“Wheat (*Triticum aestivum*) as a model plant for studying the impact of HDPE microplastics”

A DISSERTATION

SUBMITTED IN FULFILLMENT OF THE

REQUIREMENT FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

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MAY, 2021

CANDIDATE'S DECLARATION

I, Saveena, 2K20/MSCBIO/26, student of M.Sc. Biotechnology, hereby declare that the project dissertation titled **'Wheat (*Triticum aestivum*) as a model plant for studying the impact of HDPE microplastics'** 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 science, 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.

I communicated a review paper entitled **"Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and current bioremediation approaches"** in Scopus indexed journal "Journal of applied biology and biotechnology" that got accepted on **18 Feb 2022** and will be published soon. Below is the insight for the same.

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CERTIFICATE

I hereby certify that the project dissertation titled **‘Wheat (*Triticum aestivum*) as a model plant for studying the impact of HDPE microplastics**’ which is submitted by Saveena, 2K20/MSCBIO/26, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, 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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(2K20/MSCBIO/26)

Date:06-05-2022

ABSTRACT

Plastics in the water have gotten a lot of attention in the recent decade since marine pollution has revealed its consequences on aquatic ecosystems, which are visible to the general public. Plastics in the agricultural ecosystem have not had any direct consequences, but they are concerning because they can accumulate in crop plants and influence consumers via the food web. The majority of the plastics dumped in the ocean are manufactured and utilised on land. Microplastics and nanoplastics are produced and stored in substantial amounts in soil as a result of plastic degradation. Furthermore, these plastic particles pollute terrestrial ecosystems, where they may first damage biota before spreading to other habitats. Plastics have also been proven to impact soil biophysical properties as well as geochemical properties. Plastics scattering and transport in soil could straightforwardly affect plants, bringing down crop creation. In this study, high density polyethylene was chosen as example of fibre plastic residues in organic soil to evaluate effects on wheat, a terrestrial crop, in a pot experiment in a natural setting. At each pot, the polymer was introduced in concentrations of 1g and 2.5g. The findings revealed effects on wheat plant growth, biomass, and chlorophyll content. In comparison to control plants, plastics produced oxidative stress in treated plants.

Keywords: Agricultural ecosystem, Microplastics, Nano plastics, High Density Polyethylene, Terrestrial plants, Crop plants

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LIST OF ABBREVIATIONS AND SYMBOLS

mm – Millimeter

nm – Nanometer

µm – Micrometer

°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

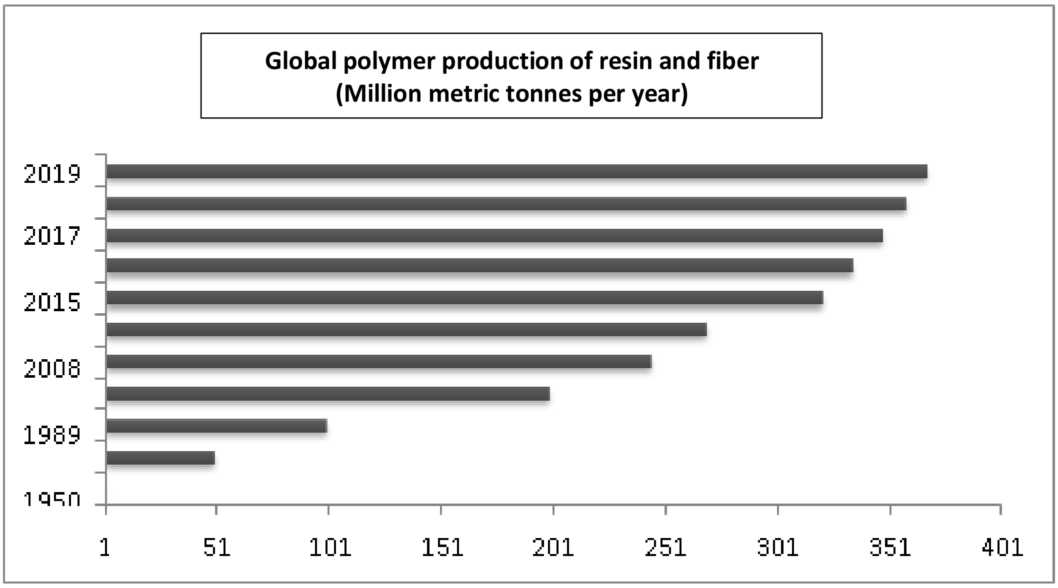
mg – Milligram

TEM – Transmission electron microscope

MDA - Malondialdehyde

Chapter 1 - Introduction

Plastic's features that make it useful for packing and manufacturing items – durability and resistance to external influences – also make it nearly impossible to totally eliminate from the environment (Zhu et al. 2019). Every year, the global manufacturing of plastics rises. Figure 1 shows that global plastic output climbed to 368 million metric tonnes in 2019 from 322 million metric tonnes in 2015 (Geyer, Jambeck, and Law 2017).



Graph 1: Plastic production from 1950 in million tonnes.

According to several reports, most plastic materials dissolve rather than deteriorate in the environment (Editor n.d.). Although plastics are long-lasting, they are susceptible to fragmentation in the environment owing to UV light and physical damage(Barnes et al. 2009). Microplastics are formed when big plastics decompose into smaller shards of less than 5mm in size.(Mammo et al. 2020) Microplastics and nanoplastics have different sizes that are currently unknown. Microplastic and nanoplastic are defined differently by different writers(Ng et al.

2018). Microplastics are particles in the nanometre (100nm to 5mm) size range, as well as sub-micrometre (100nm to 1m) and micrometre (1m to 5mm) plastics, and nanoplastics in the 1nm to 100nm size range. The prevalence of various plastic materials in maritime settings and their associated shorelines has sparked a lot of interest. Microplastic uptake by marine creatures has been studied extensively(Galloway, Cole, and Lewis 2017)(Thushari and Senevirathna 2020)(Horton et al. 2017). Microplastic has been found in the stomachs of aquatic species all across the world, according to studies. The investigation of infinitesimal plastic particles' travel past the stomach of life forms, through the food web, and between trophic levels is still in its beginning phases. While the destiny of plastics in the marine biological system is progressively surely known, the way of behaving of more modest plastic particles in the earthbound climate, especially in rural environments, stays a secret (Zang et al. 2020). So yet, critical thresholds for plastic pollution in soil have not been established, making it difficult to assess the bearing capacity of agricultural ecosystems (R. Qi et al. 2020). Two critical concerns in studying the impacts of plastics on terrestrial crop plants are whether terrestrial plants may acquire micro and nanoplastics, and if it is, how they can affect their growth (Zhu et al. 2019).

The genesis and fate of these plastic fragments in terrestrial ecosystems, particularly in terrestrial crops, have been studied recently(Rillig et al. 2019).

