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Assessment of Nanoplastic Pollution and its Associated Oxidative Stress in Local Lake Sediments

Prithipa S[1], Jayalakshmi M[2]

[1]Under Graduate Student, [2]Assistant Professor, Department of Biochemistry, Valliammal College for Women, E-9 Annanagar East, Chennai 600102. Tamilnadu, India.

***Abstract*-- The present study demonstrated the possible presence of nanoplastic particles in sediment samples through suitable extraction and staining techniques. The use of hydrogen peroxide digestion, density separation, and Congo red staining proved to be effective methods for isolating and identifying plastic particles from the sediment matrix. The observation of stained particles suggested that sediments can act as reservoirs for plastic pollutants in the environment.**

Biochemical analysis of the sediment extract revealed the presence of proteins and antioxidant enzymes such as superoxide dismutase and catalase, indicating biological activity within the sediment. The detection of lipid peroxidation products further suggested that oxidative processes may occur in the sediment environment. These results indicate that microorganisms present in sediments may respond to environmental stress through antioxidant defense mechanisms.

I. Introduction

The exponential rise in global plastic production over the past seven decades represents one of the most significant material transformations in human history. Synthetic polymers have become deeply integrated into modern economies because of their durability, versatility, chemical stability, and low manufacturing cost (Andrady, 2011).

Materials such as polyethylene, polypropylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate dominate global markets and are widely applied in packaging, agriculture, medicine, construction, transportation, electronics, and textiles. Their light weight and resistance to corrosion make them highly advantageous compared to traditional materials such as glass, wood, and metals. However, the environmental persistence that makes plastics commercially valuable also contributes to their ecological burden. Unlike biodegradable natural polymers, most synthetic plastics resist microbial degradation and remain in the environment for extended periods, accumulating in terrestrial and aquatic ecosystems (Rillig, 2012; Horton et al., 2017).

As plastic waste accumulates in the environment, it undergoes progressive fragmentation through mechanical abrasion, ultraviolet radiation, thermal oxidation, hydrolysis, and limited microbial action (Andrady, 2011).

These degradation processes do not typically result in complete mineralization but instead generate progressively smaller particles. This fragmentation leads to the formation of microplastics and nanoplastics, two emerging categories of contaminants that have become central to environmental research (Thompson et al., 2004).

Microplastics are commonly defined as plastic particles less than 5 millimeters in diameter, while nanoplastics are generally described as particles smaller than 1 micrometer. Although the exact size threshold for nanoplastics remains under scientific discussion, the defining feature of this category is its nanoscale dimension, which confers unique physicochemical properties distinct from larger plastic fragments (Gigault et al., 2018).

Microplastics can be classified as either primary or secondary. Primary microplastics are intentionally produced in microscopic form for specific industrial or commercial applications. Examples include resin pellets used in polymer manufacturing, abrasive particles for industrial cleaning, and microbeads formerly used in cosmetic products. Secondary microplastics arise from the environmental breakdown of larger plastic items such as packaging films, agricultural mulching sheets, synthetic fibers, and discarded consumer goods (Cole et al., 2011). Continuous exposure to environmental stressors reduces these materials into progressively smaller fragments, eventually producing nanoscale particles.

Nanoplastics exhibit high surface area-to-volume ratios, enhanced mobility, altered surface charge characteristics, and increased reactivity compared to larger particles, making them particularly concerning from a toxicological perspective (Gigault et al., 2018).

Although early investigations focused predominantly on marine pollution, it is now widely recognized that terrestrial ecosystems serve as major reservoirs for plastic particles (Nizzetto et al., 2016; Bläsing and Amelung, 2018).

Soils may accumulate significantly greater quantities of microplastics than oceans because of direct agricultural, industrial, and urban inputs. Agricultural soils, in particular, are exposed to multiple pathways of plastic contamination.

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The use of plastic mulching films, greenhouse covers, irrigation tubing, seed coatings, and controlled-release fertilizer encapsulations introduces plastic materials directly into farmland (Corradini et al., 2019). Over time, mechanical stress, temperature fluctuations, and ultraviolet radiation fragment these materials, leaving residues within the soil matrix.

