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Quantification of Traffic Pollution-Derived Toxic Heavy Metals and Their Impact on DNA Methylation in *Solanum tuberosum* L.

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ABSTRACT

Along with increasing population growth daily as well as vehicles, the blessing emanating from vehicles emissions and dust transmission into nature and become an air pollution. Which has a negative effect in various forms when coming in contact with growing crops along the road. The multi-abiotic agents are involved in air contamination. Transportation-related air pollution has become a potentially fatal issue. The main focus of this research is the impact of possibly toxic heavy metal pollutants from vehicle emissions on the DNA methylation status quality in crops. A comparative analysis of the levels of possibly harmful toxic heavy metal concentrations (ppm) in the crop was carried out. The sites of the very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance were chosen. The possibly harmful toxic heavy metal concentrations (ppm) evaluated were lead (Pb), mercury (Hg), nickel (Ni), zinc (Zn), arsenic (As), copper (Cu), cadmium (Cd), and chromium (Cr). Possibly harmful toxic heavy metal concentrations (ppm) and DNA methylation P values of 0.01, 0.002, 0.0001, and 0.0003 were found using statistical analysis of plant part and soil samples (P < 0.05 is considered significant, highly significant, and very highly significant). At the molecular level, DNA methylation acts as a biomarker for environmental contamination. Abiotic stress may be indicated by alterations in epigenetics. Gaining a thorough understanding of the research may help us build genetic tools to increase crop stress resistance and advance molecular plant breeding.

Keywords: *Solanum tuberosum L., environmental exposures, Toxic chemo stress, epigenetic marker, DNA methylation.*

1. INTRODUCTION

In addition to the daily increase in both the population and the number of cars on the road, automobiles also contribute to air pollution by transmitting dust and smog to the environment. Which, when it comes into contact with growing crops along the road, has a deleterious impact in a number of ways. Air pollution caused by transportation has grown to be a potentially deadly problem [1]. A global issue that affects flora is toxic abiotic agent contamination. The toxic abiotic agent can come from any source, like traffic problems, but industry and human activity are the main causes of pollution. These metals have a variety of effects on plants they alter their molecular levels. Abiotic stress-induced molecular changes are well researched. Researchers work to comprehend the stress response mechanisms and the epigenetic pathways that result in resistance to heavy metal stress [2]. Although examining the functional significance of DNA methylation in plants has been a crucial area of study in recent times. This review covers plant responses to abiotic stressors as well as DNA methylation [3]. The most recent research report states that over 40% of flora and fauna are exposed to traffic air pollution levels. The multi-abiotic agents are involved in air contamination. Transportation-related air pollution has become a potentially fatal issue. The main focus of this research was the impact of possibly harmful toxic heavy metal compounds from traffic air pollution on the DNA methylation status in plants. The extent of the toxicological role of health risks related to plants was investigated in this research. A comparative analysis of the levels of possibly harmful toxic heavy metal compounds (ppm) in crops was carried out [4].

Many reviews highlight a great deal of DNA methylation stages. Green belts can be created to reduce air pollution in pollution-stressed areas, and various approaches can be utilized to diagnose the impacts of air pollution in the future [5]. Environmental changes have a significant effect on the plant quality. Here, an advanced protocol was used to provide a detailed picture of the current research trends in the DNA methylation study. In order to gather proof of the function of DNA methylation, the comprehensive data collected was utilized to delve deeply into its principles, patterns, and mode of action [6]. The enhancement of crop stress tolerance is a major area of contemporary breeding research due to the escalation of global climate change and the growing complexity of agricultural conditions. The impact of numerous abiotic stimuli on crops and the corresponding epigenetic responses of DNA methylation is summarized in this article. Crop productivity is negatively impacted by abiotic stressors such as excessive heavy metal pollution. DNA methylation is one of the first identified and extensively researched mechanisms in these epigenetic regulatory mechanisms, which are important regulatory factors in plant stress responses. The environment plays a major role in crops' natural growth, and environmental disturbances can result in crop sterility and significant output loss. Many stresses, especially those that undergo significant alterations in DNA methylation. These results highlight the intricate processes behind crops' reaction to abiotic stress. We highlight the relevance of DNA methylation in abiotic stress tolerance and suggest that the epigenetic status be regulated to effectively improve tolerance features, thereby mitigating global climate

change [7].