Plastics to Microplastics in agricultural soil

Plastic mulching is apparently advantageous for crops as it acts as insulation film and raises crop yield, reduces weed growth, raises soil temperature, maintains sustainable water usage by crops through improving better water absorption and lower transpiration rates (H. Gao et al. 2019). These are the reasons why plastic mulching is extensively employed in agricultural fields especially in arid, semi-arid and colder mountain regions (H. Gao et al. 2019). But gradually microplastics and nano plastics are formed due to long term use of plastic mulching films, mechanical aberrations like ploughing, heavy rains, moisture of soil due to irrigation, UV- radiations from sunlight and longer heat exposure. The plastics eventually undergo weathering and keep accumulating in the soil hence polluting the soil with MPs also the increment in number of landfill sites in recent years has made soil a large microplastic sink.

CHAPTER 2 – REVIEW OF LITERATURE

2.1 *Triticum aestivum* L. – A Brief Overview

Wheat (*Triticum aestivum*) is one of the three main cereal crops consumed internationally as a staple cereal crop, alongside rice and maize. The genus Triticum contains a number of wheat species, but the most widely grown is Triticum aestivum (*T. aestivum*). In most types, the cereal grass has long, slender leaves with hollow stems. The crop grows well in tropical, subtropical, and temperate climates. Wheat can withstand extreme cold and snow, and can continue growing once the weather warms up in the spring. Rabi (winter) and spring wheat are the two main varieties of wheat crop, with winter wheat being sown from October to December and harvested in February or March. Because ordinary wheat has a high gluten content, it's ideal for creating bread and also 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.

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

Wheat grows best in cool, wet weather, with winter fog prevalent during the growing season, followed by dry, warm weather to allow for adequate grain maturation. The ideal temperature for seed germination is 20-25 degrees Celsius.

2.2 *Triticum aestivum* as model organism

Model plants are plants that have been chosen for the simplicity of studying specific biological phenomena (ecology, physiology, biochemistry, genetics) that may be generalised to other plant species, as well as their importance in biotechnology and agronomy. Small size, ease of development, short life span, small genome, and genetic manipulation capabilities are some of the characteristics that distinguish plant species as model organisms. Wheat is also one of the most widely consumed crops on the planet, making it a good candidate for research into the ecological effects of inorganic pollutants found in agricultural soils.

Figure 1. Wheat Grass

2.3 Structure and uses of High-Density Polyethylene

HDPE is an ethylene-based linear addition polymer with minimal branching. HDPE is appropriate for use as a packaging material for containers and bottles because of its great flexibility and durability to extreme temperatures, such as those found in frozen food and refrigerated environments(Selke 2019). The presence of C–C and C–H bonds in the structure give HDPE its relatively inert qualities, making it resistant to most substances except strong oxidising agents(Selke 2019).

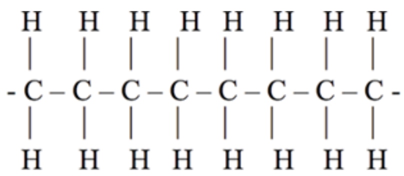


Figure 2: Polyethylene structure of High-Density Polyethylene

2.4 Potential effects of microplastics on soil texture and properties

Soil microplastics have major effect on the soil pH accompanied by increment in plant and human pathogens in the soil (Z. Li et al. 2021). MPs profoundly deteriorate the soil heath gradually. A study conducted in a home garden showed the entry of MPs into the terrestrial food chain via soil and presence of microplastics in high concentrations was detected in the faecal matter of earthworms, chickens and in the crops too (Huerta Lwanga et al. 2017). Microplastic presence in soil deteriorate biophysical properties of soil i.e., disturbing water-soil interaction, causing leaching, soil-microbe interactions. Microplastics are believed to increase the soil bulk density, disturb soil hydrological properties and physicochemical characteristics, which can implicate to difficulty in rooting of plants (De Souza MacHado et al. 2018). Apparent neurotoxicity and higher oxidative stress were observed in earthworms in the MP containing vermicompost furthermore MPs negatively impacting the pH, C/N ratio of soil (Zhong et al. 2021). MPs percolating in soil comes with heavy metals like Pd, As, Cu, Co etc., and organic pollutants in the polluted soil (Chai et al. 2020), but research on microplastics in relevance with heavy metal toxicity is very partial and therefore, requires further exploration. Further MPs can change carbon cycling in soil by changing decomposition process and also interfere in SOC determination for soil quality checking (Kim, Jeong, and An 2021). Higher cadmium mobility was seen as threat in MP polluted soil (Zhang et al. 2020).

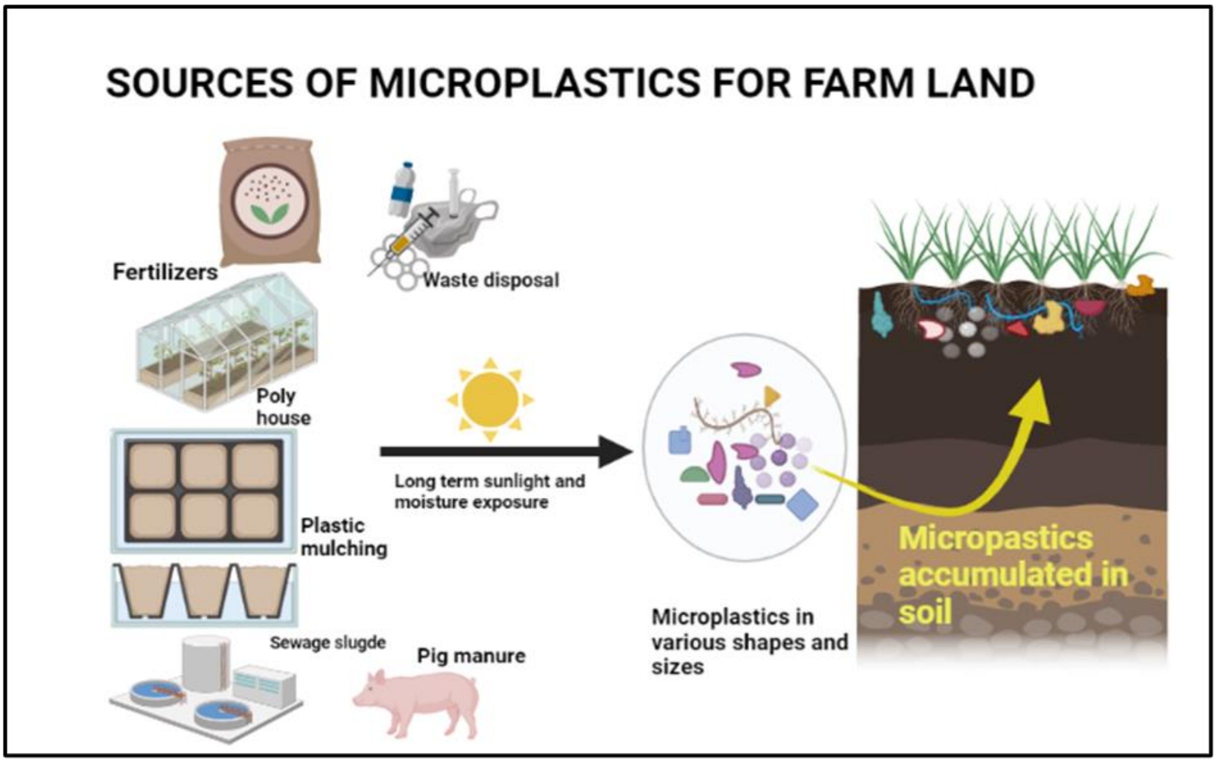


Figure 3: Different sources contributing to microplastic accretion in agricultural soil

