"Comparative analysis of Nylon 6,6 and HDPE microplastics effects on growth of wheat plant (*Triticum aestivum*)"

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT OF THE DEGREE
OF
MASTER OF TECHNOLOGY
IN
INDUSTRIAL BIOTECHNOLOGY

Submitted by
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CANDIDATE'S DECLARATION

I, Bhavika Garua, 2K19/IBT/04, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled **'Comparative analysis of Nylon 6,6 and HDPE microplastics effects on growth of wheat plant (*Triticum aestivum*)'** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.

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Date – 29-07-2021

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CERTIFICATE

I hereby certify that the project dissertation titled **'Comparative analysis of Nylon 6,6 and HDPE microplastics effects on growth of wheat plant (*Triticum aestivum*)'** which is submitted by **Bhavika Garua**, **2K19/IBT/04**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

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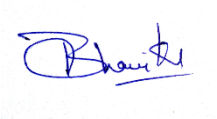

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ABSTRACT

Plastics in ocean have drawn considerable attention in last decade because the marine pollution has shown its effects in aquatic ecosystem thus, it is evident to public. Plastics in agricultural ecosystem have not shown direct effects but are also alarming as it can accumulate in crop plants and affect consumers by entering into the food web. Most of the plastics that get dumped in the ocean are produced and used on land. Through disintegration of plastics, microplastics and nanoplastics are generated and accumulated in significant quantities in soil. Further, these plastic particles contaminate terrestrial ecosystem, where these plastic particles might affect biota first and then spread to other ecosystems. Incidentally, plastics have been shown to alter biophysical and geochemical properties of soil. The dispersion and transport of plastics in soil could directly impact crop plants reducing crop yield. In this study, a pot experiment was conducted in natural environment where high density polyethylene and Nylon 6,6 were selected and used as examples of fibre and micro sized plastic residues in organic soil to study effects on wheat, terrestrial crop. The plastics were added in 1g, 2.5g and 5g concentrations in each pot. The results showed effects on growth, biomass, nitrogen and chlorophyll content of wheat plant. Plastics also caused oxidative stress in treated plants as compared to control.

Keywords - Agricultural ecosystem, Microplastics, Nylon 6,6, Polyethylene microplastic, Terrestrial plants, Crop plants

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LIST OF ABBREVIATIONS AND SYMBOLS

mm – Millimeter

nm – Nanometer

µm – Micrometer

^{o}C – Degree Celsius

HDPE – High Density Polyethylene

NaOCL - Sodium Hypochlorite

TBA - Thiobutyric acids

NBT - Nitroblue tetrazolium

NADH - Nicotinamide adenine dinucleotide

DPPH - 2,2-diphenylpicrylhydrazyl

TCA - Trichloroacetic acid

K_2SO_4 - Potassium sulphate

$CuSO_4$ - Copper sulphate

H_2SO_4 - Sulphuric acid

NaOH – Sodium hydroxide

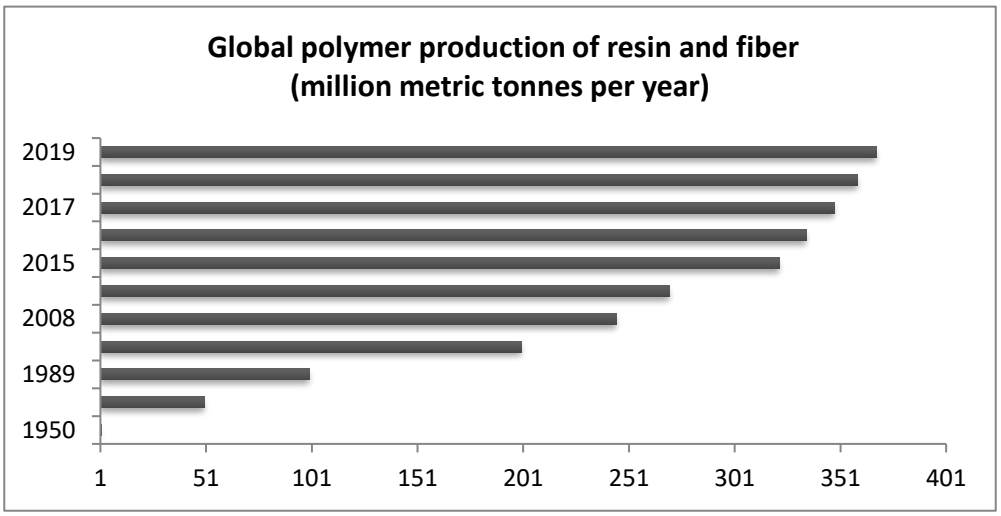
mg – Milligram

TEM – Transmission electron microscope

MDA - Malondialdehyde

CHAPTER 1 - INTRODUCTION

The properties which make plastic suitable for packaging and production of goods – durable and resistant to environmental factors- also makes it almost impossible to eradicate from environment completely. The global production of plastics increases every year. In 2019, world plastic production increased to 368 million metric tonnes from 322 million metric tonnes in 2015 shown in figure 1 (Geyer, Jambeck, and Law 2017).



Graph 1: Plastic production from 1950 in million tonnes.

Various reports have claimed that most plastic materials disintegrate rather than degrade in environment (Scott Lambert, Chris Sinclair 2014). Although plastics are durable, these are prone to fragmentation in the environment due to prolonged exposure to UV light and physical abrasion (Barnes et al. 2009). These large plastics disintegrate into smaller fragments of size less than 5mm, referred as microplastics (Mammo et al. 2020). Further deterioration of these microplastic fragments result in emergence of even smaller particles of size less than 0.1µm, commonly called as nanoplastics (Ng et al. 2018). The distinct sizes of microplastic and nanoplastics are still unknown. Different authors define microplastic and nanoplastic differently. Microplastics are generally defined as particles in the size range of nanometre (100nm to 5mm), along with sub-micrometre (100nm to 1µm) and micrometre (1µm to 5mm) plastics, and nanoplastic in the range of 1nm to 100nm.

The occurrence of different plastic materials has clinched much attention in marine

environments, and its related shoreline. Extensive research have been done on assimilation of microplastic by marine organisms (Galloway, Cole, and Lewis 2017)(Thushari and Senevirathna 2020)(Horton et al. 2017). Studies have shown presence of microplastic in the guts of aquatic organisms worldwide. Research on transport of small plastic particles beyond the gut of the organisms, entering food web and transfer between trophic levels is still in its initial stage. Whilst the fate of plastics in marine ecosystem is being progressively well studied, behavior of smaller plastic particles in terrestrial environment is somewhat obscure, especially in agricultural ecosystem (Zang et al. 2020). Critical limits for plastic contamination in soil are rarely defined so far which makes it harder to evaluate the bearing capacity of agricultural ecosystems (R. Qi et al. 2020). Whether terrestrial plants can accumulate micro and nanoplastics and if so then how these can affect their growth and consequently enter the food chain, are two crucial problems of paramount importance to study the effects of plastics on terrestrial crop plants (Zhu et al. 2019).

Recent studies have observed the origin and fate of these plastic fragments in terrestrial ecosystem especially in terrestrial crop plants (De Souza Machado et al. 2019)(Rillig et al. 2019).

CHAPTER 2 – REVIEW OF LITERATURE

2.1 *Triticum aestivum* L. – A Brief Overview

Wheat (*Triticum aestivum*) is one of the three main cereal crops, along with rice and maize, consumed globally as staple cereal crop. There are many species of wheat that together comprise the genus *Triticum* but the most commonly grown specie is (*T. aestivum*). The cereal grass consists of long slender leaves and hollow stems in most varieties. The crop can be grown in the tropical, sub-tropical and in temperate zones. Wheat can tolerate severe cold, snow and can resume growth with the start of warm spring weather. The major types of wheat crop are Rabi (winter) and spring of which winter wheat is sown from October-December and harvested in February or March. The common wheat has high gluten making it suitable for breadmaking and for pasta preparation.

Taxonomical Classification:-

Kingdom: Plantae
Subkingdom: Viridiplantae
Infrakingdom: Streptophyta
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Superorder: Lilianae
Order: Poales
Family: Poaceae.
Genus: *Triticum*
Species: *Triticum aestivum* L.

Figure 1. Wheat Grass

Botanical Name: *Triticum aestivum* L., Number of chromosomes: 7, Chromosomes in diploid cells: 42 (2n = 6X)

The optimum growth of wheat occurs in cool and moist weather with winter fog majorly during the growth period followed by dry and warm weather to enable the ripening of grain properly. The optimum temperature required for seed germination is 20-25 ^{o}C.

2.2 *Triticum aestivum* as model organism

Plants which are termed as model plants are those species which are chosen for the ease of studying particular biological phenomena (ecology, physiology, biochemistry, genetics) that can be extrapolated to other plant species and/or due its value in biotechnology and agronomy. The properties of plant species which makes it as model organism are- small size, ease of growth, short life span, small genome and capacity to manipulate genetically. Also, wheat is one of the most commonly consumed crop in the world making it suitable for studying ecological impacts of inorganic contaminants present in agricultural soils.

2.3 Structure and uses of Nylon 6,6 and High Density Polyethylene

Nylon 6,6 is a polyamide, which is a polymer obtained from the condensation of monomers which have terminal amine ($-NH_2$) groups and carboxylic acid (-COOH) groups as shown in figure (Ortega, Carter, and Ortega 2016). Nylon 6,6 is known for its high tensile strength, high melting point and thus, have high stability. This is used mostly as fabric for parachutes, waterproof swimwear, airbags, ropes, carpets etc, where fabric is susceptible to wear and tear.

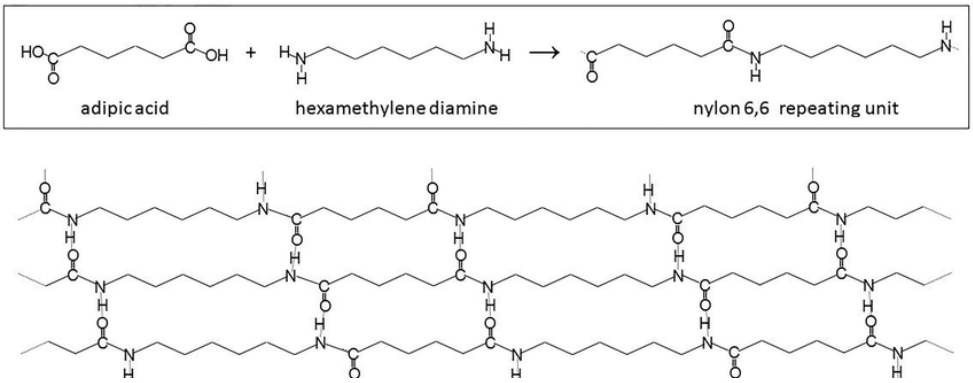


Figure 2: Condensation reaction to form Nylon 6,6 monomer and Polyamide structure

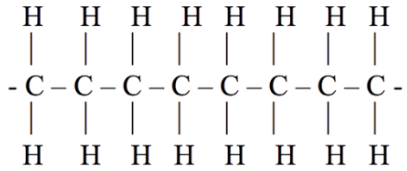


Figure 3: Polyethylene structure of High Density Polyethylene

High density polyethylene (HDPE) is a linear addition polymer of ethylene with very slight branching. HDPE has properties like high flexibility and resistance to extreme temperatures, like frozen food and refrigerated conditions which makes it suitable for use as a packaging material for containers and bottles (Selke and Hernandez 2001). The comparatively inert properties of HDPE are due to presence of C–C and C–H bonds in the structure making it resistant to most types of chemicals except strong oxidizing agents (Selke and Hernandez 2001).

