International Journal of Plant & Soil Science

Volume 36, Issue 6, Page 695-708, 2024; Article no.IJPSS.117316
ISSN: 2320-7035

A study on Effect of Ambient Air Pollution from Roadside Traffic on Qualitative and Quantitative Phytochemical Constituents of *Solanum tuberosum L.*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2024/v36i64674

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117316

Original Research Article

Received: 11/03/2024
Accepted: 14/05/2024
Published: 17/05/2024

ABSTRACT

Solanum tuberosum L. is a useful vegetable crop and a member of the Solanaceae family that yields starch molecules with a high concentration. The analysis of both qualitative and quantitative phytochemical substances was the main focus of the current investigation. We have chosen places with road traffic and without road traffic (control) for the crop comparison analysis. The qualitative phytochemical substances analysed protein, carbohydrate, iodine, phenol, tannin, flavonoids,

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Cite as: *Singh, D., Sharma, M. K., Kumar, P., & Vashistha , H. (2024). A study on Effect of Ambient Air Pollution from Roadside Traffic on Qualitative and Quantitative Phytochemical Constituents of Solanum tuberosum L. International Journal of Plant & Soil Science, 36(6), 695–708. Retrieved from https://journalijpss.com/index.php/IJPSS/article/view/4674*

saponin, glycosides, steroids, terpene, and alkaloids. The complete phenolic content exhibits a total mean value of 0.03712, surpassing the threshold of 0.03176, while the overall flavonoid content shows a total mean value of 0.04696, exceeding the threshold of 0.03764. Crops growing close to busy road traffic had lower phytochemical substances, in terms of quantity and quality. This record demonstrates the detrimental impact of air pollution caused by vehicles on crop vegetation. Crop vegetation differs between control and road traffic sites, according to data on both qualitative and quantitative phytochemical substances.

Keywords: Solanum tuberosum L.; qualitative; quantitative; phytochemical substances.

1. INTRODUCTION

Air pollution is one of the most critical environmental problems. The amount of traffic has led to air pollution being a major health hazard. Unlike other environmental problems, this type of transportation-related air pollution is mostly caused by nature, making it infamously difficult to prevent. It hurts and kills people, animals, and plants in areas with heavy traffic. Roadside trees and crops significantly increase air filtration, which lowers pollution in the environment [1]. There is more proof that recently identified microscopic contaminants, like air pollution, are causing contamination in terrestrial and aquatic ecosystems. Traffic is the primary means of transporting people and their things. Because contaminated air can bioaccumulate in food chains, breathing it in can be harmful to human health. Studies on air pollution have historically largely focused on water bodies, but more recent research has shown that land areas especially agricultural ones are widely contaminated. This is the only thorough study that we are aware of that looks at agricultural contamination in terms of its causes, effects, and mitigation techniques, despite the wealth of literature on-air pollution [2]. Moreover, pollution not only reveals its effects but also enhances our comprehension of how plants absorb it and its impact on both people and animals. We also make recommendations for future directions in the study of environmental pollution in ecosystems on land [3].

2. REVIEW Of LITERATURE

The literature review serves as a fundamental component of this study, offering a synthesis of existing knowledge and research pertinent to the subject matter. By meticulously examining a range of scholarly sources, including peer-reviewed articles, books, and reports, this review aims to provide a comprehensive overview of the current state of understanding in the field. Through this exploration, key concepts, theoretical frameworks, and methodologies employed in previous studies are critically evaluated, laying the groundwork for the present research endeavor.

It is a prominent component of numerous forest ecosystems, including urban forests and parks. The research indicates that silver birch exhibits characteristics of a pioneer plant due to its minimal requirements for habitat in the context of air pollution transport, as evidenced by the levels of specific total phenolic and flavonoid content in its leaves [4]. A Mediterranean host-spot pollution region is defined as a region with heavy traffic and industrial areas. Pomegranate trees suffered greatly from a variety of industrial airborne pollutants, including biochemical alterations that included an investigation of the overall number of flavonoids and phenolics. The total phenolic and flavonoid content varied in this study, demonstrating their total dependence on stress and polluted and non-polluted environments [5]. Through stomata, traffic air pollution is easily absorbed and transported by leaves, having detrimental effects [6]. Vehicle emissions pollution increased dramatically in tandem with the expansion of the automotive sector and the population surge. The vehicular emissions predominantly target the flora situated along highways and adjacent roadside areas. This research outlines the impact of automobile emissions on vegetation. On the other hand, it was also claimed that roadside vegetation might be able to lessen the harmful effects of automobile emissions. There was also a theory that suggested certain ways in which plants could function as bioindicators of air pollution. The paucity of studies on the impact of vehicle pollution on roadside vegetation and more especially, crops is one of the main problems. More research has been called for namely, to examine the function that nanocarbon particle pollution plays. It was stressed how crucial it is to find long-term solutions to these mounting concerns. The fruit peel of the pomegranate *Punica granatum* L. was collected from two locations close to the industrial area that had varying air quality. The first location showed the

