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Original Research

Impact of Microplastic on Roadside Vegetable Cultivation: A Case Study of Agricultural Farmland

Tanveer Ahmad[1], Hammad Hussain[1], Aroosa Sabir[2], Muhammad Irfan[3], Ayesha Ghafar[4], Muhammad Jafir[5]*, Muhammad Azeem Sabir[6], Usman Zulfiqar[7], Manal Abdulaziz Binobead[8], Bandar M. AlMunqedhi[9]**

[1]Department of Horticulture, Muhammad Nawaz Sharif University of Agriculture Multan, 66000, Pakistan
[2]Department of Zoology, Bahauddin Zakariya University Multan, Pakistan
[3]School of Environmental Science and Engineering, Tianjin University, Tianjin, 300354, People's Republic of China
[4]Department of Botany, University of Central Punjab, Constituent Punjab College Yazman Road Bahawalpur, 63100 Pakistan
[5]School of Resources and Environmental Engineering, Anhui University Hefei 230601, China
[6]Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan
[7]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[8]Department of Food Science and Nutrition, College of Agriculture Food Science, King Saud University, Riyadh, Saudi Arabia
[9]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

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Abstract

Microplastic (MPs) pollution poses a significant threat to environmental integrity, particularly in agricultural areas adjacent to roads. There is a dire need to know the occurrence of MPs on roadside vegetable farmlands and their effect on the agro-ecosystem. Therefore, the study was planned to investigate the influence of MPs on the growth and development of roadside vegetables in the city of Multan, Pakistan. Analysis indicated that fiber, microbeads, and polythene bag particles were the main types of MPs. The concentration of MPs was inversely correlated with the distance of farmlands from the road. The maximum concentration of MPs (3490-3540 items/kg) was observed in the farmlands near the road (site-1), while the minimum MPs (2698-2761 items/kg) were measured in the farmlands located away from the road (site-3). Additionally, plant analysis showed that chlorophyll contents (a & b) and ascorbic acid (AsA) contents measured in all the vegetables were directly correlated with the distance from the road respectively Chl-a [(site-1 0.07-0.17), (site-2 0.14-0.20), (site-3 0.19-0.22 mg. g^{-1} FW), Chl-b [(site-1 0.04-0.23), (site-2 0.09-0.28) (site-3 0.17-0.36 mg g^{-1} FW)], AsA (site-1 0.54-0.64), (site-2 0.95-1.13), (site-3 1.57-1.82 µmol g^{-1})] and malondialdehyde (MDA) [(site-1 6.16-14.5),

*e-mail: m.jafir@ahu.edu.cn
**e-mail: usman.zulfiqar@iub.edu.pk

(site-2 6.03-13.16), (site-3 5.16-11.33 nmol g^{-1}), superoxide dismutase (SOD) [(site-1 200-224), (site-2 175.33-199.6) (site-3 157.33-136.33 U g^{-1} FW)], hydrogen peroxide (H_2O_2) [(site-1 12.5-14.66), (site-2 6.16-10), (site-3 3.66-6.66 nmol g^{-1} FW)] and carotenoids contents [(site-1 0.058-0.099), (site-2 0.033-0.069), (site-3 0.014-0.029 mg g^{-1} FW)] were inversely correlated with the distance from the road. This study reveals the phytotoxicity of MPs for plant growth and yield as well as hazardous for animals due to their possible transfer into the food chain. Our findings highlight the detrimental effects of MP contamination on plant growth and yield, emphasizing the urgent need for mitigation strategies for food security.

Environmental Implication

Microplastics (MPs) are a burning issue of environmental pollution and contamination of land along the globe. There is a dire need to know the occurrence of MPs on roadside vegetable farmlands and their effect on the agro-ecosystem. This study reveals the phytotoxicity of MPs for plant growth and yield as well as hazards for animals due to their possible transfer into the food chain. This study's results will attract the researcher's attention to solving this problem and devising new strategies for overcoming this problem.

Keywords: microplastic, vegetable farmland, environmental pollution, Microplastic stress

Introduction

The ubiquity of microplastic (MPs) defined as plastic particles <5 mm in size has escalated due to the disintegration of large plastic debris, the breakdown of synthetic textiles, and the abrasion of plastic products [1]. Nowadays, it has attained special attention in the field of agriculture research [2, 3]. Due to the unique characteristics of plastics (portability, lightweight, and ease of carry), they are attractive for the packaging industry at large scale [4]. Plastic has become an essential component of everyone's life day by day, and the demand for plastic is also increasing every day. The expected production of plastic will increase four times by 2050 [5]. Microplastic pollution is a significant emerging issue in agroecosystems, with implications for soil and water contamination and human health. MPs have become persistent and emerging contaminants in the environment, posing potential risks to agroecosystems and food safety. They can enter agricultural soils through various pathways, such as compost application, sewage sludge, and plastic mulch. Crops can absorb them, resulting in a buildup of these substances in the edible parts of the plant. This has raised concerns about the potential impact on soil health, food safety, and human health [6-9].

Apart from the tremendous benefits of MPs, two major drawbacks are associated with plastic's massive production and usage. a) The forecast for plastic's contribution to greenhouse gas (GHG) emissions is 15% of the global carbon budget by 2050. b) Post-consumer plastic products are a significant source of marine debris and plastic pollution (World Economic Forum, 2016). The primary significant sources of microplastic are weathering and degradation of plastic products like sheets, plastic bottles, and bags [10], road traffic dust, industrial products, atmospheric deposition, laundry effluents, sedimentation from irrigation water [11], and contaminated sewage sludge [12]. 80% of all microplastic pollution comes from the earth [13].

Microplastic influence has been shown on the plant community structure and ecosystem functioning [14]. The MPs can be transported through different ways, such as wind [15] and flooding water [16] into plants. MPs directly or indirectly influence plant growth in terms of lowered germination rate, reduction in root and shoot biomass, oxidative damage, and genotoxicity [17-20]. It has been observed that MPs from the environment can accumulate in plants' shoots and leaves [21]. Absorption of MP by plant surfaces has the potential to hamper photosynthesis, which in turn negatively impacts crop development [22, 23]. It can cause physiological stress in plants through oxidative stress responses, hormonal signaling pathway disruptions, or interference with cellular metabolism, leading to growth and development compromise and reduced resilience to environmental stressors [24, 25]. A broad range of MP effects has been seen in various plant species and different plastic kinds, including stimulation of root and shoot development [26]. The cyto-genotoxicity within the root meristematic region leads to oxidative stress in plants of *Allium cepa* L. [27], and increases root elongation and biomass in *Triticum aestivum* L. crops [28]. The MPs widely distributed in agricultural soils can impact soil properties, fertility, and the microbial community, affecting soil quality and nutrient cycling. Plastic goods are manufactured and processed with various additives, such as plasticizers, flame retardants, and stabilizers, to improve their performance and functionality. These additives are gradually released into soils after prolonged exposure to the natural environment, which can negatively affect soil microbial diversity and functions [29]. MPs in soil can have positive, negative, or neutral effects on soil properties, enzyme activities,