2.4 Studies reported of microplastic effects on terrestrial plants

Studies have been carried out to observe translocation of plastics in plant tissues (Li et al. 2020)(Maity et al. 2020)(Sun et al. 2020)(Li et al. 2020)(Lian et al. 2020)(Qi et al. 2018)(Bosker et al. 2019). Probable ways of transport of micro and nano sized particles are through cell wall pores into plant cells: endocytosis, passive diffusion, facilitated diffusion, and translocation via plasmodesmata (Maity and Pramanick 2020). Nanoplastics can cross through porous plant cell wall matrix due to its small size and gets transported to endodermis by capillary action and osmotic pressure (Lin et al. 2009). Other nanoparticles can also transport through symplastic pathway in which membrane proteins help in translocation along with ions, aquaporins and are taken up by cells directly through plasma membrane or via endocytosis (Tripathi et al. 2017). Intercellular transport of such particles is mediated by plasmodesmata which connects cells together (Li et al., 2020). The stomatal openings can also be a possible route for assimilation of nanoparticles which then get translocated through the xylem tissue (Hong et al. 2014). Plasticizers released from plastic in soil can cause oxidative stress and could increase reactive oxygen species and lipid peroxidation in the wheat grain (Qi et al. 2018). Significant accumulation of plasticizers and their metabolites were found higher in grains than those of stem, leaves and root of wheat crop (Gao et al. 2019).

CHAPTER 3 – METHODOLOGY

3.1 Chemicals and Materials

- Plastic - High Density Polyethylene microplastic (40µm), Nylon 6,6 microfibers
- Chemicals- Sodium Hypochlorite (NaOCL), Thiobutyric acids (TBA), sucrose, NBT, NADH, deoxy-ribose, DPPH, Trichloroacetic acid (TCA), Nile Red dye, acetone, Potassium sulphate (K_2SO_4), Copper sulphate ($CuSO_4$), Sulphuric acid (H_2SO_4), NaOH

3.2 Study Plant

Wheat (*Triticum aestivum)* seeds were obtained from National Seeds Corporation, Pusa, New Delhi. The seeds were surface sterilized first with 0.02% Sodium hypochlorite NaOCl and then with 70% Ethanol. After sterilization seeds were repeatedly washed with distill water. The seeds were germinated first on tissue overnight with complete dark. The germinated seeds were sown in organic soil in the month of November. After emergence of seedlings, they were transplanted in different pots keeping 6 seedlings in each pot. Pots were initially irrigated twice in a week and in later stages once in every two days which was during February month. The frequency of irrigation was increased due to rise in temperature in the month of February. With respect to weight of the soil in each pot, NPK was added according to wheat NPK requirement 100, 20, 60 kg/ha which is given in the fields.

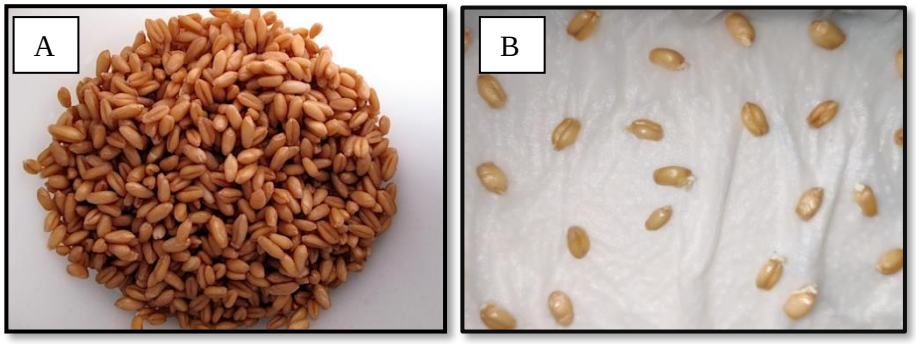


Figure 4: (A) Wheat grains and (B) Germination done in complete dark

3.3 Experiment design and growth conditions

The study had total 9 pots – 3 Control, 3 pots each of Nylon 6,6 and HDPE plastics. The pots were kept in randomized manner in natural light and conditions. To mimic

field conditions, seeds were sown in November and were harvested in the month of March.

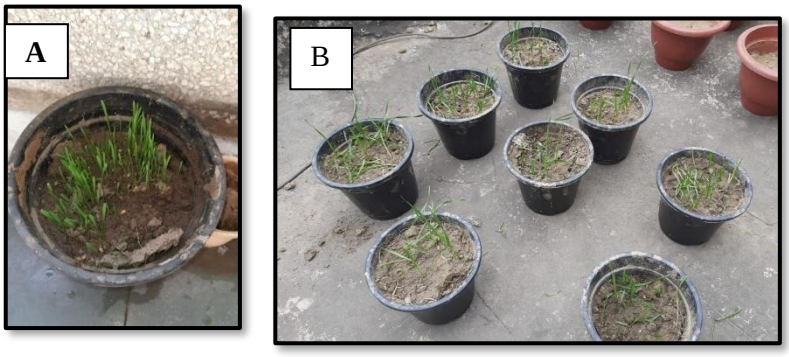


Figure 5: (A) Wheat seedlings after 1 week (B) The experimental setup in different pots, each containing 6 seedlings.

3.4 Plastic exposure

Each pot had 2.5 kg of soil. After 10 days from emergence of seedlings plastic was added except in control. Different quantities of High Density Polyethylene (HDPE) were added in 3 pots – 1g, 2.5g and 5g to achieve 0.04%, 0.1% and 0.2% (w/w) respectively. Nylon 6,6 fibres were added in 3 different pots – 1g, 2g and 2.5g to achieve 0.04%, 0.08% and 0.2% (w/w) respectively.

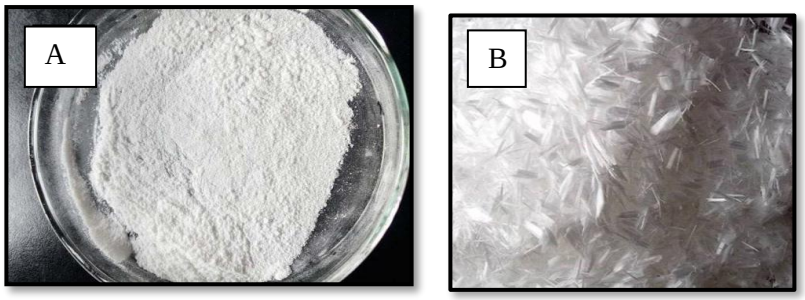


Figure 6: (A) HDPE (B) Nylon 6,6 fibers

3.5 Microplastic tagging and TEM imaging

Microplastic particles of both Nylon 6,6 and HDPE were tagged by Nile Red. 100µg/ml solution of Nile Red was made in deionised water by adding 1mg/ml solution of Nile

Red in acetone to 9ml deionised water. Dried microplastic particles were added to 100µg/ml Nile Red in DI water at a concentration of 0.5g of plastic

particles per 10ml of solution. The vials were incubated for 2h in dark and rinsed repeatedly with DI water till the supernatant becomes transparent. The particles were stored in DI water and checked by Fluorescence microscope.

Figure 7: Fluorescence Microscope

To study the uptake of microplastics in wheat plants, roots from control and HDPE treated plants were taken and washed thoroughly to remove soil particles. The samples were then given to Sophisticated Analytical Instrumentation Facility, AIIMS Delhi, for imaging by TECNAI Transmission Electron Microscope.

3.6 Measurement of wheat growth parameters

After 4 months before ripening, the wheat plants were harvested and washed thoroughly under running water to remove soil without damaging the tissue. The fresh weight and lengths of root and shoot were measured immediately using a weighing balance and ruler. The dry weight was measured by drying at 70 - 80 °c in hot air oven for 72 hours. After harvest chlorophyll, nitrogen content, DPPH scavenging activity, superoxide radical and lipid peroxidation was measured and recorded.

Figure 8. (A) Wheat plant harvested and cleaned under running water to remove soil from roots. (B) Stem of wheat plant

3.7 Chlorophyll content

Fresh leaves were collected from each pot to determine chlorophyll content. 0.5g of leaves were taken from each pot. The leaves were chopped and homogenised by adding 10ml of 80% acetone till the leaves become transparent. The extract was centrifuged at 2500 rpm for 5 minutes. Then 1ml of obtained supernatant was diluted with 9ml of 80% acetone and read at 663nm and 644nm by UV-Vis Spectrophotometer. The equations used were based on Mackinney's work and Arnon equations –

$Chl_a = 12.7\ A_{663} – 2.69\ A_{645}$

$Chl_b = 22.9\ A_{645} – 4.68\ A_{663,}$

Here A_{663} and A_{645} is absorbance at 663nm and 645nm respectively.

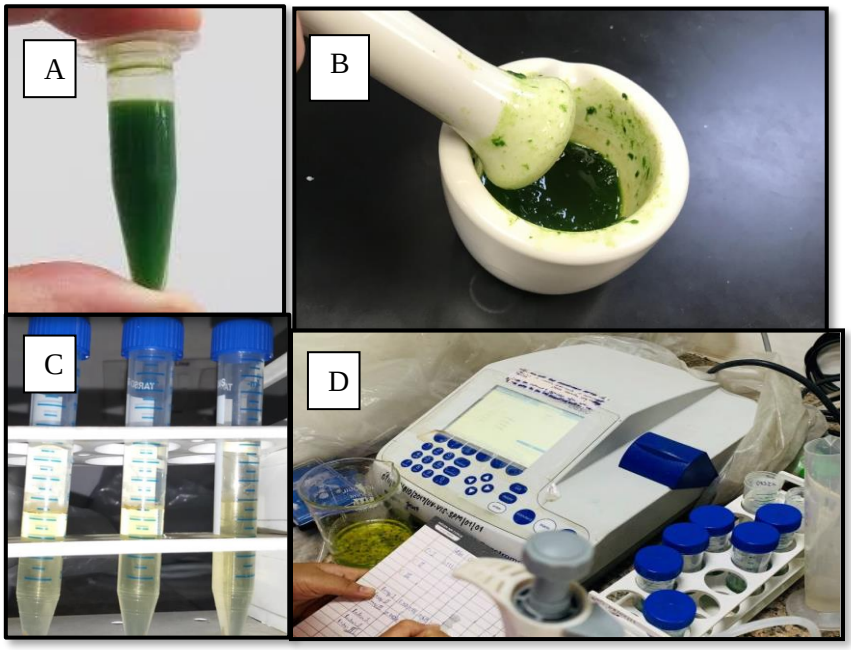


Figure 9: (A) and (B) Homogenised leaf of wheat plants with 80% acetone (C) Supernatnt from centriguged homogenized mixture diluted with 80% acetone (D) Readings taken by UV-Vis spectrometer for chlorophyll content

3.8 Nitrogen content

Nitrogen analysis was done of leaves and roots of wheat. The samples were dried at 30^{o}C for 24h. Then the dried samples were crushed to powder by mortar pestle. 0.2g of powdered sample was taken of each pot for digestion into the 250ml digestion tube. Digestion was performed on FOSS Kjeldahl digestor unit for 60 minutes with 0.2g sample,7g K_2SO_4, 0.8g $CuSO_4$.12 ml concentrated H_2SO_4 was added. Tubes were shaken gently to wet the samples. Exhaust was positioned and scrubber was turned on. Rack was removed with exhaust and left to cool for at least 15 minutes. A reagent blank was included in the digestion (all reagents added to the digestion tube except sample). Distillation was performed on FOSS Kjeltec™ 8200 unit in which 30 ml of Boric acid was added to receiver flask as receiver solution. The distillation unit adds 80 ml H_2O and 50 ml 40% NaOH to dilute the digest in the digestion tube. The distillate received was titrated with 0.1N HCl (Standardized) as titrant.