The application of sewage sludge and compost derived from municipal solid waste represents another major input pathway. Wastewater treatment plants remove a substantial fraction of microplastics from effluent, but these particles often become concentrated in sludge (Nizzetto et al., 2016).

When sludge is applied to agricultural fields as fertilizer, accumulated plastic particles are transferred to soil ecosystems. Atmospheric deposition also contributes to soil contamination. Microfibers released from synthetic textiles during washing, tire wear particles generated by vehicular traffic, and fragments from urban dust may settle onto agricultural land (Horton et al., 2017). Surface runoff and flooding events further redistribute plastic particles across landscapes, integrating them into river sediments and floodplain soils.

Once incorporated into soil systems, microplastics and nanoplastics interact with mineral particles, organic matter, soil pore spaces, and living organisms. Their presence can modify soil physical properties (de Souza Machado et al., 2018). For example, fibrous microplastics may influence soil aggregation by physically entangling mineral particles, altering aggregate stability.

Fragment-shaped particles can disrupt soil structure, affecting bulk density and porosity. Changes in porosity influence water infiltration, aeration, and root penetration. In sandy soils, microplastics may increase water retention by reducing pore connectivity, whereas in clay-rich soils they may destabilize aggregates and alter moisture dynamics (Lehmann et al., 2019).

Chemical interactions within soil further complicate the ecological implications of microplastic contamination. Plastic polymers often contain additives, including stabilizers, antioxidants, plasticizers, pigments, and flame retardants. These compounds can leach gradually into surrounding soil, introducing additional chemical stressors. Moreover, plastic surfaces exhibit hydrophobic characteristics and high adsorption capacity.

As a result, microplastics can bind heavy metals, pesticides, herbicides, and persistent organic pollutants present in soil (Rochman et al., 2013). This adsorption process can alter the mobility and bioavailability of contaminants.

Oxidative stress arises when the production of reactive oxygen species exceeds the capacity of antioxidant defense systems (Sies, 2017). Reactive oxygen species such as superoxide anions, hydrogen peroxide, and hydroxyl radicals are natural byproducts of aerobic metabolism. Under normal conditions, antioxidant enzymes including superoxide dismutase, catalase, and peroxidases maintain redox balance (Lushchak, 2011). However, exposure to environmental stressors can elevate ROS production beyond detoxification capacity. Evidence suggests that microplastics and nanoplastics can stimulate ROS generation (Bhattacharya et al., 2010; Nel et al., 2006).

Among the most significant consequences of microplastic impurity in soil is its impact on microbial communities. Soil microorganisms including bacteria, fungi, archaea, and actinomycetes play important roles in ecosystem functioning (Li et al., 2023; Wang et al., 2024). Disruption of microbial activity can thus have cascading effects on soil fertility and plant growth. One of the key mechanisms underlying microbial disturbance is oxidative stress.

Physical interactions between plastic particles and microbial cells may damage membranes or induce mechanical stress. Nanoplastics, because of their small size, may enter cells and interfere with metabolic pathways, leading to enhanced ROS production (Bhattacharya et al., 2010). Elevated ROS levels can result in lipid peroxidation, protein oxidation, and DNA damage (Halliwell and Gutteridge, 2015).These biochemical disturbances can reduce microbial biomass and alter community composition. Sensitive species may decline, while stress-tolerant organisms increase (Wiedner and Polifka, 2020).

II. Materials And Methods

Collection of the sample:

Sediment samples were collected from Pallavaram local lake. Approximately 500 g of surface sediment (0–10 cm depth) was collected from site using clean metal tools to avoid plastic contamination. The collected samples were transferred into sterile glass containers and transported to the laboratory for further analysis.

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FIG 01: SAMPLE COLLECTION AREA

Preparation of the sample:

The collected sediment samples were air-dried or oven-dried at 40–50°C until complete moisture removal.

The dried sediments were then sieved through a 5 mm mesh to remove large debris, stones, and plant materials. A known weight of the processed sediment was used for subsequent experimental analysis.

