THINK INDIA JOURNAL ISSN: 0971-1260

Vol-22-Issue-16-August-2019

Isolation and Identification of Microplastic Particles From Agricultural Soil and Its Detection By Fluorescence Microscope Technique

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The presence of microplastic in environment has been declared as an emerging pollutant. Mostly the reports have determined its presence in marine ecosystem. Limited studies were recorded from the freshwater environment and a very few based on land or soil. Analysing the microplastic contamination in soil will enlighten its impact on terrestrial ecosystem which was the aim of this study. Soil samples from agricultural land were collected from different depths to analyse its movement from surface to the deeper part of soil. Parallel to this study virgin and product plastic types were mixed in soil to calculate recovery rate of plastic particles by Air induced overflow method. FTIR spectroscopy and fluorescence microscope techniques were used for the identification and detection of plastic and its type. Polypropylene (PP) was reported with highest recovery rate of 42% among virgin plastic and among products high density polyethylene (HDPE) appeared to have highest recovery rate of 50%. The recovered particles were stained with Nile red fluorescent dye and observed under UV transilluminator. The stained particles revealed bright illuminations which further assisted in detection of microplastic particles. FTIR analysis confirmed the abundance of polyethylene in agricultural soil upto the depth of 15cm which is hazardous for the top layer organisms such as insects and annelids. Occurrence of microplastic in soil revealed a potential threat to terrestrial ecosystem. Moreover microplastic presence in soil may exhibit a pathway to contaminate groundwater aquifers as well. Besides adhering to soil particles microplastic may adsorb different contaminants in the surrounding. Therefore further research is required to focus on microplastic acting as a potential pathway for other contaminants.

Key words: Nile red, Soil contamination, Density separation, Food chain, Agriculture land

INTRODUCTION

The word plastic has been derived from the Greek word "plastikos" which means 'prepared to be formed into different shapes'. Plastics are made up of monomers that are bound together by covalent bonds. These are normally polymers of high atomic mass, and may

contain different substances to enhance their performance as well as decrease its costs. Plastic is semi natural material extracted from petroleum or oils. It is called as a polymer. It is different from natural polymers because natural polymers are derived from plants and animals where as synthetic polymers are produced in artificially in laboratory. The extreme utilization of plastics and increasing plastic waste transfer is a serious problem and the requirement for biodegradable plastics and biodegradation of plastics is needed. The mismanagement of plastics has increased its quantity in the environment.

It has been reported in Hong Kong that it is not the plastic which is toxic but the toxic chemicals which are adsorbed by it. The plastic particle being small in size resembles algae or sediments and it is taken by some species and thus it causes adverse effects on their health. So it is not the plastic which is considered toxic but the pollutants that it adsorbs. Plastics are composed of monomers (styrene, polyvinyl chloride, bisphenol-A) and additives (phthalates, nonphenol) which are hazardous and can migrate from source to media during polymerization reaction where some reactions are not complete and leave monomers that have low molecular weight. Some impurities, which are harmful and these particles migrate from the source to the media. Plastic debris is considered insert because they have small size and may comprise of monomers which are cancer causing and even estrogenic in nature and when these enter into an organism it can transfer into their tissues and cause harmful effects[1].

Natural plastic litter can experience activity like degradation and breaking down coming about because of the activity of physical, chemical, and biogenic drivers, as a result of this plastic contamination creates particles smaller than 5 mm, which are normally called as microplastics[2].

Types of degradation in plastic

Thermo-oxidative Processess: The polymer is heated to great degree high temperatures (430^{o}C). Fundamentally here the structure of the polymer gets artificially changed.

Mechanical Processes: In this process, the polymer is subjected to mechanical pressure that changes the properties of polymer.

Ultrasonic Methods: When the polymer is subjected to ultrasonic domain, the polymer chain may vibrate and get separated.

Other Chemical Methods: Here destructive synthetics, gases or fluids are utilized to disintegrate the polymer. Ozone, climatic toxins, and acids like nitric, sulfuric, and hydrochloric will strike and corrupt most polymers and cause oxidation.

Photograph oxidative Methods: The retention of radiation by polymers, or their impurities, due to daylight or high energy radiation can result in the breaking of compound bonds in polymers. This is significantly more feasible if the polymers had semiconducting properties in the noticeable or sun district light region.