microbial communities, soil animals, and plant growth. The impact of MPs depends on their type, content, size, shape, soil properties, and exposure duration[7]. Due to their persistent time in the environment, microplastics can dangerously affect living organisms. Since agricultural products play an important role in the living organism's food chain, they may cause severe health and safety issues due to the contamination of air with microplastics. The benefits of mulching such as water saving and cost-effective control of weeds [30] hide its negative effect i.e., polypropylene coating releases microplastics into the soil [31, 32]. Additionally, the exposure of crops to microplastics is significantly increased by using sludge and organic fertilizers.

Vegetables are the main component of any balanced and healthy diet. They can provide high amounts of nutritional compounds like minerals, carbohydrates, protein, vitamins, and phytohormones. It contributes to the metabolic function of the body [33-35]. Over the last decade, the demand for leafy vegetables and their products has increased day by day due to their nutritional values and health benefits [34]. Therefore, this study aimed to investigate the influence of microplastic on the growth and development of roadside vegetables, which is foundational for subsequent research. In particular, the study focused on identifying and characterizing microplastics and assessing their impact on the photosynthetic content (chlorophyll-a and b), ascorbic acid content, and antioxidant enzymes along the road of vegetable farms. This study hypothesized that microplastic contamination may significantly impact roadside vegetable cultivation in agricultural farmland, affecting plant growth, nutrient uptake, and overall crop productivity.

Materials and Methods

Sample Collection

A survey study was carried out to collect the plant as well as soil samples from different farmlands along the roadside of Muhammad Nawaz Shareef University of Agriculture Multan Pakistan. Diet-based diversified four vegetables, including spinach, cauliflower, potato, and fenugreek, were selected for the leaf sampling. Sampling farmlands (fields where the selected crops were grown) for spinach (30.14707N,71.45536E), cauliflower (30.14803N,71.45699E), potato (30.14563N,71.45004E) and fenugreek (30.14625N,71.45179E) were selected after observation of domestic garbage, nylon nets, plastic bags, polyfoams and movement of heavy traffic. Sampling sites were divided into three regions Site-1 (Near to road), Site-2 (Mid of farmland; 50 meters away from the road), and Site-3 (Far from the road or end of farmland; 100 meters away from the road). From each site, soil samples were collected at a depth of 5-10 cm, stocked into an aluminum box after removing the visible big rubbish (>5 cm), then transported to the laboratory and stored at 4ºC before analysis. Additionally, plant samples were also collected from Site-1, Site-2 and Site-3. Plant samples were taken at -80ºC for further analysis.

Microplastic Extraction

Soil samples were allowed to naturally dry after removing the large visible soil rubbish (>5 cm). The density separation method was adopted to extract MPs from soil samples following a previous method [36]. Take 100 g of soil from soil samples and mix it with 200 mL of saturated NaCl (1.19 g/cm^3, Sigma Aldrich, China) and then transfer it into a 500 mL glass beaker. By ultrasonic equipment, the mixture was shocked for five minutes and stirred with the help of an oscillator for 30 minutes. The supernatant liquid was collected after stabilizing for 24 hours into a clean beaker and filtered through filter paper (Whatman, USA). To successfully extract MPs from samples, this process was repeated three times. For the decomposition of organic matter, the solid substances on the filter paper were washed into a glass flask and digested with 100 mL of 30% H_2O_2 (Sigma Aldrich, China). The digestion liquid was placed into an incubator at 50ºC for the time of 48 hours, and every 2 hours, the solution was shaken manually for 30 seconds. The final solution was filtered with the help of filter paper. The filter paper was retained in glass dishes and dried at room temperature. A stereoscopic microscope (Optika SZM-2) was used to examine the filter membranes for the presence of MPs, and a high-resolution camera attached to computer software captured images of the particles. According to the shape of microplastic, MPs were grouped into fragments (with angular and edges), fibers (elongated), and film (soft and thin), illustrated in Fig. 1. [36, 37]. Filter particles were

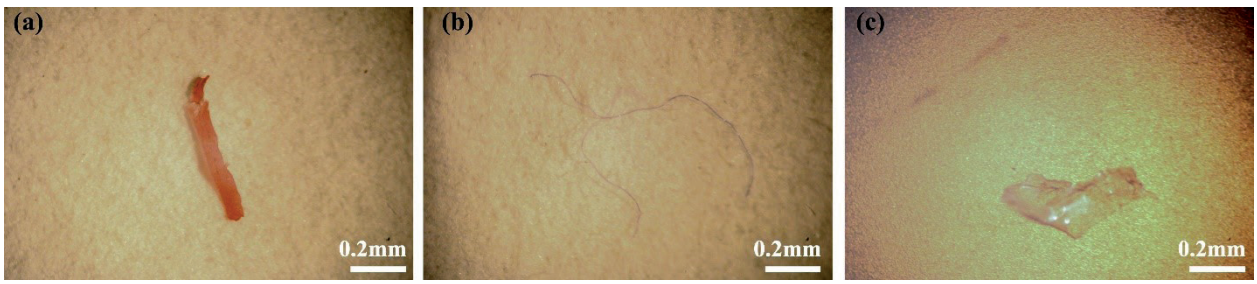

Fig. 1. Stereoscopic microscope (Optika SZM-2) MPs photographs illustrating shapes: fragment, fiber, and d film.

analyzed to determine their four distinct properties and converted into items/kg.

Chlorophyll (*a* & *b*) and Carotenoid Contents

The chlorophyll contents "a" and "b" were determined following the method described by [38]. The leaf chlorophyll contents were measured for the plants grown in plastic pots at 25 days. Fresh leaves (0.1 g) from each plant within each treatment were excised, and the chlorophylls were extracted overnight with 80 % acetone at 0.4ºC. The extracts were centrifuged at 10,000 × *g* for 5 min. The absorbance of the supernatant was read at 645, 663, and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan). The chlorophylls '*a*' and '*b*' were calculated by the following formulae:

$$\textbf{Chl. } \boldsymbol{a} = [12.7(\text{OD } 663) - 2.69 (\text{OD } 645)] \times \text{V}/1000\times \text{W}$$

$$\textbf{Chl. } \boldsymbol{b} = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times \text{V}/1000\times \text{W}$$

To quantify the carotenoid contents, predetermined acetone-dissolved samples were used. Prepared samples were centrifuged at 3000 g to segregate the plant debris from the carotenoid-containing solvent fraction. The supernatant containing the extracted carotenoids was used for spectrometric analysis. The absorbance of each extract was measured at 450 nm wavelength and followed the mathematical and statistical outline of Ur-Rehman and his colleagues [39, 40].

