

EFFECT OF INDUSTRIAL ACID RAIN ON THE BIOCHEMICAL PROPERTIES OF SOME COMMON PLANTS IN TIKRIT

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ABSTRACT

This study was conducted in Tikrit to compared between the plants used in decorating the sides of roads and public parks according to their ability to withstand the conditions of industrial acid rain in order to recommend them in afforestation industrial parks and areas of traffic density. Where different concentrations of industrial acidic rain were used (pH2, pH4, Ph6 and pH7.2) as well as five common plant species used for landscaping and road sides aspects (*Calistemon viminalis* ,*Nerium oleander*, *Olea europaea L* , *Myrtus communis* , *Dodonaea viscosa* Linn).

The plants varied in their tolerance according to the plant species and by acid precipitation concentrations of all the properties in the study [concentration of ascorbic acid (mg/g) and total chlorophyll (mg/g) and pH of the leaf extract (pH) and the relative water content of the leaves(%)].The result showed that the best plants tolerate the conditions of pollution and according to the index of APTI (Air Pollution Tolerance Induces) were (*Calistemon viminalis* > *Myrtus communis* > *Dodonaea viscosa* Linn > *Olea europaea L* > *Nerium oleander*). It was also observed that one plant has different sensitivity to the acidic industrial rain by acid concentration. Some plants in the study were sensitive to a specific concentration but were relatively tolerant or moderately tolerant at other concentrations.

Key words: Acid Rain , The Biochemical Properties , APTI

INTRODUCTION:

Although pollution of the environment can be seen as a local problem but it is a global problem. Pollutants are under the influence of many factors that do not know geographical boundaries. They are characterized by their ability to move from one location to another in the near and long term. Wind, clouds and contribute to the transfer of pollutants distant so it is illusory to imagine the individual that there is a place on the planet has not been contaminated in one way or another^[23]. Increasing acidity of natural water and soils became the hallmark of environmental degradation at present , Studies have indicated that acidic rain damage reached 30 percent of the world's forests^[15] , Rain in its equilibrium with atmospheric carbon dioxide has a pH equal to about 5.6, but the presence of sulfuric acid and nitric reduces the pH value much less, so rain with a pH of less than 5.6 is acidic rain^[25].

As most of the gaseous pollutants from car exhausts, generators, fire, oil refining, fertilizers, vegetable oils and power plants are contained in SO_2 gas, which is considered as a source of acidic rain or dry acidic drip, as well as other gases. this study is to determine the resistance of some plant species such as (*Calistemon viminalis* , *Nerium oleander* , *Olea europaea L* , *Myrtus communis* , *Dodonaea viscosa L*) Which are used for planting the sides of roads and public parks to the effect of acidic contaminants and damage that lead to theme aim of this study:

- 1-Determination of the effect of acidic rain on some biochemical parameters of plant leaves used in this study. These measures (chlorophyll, sorbic acid and relative water content, as well as pH for leaf extract).
2. Determine the degree of sensitivity of species at different pH levels of acidic rain and classify them according to the degree of resistance based on evidence of air pollution (APTI).

MATERIALS AND METHODS:

This study was conducted in Tikrit from at the site of Agriculture college / Tikrit University for a period from 15/9/2017 to 15/2/2018, to determine the resistance of different plant species to the effect of pollutants using industrial rain on seedlings of (*Calistemon viminalis* , *Nerium oleander* , *Olea europaea* L , *Myrtus communis* and *Dodonaea viscosa* L) .