The most secure approach which can be applied at large scale is natural process. Organic procedures do not damage the earth and results could be of incredible applications. Microorganisms for example microbes, parasites and actinomycetes are associated with the degradation of plastics. Plastics vigorously undergo degradation in nature by using fertilizer and soil. Carbon dioxide, water and methane are delivered through these fertilizers which aimed vigorous biodegradation. Microorganisms are capable to connect with the surface of a polymer. The extracellular chemicals emitted by the microbes cause the polymer chain to separate, prompting the arrangement of low-sub-atomic particles. These low sub-atomic particles are additionally utilized by the microorganisms as carbon which then becomes easier to get degraded

After the entry of microplastics in the environment, by means of wind, runoff of surface water, storms, etc its gets deposited in water bodies. The microplastics from fresh water reach to oceans by means of rivers but very less data is available. These plastics being smaller in size are ingested by various aquatic animals, birds and even mammals. Some contaminants also adhere with these microplastics and hence enhance pollution. Microplastics can be divided into two main types: primary and secondary microplastics. Primary microplastics are those which are directly released into the environment in small range. Whereas secondary microplastics are formed when fragmentation of large plastics occurs.

Most of the research is based on aquatic ecosystems while as very less on terrestrial ecosystem, however most of the plastic is being disposed on land. Presence of microplastics and its interactions with flora and fauna provides adverse effects on both aquatic as well as terrestrial ecosystem. Microplastics are responsible for making change in soil texture and various processes such as capillary action and pore size as well. Microplastics also cause

ISSN: 0971-1260

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changes in activity of microbes in soil by virtue of which soil functionality and organic matter might get changed[2].

MATERIALS AND MEHODOLOGY

In current work parallelly two sample used, one collected from agricultural ground and one is prepared in lab. The area of agricultural land was estimated as 4888.8889 square yards. The location (of site is near to market Maheru Phagwara Punjab, Law gate LPU) and one side is totally followed by road and other side by residential houses. The main crops cultivated from site are maize and wheat. Waste water is used for irrigation through pumping system; thereafter water is allowed to move through channels and along the surface of agricultural land. The random sampling was used for the study. Samples were collected from different depths of agriculture land at a difference of 5 centimetres each and total measuring depth of sampling was 45 centimetres.

For sample collection a solid iron hollow rod of size 70 centimetres (by measuring scale) and diameter of 2.5 centimetres (Figure A) glass bottles and measuring scale was used for sampling. Sampling starts from marking of measurement units on hollow iron rod using measuring scale in centimetres. Then incorporation of hollow rod in agricultural soil to a depth of 5 centimetres, 10 centimetres, 15 centimetres, 20 centimetres, 25 centimetres,30 centimetres, 35 centimetres, 40 centimetres and 45 centimetres respectively, and samples from each depth were collected in a glass bottles followed by weighing and drying at 60 ^{0}C in oven.

Fig. 1 A- Sampling rod

Fig. 1 B (1&2)- Showing aerator procedure for separation of particles from soil

To perform this experiment sand and soil were collected from different sites and mixed. This sample was equally separated into ten samples each weighing 102grams. Ten different types of virgin microplastics, without any additives (HDPE, LDPE, LLDPE, PA6, PA6.6. PS, PP, TPU, PVC (r), PVC (f) were cut into very small size (350micrometre- 420 micrometre).

Simultaneously ten different types of products, with additives were also cut into very small particles. From each type of microplastic (HDPE, LDPE, LLDPE, PVC (r)), PVC (f), PA6, PA 6.6, TPU, PS, PP) 50 particles were added in each soil sample (102gm). Air induced overflow method was done in order to separate the plastic particles attached to other substances. With the help of motor a pressure would be generated inside the beaker due to which the plastic particles which would be adhered to soil particles would get separated, NaCl solution would be added for density separation so that heavier particles would remain at bottom and lighter particles would float. More and more water and NaCl solution was added so that water present in the beaker would overflow into tray. Plastic particles being light would float and get collected into the tray due to overflow of water. The water from tray was again collected and transferred into another beaker and air was blown into it same way. This procedure was repeated again and again till the water in beakers became clear.
The water from tray was filtered using mesh and residue was collected and dried in the oven at 60 for 24 hours. The residue was again collected and weighed. The plastics particles which could be observed easily were separated, counted in separate petriplate and recovery rate of microplstics was calculated. Larger sediment particles were removed with the help of forceps. Smaller sediment particles which could not be recognized either as sediments or plastic were subjected to digestion process in separate petriplate. For digestion process 15ml of hydrogen peroxide was added to the sediments so as to degrade biogenic material present in the sediment for 10 days[3].

