Effect of automobile pollution on some biochemical activities of roadside plants in Misurata-Libya

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Abstract— The effect of road dust pollution was determined on chlorophyll, metals, pH, water content and dust deposition of three different plant species Ricinus communis, Pachystachys lutea and Nerium oleander growing along the roads of Misurata city-Libya. Significant changes in the level of chlorophyll “a”, chlorophyll “b” and total chlorophyll “a+b” were found in the leaves of three plant species collected from polluted sites as compared to control. Notable amount of heavy metals were recorded in the leaf samples of all plant species collected from polluted sites. pH and water content also determined and showed different results compared to unpolluted samples. Aspergillus, Penicillium, Fusarium and Rhizopus were the most frequently found genera in the plants studied in all samples. This study clearly indicated that the vehicular activities induced air pollution problem and affected on the level of some activities in plants grown nearby road side.

Keywords: Roadside plants, plant, microbial, Misurata, Libya

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INTRODUCTION

Roadside soils often contain high concentrations of metallic contamination. Acceptable particulate problem is of great concern including dust and smoke as they are mended, resulting in detrimental effect on organisms health including vegetation. Higher concentrations of heavy metals may affect the physiological behaviour of plants and damage the activities of photosynthetic enzymes and block the electron transport chain which reduced the chlorophyll contents. Dust may allow the penetration of phytotoxic gaseous pollutants and affect photosynthesis, respiration, transpiration [1,2]. Changes in pH, relative water content and species diversity of vegetation communities can be affected by dust from highways and roads [3]. Vegetation act as natural filters by depositing dust particles on their leaf surface, Susceptible and highly exposed part of a plant, thus makes an important contribution in the improvement of air quality. Leaves act as pollution receptors and decrease dust load of the air [4].

Although, there have been a considerable number of studies on the concentrations of heavy metals in roadside soil and plants, the vast majority have been carried out in developed countries with long histories of negative pollution on plants and soil but few data recorded for fungus. A large number of trees and shrubs have been identified. Leaves and leaf traits can be used as a tool as dust filter to check the rising urban dust pollution [5]. The adverse effect of gaseous and particulate pollutants could be reduced by trees, shrubs [6]. The increased dust deposition in Libya has influenced the physiological and environmental characteristic of industrial plants and its response [7]. The future research can help in reduction of dust pollution by selecting the suitable tolerant plant species. This can be take place by study the influence of dust deposition on biochemical parameters of roadside plants such as pH, metals absorption and total chlorophyll content. Although there have been a considerable number of studies on the concentrations of heavy metals in roadside soil and plants, the vast majority have been carried out in developed countries with long histories of industrialization and extensive use of leaded gasoline [8-14]. During the last few years, the number of vehicles in some Arabic cities and other places [15] mostly operated by leaded fuel, have contamination of roadside soil, plants, and air with heavy metals. The leaves of plants absorb these pollutants from the atmosphere that will effect chlorophyll contents.

Very few studies have been carried out in developing countries such as Libya and data on pollutant metal concentrations and distribution in such areas are extremely limited. Therefore, this paper aims to estimate the relationship between dust deposition and various physiochemical factors of three roadside plant species i.e. **Ricinus communis**, **Pachystachys lutea** and **Nerium oleander**. Genera of fungi which are present in leaf surface microflora in the area contaminated by dust were investigated, as well as pH, moisture, and some metals content in the polluted plants.

MATERIALS AND METHODS

Study Site:

The research work was done in Misruata zone 32.65 N (north) and longitude 14.26 E (east). It is one of the three biggest cities in Libya. In the summer, the main direction of the wind is from the desert (south) to the sea (North). However, in the winter the wind blows from north to south and from west to east. Generally speaking, the climate of the area in the summer is hot and dry. The temperature rapidly increases in this season, reaching 48°C. In the winter it is warm and rainy but rainfall is scanty. In Libya, more than 50% probability of occurrence of 100 mm rainfall amounts can be noted in sixteen meteorological stations including the Misurata study area and rainfall of 25 to 50 mm can occur. 26 millimeters is the average annual rainfall for the country. The site for sampling of dust and leaves was selected near the center of city, an important city road with high anthropogenic activities.

Plants:

Ricinus communis, Pachystachys lutea and Nerium oleander

Leaf Sampling and Physiochemical determination

Three plants of common occurrence along the roadside in Misurata-Libya were selected for the study during summer 2015 for their dust deposition and physiochemical studies. Three replicates of fully matured leaves of each species were randomly collected in early morning from the lower branches (at a height of 2 - 4 m) and were quickly transferred to the laboratory in polythene bag kept in ice box for further analysis within 24hrs of their harvesting.