One of the most popular subjects in environmental contamination studies is heavy metal pollution. Heavy metals are a serious problem that must be handled since they can have catastrophic effects on a variety of living things when present in large concentrations. The capacity of heavy metal contamination to impede DNA methylation quality is one of its most important effects. Numerous studies have demonstrated the toxicity of high quantities of heavy metals, which function as catalysts in the oxidative breakdown of biological macromolecules and generate oxidative stress that damages DNA [8]. Although epigenetic mechanisms like DNA methylation appear to be important, the molecular basis for this phenomenon is not understood. Numerous studies have demonstrated the critical role that epigenetic changes brought on by unfavorable environmental signals during sensitive developmental stages play [9]. Plants are unavoidably impacted by and react to their surroundings because of their sessile condition. Plants have so far evolved a variety of abiotic stress adaptation and control techniques. One such system is epigenetic regulation, of which DNA methylation is one of the oldest and most researched regulatory systems. It can control how the genome functions and cause plants to become resistant to and adapt to abiotic challenges. We summarize the latest research on plant DNA methylation responses to abiotic stress in this review. We also talk about stress memory that is controlled by DNA methylation, both temporarily and over an extended period of time, that may be passed on to future generations. In summary, DNA methylation in plant responses and adaptations to abiotic stressors is updated in this review [10]. Plants use epigenetic defense mechanisms, such DNA methylation, to fend against a variety of abiotic stressors, such as salt, drought, and heavy metal contamination. A chemical process known as DNA methylation modifies the structure of DNA and is crucial for controlling gene expression and plant genome defense. DNA methyltransferase enzymes, which are essential for regulating plant gene expression, catalyze the enzymatic attachment of a methyl group to the fifth carbon of cytosine in this alteration [11]. This study provides a detailed description of the detrimental effects that hazardous air pollutants have on crop vegetation. Phytochemical studies show a considerable difference in crop quality between contaminated and non-polluted sites [12]. This study examined the effects of toxic air pollution on agricultural vegetation near traffic sources because it has grown to be a major health problem [13]. By using conventional techniques to measure concentrations in sample research, the complex phytochemicals in crops systems were identified [14]. Comparative reported data on phytochemical qualities show significant crop changes between polluted and control sites. The assessment concentrated on dangerous compounds found in crops, which might have been brought on by vehicle-induced air pollution. This study examined the toxicological effects of potentially hazardous flora on air pollutants originating from automobiles [15, 16, 17].

Plant varieties developed stress-adapting processes, including DNA methylation, to survive in harsh settings. Numerous studies show that abiotic stress causes alterations in DNA methylation, which in turn causes variations in the expression levels of genes linked to stress, improving plants' ability to withstand stress. According to the present study, plants' ability to withstand abiotic stress can be improved by DNA methylation.

Additionally, we hypothesize that plant stress tolerance may be improved by methylation's interaction with other phytohormones. As a result, this review offers a deeper comprehension of the molecular response of plants to abiotic stress [17, 18]. Toxic-related hazardous metals are released uncontrollably as a result of the widespread use of nanotechnology in fields including agriculture and the environment. This has consequences for the flora and fauna. Nanotoxicity in food crops should be evaluated since it may negatively impact DNA methylation and living health [18, 19]. This epigenetic marker that probably plays a major role in determining how plants react to abiotic stress is DNA methylation [19]. The plant contains the primary important elements involved in the absorption and transport of harmful abiotic substances. The morphology of a plant's roots, especially how they adapt to their surroundings, impacts whether. For example, zinc uptake in mangrove areas adversely affected their natural adaptation due to radial oxygen loss [20]. Combining toxicology approaches strengthens the field of metals and DNA methylation, and coordinating efforts or integrating analyses across studies can lead to further advancements. The field will continue to advance with discoveries regarding the molecular underpinnings of sequence-specific epigenetic responses to metal exposure techniques [21].

Environmental traffic pollutants such as toxic abiotic stress, crop-disrupting chemicals, and airborne particulates are increasingly recognized for their potential to influence molecular function through epigenetic mechanisms. This research examines conserved pollutant-associated DNA methylation of molecular areas.

2. MATERIAL AND MATHODS

Hapur, which is between latitudes 28.730579 and 77.775879 in the northwest of the city, has a humid climate that is influenced by the monsoon, meaning that summers are hot and winters are cold [22]. In the Hapur district, Morepur is close to National Highway 235. In the Morepur place, samples were examined.

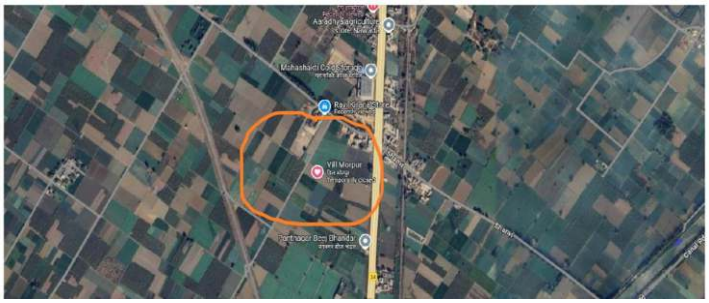

Fig. 1: The samples were studied, as shown in the Morepur map

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2.2 Acquired the crop samples

Sample places along NH-235 were selected at Morepur. The selected places are traffic road air pollution and several farms near a non-traffic road. The selected crop species for this study is *Solanum tuberosum* L. At Chaudhary Charan Singh University in Meerut, Uttar Pradesh, India, the Department of Botany used the issued sample numbers, Bot/PB/261, to confirm and authenticate the taxonomic identity of crop samples. Samples of soil, mature leaves, and mature edible components were gathered in order to assess the impact of the transportation system's air pollution.

