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Biochemical changes of *Conocarpus erectus* (combretaceae) in response to gas refinery air pollution as an air pollution indicator

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ABSTRACT: Environmental pollution consists of different types of pollutants in air, soil, and water. Due to the fact that plants can respond to environmental pollution, they can be used as bio-indicators for environmental monitoring. Air pollution in areas with oil and gas refineries nearby is an important problem. This study aims to survey the effects of gas refinery air pollutants on *Conocarpus erectus* so that it can be used for air-pollution monitoring. For this purpose, physiological indicators such as proline, protein, and malondealdehyde content as well as Air Pollution Tolerance Index (APTI value) have been used to evaluate these effects. Based on the obtained results, *Conocarpus erectus* in polluted area showed increased pH, protein, proline, malondealdehyde, and ascorbic acid contents while carotenoid quantity, total leaf chlorophyll content, and relative water content were decreased in comparison to the control plants. Therefore, it proves that air pollution affects the plant.

Keywords: Air Pollution Tolerance Index (APTI), ascorbic acid, Malondealdehyde, total leaf chlorophyll.

INTRODUCTION

Environmental pollution consists of different types of pollutants in air, soil, and water, which can be natural, like smoke from geyser that includes carbon dioxide (CO_2), Sulfur dioxide (SO_2) as well as the smoke from forests and meadows fire, etc.; however, most dangerous pollutant materials result from human activities, such as the pollution, generated from electricity power plants, refineries, cars' fuel combustion, etc. (Lakshni et al., 2009). Environmental pollutants affect different biological and non-biological events with harmful results (Abed Esfahani et al., 2013). Due to the fact that any harmful

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effect on plants will be dangerous for other organisms, monitoring plants' health is really important for environmental health surveillance (Ben Rejeb et al., 2014). Air pollution outbreaks can affect plants in different ways, including morphological, physiological, and biochemical changes. Damages in above-ground organs may happen in either a short time or a long period, like chlorosis, but plants are resistant until the injuries become effective and persistent (Ben Rejeb et al., 2014).

With regards to this subject, it is quite useful to survey and recognize particular plant species, which are capable of encountering inappropriate situations and show great sensitivity to environmental pollution, as they can be indicators to determine and then remediate the pollutants, themselves. This process, in which plants are employed to clean and eliminate environmental pollutions, is called phytoremediation (Van Aken, 2008).

Air pollution affects indicator plants, causing some changes in plant parameters such as ascorbic acid content (Agbaire & Esief Arienrhe, 2009), chlorophyll quantity (Lichtenhaler, 1987), leaf extract pH (Agbaire & Esief Arienrhe, 2009), relative water content (Liu and Ding, 2008), proline quantity (Bates et al., 1973), protein content (Lowry et al., 1951), malondealdehyde density (Davey et al., 2005), etc. Plants vary in terms of their sensitivity and response to air pollution. Those that are more sensitive, act as bio-indicators of air pollution (Lakshni et al., 2009). Air Pollution Tolerance Index (APTI) has been used to evaluate the resistance or sensitivity of plant species to air pollution, which is calculated from biochemical factors of plant samples such as total chlorophyll, ascorbic acid, pH, and relative water content (Agbaire & Esief Arienrhe, 2009).

Conocarpus erectus, a member of combretaceae family, is widespread in tropical and subtropical areas, which is found in the form of a shrub, 1.5 to 4 m tall (Bashir et al., 2015). However, it sometimes can grow up to 20 m. The extract from its leaf has been used for skin ulcer treatment, bleeding gums, etc. (Bashir et al., 2015)

The present study aims to study the effects of environmental pollutants, produced by a gas refinery in the southwest province of Khuozestan, Iran, on *Conocarpus erectus*. The results of this study can be used for air pollution monitoring and its outcome in this region.

MATERIALS AND METHODS

The studied area is located near a gas refinery, located in Khuozestan, south west of Iran. The area served as the polluted site and an unpolluted area, nearly 20 Km far from the polluted area with the same ecological properties, was selected as the control. Leaf samples from top of the sprouts were harvested on three replicates from both sites. These samples were immediately transferred on ice and then were stored at -20°C. The following parameters were investigated for these samples. All of the experiments were repeated three times for each biological factor.

The relative leaf water content (RWC) was measured in accordance with the method, described by Liu and Ding (2008), and calculated with the following equation:

$$RWC= [(FW-DW)/ (TW-DW)]\times100$$

FW=fresh weight, DW= dry weight, TW= turgid weight (Liu and Ding, 2008)

Fresh weight was calculated, using 20 fresh leaves, which were weighed after being immersing in water overnight to get TW. Subsequently, the leaves were dried at 70°C overnight and weighed to get the dry weight.

