

Preliminary investigation on the occurrence and associated risks of Microplastics, Polyaromatic Hydrocarbons in the Ansupa Wetland: A Ramsar site in Odisha, India

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Abstract

Microplastics (MPs) and polycyclic aromatic hydrocarbons (PAHs) pollution together pose significantly higher ecological and health risks in freshwater ecosystems than either pollutant alone. However, studies on the distribution and associated risks of MPs and PAHs pollution in relation to hydrological parameters are limited. The present study investigates the occurrence, spatial distribution, interrelationship of MPs, PAHs, and hydrological parameters, and their ecological risks in Ansupa Lake. Surface water samples were collected from 8 different locations and analysed using standardized physico-chemical, spectroscopic, microscopic, and chromatographic methods. The average abundance of MPs and PAHs was found to be 15.5 ± 17.3 mg/L and 0.13 ± 0.16 mg/L, respectively. MPs were extracted and characterized using a microscope and Fourier-Transformed Infrared Spectroscopy (FTIR), whereas PAHs were characterized in High Performance Liquid Chromatography (HPLC). The results revealed 10 different polymers such as Polyurethane (PU), Polyvinyl chloride (PVC), Polyethylene terephthalate (PET), Polystyrene (PS), Acrylonitrile butadiene styrene (ABS), Polypropylene (PP), Low density polyethylene (LDPE), High density polyethylene (HDPE), Polyethylene (PE), and Polyamide (PA) with highest occurrence of PET, PE, HDPE, and LDPE (100%) followed by PVC, PS, and ABS (87.5%). Twelve different types of PAHs were found among which benzo [b] fluoranthene (87.5%) showed highest % occurrence. PAHs containing 2 or 3 rings (Low molecular weight (LMW) PAHs) were detected in higher concentration as compared to PAHs containing 4, 5, and 6 rings (high molecular weight (HMW) PAHs). Source diagnostic ratio showed both petrogenic and pyrolytic origin of PAHs, with predominance of petrogenic PAHs. The principal component analysis (PCA) was carried out, and the results shown strong correlation between MPs with transparency, dissolved oxygen (DO), alkalinity, conductivity, and total dissolved solids (TDS), and PAHs with air temperature, whereas no significant relation was observed between MPs and PAHs. The pollution load index (PLI) and polymer hazard index (PHI) of MPs ranged between 1-4.35 and 25- 437587.5, respectively, depicting a low level of PLI with the presence of high-extremely hazardous polymer types. Whereas, the ecological risk assessment (ERA) of PAHs was found to be at high risk.

Keywords: MPs, PAHs, FTIR, HPLC, PLI, PHI, PCA, ERA

1. Introduction

Industrialization and population expansion has contributed towards increasing the pollutant load in the aquatic ecosystem and has become a global problem. Plastics are synthetic materials made up of long polymer chains [1]. As per recent studies, global plastic production has increased to about 359 million tons per year, but over 80 % of plastic waste enters the natural environment due to improper disposal [2]. Combination of complex physical, chemical, or biological processes can cause the breakdown of plastics into microplastics having size less than 5 mm. Microplastics exist in different forms like plastic fibers, films, or particles that create an emerging challenge to various aquatic environments with far

reaching consequences [3]. Sewage treatment plants, Industrial waste, Urbanization and agricultural runoff are considered important ways to transport land-based microplastics into freshwater and marine environments [4-5]. Lipophilic chemicals like poly aromatic hydrocarbon (PAHs) and poly chlorinated benzenes (PCBs) can easily be absorbed by microplastics from the aquatic environment which ultimately enter into organisms and causes tissue damage [6]. Absorption of PAHs by microplastics is mainly accelerated by 2 factors i.e large surface area of microplastics and the hydrophobicity of PAHs [7]. Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous and recognized for their carcinogenic, teratogenic, and genotoxic effects. PAHs are a class of

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chemicals that consist of fused aromatic rings. On the basis of the number of aromatic rings present, PAHs are classified as light or low molecular weight (LMW) PAHs (consisting of 2 or 3 aromatic rings) and heavy or high molecular weight (HMW) PAHs (consisting of more than three fused aromatic rings). The physical properties of PAHs include hydrophobic nature, low vapor pressure, high melting and boiling points, high conductivity, light sensitive, heat and corrosion resistance, and emission ability etc. The solubility of PAHs in aqueous medium decreases with increasing the number of aromatic rings. PAHs in the environment mostly come from two sources, i.e., pyrogenic and petrogenic. Pyrogenic PAHs are produced by the pyrolysis process, where organic materials undergo high temperature and low or no oxygen conditions. Petrogenic PAHs are mainly released from petroleum products under low temperature conditions [8]. Due to the presence of hydrophobic characteristic most of the polycyclic aromatic hydrocarbons (PAHs) undergoes slow biodegradation process in the natural environment and are predominantly located in soil, sediment, oily, or in association with organic matter [9]. PAHs can leach from soil into the water column, with contaminants released from industrial waste and oil spills. PAHs have different effects, and 16 priority PAHs have been recognized by USEPA due to their toxic effects. Besides their own toxicity microplastics also provide adsorption sites to other organic pollutants. Microplastics act as a carrier of PAHs, and because of their mobile nature, it can affect the distribution pattern of PAHs in the aquatic ecosystem [10]. Inside the aquatic organisms, both contaminants lead to accumulative and toxic effects resulting in their bioaccumulation in higher trophic level [11]. Ansupa is the largest freshwater lake of Odisha, which comes under the Ramsar sites. Several mechanized boats were operated mainly for recreational activities and fishing purposes. As this lake is surrounded by multiple villages and agricultural fields, PAHs and MPs contamination was expected from this lake.

However, no study was conducted on PAHs and MPs pollution in Ansupa lake. Therefore, this study was conducted to estimate the concentration of PAHs, and microplastics and to observe the correlation between PAHs, MPs, and the water quality parameters.

2. Materials and methods

2.1. Study area and sample collection

Ansupa lake the largest freshwater lakes in Odisha, also designated as a 'Wetland of National Importance'. Ansupa Lake is situated in the Banki Subdivision of Cuttack district, spanning from 85° 34' 52.697" E to 85° 37' 19.271" E longitude and 20° 26' 37.864" N to 20° 29' 59.228" N latitude. Ansupa lake has global significance as it provides habitat for a diverse group of macrophytes, numerous migratory and resident birds because of which it is also designated as a Ramsar site in October 2021. It is surrounded by various small villages fulfilling the water needs of neighboring areas and also supporting the livelihoods of local communities [12]. In recent years, freshwater ecosystem of Ansupa's lake has become threatened ecosystems that caused decline of its water coverage from 3.17 sq. km (1973) to 1.76 sq. km (2004), with its depth nearly halved by 2008 mainly due to anthropogenic pollution, overfishing, habitat destruction, climate changes, siltation, spreading of weeds, and conversion of wetlands into new agricultural fields etc. [12]. Among all the threats, pollution of the lake ecosystem with persistent organic pollutants (POPs) have emerged as a prevailing issue. Agricultural runoffs, industrial activities near the lake area, boat uses for fishing, and other anthropogenic activities have introduced such pollutants to the lake ecosystem and became major source of contaminant exposures. For the measurement of contamination level, water samples were collected from 8 sampling stations covering coordinates from 20° 27.052' N, 85° 36.447' E to 20° 26.996' N, 85° 35.923' E (Figure 1). Shallow water sampler was used for collecting water samples.

