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DETECTION AND CHARACTERISATION OF MICROPLASTICS IN ANIMAL FEED

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ABSTRACT: Microplastics (MPs) the products of plastic breakdown, are entering the environment as a result of plastic abuse, which are of size less than 5mm. Due to their ubiquitous nature, MPs have become a significant environmental concern. One alarming area of MPs contamination is their potential presence in the feed of edible animal species. Growing research suggests that MPs can enter food products and subsequently move to various trophic levels of food chains. Hence, assessing the threat of MPs contamination in animal feed is important for food security and human health. In this investigation, 36 livestock and poultry feed samples were collected from 12 different farms, MPs were detected using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimeter (DSC). The Nano particle analyser was used to determine the size distribution, and Pyrolysis-GC/MS was used to quantify MPs. According to the findings, all the feed samples contained a significant amount of Polyethylene terephthalate (PET), Polypropylene (PP), and Polyvinyl chloride (PVC) and the particle size ranged from 2.02 to 10.7 µm. Present study has given detailed information on the size distribution of MPs in animal feed, which is thought to enable them to pass through membrane barriers. From the findings it is evident that there are high chances of MPs entering animal feed due to the continuous contact of the feed with plastic-based materials. These MPs can accumulate in the tissues of animals and potentially be transferred to humans through the consumption of meat, milk, and other animal-derived products. Subsequently these MPs can finally bio-accumulate in humans and cause serious health issues.

Keywords: Feedstuff, Membrane barriers, Nanoparticles, Pyrolysis-GC/MS, Size distribution.

INTRODUCTION

The industrial revolution has escalated the economic growth of the country while depleting the social and health standards of the poverty line. The ever-racing production of daily products also creates equal and sometimes more pollutants that are discharged relentlessly into the ecosystem (Pan et al., 2022). The lagging pace between the pollutant generation rate and their degradation rate has necessitated the introduction of new and logistic methods to deal with it. The most important and serious problem in the present day is microplastics (MPs; Fadare et al., 2020). The use of various kinds of plastics as raw materials, packaging material and ingredients in many industrial sectors due to their cheaper price has made it inevitable to be avoided. The flushed MPs without any primary treatment in the environment can cause many natural imbalances thus disrupting the ecosystem (Reeves et al., 2022). Many new scientific researchers are trying to find alternatives and techniques that help in minimising and eliminating microplastic entry into our daily lives.

The pervasive nature of MPs has made it a hit spot for many scientists. MPs can be classified into two major groups depending on their origin (1) Primary MPs (2) secondary MPs (Du et al., 2020). Primary microplastics are commercially synthesised as such in small size for many industrial applications such as cosmetics, textile industries, for making fishing nets or any other filtering materials, while the secondary MPs are the result of fragmentation, decomposition or recycling of macro plastic materials. The surface characteristics of MPs encourage both heavy metal adsorption and desorption. The heavy metals trapped on to these microplastics surfaces cause additional stubbornness to them by creating complexes that are hard to decompose (Hale et al., 2020).

The contamination of water and soil with these MPs had led to a channel for them to enter the food chain (Picó and Barceló, 2019). These MPs, being in nano-micro scale can easily enter the plant and animal biological system through osmosis. The MP content gets stagnated in the food storage bodies of plants such as fruits, roots and leaves. When an animal or a human consumes these crops the amount of microplastic content multiplies depending on the quantity of intake. There they enter the bio-chain naturally (Lehel and Murphy, 2021). Moreover, the presence of MPs in animal feed is becoming prevalent these days. Fish meals are used to feed farm livestock like pigs, poultry, and fish because of their high-calorie count, good amino acid profile, and affordability. Fish contaminated with MPs can act as a source of MPs (Thiele, 2021). In addition, pig feed samples obtained from China, have shown the presence of polycarbonate (PC) and

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polyethylene (PET) (Xu, 2022). By the consumption of water contaminated with MPs, they directly enter the blood stream of any organism leading to its accumulation in the body and later creating macro lumps that obstruct the natural flow of fluids in the bio-system (Norberg-Hodge, 2006). MPs were identified in the excreta of farm animals like sheep which is a clear evidence of MP contamination in animals (Beriot, 2021). As there are very few methods and techniques available to recognise the presence of MPs a lot more research is needed in this area.

