L)	770 ± 45	1578 ± 20	IS 3025 (Part 16)	689.4	<0.001**	RPW ≠ SS
Total Alkalinity (as $CaCO_3$)(mg/L)	250 ± 12	365 ± 15	IS 3025 (Part 23)	92.6	<0.001**	RPW ≠ SS
COD (mg/L)	4979 ± 30	202 ± 12	IS 3025 (Part 58)	8420.5	<0.001**	RPW ≠ SS
BOD_5 (mg/L)	1463 ± 9	97 ± 17	IS 3025 (Part 44)	5210.7	<0.001**	RPW ≠ SS
BOD/COD Ratio	0.29	0.4	Calculation	–	–	–
TOC (mg/L)	1778	74	IS 8245	–	–	–
Phosphates (PO_4^{3-}–P) (mg/L)	14.8 ± 1.5	4.4 ± 0.5	IS 3025 (Part 31)	110.2	<0.001**	RPW ≠ SS

Conversely, the secondary sewage had relatively lower COD and turbidity with relatively high BOD concentration and therefore a high BOD/COD ratio (0.4). This is an indicator of high proportion of easy biodegradable organic materials and a large number of active microorganisms in the secondary sewage. The positive characteristics of biodegradability and balanced nutrient content render secondary sewage a microbial inoculum and co-substrate in promoting microbial acclimatization and adaptation when used together with pharmaceutical wastewater. Joss et al. [10]. TOC of the raw wastewater indicates a high concentration of organic pollutants; however, it does not suggest that the treatment system is subjected to a shock load, have also made similar observations on the use of municipal or secondary sewage as a biological seed to enhance the treatment efficiency of recalcitrant industrial effluents. Statistical analysis using one-way ANOVA demonstrated significant variation between RPW and SS across all physicochemical parameters ($p < 0.05$), with Tukey's post hoc test confirming significant pairwise differences.

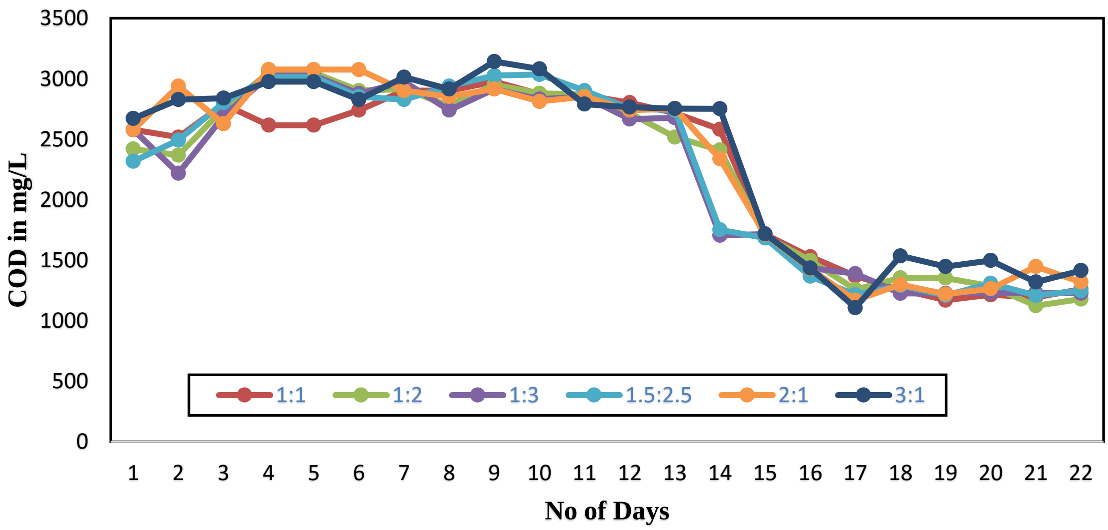
4.2 Biological Acclimatization and the Optimum Ratio Selection

Experimental investigations were carried out in a 10-liter capacity bioreactor (plastic can) for assessing the maximum biodegradability of the raw pharmaceutical wastewater in terms of the attainment of steady state measured in terms of COD values. For the above purpose, the pharmaceutical wastewater and secondary sewage were mixed in various ratios, namely 1:1, 2:1, 3:1,1.5:2.5 ,2:1 and 3:1, respectively. The above ratios were chosen based on the previous reported studies on pharmaceutical wastewater [24]. The COD stabilization profiles obtained during the biological acclimatization of the pharmaceutical wastewater using secondary sewage sludge are presented in Fig. 1. The gradual decline and eventual stabilization of COD concentration are an indication of successful microbial adaptation to dealing with complex pharmaceutical compounds. Similar behavior of acclimatization has been documented in pharmaceutical and toxic industrial effluents with increasing exposure in a step-wise manner, microbial tolerance and metabolic efficiency improved. microbial tolerance and efficiency metabolism [8,10]. The fact that was observed a "steady state" COD value between the 95th to 110th day for the 1:3 ratio indicates that a sufficient microbial density and co-metabolic substrate(s) are necessary for effective biodegradation of refractory organics.

The kinetic study revealed that the initial rate of removal was high and then gradually decreased, finally reaching a plateau state, which indicates that the process has adsorbed on the active sites and no longer the pollutants. This result confirms the assumption that the process proceeded in typical adsorption/biodegradation kinetics where the rate was constant after reaching equilibrium.