2.3 Assessment of the accumulation of potentially toxic heavy metal substances

2.3.1 Soil samples got ready for the assessment of toxic heavy metal substances

The soil samples were air-dried to remove their moisture content. Once the specimens had dried, they were crushed with a dry, sterilized pestle and mortar and then carefully filtered over a 2 mm screen. To digest three-gram sieved soil samples, a solution consisting of 3.5 milliliters of concentrated nitric acid (HNO_3) and 10 milliliters of concentrated hydrochloric acid (HCl) was used. The combinations were kept overnight unheated beneath the switch-on fume closet and then heated for two hours at 104°C the next day. Following the filtering of the digested sample with a Whatman No. 42 filter and filter paper, DW was added to a 100 ml volumetric flask. In order to perform an analysis, the solution was placed into sampling vials. Then, using the (A.A.S.), the dangerous, poisonous heavy metal compound concentrations (ppm) in soil samples were determined [23].

2.3.2 Crop samples got ready for the assessment of toxic heavy metal substances

A digital electronic scale was used to weigh fresh crop samples, which included mature leaves and mature edible components. The samples were separated and dried completely with a hot air tool set at 70°C for 48 hours.

The dried crop samples were ground into a fine powder in a mechanical grinder. For the initial breakdown, one gram of each sample of mature leaves and mature edible part was put in 150 ml conical flask, and 15 ml of a di-acid mixture (nitric acid, HNO_3, perchloric acid, and $HClO_4$) was added. The mixture was then left to stand all night long. The digesting process was finished by heating conical flasks on a hot plate after the materials had partially broken down. After the ingredients were digested and filtered through Whatman No. 42 filter paper, the final volume was measured out to 50 ml in volumetric flasks and cleaned with D.W. Then, using the (A.A.S.), the dangerous, poisonous heavy metal compound concentrations (ppm) in mature leaves and mature edible part samples were determined [23].

2.4 DNA extraction from crop samples

The DNA was isolated using the method described. In summary, 1200 µL of extraction buffer (0.5 M sodium chloride, 1% SDS) was used to homogenize 50 mg of the dried powdered leaf sample. The supernatant was extracted from the homogenate after it had been centrifuged for four minutes at room temperature at 13,500 rpm. After being reconstituted with an equivalent volume of isopropanol, the quality liquid was set on ice for five minutes. The mixture was centrifuged again at 13,500 rpm for four minutes at room temperature, and the supernatant was then removed. After again centrifuging the DNA pellet for two minutes at room temperature at 13,500 rpm, it was reconstituted with 70% ethanol. The DNA pellet was dissolved in Dh_2O following a period of air drying. The material was stored at 20°C. The material was measured using a spectrophotometer [24].

2.4.1 DNA methylation

The DNA methylation profile was ascertained using a commercial Enzyme-Linked Immunosorbent Assay-based kit (5Mc DNA Enzyme-Linked Immunosorbent Assay Kit (Zymo Research, D5326)) in compliance with the manufacturer's guidelines. First, two hundred ng of genomic DNA and calibrators were adjusted to one hundred µL using a 5-Mc coating buffer, and then they were denatured for five minutes at 98°C. Samples were immediately placed on ice, after all. Following the denaturation process, the DNA samples were incubated on the well strips for an hour at 37°C. Each well was washed three times with 200 µL of the five Mc Enzyme-Linked Immunosorbent Assay buffer after the incubation period. After the reaction, 100 ml of the antibody mixture was added to each well, and an additional hour of incubation was conducted. At the end of the incubation time, 200 ml of 5Mc ELISA buffer was used to clean each well. A spectrophotometer was used to analyze the reaction 30 to 60 minutes after 100 microliters of HRP developer were added to start the color development process. A microplate spectrophotometer (Multiscan-GO, Thermo Scientific) set to 405 nm absorbance was used to measure the color development.

2.5 Statistical Analysis

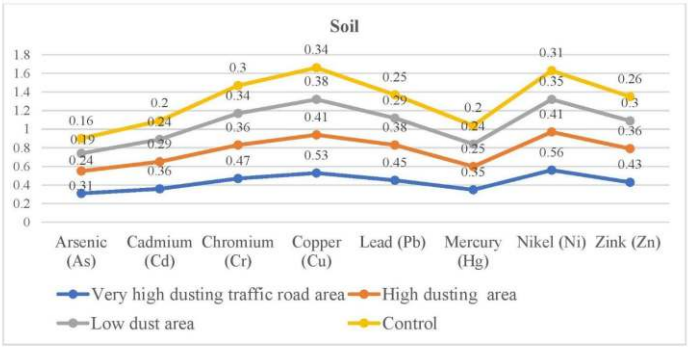
A single-factor analysis of variance (ANOVA) was used to examine different sample groups. In accordance with the criteria described by Gomez (1984), the considered recorded data P = 0.01, 0.002, 0.0001, and 0.0003 ($P < 0.05$ is regarded as significant, highly significant, and very highly significant) are the values [14].