The major victims of this microplastic pollution are the aquatic plants and animals. Especially, fish that consume MPs through water have major respiratory and reproductive issues (Wang et al., 2020). The fish have special respiratory routes that involves transportation of water through the canals of their gills allowing exchange of gases. The accumulation of these MPs in these narrow canalised system makes it hard for the water flow (Koongolla et al., 2020). Apart from the obstruction of the respiratory gills, the consumption of these MPs effects the reproductive capacity of many microorganisms and fishes in the aquatic system that effects the overall balance in the ecosystem (Jin et al., 2022).

MATERIALS AND METHODS

Sample collection

All the animal feeds (cow, pig, chicken, and fish) samples were collected from different livestock farms located in and nearby locations of Visakhapatnam. A total of 36 feed samples (100g) were collected from 12 different livestock farms (cow feed A,B,C, pig feed D,E,F, chicken feed G,H,I, fish feed J,K,L). All the samples were carefully weighed and gathered in sterile glass bottles which were pre-cleaned and stored in a dry place until further analysis. The details of farms and the composition of feed was given in Table 1.

Table 1 - Composition of various animal feeds used in the study.

Animal	Farm	Composition
COW	A	Corn, straw, whole cotton seed, vitamins and minerals mix
	B	Basal grain, chopped hay, soybean hulls, vitamins
	C	Corn silage, crushed maize, wheat bran, mineral mixture
PIG	D	Soybean meal, meat and bone meal, wheat, and barley.
	E	Wheat, maize, wheat bran, peas
	F	Palm kernel meal, rice bran, maize cassava
CHICKEN	G	Soya, maize, bone meal fish meal, growth premixes
	H	Corn gluten meal, soybean oil, limestone mineral mixes
	I	Soy protein concentrate, crude fibre, mineral, and vitamin premix
FISH	J	Palm oil, groundnut cake and maize powder, bone meal, premix
	K	Mustard oil cake, poultry by-products, bone meal, vitamin mixes
	L	Oyster shell meal, soybean grits, mustard oil cake, mineral, and vitamin mixes

Processing feed samples

At the time of collection, the feed samples were in various forms (pellets, powder). The pellet form samples were finely ground and homogenized using a mortar and pestle. All the ground feed samples were oven dried to remove any moisture that might have been present. Stainless steel sieves (mesh size 5mm) were used for primary filtration to remove large-sized coarse particles which might obstruct the vacuum filtration process. In secondary filtration, the samples were passed through a glass fibre filter with a 20 mm diameter and 2μm mesh size. The MPs were separated from the samples using Accelerated solvent extraction (van der Veen et al., 2022) with tetrahydrofuran and methanol. Prior to transferring the filter papers to a pyrolysis cup with residue they were placed in clean petri dishes, and dried at 45°C in an oven for 4hrs.

Characterisation of MPs

The chemical composition of the particles was determined using FT-IR (Fourier transform infrared) in the region of 4000 to 400 cm^{-1} by Bruker and the thermal characteristics (melting points) of the polymers were assessed using DSC (Differential scanning colorimeter) STA7300 Thermal Analysis System. A technique that included heating from 30 to 300°C, cooling to 30°C, and then heating again to 300°C with nitrogen flow rate of 100 mL/min^{-1} was used for the analysis.

Surface Morphology and size analysis

The topography of the MPs was determined using Field Emission Scanning Electron Microscopy (FESEM, Carlzeiss Ultra 55). The average size of the extracted particles was obtained using Nano Particle Size Analyser (NPA), HORIBA SZ-100.

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Quantification using Pyrolysis - GC/MS