(a)

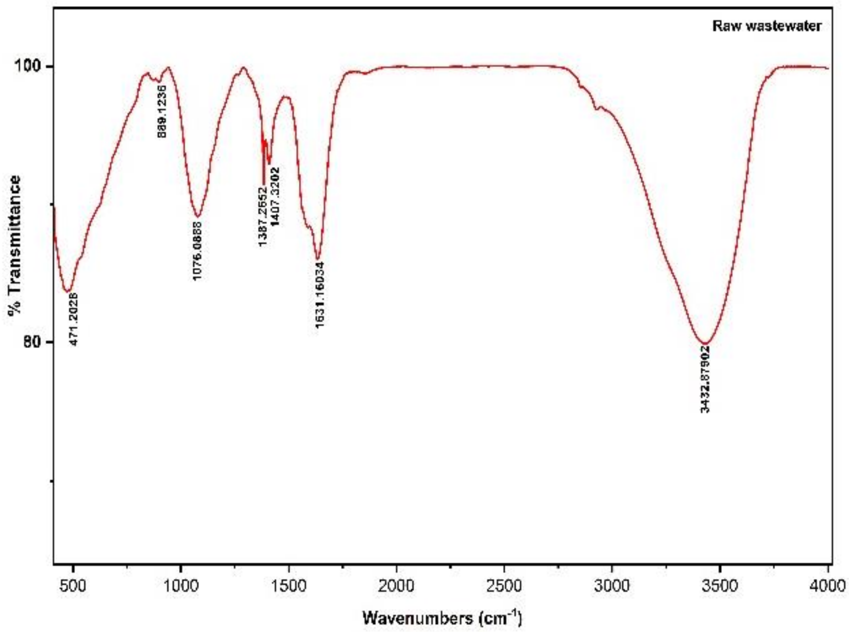


(b)

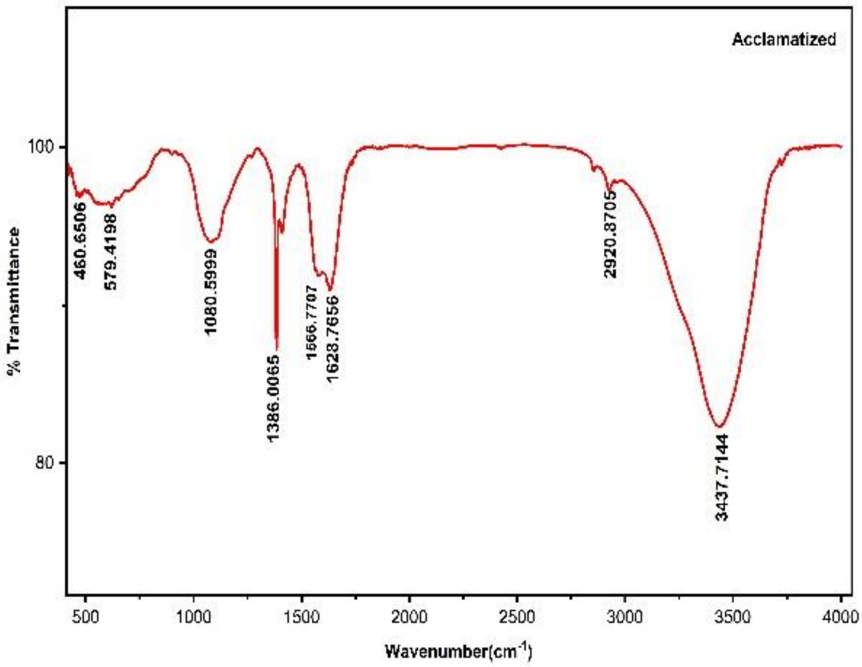
Fig. 1. (a) Photographic view of Bio reactors for different ratios, of pharmaceutical wastewater and Secondary Sewage from 0th to 110th day (acclimatization Process) (b)variation of COD for different ratios of pharmaceutical wastewater and Secondary Sewage from 0th to 110th day

4.3 Spectroscopic Indication of Biodegradation

The Fourier Transform Infrared (FTIR) spectra of the samples collected before and after the acclimatization process are shown in Fig. 2. The raw pharmaceutical wastewater showed prominent absorption bands corresponding to aliphatic C-H stretching mode, aromatic C=C stretching mode, carbonyl (C=O), and nitro (-NO2) functional groups due to the existence of diverse pharmaceutical compounds and complex organic structures. After the process of biological acclimatization, a significant decline in the intensity of aromatic C=C functional groups, carbon was observed, providing evidence for the partial biological degradation of organic constituents and destruction of complex molecular structures. Similar reductions in FTIR peak intensities in the course of the biological treatment of pharmaceutical wastewater have also been reported by Singh Rana et al. [9] and Wang et al. [11], verifying the susceptibility of certain organic moieties to microbial degradation.