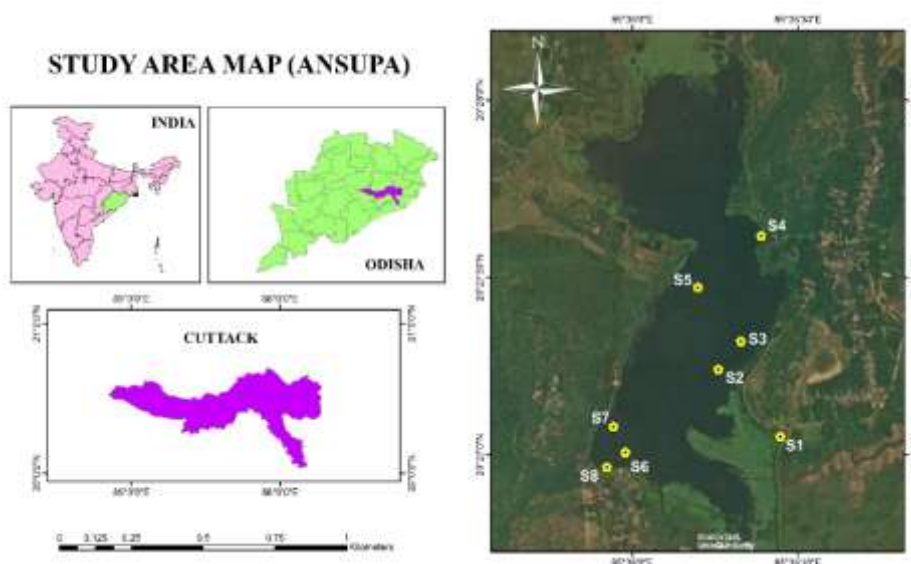


Figure 1. Study area showing stations location of Ansupa lake.

2.2. Analysis of hydrological parameters

Water temperature and air temperature was measured using 0.01C precision thermometer. P^H was measured using pocket P^H meter (Thermo-scientific). Transparency was measured with the help of Secchi disc. Dissolved oxygen of water samples were measured using Winkler's method [13]. Estimation of other physicochemical parameters was carried out as per standard methods. Dissolved oxygen was analyzed by modified Winkler's method, alkalinity, hardness, nitrate, nitrite and phosphate were estimated by titrimetric method. All the physicochemical parameters were measured using standard protocols of Strickland and Parsons 1972.

2.3. Analysis of polyaromatic hydrocarbon (PAHs)

2.3.1. Quantification of PAHs

Filtration of water samples were done using Whatman no. 1 filter paper and liquid-liquid extraction method was used. Separation was done using a glass separating funnel containing n-Hexane and DCM (dichloromethane) in 1:1 ratio [14]. The mixture was set aside for 20 minutes for the separation of polar and non-polar phases. The polar layer, consisting of water was discarded and the non-polar layer containing the organic component was collected in a beaker and dried completely at room temperature. This was followed by the addition of 1 ml of THF (tetrahydrofuran) to the dried beaker, 10 min centrifugation (3000 rpm) and filtration using a 0.45 μ syringe filter. Then the extract was loaded on HPLC for quantification of PAHs. For standard preparation Sixteen individual PAHs including Naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flr), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene

(Pyr), benzo(a)anthracene (BaA), chrysene (Cry), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DahA), benzo(g,h,i)perylene (BghiP) and indeno(1,2,3-cd)pyrene (InP) were purchased from Sigma Aldrich having 99.9% of purity. Stock solution of PAHs was prepared in acetonitrile solution. PAHs of unknown samples were quantified using peak areas of standard curve. All the solvents used during separation were of HPLC grade. Separation of all identified PAHs were carried out in a C18 column having length 250 mm, diameter 4.6 mm and 5 μ m of film thickness as described in our previous work [15]. For the separation of PAHs flow rate was maintained at a speed of 1.5 ml/minute.

2.3.2. Source determination of PAHs

PAHs Molecular diagnostic ratio (MDR) or PAHs isomeric ratio (PIR) such as Flr/Pyr and Flr / Flr + Pyr, Ant/Phe + Ant and LMW/HMW ratios have been used worldwide for source identification of PAHs [16]. Among all ratios, Flr / Flr + Pyr and Ant / Phe + Ant was commonly used. The ratio Flr/Pyr showed a changeover point of 1 where the ratio ≥ 1 indicated PAHs sources of pyrolytic origin whereas that of < 1 indicated sources of petrogenic origin. Flr / Flr + Pyr ratio ≥ 0.5 showed pyrolytic or combustion derived sources and the ratio < 0.5 indicated pollutants mainly originated from petroleum derivatives [15]. The ratio Ant/Phe + Ant ≥ 0.1 indicated pollution originated due to incomplete combustion and values < 0.1 showed indication of petroleum pollution source [17]. Similarly, in case of LMW/HMW ratio, the value greater than 1 showed PAHs originated from petrogenic sources and that of less than 1 showed PAHs from pyrolytic sources. PAHs molecular diagnostic ratio was calculated for each station by

using ratios of specific PAHs which are represented in table.

2.3.3. Risk assessment of PAHs

Risk quotient (RQ) was calculated to understand the impact of PAHs on lake inhabiting organisms [18]. Here RQ value was calculated by dividing the average value of individual PAHs to the quality value (QV) of that particular PAHs as given in our previous work [15].

$$RQ = \frac{C_{PAHs}}{C_{QV}}$$

Where C_{PAHs} =Average concentration of individual PAH (ng/L)

C_{QV} = Quality value of individual PAHs

As there is no such data reported for quality values, negligible concentration (NC) and maximum permissible concentration (MPC) reported by Cao *et al.*, 2010 and Kalf *et al.*, 1997 were used in place of quality value [19-20].

$$RQ_{NCs} = \frac{C_{PAHs}}{C_{QV}(NCs)}$$

$$RQ_{MPCs} = \frac{C_{PAHs}}{C_{QV}(MPCs)}$$

Here $C_{QV}(NCs)$ represents quality value of NCs of individual PAHs and $C_{QV}(MPCs)$ represents quality value of MPCs in water sample. Risk classification of individual PAHs and Σ PAHs were presented in Table 1. [21-22].

Table 1. Risk calculation table of individual PAHs and Σ PAHs based on RQ values.