2.5 Studies reported of microplastic effects on terrestrial plants

Studies on the translocation of plastics in plant tissues have been conducted(L. Li, Luo, Li, et al. 2020)(Maity et al. 2020)(L. Li, Luo, Peijnenburg, et al. 2020a)(Sun et al. 2020)(Lian et al. 2020)(Y. Qi et al. 2018)(Bosker et al. 2019). Endocytosis, passive diffusion, assisted diffusion, and translocation via plasmodesmata are all possible mechanisms for micro and nanosized particles to enter plant cells through cell wall pores(Maity et al. 2020). Because of their small size, nano plastics can pass through permeable plant cell walls and are delivered to the endodermis by capillary action and osmotic pressure(Lin et al. 2009). Other nanoparticles can be transported by the symplastic pathway, in which membrane proteins, ions, and aquaporins aid in translocation (Tripathi et al. 2017). Plasmodium-driven intercellular particle transport is mediated by plasmodesmata, which join cells(L. Li, Luo, Peijnenburg, et al. 2020a). Stomatal apertures could also be a mechanism for nanoparticles to be assimilated and then translocated through the xylem tissue(Hong et al. 2014). Plasticizers released into the soil by plastic can cause oxidative stress in wheat grains, increasing reactive oxygen species and lipid peroxidation(Y. Qi et al. 2018). Plasticizers were detected in greater concentrations in grains of wheat than in the root, stem, and leaves(M. Gao et al. 2019).

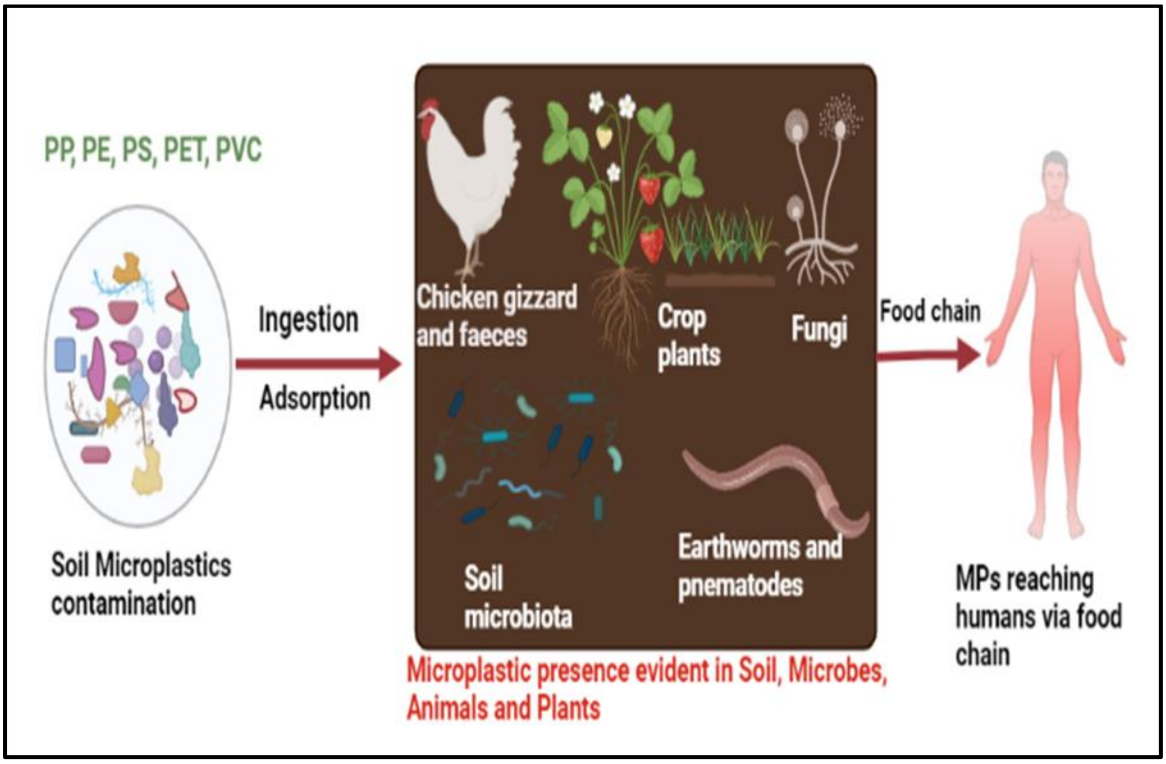


Figure 4: Microplastics in terrestrial ecosystem

2.6 Microplastics Effects on Plants and Food Crops

Several studies on interaction between soil microplastics and plants have shown the negative effects of MPs. A clear uptake by roots and localization of MPs occurs in plants via Apo-plastic pathway as demonstrated by employing fluorescent MP particles (L. Li, Luo, Peijnenburg, et al. 2020b), also MPs cause blocking of intercellular connections in roots thereby lowering the nutrient transfer and decreased biomass, lower catalase activation in higher plants and declined growth at higher MP concentrations (Jiang et al. 2019).

Higher arsenic concentration was detected in rice seedlings under exogenous microplastic presence leading to a higher ROS and lower Rubisco function and production thereby disturbing the normal photosynthesis process (Dong et al. 2020). They are also found to be inhibiting uptake of nutrients via roots of rice plants thereby lowering biomass and yield decrement. Polyethylene microplastics in soil cause lower metal ion (Pb and Zn) adsorption thereby lowering the metal bioavailability (M. Li et al. 2021).