contaminated site, which is situated in the industrial sector near the oasis. The Control site, which was 37 km from the industrial region, was the second site mentioned. The total phenolic and flavonoid content of pomegranate fruit peel methanol extract was identified and measured [7]. There are many separate phytochemicals found in plants [8,9]. Total phenolic and flavonoid levels, among other phytochemical components, were detected and quantified in the peel extract, according to the article. Comparing the peel retrieved from the contaminated location to the control site, there was a higher total phenolic and flavonoid content [10]. Nowadays, traffic air pollution, dust, or Particulate Matter (PM), is one of the primary issues affecting human health and crops, owing mostly to the rapid development of industrial activity and road traffic [11]. Research has indicated that there are differences in the total flavonoid content between the two extracts obtained from the polluted and unpolluted *N. oleander* L. plants [12]. Numerous effects of anthropogenic pollution can be seen in plants and the air. Monitoring pollution via bioindicators, or compounds found in living organisms like plants, could prove to be a useful approach to environmental monitoring. The bioindicators of residential and industrial zones in Sadat City, Egypt, were scrutinized. Phenolic and flavonoid constituents were characterized via spectrophotometric analysis of *Bougainvillea glabra* L. leaves [13]. In leaves, it was discovered that the industrial zone had significantly higher concentrations of flavonoids, which are phenolic compounds, than the residential zone. The previous investigation revealed that pollution significantly influences the total levels of phenolic and flavonoid compounds in *B. glabra* L. plants highlighting the negative effects of pollution on environmental health and opening the door for the use of plants as bioindicators [13]. *Portulaca oleracea* L. underwent quantitative phytochemical analysis for several key characteristics, including alkaloids, flavonoids, tannins, proteins, and saponins. The leaf samples were collected from two areas a roadside spot that was prone to air pollution from automobile tailpipes, and an unpolluted garden. Leaf samples taken from contaminated areas also showed signs of nutrient stress, water stress, and high-temperature stress. The leaf sample contains high percentages of phytochemical substances such as tannins, alkaloids, flavonoids, and saponins, as well as nutritional component protein. Compared to samples from garden settings, those from contaminated sites had noticeably higher percentage values. The research shows that *P. oleracea* L. may grow in wastelands that are stressed by fertilizer, water, traffic, and air while also displaying reasonably high amounts of phytoconstituents [14]. Examining the effects of differing exposure levels to road dust on the phytochemical composition of *Barleria dinteri* L., a traditional African plant, involved collecting samples from two specific locations within the study area. The test sample originated near a dusty road, while the control sample was retrieved from a more distant location. Using spectrophotometry, the total phenolic, tannin, flavonoid, and saponin contents in the sample extracts were also quantitatively examined. The qualitative analytical results showed that there was a substantial difference in the phytochemical contents of the extracts from the test and control samples. Quantitative analysis revealed that the total tannin, total flavonoid, total phenolic, and total saponin concentrations in leaf extracts from the experimental sample were higher than those from the control sample. The root test sample had higher total phenolic and flavonoid levels, much as the control sample had higher total tannin levels. The results indicate that exposure to road dust pollution has a moderate effect on the quality of the phytochemicals held by the samples of plant leaves and roots, despite a substantial quantitative influence in the phytochemicals. The previous study indicates that *B. dinteri* L. exhibits increased accumulation of phytochemicals, particularly in its leaves, attributed to road dust pollution [15]. Conversely, the present study endeavors to evaluate the impact of various environmental factors on the phytochemical compositions of *Solanum tuberosum* L. Plant extracts obtained from the aerial portions of the plants were used to test the phytochemical components compared to the extracts from non-polluted, the phytochemical levels were different in the plant extracts from the two plants grown in the polluted sites [16]. The main important objective is to evaluate the phytochemical substances of *Solanum tuberosum* L. in road traffic and non-road traffic situations (control).

3. MATERIALS AND METHODS

3.1 Study area

Hapur is located in Uttar Pradesh northwest. Hapur experiences cold winters and hot summers because of its humid climate, which is influenced by the monsoon and extends from latitude 28.730579 to longitude 77.775879 [17].

3.2 Collection of Crop Sample

Near Morepur on NH-235, two sites were chosen for the crop sample, traffic and non-traffic. The control site is 1000 meters separated from the traffic road site. *Solanum tuberosum* L. was the crop species used in this investigation. The C.C.S. University, Meerut, Uttar Pradesh, India in the Department of Botany, identified and recognized the crop sample taxonomically. Bot/PB/261 is the sample number.

3.3 Consideration of the Air Quality Index at the Sampling Site

The series 500 (S500) gas monitoring equipment from Aeroqual (Hapur district, NH-235), was utilized to measure the concentrations of CO, NO, NO2, SO2, O3, and UV at the different sample locations. Every day from 7 a.m. to 3 p.m., the air quality at every location was monitored for a recorded period.

3.4 Standing Preparation to Extract a Solvent

The Soxhlet extraction technique was employed to extract powdered leaves. The procedure involved using separate batches of 250 milliliters each of various solvents to extract 1 gram of uniformly dispersed plant powder contained within a thimble. Methanol served as the selected solvent for this extraction process. When the solvent in the extractor syphon tube no longer has colour, the extraction process is considered complete, which happens after 24 hours. The extract was subsequently heated in a beaker over a hot plate that was set at 64.7 ºC to distil it from its solvent. To prepare the extract of the leaves for phytochemical analysis, it was chilled to 4ºC.

3.4 Analysis of Phytochemical Substances

3.4.1 Qualitative analysis

The usual methods listed below were used to investigate if the extract contained any bioactive compounds [18-20].

3.4.1.1 Test for proteins

3.4.1.1.1 Biuret test

The Biuret test is a chemical test used to detect the presence of proteins in a sample. It relies on the reaction between the peptide bonds in proteins and copper (II) ions in an alkaline solution. When the Biuret reagent, typically a solution of copper (II) sulfate and sodium hydroxide, is added to a protein-containing sample, a violet color change occurs if proteins are present. This color change is due to the coordination of copper ions with the peptide bonds in proteins, forming a colored complex. The Biuret test is commonly used in biochemical analysis to determine the protein content in food and biological samples [18-20].

3.4.1.2 Test for carbohydrates

3.4.1.2.1 Fehling's test

The leaf extract was mixed with two milliliters of Fehling A and Fehling B reagents, each in an equal volume, and heated gradually until the mixture boiled. At the base of the tube, a brick-red precipitate would form when reducing sugars were present [18-20].

3.4.1.2.2 Benedict's test

When two milliliters of Benedict's reagent and leaf extract were combined and boiled, a reddish-brown precipitate formed, indicating the presence of carbohydrates [18-20].

3.4.1.2.3 Molisch's test

The mixture of leaf extract and two milliliters of Molisch's reagent were ready after giving it a good shake. The concentrated H_2SO_4 (02 milliliters) had to be gently poured down the side of the tube next. Carbohydrates were detected by the formation of a violet ring during the interphase [18-20].

3.4.1.3 Iodine test

The leaf extract was mixed with two milliliters of iodine solution. The hue changed to a deep blue or purple when the carbohydrate was present [18-20].

3.4.1.4 Test for phenols and tannins

The mixture contained the leaf extract and two milliliters of a 2% $FeCl_3$ solution. A blue-green or even black colour indicated the presence of tannins and phenols [18-20].

3.4.1.5 Test for flavonoids

3.4.1.5.1 Shinoda test

After mixing a tiny amount of magnesium ribbon bits and leaf extract, drops of strong hydrochloric

acid were added. A few minutes later, a reddish-pink colour appeared, indicating the presence of flavonoids [18-20].

3.4.1.5.2 Alkaline reagent test

A 2% NaOH solution was added to two milliliters to prepare the leaf extract. The intense yellow colour that had been produced turned colorless when a few drops of diluted acid were added, suggesting the presence of flavonoids [18-20].