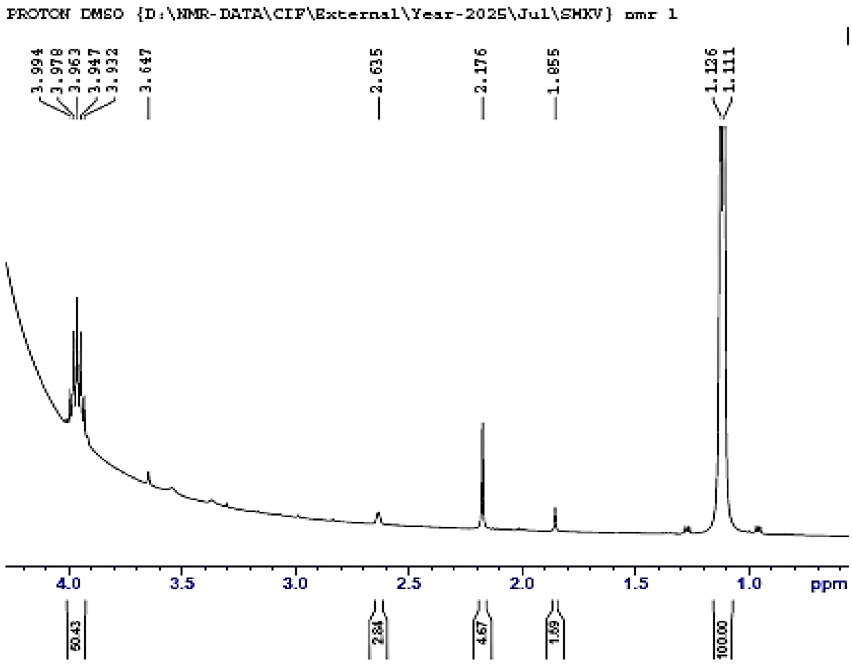
(a)



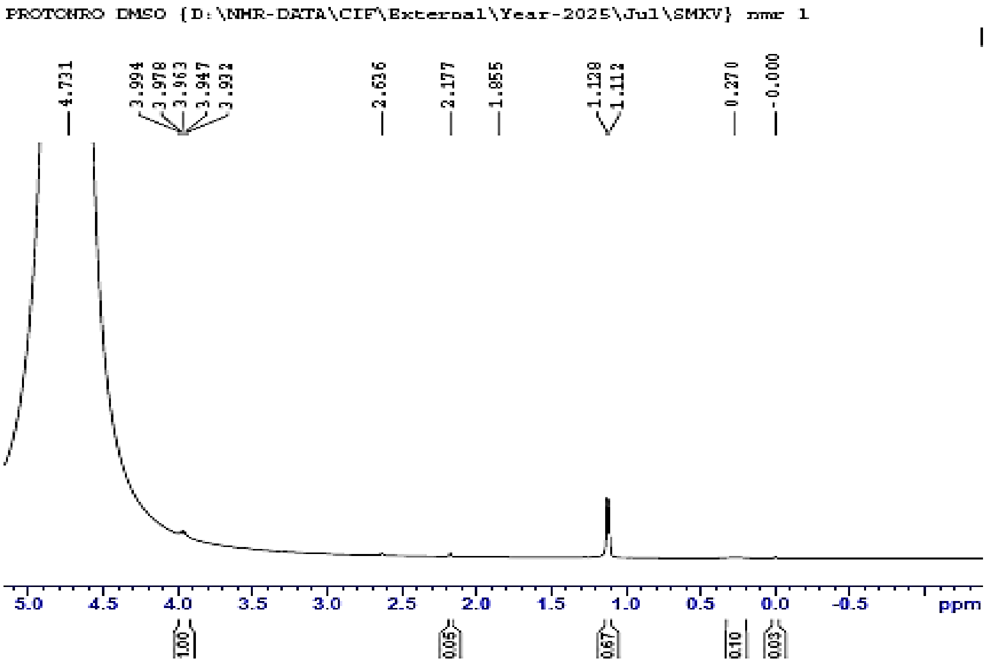
(b)

Fig. 2. Comparative FTIR spectroscopy showing (a) before and (b) after acclimatization of pharmaceutical wastewater using secondary sewage treatment

Despite the overall decrease in peak intensities, residual absorption bands corresponding to carbonyl and nitro functional groups remained after acclimatization, implying incomplete mineralization of recalcitrant pharmaceutical compounds. The robustness of these functional groups is typical for antibiotic-rich and chemically complex wastewaters wherein a high-level of resistance of aromatic as well as nitrogen-containing structures are found to complete biochemical degradation under aerobic conditions [12]. These observations suggest that biological acclimatization, where the organic load is reduced, is not the sole means of achieving complete degradation of all of the pharmaceutical residues.



(a)



(b)

Fig. 3. photographic view of NMR spectroscopy showing (a) before vs (b) after Acclimatization of pharmaceutical wastewater using secondary sewage treatment

The NMR spectra (Fig.3) further supported the results from the FTIR in terms of the information on molecular-level transformations that occur during acclimatization. The weakening and partial disappearance of proton signals corresponding to aliphatic and nitrogen-containing heterocyclic structures suggest the modification of the structure and the fragmentation of pharmaceutical molecules, and not destruction. Such partial degradation pathways during aerobic biological treatment have been reported extensively, and complex pharmaceutical compounds are degraded into simpler intermediates, which need further physicochemical treatment for complete removal [13]. The combination of FTIR and NMR analyses, therefore supports the efficiency of the acclimatization process while at the same time underscoring the need for further treatment approach in the form of a hybrid solution.

4.4 Effect of Natural Coagulants on Physicochemical Parameters

The variation of pH and turbidity with the hydraulic retention time (HRT), as shown in Fig. 4, evidences the effectiveness of the natural coagulants to promote the destabilization of suspended and colloidal particles from combined coagulative mechanisms of charge neutralization, adsorption and inter-particle bridging. A gradual pH stabilization towards near-neutral values was observed with increasing HRT, which suggests that no adverse acidic or alkaline conditions were established by the coagulation process, which is favorable for subsequent treatment and discharge. The resulting turbidity was significantly reduced with the increase of HRT due to improved settling efficiency under prolonged quiescent conditions. It has been previously stated that plant-based coagulants are more effective in removing turbidity, especially Moringa oleifera, because of the presence of water-soluble cationic proteins and natural polyelectrolytes that are effective in neutralization of negatively charged particles and flocculation [14, 15].