Leaf extract pH was measured based on the Agbaire method (Agbaire, 2009). Five grams of the fresh leaves were homogenized with 10 ml of deionized water, then to be filtered. The pH was then measured.

The total amount of chlorophyll (T) was measured, based on Lichtenhaler (1987) method (Lichtenhaler, 1987). For this purpose, 0.2 gr of fresh leaves were powdered and homogenized with 10 ml of 80% acetone (Merck, Germany). Eventually, the total volume of the extract was adjusted to 15 ml. Following centrifugation at 6000 g for 20 min, the absorbance of supernatants was measured at 470, 646, and 663 nm against 80% acetone as the negative control. The results were calculated based on the following equations:

$Chla\ (\mu g/l)=12.26A_{663}-2.79A_{646}$
$Chlb\ (\mu g/l)=21.50A_{646}-5.10A_{663}$
$ChlTotal\ (\mu g/l) = Chla+ Chlb$

To measure ascorbic acid content (AA), 1 g of fresh leaves was homogenized in 5% metaphosphoric acid (Merck, Germany) solution. The homogenized leaves were centrifuged at 16000 g for 25 min at 4°C and the collected supernatants were gathered, their absorbance, measured at 520 nm (Smirnoff, 2000).

APTI was determined, based on Liu and Ding Method (2008). Its equation is as follows:

$APTI= [A\ (T+P)] + R]/10$

A=Ascorbic acid content ($^{mg}/_{g}$ DW), T= total chlorophyll ($^{mg}/_{g}$ DW), P= pH of leaf extract, and R= relative water content of leaf (%RWC) (Liu and Ding, 2008).

In order to determine the significant differences between polluted and control sites, the data were statistically analyzed, using the statistical software MSTATC.

Proline content of leaves was measured using Bates method (Bates et al., 1973), according to which 0.5 g of fresh leaves was homogenized in 10 ml sulfosalisilic acid 3% (v/w) (Merck, Germany). The homogenized leaves were centrifuged (16000 g, 20 min) and 2 ml of the obtained supernatant was mixed with 2 ml Ninhydrin (Merck, Germany), 2 ml acetic acid (Merck, Germany), and 4 ml Toluen (Merck, Germany) in sequential steps. Their absorbance was finally measured at 520 nm (Bates et al., 1973).

In order to measure the protein content, an extract was prepared as follows: frozen leaves (0.5 g) were homogenized in 10 ml of 0.1 M potassium phosphate buffer (pH 7.4) at 4°C and the homogenate was centrifuged at 15000 rpm for 20 min at 4°C. The supernatant was then stored at -70°C. Extracts were used for protein quantification through measurement at 660 nm, according to Lowry Method with bovine serum albumin as standard (Davey et al., 2005).

To determine malondealdehyde content, one ml of leaf extract was added to the same amount of 0.5% thiobarbituric acid, containing 20% thrichlroacetic acid. After centrifugation, the absorbance of sample was measured at 532 nm (proprietary) and 600 nm (non proprietary) (Van Aken, 2008).

RESULTS AND DISCUSSION

As a result of this study, it was observed that some morphological and biochemical indicators were different in polluted and control sites. Some of these indicators are as follows:

The pH of the leaf extract was increased (from 4.79 to 4.84) in pollutant exposured plants, though this increase was not statistically significant. The result, obtained for relative water content (%RWC), showed that it had decreased significantly from 64.75% in the control site to 61.19% in the polluted region. The Total Chlorophyll Content (TCH) in the control site (1.42 $^{mg}/_{gr}$ FW), related to the polluted site (0.55 $^{mg}/_{gr}$ FW), had significantly decreased (P value = 0.05) and the deal of carotenoid pigment declined from 0.25 ($^{mg}/_{gr}$. FW) to 0.21 ($^{mg}/_{gr}$. FW), though it was not significant. Furthermore, ascorbic acid content varied from 0.56 ($^{mg}/_{gr}$ FW) in the control site to 11.58 ($^{mg}/_{gr}$ FW) in the polluted region. This difference was also significant (P value =

0.01). Moreover, in response to air pollution stress, proline content of the leaves ascended significantly from 20.58 ($^{mg}/_{gr}$ FW) to 76.06 ($^{mg}/_{gr}$ FW) (P value 0.01). Also, the total protein content of leaves was significantly increased from 22.66 ($^{mg}/_{gr}$ FW) to 67.15 ($^{mg}/_{gr}$ FW) (P value 0.01) and finally the amount of malondealdehyde of leaves ascended from 5.49 ($^{mM}/_{g}$ FW) to 8.87 ($^{mM}/_{g}$ FW) in response to air pollution (P value = 0.01; Table 1).