After the process of digestion this solution was filtered using watmans filter paper and then it was subjected to centrifugation at 3000r.p.m for 15 minutes. After centrifugation the supernatant was collected and checked for presence of any microplastic particle. The recovered particles were counted and their rate was calculated. The recovered particles were stained with Nile Red dye. Nile red stock solution was prepared by dissolving 0.05mg of Nile red in 1ml acetone and was stored in eppendorf tube at 4^0C. This stock solution was kept overnight for ageing for 24 hours and also for 48 hours. This stock solution was then diluted 10 times in n-hexane to make NR working solution. This working solution was used to stain all the recovered particles. After the staining of particles, they were air dried and left for 24 hours[4]. After 24 hours the stained particles were observed under UV transilluminator for fluorescence. Different particles were observed to emit different fluorescence and different particles were observed to have different capacity of retaining

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Nile red.

Table: List of plastic products taken for analysis

Types of plastic	Products selected for analysis
HDPE	Bottle cap
LDPE	Carry bag
LLDPE	Packaging material
PS	Disposable items
PP	Tips of micro pipette
TPU	Mobile cover
PVC (r)	Shampoo bottle
PVC(f)	Electric pipe
PA6	Tooth brush
PA 6.6	Rope

Along with Nile red attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) is also performed for confirmation of particles.

Results and discussion

Agricultural land:

Samples from different soil depths of agricultural land were collected from different areas (A, B, C, D and E) of agricultural site (Figure 2).

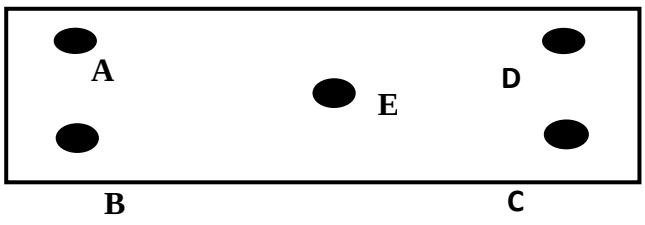

Figure 2: Diagrammatically representation of sampling site and different area of sampling. Rectangle: Representing Agricultural land (Sampling Site). Black dots: Representing Sampling areas within Agricultural land.

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Each soil sample of different depths were analysed for presence of plastic particles visually and four particles were collected by forceps; three blue coloured particles and one white coloured particle. Out of which two blue coloured were collected from the sample depth of 05 centimetres, one white particle from the sample depth of 10 centimetres and one blue coloured particle from the sample depth of 15 centimetres (Figure 2). These particles were assigned with different alphabets (A, B, C and D) because the nature of these collected particles were not identified yet. To confirm whether these particles are of plastic polymers or not hot needle test was followed.

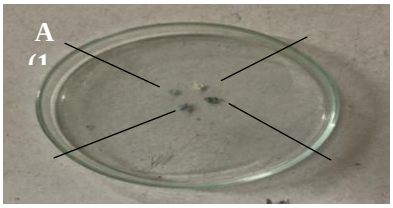

Figure 2: Particles collected from samples of different depths during visual separation. Size of particles: A (1.22µm), B (1.09µm), C (1.62µm) and D (0.90µm).

Hot needle test:

The collected particles were brought in contact with hot needle. When the particles were in contact with hot needle, they get deformed in their appearance such as reduction in size and change in shape or we can say that shrinkage of particles were observed. This phenomenon confirms the particles nature as plastics (Figure 3).