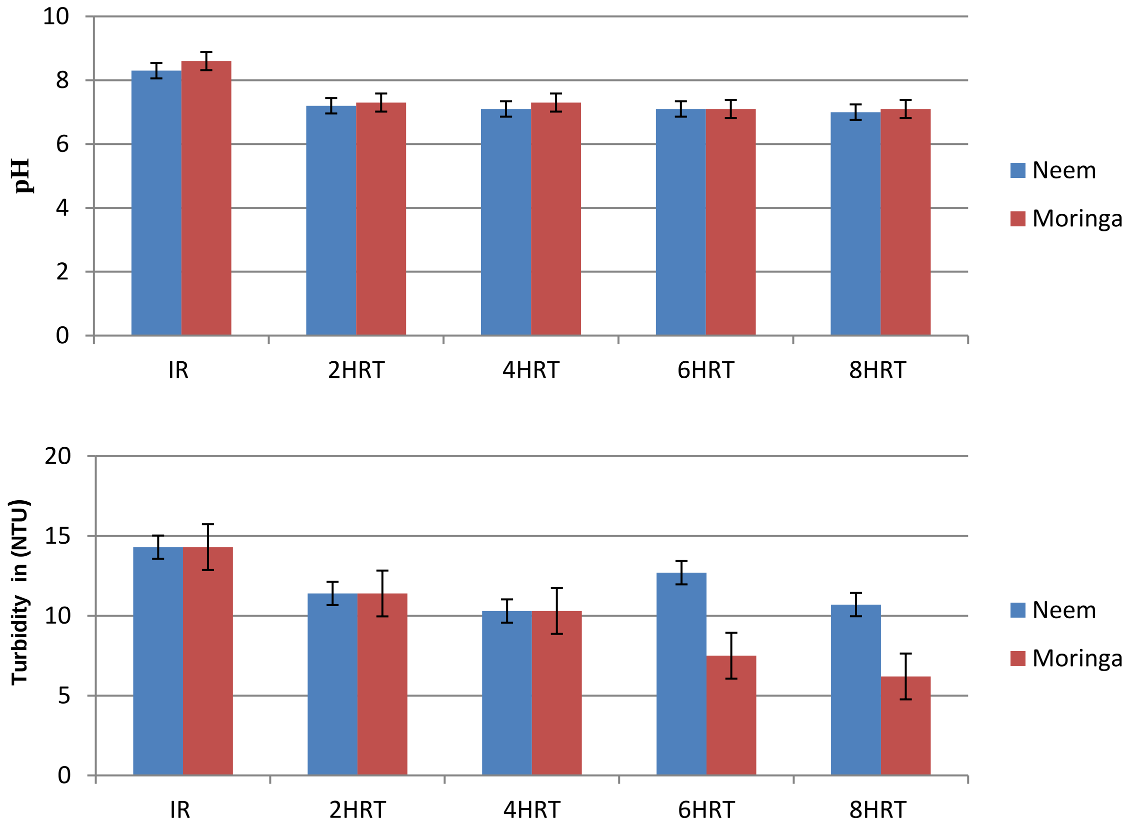


Fig. 4. Variation of pH and Turbidity in the Treatment of Acclimatization Pharmaceutical wastewater by using Natural coagulants at different HRT

** Note: IR – Initial Reading*

The decrease in total dissolved solids (TDS) and total alkalinity with HRT increase (Fig. 5) further supports the importance of adsorption and ion exchange mechanisms during the coagulation-flocculation process. The gradual diminishing of alkalinity appears to be indicative of the elimination of bicarbonate and carbonate ions by co-precipitation and absorption on surface complex sites of coagulants. Similar trends have been observed in natural coagulant-based wastewater treatment studies in which a longer retention time increased the interaction between pollutants and coagulants and overall treatment efficiency [16].