Table 1. Results of measured indicators of *Conocarpus erectus*

R (%)	pH	TCH ($mg.g^{-1}$ FW)	A ($mg.g^{-1}$ FW)	Car ($mg.g^{1}$FW)	Pl ($mg.g^{1}$FW)	Pt ($mg.g^{1}$FW)	M (mM/g.FW)	Area
64.75	4.79	1.42	0.56	0.25	20.58	22.66	5.49	clean
61.19	4.84	0.55 *	11.58 **	0.21	76.06**	67.15**	8.87**	polluted

R: Relative Water Content (RWC); TCH: Total chlorophyll; A: Ascorbic acid content; Car: Carotenoid; Pl: Prolin; Pt: Protein; M: Malondealdehyde; * and ** means significant level of 0.05 and 0.01 respectively

APTI was also calculated to assess sensitivity of the studied plant to air pollution exposure. In this survey the amount of this parameter was 7.85. This plant is presumed to be grouped among sensitive plants, according to the plant division based on the reports of Lakshni et al. (2009).

Table 2. Plants division according to APTI

APTI value	Response
30 to 100	Tolerant
29 to 17	Intermediate
16 to 1	Sensitive
<1	Very sensitive

Plants usually show physiological changes before morphological injuries in leaves against air pollution (Agbaire & Esief Arienrhe, 2009).

Different plants show different sensitivity to air pollutants along with considerable defects in their growth and biological behavior; however, via their defensive mechanisms, they can resist environmental stresses, hence having an increased survival chance (Ben Rejeb et al., 2014).

It was found in this research that the total chlorophyll content was decreased in polluted plant (P< 0.05), thus its carotenoid pigment decreased too.

Chlorosis and decreased chlorophyll is one of the results of pollution stress in plants sensitive to gas pollution, leading to pigment loss in the leaf. For example in Giri survey there was a lack of photosynthetic capacity (Giri et al., 2013).

Moreover, lack of chlorophyll can be due to high degree of chlorophylase activity or increased chlorophyll oxigenase gene expression (Tripathi & Gautam, 2007).

In this survey, the effect of free oxygen radicals on pigments appeared to be effective, because our plant has had its proline and ascorbic acid content increase (Tripathi & Gautam, 2007). Among the pollution effects on plants, loss of cellular lipids and fatty acid peroxidation can be mentioned. In this research, polluted leaf samples have a higher amount of ascorbic acid content (P<0.01). Furthermore, it was observed that the pH of leaf extract of polluted *Conocarpus erectus* was increased, but it was not significant.

Ascorbic acid is a natural antioxidant in plants that can act with oxygen peroxide and preserve carotenoids in response to different stresses (Bates et al., 1973).

During pollution stress, ascorbic acid provides cell membrane stability. Increased ascorbic acid content can be the consequence of oxidative stress. The pH is one of the parameters, involved in APTI calculation, which is the element of ascorbic acid formation from hexose sugars. When the quantity of pH becomes more, the chance of this conversion increases as well; besides low amount of pH has a direct relation with the increase of plants sensitivity to air pollution. Also, total deal of chlorophyll

indicates ascorbic acid formation. The samples' RWC% in this experiment declined in relation with the pollution ($P<0.05$), showing that the plant endures the situation with leaf surface decline. If the amount of water volatilization from leaf surface decreases, plant cannot live normally, because in this situation, plant has lost its force to raise water from root to shoot for photosynthesis; therefore, it cannot transmit the minerals to leaves or cool the leaves. So, preservation of RWC in plants can make them resistant to pollution (Li et al., 2009).

In this assessment, the deal of leaf proline ascended significantly ($P<0.01$), perhaps as a result of one of these reasons: either our plant has prevented proline oxidation to other soluble compounds or in response to stress and absisic acid content increase it may have increased proline biosynthesis (Tripathi & Gautam, 2007).

Total protein content in plants include phytochelates, homo phytochelates, metaloproteins, pathogen related proteins, HSP, and enzymes. Probably increase in the quantity of proteins in stress situations is due to change of gene expression pattern for raising plant endurance (Liu & Ding, 2008). Also, protein synthesis changes to decrease polysomes formation (Lichtenhaler, 1987), and it is said that protein increase can be as a result of plant's ability to absorb NOx gases from them environment, storing them in the form of proteins (Li et al., 2009). Finally, malondealdehyde as an instance of fatty acid in this research has increased in response to stress ($P<0.01$). It seems that there is a relation between lipid peroxidation and proline aggregation in plants' surface with different kinds of stresses. If so, aggregation of proline can play an important role in preventing lipid peroxidation (Hayat et al., 2012).

CONCLUSION

As a result of this study it can be concluded that, *Conocarpus erectus* is a sensitive plant to air pollution. Interestingly, this plant can be used for persistent monitoring of performance of pollution control and reduction plans.

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