According to a previously described and approved procedure (Leslie et al. 2022 and van der Veen et al. 2022), the microplastic content of each sample was examined. The multi shot pyrolysis unit EGA/PY-3030D was used for the analysis in "double shot" mode. The sample was initially put into the pyrolyzer unit at 100°C and heated to 300°C at a rate of 50°C/min. The GC/MS measurement for any volatile substances on the filter began after the sample was withdrawn since they thermally desorb between 100° and 300°C. A 30-meter long, 0.25 millimetre thick, 0.25-micrometer Ultra Alloy-5 column was installed in the GC/MS (Agilent 6890 GC and 5975C MS). Measurements were carried out in split mode (1:50 split ratio) and full scan mode (m/z 33–500). On the GC column, the chemicals were recovered. The column was designed to operate for a total of 20 minutes, rising from 40°C (2 min) at a rate of 20°C/min to 360°C, then holding at 360°C for 2 min. The pyrolyzer was heated to 600°C following the thermal desorption stage, and the filter was reintroduced (1min) for the following measurement (pyrolysis). The column was configured to run for 20 min, going from 40°C (2 min) at a rate of 40°C/min to 360 °C (2 min). The components that are volatilized at 300°C are the substances that are desorbed in the first run (or "shot") and may comprise unpolymerized monomers, additives, and other sorbed chemicals. With the exception of PET, where the derivatization product already forms at 300°C and the results from both the first and second shots were combined, any monomers (such as benzene or styrene) that may have been present during this run were not taken into consideration when calculating the concentrations of plastic particles. The other polymer concentrations linked to the polymers were determined using the pyrolysis second "shot" chromatograms.

RESULTS AND DISCUSSION

In this investigation, a total of 36 animal feed samples, including nine samples each of cow, pig, chicken, and fish feed, were gathered. All the tested feed samples were found to contain MPs, which was confirmed using FTIR and DSC, but the concentration and type of polymer identified varied among different feed samples and farms which can be seen in fig.4. While some of the feed were found to contain plastic particles which are visible to naked eyes, these macroplastics were separated during primary filtration process. Ten procedural blank samples were collected, analysed, and compared to the feed samples in the same series and all the experiments were performed in triplicates.

Characterisation of MPs

Identification of the MPs is essential, hence FTIR seems to be a preferable method for examining their properties with respect to functional groups. From the FTIR spectra (figure 2), functional groups specific to each polymer were observed. Polymers with peaks 1860, 1766, 1240 and 720 were confirmed as PET, samples which showed peaks 2898, 1415, 1258, 736 and 600 were identified as PVC, and polymers with peaks 2920, 1719, 1170, 1454, 970 were detected as PP. The outcomes of DSC (figure1) were in accordance with the results of FTIR. The endothermic peaks represented in the graph indicate the melting points of the respective polymers, while the ascending points indicate the exothermic reactions. A slight shift can be seen in the melting points of PP and PET with peak temperatures 160±3°C and 260±3°C, respectively, but the endothermic peaks acquired in this study were in congruence with the data available in the literature (Guaita et al., 1985). The thermolytic behaviours of plastics are reportedly affected by thermolysis conditions and the status of plastic particles such as additives, particle size, degree of polymerization, and crystallinity (Guaita et al., 1985; Choi et al., 2021). These findings confirm that PP and PET were the most widely utilised and discarded polymers, which is incompetence with other recent studies that demonstrated their pervasive prevalence in cattle and poultry farms globally (Geyer et al., 2017). Plastic bottles, polythene bags and disposable plastic items can be a possible source of PET in the feed samples because PET was found in significant amounts in all the samples (Peez et al., 2019).

Quantification, size analysis and surface morphology

The results of Py-GC/MS show that each sample had a different distribution of polymer types and concentrations. The highest concentration of PET was 693μg while PP and PVC were in the range of 597μg and 553μg respectively (table 2a-2b). In a single sample, up to three different types of polymers were discovered. When compared among all the farms (cow, pig, chicken and fish) the highest concentration of microplastics was extracted from the samples of chicken farms (G, H, I) then followed by the fish farms (J, K, L) which can be observed from Figure 4. Microplastic particles ranged in size from 2.02 to 10.7 μm, with a higher proportion of those in the 3-6 μm size range compared to other ranges. Based on the outcomes these particles can have the ability to enter the blood stream by crossing the intestinal membrane barrier (Luo et al., 2019). According to literature MPs can occur in different shapes like fibres, fragments, pellets etc., among which MP fibres were found to be more toxic and damaging compared to other shapes of MPs (Thornton Hampton, 2022). In the present study, to know the physical form and shape of extracted MPs, they were observed under the Scanning electron microscope. Few Scanning electron microscopic images of the extracted MPs from various farms (Figure 3) exhibited fibre-like MPs with surface fractures and disrupted structures. The disintegration of the fibres suggests that these plastics might have undergone mechanical weathering. The surface properties of a polymer play a key role in determining its adsorption abilities. The surface morphology of the extracted MPs may facilitate the adsorption of heavy metals, toxic chemicals and microorganisms from their surrounding environment onto their surface further increasing the risk of these contaminants.