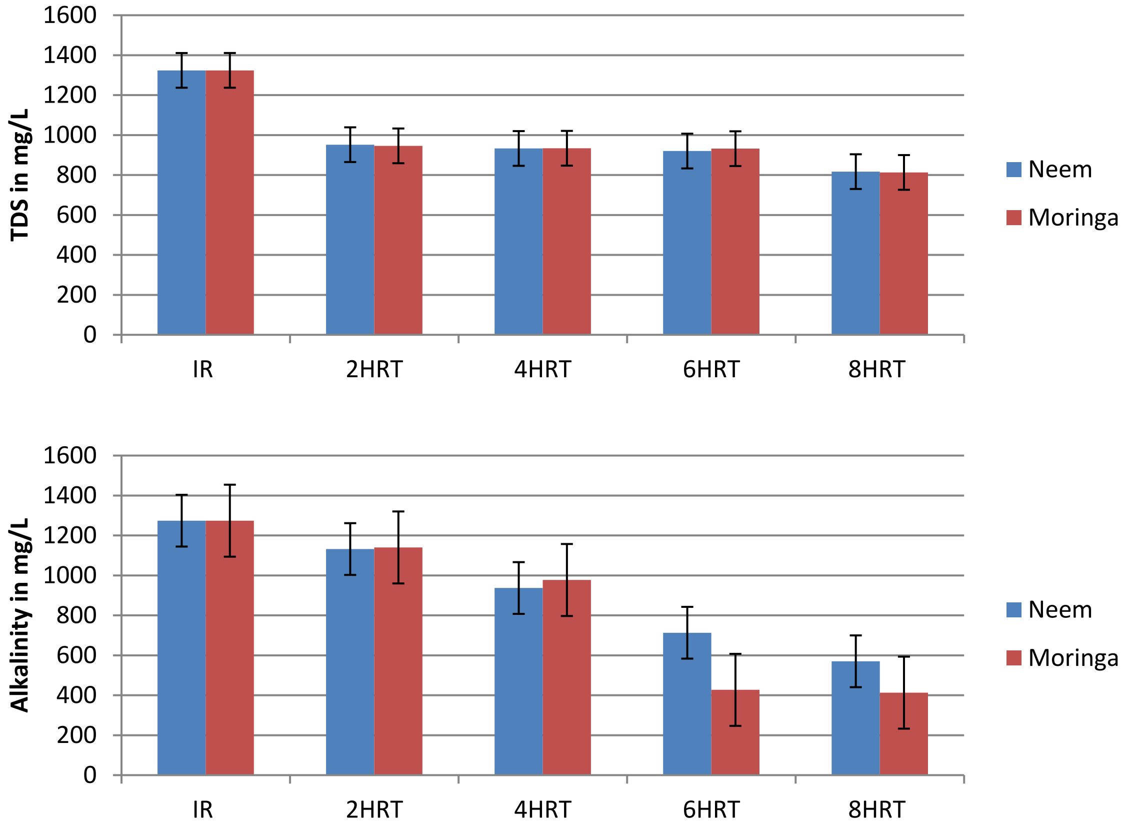
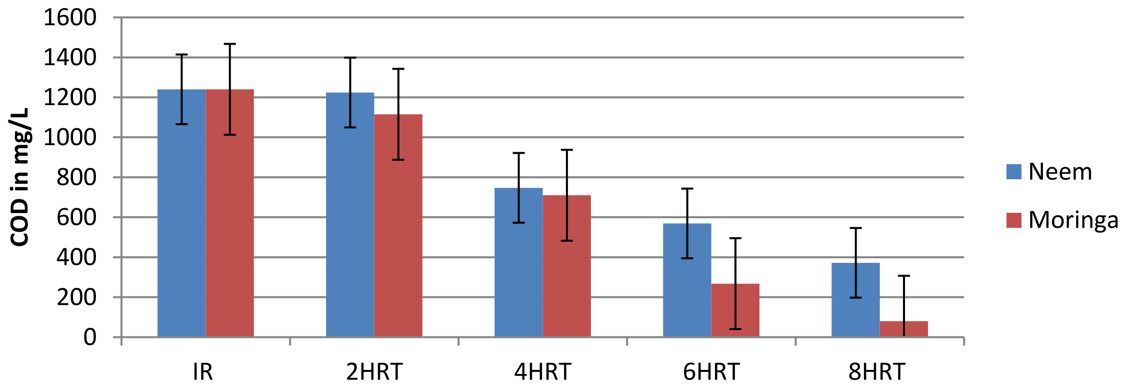


Fig. 5. Variation of TDS and Total Alkalinity in the Treatment of Acclimatization Pharmaceutical wastewater by using Natural coagulants at different HRT

4.5 COD Removal Mechanisms and Phosphate Removal Mechanisms

The significant decrease of chemical oxygen demand (COD) and phosphate concentrations, together with an increase of the hydraulic retention time (HRT), as it may be seen in Fig. 6, are characteristic of the synergy of biological acclimatization and natural coagulation-flocculation. The acclimatization procedure decreased the toxicity and the complexity of pharmaceutical wastewater, thereby improving the effectiveness of the ensuing physicochemical treatment. Among the coagulants tested, the Moringa leaf powder-based treatment resulted in better efficiency of COD removal of 94%, is shows the high reduction were compared with the removal percentage of hospital and pharmaceutical wastewater reported in [25].



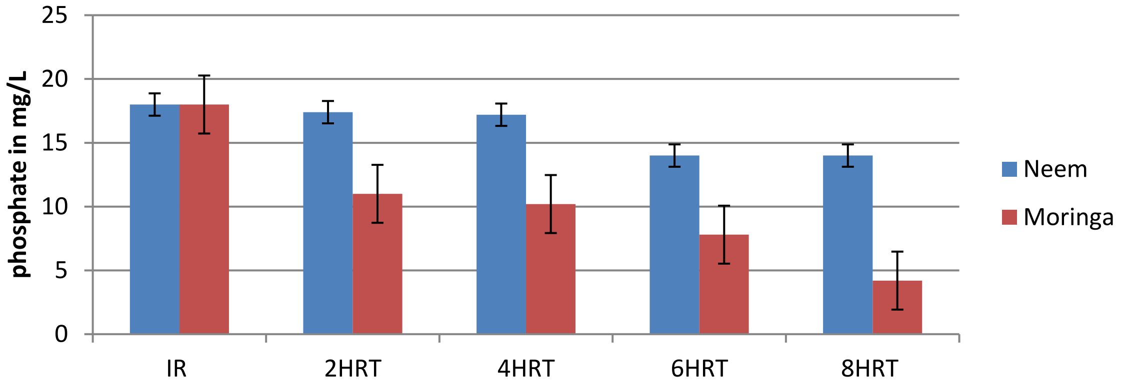


Fig. 6. Variation of COD and Phosphate in the Treatment of Acclimatization Pharmaceutical wastewater by using Natural coagulants at different HRT

Which might be due to the existence of bioactive water-soluble cationic proteins involved in charge neutralization, adsorption, and the interparticle bridging process of organic pollutants [14,16]. Prolonged HRT also allowed for better interactions between the pollutants and coagulant active sites, leading to improved floc formation and settling.