3.4.1.6 Test for saponins

3.4.1.6.1 Foam test

A few drops of each plant extract were dissolved in various solvents, diluted to a volume of 25 millilitres in distilled water, and vigorously stirred for almost ten minutes. The extract's ability to form layer foam suggested the presence of saponins. The 5 milliliters of distilled water were shaken hard and then the liquid was let to settle in a test tube with leaf extract. The presence of saponins was assumed because stable foam developed [18-20].

3.4.1.7 Test for glycosides

3.4.1.7.1 Liebermann's test

The leaf extract was mixed with two milliliters of acetic acid and two milliliters of chloroform. Ice was used to rock-cool the mixture. With extreme caution, H_2SO_4 was added. A shift in colour from violet to blue to green indicated the existence of the steroidal nucleus, or the glycine portion of the glycoside [18-20].

3.4.1.7.2 Salkowski's test

The leaf extract was mixed with two milliliters of chloroform. After that, 2 milliliters of concentrated H_2SO_4 were cautiously added and steadily stirred. A reddish-brown hue indicates the existence of the glycoside, also known as the steroidal ring [18-20].

3.4.1.7.3 Keller-kilani test

The leaf extract was combined with two milliliters of glacial acetic acid and one or two drops of a 2% $FeCl_3$ solution. To the combination, two milliliters of concentrated H_2SO_4 were added in a different test tube. At interphase, a brown ring would appear if cardiac glycosides were present [18-20].

3.4.1.8 Test for steroid

The 2 milliliters of chloroform that had already been mixed with crude extract were treated side by side with concentrated H_2SO_4. In the presence of steroids, the lower layer of chloroform turned red. Two milliliters of chloroform were combined with the crude extract in an independent experiment. The mixture was then mixed with two milliliters of concentrated H_2SO_4 and acetic acid. The emergence of a greenish colour indicated the presence of steroids [18-20].

3.4.1.9 Test for terpenoids

After dissolving the leaf extract in two milliliters of chloroform, it was dried. Two milliliters of concentrated H_2SO_4 were added to this, and it was heated for approximately two minutes. There was a greyish colour due to the terpenoids [18-20].

3.4.1.10 Test for alkaloids

3.4.1.10.1 Mayers test

For every millilitre of plant extract diluted in various solvents, a few drops of reagents solution were added. Alkaloids were present when a pale or cream hue formed.

3.4.1.10.2 Hager's test

0.5 ml of each plant extract diluted in various solvents was mixed with a few drops of Hager's reagent solution. The precipitate's yellow appearance suggested that alkaloids were present in the extracts.

3.4.1.10.3 Tannic test

0.5 ml of each plant extract diluted in various solvents was mixed with a few drops of 10% tannic acid. The presence of alkaloids in the extracts was revealed by the formation of a buff-coloured precipitate.
The 2 milliliters of 1% hydrochloric acid combined with a slow-heating leaf extract combination were used. Then, Wagner's and Mayer's reagents were added to the mixture. The turbidity of the precipitate that was formed indicated the presence of alkaloids [18-20].

3.4.2 Quantitative analysis

The method used determined the quantitative phytochemical analysis [21].

3.4.2.1 Total phenolic content

The modified Folin-Ciocalteu reagent method was employed to quantify the phenolic content within the aqueous extract. A volume of 1 milliliter of plant extract was combined with 2 milliliters of a 2% Na_2CO_3 solution and 2.5 milliliters of a 10% Folin-Ciocalteu reagent. The resulting mixture underwent incubation at room temperature for 15 minutes. Subsequently, the absorbance of the sample was measured at 765 nm. Gallic acid (1 mg/ml) served as the reference standard. To ensure precision, the experiment was repeated three times. Utilizing the standard curve generated from gallic acid, the gallic acid equivalent (mg/g of extracted material) which was used to show the results [21].

3.4.2.2 Total flavonoid content

3.4.2.2.1 Alkaline reagent test

After dissolving 1 millilitre of each herbal extract (leaf, stem, and root) in various solvents, a little amount of NaOH solution was added. The appearance of a yellow hue that vanished when diluted acid was added suggested the presence of flavonoids.

3.4.2.2.2 The ferric chloride test (FeCl3)

The presence of flavonoids was revealed by the formation of a blackish precipitate when a few drops of $FeCl_3$ were added to the herbal extracts. A solution was prepared and allowed to stand at room temperature for thirty minutes, comprising 1 milliliter of plant extract, 3 milliliters of methanol, 2 milliliters of 10% aluminum chloride, 1 milliliter of potassium acetate, and 5 milliliters of distilled water. The absorbance was measured at 420 nm. Quercetin (1 mg/ml) served as the reference standard. Utilizing the standard curve generated from quercetin, the concentration of flavonoids in the isolated product was determined and expressed as mg/g of quercetin equivalent [21].

3.5 Statistical Analysis

A t-test was utilized to analyses the plant samples. The established technique revealed that the values of 0.0001 had a significant difference [22].

4. RESULTS

4.1 Evaluating the Air Quality at the Sampling Sites

Figs. 1 and 2 show the concentrations of CO, NO, NO_2, SO_2, O_3, and UV at the major road traffic and control sites respectively, it was recorded that, compared to the control sites, the air quality concentrations on the road traffic were higher. Between the road traffic and the control sites was a statistically significant difference in the mean of the total air quality index values (159.1<255.4).

4.2 Qualitative Analysis

Tables 1 and 2, showed qualitative analysis (protein, carbohydrate, iodine, phenol, tannin, flavonoids, saponin, glycosides, steroid, terpene, and alkaloid) demonstrates that throughout the observation period, the quality of qualitative phytochemical substances was shown to be bad under the road traffic. The quality of qualitative phytochemical substances was seen to be better for the crop growing away from road traffic.