In addition to changing the soil properties like pH and causing soil acidification, LDPE and biodegradable plastic mulching film accumulation in soil causes disturbances in wheat rhizosphere thereby disturbing their structure and strongly effecting wheat growth (Y. Qi et al. 2020). Significant decrement in Mineral levels of Mg, Ca and Fe alongside metabolite production was detected in in cucumber fruits in presence of PSNPs (Polystyrene nano plastics) and these nano plastic particles were absorbed via roots and after accumulation in root they were distributed across leaves and fruits (Z. Li et al. 2021). Microplastic pollution in soil adversely effects photosynthesis as observed in Lettuce plants that showed a reduced superoxide dismutase activity and dysregulated photosynthetic activity and electron transfer hurdles induced by two types of PVC microplastics PVC-a and PVC-b (Z. Li et al. 2020). MPs cause delay in seed germination and slower root growth as they accumulate at the germinal pore of seeds acting as physical blockers (Bosker et al. 2019). Soil being porous in nature allows microplastics to migrate to deeper soil layers via run-off and this can highly effect root growth, water absorption efficiency, root movement and so on (Hou et al. 2020).

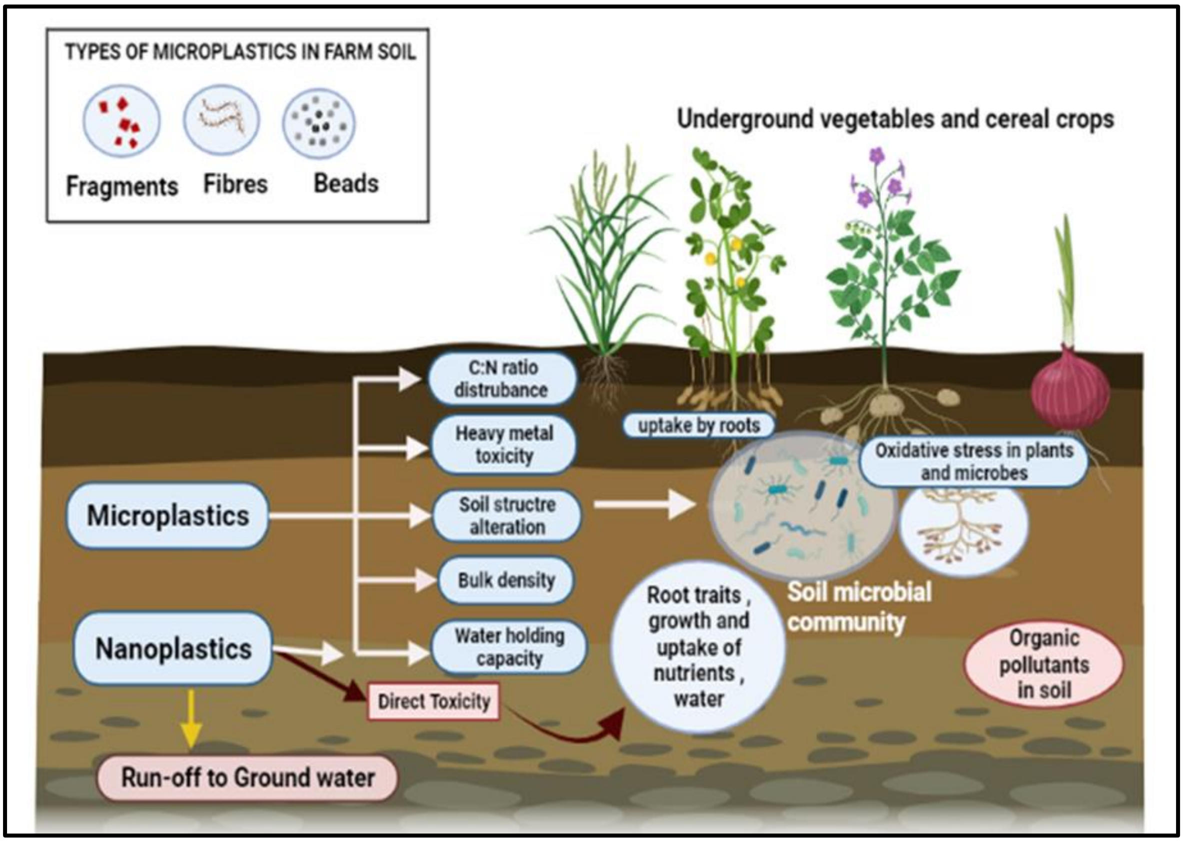


Figure 5: Conceptual diagrammatic representation of possible effects of microplastics on plant growth and soil properties

CHAPTER 3 – METHODOLOGY

3.1 Chemicals and Materials

- Plastic - High Density Polyethylene microplastic (40µm),
- Chemicals- Sodium Hypochlorite (NaOCL), Thiobutyric acids (TBA), sucrose, NBT, NADH, deoxy-ribose, DPPH, Trichloroacetic acid (TCA), Nile Red dye, acetone, Potassium sulphate (K2SO4), Copper sulphate (CuSO4), Sulphuric acid (H2SO4), NaOH

3.2 Study Plant

National Seeds Corporation, Pusa, New Delhi, provided wheat (*Triticum aestivum*) seeds. The seeds were first surface sterilised with 0.02 percent sodium hypochlorite NaOCl, followed by 70 percent ethanol. Seeds were regularly washed with distil water after sterilisation. The seeds were first germinated overnight on tissue in total darkness. In November, the germinated seeds were planted in organic soil. Seedlings were moved into different pots after emergence, with six seedlings in each pot. During the month of February, the pots were irrigated twice a week at first, and then once every two days. Because to the warmer weather in February, the irrigation frequency was increased. NPK was added to each pot based on the weight of the soil in each pot and the wheat NPK requirement of 100, 20, and 60 kg/ha as stated in the fields.

A. **B.**

Figure 6: (A) Wheat grains and (B) Germination done in complete dark

3.3 Experiment design and growth conditions

The investigation used a total of six pots: three control pots and three pots each of HDPE plastics. In natural light and circumstances, the pots were kept in a randomised order. Seeds were planted in November and harvested in March to simulate field conditions.

3.4 Plastic exposure

There was 2.5 kilograms of soil in each pot. Plastic was introduced after 10 days following seedling emergence, except in the control group. In two pots, different amounts of High-Density Polyethylene (HDPE) were added to reach 0.04 percent and 0.1 percent(w/w) accordingly.

Figure 7: HDPE in powder form

3.5 Microplastic tagging

Nile Red was used to tag HDPE microplastic particles. In deionized water, a 100g/ml Nile Red solution was generated by adding 1mg/ml Nile Red in acetone to 9ml deionized water. At a concentration of 0.5g of microplastic particles per 10ml of solution, dried microplastic particles were added to 100g/ml Nile Red in DI water. The vials were incubated in the dark for 2 hours before being washed with DI water several times until the supernatant became translucent. The particles were kept in DI water and examined using a fluorescence microscope. Roots from control and HDPE-treated wheat plants were removed and carefully cleansed to eliminate soil particles in order to examine microplastic uptake in wheat plants.

3.6 Measurement of wheat growth parameters

The wheat plants were taken 2 months before ripening and rinsed extensively under running water to remove soil without injuring the tissue. Using a weighing scale and a ruler, the new weight and lengths were estimated immediately, of both the root and shoot. The dry weight was estimated by keeping them for 72 hours at 70-80 degrees Celsius in a hot air oven, called drying. Chlorophyll, nitrogen content, DPPH scavenging activity, superoxide radical, and lipid peroxidation were all measured and reported after the harvest.