Determination of Ascorbic Acid Contents

Ascorbic acid contents were determined following [41]. Fresh leaf material (0.25 g) was homogenized in 10 mL 6% trichloroacetic acid (TCA) solution. Four milliliters of the extract were reacted with 2 mL (2%) of dinitrophenyl hydrazine in an acidic medium. Afterward, one drop of thiourea (10%) was prepared in 70% ethanol and added to the reaction mixture. The reaction mixture was boiled in a water bath for 20 min. After cooling the mixture (at 0°C), 5 mL of 80% H_2SO_4 (v/v) was added to it. The absorbance of the colored mixture after the reaction was read at 530 nm. The concentration of ascorbic acid was worked out from a standard curve using a series of standard solutions (50-300 ppm) of ascorbic acid.

Analysis of Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined following the method of [42]. The method was based on the principle of photochemical reduction inhibition of nitroblue tetrazolium (NBT) at 560 nm. SOD activity was determined by adding the enzymatic extract 50 µl to a solution having 50 µM NBT, 1.3 µ*M* vitamin B2 (riboflavin), 13 m*M* methionine, 75 n*M* EDTA, and 50 m*M* phosphate buffer (pH 7.8). The reaction solution was placed below a light source of 30 W fluorescent in a chamber having an inner side coated with aluminum. The reaction was initiated by switching on the fluorescent lamps for 15 min and stopping the reaction by turning off the lamp. In the photoreduction of NBT, the blue formazone produced was measured as absorbance at 560 nm using a UV-visible spectrophotometer. The reaction mixture without enzyme extract and other samples were taken as the control. One unit of SOD was defined as the amount of the enzyme needed to cause 50% inhibition of the rate of NBT reduction in comparison with tubes without the enzyme extract.

Malondialdehyde (MDA) Content Determination

Salt-induced oxidative damage (membrane lipid peroxidation) was assessed by measuring the amount of malondialdehyde in tissue as described by [43] with minor modifications. Leaf samples of 1.0 g were homogenized in 3 mL of 0.1 % (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 20000 x *g* for 15 min. Three mL of 0.5 % thiobarbituric acid (TBA) prepared in 20 % TCA was added to 0.5 mL of the supernatant. The mixture was heated at 95ºC in a water bath for 50 min. The reaction was stopped by cooling the tubes in chilled water. Then, the samples were centrifuged at 10,000 × *g* for 10 min, and the absorbance of the supernatant read at 532 and 600 nm. The MDA concentration was calculated as the difference in absorbance at 600 and 532 nm using the following formula:

$$\text{MDA level (nmol)} = \Delta (\text{A } 532 \text{ nm} - \text{A } 600 \text{ nm})/1.56\times 105$$

The absorption coefficient for calculating MDA is 156 $mmol^{-1}cm^{-1}$.

Estimation of Total Hydrogen Peroxide Contents

The trichloroacetic acid protocol was followed by [44] to estimate hydrogen peroxide contents. Grinding the samples in pestle and mortar was used to extract the samples 0.1% (v/v) TCA. After centrifuging the sample to the 0.5 ml of supernatant, 0.5 mL of potassium phosphate buffer and 1 mL of potassium iodide were added. Then, a Pico drop was used to measure the absorption at a wavelength of 390 nm.

Data Analysis

The collected data were analyzed via Tukey HSD and two-way ANOVA to compare the different means of various treatments at a significance level of $\alpha = 0.05$. Statistics 8.1 software was used to perform the statistical analysis.

Results

Distribution of Microplastic Abundance

In the present survey study, the results of soil analysis indicated a significant difference ($p<0.05$) in microplastic concentration according to the location of farmland. Tukey's HSD test was performed for a mean comparison of MP concentration in soil samples collected from different sites, and results indicated the highest concentration of MPs in those samples collected to the nearest road (site-1). The MP concentration was decreased by increasing the distance from the road and the microplastic concentration range (3540-2698 items/kg). The increasing order of MP concentration (site-1>site-2>site-3) was observed in different farmland samples. The MPs concentration were recorded in crops including potato, fenugreek, spinach, and cauliflower respectively [(site-1 (3540 items/kg), site-2 (3051 items/kg), site-3 (2761 items/kg)] [(site-1 (3510 items/kg), site-2 (3031 items/kg), site-3 (2698 items/kg)], [(site-1 (3495 items/kg), site-2 (3029 items/kg), site-3 (2711 items/kg)], [(site-1 (3490 items/kg), site-2 (3035 items/kg), site-3 (2721 items/kg)] (Fig. 2).

Influence of Mps on Photosynthesis Pigment (Chl-*A* and Chl-*B*)

The results of photosynthetic pigment (Chl-a) in crops indicated a significant difference ($p<0.05$) among the different sites of farmlands while there was a non-significant difference among different crops. For the mean comparison of Chl-a contents, Tukey's HSD test was performed, and results indicated the lowest contents of Chl-a in those samples that were collected from nearest to the road (site-1) of different farmlands. Increasing order of Chl-a contents (site-1<site-2<site3) was observed among the sites of different farmlands. As the distance of the sampling site increased from the road, higher chlorophyll contents were observed. Chl-a contents in crops including fenugreek, cauliflower, potato and spinach were recorded as ([(site-1 0.1798, site-2 0.2007, site-3 0.2222)], ([(site-1 0.1097, site-2 0.1916, site-3 0.1966)], ([(site-1 0.0915, site-2 0.1419, site-3 0.1915)], ([(site-1 0.0775, site-2 0.176, site-3 0.198)] respectively (Fig. 3). Similarly, Chl-b content results indicated the lowest chlorophyll-b content in those collected samples that were nearest to the road (site-1) of different farmlands. Among the sites of different farmland, the increasing order of Chl-b (site-1<site2<site3) was observed. The maximum chlorophyll contents were observed in those samples which were collected from the distant road. The Chl-b content was recorded in various crops including fenugreek, cauliflower, spinach, potato, ([(site-1 0.2354 mg g^{-1} FW, site-2 0.284 mg g^{-1} FW, site-3 0.3694 mg g^{-1} FW)], [(site-1 0.056 mg g^{-1} FW, site-2 0.108 mg g^{-1} FW, site-3 0.277 mg g^{-1} FW)], [(site-1 0.097 mg g^{-1} FW, site-2 0.1729 mg.g^{-1} FW, site-3 0.2356 mg g^{-1} FW)], [(site-1 0.048 mg g^{-1}FW, site-2 0.097 mg g^{-1} FW, site-3 0.1749 mg g^{-1} FW)] respectively (Fig. 4).