3. RESULT

3.1 Buildup of possibly harmful toxic heavy metal compounds: -

3.1.1 Assessment of possibly harmful toxic heavy metal compounds in soil: -

During the duration of the inspection, concentrations (ppm) of possibly harmful toxic heavy metal compounds for example, arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) were found in the soil at the following sites: very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance. It made an obvious variation in the concentrations (ppm) of these possibly harmful toxic heavy metal compounds across the multisite, according to the overall concentrations (ppm) values for arsenic (0.31 > 0.24 > 0.19 > 0.16 ±), cadmium (0.36 > 0.26 > 0.24 > 0.20 ±), chromium (0.47 > 0.36 > 0.34 > 0.30 ±), copper (0.53 > 0.41 > 0.38 > 0.34 ±), lead (0.45 > 0.38 > 0.29 > 0.25 ±), mercury (0.35 > 0.25 > 0.24 > 0.20 ±), nickel (0.56 > 0.41 > 0.35 > 0.31 ±), and zinc (0.43 > 0.36 > 0.30 > 0.26 ±) at show fig 2. The inspection of the statistical analysis show that the amount of possibly harmful toxic heavy metal compounds (ppm) varied between the multisite by P=0.0001 (P<0.05 is regarded as very highly significance). In Fig. 3, possibly harmful toxic heavy metal compounds statistical mean values varied from (0.43 > 0.33 > 0.29 > 0.25 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters.



Significant at: P=0.0001 (P<0.05 is regarded as very highly significant)

Fig. 2: Levels of concentrations (ppm) of possibly harmful toxic heavy metal compounds in soil at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

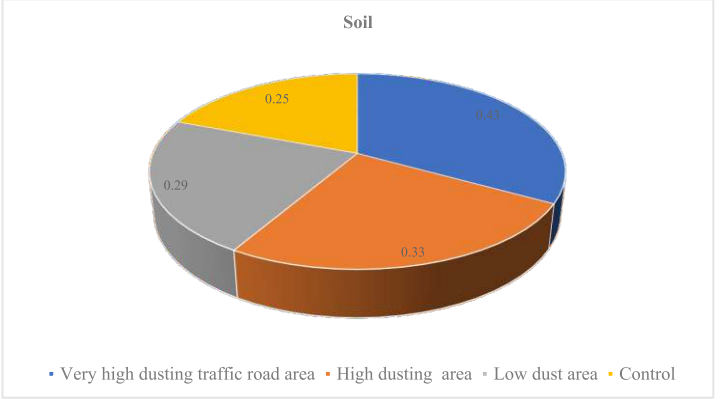
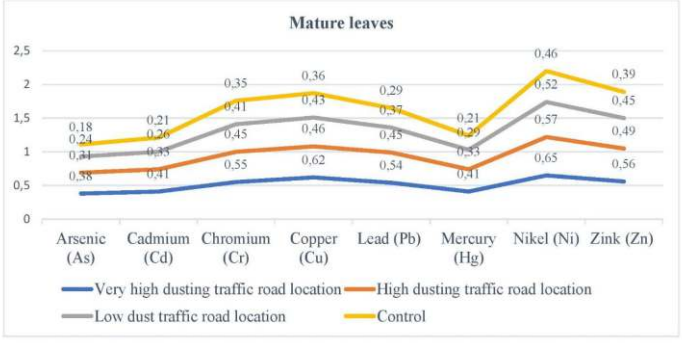


Fig. 3: Levels of mean value of possibly harmful toxic heavy metal compounds in soil at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

3.1.2 Assessment of possibly harmful toxic heavy metal compounds in mature leaf

During the duration of the inspection, concentrations (ppm) of possibly harmful toxic heavy metal compounds for example, arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) were found in the mature leaves at the following sites: very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance. It made an obvious variation in the concentrations (ppm) of these possibly harmful toxic heavy metal compounds across the multisite, according to the overall concentrations (ppm) values for arsenic (0.38 > 0.31 > 0.24 > 0.18 ±), cadmium (0.40 > 0.33 > 0.27 > 0.23 ±), chromium (0.54 > 0.47 > 0.41 > 0.38 ±), copper (0.62 > 0.48 > 0.41 > 0.35 ±), lead (0.54 > 0.44 > 0.39 > 0.30 ±), mercury (0.41 > 0.31 > 0.27 > 0.24 ±), nickel (0.64 > 0.56 > 0.51 > 0.45 ±), and zinc (0.56 > 0.50 > 0.45 > 0.38 ±) at show fig 4. The inspection of the statistical analysis show that the amount of possibly harmful toxic heavy metal compounds (ppm) varied between the multisite by P=0.002 (P<0.05 is regarded as highly significance). In Fig. 5, possibly harmful toxic heavy metal compounds statistical mean values varied from (0.51 > 0.42 > 0.36 > 0.31 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters.