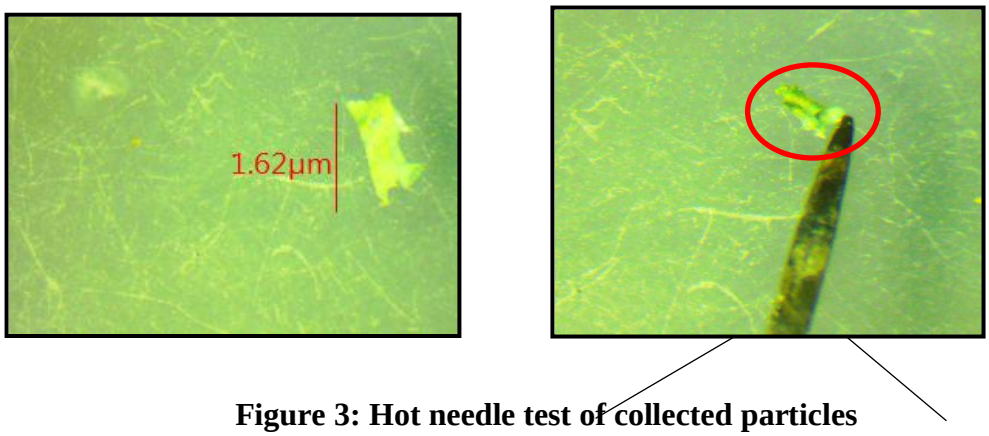

Figure 3: Hot needle test of collected particles

After Organic digestion by hydrogen peroxide (30% H_2O_2), the organic matter present in samples were degraded after seven days (Figure 4). Organic matter present in each sample depths were entirely degraded while the remaining matter undergoes certain visual changes

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such as get turn into transparent or discolouration. The organic matter present in samples was mostly from plant origin.

Figure 4A: Before digestion

Figure 4B: After digestion

Figure 4: Organic digestion of sample by H_2O_2

The collected and digested particles were subjected to FTIR (8400S, SHIMADZU CORPORATION) in order to specify the type of polymer present. The all four collected particles A, B, C and D were identified as polyethylene plastics because the spectra obtained from FTIR of collected particles showed presence of typical characteristic peaks around 2920 and 2850 cm^{-1} (CH_2 stretching), 1460 and 1473 cm^{-1} (CH_2 bending), 720 and 730 cm^{-1} (CH_2 rocking). Thus the all four collected particles confirm the presence of polyethylene group. In order to validate the result, the IR spectra of all four collected particles were merged with the virgin polyethylene and are identified as polyethylene.

Similarly the residue recovered from different depths (05 cm, 10 cm, 15 cm, 20 cm, 25 cm, 30 cm, 35cm, 40 cm and 45 cm) of agricultural soil samples after organic digestion and density separation were analyzed by using the Fourier-transform-infrared-spectroscopy (FTIR-8400S,) technique. The residues of sample depth 05 cm, 10 cm and 15 cm were obtained with the peaks at 2920 and 2850 cm^{-1} (CH_2 stretching), 1460 and 1473 cm^{-1}(CH_2 bending) and 720 and 730 cm^{-1} (CH_2 rocking). Thus this provides the confirmation about the presence of polyethylene in samples of depth 05 cm, 10 cm, and 15 cm. The encircled broad peaks in sample spectra are expected due to presence of different functional groups such as Alcohol (O-H stretching), Amine (N-H stretching, N-H bending), Alkene (C=C stretching), Carbonyl group (C=O stretching), Amide (C=O stretching, N-H bending), Alkyl Halide (C-

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F stretching), Ether (C-O stretching) and Ester (C-O stretching). The overlapping of IR spectra of 05 cm, 10 cm and 15 cm with virgin polyethylene confirms presence of polyethylene in their residues.

While as the IR spectra of sample depths from 20 cm to 45 cm were not analyzed as polyethylene because only two peaks at 2920 and 2850 cm^{-1} (CH_2 stretching) were same as that of polyethylene and remaining typical characteristics peaks of polyethylene at 1460 and 1473 cm^{-1}(CH_2 bending) and 720 and 730 cm^{-1} (CH_2 rocking) were absent. The overlapping of 20 cm sample depth with virgin polyethylene confirms it as non-identical polyethylene group and that of 40 cm.

Polyethylene type of plastics has vast range of application from very small scale to very large scale and has been considered most common type of plastic used nowadays and then obviously polyethylene contamination would be common. Apart from this survey of sampling site demonstrated that the sample site is near to market and residential houses. The products in market are totally bound to polyethylene (packaging) even common type of plastic contamination from homes is from polyethylene. Hence the chances of polyethylene contamination would be more as compared to other types of plastics no doubt others would be but the site would be more prone to polyethylene.