FIG 02: SEDIMENT SAMPLE

Organic Matter Digestion:

The collected supernatant containing suspected plastic particles was treated with 30% hydrogen peroxide (H_2O_2) and incubated at 50–60°C until complete digestion of organic matter.

The treated solution was then filtered using 0.45 µm membrane filters to collect the remaining particles.

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FIG 03: COMPLETE DIGESTION

Centrifugation and Separation of Sediment Particles

After the digestion of organic matter, the treated sample mixture was transferred into centrifuge tubes. The tubes were placed in a centrifuge and spin at 3000 rpm for 10 minutes. Centrifugation allowed the separation of solid sediment particles from the liquid phase.

Density Separation of Nanoplastics

To isolate lighter particles such as nanoplastics or other low-density materials, the sediment pellet was resuspended in saturated sodium chloride (NaCl) solution. Sodium chloride solution provides a high-density medium that allows lighter particles to float while heavier sediment particles settle at the bottom. The suspension was mixed thoroughly and centrifuged again at 3000 rpm for 10 minutes. After centrifugation, the floating supernatant containing lighter particles was carefully collected without disturbing the settled sediment.

Congo Red Staining

The filtered particles were stained using Congo Red solution (0.1%) and incubated for 20–30 minutes in the dark. Congo red selectively binds to the cellulose paper, giving a bright red background to identify the plastic particles as transparent.

Microscopic Analysis

The stained membrane filters were examined under fluorescence microscopy or optical microscopy. Plastic particles were identified based on their fluorescence properties, size, morphology, and color. The abundance of micro- and nanoplastics was expressed as number of particles per gram of dry sediment.

Preparation of Sediment Extract for Biochemical Analysis

Approximately 5–10 g of sediment sample was homogenized in phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 minutes. The supernatant obtained after centrifugation was collected and used for biochemical assays.

Biochemical Parameter Analysis

Protein Estimation by Bradford Assay

- For the assay, 50 µl of sediment extract was taken in a test tube. To this, 500 µl of Bradford reagent was added. The mixture was gently mixed to ensure proper interaction between the dye and the proteins.
- The reaction mixture was allowed to stand at room temperature for about 5 minutes for color development. After incubation, the absorbance was measured at 595 nm using a spectrophotometer.

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- A standard curve was prepared using known concentrations of bovine serum albumin (BSA). The protein concentration in the sediment extract was calculated by comparing the absorbance values with the standard curve.

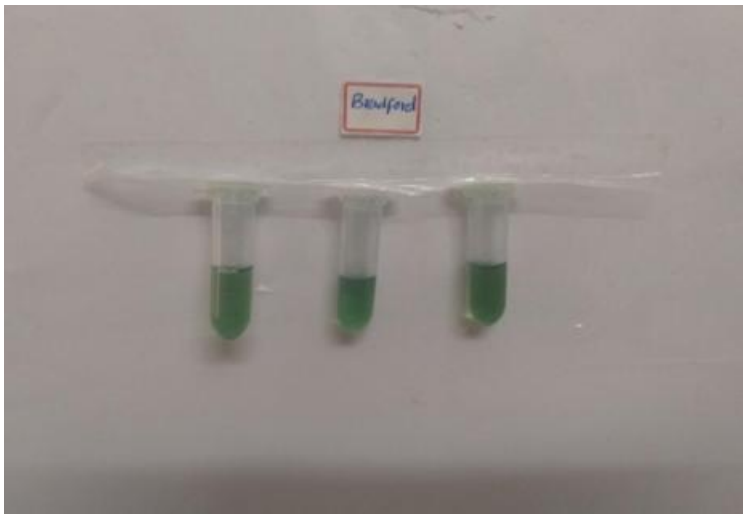

FIG 04: BRADFORD

Superoxide Dismutase (SOD) Assay:

The reaction mixture contains

1. 50 µl of sediment extract
2. 100 µl of EDTA solution
3. 250 µl of distilled water
4. 200 µl of epinephrine solution

- The mixture was gently mixed and the reaction was allowed to proceed. The absorbance of the reaction mixture was measured at 480 nm using a spectrophotometer.
- The change in absorbance indicated the activity of the SOD enzyme present in the sample.