The industrial acidic rain was prepared from a mixture of sulfuric acid and nitric (1Normality) 70 : 30, This mixture was used in addition to distilled water to obtain different levels of pH used in the study (pH2 , pH4 , pH6 , pH7.2) Eight times spray were used in this study (One per week) and 125 ml for each seedling. The spraying process was stopped when some seedlings began to die at high acidic concentration for fear of loss of experimental parameters

VITAL INDICATORS:

A manual for carrying air pollution (APTI) was calculated by estimating

1- leaves Content of ascorbic acid: (mg / g)

Depending on the method followed [13] , several soft leaves were collected from each replication and cleaned thoroughly from any suspended material. Each leaf was cut into small pieces and weighed 1 g of all crushed leaves and crushed with 5 mL of pre-prepared oxyzalic acid until it became a paste and then moved all the contents of the ceramic mortar to the bottles of the centrifuge and at the speed of 4000 cycles / minutes and for ten minutes, was obtained a solution, a clear, took 1 ml and was calibrated withwith a solution of dye 2-6 - Dichlorophenol - indophenol concentrate and continue to add the dye solution to the solution and stop. The point at which the pink color appears and then the amount of dye added in millimeters was calculated by the proportion between the added amount of the dye solution to the solution and the quantity added by the same dye to the pre-prepared ascorbic acid solution at a known concentration. The content of the ascorbic acid was evaluated in each replicate plant species under study.

2-Total chlorophyll content of the leaf : (mg / g)

According to the method described by^[15] The soft leaves were washed with distilled water, then dried and cut into small pieces, weighing 1 g of cut plant tissue and placed in a clean mortar and then washes by acetone concentration of 80% for 15 minutes until the color of the fabric powder was diluted to yellowish white. The filtered green liquid was then separated from the centrifuge fabric at a speed of 2500 cycles / minutes for 3 minutes. A well-known chlorophyll volume was obtained. Using a spectrophotometer, the absorption reading was recorded on the 663 and 645 nm calculated the amount of chlorophyll a and b using the following two equations of mg / g and as follows:

$$\text{Chlorophyll a} = [12.7 (D_{663}) - 2.69 (D_{645})] \times V_{\text{mg}} / 1000 W$$

$$\text{Chlorophyll b} = [22.9 (D_{645}) - 4.68 (D_{663})] \times V_{\text{mg}} / 1000 W$$

whereas:

V_{mg} : The final volume of chlorophyll extracted after separation with the centrifuge

D : Read absorption of chlorophyll extracted

W : Fresh Weight (g)

By collecting the amount of chlorophyll (a + b) total chlorophyll (TCH) is estimated to be by Mg/g (Soft Weight)

$$\text{TCH} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

3-Determination of leaf extract (pH):

Using the method used by ^[19]. The collected leaves were cleaned from any stuck and then washed with the electric mixer and took the weight of 5 g of the washed sample, added 50 ml from distilled water, then mixed using the centrifuge speed of 5000 cycles for 10 mints, after that measured the pH of extract.

4-Determination of relative water content of leaves (RWC)

The fresh weight (FW) was obtained immediately after collection. Then, it was submerged with distilled water for 24 hours. Then dried from the wet by filter paper to obtain the turgid weight (TW), dried on 70 ° C until its weight remained constant and weighed again for dry weight (DW). Finally, the relative water content of the leaves (RWC) was calculated from the equation described by ^[7] expressed as a percentage and as follows:

$$RWC\% = \{(FW-DW) / (TW-DW)\} \times 100$$

FW: Fresh weight

DW: Dry weight

TW: Turgid weight

5- Air pollution tolerance indices calculation:

The above four biomarkers were calculated according to the formula described earlier, ^[8] for the manual of tolerant air pollution (APTI) in the species under study as follows:

$$APTI = \{ A (T+P) R \} / 10$$

whereas :

A : Ascorbic acid (mg / g)

T : Total chlorophyll (mg / g)

P : pH of the leaf extract (pH)

R : Relative water content of leaves (%)

Based on the calculated values of APTI and based on the method followed by ^[11] in their classification of plants according to their degree of tolerance to air pollutants, the plant species were classified into four degrees of tolerance:

- 1- Tolerance : $SD + APTI \text{ Mean} < APTI$
- 2- Relatively tolerant : $SD + APTI \text{ Mean} > APTI > APTI \text{ Mean}$
- 3- moderate tolerances : $APTI \text{ Mean} > APTI > APTI \text{ Mean} - SD$
- 4- sensitive : $APTI \text{ Mean} - SD > APTI$

Where .