Phosphate removal shows a similar tendency and points out the removal of anionic phosphate species by adsorption to positively charged functional groups and co-precipitation in the formed flocs. The results of the overall percentage removal efficiencies of Phosphate and the removal efficiencies of COD are summarized in Fig. 7 which shows a progressive increase with respect to HRT, which proves the importance of adequate contact time for good treatment performance. These trends are consistent with previous research works on hybrid bio coagulation systems for high-strength industrial wastewater, which report enhanced removal efficiencies when biological pretreatment of wastewater is combined with coagulation-flocculation processes [8].

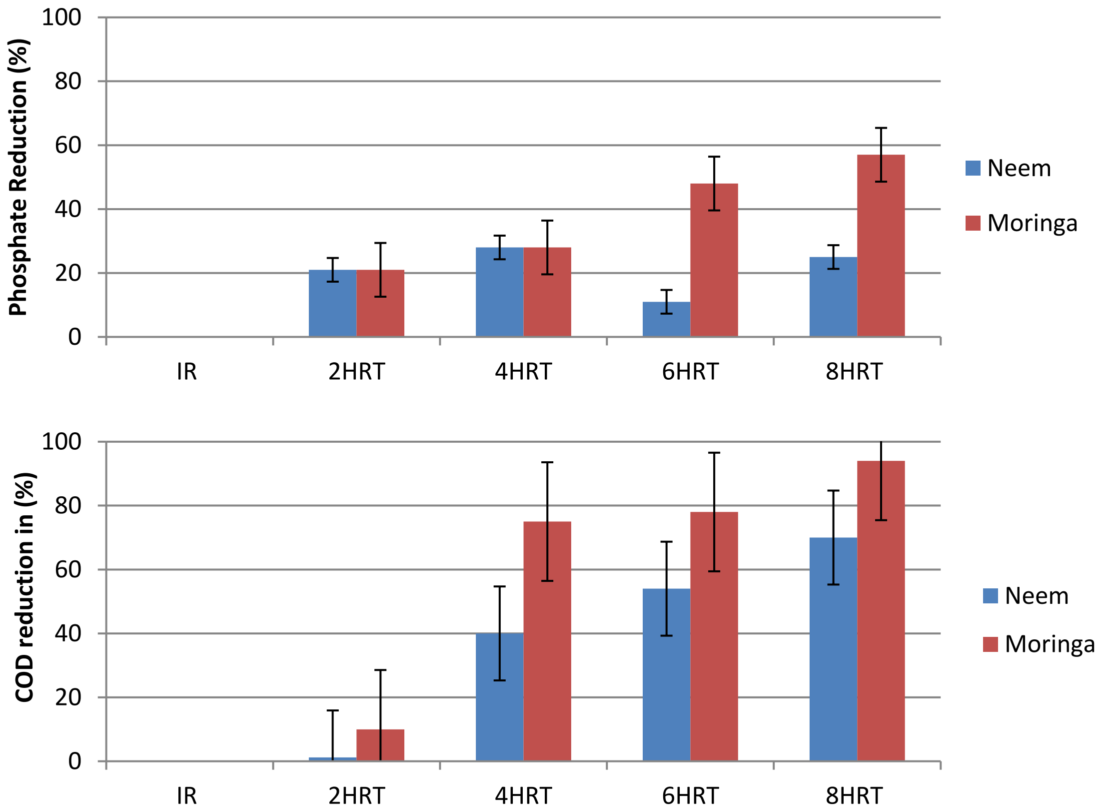
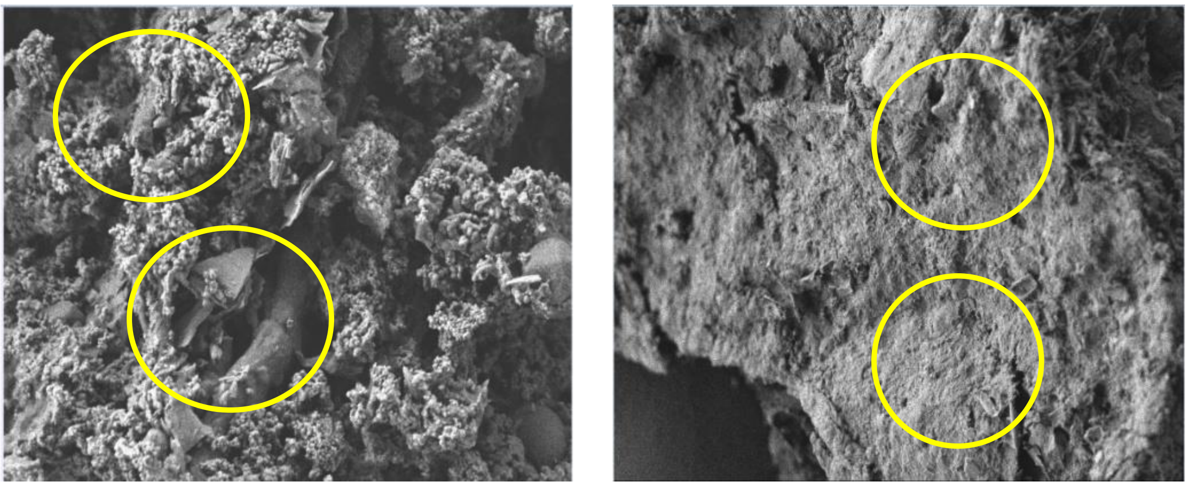