FIG 05: SUPEROXIDE DISMUTASE

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Catalase (CAT) Assay:

- For the catalase assay, 50 µl of sediment extract was mixed with 1 ml of hydrogen peroxide solution.
- The reaction was initiated by adding hydrogen peroxide to the sample.
- The mixture was incubated briefly and the decrease in absorbance was measured at 440 nm using a spectrophotometer.
- The rate of decrease in absorbance was used as an indicator of catalase activity.

FIG 06: CATALASE

Lipid Peroxidation (LPO) Assay:

Lipid peroxidation levels were determined using the FOX (Ferrous Oxidation-Xylenol Orange) reagent. The reagent was prepared using xylenol orange (100 µL), 11 mM butylated hydroxytoluene, 25 mM sulfuric acid, 25 µM ferrous sulphate, 90 mL methanol, and 10 mL of 250 mM sulfuric acid.

To 50 µL of protein sample, 0.9 mL of FOX reagent was added and the mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 560 nm using a UV–Visible spectrophotometer against a reagent blank. The amount of hydroperoxides formed was calculated using the molar extinction coefficient of $4.6 \times 10^4\ M^{-1}\ cm^{-1}$ and expressed as nmole per gram of tissue.

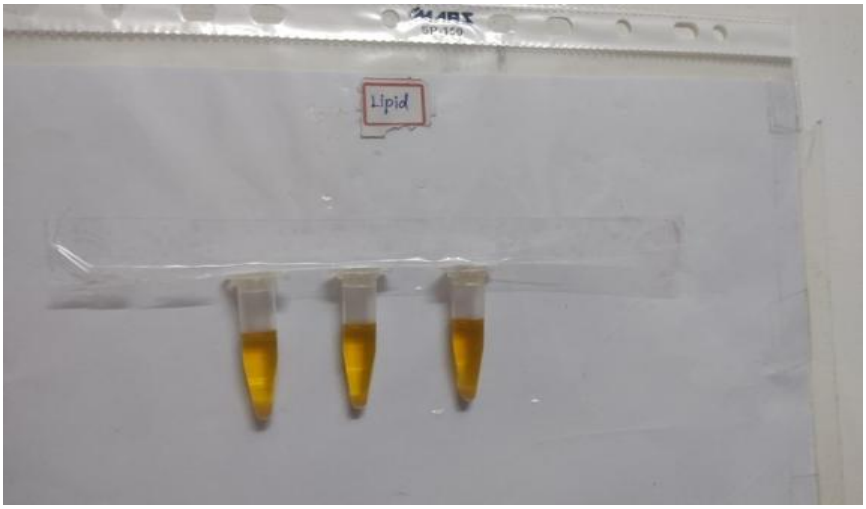

FIG 07: LIPID PEROXIDATION

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Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Correlation analysis was conducted to evaluate the relationship between nanoplastic abundance and biochemical parameters.

III. RESULTS

Detection of Nanoplastic Particles in Sediment Samples and Microscopic evaluation:

Density separation followed by filtration and Congo red staining enabled the successful detection of nanoplastic particles in river sediment samples. Under fluorescence microscopy, stained particles exhibited distinct fluorescence signals, confirming the presence of plastic materials within the sediment matrix.