Leaf dust collection:

The amount of dust was collected as described by [4]. They calculated by taking the initial and final weight of beaker in which the leaf samples were washed. Calculated formula was used in this process as follows:

$$W = W2 – W1/ A$$

Where, W = Dust content (mg/cm2), W1 = Weight of beaker without dust, W2 = Weight of beaker with dust A = Total area of leaf in cm^2.

Leaf Extract pH:
Leaf sample (0.5 g) was crushed and homogenized in 50ml deionized water, the mixture was centrifuged and supernatant was collected for detection of pH as described by [4].

Relative Leaf Water Content (RWC):
The method described by [4], was followed to determine RWC based on the formula, RWC= (wf – wd) x 100 / (wt – wd). Where, w fresh wt of the leaf. wt-turgid weight of the leaf after immersing into water overnight. wd-dry weight of the leaf. 0.5 g of leaf pieces were placed in a dryer at 240^C for 24 hr.

Leaf length:
Because of Leaf area is an indicator of crop growth and productivity and therefore, accurate measurements of leaf area (LA) are essential to understand the interaction between crop growth and environment , each leaf was placed onto an A4 sheet and was photocopied. Actual leaflet areas were measured using a slide rule [16] .

Plant metals Analysis
Because of the high content of metals in polluted air , the concentration of some metals were measured in leaves from each station. Metals were determined using the method of [17].

Total Chlorophyll Content :
This was done according to the method described by [4]. 0.25 g of fresh leaves were blended and then extracted with 10 ml of80% acetone and left for 15 min. The liquid portion was decanted into another text tube and centrifuged at 2,500 rpm for 3 min. The supernatant was then collected and the absorbance was then taken at 645 nm and 663 nm using a spectrophotometer. Calculations were made using the formula below:

$$\text{Chlorophyll a} = 12.7DX663 – 2.69DX645 \times V/1000W \text{ mg/g.}$$
$$\text{Chlorophyll b} = 22.9DX645 – 4.68DX663 \times V/1000W \text{ mg/g.}$$

Ch = chlorophyll a + b mg/Dx = Absorbance of the extract at the wave length nm.
V = total volume of the chlorophyll solution (ml),and W = weight of the tissue extract (g).

Fungal Isolation
Fungi were isolated from leaves as described by Rai and [18] and [19] with slight changes.

Statistical Analysis
Data were analyzed using SPSS. For some experiments, there were three replicates per site. Three to six replicates (cultures) from each site were used in the analysis of data on isolation of fungi from plant leaves. Using one-way classification, analysis of variance (ANOVA) of the polluted and control plant samples was undertaken. Throughout $P \leq 0.05$

was used to define statistical significance mean separations were carried out using Duncan’s test. Microsoft Excel was used to produce graphs.

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RESULTS AND DISCUSSION

A. Leaf pH

The results in this paper show that pH values in the all samples, were significantly lower in the polluted and unpolluted sites (P > 0.01) (Table 1). It ranged between 2.0 to 3.5 and were more acidic. This condition may be due to presence of an acidic pollutant that shifts the cell [4].

B. Leaf dust collection:

Dust deposition capacity varied from a maximum in **Ricinus communis**,to a minimum in **Nerium oleander**. The trend of dust deposition among the species was **Ricinus communis**, **Pachystachys lutea** and **Nerium oleander**. Their quantity was significantly greater 0.40 , 0.29 , 0.005g at **Ricinus communis**, **Pachystachys lutea** and **Nerium oleander** respectively whereas, the unpolluted leaves were between 0.002-0.004 g. (Table 2). Dust

Table 1. Measured pH Of Plant Samples, ***Ricinus communis, Pachystachys lutea* and *Nerium oleander*** From Polluted Area And Unpolluted Area Values Within A Row Followed By The Same Letter Are Not Significantly Different At P = 0.05 According To Duncan’s Test.

Plant	Polluted area	Unpolluted area
R. communis	2.3 a	**2.5 b**
P. lutea	**3.5 b**	**2.0 a**
N.oleander	**3.0 b**	**3.0 b**

deposition capacity of plants depends on their surface geometry, phyllotaxy and leaf external characteristics such as presence/absence of hairs, cuticle, length of petiole, height and canopy [20-22].