Significant at: P=0.002 (P<0.05 is regarded highly significant)

Fig. 4: Levels of concentrations (ppm) of possibly harmful toxic heavy metal compounds in mature leaves at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

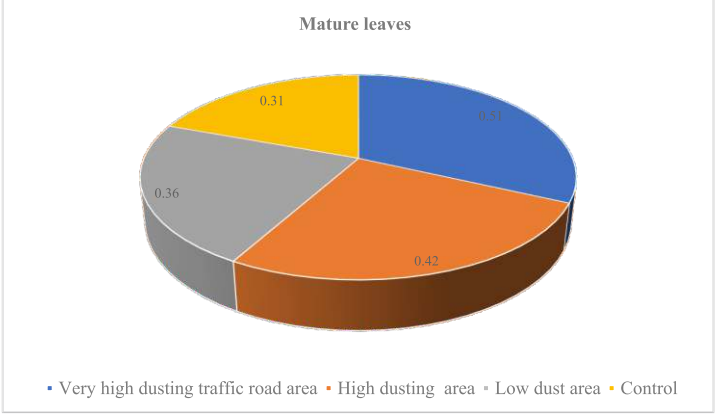
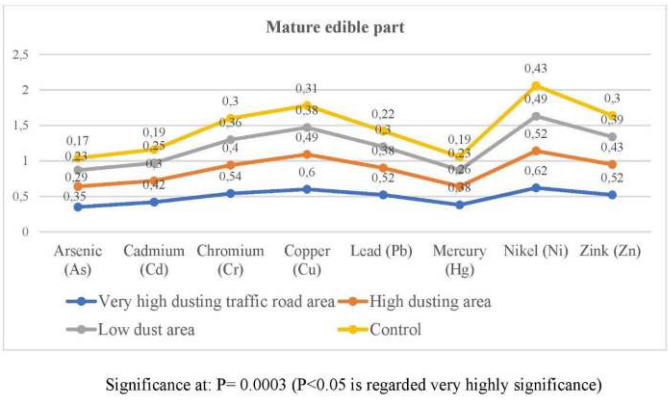


Fig. 5: Levels of mean value of possibly harmful toxic heavy metal compounds in mature leaves at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

4.1.3 Assessment of possibly harmful toxic heavy metal compounds in mature edible part

During the duration of the inspection, concentrations (ppm) of possibly harmful toxic heavy metal compounds for example, arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) were found in the mature edible part at the following sites: very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance. It made an obvious variation in the concentrations (ppm) of these possibly harmful toxic heavy metal compounds across the multisite, according to the overall concentrations (ppm) values for arsenic (0.35 > 0.29 > 0.23 > 0.17 ±), cadmium (0.42 > 0.30 > 0.25 > 0.19 ±), chromium (0.54 > 0.40 > 0.36 > 0.30 ±), copper (0.60 > 0.49 > 0.38 > 0.31 ±), lead (0.52 > 0.38 > 0.30 > 0.22 ±), mercury (0.38 > 0.26 > 0.23 > 0.19 ±), nickel (0.62 > 0.52 > 0.49 > 0.43 ±), and zinc (0.52 > 0.43 > 0.39 > 0.30 ±) at show fig 6. The inspection of the statistical analysis show that the amount of possibly harmful toxic heavy metal compounds (ppm) varied between the multisite by P=0.0003 (P<0.05 is regarded as very highly significance). In Fig. 7, possibly harmful toxic heavy metal compounds statistical mean values varied from (0.49 > 0.38 > 0.32 > 0.26 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters.



Significance at: P= 0.0003 (P<0.05 is regarded very highly significance)

Fig. 6: Levels of concentrations (ppm) of possibly harmful toxic heavy metal compounds in mature leaves at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

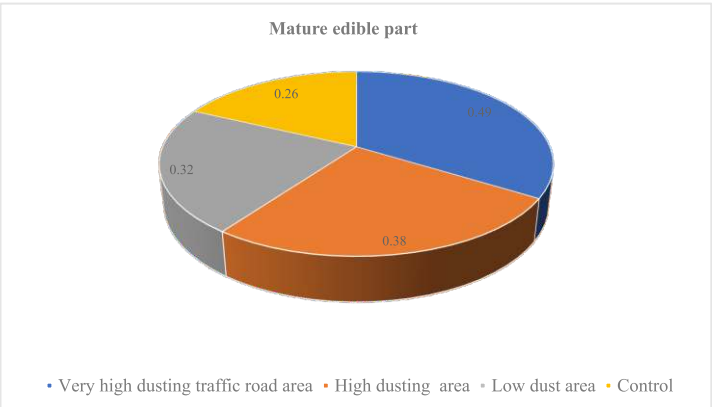
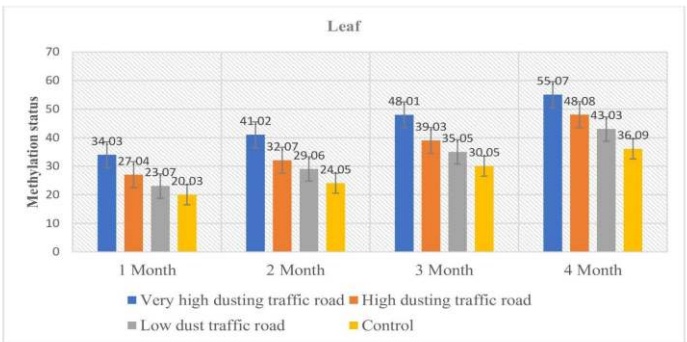