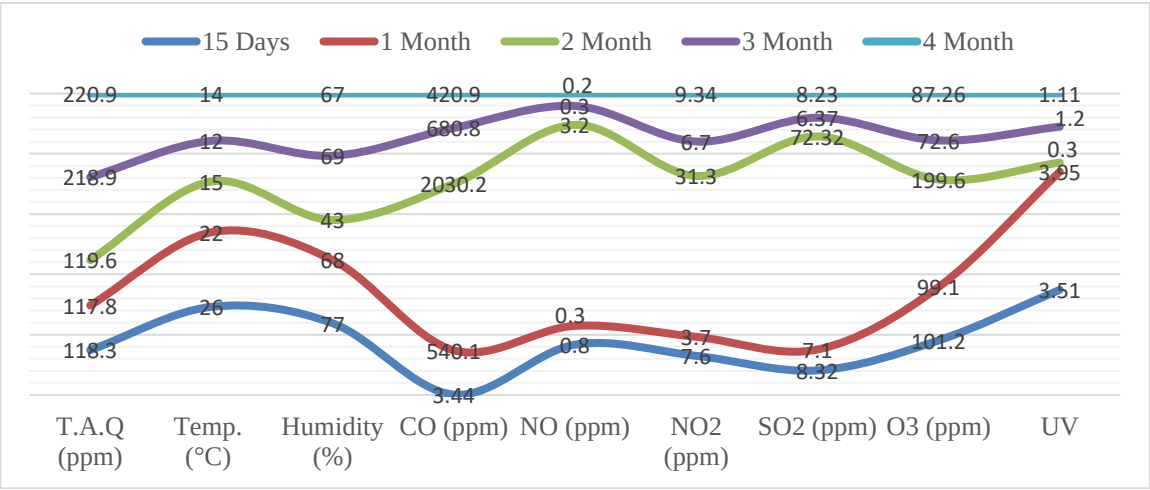


Fig. 1. Variable gas concentrations at the under-control site

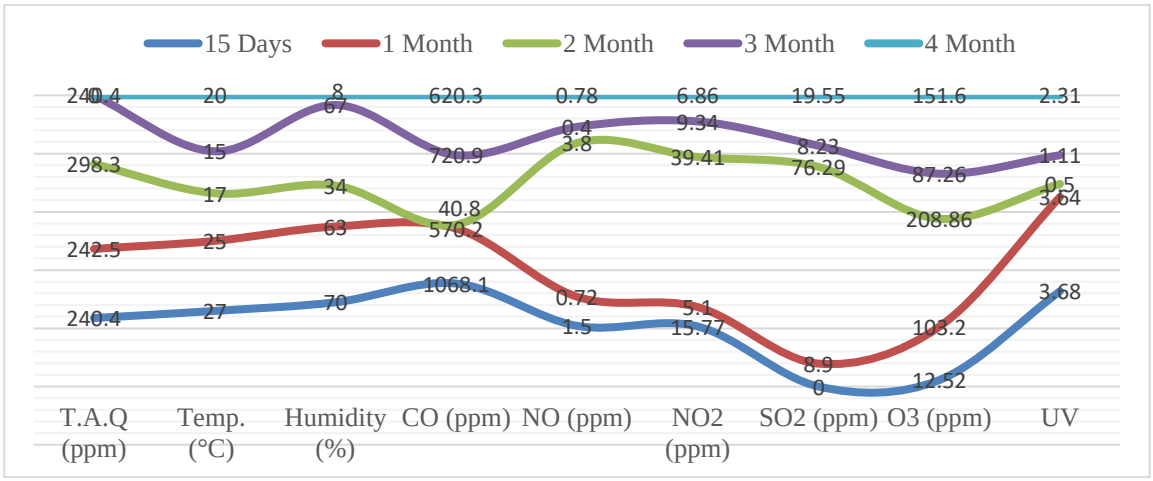


Fig. 2. Variable gas concentrations at the under-road traffic site

Table 1. The qualitative substances under the Control site

Qualitative Substances	15 Days	1 Month	2 Month	3 Month	4 Month
Protein	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Iodine	-	+	+	+	+
Phenol	+	+	+	+	+
Tannin	-	+	+	+	+
Flavonoids	+	+	+	+	+
Saponin	-	+	+	+	+
Glycosides	+	+	+	+	+
Steroid	+	+	+	+	+
Terpene	+	+	+	+	+
Alkaloid	+	+	+	+	+

Table 2. The qualitative substances under the road traffic site

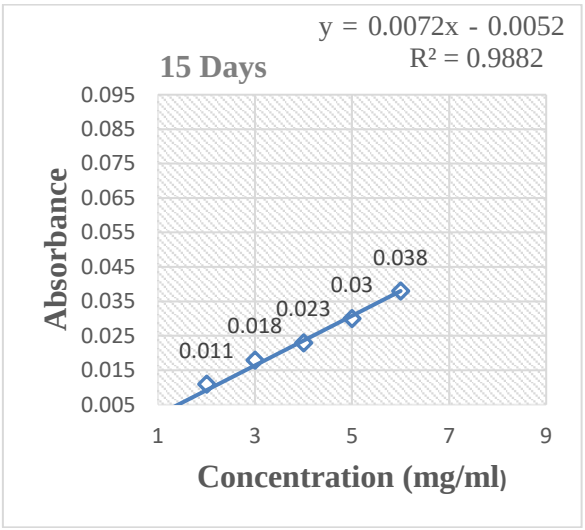
Qualitative Substances	15 Days	1 Month	2 Month	3 Month	4 Month
Protein	-	-	+	+	+
Carbohydrate	+	+	-	+	+
Iodine	-	-	+	-	-
Phenol	-	-	+	-	+
Tannin	-	+	-	-	-
Flavonoids	-	-	+	-	+
Saponin	-	+	+	+	-
Glycosides	+	-	+	+	+
Steroid	+	-	-	+	-
Terpene	+	-	+	+	-
Alkaloid	-		-	+	-

4.3 Quantitative Analysis

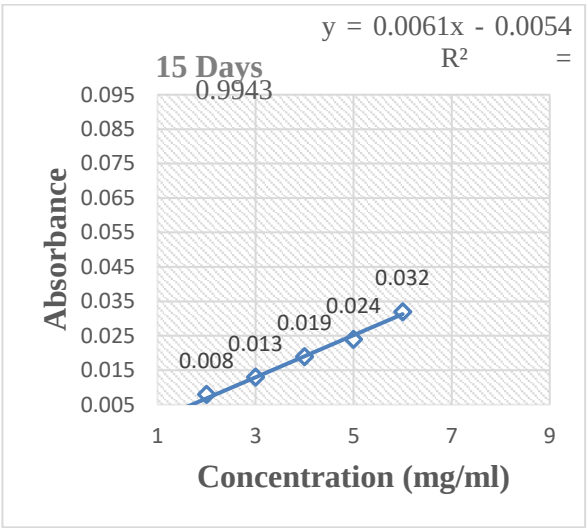
4.3.1 Total phenolic content

Figs. 3 and 4 showed that during the investigation, we were able to show that crops planted close to busy road traffic had lower levels of these quantitative phytochemical substances, whereas the control sites had higher levels of total phenolic content values. Research indicates that, whereas these characteristics were lacking from the road traffic, they were present in the leaves of the control sites. With a total mean value of (0.03712 > 0.03176), the results demonstrate that the total phenolic content levels at the road traffic and the