% Nitrogen and % Protein was calculated by following equations -

$$\% N = (T-B) \times N \times \mathbf{14.007 \times 100 / \text{ weight of sample in mg}}$$

T = Sample titration, B = Blank titration, N = Normality of titrant

$$\% \text{ Protein} = N \times F$$

F = Protein factor = 5.27 for Wheat

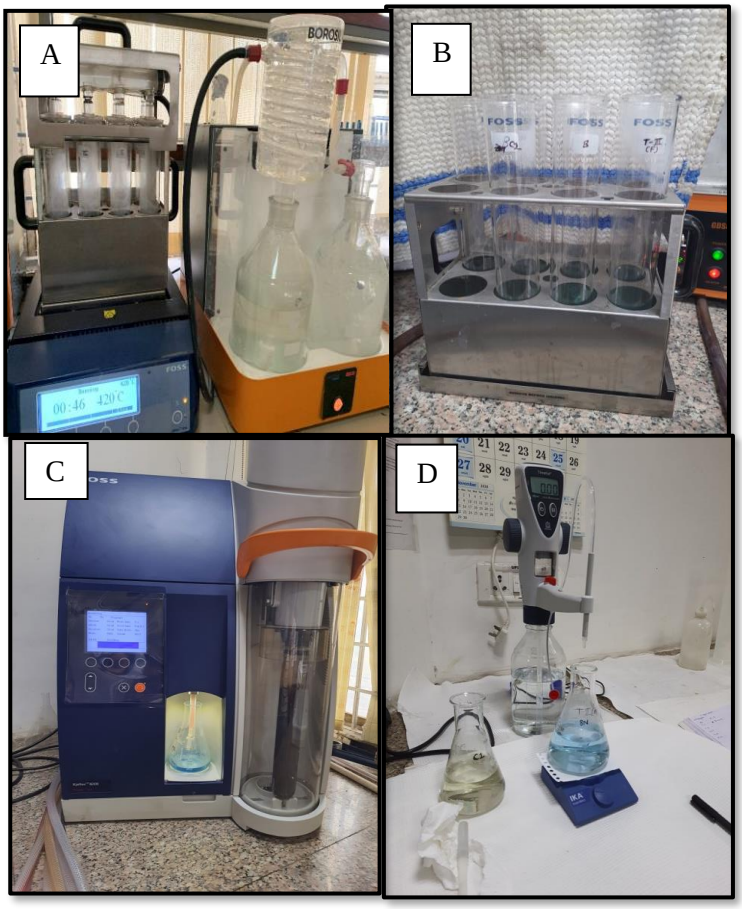


Figure 10: (A) FOSS kjeldahl digester unit (B) Distillation tubes (C) Protein Distillation Unit (FOSS KJELTEC 8200) (D) Titration with table-top burette

3.9 DPPH free radical scavenging activity

DPPH is stable free radical which mimics reactive oxygen species present in cells. The DPPH solution in methanol which is its oxidized form is deep blue colour but when reduced by antioxidants it turns colourless. Wheat roots were taken as sample, about 100 mg, which was then homogenized and centrifuged and the supernatant was collected. To 1.5 ml of supernatant, 500 µl of DPPH (60 µM in methanol) solution was mixed and incubated for 30 minutes in dark at room temperature. After incubation absorbance was taken in UV-VIS spectrophotometer at 517nm. The percentage of DPPH free radical scavenged by antioxidants was calculated as following-

the percentage of DPPH scavenged (%) = (Ao –As/Ao)*100.

Where,

Ao = absorbance of blank

As = absorbance of sample.

Results were expressed as %DPPH scavenged/mg of tissue (Maity et al. 2020)

3.10 Superoxide radical

The superoxide radical can be measured by the formation of blue monoformazan by NBT reduction Kiba et al., 1997 [48]. The wheat roots, about 100 mg, were homogenized in 4 ml of TCA (0.1%). The supernatant of homogenised mixture after centrifugation was incubated with 3 ml of 50 mM Tris HCl of pH-6.5, containing 0.2 mM NBT, 250 mM sucrose, and 0.2 mM NADH for 24h at 25˚C in dark. The absorbance was taken at 530nm by UV-Vis spectrometer. The extinction coefficient ε taken was 12.8 L/mol.cm. The results obtained were expressed as μM of NBT reduced per mg of fresh tissue.

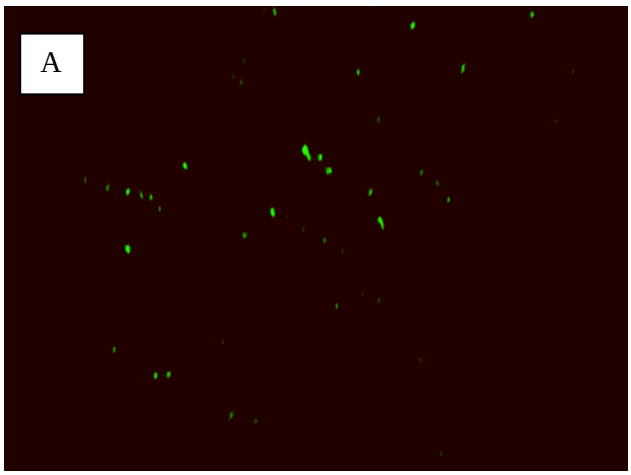
3.11 Lipid peroxidation

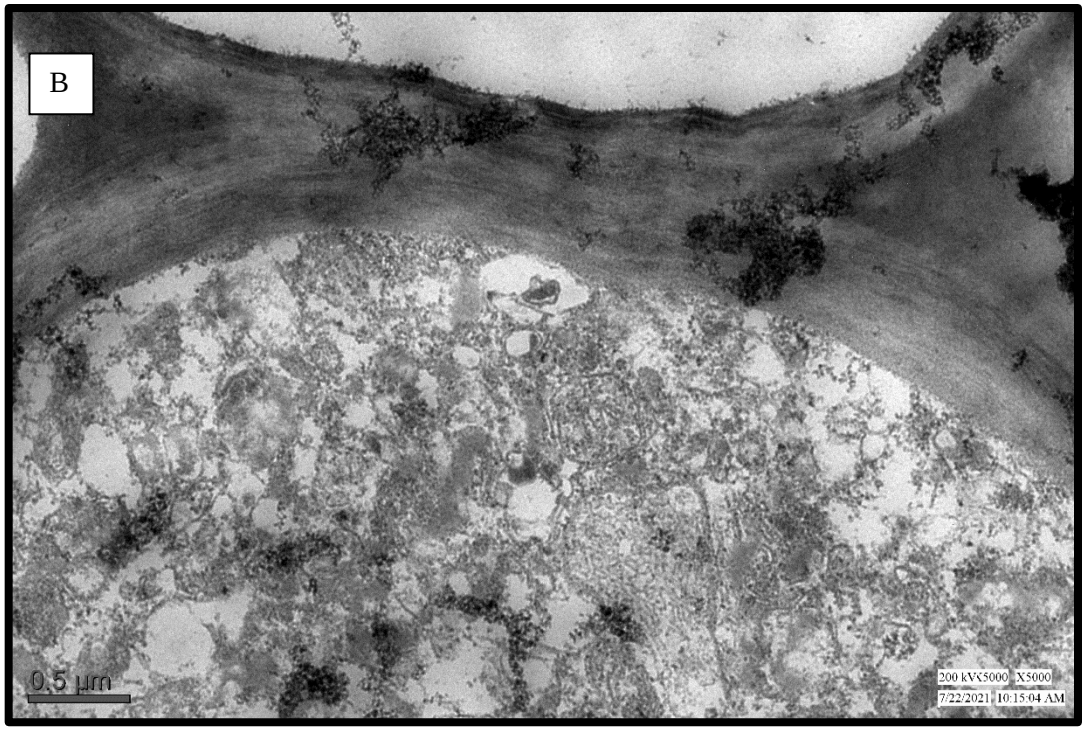
Heath and Packer 1968. [50] 100 mg of wheat roots was taken as sample which was then homogenized in 4 ml of solution made with 0.5% TBA and 20% TCA on ice. The sample was then incubated at 95˚C for 30 min and rapidly cooled for 10 min on ice in order to stop the reaction. It was centrifuged at 1000 rpm for 10 min. The supernatant was then read at 532 nm and 600nm for TBA-MDA complex and non-specific absorbance (respectively) by UV-VIS spectrophotometer. The MDA content formed was then calculated with molar extinction coefficient 155 mM-1 cm-1. The results obtained were expressed as μM of MDA per ml of sample.

CHAPTER 4 – RESULTS AND DICUSSION

4.1 Microplastic tagging and TEM micrograph

The HDPE and Nylon microplastics were tagged with fluorescent dye Nile Red. The chemical incorporation of dye was confirmed by fluorescent microscopy.