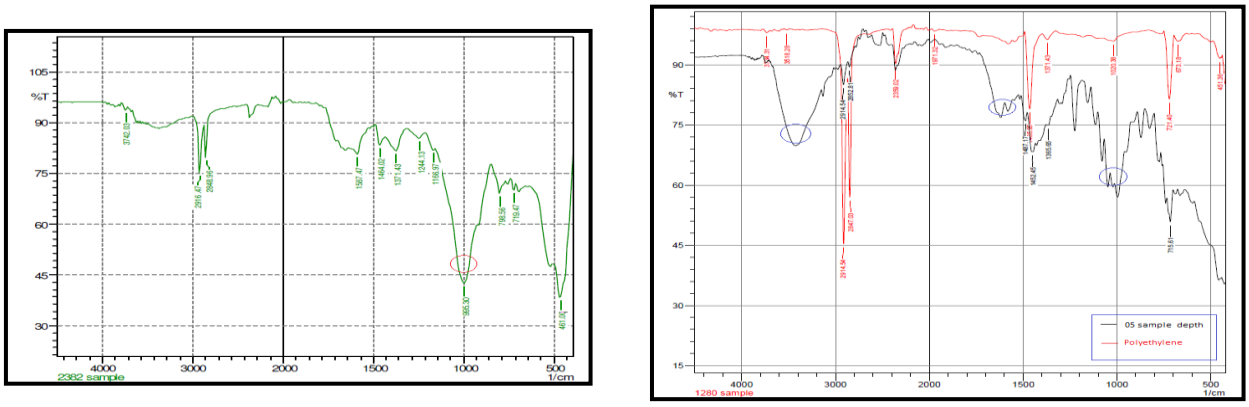

Figure 5 A : IR spectrum of particle A collected from samples during visual separation **B:** Virgin polyethylene merged with spectrum of residues collected from sample depth of 05 cm.

The particles selected to be used in our experiment were of 10 different types of plastics, virgin form as well as their products they were: high density polyethylene (HDPE), low density polyethylene (LDPE), linear low density polyethylene (LLDPE), polypropylene

(PP), polystyrene (PS), polyvinyl chloride rigid (PVC(r)), polyvinyl chloride flexible (PVC(f)), polyamide.6 (PA 6), polyamide 6.6 (PA 6.6), thermoplastic polyurethane (TPU). These plastics were selected because they are most commonly used plastics in daily lives.

The virgin plastic particles were brought from industry HI TECH POLYMER PRODUCTS, Ludhiana, and their products were brought from market as they are easily available. The particles both virgin as well as their products were cut into small pieces (size = 0.394; weight = 0.77).

Among each type of plastic 50 particles were mixed in 102gm of soil sample separately. Soil sample was confirmed contamination free as well as plastic free by visual observation before mixing plastic in it. Then each sample was subjected to density separation method by adding NaCl solution (26%) and water. So that plastic particles being lighter float on surface of water and heavier particles remain at the bottom.

Plastic particles were attached to particles of soil, in order to separate them Air induced overflow method was done using motors, it induced bubbles in the water that enforced plastic particles to separate from soil. The plastic particles that float at the surface of water were separated by filtration process and then by visual observation. The residue from filtration process was subjected to organic digestion in 30% hydrogen peroxide (15ml) for 15 days. After 15 days biogenic material was found to be degraded and remainder was filtered and centrifuged. The supernatant was then filtered and then centrifuged. It was then examined for presence of any microplastic in it, after drying by visual observation.

All the recovered particles were counted. It was found that among virgin plastic particles and in products recovery rate was found to be different. Among virgin plastic particles polypropylene (PP) was calculated to have highest recovery rate i.e 42% and polyamide 6.6(PA6.6) was calculated to have lowest recovery rate i.e 10% (Table 1). Among products high density polyethylene(HDPE) was calculated to have highest recovery rate i.e 50% and polyvinyl chloride rigid (PVC(r)) was calculated to have lowest recovery rate i.e 12% (Table 2).