Individual PAHs			Σ PAHs		
RQ _{NCs}	RQ _{MPCs}	Risk level	RQ _{ΣPAHs(NCs)}	RQ _{ΣPAHs(MPCs)}	Risk level
<1	<1	Risk free	<1	<1	Risk free
≥1	<1	Moderate risk	≥1;<800	<1	Low risk
≥1	≥1	High risk	≥800	<1	Moderate risk-I
			<800	≥1	Moderate risk- II
			≥800	≥1	High risk

2.4. Analysis of MPs:

Water samples were sieved and the sieved residues were collected using distilled water. The organic matter present in the collected samples were first oxidized using hydrogen peroxide and then separation of microplastics were carried out by increasing density of the solution using NaCl. The solution was passed through 1µm sieve to separate microplastics, and collected in petri-dish. The obtained microplastics were dried, followed by microscopic examination and FTIR analysis. The dried slides were examined and photographed under Nikon microscope equipped with a camera at 10X magnification and fluorescent microscope stained with Nile red (SRL-47353) solution for the visualization of microplastics. Fourier-Transformed Infrared Spectroscopy (FTIR) is an absorption-based technique that measures the transmitted radiation from the sample following the illumination of IR rays to provide chemical fingerprint of the same. The KBr pellets were prepared using dried samples and KBr (SRL-13873). The pellet formed was

then examined in FTIR spectroscopy to characterize polymers according to their chemical nature.

2.4.1. Risk assessment of microplastics:

The pollution load index (PLI) was calculated to assess the level of microplastic contamination in water samples collected from the study area [23]. PLI was calculated using formula given by Tomlinson *et al.* (1980), Ranjani *et al.* (2021) [24-25]. PLI is described as:

$$CF_i = \frac{C_i}{C_{oi}}$$

$$PLI = \sqrt{CF_i}$$

Where, C_i is microplastic concentration in each species, and C_{oi} is the background microplastic concentration, which is the lowest concentration of MPs. The PLI score obtained was compared to reference values for identifying risk category (Table 2).

Table 2. Showing PLI scores and their hazard and risk categories.

PLI	Hazard Category	Risk category
< 10	I	Minor
10 to 20	II	High
20 to 30	III	Danger
>30	IV	Extremely danger

Polymer hazard index (PHI) of MPs in the water samples was calculated considering concentration, polymer type and their hazard score [26-27]. Calculation of PHI was done by using the following formula:

$$PHI = \sum P_n \times S_n$$

Where, P_n is the percentage of polymer types, and S_n is the hazard score. The scores were calculated following standard method given by Lithner et al. (2011) [27].

3. RESULT

3.1. Analysis of hydrological parameters

In our study all physicochemical parameters like air temperature, water temperature, transparency, DO, alkalinity, hardness, conductivity, TDS and pH showed significant variation among all the sampling

stations. Physico-chemical parameters with its range, mean and standard deviation are represented in Table 3. Air temperature was observed in the range of 28 to 37 °C whereas water temperature varies between 26 to 29 °C. Measured transparency showed fluctuation between 0.3 to 1.5 m with an average value of 0.55 m. P^H value varies from 7 to 8 which indicated alkaline nature of Ansupa lake. Average DO of Ansupa lake was calculated as 6.56 mg/ L. Alkalinity of water sample ranged between 48 mg/L to 66 mg/L. Conductivity and TDS was observed in the range of 23.16 to 25.36 μ S and 11.06 to 16.45 mg/L respectively. Nutrients like nitrite, nitrate and phosphate present in water samples were observed in the range of 0.007 to 0.027 mg/L, 0.049 to 0.153 mg/L and 0.001 to 0.004 mg/L with an average value of 0.01 mg/L, 0.05 mg/L, and 0.002 mg/L respectively.

Table 3. Physicochemical parameters with its range, mean value and standard deviation.

Parameters	Range	Mean	Standard deviation
AT (°C)	28-37	31.50	3.46
WT (°C)	26-29	27.75	1.16
pH	7-8	7.45	0.29
Transparency (m)	0.3-1.5	0.96	0.55
DO (mg/L)	2-9.3	6.56	2.62
Alkalinity (mg/L)	48-66	62.00	5.98
Hardness (mg/L)	60-74	66.50	4.11
Conductivity (μ s)	23.16-25.36	23.26	2.50
TDS (mg/L)	11.06-16.23	14.89	1.60
Nitrite (mg/L)	0.007-0.027	0.02	0.01
Nitrate (mg/L)	0.049-0.153	0.10	0.05
Total phosphate (mg/L)	0.001-0.004	0.003	0.002

3.2. Analysis of PAHs present in water samples

3.2.1. Quantification of PAHs

A total of 12 PAHs were observed in surface water samples covering 8 stations of Ansupa lake. In water samples total PAHs concentration ranged from 0.001 to 0.486 mg/l, with an average of 0.128 mg/L. The highest total PAHs concentration was observed at S6 followed by S5, S8, S7, S3, S2, S1 and S4 respectively. Station wise total PAHs concentration with ring composition profile are represented in Figure 2. Majority of the sampling stations showed the dominance of 3 ring PAHs, whereas 4 ring PAHs were

observed at S1 and S4, followed by 5 ring PAHs at S2. The order of PAHs distribution based on the ring structure at sampling sites was in the order of 3 ring > 4 ring > 5 ring > 2 ring respectively (figure 2). In 5 stations the concentration of LMW PAHs (including 2 and 3 ring PAHs) was observed to be high whereas in 3 stations the concentration of HMW PAHs showed dominance. For all the sampling stations, the percentage occurrence of individual PAHs was calculated and represented in figure 3. The result showed B(b)F and B(k)F was present in maximum stations having percentage occurrence of 87.5% and 75 respectively.

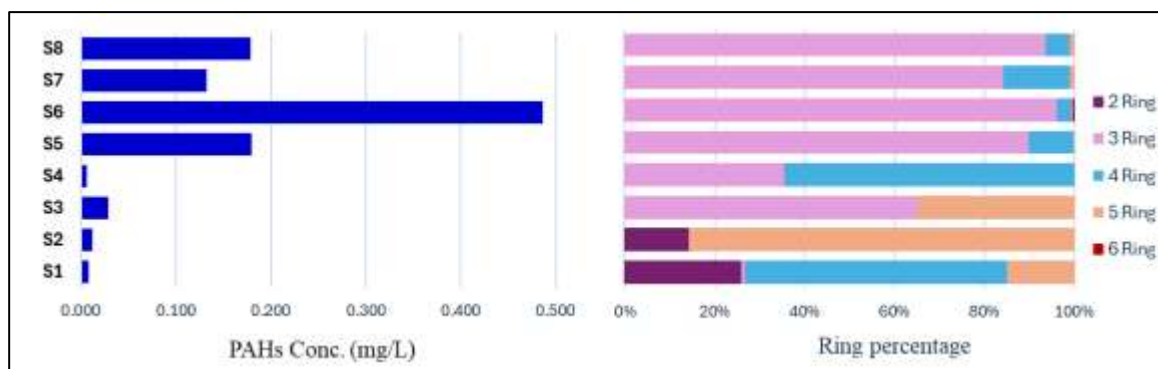


Figure 2. Station wise representation of total PAHs concentration with its ring composition profile.