A

B

C

Figure 8: (A) Wheat plants growing in different concentrations of HDPE (B) Wheat plant harvested and cleaned under running water to remove soil from roots. (C) Stem of wheat plant

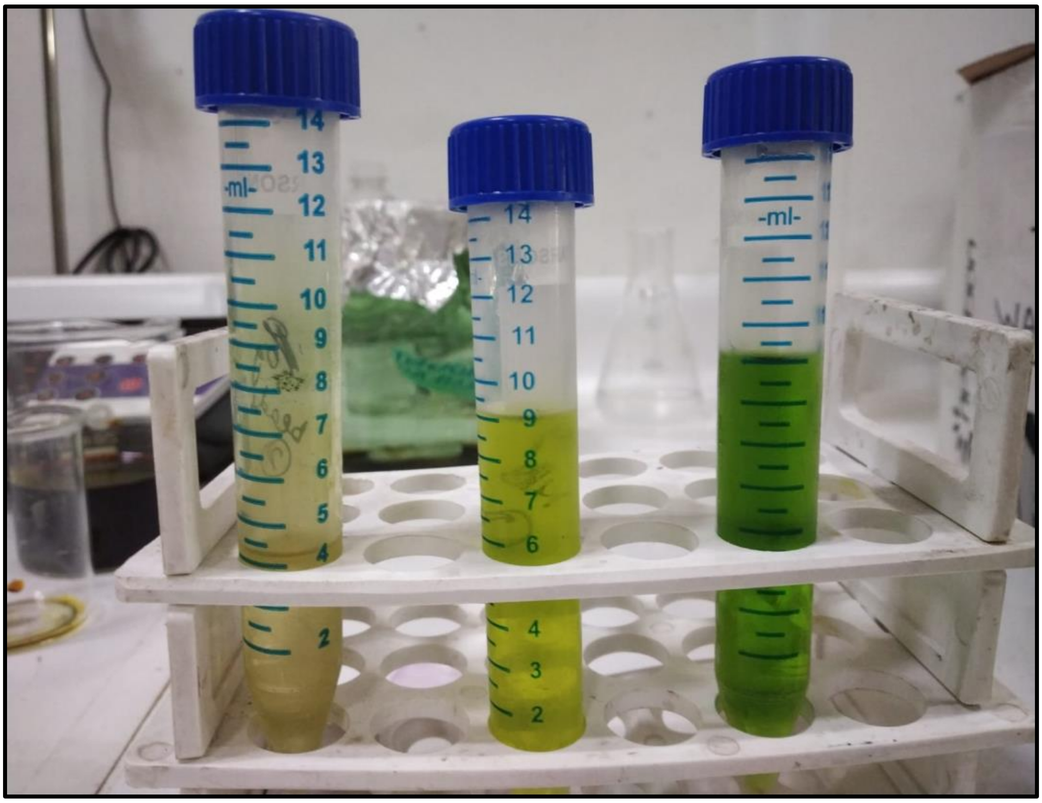
3.7 Chlorophyll content

To determine chlorophyll concentration, fresh leaves were gathered from each pot. Each pot yielded 0.5 grammes of leaves. The leaves were cut and homogenised with ten millilitres of 80 percent acetone until they became translucent. The extract was centrifuged for 5 minutes at 2500 rpm. Then, using a UV-Vis Spectrophotometer, 1ml of the obtained supernatant was diluted with 9ml of 80 percent acetone and read at 663nm and 644nm. Mackinney's work and Arnon equations were used to create the equations.

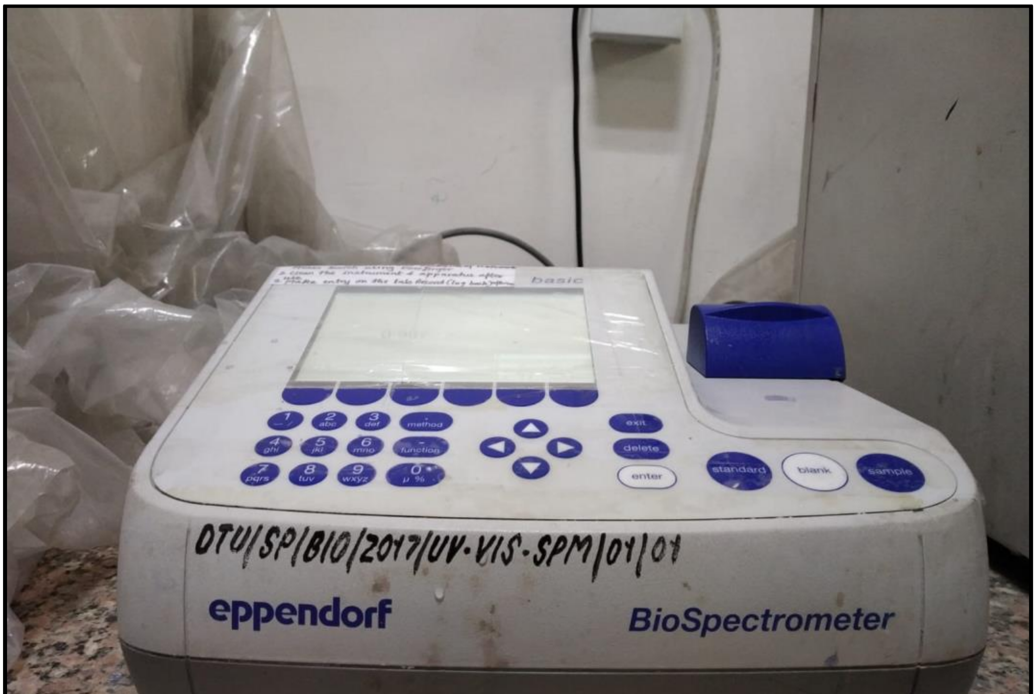
Chla = 12.7 A_{663} – 2.69 A_{645}

Chlb = 22.9 A_{645} – 4.68 A_{663},

Figure 9: (A) Homogenised leaf of wheat plants with 80% acetone



(B) Supernatant from centrifuged homogenized mixture diluted with 80% acetone



(C) Readings taken by UV-Vis spectrometer for chlorophyll content

3.8 DPPH free radical scavenging activity

DPPH is a persistent free radical that resembles the reactive oxygen species found in living cells. The oxidised form of DPPH in methanol is a deep blue colour, but when reduced by antioxidants, it becomes colourless. About 100 mg of wheat roots were taken as a sample, which was then homogenised and centrifuged, with the supernatant collected. 500 l of DPPH (60 M in methanol) solution was added to 1.5 ml of supernatant and incubated for 30 minutes at room temperature in the dark. After the process of incubation, at the wavelength of 517nm in a UV-VIS spectrophotometer, measured the absorbance. The proportion of DPPH free radicals scavenged (percent) = (Ao –As/Ao) *100 was used to compute the percentage of DPPH free radicals scavenged by antioxidants.