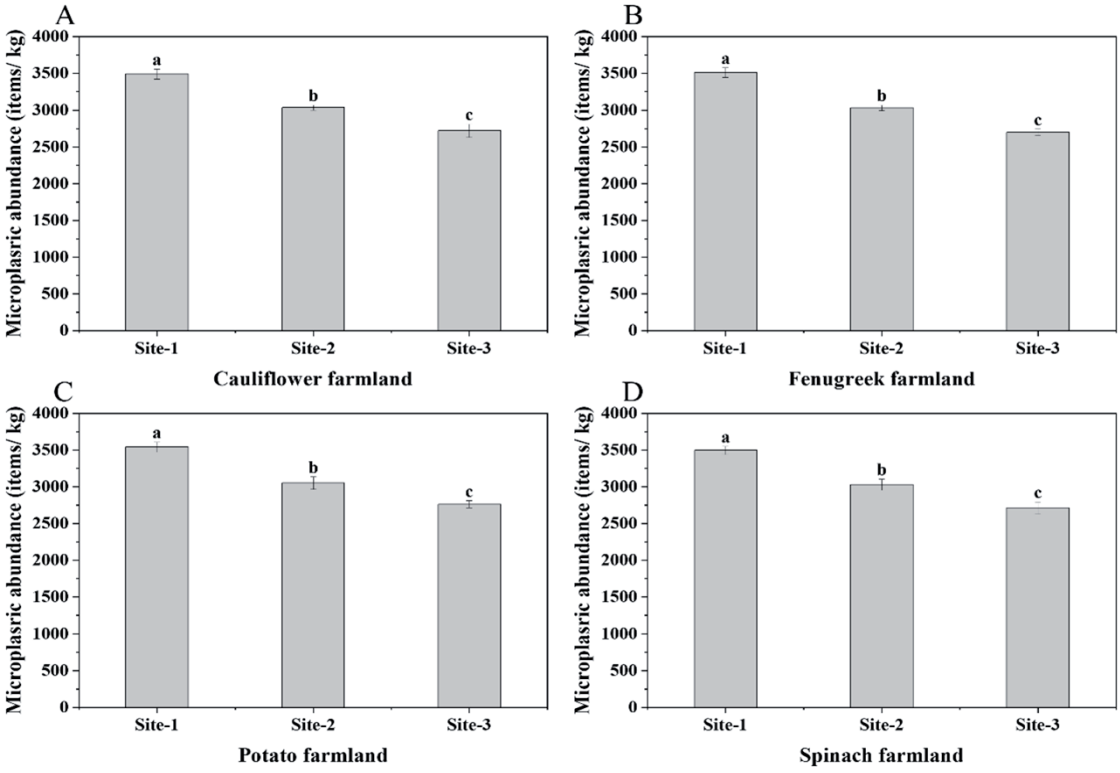

Fig. 2. Presence of microplastic in different farmlands alongside the road in Multan, Pakistan.

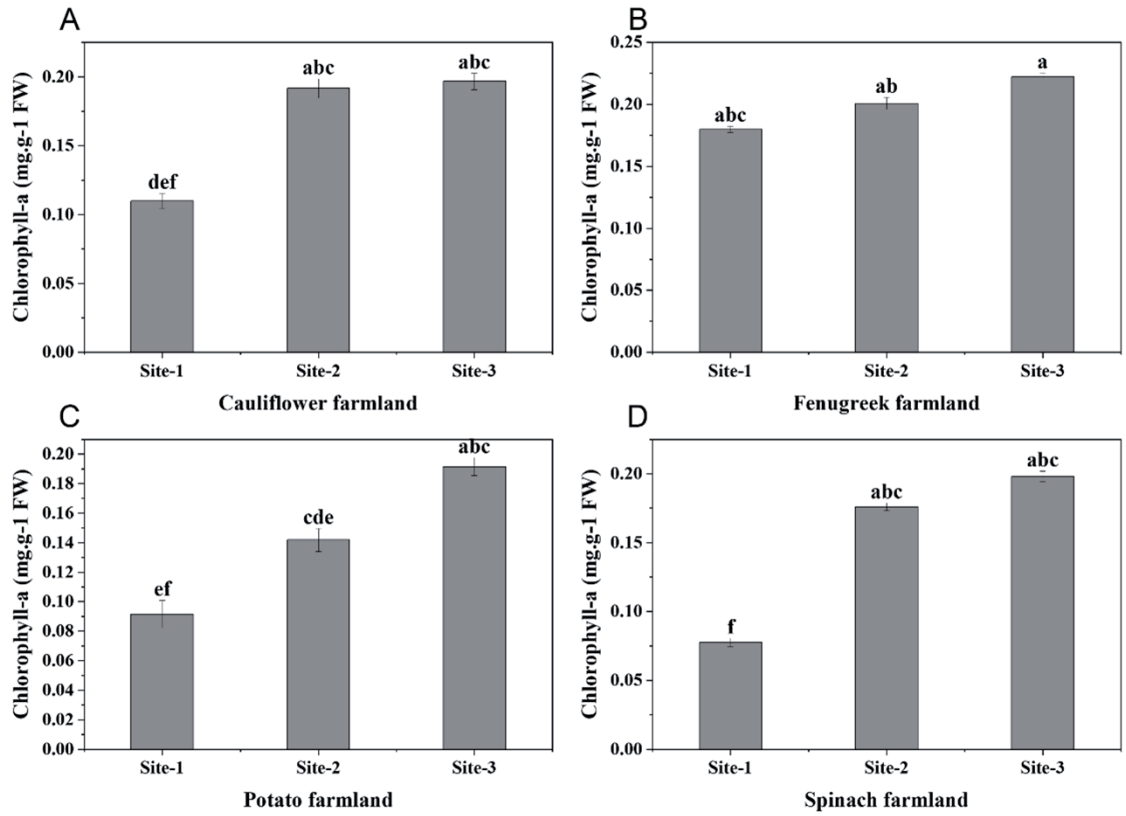

Fig. 3. Chlorophyll-a pigment of different farmland samples alongside the road in Multan, Pakistan.