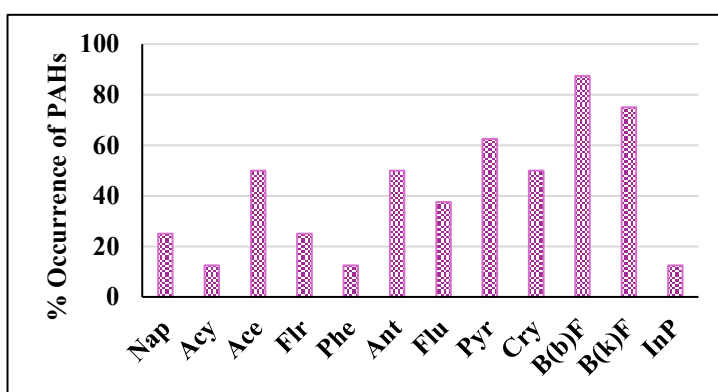


Figure 3. Percentage occurrence of all the identified PAHs among all the sampling stations.

3.2.2. Source determination of PAHs

PAHs molecular diagnostic ratio was calculated for each station by using ratios of specific PAHs which are represented in Table 4. In our result PAHs present in S5, S6, S7 and S8 are originated from petrogenic

sources. PAHs present in S2, and S4 were originated from pyrolytic sources whereas PAHs present at S1 and S3 are originated from combined sources i.e. from both petrogenic and pyrolytic sources (Table 4).

Table 4. Molecular diagnostic ratio for identification of PAHs pollution emission source. - represents PAHs that are present below detection limit.

PAHs RATIO	S1	S2	S3	S4	S5	S6	S7	S8	Petrogenic	Pyrolytic
Flu/Pyr	0.18	-	-	-	0.02	0	0	0	≤ 1	>1
Flu/(Flu+Pyr)	0.18	-	-	-	0.023	0	0	0	≤ 0.5	> 0.5
Ant/(Phe+Ant)	1	-	1	1	0	1	0	0	≤ 0.1	> 0.1
LMW/HMW	0.36	0.16	1.87	0.55	8.89	24.27	5.32	14.65	HIGH	LOW

3.2.3. Ecological risk assessment of PAHs.

The calculated value of RQ_{NCs} and RQ_{MPCs} for all the observed PAHs along with the mean value of RQ_{NCs} and RQ_{MPCs} are represented in Table 5. Then the obtained RQ_{NCs} and RQ_{MPCs} values were compared with the standardized risk classification table (Table 1.) for the determination of risk category. From table 5, the observed RQ_{NCs} values of all PAHs was greater than 1 whereas the RQ_{MPCs} value of maximum PAHs was greater than 1 indicating the presence of high ecological risk of these PAHs. Nap, Phe, flu and Cry

showed RQ_{MPCs} value of less than 1 showing moderate ecological risk to the aquatic organisms whereas the RQ_{MPCs} values for remaining PAHs was greater than 1 showing high ecological risk. For all sampling stations RQ_{NCs} value for $\sum PAHs$ was 1375.08 which was greater than 800 and RQ_{MPCs} value for $\sum PAHs$ was 137508.26 i.e. greater than 1 indicating high ecological risk associated with the water samples.

Table 5. Ecological risk assessment (ERA) of all the detected PAHs and summation of PAHs based on RQ values.

PAHs	Average (ng/L)	NCs	MPCs	RQ _{NCs}	RQ _{MPCs}	Ecological Assessment Individual PAH)	Risk of
Naphthalene	350.91	12	1200	29.24	0.29	Moderate risk	
Acenaphthylene	1724.55	0.7	70	2463.64	24.64	High risk	
Acenaphthene	82169.09	0.7	70	117384.41	1173.84	High risk	
Fluorene	263.64	0.7	70	376.62	3.77	High risk	
Phenanthrene	13.64	3	300	4.55	0.05	Moderate risk	
Anthracene	76.36	0.7	70	109.09	1.09	High risk	
Fluoranthene	138.18	3	300	46.06	0.46	Moderate risk	
Pyrene	5988.18	0.7	70	8554.55	85.55	High risk	
Chrysene	90.00	3.4	340	26.47	0.26	Moderate risk	
Benzo(b) fluoranthene	261.82	0.1	10	2618.18	26.18	High risk	
Benzo(k) fluoranthene	2152.73	0.4	40	5381.82	53.82	High risk	
Indeno(1,2,3-cd) pyrene	205.45	0.4	40	513.64	5.14	High risk	
ERA of Σ PAHs				137508.26	1375.08	High risk	

3.3. Analysis of microplastics

3.3.1. Abundance and visualization of microplastics:

The average concentration of MPs in water sample collected from Ansupa Lake was 279 particles/L which varied from 54 particles/L in station-3 (S3) to 1026 particles/L at S1 (Fig- A). The lower MPs concentration was observed in S3, S5, and S6 which were 54 particles/L, 72 particles/L, and 126 particles/L

respectively. The MPs concentration in S8, and S7 was 216 particles/L, and 270 particles/L. Similar concentration was observed in S2 & S4, which were 234 particles/L. Microplastics are found in different forms such as fragment, fibre, film, foam, pellet, and bead [40]. The extracted microplastics were observed under compound microscope and depicted in figure 5. The extracted MPs were stained with Nile red (figure 6) which specifically binds to the hydrophobic particles on MPs polymer.