Table 1: Percentage of recovered particles in virgin plastics after adding them to soil:

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Particles	No of particles added in soil	No of recovered particles	Percentage of recovered particles
HDPE	50	14	28%
LDPE	50	15	30%
LLDPE	50	11	22%
PS	50	12	24%
PP	50	21	42% **high recovery
TPU	50	17	34%
PA6	50	14	28%
PA 6.6	50	05	10% *low recovery
PVC(r)	50	09	18%
PVC (f)	50	06	12%

Table 2: Percentage of recovered particles in product particles after adding them in soil:

Particle	No of particles added in soil	No of recovered particles	Percentage of recovered particles
HDPE	50	25	50% ****high recovery**
LDPE	50	19	38%
LLDPE	50	08	16%
PS	50	07	14%
PP	50	09	18%
TPU	50	11	22%
PA6	50	09	18%
PA6.6	50	12	24%
PVC (r)	50	06	12% ***low recovery**
PVC (f)	50	07	14%

Recovered particles were stained with Nile red, which a fluorescent dye (0.05mg NR in 1ml

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acetone) and was used to recolor polymer particles. After staining Nile particles were air dried and left for 24 hours. After 24 hours these stained particles were observed under UV transilluminator. All particles were stained by Nile red by diluting it 5 times as well as without diluting it. The dye was kept for ageing for 24hours and 49hours in both cases.

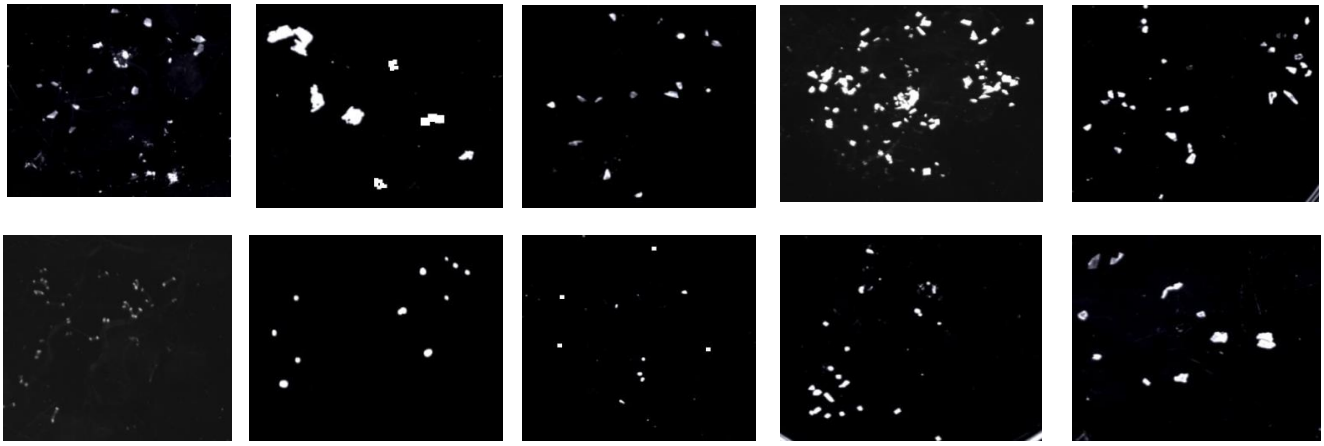

Figure 3: Images of products stain with Nile red and their observation under UV transilluminator (24 hour ageing)- A = HDPE ; B = LLDPE ; C = LDPE ; D = PA 6.6 ; E = PA.6 ; F = PP ; G = PS ; H = PVC (r) ; I = TPU ; J = PVC (f)

The product particles where again stained with Nile red dye without diluting it and observed under UV tansilluminator. All the particles exhibited illumination but low density polyethylene (LDPE) was found to exhibit less illumination than rest of the plastic particles