Table 2. Measured dust deposition (g) of plant samples, from polluted area and unpolluted area values within a row followed by the same letter are not significantly different at p = 0.05 according to Duncan's test.

Plants	Polluted area	Unpolluted area
R. communis	0.40 a	**0.003 b**
P. lutea	**0.29 a**	**0.004 b**
N.oleander	**0.005 a**	**0.05 b**

C. Relative Leaf Water Content (RWC):

Leaf moisture was measured for all contaminated samples and unpolluted samples (TABLE 3). The results show highly significant differences between **Ricinus communis** in polluted area in comparison to unpolluted area (P > 0.01). The water content of other leaves from polluted and unpolluted area sites were not significantly different (P <0.05) (TABLE 3). High volume of water in **Ricinus communis** even in polluted area may be to a large **Leaf** length and area. In addition, the **Ricinus communis** leaves may absorbed greatly water due to high dust deposition. In **Pachystachys lutea** and **Nerium oleander** Relative Water Content is least may be due to lower availability of water in soil along with high transpiration rate [4] .

Table 3. Measured water content (%) of plant samples, from polluted area and unpolluted area values within a row followed by the same letter are not significantly different at p = 0.05 according to Duncan's test.

Plants	Polluted area	Unpolluted area
R. communis	49.6 a	17.4 b
P. lutea	15.2 a	15. 4 a
N.oleander	25.0 a	26.2a

D. Leaf length and area:

There were no significant differences in Leaf length and area between polluted and unpolluted area in **Ricinus communis** and **Pachystachys lutea** ($P < 0.05$), whereas the leaves area of **Nerium oleander** showed a significant differences ($P > 0.05$) and this area showed twice. In addition, no significant differences in **Leaf length** between all samples in polluted and unpolluted area except **Nerium oleander.** These results similar to [23].

Table 4. Measured leaf length and area (cm) of plant samples, from polluted area and unpolluted area values within a row followed by the same letter are not significantly different at p = 0.05 according to duncan's test.

Plants	**Polluted area**		**Unpolluted area**	
	length	area	length	Area
R . communis	14.9 a	65.8a	12.3a	60.4 a
P. lutea	6.3 a	64.3a	6.7a	49.3a
N. oleander	10. 5a	54.6a	14.5a	90.9 b

H. Leaf metals analysis:

The results showed in Table V that the cadmium investigated appeared significant difference ($p > 0.05$) between polluted and unpolluted leaves except **Pachystachys lutea**. Statistically, however all leaves except **Nerium oleander** indicated a significant difference in Pb content between polluted and unpolluted samples. The Cu and Zn showed no significant difference at all samples. Theses metals recorded significant difference ($p < 0.05$) between groups. High accumulation of Pb and Zn were observed an all samples compared to other metals investigated. This is in same trend with [15]. The selectivity of different plants for heavy metals is different, but rough comparison of heavy metals in plants with other This could be due to different plant types and different number of vehicles per day in those places.

Table 5. Measured metals content (ppm) of plant samples (rc) **ricinus communis**, (pl)) **pachystachys lutea** (n.o) **nerium oleander**, from polluted area and unpolluted area values within a row followed by the same letter are not significantly different at p = 0.05 according to Duncan's test.

PLANTS	POLLUTED AREA				UNPOLLUTED ARE			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
R.C	0.04a	0.16a	0.22a	0.26a	0.10 b	0.4a0	0.04 b	0.71a
P.L	0.07a	0.80a	0.18 a	0.70a	0.06a	0.60a	0.04 b	0.51a
N.O	0.09a	0.21a	0.08a	0.23a	0.10b	0.30a	0.05 a	0.40a

E. FUNGI ISOLATED FROM PLANT

There was a large increase in the total number of fungi isolated in this study. Fifteen species belonging to four genera of fungi were recovered from all leaves. Aspergillus, Penicillium, Fusarium and Rhizopus were the most frequently found genera in the plants studied in both polluted and unpolluted locations (Table 6). When fungi were isolated from olive leaves that had been exposed to cement dust for a long time, the most common colonies isolated were Aspergillus, Fusarium, Mucor, and Penicillium [24]. Such fungi presented in all polluted and unpolluted sites were Aspergillus niger and Rhizopus sp. It suggested a certain adaptation of this fungus to such environmental stress. A. niger was noted as one of the most widespread species in soil and plants contaminated by dust [25,26].