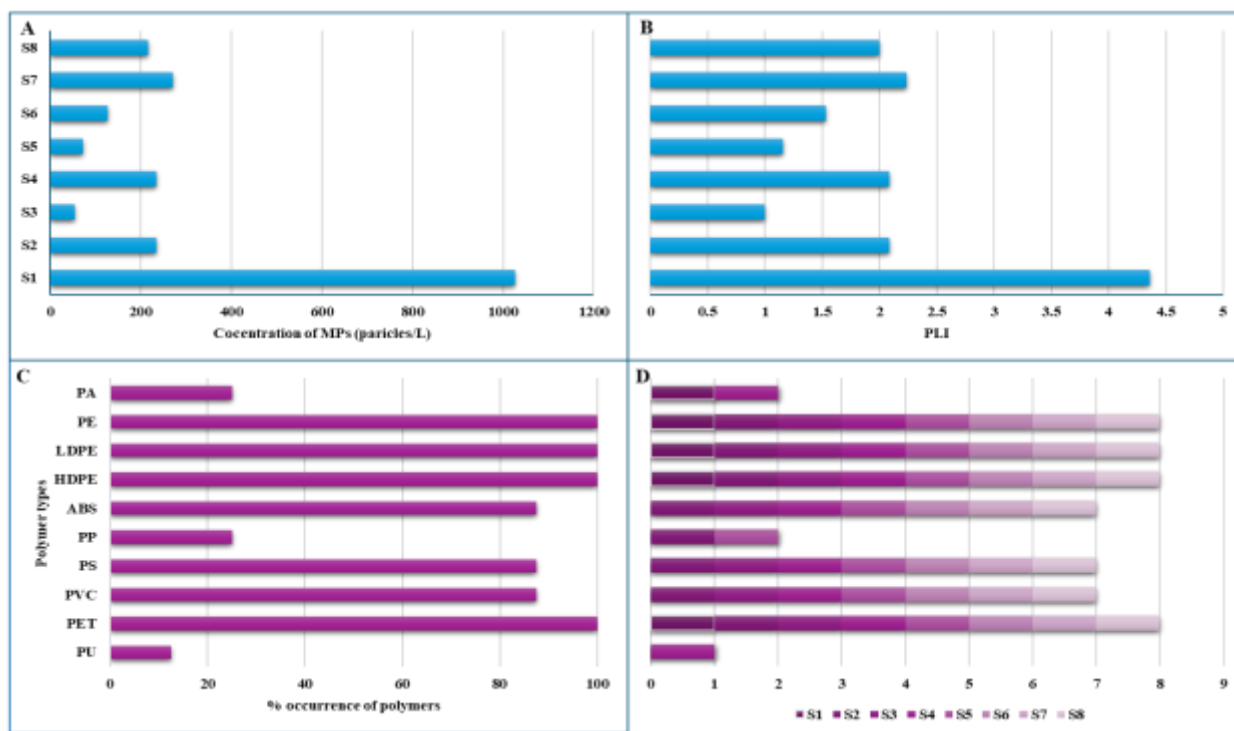


Figure 4. Showing A. Microplastics concentration, B. PLI scores, C. % occurrence of polymers, D. presence of polymers according to stations in water samples collected from 8 stations.

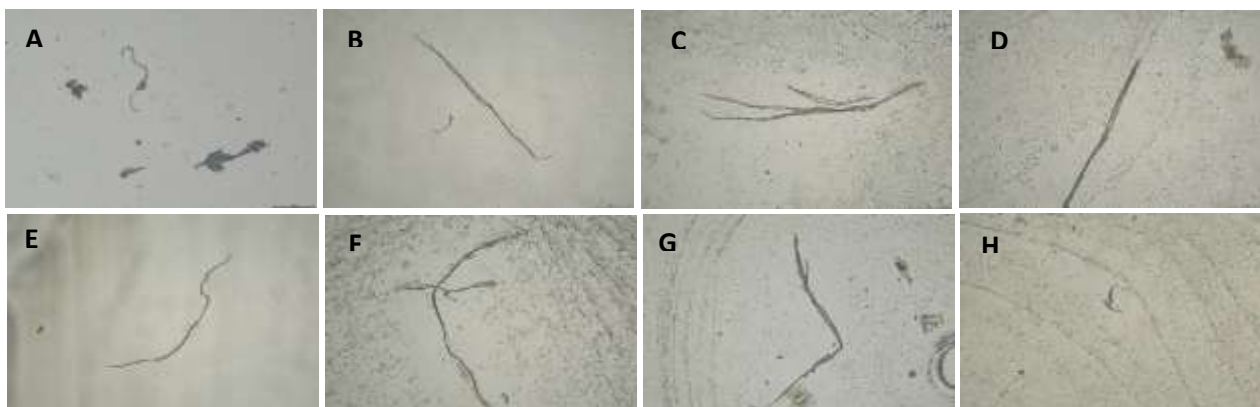


Figure 5. Compound microscopic image of microplastics in water samples collected from 8 stations (A-H corresponds to images from S1 to S8 respectively).

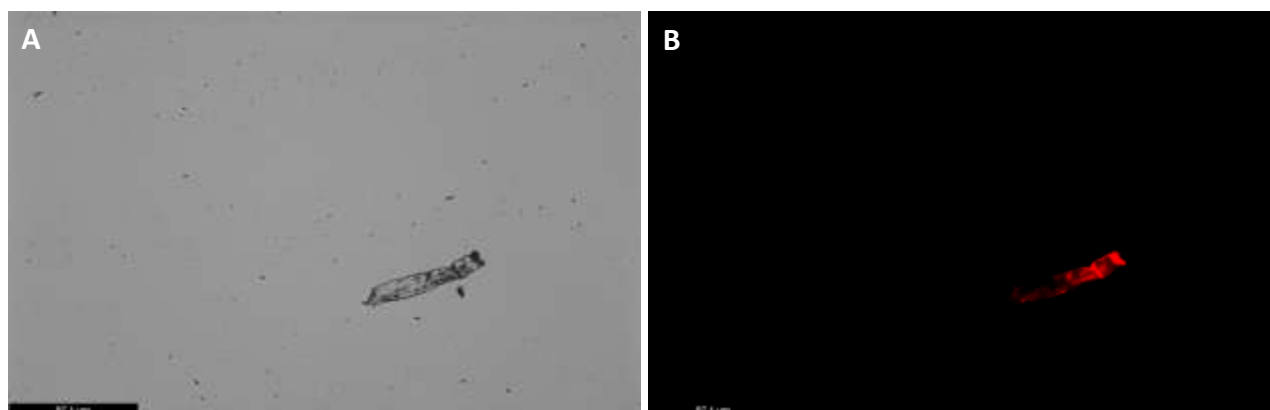


Figure 6. Fluorescent microscopic image of MP in water sample with Nile red stain (A- Bright field image & B- Fluorescent image of MP).