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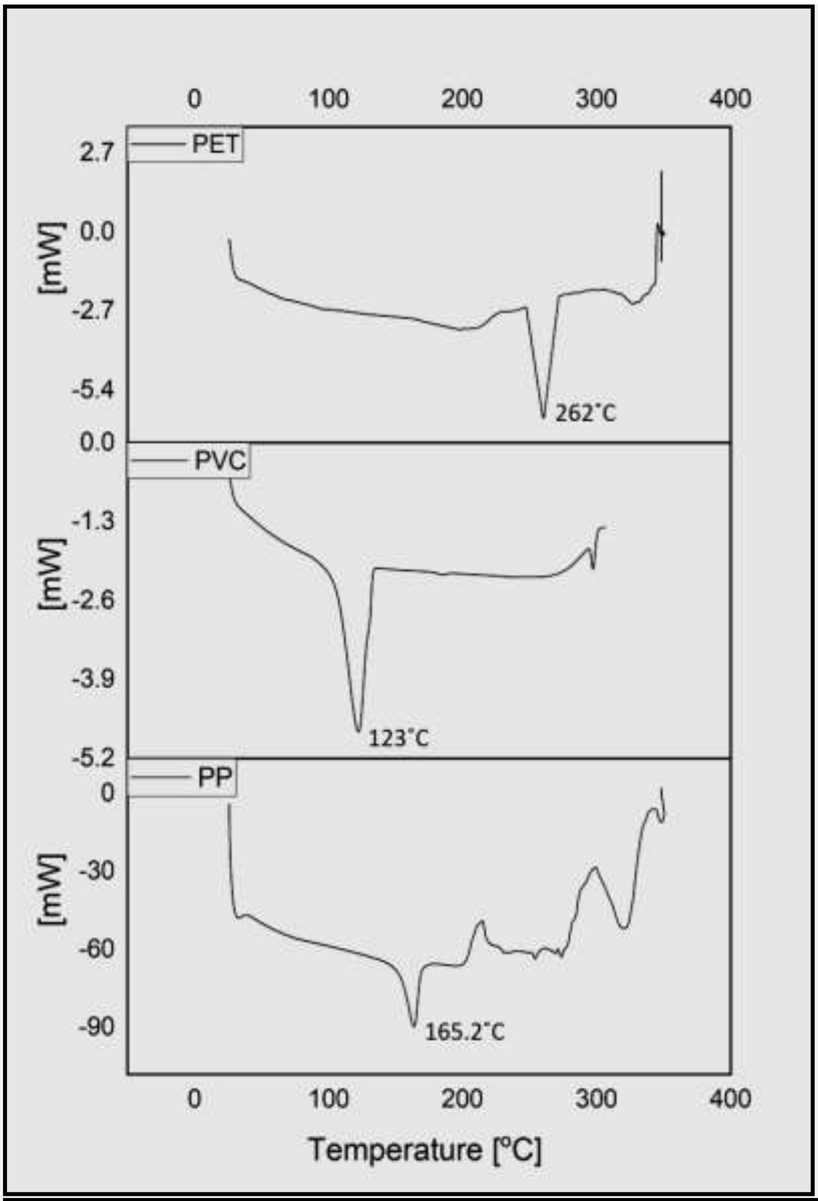

Figure 1 - DSC curves of polymers illustrating the melting points of MPs detected in the feed samples.