APTI : Directory values carry calculated air pollution.

APTI Mean : The arithmetic mean of calculated APTI values for all plant species in the study.

SD : The arithmetic mean of the total deviation of the calculated values of all plant species in the study.

The values of the APTI values for each degree of tolerance are calculated by the arithmetic mean of all calculated APTI values and the total mean deviation of (SD) for all plant species studied.

STATISTICAL ANALYSIS

The experiment layout was a Randomized Complete Block Design (RCBD) Replicated 3 time with 3 samples. data were subjected to two ways analysis of

variance . statistical analyses were performed using SAS statistical software (version 9) data were analyzed by analyses of variance (ANOVA) and treatment means were compared using Duncan's at $p \leq 0.05$

Factor 1: Concentration of acid solution at four levels:

- 1- PH 7.2 (Control) (T1)
- 2- PH 6 (T2)
- 3- PH 4 (T3)
- 4- PH 2 (T4)

Second Factor: The plant has five levels:

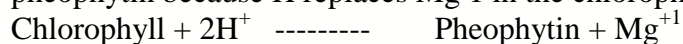
- 1- *Calistemon viminalis* (S1)
- 2- *Nerium oleander* (S2)
- 3- *Olea europaea* L (S3)
- 4- *Myrtus communis* (S4)
- 5- *Dodonaea viscosa* L (S5)

To have (60) experimental unit (5*4*3)

RESULTS AND DISCUSSION :

1 - Total chlorophyll in the leaves (TCH) mg/g

The plant content of chlorophyll helps to increase the effect of photosynthesis and the growth and development of biomass^[19]. Table (1) indicates that there were significant differences between the treatments. The treatment (T1) gave the highest concentration of chlorophyll 1.63 mg, While the treatment (T4) gave lowest concentration 0.39 mg . ,There were not different between (T2 and T3) treatments , the concentration in then reach up (0.74 and 0.65) respectively . The reason may be due to the fact that substances entering the plant through acidic rain are working to convert chlorophyll to pheophytin because H replaces Mg 1 in the chlorophyll molecule by equation



This depends mainly on the concentration of nitrogen in the leaves^[20] . The high concentration of nitrogen in the treatment (T4) from the acidic concentration high pH2 led to the reduction of the concentration of total chlorophyll . This result agree with the^[11] decrease in the concentration of chlorophyll in polluted sites compared to non-polluted sites from(32.76 to 29.63) in the study of the determination of chlorophyll in one of the Eucalyptus species growing in two sites of the city of Quetta of Pakistan, one polluted and the other uncontaminated. This results was within the range reported by^[21] , in their study in Iran on Eucalyptus trees, Cypress and the Eastern Calf . The concentration of chlorophyll (2.98, 1.76 and 1.63). mg/g respectively. and According to^[13] found increase in acidic concentration led to reduce in total chlorophyll.

There was a significant difference in the total amount of chlorophyll in the leaves .The highest concentration in the plant type (S1) was 1.40 mg/g, while the lowest concentration was in the treatment (S4) which reached 0.66 mg/g, while as there not significantly different among S1, S2, and S3 in chlorophyll concentration was 0.79, 0.79 and 0.101 mg/g, respectively. This result agrees with^[14] noted that the leaves of plant species have a difference in their total chlorophyll content. The researcher attributed the cause of variation to the differences in the physiological and morphological of leaves. . This results also agree with the^[15] pointed out that concentration of chlorophyll in the leaves varied from 1.44 to 6.9 mg/g among deference plants .

Treatment (T1 X S4) gave highest concentration 1.89 mg/g, while the lowest concentration of chlorophyll in the treatment (T4 X S5) 0.32 mg/g.