Fig. 7: Levels of mean value of possibly harmful toxic heavy metal compounds in mature leaves at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

3.2 Assessment of DNA methylation: -

The inspection duration, the DNA methylation status was detected in the mature leaf at the sites of the very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance. At the sites with very high dusting traffic roads, high dusting traffic roads at a 500-meter distance, low dust traffic roads at a 1000-meter distance, and control at a 1500-meter distance, the DNA methylation status ranged differently in fig. 8. According to inspection, the major DNA methylation status was arranged in descending order 1 month: (34.03 > 27.04 > 23.07 > 20.03 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 2 month: (41.02 > 32.07 > 29.06 > 24.05 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 3 month: (48.01 > 39.03 > 35.05 > 30.05 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 4 month: (55.07 > 48.08 > 43.03 > 36.09 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters). The inspection of the statistical analysis show that the amount of DNA methylation status varied between the multisite by P=0.01 (P<0.05 is regarded as significance). In Fig. 9, DNA methylation status statistical total mean values varied from (44.53 > 36.55 > 32.55 > 27.05 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters.



Significant at: P=0.01 (P<0.05 is regarded significant)

Fig. 8: The levels of DNA methylation status in the leaf at the sites of the very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

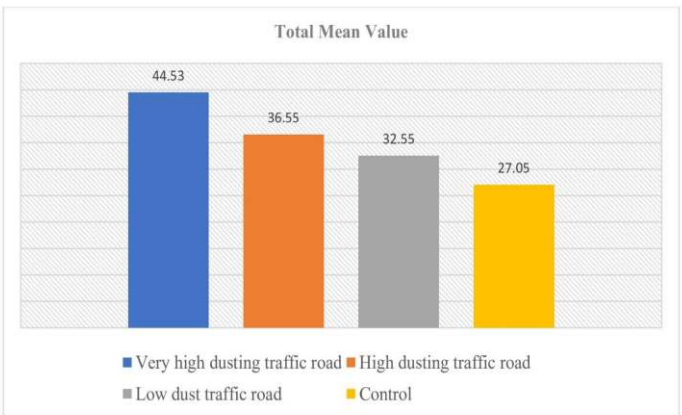


Fig. 9: Levels of total mean value of DNA methylation status in leaf at sites of very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance.

4. DISCUSSION

Possibly harmful toxic heavy metal at the farm and without-traffic road (control site) near the with traffic road site, the observation period with-traffic road, and without-traffic road sites have the greatest and lowest quantities of possibly harmful toxic heavy metal, respectively, as shown. The concentrations of quality of the air at the with-traffic road were found to be more than those at the without-traffic road sites. The findings of the statistical analysis show that the amount of possibly harmful toxic heavy metal (ppm) varied between the multisites by P=0.0001 (P<0.05 is regarded as very highly significant). In the soil sample, possibly harmful toxic heavy metal statistical mean values varied from (0.43 > 0.33 > 0.29 > 0.25 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The findings of the statistical analysis show that the amount of possibly harmful toxic heavy metal (ppm) varied between the multisites by P=0.002 (P<0.05 is regarded as highly significant). In the mature leaves sample, possibly harmful toxic heavy metal statistical mean values varied from (0.51 > 0.42 > 0.36 > 0.31 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The findings of the statistical analysis show that the amount of possibly harmful toxic heavy metal (ppm) varied between the multisites by P=0.0003 (P<0.05 is regarded as very highly significant). In the mature edible part, possibly harmful toxic heavy metal statistical mean values varied from (0.49 > 0.38 > 0.32 > 0.26 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The finding, DNA methylation status was detected in the mature leaf at the sites of the very high dusting traffic road, high dusting traffic road 500 meters distance, low dust traffic road 1000 meters distance, and control 1500 meters distance. At the sites with very high dusting traffic roads, high dusting traffic roads at a 500-meter distance, low dust traffic roads at a 1000-meter distance, and control at a 1500-meter distance, the DNA methylation status ranged differently. According to finding, the major DNA methylation status was arranged in descending order 1 month: (34.03 > 27.04 > 23.07 > 20.03 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 2 month: (41.02 > 32.07 > 29.06 > 24.05 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 3 month: (48.01 > 39.03 > 35.05 > 30.05 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters), 4 month: (55.07 > 48.08 > 43.03 > 36.09 ± in very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters). The findings of the statistical analysis show that the amount of DNA methylation status varied between the multisites by P=0.01 (P<0.05 is regarded as significant). The DNA methylation status statistical total mean values varied from (44.53 > 36.55 > 32.55 > 27.05 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The DNA methylation rate was high in traffic road sites, and the DNA methylation rate was low in the control site. A change of epigenetic marker from such a record data can be seen by changing. The abiotic stress can be detected by the change of epigenetic markers.