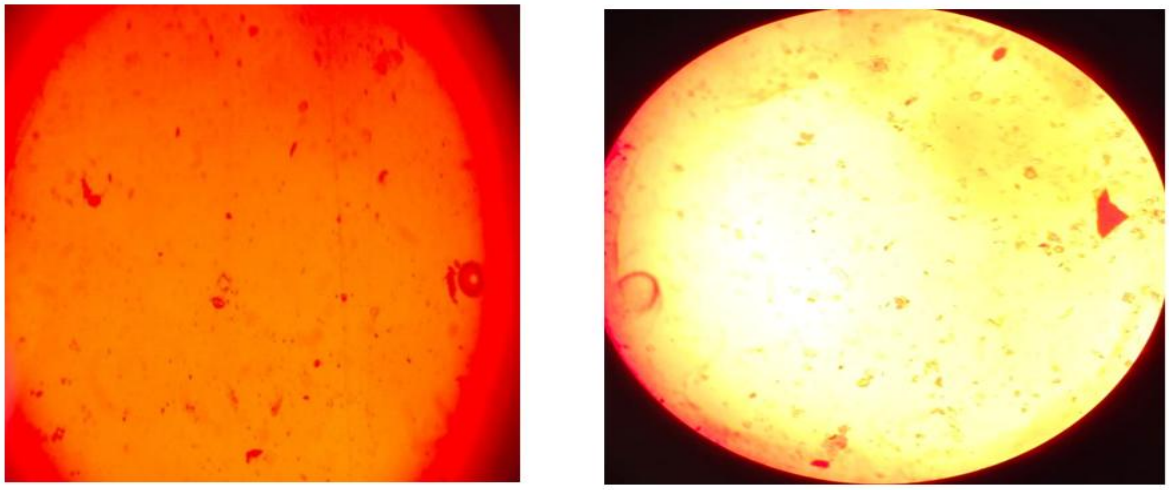

FIG 08: CONGO RED STAINING

Microscopic examination revealed the presence of different particle morphologies including fibers, fragments, and irregularly shaped particles. These particles varied in size, indicating the presence of both microplastics and nanoplastics within the sediment samples.

Biochemical Analysis Of Sediment Extract

Biochemical assays performed on sediment extracts indicated measurable levels of total protein, antioxidant enzyme activities, and oxidative stress markers.

Moderate lipid peroxidation levels (TBARS) suggested oxidative stress in sediments with higher nanoplastic contamination. Additionally, variations in catalase and superoxide dismutase activities were observed, indicating possible stress responses in sediment-associated microbial communities. The values are so far apart, a logarithmic scale was used on the y-axis so that the smaller bars for Catalase and LPO are still visible next to the much larger Protein bar which was shown in the Figure 09.

Table 01: Biochemical Analysis Of Sediment Extract

Assay	Parameter	Result	Units
Bradford	Protein Concentration	121	ug/mL
SOD	Activity	30.129	U/mL
Catalase	Specific Activity	1.644	U/mg protein
LPO	Level	2.3214	nmol/g protein

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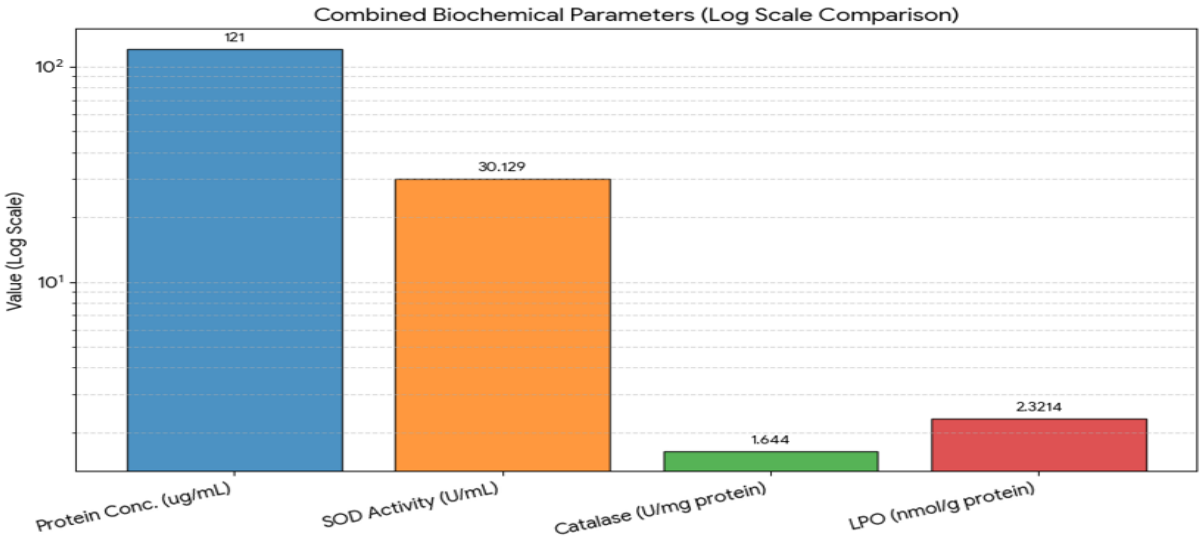