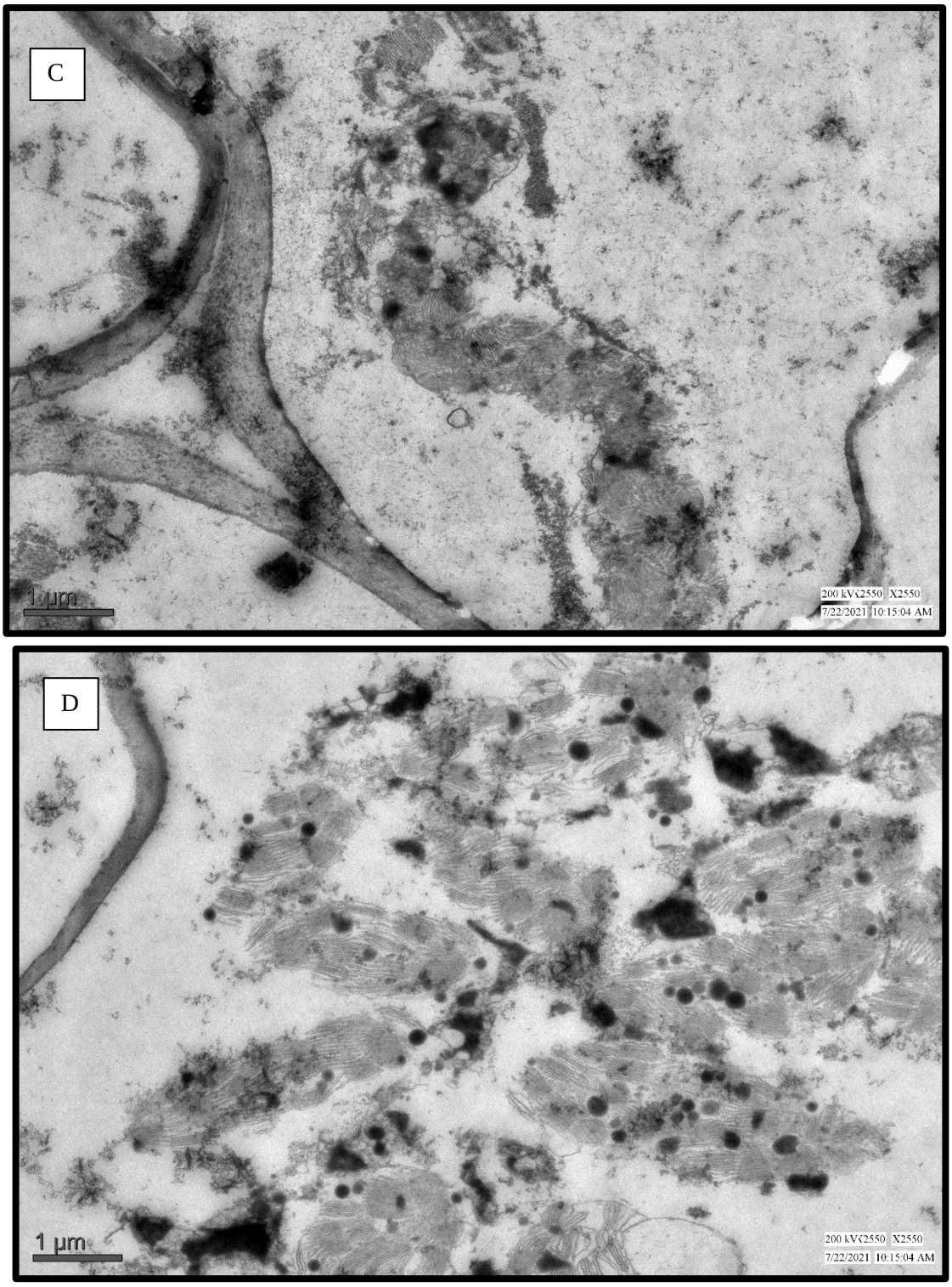
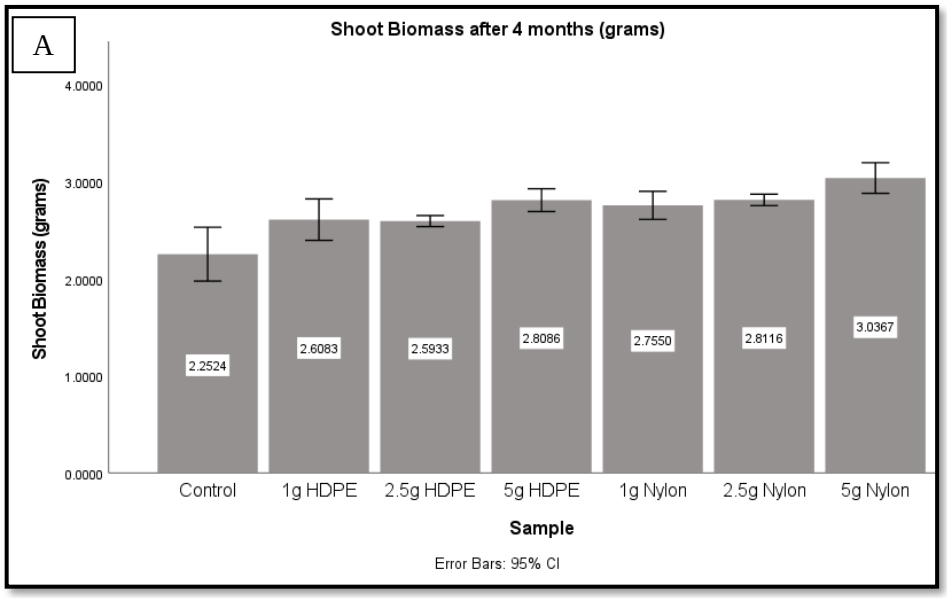
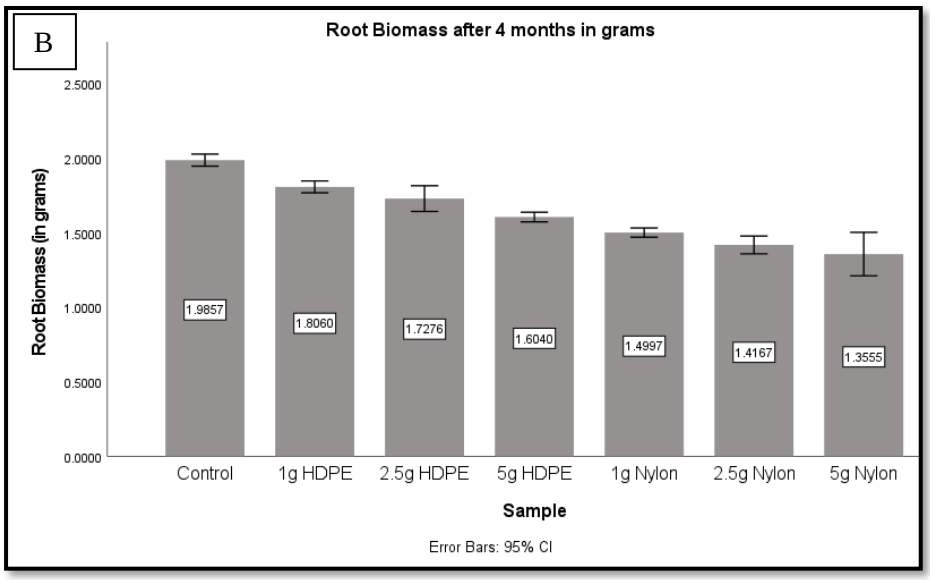


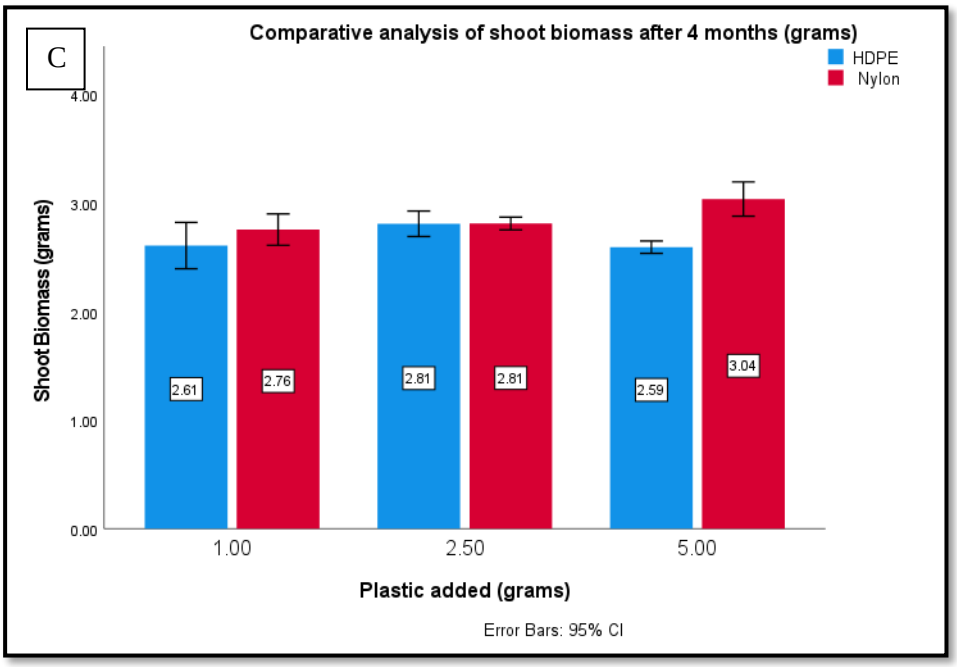
Figure 11. (A) Tagged microplastic imaged through Fluorescent microscope. (B-D) TEM micrographs of wheat plant roots after 25 days from sowing. (B) Micrograph of wheat plant root from control untreated plants (C and D) Micrograph of HDPE treated wheat plant roots. When compared with untreated wheat plant roots i.e. control (B), (C and D) clearly shows presence of tagged microplastic particles (dark globular structures) around. These results show internalization of tagged microplastics in root hair cells.