The change of epigenetic markers can cause reduced growth of cytosine methylation present in the base of DNA molecules. This was the cause of the traffic-related toxic air quality and possibly harmful toxic heavy metal, which was observed to differ between the control site and the farm near the traffic road.

The finding of the statistical analysis show that the amount of potentially dangerous toxic heavy metal substances (ppm) varied between the multisite by P=0.004 (P<0.05 is regarded as highly significant). In the soil sample, potentially dangerous toxic heavy metal substances statistical mean values varied from (0.42 > 0.31 > 0.29 > 0.26 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The findings of the statistical analysis show that the amount of potentially dangerous toxic heavy metal substances (ppm) varied between the multisites by P=0.001 (P<0.05 is regarded as highly significance). In the mature leaves sample, potentially dangerous toxic heavy metal substances statistical mean values varied from (0.51 > 0.42 > 0.37 > 0.30 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The findings of the statistical analysis show that the amount of potentially dangerous toxic heavy metal substances (ppm) varied between the multisites by P=0.0002 (P<0.05 is regarded as highly significance). In mature edible part, potentially dangerous toxic heavy metal substances statistical mean values varied from (0.49 > 0.37 > 0.32 > 0.26 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The findings of the statistical analysis show that the amount of DNA methylation status varied between the multisite by P=0.004 (P 40.04 > 35.03 > 31.05 ±) at the very high dusting traffic road, high dusting traffic road 500 meters, low dusting traffic road 1000 meters, and control 1500 meters. The DNA methylation rate was high in traffic road sites, and the DNA methylation rate was low in the control site [17].

Among epigenetic regulators, methylation is the most well-understood process, especially concerning the C (cytosine) base, which is found in specific locations within the DNA molecule. The corpus of recent research indicates that cytosine methylation is associated with various genetic processes, including molecular and genetic factors [25]. As previously stated, when exposed to abiotic chemicals, the different types usually display hereditary feature variability at DNA methylation sites together [26]. High-density chemical elements known as heavy metals have the potential to be harmful or hazardous even in small amounts. Due to automobile emissions and industrial processes, they are extensively dispersed throughout the environment as a result of air pollution. The findings showed that aromatic plants' genomes are affected genotoxically by high concentrations of heavy metals. Four distinct methylation patterns were identified by epigenetic analysis, with the maximum overall methylation rate of 95.40% recorded at a concentration of 20 mg L−1 and the lowest rate of 92.30% recorded at 160 mg L−1. Furthermore, the highest non-methylation percentage was found at 80 mg L−1. These findings imply that alterations in methylation patterns may be a crucial defense against the toxicity of heavy metals. Additionally, aromatics can be utilized as a biomarker to identify pollution in heavy metal-contaminated soils [27].

The ANOVA test was used to analyze the data, and differences were assessed at $p < 0.05$.

The results of the experiment showed that the amounts of abiotic agents were multi-amount in plants and soils, respectively. There is a substantial metal load in the soil and flora along the roadsides, which has been linked to vehicle emissions. The quantity of abiotic agents in the soil and other crops grown near the road was measured by a survey [28]. The impact of these metals on crops at the molecular level was also evaluated. A sample of dust, soil, and plants was taken from Faisalabad's Millat Road. At four distinct locations 10, 30, and 60 meters from the road, samples were taken. Each location was five kilometers apart. Consequently, as. The amounts of abiotic agents on dust, soil, and crops reduced as the road grew farther away ($P \geq 5$). Abiotic agents in crops were responsible for the increased concentrations of HMs on dust and soil (10 meters from the road). Similarly, higher metal concentrations were observed in crops growing near the road [29]. In order to follow the environmental stressors caused by the accumulation of heavy metals and harmful organic compounds, we looked at the epigenetic changes in the crop species. The changes in DNAm (DNA methylation) in the crop-environment relationship. The vegetative form was collected in two different geographic locations: one near the major road (MR) and the other in the forest region (FS). DNAm rates were 10.41±2.009 in MR and 23.37±2.94 in FS. The finding of the statistical analysis shows that the amount of DNA methylation status varied between the two locations ($P < 0.005$). The only thing that separated the two samples was the traffic-related pollutants. The results suggest that automotive pollution induces epigenetic changes in plant species, particularly DNA methylation, which could be a helpful biomarker for assessing the danger of pollution from vehicle traffic. The findings demonstrated that Pb and Cd stress had an impact on the plant DNA methylation profile. The levels of DNA methylation in the control check (CK), Pb stress group, and Cd stress group (CK 46.96%, Pb 48.23%, and Cd 48.1%) did not differ significantly. On the other hand, the hemi-methylation levels of the Pb stress group (19.89%) and Cd stress group (27.85%) were greater than the control (13.04%), while the full-methylation levels of the Pb stress group (28.34%) and Cd stress group (20.25%) were lower than the control (33.91%) [30, 31].