FIG 09: ASSAY ACTIVITY

Correlation Between Nanoplastics and Biochemical Markers

Statistical analysis showed that as the amount of nanoplastics increased, the level of oxidative stress also increased. Changes in enzyme activity indicated that microbial metabolic functions were affected in the contaminated sediments.

IV. Discussion

The present study aimed to detect nanoplastic particles in sediment samples and to evaluate certain biochemical indicators related to oxidative stress. Sediments are known to act as important reservoirs for various environmental pollutants, including plastic debris (Wright et al., 2013; Horton et al., 2017). Over time, larger plastic materials degrade into smaller particles such as microplastics and nanoplastics, which can accumulate in sediment layers (Andrady, 2011; Zhang et al., 2021).

In this study, the treatment of sediment samples with hydrogen peroxide helped remove organic matter, while density separation using saturated sodium chloride solution allowed lighter particles to be isolated from heavier sediment components (Masura et al., 2015; Hurley et al., 2018). The use of congo red staining enabled the visualization of plastic particles, and the observation of stained particles indicated the possible presence of nanoplastics in the sediment sample (Hidalgo-Ruz et al., 2012).

Biochemical analysis of the sediment extract showed measurable protein content, suggesting the presence of microbial and biological materials within the sediment. The activities of antioxidant enzymes such as superoxide dismutase and catalase were also detected, indicating that microorganisms present in the sediment may possess defense mechanisms against oxidative stress (Lushchak, 2011; Apel and Hirt, 2004). In addition, lipid peroxidation analysis suggested the occurrence of oxidative processes in the sample (Halliwell and Gutteridge, 2015).

Exposure to microplastics and nanoplastics has been reported to induce oxidative stress in biological systems through the generation of reactive oxygen species (ROS) (Bhattacharya et al., 2010; Nel et al., 2006). Similar findings have been reported in recent studies where nanoplastics altered antioxidant enzyme activities and increased lipid peroxidation levels in organisms (Jovanovic et al., 2018; Wang et al., 2024). Furthermore, microplastics present in sediments may influence microbial community structure and enzyme activities, thereby affecting nutrient cycling and ecosystem functioning (de Souza Machado et al., 2018; Fei et al., 2020; Li et al., 2023).

Overall, these findings highlight that sediment environments not only accumulate nanoplastic particles but also contain active biological systems that respond to environmental stress conditions. The interaction between nanoplastics and sediment-associated microorganisms may have significant ecological implications, particularly in terms of oxidative stress, enzyme activity, and overall ecosystem health (Wang et al., 2024; Zhang et al., 2022).

V. Conclusion

The present study demonstrated the possible presence of nanoplastic particles in sediment samples through suitable extraction and staining techniques. The use of hydrogen peroxide digestion, density separation, and Congo red staining proved to be effective methods for isolating and identifying plastic particles from the sediment matrix. The observation of stained particles suggested that sediments can act as reservoirs for plastic pollutants in the environment.

Biochemical analysis of the sediment extract revealed the presence of proteins and antioxidant enzymes such as superoxide dismutase and catalase, indicating biological activity within the sediment. The detection of lipid peroxidation products further suggested that oxidative processes may occur in the sediment environment. These results indicate that microorganisms present in sediments may respond to environmental stress through antioxidant defense mechanisms.

Overall, the findings highlight the interaction between environmental pollution and biological processes in sediment ecosystems. Monitoring nanoplastic contamination and related biochemical responses is important for understanding the potential ecological impact of plastic pollution in aquatic environments.

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