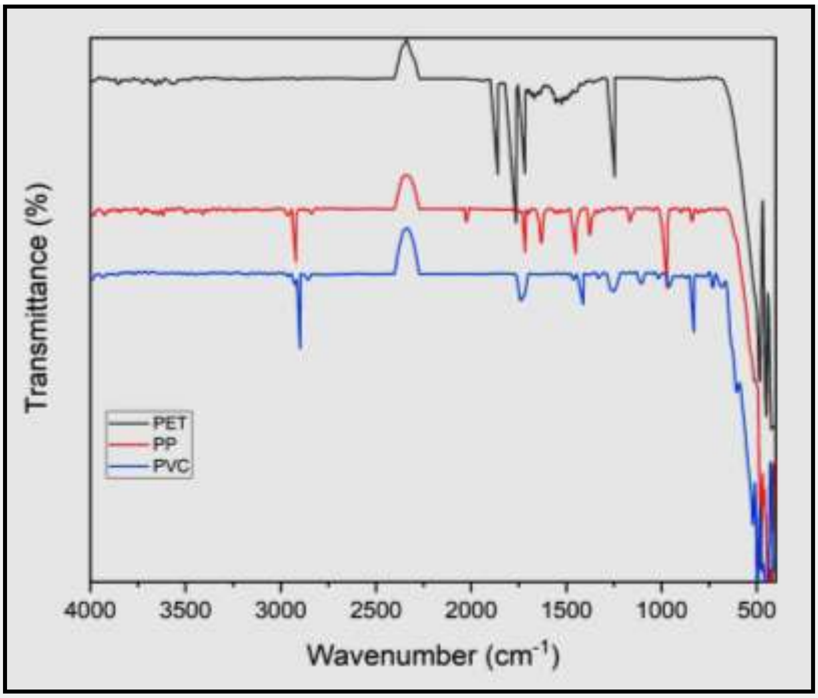

Figure 2 - FTIR Spectra of polymers identified in the feed samples

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Table 2a - Concentration (µg/g) and size of MPs in feed samples cow feed (A-C) and pig feed (D-F)						
Sample No.	Feed type	Farm	PP	PET	PVC	Size (µm)
1	Powder	A1	163	173	–	2.1
2	Powder	A2	164.7	171.9	–	2.0
3	Powder	A3	165.9	172	–	2.02
4	Powder	B1	–	89	601.3	9.24
5	Powder	B2	–	88.6	600.6	9.0
6	Powder	B3	–	87.8	602	8.9
7	Powder	C1	245	325	482.6	11.0
8	Powder	C2	246.6	324.4	483	10.70
9	Powder	C3	247.2	325.8	483.8	10.1
10	Pellet	D1	98	237.3	95.9	7.0
11	Pellet	D2	97	236	96.7	7.1
12	Pellet	D3	99.6	236.7	97.1	6.93
13	Pellet	E1	322	156	238	6.0
14	Pellet	E2	320.9	157.5	238	6.44
15	Pellet	E3	321.5	156.7	238	5.44
16	Pellet	F1	–	586.8	–	8.93
17	Pellet	F2	–	587.6	–	8.02
18	Pellet	F3	–	588.1	–	7.8

PP- Polypropylene; PET- Polyethylene terephthalate; PVC- Polyvinyl Chloride

Table 2b - Concentration (µg/g) and size of MPs in feed samples chicken feed (G-I) and fish feed (J-L)						
Sample No.	Feed type	Farm	PP	PET	PVC	Size (µm)
19	Powder	G1	84.9	693	366	10.61
20	Powder	G2	83.5	691	367.8	9.6
21	Powder	G3	84	693.5	367	10.0
22	Powder	H1	–	348	420	9.43
23	Powder	H2	–	348.5	421	9.0
24	Powder	H3	–	347	420.8	8.93
25	Powder	I1	351	161	340	7.65
26	Powder	I2	351.9	163	341	7.0
27	Powder	I3	350	162	341.6	8.0
28	Grain	J1	157.3	93	257	9.84
29	Grain	J2	158.3	93	2576.9	8.84
30	Grain	J3	158	92.1	258.2	9.0
31	Grain	K1	597	519	–	7.67
32	Grain	K2	596	519	–	7.09
33	Grain	K3	596.4	518	–	6.67
34	Grain	L1	–	319	158	8.02
35	Grain	L2	–	565	158.4	8.9
36	Grain	L3	–	288	157	9.0