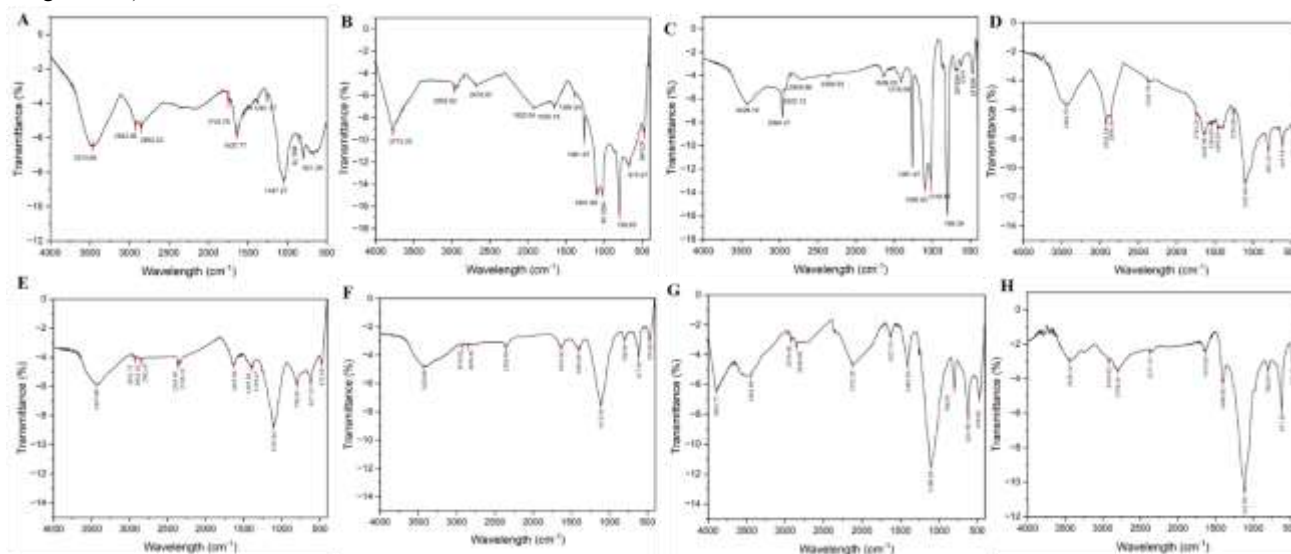


Figure 7. FTIR graphs of extracted microplastics in water samples (A-H corresponds to S1-S8 respectively) collected from 8 stations.

3.3.2. Polymer characterization:

The extracted microplastics were analyzed in FTIR to characterize the polymer types present in the samples (Figure 7). The obtained characteristic peaks were analyzed for eleven different polymers such as Polystyrene (PS), Polypropylene (PP), Polyethylene (PE), Polyurethane (PU), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Acrylonitrile butadiene styrene (ABS), High density polyethylene

(HDPE), Low density polyethylene (LDPE), and Polyamide (PA). From the above analysis, the % occurrence of PET, PE, HDPE, and LDPE were found to be highest (100%) followed by PVC, PS, and ABS (87.5%) at all the stations (figure 4). The % occurrence of PP and PA was found to be 25%, whereas the lowest polymer types were PU with occurrence percentage 12.5%.

Table 6. Showing PHI of polymers and their hazard score along with risk level.

Polymer Type	PHI	Hazard Score	Risk Level
ABS	573300	V	Extremely danger
PVC	437587.5		
PU	92300		
PS	2625		
PA	1250		
HDPE	1100		
LDPE	1100		
PET	400	IV	Danger
PE	275	III	High
PP	25		

3.3.3. Risk assessment:

The PLI range of microplastics (MPs) (1- 4.35) indicate hazard category as HC-I and PHI was higher in ABS (573300), PVC (437587.5), PU (92300), PS (2625), PA (1250), HDPE, and LDPE (1100), which comes under the hazard category V suggesting extremely dangerous MPs. The PHI of PET and PE were 400 and 275 respectively, coming under hazard category IV. Meanwhile, PP (25) came under hazard category III.

3.4. Correlation matrix:

Positive correlation was observed between MPs abundance and three parameters such as water temperature (WT) ($r=0.177$), nitrite ($r=0.155$), and phosphate ($r=0.337$). Except the former three all other parameters were negatively correlated with MPs, likely air temperature (AT) ($r=0.352$), pH ($r=0.329$), transparency ($r=0.496$), DO ($r=0.696$), alkalinity ($r=0.971$), conductivity ($r=0.928$), TDS ($r=0.928$), hardness ($r=0.525$), nitrate ($r=0.194$), and concentration of PAHs ($r=0.350$). Meanwhile, PAHs

abundance was positively correlated with WT ($r=0.421$), AT ($r=0.661$), transparency ($r=0.461$), DO ($r=0.361$), alkalinity ($r=0.349$), conductivity ($r=0.235$), TDS ($r=0.234$), hardness ($r=0.257$), nitrite

($r=0.108$), and nitrate ($r=0.063$), while negatively related to pH ($r=0.027$), phosphate ($r=0.468$), and MPs ($r=0.350$) (figure 8).

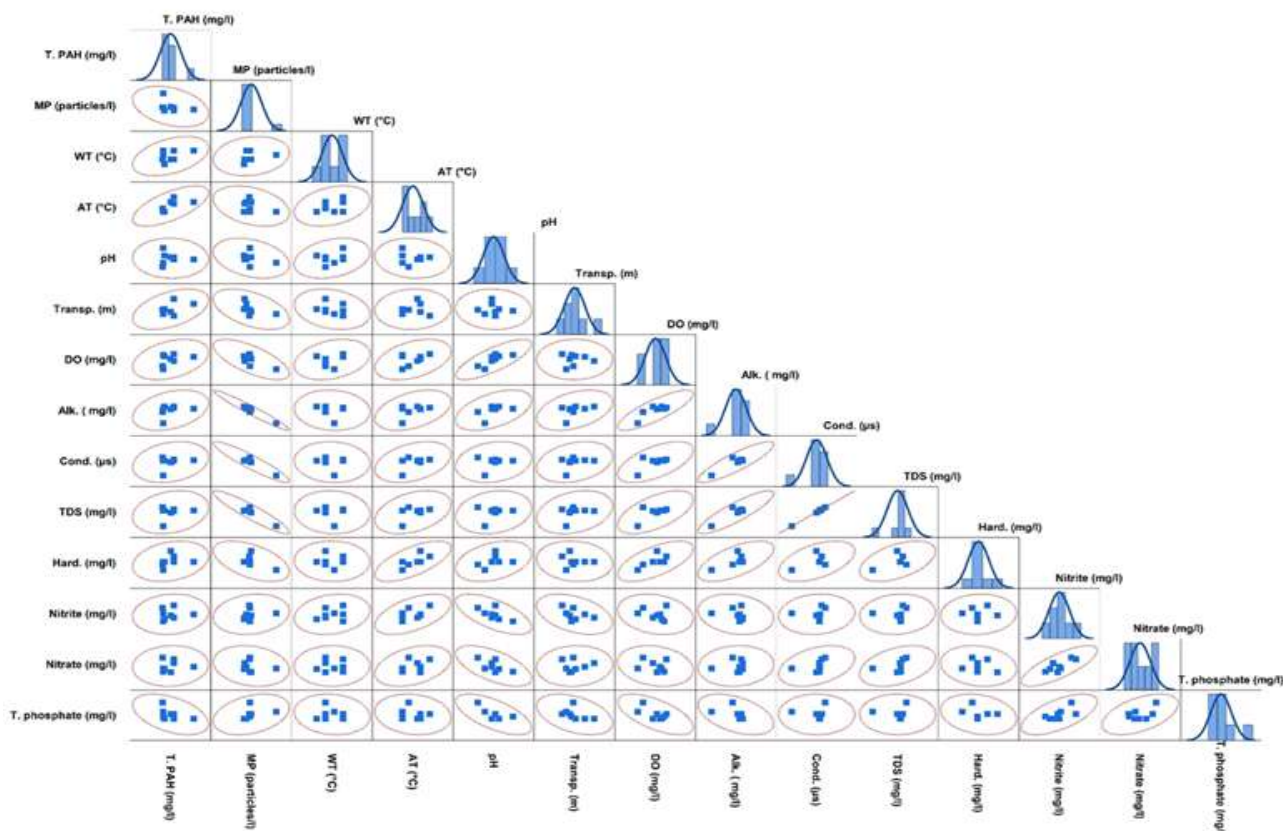


Figure 8. Scatter matrix plot showing correlation between PAHs, MPs with all physicochemical parameters.