Table 6. Summary of fungal colonies isolated from plant samples studied (rc) **Ricinus communis**,(pl) **Pachystachys lutea** (n.o) Nerium oleander (leaf discs) from polluted area (pa) and unpolluted area (ua).

FUNGUS	RC		PL		NO	
	PA	UA	PA	UA	PA	UA
Aspergillus flavus	29	6	5	4	11	2
Aspergillus niger	130	44	103	32	93	25
Aspergillus ochraceus	4	0	0	0	0	0
Aspergillus sp_1	2	0	0	0	0	0
Aspergillus sp_2	0	0	4	0	0	0
Aspergillus sp_3	0	2	0	0	0	0
Fusarium oxysporum	2	0	0	0	0	0
Fusarium sp_1	2	0	0	0	0	0
Fusarium sp_2	3	0	0	0	0	0
Fusarium sp_3	2	0	0	0	4	0
Fusarium sp_4	2	0	0	0	2	0
Penicillium chrysogenum	4	0	5	0	4	0
Penicillium sp_1	0	0	0	0	10	0
Penicillium sp_2	0	12	0	0	0	0
Rhizopus sp_1	15	16	3	0	50	25
Total number of species	11	5	5	2	7	3
Total number of genera	4	3	3	1	3	2
Total number of colonies	195	80	32	36	174	52

0 - No growth

F. Total Chlorophyll Content (TCH):

Chlorophyll plays an important role in plant growth. Variation in chlorophyll content has been used in many studies in order to investigate the effects of pollutants on plants total chlorophyll content of selected plant species under study is shown in Table VII. All the plant species exhibited maximum chlorophyll content at all samples. This chlorophyll showed significant differences ($P > 0.05$) at all samples except **Pachystachys lutea** at A, B and A+B between polluted and unpolluted samples. The variation in chlorophyll content of selected plants may be due to the dust particles [7,21]. Dust accumulation causes severe damage in the soil and may also affected photosynthetic apparatus [7,27]. Deposited dust on the surface of leaf alters its optical properties particularly the surface reflectance in the visible and short wave infrared radiation range [28 - 31]. This study clearly indicated that the vehicular activities induced air pollution problem and affected on the level of chlorophyll pigments in trees which were exposed to road side pollution. This might be due to long time deposition of dust on plane leaves

Table 7. Measured chlorophyll content (gm) of plant samples(rc) **ricinus communis**, (pl) **pachystachys lutea** (n.o) **nerium oleander**, from polluted area and unpolluted area values within a row followed by the same letter are not significantly different at $p = 0.05$ according to Duncan's test

PLANTS	POLLUTED AREA			UNPOLLUTED ARE		
	A	B	A+B	A	B	A+B
R..C	16a	20a	36a	7b	7b	14b
P.L	9a	4a	13a	6a	1a	7a
N.O	12a	10a	22a	4b	1b	5b

CONCLUSION

From this study it can be concluded that tested plant species were affected by pollution, the results clearly indicated that the vehicular activities induced air pollution problem. Furthermore, some physical and biological characteristics, such chlorophyll pigments, pH, water, metals and fungal content, in plants that exposed to road side pollution significantly affect when compared with their control.

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تأثير ملوثات المركبات علي بعض النشاطات الكيموحيوية لبعض النباتات بجانب الطرق في مصراتة

عبدالمجيد مليطان و أم كلثوم الصل و منيه القايد و سارة أبوغولة و أمينة العاتي

قسم النبات، كلية العلوم، جامعة مصراتة، مصراتة، ليبيا

الخلاصة:

تأثير التلوث بغبار الطرق علي محتوي اليخضور، المعادن، قيمة الأس الهيدروجيني، المحتوي المائي و الفطريات المرافقة علي ثلاث انواع من النباتات وهي الخروع، المصاص والدفلة. أظهرت النتائج تغيرات في كمية اليخضور مقارنة بنباتات الشاهد من مناطق بعيدة عن التلوث. كما سجلت نسبة عالية من المعادن، ولوحظ تغير في قيمة الأس الهيدروجيني والمحتوي المائي أيضا. تم عزل العديد من الفطريات وكان أكثرها سيادة فطر الأسبرجلس والبنسيليوم والفيوزاريم وعفن الخبز. ومن خلال هذه النتائج المتحصل عليها ربما يتضح التأثير السلبي لعوادم السيارات علي نمو النباتات قيد الدراسة.

الكلمات المفتاحية: جانب الطرق، نبات، ميكروبي، مصراتة، ليبيا