Where,

Ao is the absorbance of blank

As is the absorbance of sample.

Results were showed as %DPPH scavenged/mg of tissue (Maity and Pramanick 2020)

3.9 Lipid peroxidation

On ice, 100 mg of wheat roots were homogenised in a solution(4ml) having 0.5% TBA and 20% TCA. To stop the reaction, the sample was incubated at 95°C for 30 minutes before being abruptly cooled for 10 minutes on ice. It was centrifuged for 10 minutes at 1000 rpm. TBA-MDA complex and non-specific absorbance (respectively) were measured in the supernatant using a UV-VIS spectrophotometer at 532 nm and 600 nm. With a molar extinction value of 155 mM-1 cm-1, the MDA content produced was determined. The results were represented as milligrammes of MDA per millilitre of sample.

CHAPTER 4 – RESULTS AND DICUSSION

4.1 Microplastic tagging

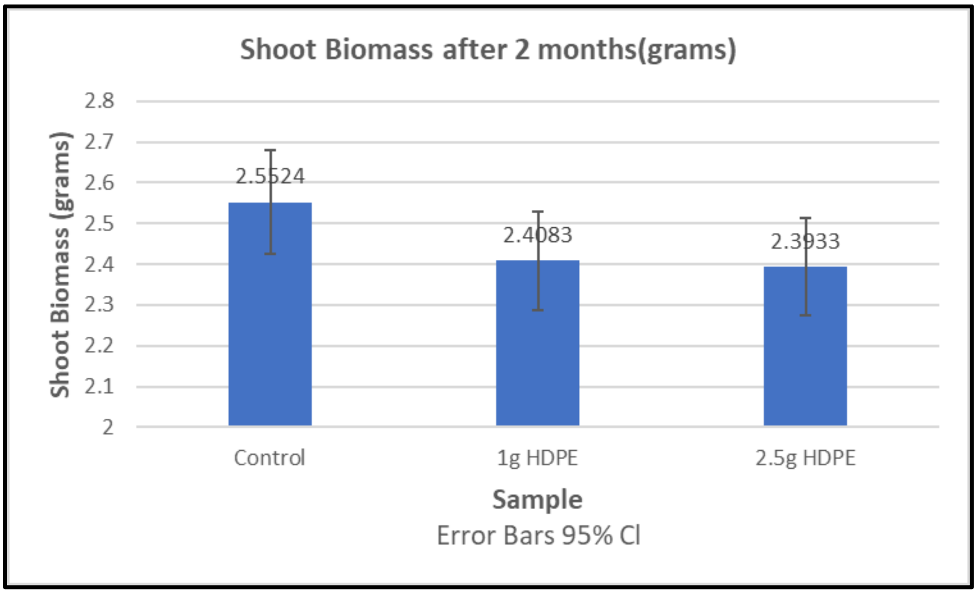
The fluorescent dye Nile Red was used to label the HDPE microplastics. Fluorescent microscopy revealed the dye's chemical assimilation.

Figure 10. Tagged microplastic imaged through fluorescent microscope

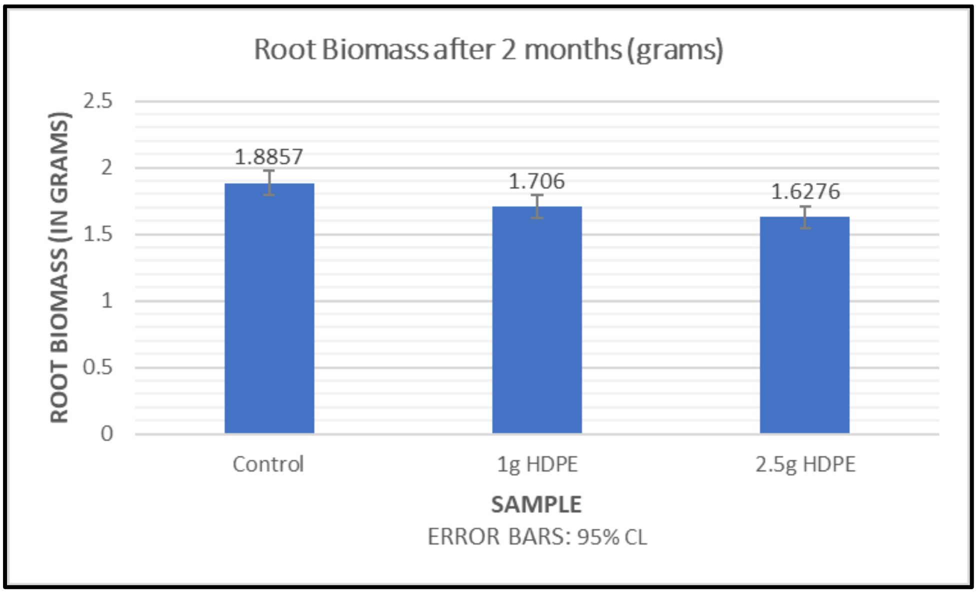
Visualizing the root cross - section under fluorescence microscopy, it could be observed that the tagged microplastics were transported across the xylem tissues as shown in red colour. These microplastics could further be assimilated in the phloem tissues of the plant.

4.2 Shoot and Root Biomass

A.



B.



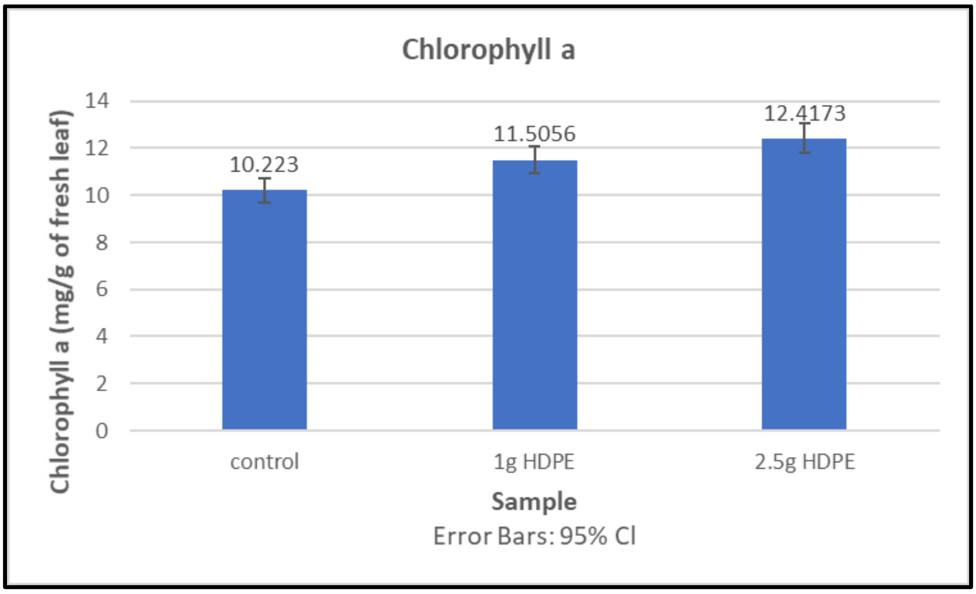
Graph 2: Effects of HDPE addition to (A) and (B) Shoot and Root biomass of wheat after 2 months of sowing.