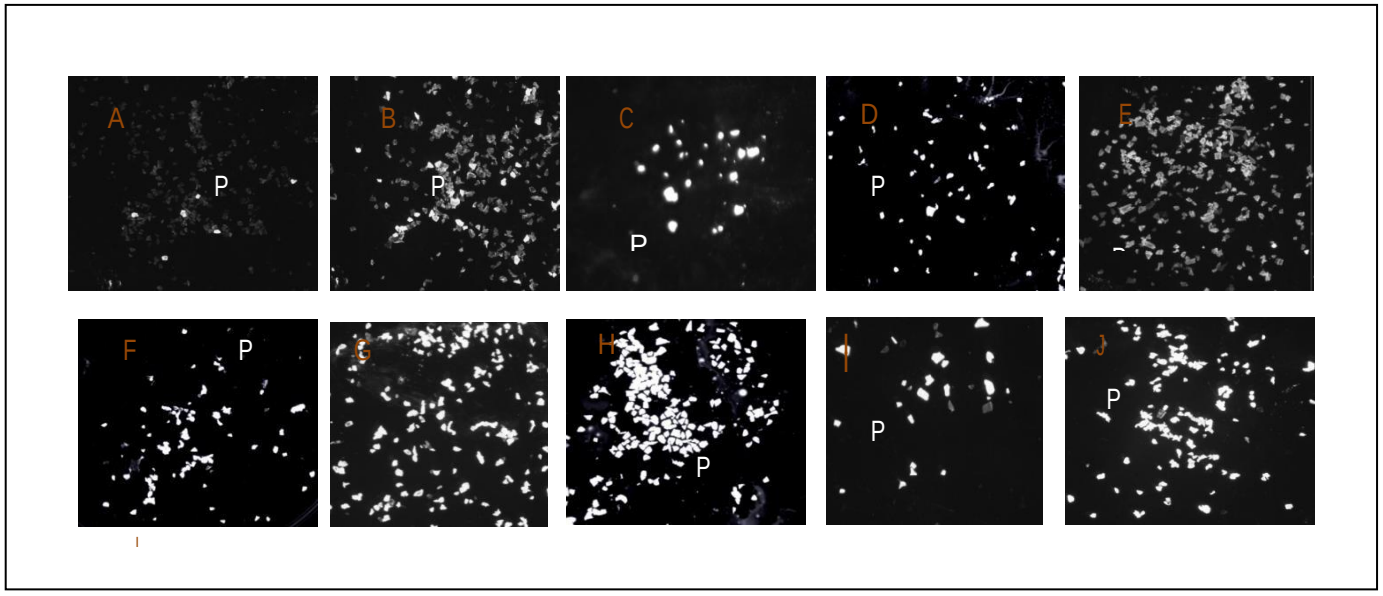

Fig 2: Images of virgin plastic particles stained with Nile red dye without dilution and their observation under UV transilluminator (24 hour ageing).
A = LDPE; B = LLDPE ; C = HDPE; D = PA.6; E = PA6.6; F = PP; G = PS; H = PVC(f); I = PVC(r); J = TPU

The virgin plastic particles when stained with Nile red dye without dilution after observing under UV transilluminator, all the particles exhibited illumination but low density polyethylene (LDPE) exhibited lowest illumination then rest of the particles.

Microplastics present in soil are attached to particles of soil. When soil sample is collected microplastics being very small in size cannot be identified easily. The soil sample containing sediments as well as microplastics is subjected to density separation so that microplastics will be separated from the soil. NaCl was used for density separation in order to decrease the mass of heavier sediment particles. Besides NaCl water was also added to the soil[5]. Air induced overflow method was used which forms bubbles in the solution and that enforced lighter particles to get separated from the soil particles and float over the top layer of water. NaCl was used because it is easily available and cost effective, besides it is not harmful to environment. The maximum density that is gained after using NaCl is 1.2gm/cm3. Polymers like PVC, HDPE, PA have good amount of additives mixed to them, so these polymers go beyond such density. In case of light weight particles density could be raised due to existence of additives etc. The density of NaCl is less to permit floatation of such particles. In some studies NaI was used for density separation for polymers (along with those that consists of additives) from those substances that have larger density[3].

The process of density separation was followed by another process in which biogenic material was digested using hydrogen peroxide (H_2O_2). Various other chemicals were also used like HCL, NaOH, but they were dismiss small amount of biogenic material. Hydrogen peroxide was considered efficient for dissolving 50% of biogenic materials. After the biogenic material was completely dismissed by H_2O_2, and there was no change in the conformation of polymers it was concluded that polymers are resistant to H_2O_2 [3] It has been found that after treatment with H_2O_2 the plastic particles became colorless and transparent, but in nature various microplastics have been found that are transparent. So visual analysis could be difficult and even incorrect task for identification of microplastics[5].

It is difficult to find small particles that resemble plastic from sediments, so it is very tough task

to identify them. The particles recovered from the soil are small in size and sometimes these particles are shielded by other substances (not plastic). So in such cases Nile red dye is more efficient in pointing out these masked microplastics. For staining polymer particles Nile red dye was used. Neutral lipids are also stained by Nile red dye[6]. The particles recoloured using Nile red was then observed under fluorescence microscope.