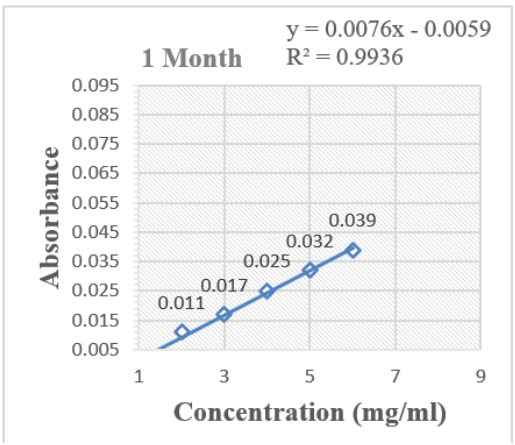
control sites differed substantially. The control and road traffic site data showed a statistically significant difference (Variance < 0.0001).



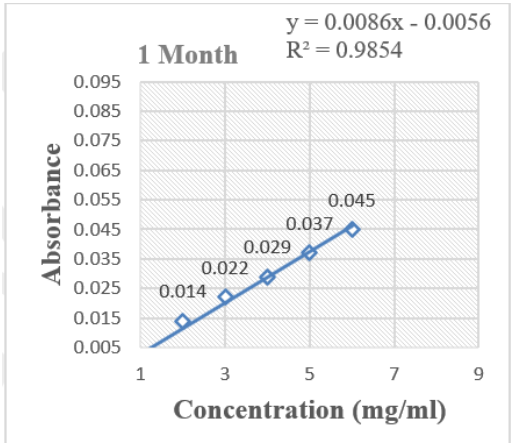
A - C.S 15 Days



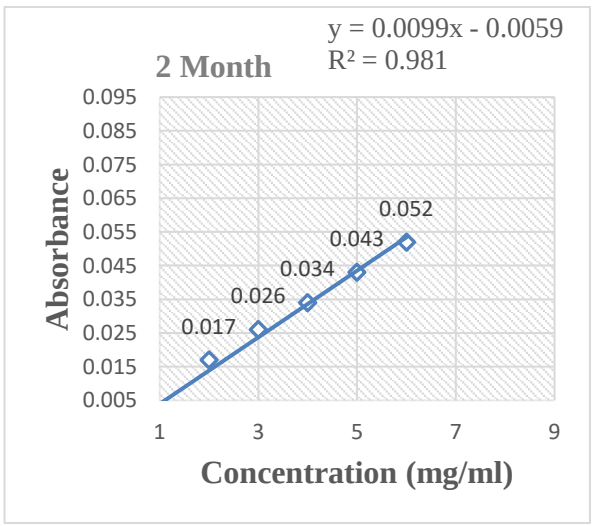
B - R.T. S 15 Day



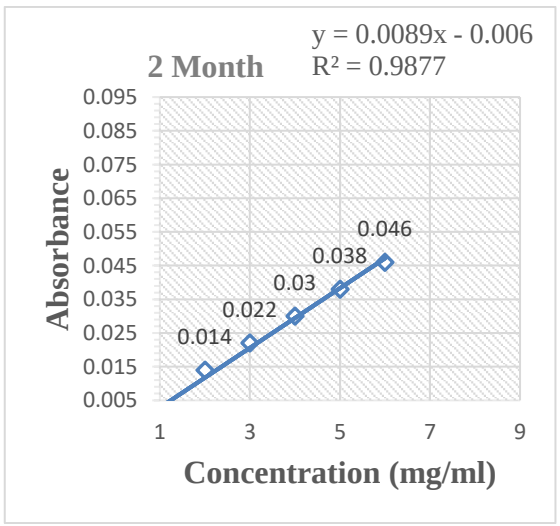
C - C.S 1 Month



D - R.T. S 1 Month



E - C.S 2 Month



F - R.T.S 2 Month

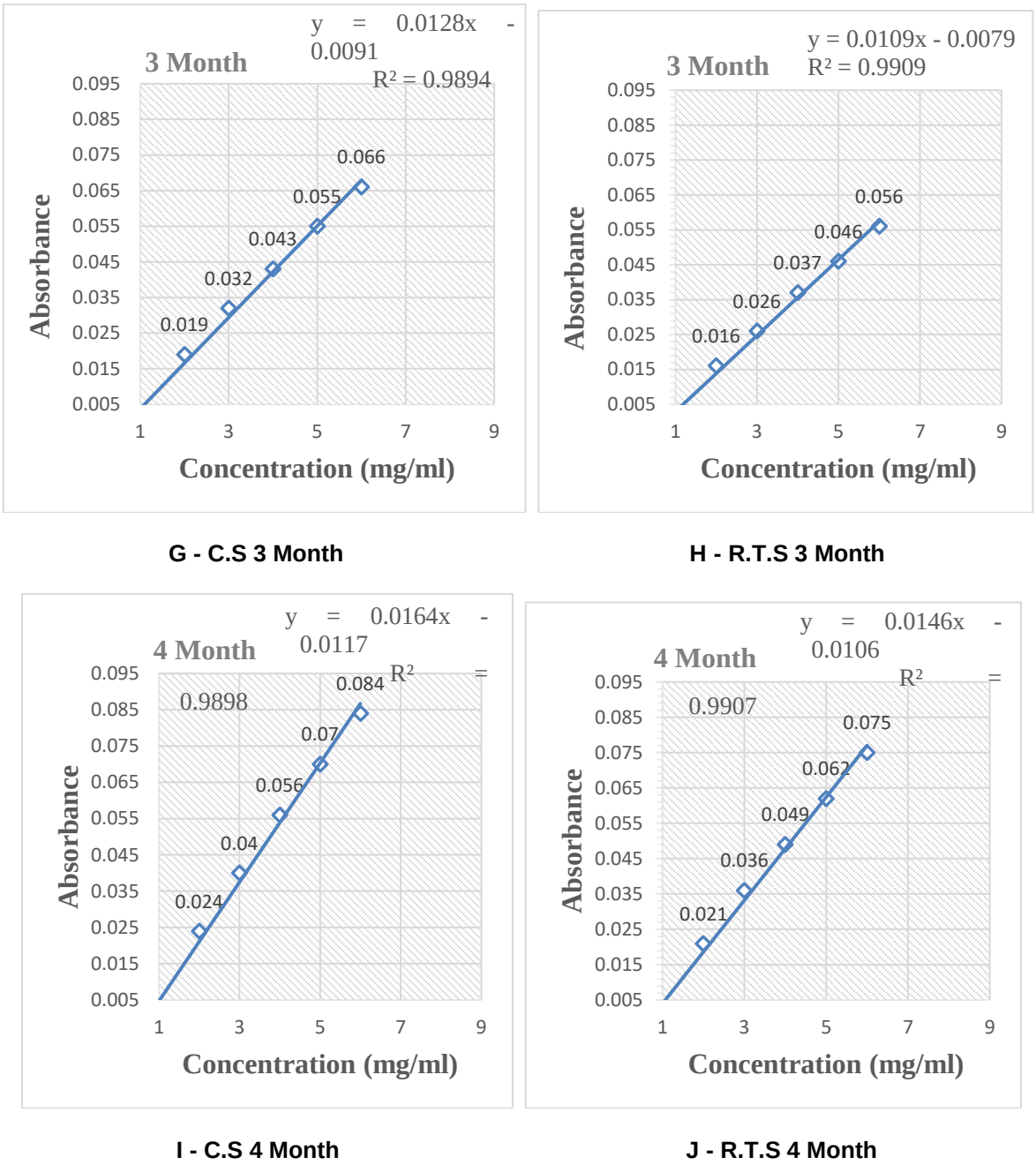


Fig. 3. The standard curve showing the total phenolic content value at various intervals under the control and road traffic sites

C.S – Control Site; R.T.S –Road Traffic Site

4.3.2 Total flavonoid content