Although there was no significant increase in shoot and root biomass, there was a difference between the highest concentration treated wheat plants and the control wheat plants as shown in graph. In both the cases as increased the concentration of HDPE from 1g to 2.5g, the shoot and root biomass was decreased.

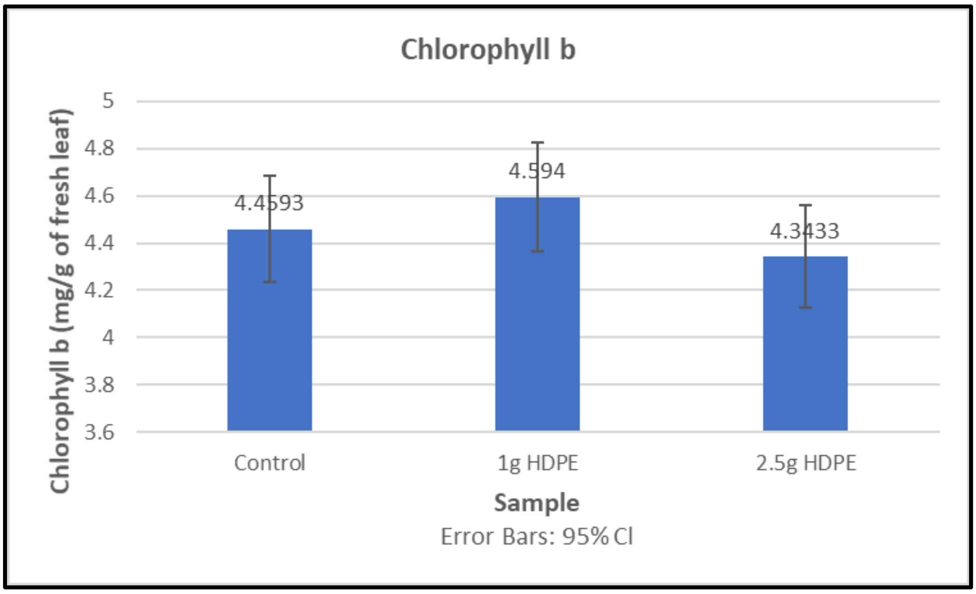
The results from the present study demonstrate that plastics particles adsorb particularly on the root hairs. Exposure to plastic particles results in short-term and transient effects on germination rate and root growth.

4.3 Chlorophyll content

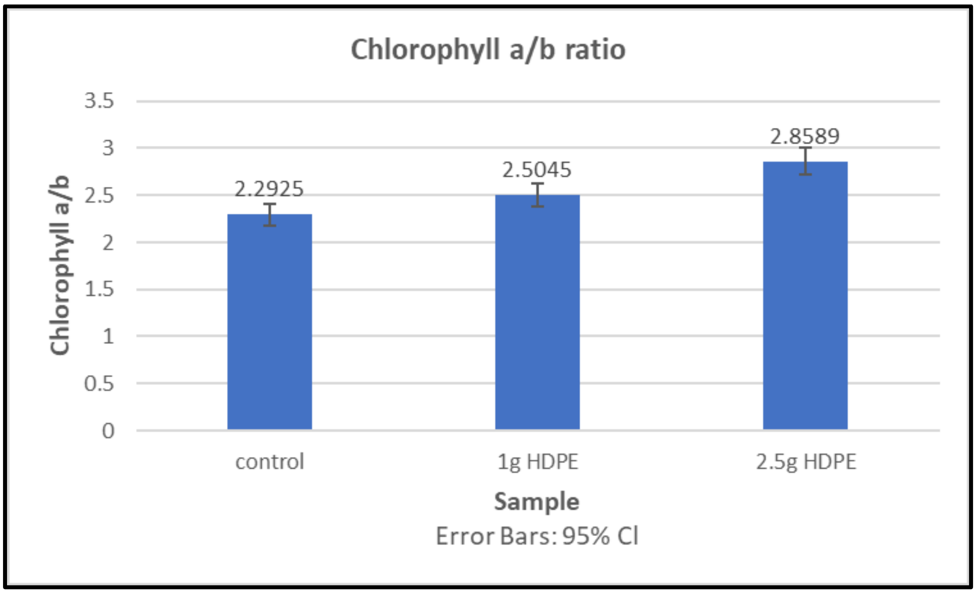
A.



B.



C.

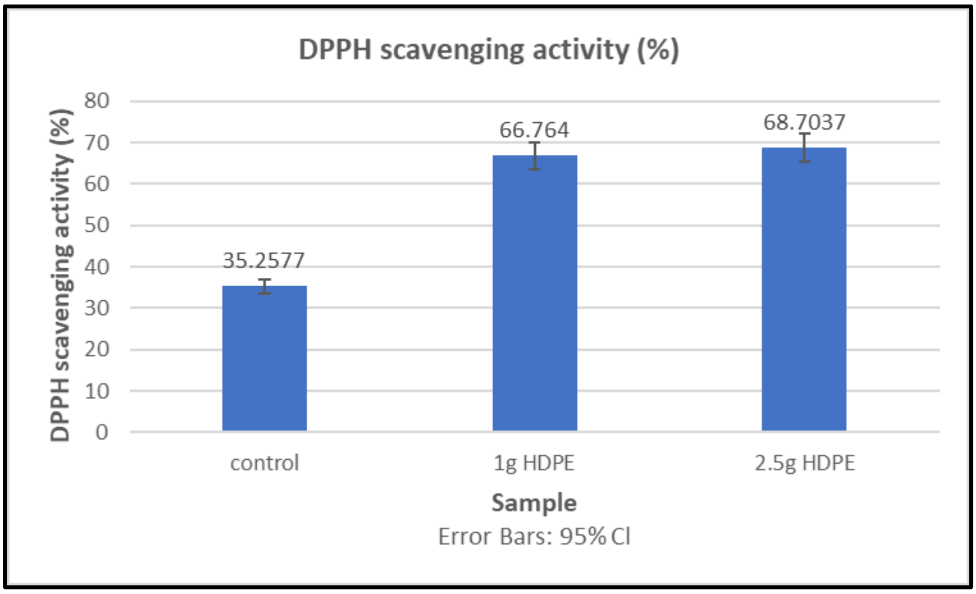


Graph 3: Effects of HDPE addition to (A) and (B) Chlorophyll a and chlorophyll b content respectively (C) Ratio of chlorophyll a to chlorophyll b