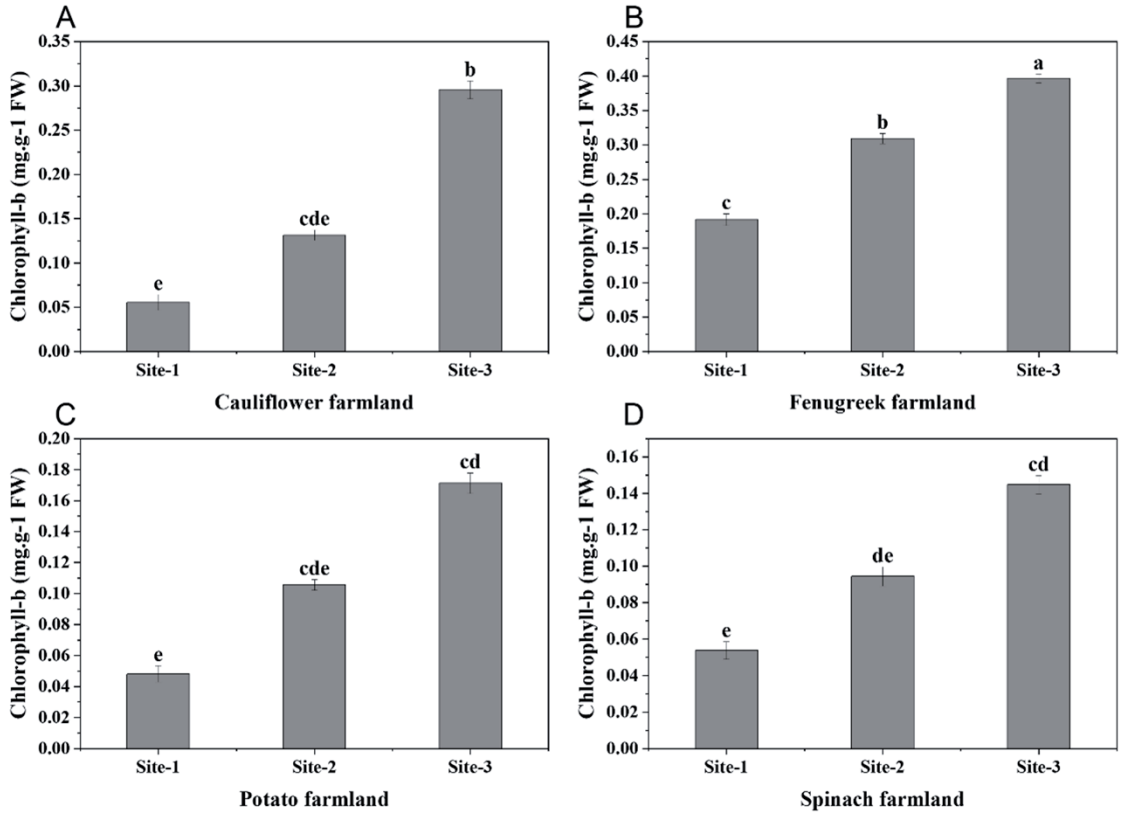

Fig. 4. Chlorophyll-b pigment of different farmland samples alongside the road in Multan, Pakistan.

Impact of Carotenoid Content Regarding MP Stress

The results of carotenoid content indicated a significant difference ($p<0.05$) in different farmland sites, while non-significant difference among different crops. Tukey's HSD test was performed for a mean comparison of carotenoid content, and results indicated the highest carotenoid content was recorded in those samples collected from the near to road (site-1). As the distance of the sampling site increased from the road, the lowest carotenoid contents were observed. The increasing order of different farmland sites (site-1>site-2>site3) was observed. The carotenoid content in crops including potato, cauliflower, fenugreek, and spinach was recorded as [(site-1 0.0992 mg g^{-1} FW, site-2 0.054 mg g^{-1} FW, site-3 0.0292 mg g^{-1} FW)], [(site-1 0.0966 mg g^{-1} FW, site-2 0.0694 mg g^{-1} FW, site-3 0.0275 mg g^{-1} FW)], [(site-1 0.079 mg g^{-1} FW, site-2 0.059 mg g^{-1} FW, site-3 0.0438 mg g^{-1} FW)], [(site-1 0.0586 mg g^{-1} FW, site-2 0.0331 mg g^{-1} FW, site-3 0.0143 mg g^{-1} FW)], respectively (Table 1).

Impact of Ascorbic Acid Content Regarding Mps Stress

The results of ascorbic acid content showed statistically significant differences ($p<0.05$) in different sites of farmlands, while non-significant differences among different crops. For the mean comparison of AsA, Tukey's HSD test was performed, and results indicated the highest AsA contents were recorded in those samples which were collected from a distance to roads (site-3). The lowest AsA content was observed in the nearest road samples (sit-1). The increasing order of different farmland sites (site-1<site-2<site3) was observed. The ascorbic acid content in crops included spinach, cauliflower, fenugreek, potato was recorded as [(site-1 0.6417 µmol/g, site-2 1.1333 µmol/g, site-3 1.8233 µmol/g)], [(site-1 0.5673 µmol/g, site-2 1.0803 µmol/g, site-3 1.7 µmol/g)], [(site-1 0.5413 µmol/g, site-2 0.956 µmol/g, site-3 1.577 µmol/g)], [(site-1 0.5413 µmol/g, site-2 0.956 µmol/g, site-3 1.577 µmol/g)], respectively (Fig. 5).

Superoxide Dismutase (SOD) Contents

Superoxide dismutase (SOD) content results indicated a significant difference ($p<0.05$) in crop samples that were collected among the different farmlands, while the non-significant difference among different crops (Table 2). The highest value of SOD content was observed in the samples that were collected from nearest to the road (site-1), and the lowest SOD value was recorded by increasing the distance from the roadside site. The increasing trend of SOD content in different farmland sites (site-1>site-2>site3) was observed. The SOD content was recorded in various crops, including spinach, cauliflower, fenugreek, potato, [(site-1 224 U/g FW, site-2 199.67 U/g FW, site-3 157.33 U/g FW)], [(site-1 220 U/g FW, site-2 199 U/g FW, site-3 157.33 U/g FW)], [(site-1 213.67 U/g FW, site-2 181 U/g FW, site-3 136.33 U/g FW)], [(site-1 200 U/g FW, site-2 175.33 U/g FW, site-3 144 U/g FW)], respectively (Fig. 6).