Figs. 5 and 6 showed that during the investigation, we were able to show that crops planted close to busy road traffic had lower levels of these quantitative phytochemical substances, whereas the control sites had higher levels of total phenolic content values. Research indicates that, whereas these characteristics were lacking from the road traffic, they were present in the leaves of the control sites. With a total mean value of (0.04696 > 0.03764), the results demonstrate that the total flavonoid content levels at the road traffic and the control sites differed substantially. The control and road traffic site data showed a statistically significant difference (Variance < 0.0001).

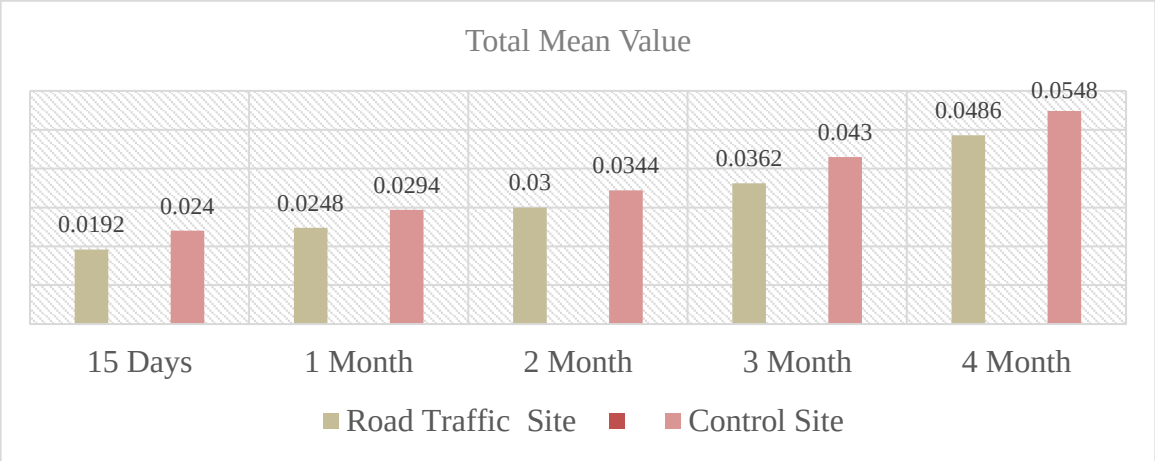
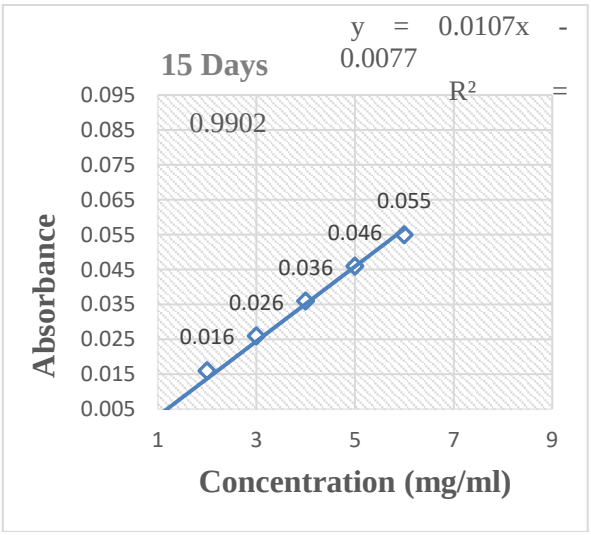
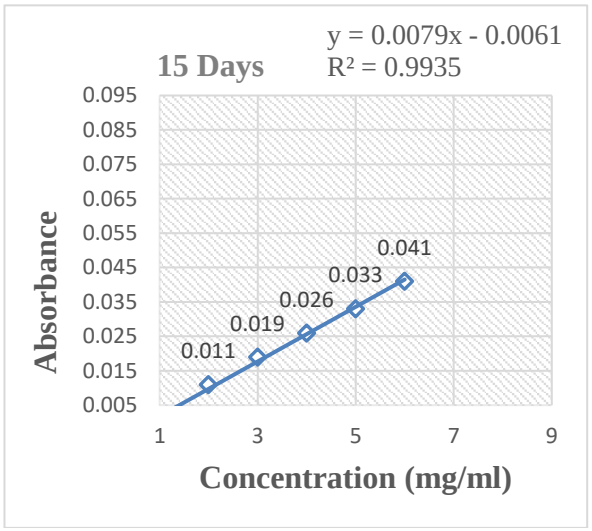