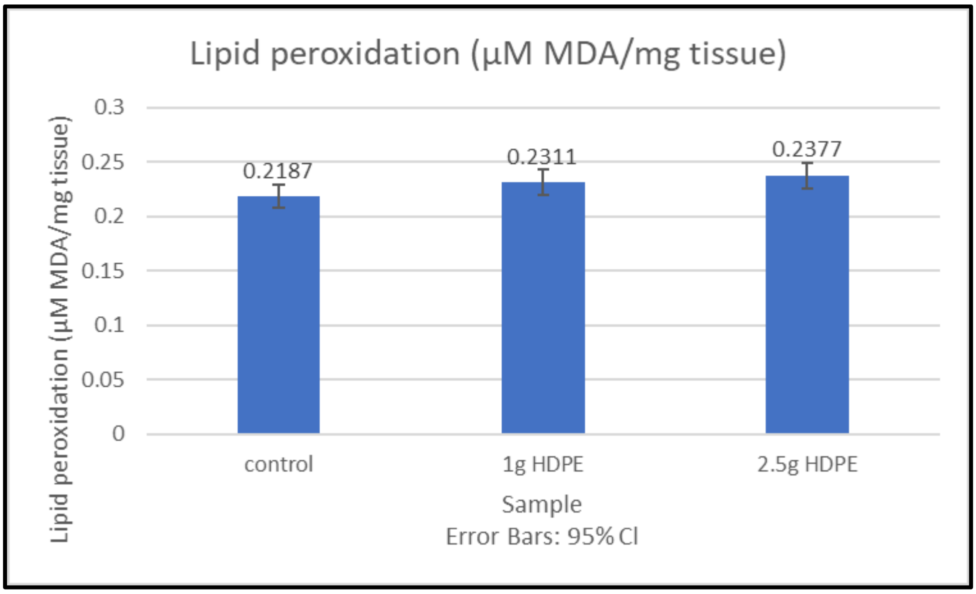
The chlorophyll a and chlorophyll b contents of wheat leaves from treated and control samples did not differ significantly, but the ratio of chlorophyll a to chlorophyll b increased in response to increasing microplastic concentrations when compared to control. The ratio of Chlorophyll a to Chlorophyll b is a crucial characteristic for photosynthetic activity, and its variations indicate plant stress. As a result, the increased ratio in this study indicates a considerable suppression of synthesis of chlorophyll b in response to addition of microplastic. Chlorophyll b is a pigment that promotes photosynthetic efficiency in plants and is also significant in the primary production of grassland in agricultural environments.

4.4 Oxidative stress analysis

A.



B.



Graph 4: Effects of HDPE addition on (A) DPPH scavenging activity (B) Lipid peroxidation

Because the percent of DPPH scavenging activity is proportional to the total antioxidants in the system, an increase in the percent of DPPH scavenging activity with an increase in plastic concentration suggests oxidative stress in the plants depicted in the graph. Similarly, a rise in lipid peroxidation, as measured by µM MDA/mg tissue, indicates the damaging effects of oxidative stress, which are caused by ROS interacting with DNA, lipids and proteins in cells. The development of TBARS (thiobutyric acid reactive substance) in a lipid peroxidation assay is a biomarker of lipid peroxidation oxidation stress. Lipid peroxidation in a cell is one of the most dangerous consequences of oxidative stress, as a toxicant destroys the cell membrane, potentially increasing permeability to outside agents. The physiological homeostasis of a cell is maintained by an established equilibrium between the ROS and antioxidant systems. As indicated in the results, adding plastic to soil enhanced the concentration of DPPH radical scavenging activity compared to the control, implying that plastic, like inorganic pollutant in soil, has the ability to induce ROS. This could lead to a shift in ROS balance within the cell, resulting in biomolecule oxidation and, ultimately, cellular damage.

CHAPTER 5 – CONCLUSION

Using wheat (*Triticum aestivum*) as a model system, this study adds to the growing body of evidence regarding microplastics' potential negative consequences in agricultural ecosystems. Such negative effects in agricultural environments could impair all out creation and nature of crop plants by lessening plant development and changing the dirt supplement climate, as well as have further ramifications for consumers through uptake and accumulation in plant tissues. With the widespread utilization of plastics in different businesses, numerous new advances have been created to make plastics stronger, resulting in an expansion in plastics in the environment and expanded natural worries from contamination. The investigation of plastics in the farming biological system and the hidden components of harvest plant take-up is still in its beginning phases. This information can help with deciding the degree of long-haul openness as well as future exploration on other rural plants. This knowledge can aid in the creation of management strategies for plastic pollution and emission reduction in agricultural ecosystems, as well as in the development of management strategies for people as consumers.

CHAPTER 6 – REFERNCES

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Review paper acceptance letter

February 17, 2022

Dear Jai Gopal Sharma

I am pleased to inform you that your manuscript titled "Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction, and current bioremediation approaches" (Manuscript Number: JABB-2021-12-803) is accepted for publication in the Journal of Applied Biology & Biotechnology.

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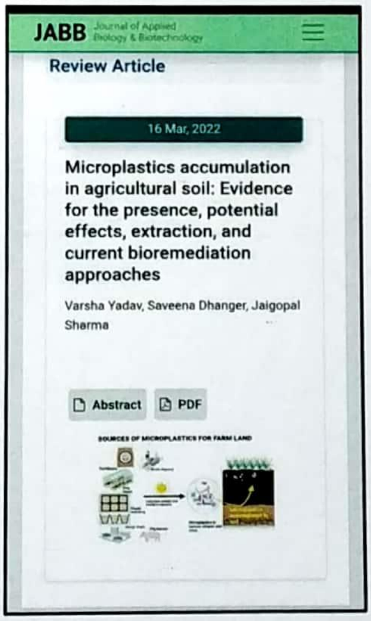
CANDIDATE'S DECLARATION

I, Saveena, 2K20/MSCBIO/26, student of M.Sc. Biotechnology, hereby declare that the project dissertation titled **'Wheat (*Triticum aestivum*) as a model plant for studying the impact of HDPE microplastics'** 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 science, 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.

I communicated a review paper entitled **"Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and current bioremediation approaches"** in Scopus indexed journal "Journal of applied biology and biotechnology" that got accepted on **18 Feb 2022** and will be published soon. Below is the insight for the same.



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CERTIFICATE

I hereby certify that the project dissertation titled **'Wheat (*Triticum aestivum*) as a model plant for studying the impact of HDPE microplastics'** which is submitted by Saveena, 2K20/MSCBIO/26, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, 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.

05/05/2022

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