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Morphological, physiological and biochemical facets of *Ricinus communis* and *Ficus racemosa* plants grown at three different sites

Bhiolina Bharadwaj[1], Karishma Sahu[1], Neetam Kumari[1], Shalini Sharma[1], Tamanna[1], Reema Mishra[1], Geeta[1], Renu Soni[1*]

[1]*Department of Botany, Gargi college, University of Delhi, New Delhi, 110049*

[*] Corresponding author- Dr. Renu Soni- renu_mundhara@yahoo.co.in

Abstract

In the present study, changes occurring in plants of *Ricinus communis* and *Ficus racemosa* collected from three different polluted sites (Sites 1, 2 and 3) have been investigated. Changes in the physical appearances of leaves, physiological (relative water content) and biochemical analysis (proline content, sugar content and catalase activity) were analyzed. Air Pollution Tolerance Index was calculated after computing pH, ascorbic acid, chlorophyll and relative moisture content. At site 2 and 3, higher APTI values in *F. racemosa* and *R communis* was observed when compared with site 1. Site 1 had low air quality index than site 2 and 3 which led us to infer that both the plants display higher degree of tolerance against pollution and thus seems to be a better choice for phytoremediation. From these analyses, it can also be established that pollution plays a substantial role in influencing the morphological, physiological and biochemical features of plants.

Keywords: Air Pollution Tolerance Index, Air Quality Index, Ascorbic acid content, Chlorophyll content, Pollution, Relative moisture content

Abbreviations: APTI: Air Pollution Tolerance
Index RWC: Relative Water Content

Introduction

In metropolitan areas, rapid industrialization and automobile traffic precedes to pollution of air by adding toxic gases into the atmosphere (Seyyednejad et al. 2011). Other factors like combustion of natural gas, firewood, or deposition of waste products also leads to air pollution (Falusi et al. 2016). These anthropogenic activities release pollutants such as oxides of sulphur (SO_x), oxides of nitrogen (NO_x), carbon monoxide (CO), hydrogen sulfide (H_2S), and particulate matter, toxic metals, organic molecules, and radioactive isotopes into the atmosphere and pollute the environment (Ali 1993). Plants are presumably as susceptible to pollution as humans. Plants are immobile and are exposed to air pollutants constantly and each plant responds distinctly. These pollutants directly or indirectly affect the plants negatively, can reach the food chain and affect the whole ecosystem (Seyyednejad et al. 2011; Benguzzi et al. 2013). Studies have also mentioned that the response (resistance or susceptibility) of plants to air pollutants depends upon the interaction of plants with its surrounding environment (Verma and Singh 2006; Shyam et al. 2008). Plants undergo various morphological, physiological as well as biochemical alterations in the existence of air pollutants (Gheorghe and Ion 2011; Falusi et al. 2016; Uka et al. 2017). Plants response to air pollution can be studied by computing the Air Pollution Tolerance Index (APTI) which is based on four parameters namely pH, ascorbic acid, chlorophyll and relative moisture content (Singh and Rao 1983). APTI is a species reliant index which indicates how a plant encounters different stresses existing in the environment. Plant species with higher APTI values are more resistant to air pollution when contrasted with the ones with lower values. So it is important to study APTI of plant species before carrying out the plantation in polluted or roadside areas. It is a well-established method that can be used to screen sensitive along with tolerant plants which can be further used as indicator plants (Rai and Panda 2015).

We have directed our analysis towards studying the consequence of pollution on morphological, physiological and biochemical characteristics of *R. communis* (commonly called as castor bean) belonging to family Euphorbiaceae and *F. racemosa* (commonly named cluster fig) belonging to family Moraceae collected from three different areas having varied pollution levels.

Material and Methods

Sample collection

Plant materials (leaf and stem samples) of *R. communis* and *F. racemosa* were used for the present investigation. Samples were procured from August 2018 to February 2019 from three locations (i.e. Gargi College as site 1, Faridabad as site 2, Okhla as site 3) at regular intervals. Locations were selected based on increasing Air Quality Index (AQI) and difference in their environmental conditions (Table 1). The collected samples were kept in refrigerator at 4°C until needed.