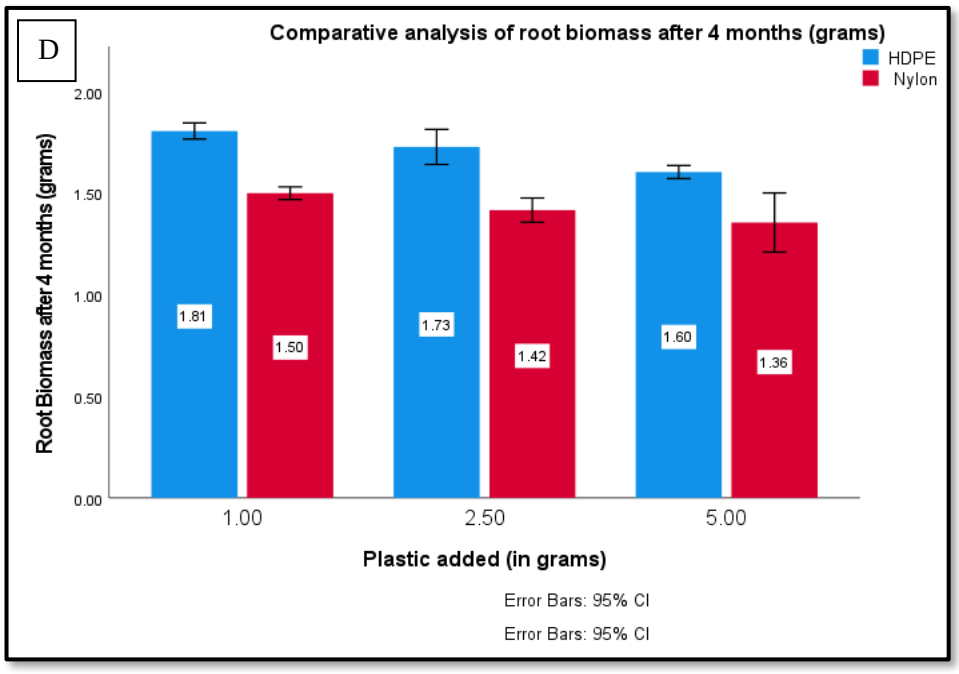
The TEM micrographs of HDPE treated wheat plant roots shows presence of tagged microplastics as dark globular structures around which were not present in sections from untreated wheat plant roots. This confirms uptake of HDPE microplastics by root cells. This uptake mechanism is also observed in other plants where it has been shown that diffusion through apical meristem tissue which is porous due to active cell division in young seedlings (Li, Luo, Peijnenburg, et al. 2020). However, the cell wall pores and intercellular plasmodesmata have diameters that are smaller than HDPE microplastics used in this study. Thus, the uptake can be explained through the fact that Casparian band around epidermal layers of intact apical root is not fully developed. Then these particles got transported through intercellular space via apoplastic transport. Once the particles come inside the central cylinder, they move towards aerial parts of the plant through vascular system of xylem tissue in transpiration stream (Lian et al. 2020). Li et al. hypothesised transport of plastic particles into the plant roots via crack entry mode; entire lateral root cap and the root apical meristem of wheat and lettuce, further to shoots through apoplastic transport (Li, et al., 2020). The apoplast comprise of all beyond the plasmalemma including intermicellar and interfibrillar space and xylem stretches to the rhizoplane and cuticle (Sattelmacher 2009). The Casparian band in root endodermis does not allow transport of water and chemicals into root stele and act as physical barrier. However, in areas where endodermal cells are not mature and at the secondary root initiation sites, this Casparian band is found discontinuous. Discontinuous areas where active cell division is observed in apical meristem can allow transport of plastics unhindered as these areas are entry point for plant pathogens known as crack entry mode (Li et al., 2020).

4.2 Shoot and Root Biomass





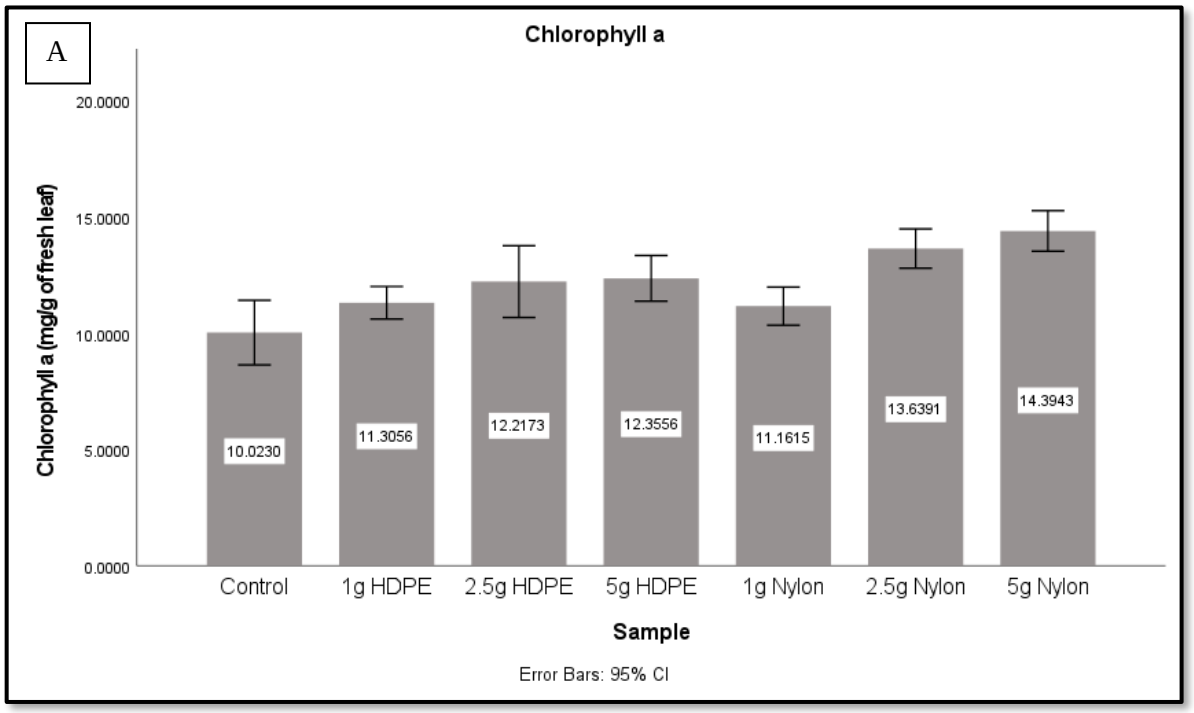




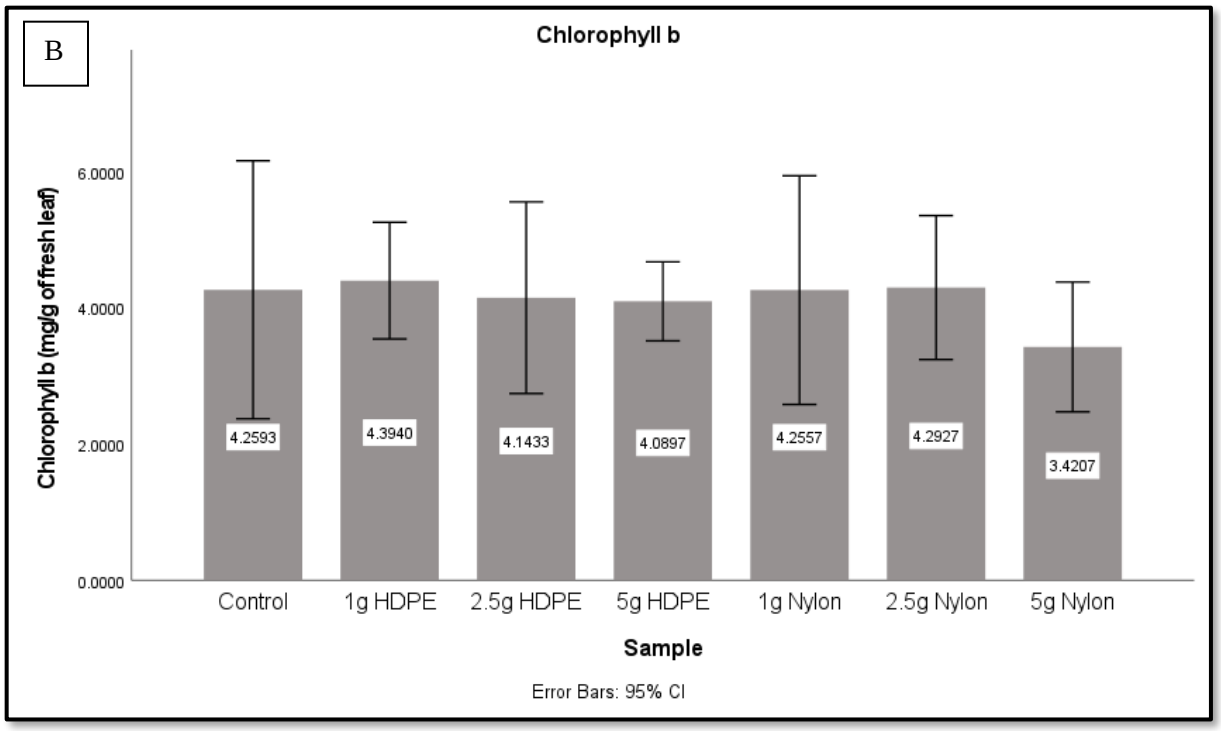
Graph 2: Effects of HDPE and Nylon addition to (A) and (B) Shoot and Root biomass of wheat after 4 months of sowing (C) and (D) Comparasion of shoot and root biomass of wheat treated with HDPE and Nylon

There was no significant increase in shoot and root biomass but the highest concentration treated wheat plants showed difference as compared to the control wheat plants in both plastic treatments as shown in graph 2. When compared the two plastic effects on shoot and root biomass, shoot biomass increased of nylon treated plants as compared to HDPE whereas root biomass decreased after 4 months. This can be due to increase nitrogen in soil increasing leaf. Also as reported by previous studies, soil characteristics are altered changing properties like water holding capacity and soil porosity which can be a probable reason for decrease in root biomass (De Souza Machado et al. 2019). Also, decrease in root growth is directly related to the presence of a toxicant which is related with the inhibition of root apical meristem activity (Maity et al. 2020).