PP- Polypropylene; PET- Polyethylene terephthalate; PVC- Polyvinyl Chloride

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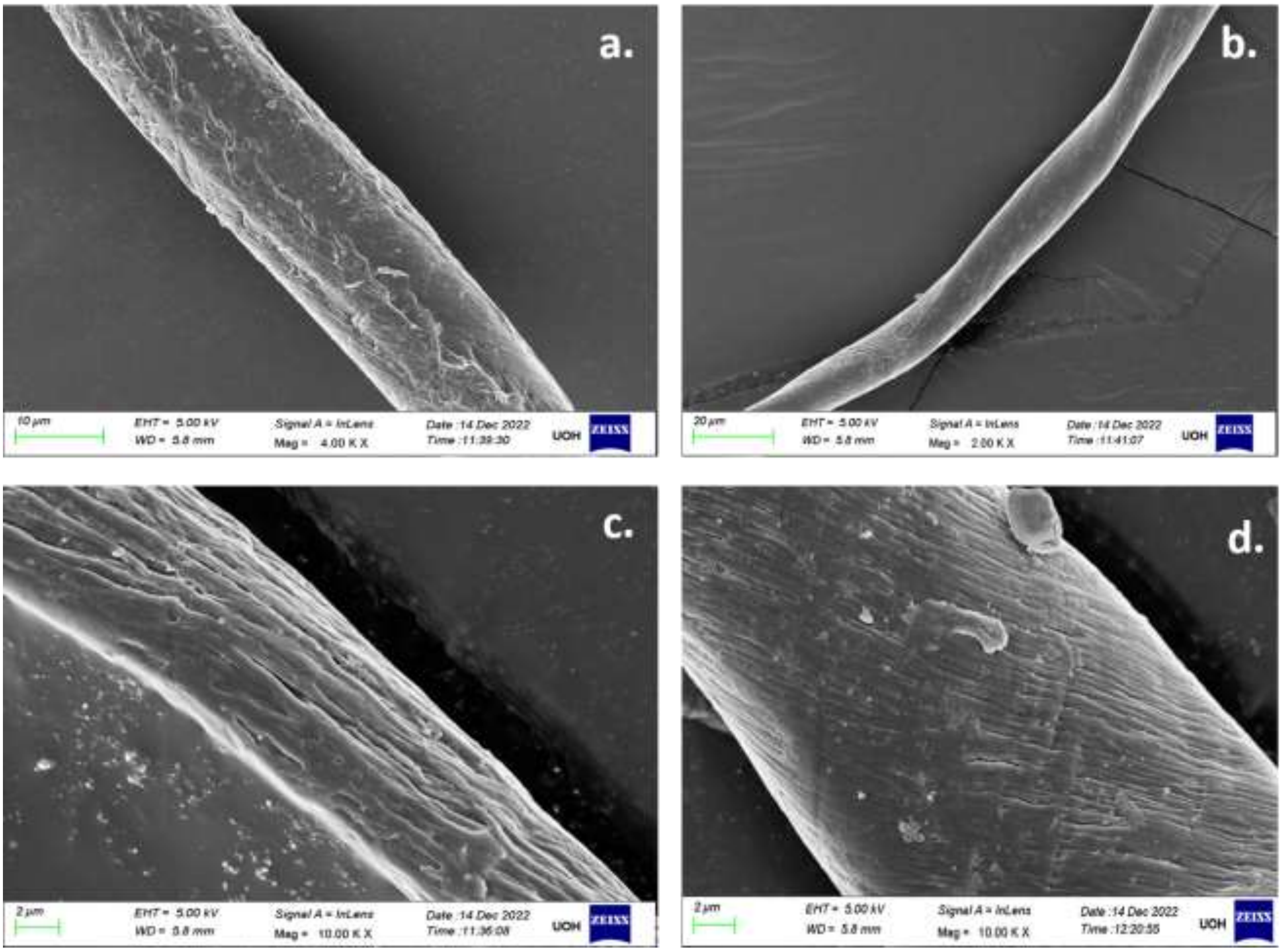

Figure 3 - SEM images of microplastics detected in the feed samples from various animal farms, a) Cow farms b) Pig farms c) Chicken farms and d) Fish farms.