Table 1. A comparative description of different sites used for sample collection

Site codes	Geographic coordinates	Average AQI (ppm) Data adapted from (https://cpcb.nic.in/)	Site description
Site 1 (Control)	28.5506° N 77.2148° E	83 – 223	Botanical garden of Gargi College (artificial environment with regular supply of water and care)
Site 2	28.4089° N 77.3178° E	131 - 237	Roadside industrial Faridabad (polluted environment, vehicular pollution being significant)
Site 3	28.5358° N 77.2764° E	125 - 276	Landfill area in Okhla (highly polluted area with huge amount of waste disposal and vehicular pollution)

Morphological analysis

Colour, texture, and physical appearance of the leaves were analysed via naked eyes.

Physiological and biochemical analysis

Proline content was assessed by ninhydrin method given by Bates et al. (1973). and sugar content was calculated following protocol given by Hodge (1962). For calculating the catalase activity, protocol given by Euler (1927) was followed.

APTI (Air Pollution Tolerance Index)

APTI was calculated by estimating total chlorophyll, pH, relative water content (RWC) and ascorbic acid content in the collected leaf samples. Chlorophyll content was analysed by destructive method with the methodology described by Hiscox and Israelstam (1979), concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using equations given by (Arnon 1949) which are mentioned below

$$\text{Chlorophyll 'a'} = \frac{12.7\,(A663) - 2.69\,(A645)}{1000 \times w \times a} \times V$$

$$\text{Chlorophyll 'b'} = \frac{22.9\,(A645) - 4.68\,(A663)}{1000 \times w \times a} \times V$$

$$\text{Total Chlorophyll} = \frac{20.2\,(A645) + 8.02\,(A663)}{1000 \times w \times a} \times V$$

Where,

A = Absorbance at specific wavelength (645nm, 653nm)

V = Final volume of the chlorophyll extract (ml)

W = Fresh weight of the sample (g)

The pH was measured with a digital pH meter (calibrated with distilled water at pH 7). The electrode of pH meter was immersed in the leaf extracts and readings were noted.

For the estimation of RWC, 10 small equally sized discs of fresh leaf samples were weighed (fresh weight), submerged in water for four hours and weighed again (turgid weight). Then they were oven dried and finally weighed again (dry weight). Following formula was applied for the calculation of the % of RWC:

RWC (%) = (Fresh weight - Dry weight / Turgid weight - Dry weight) × 100

Ascorbic acid content was estimated by using 2, 6- dichlorophenol indophenol (DCPIP) dye following the method suggested by Sadasivam and Manickam (1996).

By combining these parameters, APTI was calculated by following formula given by Singh et al. (1991):

$$\text{APTI} = [A\,(T+P) + R]/10$$

where,

A = Ascorbic acid content (mg/g fresh weight)

T = Total chlorophyll content (mg/g fresh weight)

P = pH of leaf extract

R = RWC of leaf tissue (%)

RESULT AND DISCUSSION

AQI tells us the quality of air on a daily basis. Higher AQI indicates greater level of air pollution. Six categories of AQI are good, satisfactory, moderately polluted, poor, very poor, and severe (Sharma et al. 2019). For collection of plant samples of *R. communis* and *F. racemosa* three sites were chosen based on AQI. Site 1 had AQI ranging from 83 – 223 (satisfactory to moderately polluted), site 2 had 131 – 237 (moderately polluted) and site 3 had 125 – 276 (moderately polluted-poor) (Sharma et al. 2019). A comparative description of different sites is given in table 1. Different morphological, physiological and biochemical studies investigated are discussed in the following sections.

Morphological studies

The most sensitive part of plant which gets affected by pollutants existing in the air is the leaf. The vast majority of the physiological processes occurring in plants are associated to the leaf. Leaves are used as an indicator of pollution as they absorb as well as filter the air pollutants (Willekens et al. 1994; Freer-Smith et al. 2004; Waugh et al. 2006; Leghari and Zaidi 2013). Air pollutants can have directly or indirectly damaging effects on plant's photosynthesis, stomatal functioning, longevity, reproduction and can change plant and environment relationships (Gheorghe and Ion 2011).