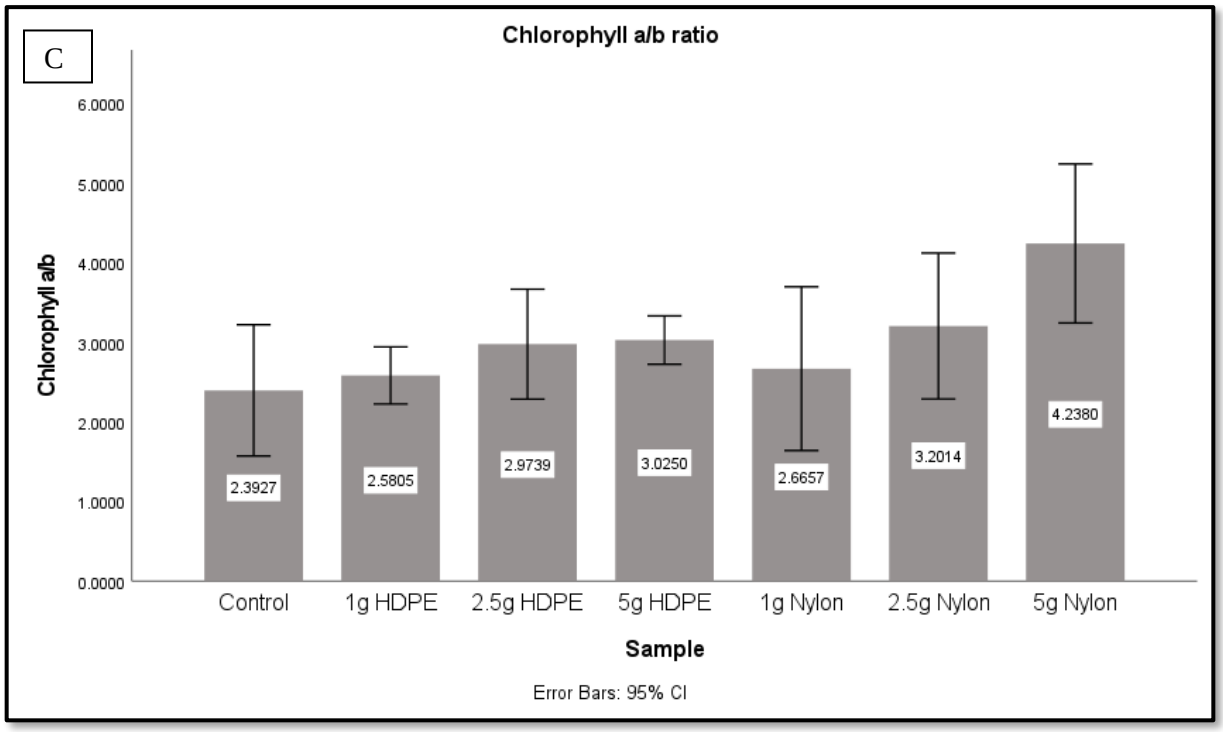
4.3 Chlorophyll content

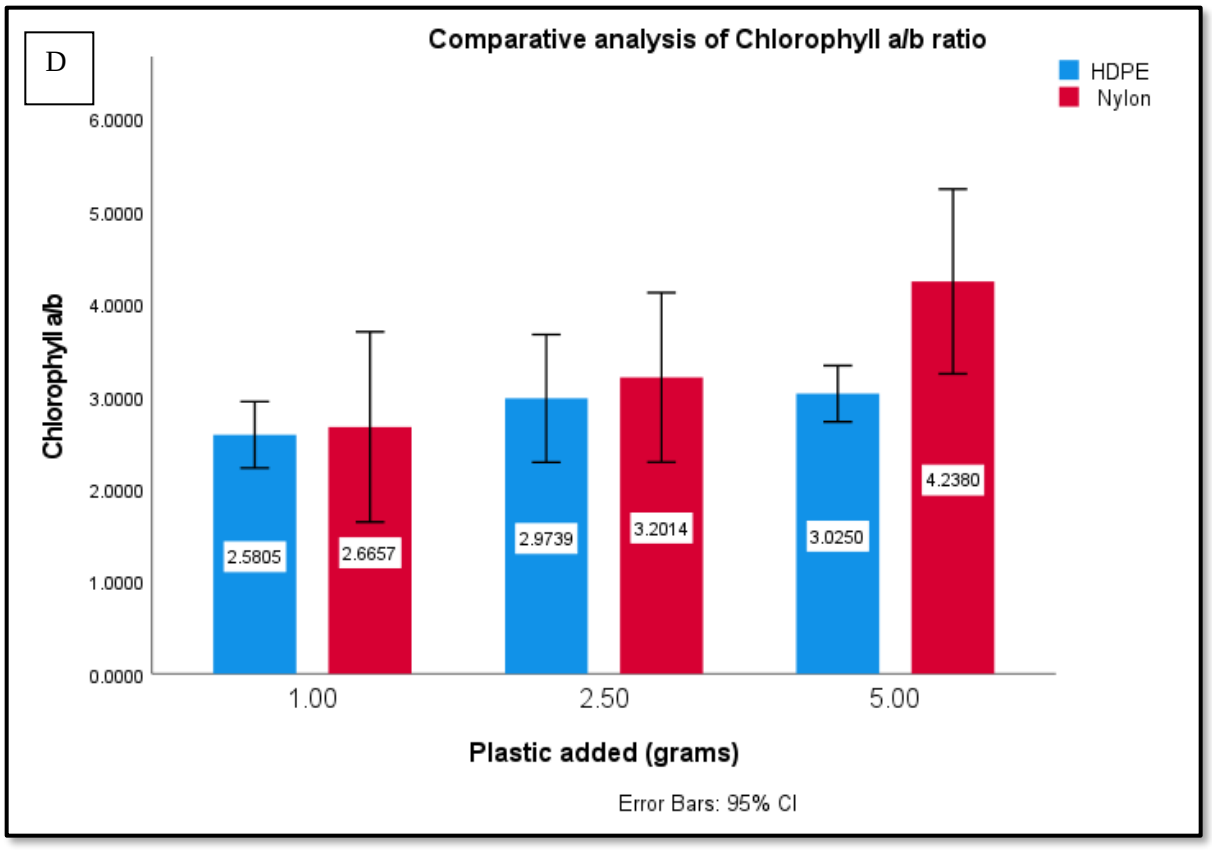


B


Chlorophyll b
Chlorophyll b (mg/g of fresh leaf)
6.0000
4.0000
2.0000
0.0000
4.2593
4.3940
4.1433
4.0897
4.2557
4.2927
3.4207
Control
1g HDPE
2.5g HDPE
5g HDPE
1g Nylon
2.5g Nylon
5g Nylon
Sample
Error Bars: 95% CI

C


Chlorophyll a/b ratio
Chlorophyll a/b
6.0000
5.0000
4.0000
3.0000
2.0000
1.0000
0.0000
2.3927
2.5805
2.9739
3.0250
2.6657
3.2014
4.2380
Control
1g HDPE
2.5g HDPE
5g HDPE
1g Nylon
2.5g Nylon
5g Nylon
Sample
Error Bars: 95% CI

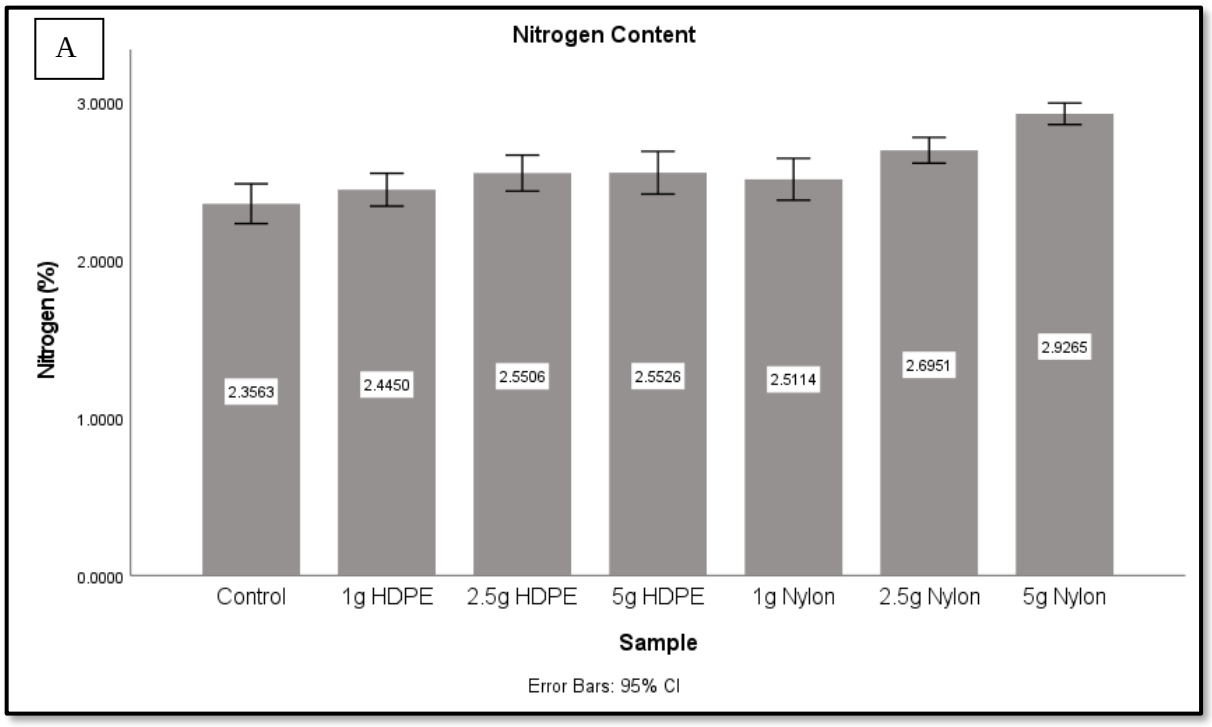


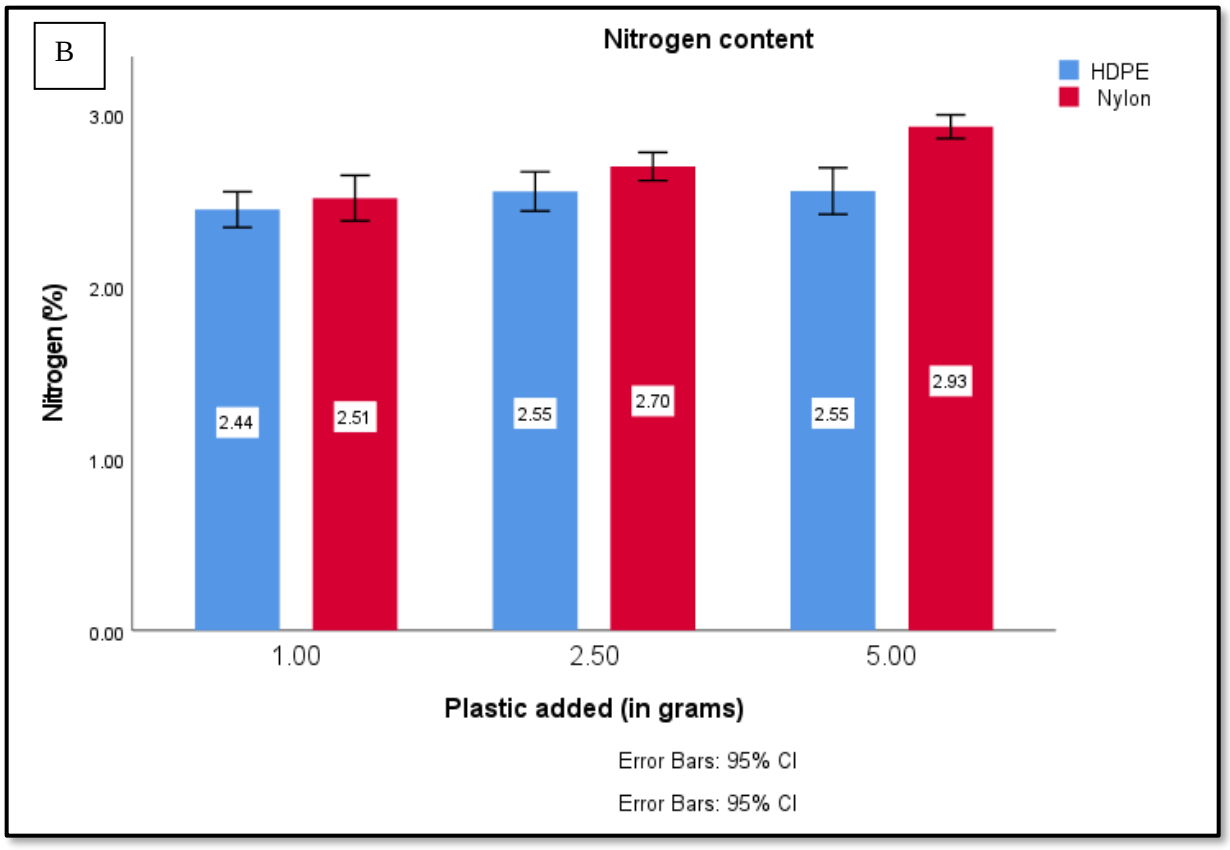
Graph 3: Effects of HDPE and Nylon addition to (A) and (B) Chlorophyll a and chlorophyll b content respectively (C) Ratio of chlorophyll a to chlorophyll b (D) Comparasion of ratio of chlorophyll a to chlorophyll b of wheat treated with HDPE and Nylon