Though little research has examined agricultural epigenetics in relation to cadmium exposure, epigenetics plays a significant role in crop tolerance to abiotic stresses. To examine the impact of cadmium exposure on crop genomic DNA methylation levels and patterns using methylation-sensitive amplified polymorphism (MSAP) analysis, hydroponically grown crop seedlings were treated with hydroponic nutrient solution containing different concentrations of CdCl2 (0.5, 5, and 15 mg L−1). Overall, the findings showed that crop seedlings under cadmium stress had altered genomic DNA methylation levels and patterns, with both methylation level and methylation polymorphism rate rising in a dose-dependent way with increasing cadmium concentration [26]. The genomic DNA of the control (CK), S1 (0.5 mg L−T CdCl2), S2 (5 mg L−1 CdCl2), and S3 (15 mg L−1 CdCl2) groups had overall methylation rates of 27.92%, 29.78%, 33.2%, and 35.79%, respectively, as well as methylation polymorphism rates of 14.46%, 16.57%, and 18.15%, respectively. Therefore, enhanced genomic DNA methylation was the primary cause of methylation alterations brought on by crop cadmium stress. These findings suggest that the crop increases both the amount of genomic DNA methylation and the rate of genomic DNA methylation to withstand cadmium stress.

This work offers a systematic basis for examining the molecular pathways that underlie the response of soybeans to cadmium stress [30]. Several lines of study suggest that DNA methylation may be a biomarker that evaluates toxicity. In this study, we examined the effects of anthropogenic sources and transportation pollutants on the levels of natural DNA methylation in all relevant regions. The results of our study clearly show that pollution induces epigenetic modifications, and global methylation status can be used to determine the degree of risk [32].

Several distinct DNA methylation marker sites have been identified in plants under a variety of hazardous stress situations [33]. The crops under heavy metal stress exhibit changes in DNA methylation. The goal is to evaluate the level of pollution and health concerns associated with multi-HMs in three commonly consumed vegetables near any roadway. This suggests that automobile emissions, commercial waste, gas stations, and agriculture are typical human-caused sources of these elements [34].

5. CONCLUSION

The impact of possibly toxic heavy metal pollutants from vehicle emissions on the DNA methylation status quality in *Solanum tuberosum* L. in this research. This research has demonstrated the significance of cytosine DNA methylation in crop responses and adaptations to diverse abiotic stressors. DNA methylation was shown to be higher in crops grown close to heavily frequented regions and lower in crops grown further away. DNA methylation data varied, suggesting epigenetic shifts. Epigenetic changes may indicate the existence of abiotic stress. A decrease in methylation levels may be facilitated by the presence of cytosine in DNA. The farm next to the heavily frequented region showed greater amounts of these hazardous compounds, according to recorded data showing potentially hazardous quantities of toxic HMs and gases at a site compared to a site without traffic. Because there are pits close to the busy location that fill with water when it rains, the pollution created by passing cars combines with the water in these pits. When water fills a pit, the water turns hazardous and penetrates the farm. The farm's soil therefore, turns poisonous. The findings demonstrate that these pollutants are absorbed by crops through their root systems and leaf stomata and that the concentrations of these pollutants are much higher in crops located near dust-impacted, traffic-polluted areas than in control areas that are farther away from traffic sources. This was the reason why the control site and the farm close to the with-traffic site had different levels of traffic-related air pollution. These crops have high concentrations of hazardous heavy metals (HMs), which function as poisonous substances that endanger plant and animal life. Additionally, our research indicated that the use of heavy metal-based herbicides in crop cultivation should be done carefully since high quantities of heavy metals can be genotoxic to crop plants. Furthermore, the buildup of these toxins in portions of edible crops poses serious health risks to people, and crops intended for animal feed also contribute to the introduction of these pollutants, upsetting livestock health systems.

6. FUTURE SCOPE

This research also includes a record of qualitative and quantitative data. This data may be essential for pinpointing the precise effects of emissions on different crop species and improving our understanding of those effects.

This might result in further studies utilizing the data from these discoveries. Such research could help evaluate the environmental hazards that automobile air pollution poses. This may entail particular studies that use molecular information obtained from these sources. Such data demonstrates changes in epigenetics. Abiotic stress may be indicated by alterations in epigenetics. Pollution can be evaluated using DNA methylation status. At the molecular level, DNA methylation acts as a biomarker for environmental contamination. Gaining a thorough understanding of the pathways may help us build genetic tools to increase crop stress resistance and advance molecular plant breeding. The exact creation of artificial epigenomic diversity and site-specific epigenetic alterations may be made possible by the development of epigenome editing techniques. The target-specific epigenetic engineering and other techniques will all help with future epigenetic breeding initiatives. Transportation-related air pollution has become a major health risk. Numerous successful tactics have been used to reduce traffic-related air pollution. Roadside vegetation, building dust barriers along roads, promoting the use of compressed natural gas (CNG) vehicles, encouraging the adoption of electric vehicles, reducing the overall number of vehicles, encouraging vehicle usage only when necessary, using genetic technology, using greenhouses for crop production, and planting crops farther away from major roads are some of these strategies.

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8. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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