Fig.1: Morphological variations in leaves of *Ricinus communis* collected from various sites

Fig.2: Morphological variations in leaves of *Ficus racemosa* collected from various sites

For morphological analysis, colour and texture of the leaves were observed via naked eyes. As depicted in the Fig.1 and Fig.2, leaves collected from site 1 had shiny green appearance (in both *sp.*) and were healthy and soft textured. Leaves collected from site 3 were rough and had a brown appearance suggesting their unhealthy nature.

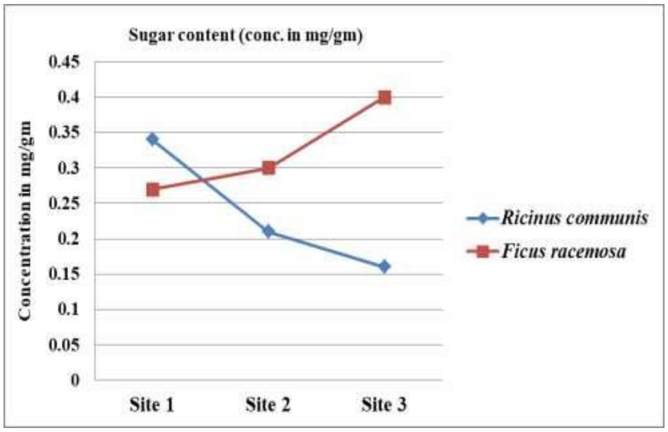
From the above study, it can be inferred that pollution considerably affects the morphological characteristics and properties of *R. communis and F. racemosa*. Leaves of both the plants collected from site 3 (polluted) displayed leaf necrosis while the leaf surface of plants collected from site 1 (control) did not exhibit such features. In polluted populations, fading of dark green colour and chlorosis of leaves with apertures on both ventral and dorsal surfaces were detected in comparison to non-polluted populations. Gheorghe and Ion (2011) have also reported that air pollutants can cause chlorosis as well necrosis in leaves.

Physiological and biochemical analysis

Plants when exposed to fluctuating environment undergo physiological and biochemical changes. These changes help them to cope up the extreme environmental conditions (Escobedo et al. 2008). Different biochemical characteristics like sugar, proline, chlorophyll, ascorbic acid, enzymes like catalase, superoxide dismutase gets altered by pollution (Saxena and Kulshrestha 2016). In the present communication, we have investigated total chlorophyll, ascorbic acid, relative water content and pH to detect the tolerance levels of the two plant species. All these parameters are significant in calculating APTI.

Effect of pollution on sugar content

Sugar is significant constituent and root source of energy for plants, synthesized during photosynthesis and broken-down during respiration (Tiwari et al. 2006). Soluble sugars play a protective part of osmoprotectant and cryoprotectant against different stresses and are important for maintaining the plant structure (Saxena and Kulshrestha 2016; Finkelstein and Gibson 2001). The amount of soluble sugars indicates the physiological state of a plant and ultimately governs the tolerance of a plant to air pollution (Seyyednejad et al. 2011). Accumulation or reduction of soluble sugar content is also dependent on the sensitivity of the plants to air pollution (Prado et al. 2000).



Graph 1: Sugar content analysis of *Ricinus communis* and *Ficus racemosa* plants

Present study has shown a great deal of deviation in the levels of sugar in both the plant species. As seen in graph 1, *R. communis* shows a maximum sugar content value of 0.34 mg/gm at site 1 and the values decreased with increase in air pollution i.e. 0.21mg/gm and 0.16 mg/gm at site 2 and site 3 respectively which can be ascribed to the increased rate of respiration. Wilkinson and Wilkinson (2010) also reported similar results and inferred that as the leaf surfaces are covered with air pollutants which lead to shading effect. This shading effect might lead to blocking of stomata which in turn reduces carbon dioxide (CO_2) availability in leaves and inhibits carbon fixation. Other studies also reported decline in sugar content with increase in pollution and attributed it to photosynthetic inhibition or increased energy requisite (Rai et al. 2013; Agbaire 2016). Kameswaran et al. (2019) have suggested that the polluted gases in the environment also lead to reduction of soluble sugars in leaves.