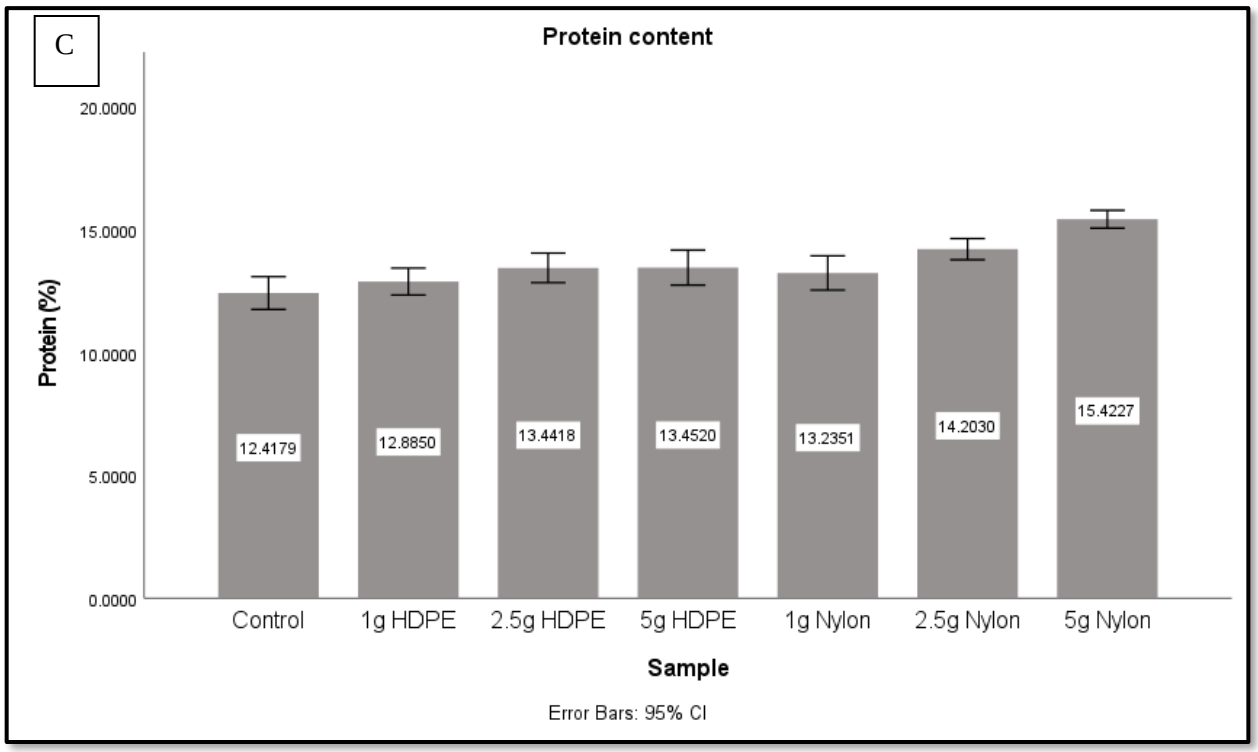
There was not much difference in the chlorophyll a and chlorophyll b contents of wheat leaves of treated and control sample shown in graph 3 but interestingly the ratio of Chlorophyll a is to Chlorophyll b increased in response to higher concentration of microplastics as compared to control, regardless of the type. The ratio of Chlorophyll a to Chlorophyll b is an important parameter for photosynthetic activity and its deviations is indicative of stress in plants (Siddiqui, Al-Whaibi, and Mohammad 2015). Thus, the increase in ratio in this study suggests a significant inhibition of synthesis of chlorophyll b with respect to microplastic addition. The pigment Chlorophyll b improves the efficiency of photosynthesis in plants (Katz et al. 1978) and also plays an important part in primary production of grassland in agricultural ecosystems. One of the recent studies reported that polyamide (nylon contains polyamide) present in soil increased nitrogen content in spring onion leaves which was explained by possibility of polyamide being a source of nitrogen when

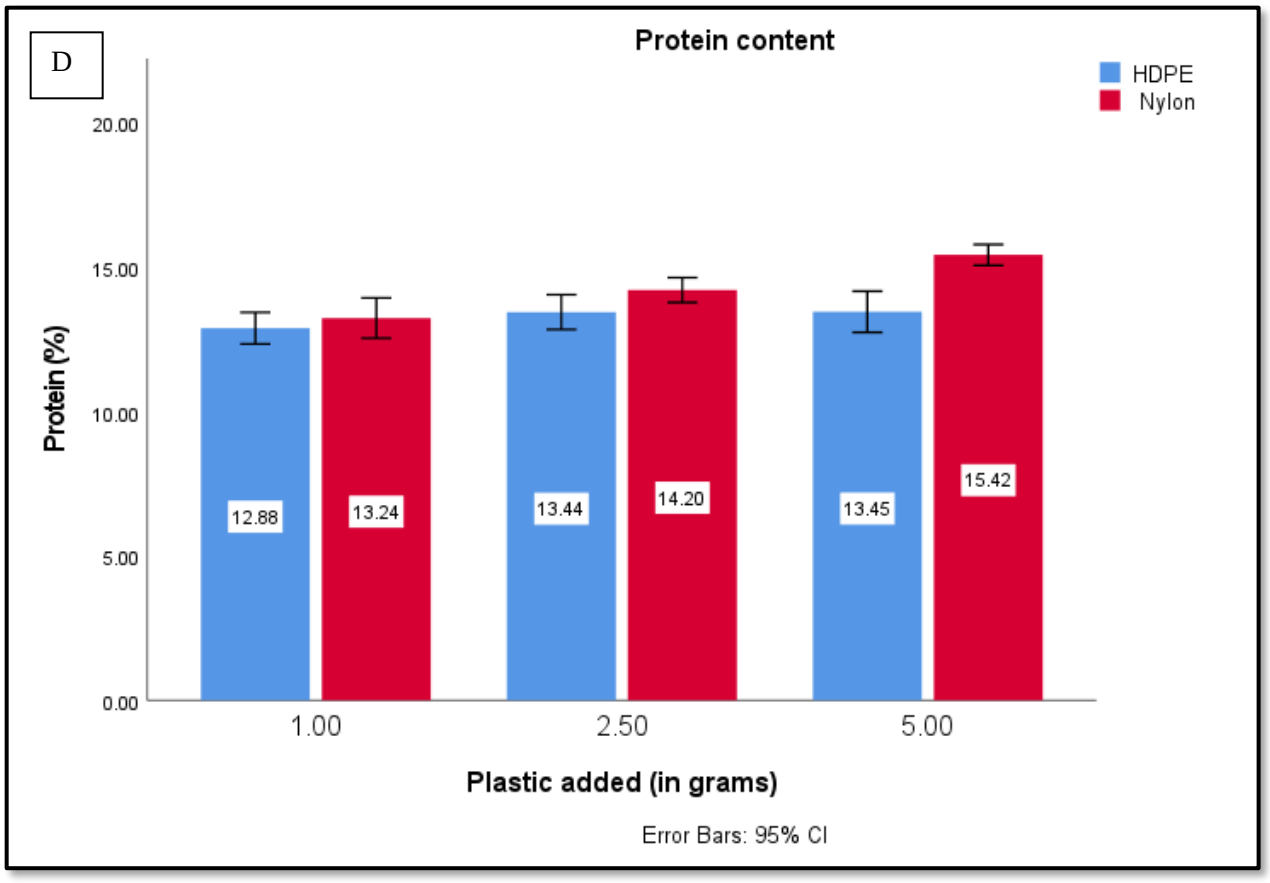
disintegrated within soil whereas polymers HDPE have no nitrogen in their structure (Boots, Russell, and Green 2019).

4.4 Nitrogen and protein content




B
Nitrogen content
HDPE
Nylon
3.00
2.00
1.00
0.00
Nitrogen (%)
2.44
2.51
2.55
2.70
2.55
2.93
1.00
2.50
5.00
Plastic added (in grams)
Error Bars: 95% CI
Error Bars: 95% CI

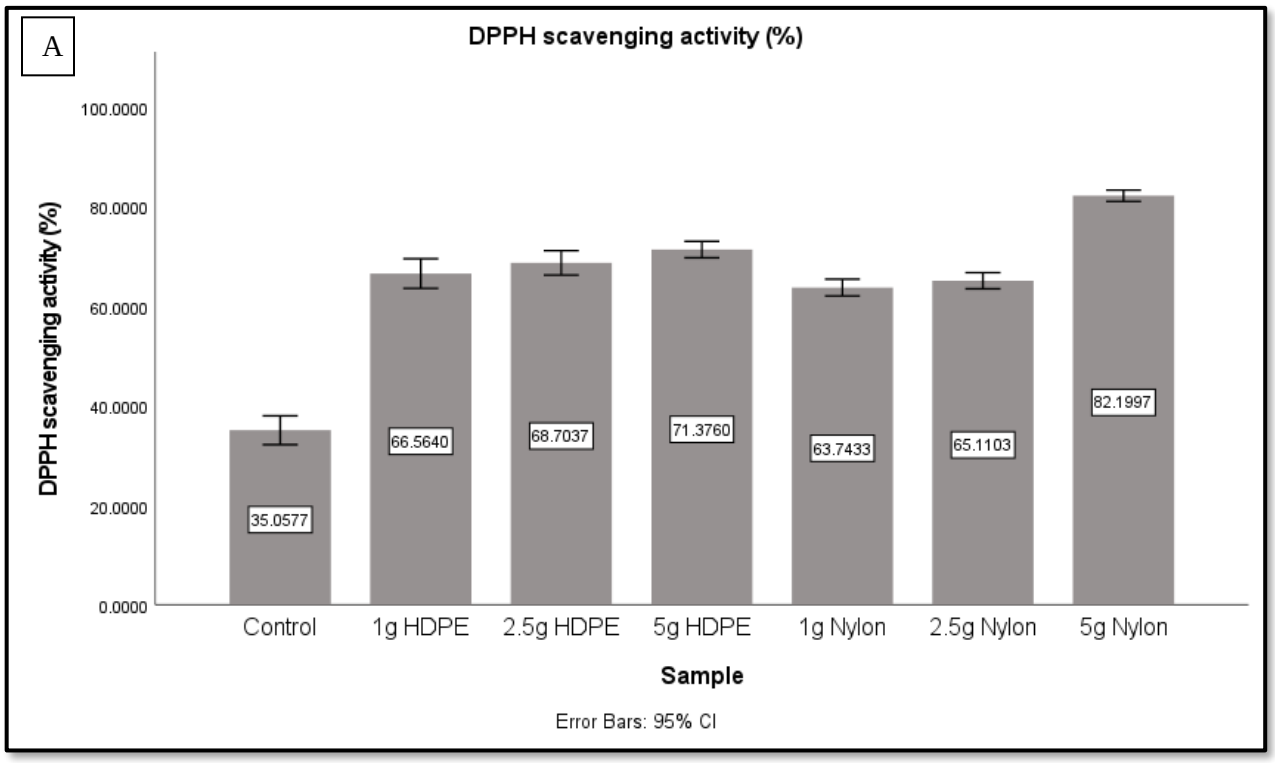

C
Protein content
20.0000
15.0000
10.0000
5.0000
0.0000
Protein (%)
12.4179
12.8850
13.4418
13.4520
13.2351
14.2030
15.4227
Control
1g HDPE
2.5g HDPE
5g HDPE
1g Nylon
2.5g Nylon
5g Nylon
Sample
Error Bars: 95% CI