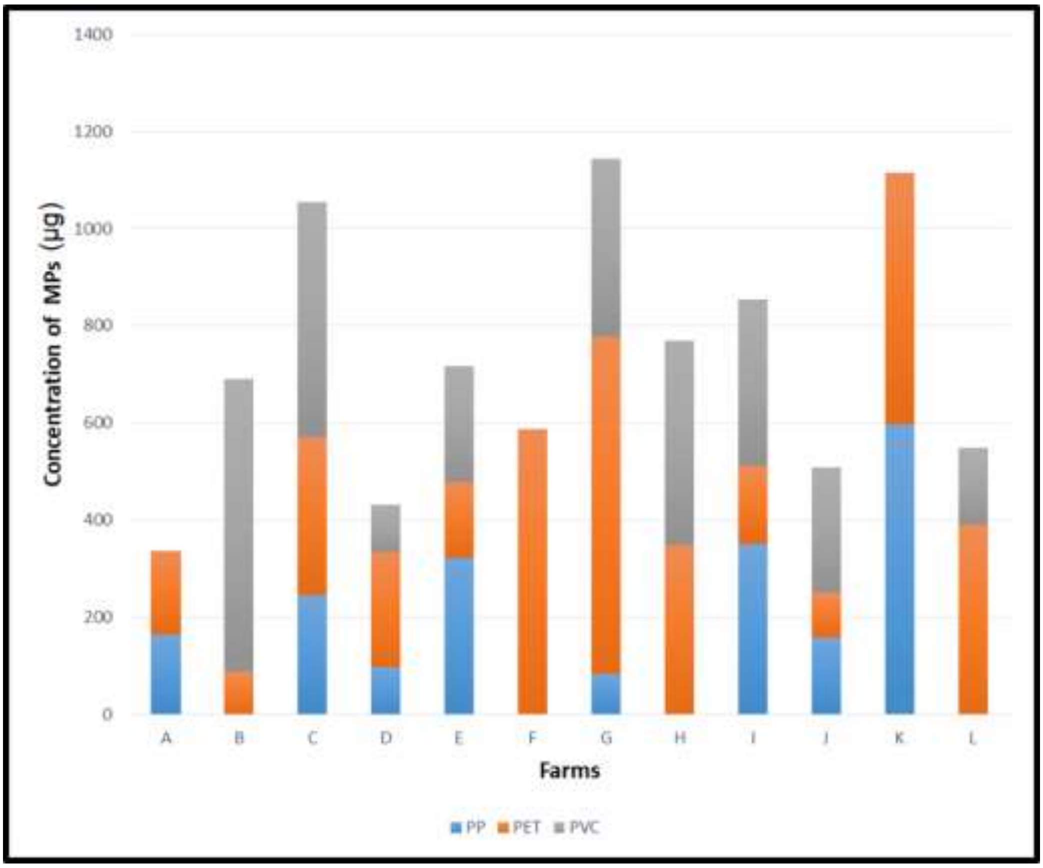

Figure 4 - Graphical representation of concentration of different types of polymers detected in all the farms (A-L)

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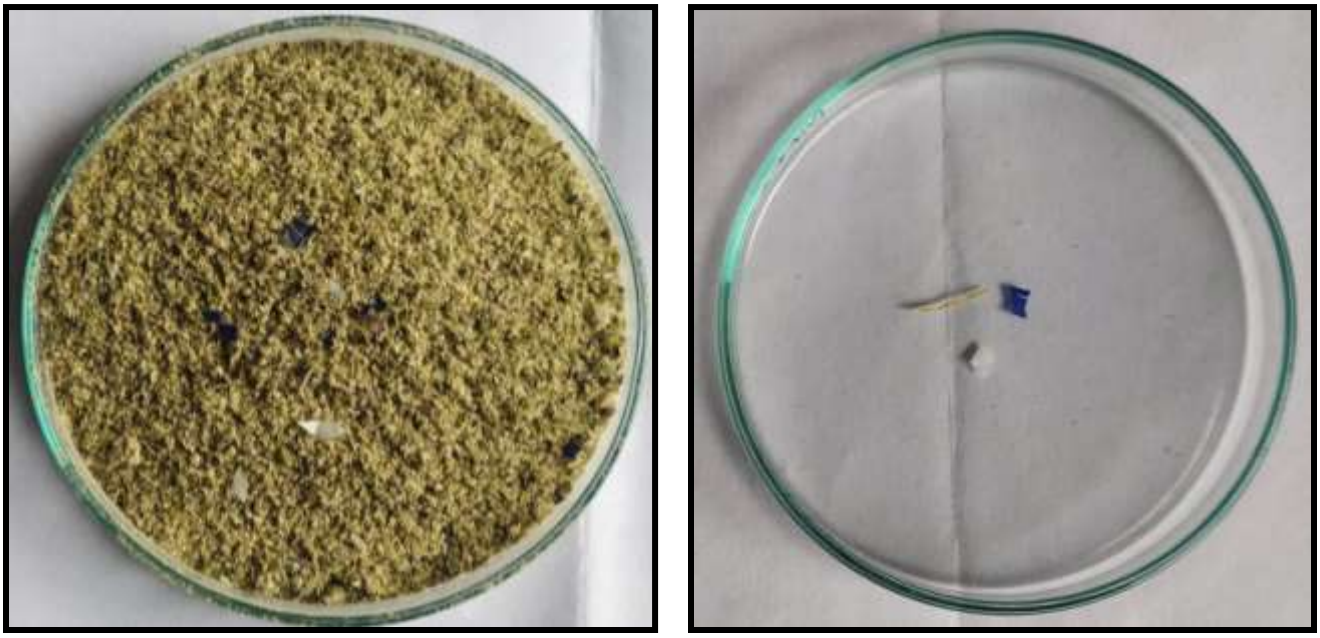