Table (1) Effect of pH , species and interaction between them on total chlorophyll in leaves (mg/g)

Species	S1	S2	S3	S4	S5	Effect of concentration (pH)
T1	1.59 ab	1.73 ab	1.38 b	1.89 a	1.57 ab	1.63 a
T2	0.76 c	0.56 c	0.78 c	1.23 b	0.41 c	0.74 b
T3	1.39 b	0.45 c	0.67 c	0.44 c	0.35 c	0.65 b
T4	0.42 c	0.40 c	0.31 c	0.51 c	0.32 c	0.39 c
Effect of species	1.40 a	0.79 bc	0.79 bc	0.101 ab	0.66 c	

* Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

2- Relative water content of leaves (RWC) %

The relative water content helps the plant maintain physiological balance when exposed to air pollutants^[23]. The decrease in the water content of the leaves, which increases the concentration of acid^[17].

Table (2) shows the treatment (T2) where the highest water content of leaves 80.54%, but there was not significant different with treatment (T3), while the treatment (T4) gave less water content of leaves 74.13% . This is due to the effect of acidity on the cellular wall in terms of permeability, which contributed to the loss of water content. in this regard Silva and^[18] reported that acidity was affected on the leaves of plants and mainly on the skin cells causing damage on the cuticle lyre^[19] notice that water content reduced by increasing acidity.

on the other hand, (S1) plant gave highs value for concentration of relative water content 84.58%, while there were not significantly different among the plant species (S3, S4 and S5). The reason these might be due to differential behavior of species to acidity (according to the plant species and the anatomical composition of the leaf was also expressed in terms of number of holes and thickness of the cuticle layer and the presence of trichomes on the leaves) . The species vary in their water content due to the different pollution conditions surrounding these species, where the plants increase their water content in order to maintain their physiological equilibrium under these conditions, which are usually highly polluted^[20] . As per^[21] , these values were whitin range from 39.25 to 96.17%

In different locations.

As seen in Table (2). A significant increase was observed in relative water content with (T3 x S1) treatment 87.96%. By contrast, relative water content in (T1 x S1) treatment reached 59.90%.

Table (2)) Effect of pH , species and interaction between them on Relative water content (%)

Species →	S1	S2	S3	S4	S5	Effect of concentration (pH)
Concentration of PH ↓						
T1	87.36 a	59.90 g	68.40 ef	78.72 abcd	85.06	75.94 bc
T2	87.38 a	80.00 abcd	74.28 cdef	85.20 a	75.84 bcde	80.54 a
T3	87.96 a	76.67 bcde	79.93 abcd	83.96 abc	64.89 gf	78.68 ab
T4	75.36 bcde	72.32 def	74.04 cdef	71.58 def	77.33 abcde	74.13 c
Effect of species	84.58 a	72.22 c	74.16 c	79.86 bc	75.78 bc	

* Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

2- Ascorbic acid(mg / g) :

Ascorbic acid is an important biochemical indicator for the study of susceptibility of plants to tolerance. Its concentration is directly proportional to these conditions. Its high concentration in plant leaves indicates the plant's tolerance to air pollutants while its low concentration in some plant species indicates constant exposure to air pollutants ^[20]. Table (3) showed significant increase in acid concentration by T4 (0.70) mg/g, while the lowest concentration in T1 0.46 mg/g but it was not significantly different with T2 , 0.50 mg/g. The finding of ^[4] agreed with this, the amount of ascorbic acid increases with the concentration of pollutants as the amount of ascorbic acid (0.29 - 0.47) mg / g. Also, cording ^[22], showed that the concentration of ascorbic acid was higher in the contaminated sites (3 mg / g) than the less polluted sites (2.3 mg / g) in their study comparing the concentration of ascorbic acid in *Syzygium cumini* trees of the selected Asian family in two locations near the roadside (1) km from the first site. As for the effect of the type on the concentration of acid, the highest concentration of acid in the treatment (S5), which did not differ significantly from the treatment (S1), (0.75, 0.73) mg/g, respectively. Were as the lowest concentration was in the treatment (S4) 0.40 mg/g. This result agrees with ^[15].The highest treatment concentration (T4 X S5) 0.98 mg/g, while the lowest concentration (T1 X S4) 0.27 mg/g.