Fig. 7. Variation in % of reduction in turbidity and COD in the Treatment of Acclimatization of Pharmaceutical wastewater by using Natural coagulants at different HRT

4.6 SEM-EDX Indication of Pollutant Removal

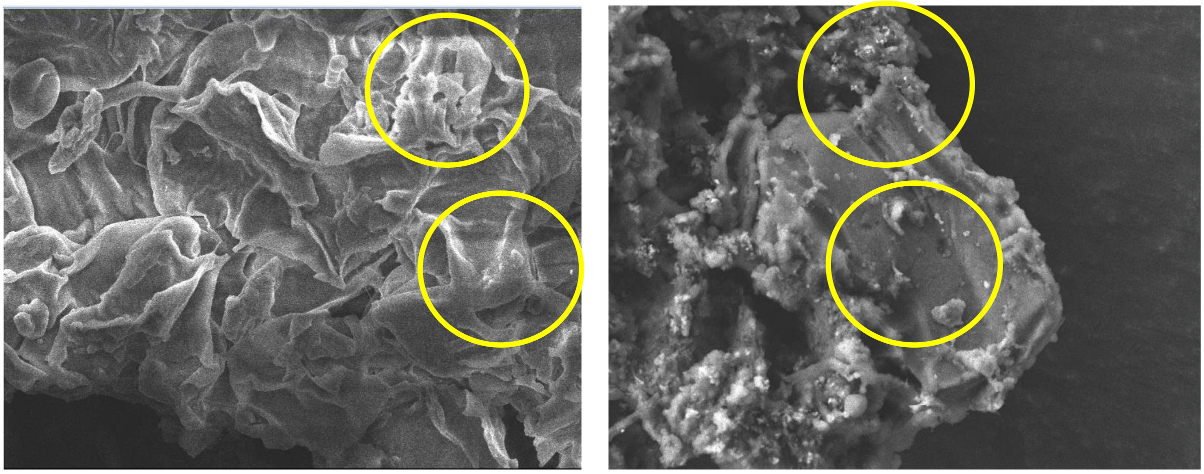
The FE-SEM micrographs of the raw and treated Sludge (Fig. 8 and Fig. 9) showed significant morphological changes after treatment in the form of an increase in surface roughness, pore formation, and the formation of irregular floc structures. These surface modifications imply that there is an effective amount of adsorption and entrapment of pollutants in the floc matrix, which will be beneficial for improved settling and removal efficiency. The porous and heterogeneous surface morphology seen after treatment indicates improved contact between the constituents of wastewater and active site present on both the acclimatized sludge and natural coagulant surface.



(a) (b)

Fig. 8. Photographic view of FE- SEM image for (a) Raw Pharmaceutical Wastewater and (b) acclimatized pharmaceutical wastewater

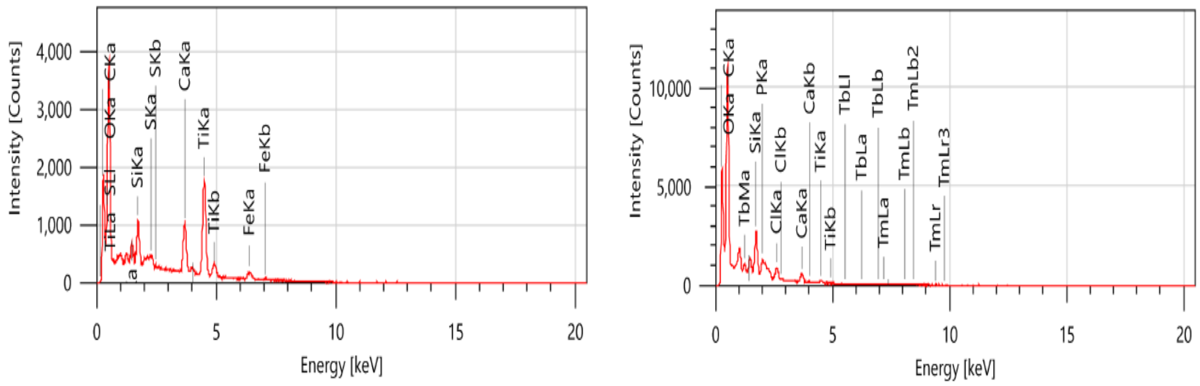
The FE-SEM image of the raw wastewater reveals the presence of both organic and inorganic particles, along with indications of undigested materials. Further, it also indicates the presence of 'dense micro-aggregates'. After acclimatization the FE-SEM image indicates a 'potential microbial activity', or degradation having taken place. Further, 'structural breakdown' and the presences of voids, indicates the presence of biological activity.