Figure 5 - Macroplastic particles were observed in the few samples, which were separated during the process of sieving

Sources of MPs into feed and their health effects

There can be numerous ways in which MPs enter the livestock feed. The main entry pathways for MPs into grazing-based and mixed livestock production systems have been identified as plastic mulching, fragmentation of plastic wastes, and stream water discharge (Ramachandraiah et al., 2022). MP pollution in farmyards was extensive, which may be related to a lack of trained cleaning personnel. Additionally, the inner surface of feedbags is made of PET, while their exterior is constructed of PP. Some animal farms use liquid feeding systems, which deliver the feed through PVC troughs or pipelines (De Lange et al., 2006). On the other hand, feed mixers, transporting vehicles, feed storage tanks, and other anthropogenic activities can contribute to the MP contamination of feed. A recent study suggested that MPs might also reside in the digestive system, and given the co-occurrence of PP, PE, and PR MPs in animal manure and feed, it is plausible that these MPs could be passed out by the cattle when they consume contaminated feed thereby polluting the soil (Stock et al., 2020). There have been several irreversible, long-term health impacts linked to MPs. The digestive gland's oxidative balance was altered in the mussel *Mytilus spp.* after exposure to polystyrene micro beads (2 and 6 m, 32 g/L, for seven days; decreased catalase and glutathione reductase activities, as well as lowered lipid peroxidation (Paul-Pont et al., 2016). Furthermore, numerous animal studies indicate that ingesting microplastics impairs crucial intestinal processes like the maintenance of the gut barrier and the maintenance of the gut microbiota. These plastic-associated abnormalities may increase immunological, inflammatory, and metabolic ailments, given the multifunctional nature of the intestinal system.

Microplastic exposure has been associated with immune system dysfunction in a number of investigations involving invertebrates. The intestinal lamina propria and the draining mesenteric lymph node are home to myeloid cells, innate lymphoid cells, and T cells, which are crucial fundamental units of the immune system. On exposure to MPs serious damage to these cells was observed. When exposed to polystyrene particles (500 nm and 30 μm), hemocytes showed several abnormalities, including a significant drop in cell count and phagocytosis activity, as well as various alterations in immunological markers related to oxidative stress, apoptosis, and inflammatory response (Shi et al., 2020 and Tang et al., 2020). The dominance of plastics in our daily lives is attributed to chronic, continuous exposure to MPs, according to the growing body of research.

CONCLUSION

One of the potential absorption pathways is through food or feeding. Based on the outcomes of the present study it was understood that severe MP contamination occurred in cattle and poultry farms, mirroring the situation in the land and aquatic environment. Among all the feed samples PET was very predominant. MPs were discovered in all 36 feed samples with an average size ranging between 2.02-10.7 μm. The consumption of feed contaminated with MPs is not only harmful to animals but also to humans (tertiary consumers), thereby leading to bioaccumulation of MPs in the food chains. Thus there is an immediate need to implement modern strategies to prevent the MPs issue from getting worse.

DECLARATIONS

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Authors' contribution

Sharon Sushma carried out major part of experiments, analysis, and assisted in data curation. Akkina Rajani chowdary is involved in data curation, analysis, overall supervision of the entire research, and prepared the manuscript.

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Competing interest

The authors declare that there is no competing interest to this research publication.

Ethics committee approval

Not applicable.

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