Table (3) Effect of pH , species and interaction between them on Concentration of ascorbic acid (mg / g) in tree leaves .

Species →	S1	S2	S3	S4	S5	Effect of concentration (pH)
Concentration ↓ pH						
T1	0.59 def	0.36 ghi	0.44 hgif	0.27 j	0.67 cde	0.46 c
T2	0.71 bcd	0.37 ghij	0.47 fghi	0.35 ij	0.66 cde	0.51 c
T3	0.80 bc	0.52 efgh	0.58 def	0.52 efg	0.71 bcd	0.62 b
T4	0.83 b	0.56 def	0.70 bcd	0.45 fghi	0.98 a	0.70 a
Effect of species	0.73 a	0.45 b	0.54 b	0.40 c	0.75 a	

* Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

4-The pH of leaf extract:

Table (4). indicates the highest pH value is 6.62 in the T2 treatment, while pH the lowest pH value was in (T4) treatment, which did not differ significantly with the treatments (T3 and T1) In study for ^[19] about effect of industrial acid rain on the different ages of Eucalyptus seedlings and *Milia azedarach*, the pH values decreased in the high acid concentration (pH2) and increased with increasing concentration. The pH values ranged between (5.68 - 6.10). also ⁵¹ pointed out in his study about the effect of acid contaminants on the concentration of pH in trees planted on the sides of the roads where the concentration of pH (between 5.59 - 5.80). (S2) treatment gave 6.40 which did not differ significantly with the treatments (S1 and S5), but (S3) treatment gave the lowest value 6.12, which did not differ significantly with (S4) treatment 6.17 (Tabl, 4). Plants differ in their pH depending on the type of contaminants they are exposed to. The presence of acid pollutants at a given location leads to a decrease in the pH of the plants of that site. It has been found that the pH has decreased in sensitive species for pollution-tolerant species^{20]} . ^[23], reported that pH values varying from 5.2 to 6.4. ^[24], found that pH values in Eucalyptus trees, Kazurina and white berries (5, 5.2 and 6.5), respectively. The interaction between T2 and S2 treatment gave highest value 6.95, by contrast the intraction T3 and S4 gave lowest value 5.80.

Table (4) pH in tree leaves for concentration of pH, types and interactions between them

Species →	S1	S2	S3	S4	S5	Effect of concentration (pH)
Concentration of pH ↓						
T1	6.06 ef	6.27 cdef	6.28 cde	6.33 bcde	6.37 bcde	6.26 b
T2	6.77 ab	6.95 a	6.15 def	6.59 abcd	6.68 abc	6.62 a
T3	6.21 def	6.28 cde	6.13def	5.80 f	6.10 ef	6.10 b
T4	6.33 bcde	6.11 ef	5.92 ef	5.98 ef	6.68 abc	6.10 b
Effect of species	6.34 ab	6.40 a	6.12 c	6.17 bc	6.33 ab	

* Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

5-Air pollution tolerance index (APTI) :

APTI is the optimum method in agricultural engineering to plant areas contaminated by industrial pollution. APTI is used by agronomists to select plant species tolerated for atmospheric pollution^[13]. Based on the calculated APTI values and according to^[11], plant species were classified in this study

1. APTI-tolerant < 9.05 .
 2. Relatively tolerant $7.12 < \text{APTI} < 9.05$.
 3. Medium of Endurance $7.12 < \text{APTI} < 8.13$.
- Sensitive

Table (5) APTI index of tree leaves for pH concentrations, types and interactions between them