(a) (b)

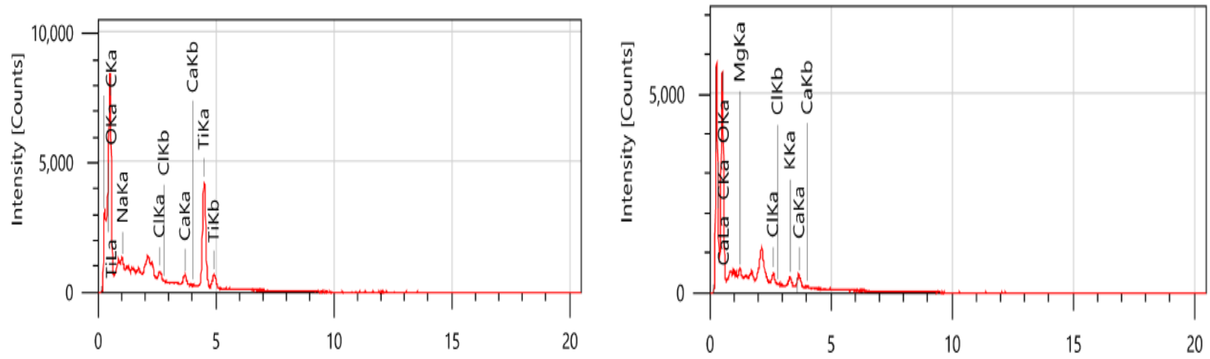
Fig. 9. Photographic view of FE - SEM image of (a) Moringa Leaves before Treatment (b) Moringa Leaves Treated with acclimatization pharmaceutical wastewater

Corresponding EDX spectra (Fig. 10, Fig. 11 and Fig. 12) (a) and (b) further supported the findings of the above observations, by showing significant changes in elemental composition after treatment. An increase in the inorganic elements with an increase in carbon and oxygen signals are indicators of successful sequestration of organic and inorganic contaminants to the sludge and coagulant matrices.



(a) (b)

Fig. 10. Photographic view of EDX image for (a) Raw Pharmaceutical Wastewater and (b) acclimatization pharmaceutical wastewater



(a) (b)

Fig 11: Photographic view of EDX image of (a) Moringa Leaves before Treatment and (b) Moringa Leaves Treated with Acclimatization Pharmaceutical wastewater

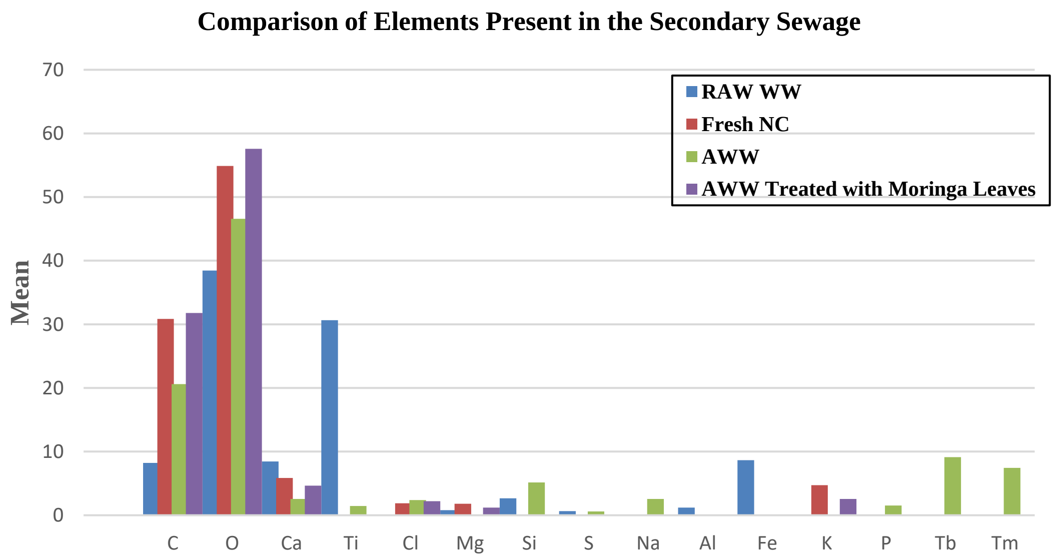


Fig. 12. Variation of Elements present in the sludge analyzed by EDX in the Treatment of Acclimatization Pharmaceutical wastewater by using Natural coagulants

Note: RAW WW- Raw wastewater, NC- Natural Coagulant, AWW- Acclimatized wastewater, Acclimatized wastewater treated with Moringa Leaves.

These elemental transitions provide empirical evidence for the specific roles of adsorption, surface complexation, and co-precipitation mechanisms in the overall removal of pollutants from the aqueous phase. Morphological transformations and changes in element redistribution have also been observed for natural coagulant-based wastewater treatment systems and confirm the use of plant-derived coagulants in enhancing pollutant removal due to coagulation [16, 17, 22].

5. Conclusion

Industrial pharmaceutical wastewater was acclimatized using secondary sewage sludge at varying ratios in a 10 L bioreactor over a period of 95–110 days to enhance the breakdown of complex compounds. Among the tested conditions, the 1:3 ratio (raw pharmaceutical wastewater to secondary sewage) showed optimal performance, with Chemical Oxygen Demand (COD) reaching a steady state by the 110th day. Following acclimatization, a 50:50 blend of pharmaceutical wastewater and acclimatized effluent was treated using various leaf-based natural coagulants. Among them, Moringa oleifera (150 µm particle size) demonstrated superior performance, achieving up to 94% COD reduction and 71% turbidity removal at an 8-hour settling time. FE-SEM analysis revealed significant changes in surface morphology before and after treatment, while EDX confirmed variations in elemental composition during acclimatization and treatment. Compared to neem (Azadirachta indica), which achieved only 70% COD and 30% turbidity removal, Moringa oleifera exhibited higher adsorption efficiency. FTIR analysis indicated effective degradation of aliphatic compounds and partial degradation of persistent pharmaceutical groups such as C=O, NO_2, and C–O. The use of natural coagulants provides a cost-effective and eco-friendly alternative to conventional chemical treatments, with the added potential of utilizing the resulting sludge as a bio-fertilizer, supporting sustainable wastewater management.

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