Fig. 4. The total mean values at the level of total phenolic content that were calculated for the under-road traffic and control sites

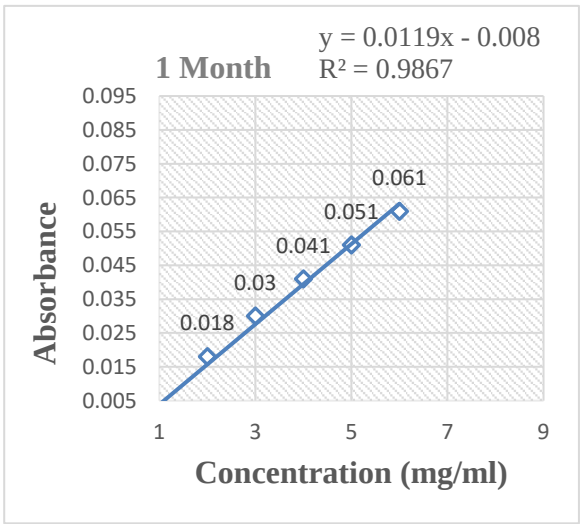
Significant at: Variance = 0.0001 (Variance < 0.05 is considered very high significant)



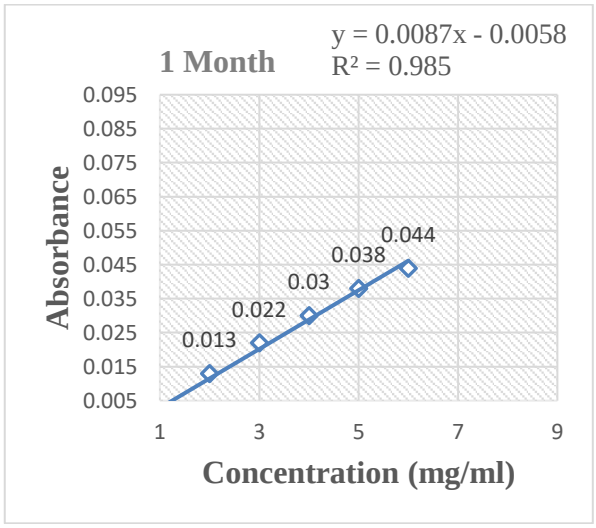
A - C.S 15 Days



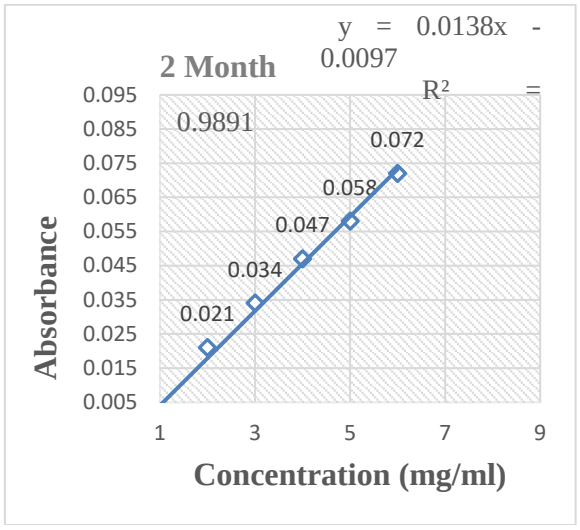
B - R.T. S 15 Day



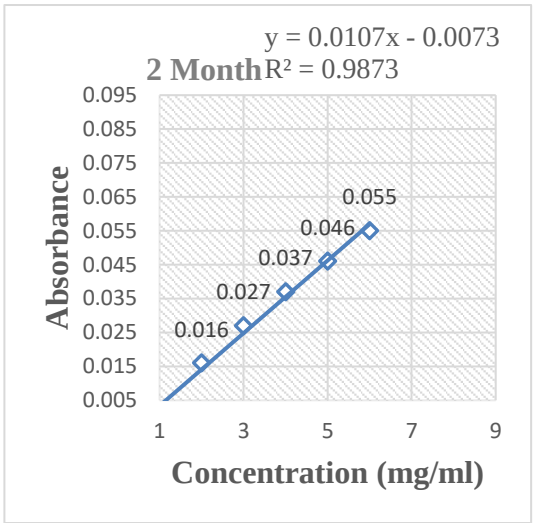
C - C.S 1 Month



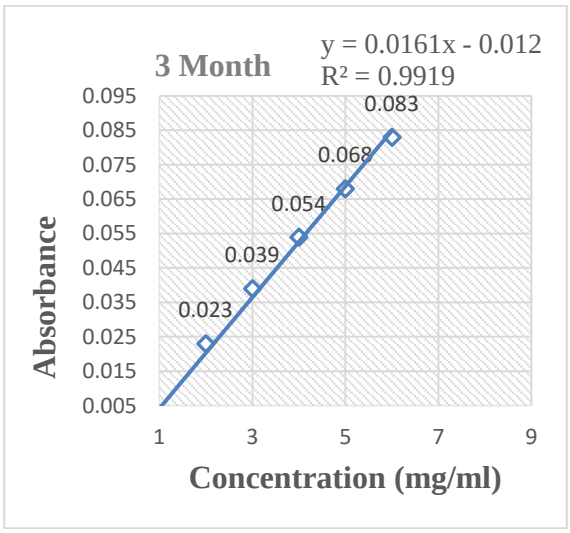
D - R.T. S 1 Month



E - C.S 2 Month



F - R.T.S 2 Month



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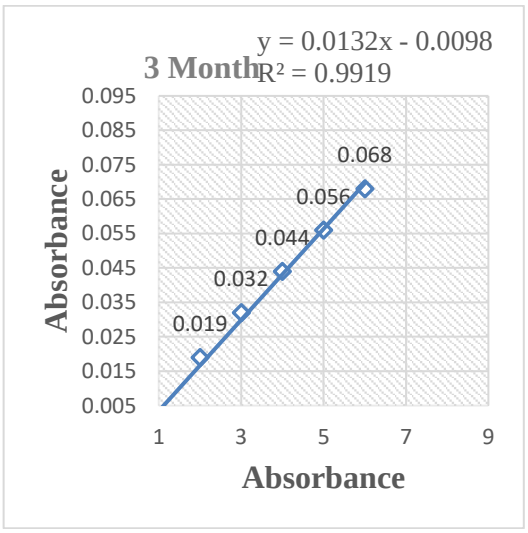
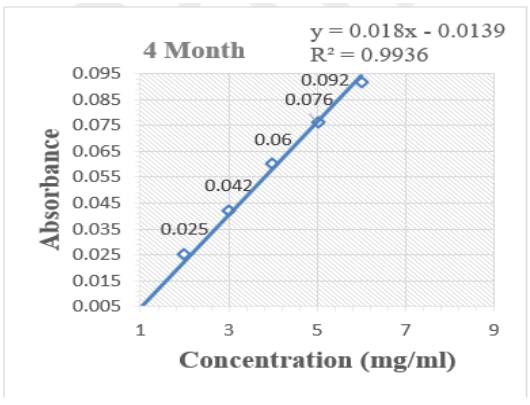
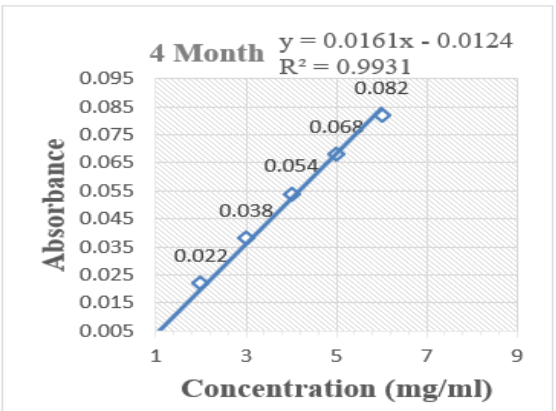


H - R.T.S 3 Month



I - C.S 4 Month



J - R.T.S 4 Month

Fig. 5. The standard curve showing the total flavonoid content value at various intervals under the control and road traffic sites

C.S – Control Site; R.T.S – Road Traffic Site

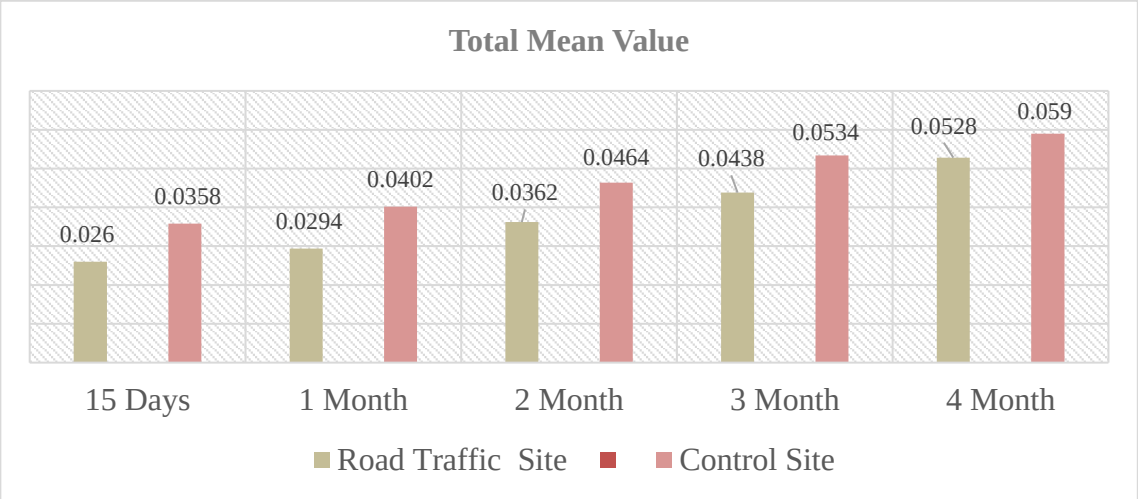


Fig. 6. The total mean values at the level of total flavonoid content that were calculated for the under-road traffic and control sites

Significant at: Variance = 0.0001 (Variance < 0.05 is considered very high significant)

5. DISCUSSION

The observed concentrations of carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), and ultraviolet (UV) radiation at prominent road traffic and control locations revealed elevated air quality levels at the road traffic sites in comparison to the control sites. Between the road traffic and the control sites was a statistically significant difference in the mean of the total air quality index values (159.1<255.4). The qualitative analysis (protein, carbohydrate, iodine, phenol, tannin, flavonoids, saponin, glycosides, steroid, terpene, and alkaloid) demonstrates that throughout the observation period, the quality of qualitative phytochemical substances was shown to be bad under the road traffic. The quality of qualitative phytochemical substances was seen to be better for the crop growing away from road traffic. The record showed that the qualitative substances analysis (protein, carbohydrate, iodine, phenol, tannin, flavonoids, saponin, glycosides, steroid, terpene, and alkaloid) the quality of qualitative phytochemical substances is seen to be negative in the crop growing on the