Nile red should be added to solvent that is suitable for it. Otherwise it would damage the filter paper on which particles are stained. After Nile red stock solution was prepared in acetone, it was used in 2 ways:

- Without dilution.
- After 5 times dilution in n-hexane.

The particles stained with Nile red exhibited bright illuminations. The polymers after staining were observed in green filter under fluorescent microscope.

Eosin B, Rose Bengal and NR are some dyes that were tried for their capacity to absorb to plastics. NR was selected since it was the best among all in case of adsorption and fluorescence power. Utilizing higher amount of dye, the fluorescence power of the colored particles was increased. Nile red has solvatochromic nature and it shifts its fluorescence outflow range red as the limit of the solvent increases. Due to solvatochromic nature of Nile red, it produces distinct colors for various kinds of polymers. This enables microplastics to be categorized by polymer difference[7]. Nile red enables fast and cheap assessment of microplastics in any sample[4]. The power of fluorescence is influenced by solvent that has been used in the process of extraction [6]. In this study the solvents used for assessment of microplastics were acetone and n-hexane [4]. In recent studies n- hexane was used and percentage of recovered particles was 98 %.(LDPE).

In our results the highest recovery rate among virgin plastics were shown by polypropylene(PP) i.e 42% and lowest recovery rate was observed in polyamide 6.6(PA 6.6) i.e 10 %. Similarly in case of products the highest recovery rate was shown by high density polyethylene (HDPE) i.e 50% and lowest recovery rate was observed in polyvinyl chloride (rough) (PVC r) i.e 12%. The less recovery rate of particles as compared to previous studies may be due to larger size of mesh or improper density separation method.

The particles after staining with Nile red exhibited fluorescence when observed under UV, this is because plastic particles have capacity to absorb this dye.

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Conclusion

Microplastics of polyethylene type of plastics were detected up to the depth of 15 cm (05-15 cm) from soil samples of the agricultural land (Maheru, Phagwara, Punjab). Therefore microplastics were found in the uppermost layer of soil called top soil. Further analysis to extract microplastics is needed to identify rest type of microplastics. Microplastics are having potential threat to agricultural productivity which in turn is linked to different food chains therefore the trophic transfer of microplastics would be present. Microplastics presence enhances the migration of pollutants horizontally as well as vertically in agricultural soil. The presence of microplastics in agricultural land alarms that microplastics can invade such areas which are of highly concern. Waste water and sludge comprises of microplastics as well as other environmental toxins which are potential hazardous to soil environment. Current studies do not provide clear information about the effects of microplastics on composition of soil community as well as on root colonizer microbes' therefore precise research is needed to investigate it.

This work can be served as base for future study of microplastics on agricultural land and how to detect microplastics from agricultural soil for future studies. Detection of microplastics in soil provides information about the various threats caused to several organisms present in the soil. This study also provides the first clue towards the presence of microplastics in the agricultural fields which can be used for evaluating the soil productivity and fertility.

References

[1] C. M. Rochman *et al.*, "Rethinking microplastics as a diverse contaminant suite," *Environmental Toxicology and Chemistry*. 2019.

[2] A. A. de Souza Machado, W. Kloas, C. Zarfl, S. Hempel, and M. C. Rillig, "Microplastics as an emerging threat to terrestrial ecosystems," *Global Change Biology*. 2018.

[3] M. T. Nuelle, J. H. Dekiff, D. Remy, and E. Fries, "A new analytical approach for monitoring microplastics in marine sediments," *Environ. Pollut.*, 2014.

[4] W. J. Shim, Y. K. Song, S. H. Hong, and M. Jang, "Identification and quantification of microplastics using Nile Red staining," *Mar. Pollut. Bull.*, 2016.

[5] V. Hidalgo-Ruz, L. Gutow, R. C. Thompson, and M. Thiel, "Microplastics in the marine environment: A review of the methods used for identification and quantification," *Environ. Sci. Technol.*, 2012.

[6] P. Greenspan and S. D. Fowler, "Spectrofluorometric studies of the lipid probe, nile red," *J. Lipid Res.*, 1985.

[7] T. Maes, R. Jessop, N. Wellner, K. Haupt, and A. G. Mayes, "A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red," *Sci. Rep.*, 2017.