Species →	S1	S2	S3	S4	S5	Effect of concentration (pH)
Concentration of pH ↓						
T1	9.20 ± 0.26 ab	6.28 ± 1.0 h	7.18 ± 0.56 fgh	8.09 ± 0.20 cdef	9.04 ± 0.25 abc	7.95 ± 1.24 b
T2	9.27 ± 0.38 ab	8.28 ± 0.40 bcde	7.75 ± 1.1defg	8.79 ± 0.72 abcd	8.05 ± 0.29 cdef	8.43 ± 0.60 a
T3	9.40 ± 0.48 a	8.01 ± 0.09 cdef	8.39 ± 0.40 abcde	8.72 ± 0.21 abcd	6.95 ± 0.21 hg	8.29 ± 0.90 ab
T4	8.10 ± 0.33 cdef	7.60 ± 0.40 efg	7.84 ± 0.65 defg	7.45 ± 0.35 efg	8.37 ± 0.40 abcd	7.87 ± 0.37 b
Effect of species	8.99 ± 0.60 a	7.54 ± 0.88 c	7.79 ± 0.49 bc	8.26 ± 0.62 b	8.10 ± 0.87 b	8.13 ± 0.92

* Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

Table (5) indicates that there were significant differences in the effect of the treatments. (T2) treatment gave the highest value of the index (APTI) 8.43, which did not differ significantly with the treatment (T3), while (T4) treatment gave the lowest value of the index (APTI) 7.87, which did not differ significantly with the treatment (T1). This results agree with ^[19] reported that increase of acid concentration in acidic rain led to increase of (APTI) between 7.81-9.17.

Table (5) shows that the species in the study have different effects on the value of the (APTI). treatment (S1) gave the highest value of the index reached 8.99, while treatment (S2) gave the lowest value 7.54. Whereas, there were not significantly difference among (S3,S4 and S5) 7.79, 8.26, and 8.10, respectively. Therefore, the treatment (S1) is the best plant species in terms of resistance to acid contamination conditions at different concentrations. Whereas, (S2) treatment is the tolerance lowest for acidic pollution conditions. Due to the difference in the sensitivity of the plants to the pollutant content of the leaves, which showed the difference in the content of their leaves of ascorbic acid, total chlorophyll, relative moisture content and concentration of the hydrogen ion when discussing these biochemical indicators separately during the current study. Also because the variation in (APTI) levels led to differences in the pollution^[25]. this results agreed with ^[15] reported that the plants are affected differently by acid contaminants. ^[19] found that the plants vary according to age and species in their susceptibility to tolerance the pollution conditions with acid contaminants. ^[13] reported that variation in the susceptibility of plants to contain air pollutants and their accumulation in their tissues. Table 5. shows the value of the (APTI) with treatment (T3 X S1) was highest value 9.40, while the lowest value of the index (APTI) with treatment (T1 X S2) 6.28.

Recommendations

As per ^[10] classification and within ranges above will be *Calistemon viminalis* and *Myrtus communis Nerium oleander* are trelatively tolerance for pollution. Whereas, *Olea europaea L.* and *Dodonaea viscosa Linn* are partly of pollution tolerances. ^[20] indicated that plants with higher APTI values are tolerant of air pollution. Species that have low APTI values are sensitive to air pollutants. ^[20] that the variation in tolerance of the air pollution differ from one plant to other depending on the ability of the plant to withstand the effect of pollutants without any external damage. The degree of sensitivity in each plant varied according to the levels of treatment of different industrial acidic rain, ranging from (sensitive - tolerant) and according to Acid concentration and plant abilty to pollution resistance.

Table 6: Sensitivity of species to air pollution according to pH and species concentrations as per ^[10] classification.

Species →	S1	S2	S3	S4	S5
Concentration of pH ↓					
T1	T	S	IT	IT	RT
T2	T	RT	IT	RT	IT
T3	T	IT	RT	RT	S
T4	IT	IT	IT	IT	RT
Effect Species	RT	IT	IT	RT	IT

* (T): Pollutant tolerant and (RT): relatively tolerant and IT: moderate tolerances and (S): sensitive to pollution.

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