roadside, control site the quality of qualitative phytochemical substances is seen to be positive in the crop growing away from the road. The recorded quantitative data were analyzed as a total mean value of total phenolic content (0.03712 > 0.03176) and the total mean value of total flavonoid content (0.04696 > 0.03764) between the control and road traffic sites. The control and road traffic site data were statistically significant the total phenolic content (Variance < 0.0001) and total flavonoid content (Variance < 0.0001).

6. CONCLUSION

The outcomes of the research substantiate those diverse manifestations of vehicular road traffic congestion precipitate air pollution in the road traffic environment, eliciting deleterious consequences. In addition to the fact that these objective qualities showed that crops are adversely affected by some gases (CO, NO, NO_2, SO_2, O_3, and UV), agricultural air pollution is currently a significant health hazard. The quality of both qualitative and quantitative phytochemical substances is shown to be better in the crop growing away from the road traffic and is observed to be less in the crop growing on the road traffic site.

7. FUTURE PERSPECTIVES

Outlined are the primary objectives of the analysis aimed at discerning and comprehending the effects of emissions on diverse plant species. The acquisition of qualitative and quantitative data is crucial for accurately pinpointing the specific impacts of plant emissions. This could be useful in assessing the environmental pollution risk. It is important to ensure *S. tuberosum* L. leaves are also good fodder for livestock and there should be security of edible parts. Roadside crops are negatively impacted by traffic air pollution. To prevent pollution caused by road traffic, trees should be planted on both sites of the road. Fewer vehicles should be utilized,

electric vehicles should be used, and crops should be grown farther away from busy roads.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Botany at J.V. College in Baraut, Baghpat, Uttar Pradesh, India, for providing the necessary lab space for this work. Additionally, the Department staff deserves recognition from the authors for their invaluable assistance with several analyses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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