4. Discussion

4.1. Physico-chemical parameters

All physicochemical parameters varied among all the sampling stations. pH range (7-8) of Ansupa lake indicated its slight alkaline nature, which was also supported by its alkalinity range, i.e., 46 -66 mg/L. The pH range obtained in our study, i.e., 7 to 8, was within the range obtained by Panda et al., 2016 [28] in Ansupa lake, i.e., 7-7.9. In water bodies, dissolved oxygen concentration is regulated by the amount of oxygen dissolution in water and photosynthetic activity, which ultimately depends upon the water temperature, water agitation, types and number of aquatic plants, light penetration, and amount of dissolved or suspended solids. DO levels between 5-8 mg/L are satisfactory for the survival and growth of aquatic organisms. In the present study, minimum and maximum DO values ranged between 2 mg/L (at S1) and 9.3 mg/L (S8). In a lake ecosystem, both organic and inorganic phosphorus remain either as dissolved state in water or remains attached to particles present

in the water column. Total phosphate value in our study was observed to be very low because of water column stability that inhibits vertical mixing of phosphate from the sediment [29]. The minimum and maximum range of phosphate concentration obtained in our study (0.001 to 0.008 mg/L) was lower than the range reported by Panda et al., 2016 where they observed a range of 0.012 to 0.094 mg/L [28], and Behera and Bhatta, 2021 observed average phosphate value of 0.254 mg/L in Ansupa lake [30]. Nitrate concentration observed in this study was high as compared to phosphate because phosphate gets absorbed to bottom sediment. Nitrate in Ansupa lake ranged between 0.049 and 0.153 mg/L, which was lower than the maximum permissible limit of nitrogen concentration, i.e., 0.48 mg/L [28]. The average TDS concentration at all the sampling stations was observed as 14.89 mg/L, which was very low as compared to the EPA-reported permissible limit of 500 mg/L.

4.2. PAHs distribution, source and ecological risk assessment.

Observed PAHs concentration in our study was higher than the result obtained by Meng et al., 2019 [31], in great lakes of China (4.0 to 12,970.8 ng/L), and Zhao et al., 2015 [32] in Taihu lake of China where they observed PAHs concentration of 45.40 to 232.74 ng/L during winter and 49.53 to 197.72 ng/L during summer [32]. Highest PAHs concentration was observed at S5, S6, S7 and S8 which were present near the zone of high recreational boating activities. High concentration of PAHs in this station might be due to petroleum derived compound and black smoke that are released from various small motor boats along with PAHs from nearby agricultural lands. The lowest concentration of PAHs at S4 (weedy areas) may be attributed to the presence of macrophytes, such as hydrilla and water hyacinth, which play a major role in the natural remediation of toxic heavy metals and pollutants from water. Due to the cleaning property of macrophytes, they play an important role in maintaining the stability of the littoral zone of the lacustrine ecosystem [33]. Low molecular weight 2 and 3 ring followed by 4 ring PAHs were detected at higher concentration as compared to 5 and 6 ring high molecular weight PAHs. Dominance of LMW PAHs in water samples may be attributed to their high aqueous solubility and high vapor pressure [34]. A similar type of results was obtained by Kurwadkar et al., 2022, where they observed dominance of LMW and MMW PAHs in the surface water sample of the Mahanadi River basin [35]. In contrast, HMW PAHs were present in high concentration at S1, S2, and S4 because of low water depth in these stations, which resulted in resorption or leaching of HMW PAHs from sediment to surface water during vertical mixing. The highest percentage occurrence of B(b)F (87.5%) might be attributed to its potential sources like fossil fuel combustion, vehicle emissions, wastewater released from nearby areas, pesticide and fertilizer use, chemical and dye manufacturing, etc. [36]. Analysis of samples indicates both petrogenic and pyrolytic PAHs sources, and petrogenic PAHs present at S5, S6, S7, and S8 may be due to the release of petroleum products and smoke from small motor boats operating very close to the docking area of the lake. The presence of petrogenic PAHs in these stations was also supported by the presence of high concentrations of LMW PAHs. LMW PAHs are originated mainly from petrogenic sources and may cause acute toxicity to the surrounding organisms [37]. Pyrolytic PAHs from S2 and S4 may be attributed to anthropogenic activities or combustion related emission done by these areas. Overall, PAHs pollution in Ansupa lake is carried out by both petrogenic and pyrolytic sources, which is also supported by Berríos et al., 2025 and Nayak et al., 2025 [38, 15]. Ramsar sampling site is contaminated by mixed source of PAHs pollution, where oil leakage and combustion-based emission contributed the maximum towards petrogenic and pyrolytic sources, respectively [38]. The results (Table 5.) show RQ_{MPCs} value greater than 1 which indicate that there is a

certain ecological risk to the organisms. RQ_{MPCs} value for Naphthalene, Phenanthrene, fluoranthene and chrysene were less than 1 which suggested that risk caused by these compounds might be low or negligible. Result from ERA table indicate that the ecological risk for Σ PAHs is high at Ansupa lake having ΣRQ_{NCs} value greater than 800. Therefore, strict management measures, particularly for 3 ring and 4 ring PAHs along with certain remedial approach must be taken to control PAHs pollution in this Ramsar site.

4.3. Analysis, polymer characterization and risk assessment of MPs.

The transport of microplastic particles is dependent on their shape, size, density, velocity of water, hydrodynamic mixing of water, and wind. The specific density, buoyancy and mobility of the polymers also play a major role in determining their spatial position. In general, high-density particles settle down at the bottom whereas, low density particles float on the surface of water. The water depth at S1 was very low with high anthropogenic activity which might be the contributing reason for higher accumulation of MPs [39]. S3 was distantly located from the area of human endeavors with high water level column supporting low accumulation of MPs. The possible sources of these MPs are land-based sources such as littering, agricultural and house runoff, waste from industries, rain, surface runoffs, and atmospheric deposition through wind [40].