Malondialdehyde (MDA) Contents

The significant difference ($p<0.05$) observed in MDA content results in crop samples collected from different farmland sites, while non-significant difference among different crops. Tukey's HSD test was performed for a mean comparison of MDA content, and results indicated the highest content of MDA samples collected from nearest to the road (site-1) of different farmlands. The increasing order among different sites of farmlands (site-1>site-2>site3) was observed regarding MDA content. The MDA content was decreased by the increasing distance to the road. MDA content in crops including potato, spinach, cauliflower, fenugreek was recorded as [(site-1 14.5 nmol g^{-1}, site-2 13.167 nmol g^{-1}, site-3 11.333 nmol g^{-1})], [(site-1 12.833 nmol g^{-1}, site-2 11.5 nmol g^{-1}, site-3 11 nmol g^{-1})], [(site-1 12.167 nmol g^{-1}, site-2 11 nmol g^{-1}, site-3 10.833 nmol g^{-1})], [(site-1 6.167 nmol g^{-1}, site-2 6.033 nmol g^{-1}, site-3 5.167 nmol g^{-1})] respectively (Table 3).

Table 1. H_2O_2 content (nmol g^{-1} FW) of different farmland samples alongside the road in Multan, Pakistan.

Farmlands	Site-1	Site-2	Site-3	p-value
Cauliflower	14.333±**0.44**^{ab}	10±0.29^{c}	6.333±0.60^{f}	<0.001
Fenugreek	12.5±0.29^{b}	6.167±0.44^{f}	3.667±0.17^{g}	<0.001
Potato	14.667±0.44^{a}	9.167±0.44^{cd}	6.667±0.17^{ef}	<0.001
Spinach	12.833±0.44^{ab}	7.833±0.44^{def}	3.833±0.44^{g}	<0.001

Note: Site-1: (near to road farmlands), Site-2: (Mid of farmland; 50 meters away from the road), Site-3: (Far from road or end of farmland; 100 meters away from the road). For each farmland, means±standard error followed by different lowercase letters (a, b, c, d, e, f, g) in a column indicate significant differences in different sites ($p<0.05$).

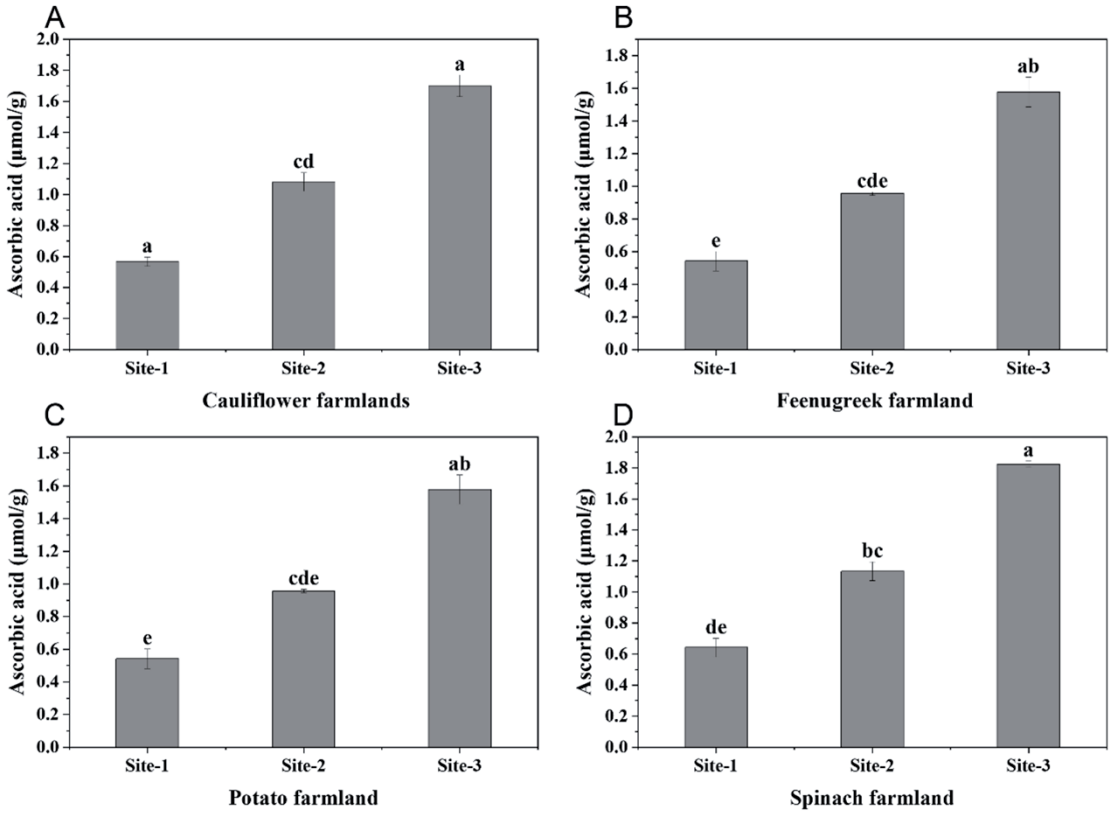

Fig. 5. Ascorbic acid content of different farmland samples alongside the road in Multan, Pakistan.

Estimation of Hydrogen Peroxide (H_2O_2)

The hydrogen peroxide (H_2O_2) showed a statistically significant difference ($p<0.05$) in different sites of farmlands, while there was a non-significant difference among different crops. Tukey's HSD test was performed for a mean comparison of H_2O_2 content; the highest H_2O_2 content was observed in those samples that were collected nearest the road (site-1). As the distance of the sampling site increased from the road, the lowest H2O2 content was observed. Among the sites of different farmlands, increasing order of H_2O_2 content (site-1>site-2>site3) was recorded. The H_2O_2 content in crops included potato, cauliflower, spinach, fenugreek was recorded as [(site-1 14.667 nmol g^{-1} FW, site-2 9.167 nmol g^{-1} FW, site-3 6.667 nmol g^{-1} FW)], [(site-1 14.333 nmol g^{-1} FW, site-2 10 nmol g^{-1} FW, site-3 6.333 nmol g^{-1} FW)], [(site-1 12.833 nmol g^{-1} FW, site-2 7.833 nmol g^{-1} FW, site-3 3.833 nmol g^{-1} FW)], [(site-1 12.5 nmol g^{-1} FW, site-2 6.167 nmol g^{-1} FW, site-3 3.667 nmol g^{-1} FW)], respectively (Table 1).

Discussion

In suburban vegetable soil areas of Shanghai, the concentration of MPs was observed (62.50e78.00 items/kg) [36]. In Shanghai, 10.3±2.2 items/kg were observed in the rice-fish co-culture ecosystem [45]. In southeast Germany, agricultural farmland soil, 0.34e0.36 MPs particles per kg in dry weight of soil [46]. Besides, MPs were also found in other types of soil like industrial soil

Table 2. Carotenoid content (µmol/g) of different farmland samples alongside the road in Multan, Pakistan.