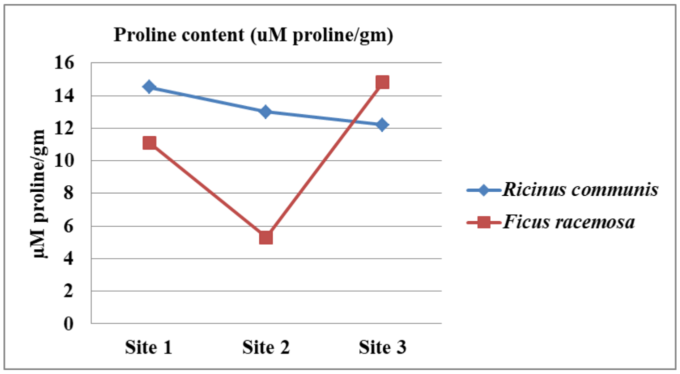
On the contrary, *F. racemosa* showed rise in sugar content with increase in pollution and displayed 0.27 mg/gm, 0.30 mg/gm and maximum value of 0.40 mg/gm at site 1, site 2 and site 3 respectively (Graph 1). It has been reported that in response to diverse environmental stresses such as air pollution, accumulation of sugars take place in various parts of the plants (Prado et al. 2000) and plays a defensive role against stress (Finkelstein and Gibson 2001). Investigations have also shown that more resistant plant species show accumulation of soluble sugar as observed *in F. racemosa* (Kameli and Losel. 1993; Keller and Ludlow 1993). Seyyednejad et al. (2009) also reported increase in sugar content with increase in pollution.

Proline content in response to air pollution

Proline is an amino acid that works as an osmolyte and has been reported to accumulate in plants under stress and prevents degradation of proteins (Khedhar amd Gadge 2014). Different reports have stated higher accretion of proline in stress resistant plants (Liang et al. 2013; Sabri et al. 2014). Enhanced proline levels in plant can be used as an indicator to monitor vehicular pollution (Patidar et al. 2016).

In *F. racemosa*, higher levels of proline with regard to increased pollution was detected with values ranging from 11.1 μM proline/gm at site

1 to the highest proline content of 14.8 µM proline/gm at site 3 i.e. in the polluted population (Graph 2). Increase in the proline concentration in the plants grown at the polluted site had been observed by different researchers (Seyyednejad et al. 2009; Agbaire 2016; Sanaeirad et al. 2017). Air pollutants deplete the cellular lipids and induce the peroxidation of polyunsaturated fatty acids, ultimately leading to accumulation of proline (Tiwari et al. 2006).



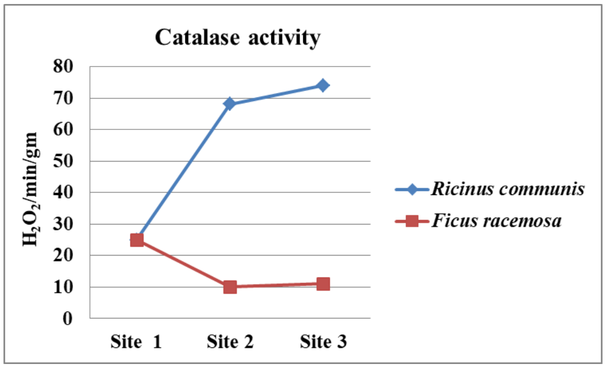
Graph 2: Proline content analysis of *Ricinus communis* and *Ficus racemosa* plants

No significant change in proline content was seen in *Ricinus* (Graph 2). This could be because of the fact that during sampling time, a significant amount of proline has already been used up in the growth of reproductive structures in *Ricinus* (Bora 2014).