Fibre type of microplastics was the most dominating form of microplastics observed in the present study. Similar results have been obtained by Negrete Velasco et al. (2020) in lake in Switzerland (Sassolo) [41], and Kaliszewicz et al. (2020) in fresh water of Poland [42]. Likewise, Uurasjärvi et al., (2020) reported occurrence of 64% fibre form of MPs in northern European lake [43]. Their possible sources of fiber form of MPs in fresh water ecosystems could be textiles, plastic bags, laundering, fishing nets, and plastic packaging, which could have been a possible explanation for the dominance of fiber type in the present study [44]. The spectra obtained (through FTIR analysis) from the present study were compared to characteristic peaks reported by Nandiyanto et al. (2023), Strong. (2021), and Jung et al. (2018) [45-47]. In the IR spectra, C-H stretching ($2964-2923\text{ cm}^{-1}$ for asymmetric) and C-H stretching ($2855-2848\text{ cm}^{-1}$ for symmetric) were common in PU, PS, PP, ABS, PE, HDPE, LDPE, and PA, whereas the N-H stretch around 3400 cm^{-1} was found in PA in S4. Spectra of PE, HDPE and LDPE showed absorbance band at same wave number as $1459-1400\text{ cm}^{-1}$ represents CH_2 bending, and CH_2 rocking was found at $856-612\text{ cm}^{-1}$. The C=O stretching present in PU, PET, and PA fell under $1743-1622\text{ cm}^{-1}$ whereas the C-O stretch was found at $1261-1019\text{ cm}^{-1}$. Characteristic absorption bands for PVC

were C-C stretching, and C-Cl stretching ($612\text{-}670\text{ cm}^{-1}$). PA have C=C stretch ($1655\text{-}1622\text{ cm}^{-1}$) and imide ring of carbonyl (1743 cm^{-1}) in S1&4 only [48].

From the above characterization the % occurrence was found to be highest for polymers such as PE, PS, HDPE, LDPE, PVC, PET, and ABS in water samples. The high concentration of PS and PE was also reported by Mao et al., (2020) on surface water of Wuliangshuai Lake, north China [49], PE and PP by Xiong et al., (2018) in Qinghai Lake, China [50]. Similarly, Uurasjärvi et al., (2020) reported PE, PP, and PET majority in northern European lake [43]. The sources of PE and its derivatives in Ansupa Lake could be the most commonly used food packages, bottles, and rigid plastics [51]. The common sources of PVC are rain coats, bottles, shoes, seat covers, plastic film, cups, and curtains among which the possible sources of contamination in Ansupa Lake could be breakdown products of pipes, bottles, packaging, and windblown dust. The electronic wastes, toys, pipes, house-building, refrigerator liners, and auto-parts can lead to ABS contamination while for PS packaging and disposable plastics act as the source. In the current study the sources of ABS might be the house hold wastes having electronic wastes, toys, and building equipment whereas, the sources of PS might be disposable plastic packaging. The sources of PP are fishing nets, pipes, carpets, textile floor coverings, plastic tools, and sports wears [52]. As the settlement around the lake depends upon fisheries, the PP polymer might have leach out from fishing nets. The commonly occurring PA contaminants are seats, door knobs, electronic materials [53]. From which electronic wastes in urban runoffs could contribute to PA contamination in the Ansupa Lake. For the estimation of level of MPs contamination in water bodies, PLI was used worldwide. It is a standard monitoring method to depict the pollution load in different study stations [54]. PLI values were found to be below 10, indicating the Hazard level as I (Table.1). Based on the PLI values the sampled site shows low contamination of MPs. MPs abundance is associated with human lifestyle, population density, economic status, industrialization, fishery development, and anthropogenic activities [55].

Microplastics are composed of different type of polymers. These plastic polymers are formed of repeating monomers. Although plastics are inert compounds, there are several hazardous additives are often found attached to environmental MPs, which include plasticizers, PAHs, colorants, stabilizers, fillers, flame retardants, and sorbed organic and metal pollutants [56]. During weathering of MPs these chemicals can leach out to the environment threatening environmental health. To calculate hazard index PHI values are used and the same has been applied in our study. Using PHI values microplastic

contamination risk at Ansupa Lake was calculated and grouped under three levels of hazard assessment i.e III, IV, and V (Table.1). Even though the hazard score of polymers like PA, PP, HDPE, LDPE, PS, PET and PE are low yet they contribute to high PHI values. The MPs pollution in the present study areas follows serious risk category due to presence of MPs with high hazard score like PU, PVC, and ABS.

Strong negative correlation was observed between abundance of MPs and DO ($r=0.696$), alkalinity ($r=0.971$), conductivity ($r=0.928$), TDS ($r=0.928$), and hardness ($r=0.525$). MPs increase the pollutant load in the water which triggers consumption of oxygen for degradation, decreasing DO of the water [57]. Furthermore, MPs can adhere to microbial community and larger MPs can interfere with microbial photosynthesis decreasing their growth and DO level [58]. In the present study DO was found to be negatively affecting the MPs concentration which was also observed in in the rivers of Japan, and the Brantas River, East Java, Indonesia [59]. TDS is total dissolved organic and inorganic solids such as salts, minerals, and metals present in the water. MPs can absorb organic and inorganic pollutants like POPs, HMs, and PAHs through hydrophobic interaction which in turn reduce the dissolved organic solids in the water [60]. Which supports the negative relation between MPs and TDS. Similar results have been obtained in Progo River, Indonesia [61]. From the correlation matrix it was observed that MPs showed strong negative correlation with DO, alkalinity, conductivity and TDS. Buwono et al., (2021) also stated high solubility of fiber form of MPs with water having low TDS and conductivity. Mahagamage et al., (2025) also observed a negative correlation between MPs and alkalinity as well as between MPs and TDS. The results indicate that sites having higher microplastic load might show higher organic contamination decreasing the overall quality of water. Wetlands are crucial ecosystems that provide livelihood for people besides supporting rich biodiversity. The area of Ansupa lake has reduced from 320 hectares to 231 hectares [62]. Management of Ansupa lake is required as the lake recharges the surrounding ground water level. Groundwater level decreases whenever Ansupa Lake shows more siltation, growth of weeds and algae. Ansupa lake not only supports aquatic biodiversity but also migratory birds. The primary problem is soil erosion, leaching and decrease in the depth of the lake. Soil erosion can be minimized by increasing plantation drive and removal of weeds at regular intervals [12]. Hence regular monitoring can provide information about the

water quality status and biodiversity leading to precision based policies to strengthen the ecosystem.

5. Conclusion

Ansupa wetland is significant as it is the largest freshwater lake and supports rich biodiversity. It also acts as an important source of livelihoods of local communities who depend on fishing. Due to increase in eco-tourism and other anthropogenic activities, microplastic as well as polyaromatic hydrocarbon load is increasing in the oxbow lake, which will impact the biodiversity and water quality. Microplastics and Polyaromatic hydrocarbons might be entering the wetland through wastewater discharge, agricultural runoffs from nearby rice fields, and degradation of plastic litter due to tourism. The sampled wetland confirms the presence of both the pollutants capable of impacting aquatic fauna and flora through accumulation in the food chain. The base line data generated through the present study will help in strengthening the restoration strategy and decreasing the pollutant load. PAHs can bind to microplastics and increase the severity of the contaminant. The need of the hour is to implement various mitigation strategies and also adhere to the existing measures to address the emerging threat to Ansupa wetland.

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