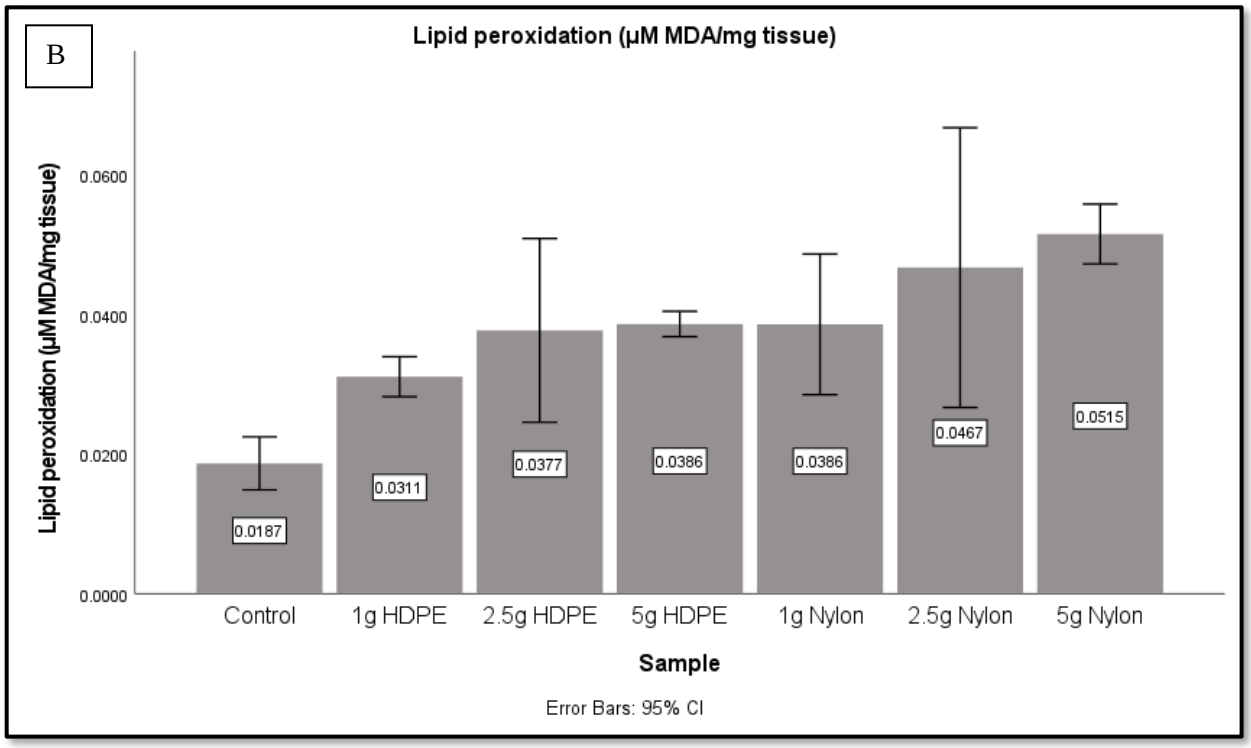


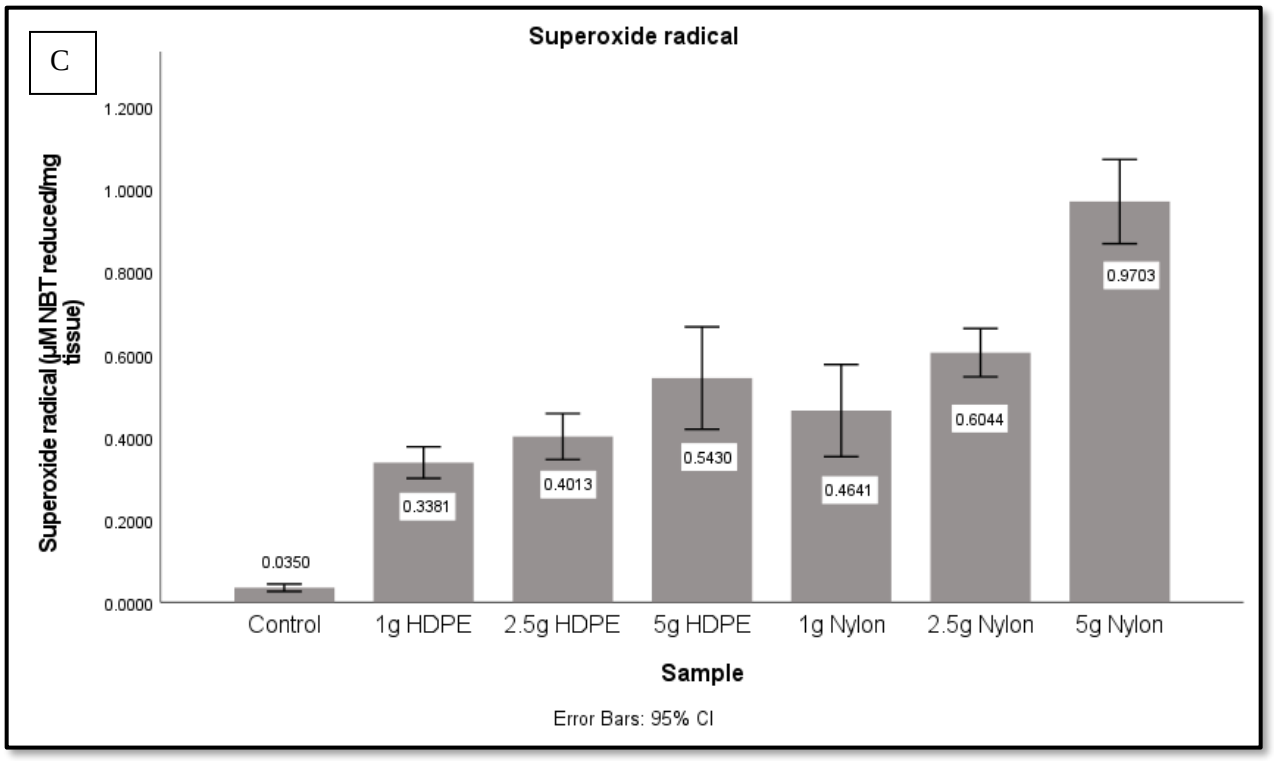
Graph 4: Effects of HDPE and Nylon addition on (A) Nitrogen content expressed as % (C) Protein content expressed as %. (B) and (D) (respectively)Comparative analysis of nitrogen and protein content of HDPE and Nylon

Particularly for Nylon treated wheat plants, nitrogen increased as compared to control and HDPE. These effects could be explained by the enrichment of soil with nitrogen due to Nylon as discussed in above section. Polyamide or Nylon production reaction involves polymerization of amines and carboxylic acids (Palmer 2001). The monomers of such polymers could leach out into the soil which work as fertilizer (De Souza Machado et al. 2019). Moreover, nitrogen supply to plants can increase leaf growth, in this manner it influences photosynthesis and chlorophyll content (Bojović and Marković 2009).

4.5 Oxidative stress analysis







Graph 5: Effects of HDPE and Nylon addition on (A) DPPH scavenging activity (B) Lipid peroxidation (C) Superoxide radical

The % of DPPH scavenging activity is directly related to the total antioxidants of the system thus, increase in % of DPPH scavenging activity with increase in plastic concentration indicates oxidative stress in plants shown in graph 5. Similarly increase in lipid peroxidation measured as µM MDA/mg tissue shows toxic effects of oxidative stress which due to the interaction of reactive oxygen species (ROS) with biomolecules (DNA, lipid, and proteins) of cells. The formation of this MDA in lipid peroxidation assay, a thiobutyric acid reactive substance (TBARS) is a biomarker of lipid peroxidation oxidation stress. The lipid peroxidation in a cell is one of the most serious effects of oxidative stress due to toxicant that damages cell membrane which could further increase permeability of cell membrane to foreign agents (Olszewska-Słonina et al. 2011). Normally in cell, there is an established equilibrium between ROS and antioxidant system which maintains the physiological homeostasis (Maity et al. 2020). The plastic added in soil increased

the concentration of superoxide and DPPH radical scavenging activity compared to control as shown in results suggesting ROS inducing potential of plastic like inorganic contaminant in soil. This could further cause shift in ROS equilibrium within the cell leading to the oxidation of biomolecules, eventually could cause cellular damages (Olszewska-Słonina et al. 2011).

Different type of microplastics affect wheat growth differently as Nylon has shown significant difference in results obtained as compared to control while HDPE in the concentrations treated in this study did not show observable effects. The shape of microplastic might be a reason of such effects as HDPE taken in this study was of smaller size than Nylon thus, other chemicals released due to partial degradation of Nylon was more than HDPE as shown by results. Presence of plastics having chemicals or compounds that can function analogous to compounds found in fertilizer in their structure can increase nutrients available to plants in agricultural soils. In previously reported pot studies, the addition of plastics was about 1% w/w of soil but the amount added in this study was less than 1% . Moreover, the effects were similar for Nylon (polyamide) plastic fiber Though the amount of plastic added is less in this study: 0.04%, 0.08% and 0.2% (w/w) of soil, these findings require more study of nutrient analysis of the soil after addition of such plastics which can further infer the increase and decrease of nutrients in soil available for plant growth.

CHAPTER 5 – CONCLUSION

This study shows further evidence for potential detrimental effects of microplastics in agricultural ecosystems by using wheat (*Triticum aestivum)* as a model system. In agricultural ecosystems, such detrimental effects might affect the overall yield and quality of crop plants by affecting plant growth and altering the soil nutrient environment and can have further potential implications for consumers through the uptake and accumulation in plant tissues. With continuous use of plastics in different fields, many new technologies have been introduced making plastics more durable which will further increase plastics in ecosystem and ecological risks from its pollution. The research of presence of plastics in the agricultural ecosystem and underlying mechanism of uptake by crop plants is still in its infancy. This information can help in understanding of extent of long-term exposure and future studies on other crop plants. This understanding can help in development of management strategies for plastic pollution and emission curb to agricultural ecosystems and further to humans as consumers.

CHAPTER 6 – REFERNCES

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