Farmlands	Site-1	Site-2	Site-3	p-value
Cauliflower	0.0966±0.004^{a}	0.0694±0.004^{bc}	0.0292±0.007^{fg}	<0.001
Fenugreek	0.079±0.003^{a}	0.059±0.001^{cd}	0.0438±0.002^{def}	<0.001
Potato	0.0992±0.005^{a}	0.054±0.004^{cd}	0.0292±0.002^{fg}	<0.001
Spinach	0.0586±0.002^{cd}	0.0331±0.003^{efg}	0.0143±0.002^{g}	<0.001

Note: Site-1: (near to road farmlands), Site-2: (Mid of farmland; 50 meters away from the road), Site-3: (Far from road or end of farmland; 100 meters away from the road). For each farmland, means±standard error followed by different lowercase letters (a, b, c, d, e, f, g) in a column indicate significant differences in different sites ($p<0.05$).

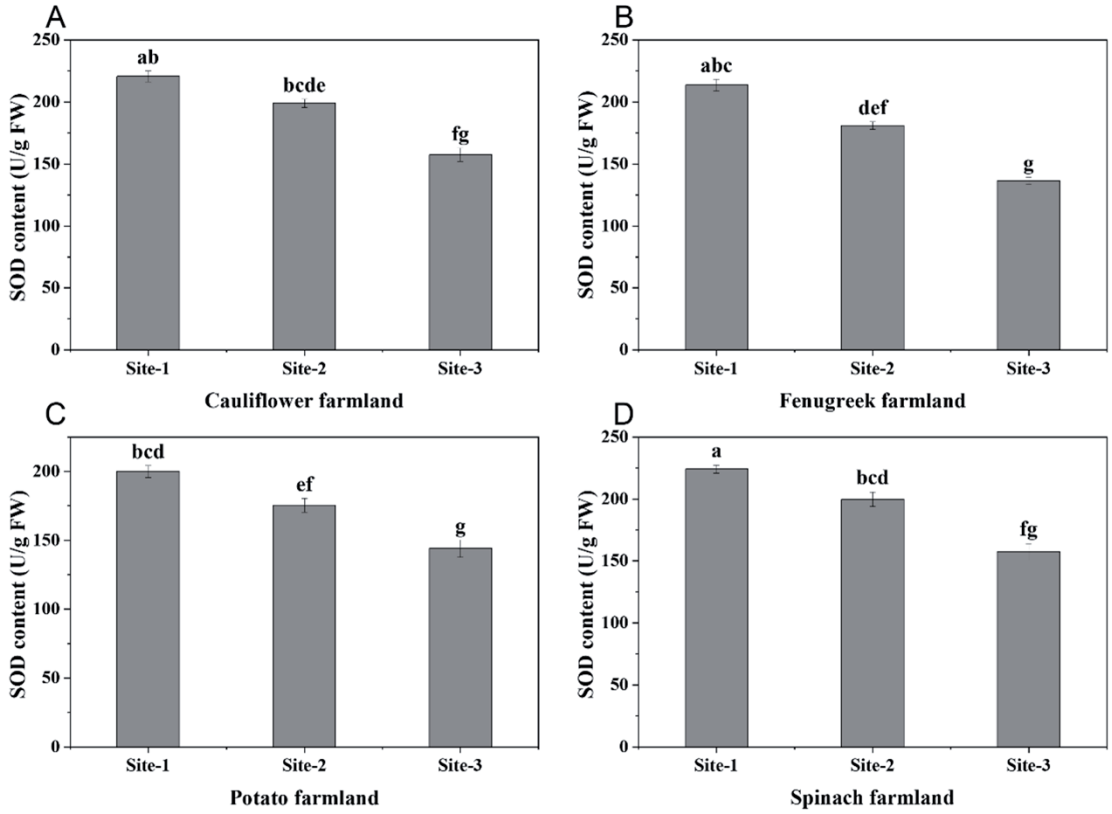

Fig. 6. SOD content of different farmland samples alongside the road in Multan, Pakistan.

[47] and floodplain soil [48]. There are several factors involved in the diversification of MP abundance in different regions, such as sampling sites, cultivation, and fertilization. Additionally, the content of plastic and pollution sources also contribute to the dissemination of MP pollution. MPs have been detected by the application of MPs [49-51]. Near the sample sites of suburban highways, there are a large number of establishments, such as car washes and building material stores that may dispose of plastic trash and discharge wastewater containing MPs into the vegetable farmlands. The green high-density polyethylene nets cover the bare land. After prolonged exposure to sunshine, the green nets may disintegrate into microscopic fibers by photooxidation and subsequently scatter throughout the soil. In recent years, tire tread particles have been recognized as the environmental MP source. Due to the frictional interaction between the tire's forerunners and the road surface, these particles are released into the surroundings [52-54]. In residential areas, enormous volumes of plastic bags, disposable lunch boxes, and foam boxes were discovered. Due to high population densities and human activities, the discharge of domestic sewage and household waste containing extensive plastic decomposition might be the primary source of MP pollution in vegetable farmland near residential areas [53]. MPs have been added to many personal care products like hand cleaners, facial cleaners, and toothpaste which enter into the environment via domestic sewage [11]. It was claimed that around 6% of liquid skin-washing solutions in the European Union, Switzerland, and Norway included MPs, of which 93%

Table 3. MDA content (nmol g^{-1}) of different farmlands samples alongside the road city Multan, Pakistan.

Farmlands	Site-1	Site-2	Site-3	p-value
Cauliflower	12.167±0.83^{ab}	11±0.60^{b}	10.833±0.29^{b}	<0.001
Fenugreek	6.167±0.32^{c}	6.033±**0.44**^{c}	5.167±0.44^{c}	<0.001
Potato	14.5±**0.29**^{a}	13.167±0.44^{ab}	11.333±0.60^{b}	<0.001
Spinach	12.833±0.73^{ab}	11.5±0.76^{b}	11±0.29^{b}	<0.001

Note: Site-1: (near to road farmlands), Site-2: (Mid of farmland; 50 meters away from the road), Site-3: (Far from road or end of farmland; 100 meters away from the road). For each farmland, means±standard error followed by different lowercase letters (a, b, c) in a column indicate significant differences in different sites ($p<0.05$).

were manufactured from PE [55]. Moreover, synthetic textiles are significant sources of MPs that have been discovered in ecosystems across the world [56-58]. Polyamide, polyester, and acrylic are the three most common synthetic polymers used in textiles worldwide. They can emit airborne and particulate matter, even when used commonly [10]. According to [12], there were 0.08 kg per year per capita air-borne textile microfibers in dust settling on household surfaces and 0.12 kg per year per capita fibers in laundry effluent in Norwegian homes. In our study, the soil of site-1 was more serious rather than that of site-2 and site-3 regarding MPs contamination. Suburban roads are known for their high traffic flow, broad dispersion surface, and high mobility. There are a variety of different sources of MP contamination that have the potential to get into soils through surface runoff, atmospheric deposition, and other pathways [10, 11].

The findings of this study show that Malondialdehyde (MDA) and superoxide dismutase (SOD) content were observed with the 2highest value in site-1 samples as compared to site-2 and site-3. Enzymatic and non-enzymatic antioxidants in plants are crucial in neutralizing reactive oxygen species to prevent oxidative damage [59]. SOD is the first enzyme to combat oxygen-free radicals [60]. When plants are exposed to stress, carotenoids play a critical role in their antioxidant systems and photosynthesis [61]. Generally, elevated ROS levels may induce cell membrane lipid peroxidation, which is often accompanied by an increase in MDA concentration (one of the final products of membrane lipid peroxidation) [62, 63]. Consequently, MDA content can be exploited as a significant indication of plant cell membrane peroxidation under stress [64]. In our experiment, the primary non-enzymatic antioxidant carotenoid and enzymatic antioxidant SOD were significantly higher in site-1 samples as compared to other sampling sites (site-2, site-3). We believe it's due to nanoplastic pollution that is transported through dust particles.

Plant growth and development are reduced by MP stress due to physical obstruction and reduced soil productivity. MPs enter the shoot through xylem vessels due to the transpiration pull and accumulate in the leaves [65]. After root accumulation, MPs can enter stems and leaves via the apoplastic pathway [66]. However, factors such as microplastic composition, geometry, root surface area and volume, cell membrane potential, and xylem properties affect MP translocation from roots to leaves via shoot. Microplastic exposure can cause plant cellular damage by inducing oxidative stress and generating reactive oxygen species (ROS). This can impact various physiological processes, such as photosynthesis, respiration, and cell metabolism. Ultimately, it can affect the growth and productivity of plants. Microplastics in soil can negatively impact plant growth and development, including physical growth reduction, interference with nutrient and water uptake, drought induction, and photosynthesis disruption. Additionally, Microplastics induce oxidative stress, altering plant antioxidant defense and metabolic pathways, compromising plant health and productivity [24, 67-70]. The reduction in physical growth caused by MPs is followed by changes in essential physiological functions, including photosynthesis, ionic homeostasis, redox control, and hormone regulation. Any alteration to these mechanisms diminishes crop growth as plants are vulnerable to stressful conditions. MP stress led to reduced biomass production and shoot and leaf growth in plants. Photosynthesis is a vital process in plants that produces oxygen and energy in the form of sugars. The process depends on various factors, such as the biosynthesis of photosynthetic pigments, chlorophyll fluorescence, leaf gas exchange, ionic homeostasis, and redox regulation. However, MP stress can negatively affect these factors, leading to a reduction in photosynthesis in plants. These factors are negatively regulated by MP stress, which consequently inhibits photosynthesis in plants [71]. PS MPs application decreased carotenoids, chlorophyll a, and b by 12.5%, 9.1%, and 8.7%, respectively. This indicates that PS MP stress is one of the factors that reduces shoot dry weight, shoot height, and leaf area in lettuce (*Lactuca sativa* L.) [72]. In various plant species, including tomato, cucumber (*Cucumis sativus* L.), cabbage (*Brassica oleracea* L.), lettuce, and pakchoi (*Brassica rapa* L.), the use of PAN MPs resulted in a decrease in the growth of the plants, due to a negative correlation with the levels of chlorophyll a and b in the leaves [73]. In fact, the findings of this experiment illustrated a decrease in chlorophyll content (Chl-*a* & Chl-*b*) in sampling site-1 crops as compared to others (sit-2 and site-3). It is due to exogenous pollutants that block Chlorophyll synthesis, damage the chloroplast, and trigger ROS intracellular accumulation [74]. Microplastic concentrations of 250 mg L^{-1} were shown to impede photosynthesis in microalgae, with the degree of inhibition increasing as the particle size dropped [75]. The result of this experiment seems to be our study that demonstrates a decreased chlorophyll content (Chl-*a*, Chl-*b*) [59].

The results obtained in this study showed that H_2O_2 production was higher in all crops sampling sites-1 as compared to site-2 and site-3. The H_2O_2 production trend following site-1 > site-2 > site-3. The high production of H_2O_2 in plants has been attributed to its function as a signaling molecule and to various enzymes that use it as a substrate [76, 77]. Therefore, they trigger the production of low molecular weight compounds with antioxidant effects [78, 79]. The ascorbate and glutathione play a key role, as they react fast with hydrogen peroxide via particular enzymes, the peroxidases, while reductases restore their oxidized states. Recent research [26, 80-82] has not investigated the physiological impact of microplastics on exposed plants. They just focused only on movement, fate, and consequences of MPs on the soil. In other words, it is unknown whether the addition of microplastics to soil has an influence on the generation of reactive oxygen species and antioxidants in plants.

Additionally, is it accurate to state that microplastic is considered an "abiotic stress" factor? When plants are exposed to stressful environments, the increase in ROS production is the key response of plants. There are several reactions involved in producing ROS in plants, in which oxygen (O2) undergoes reduction to hydrogen peroxide (H_2O_2) or superoxide. To convert superoxide to H_2O_2, the enzyme superoxide dismutase (SODs) aids in the process [76].

Conclusions

The different vegetable farmlands in the Pakistan city of Multan are contaminated by microplastic. The concentration of MPs near roadside farmlands is maximum as compared to those distant from the road, which implies a negative impact on the growth of various vegetables on the roadside. Different forms and shapes of microplastic were observed in vegetable farmlands, but micro-spheric were the primary contaminants. The dominant sources of microplastic in different vegetable farmlands were large traffic flow, agricultural activities, and domestic waste. Microplastic may have an adverse impact on vegetable growth, soil contamination, and environmental pollution as well. This study helps us better understand MP pollution's impact on the growth of different vegetables in various vegetable farmlands. This article provides significant references for further research about the risk of microplastic in agroecosystems**.** Further studies are needed to develop and implement strategies to mitigate microplastic pollution from agricultural lands. Such measures may encompass alternative agricultural techniques and enhanced waste management protocols for plastic.

Declarations of Interest Statement

Ethical Approval

All authors approve this manuscript for submission to the Polish Journal of Environmental Studies

Consent to Participate

All authors participate in the preparation of the manuscript.

Conflict of Interest

All authors declare that they have no conflict of interest.

Availability of Data and Materials

The raw data are available and will be provided on demand.

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