Air pollution and catalase activity

Air pollutants have the capacity to enter into the plant cells through stomata and results in enhanced level of reactive oxygen species (ROS) which damages the plant cells (Hippeli and Elstner 1996; Shannigrahi et al. 2004; Uka et al. 2017). In response, plant cells have developed different antioxidant mechanisms to reduce this effect and catalase activity is one such response exhibited by plants (Kangasjärvi et al. 1994; Pell et al. 1997). Catalases, the most common tetrameric enzymes in living organisms that breaks hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) and are responsible for ROS detoxification under stressed conditions (Chelikani et al. 2004). Present investigation in *Ricinus* showed enhanced catalase activity with increment in pollution levels with 25, 68 and 74 unit H_2O_2/min/gm at site 1, site 2 and site 3 respectively (Graph 3). Other researches have also stated enhanced catalase activity with increased rate of pollution (Wilhelm Filho et al. 2001; Martinez-Dominguez et al. 2008). Ghorbanli et al. (2007) also reported increase in catalase activity in *Nerium oleander* and *Robinia pseudoacacia* plants collected from polluted site. Thus catalase plays a significant role in scavenging the oxide radicals and ameliorates negative effects caused by air pollution.

Graph 3: Estimation of catalase activity in *Ricinus communis* and *Ficus racemosa* plants



Decrease in catalase activity was reported in *F. racemosa* (Graph 3) which might be due to the damaging effects caused by the pollutants. Study by Castillo (1987) also reported less catalase activity in leaves of *Ficus* plant grown at polluted area.

APTI analysis

Abiotic and biotic stress conditions are detrimental to plants and affect their growth, metabolic activities and yield (Pandey et al. 2017; Ahmad et al. 2019). APTI is the capacity of plants to manage the air pollution. Susceptible as well as tolerant plants can be evaluated by calculating APTI. Four parameters namely total chlorophyll content, ascorbic acid value, pH value and RWC were measured to compute APTI.

Chlorophyll is a vital photoreceptor in plants and is indicative of plant metabolism as well as growth. It differs from species to species and is significantly affected by environmental stresses. Any change in chlorophyll content can considerably affect the morphological and physiological status of plants. In *F. racemosa*, a reduction in the total chlorophyll levels was detected at site 3. Decline in chlorophyll content could be due to deactivation of photosynthesis (Agbaire and Esiefarienhre 2009), increased chlorophyll degradation or damaged chloroplasts (Rai and Panda 2015). The decline in chlorophyll levels could also be due to deposition of particulate matter on the surface of leaves which ultimately disturbs the photosynthetic activity of plants (Kalyani and Charya Singara 1995; Karmakar et al. 2016). It also indicates that in plants air pollutants majorly attack the chloroplasts (Tripathi and Gautam 2007). Other researchers have also observed decline in chlorophyll levels with enhanced air pollution (Mir et al. 2008; Jyothi and Jaya 2010).

In *R. communis*, maximum total chlorophyll content was observed at site 3. This could be firstly because polluted soil where *Ricinus* was growing contains high level of nitrate (data not shown) which when absorbed by the roots and transported to leaves can stimulate chlorophyll synthesis (reported in their studies by Bondada and Syvertsen 2003; Wu et al. 2006) and secondly due to tolerant nature of *Ricinus* plants as reported by other researchers (Patel and Hina 2011; Jabeen 2019). Thus, measurement of chlorophyll is an important tool used to examine the effect of air pollution on the plants.

Ascorbic acid acts as a crucial antioxidant and reductant present in different cellular compartments and confers stress tolerance to plants. Reducing activity of ascorbic acid has been shown to be directly proportional to its concentration (Agbaire and Esiefarienrhe 2009). Reports have suggested that plants with better defense mechanisms show an increase in leaf ascorbic acid (Cheng et al. 2007; Singare and Talpade 2013). Its synthesis and reducing activity is dependent on pH, being less at lower pH and vice versa. High pH enhances the conversion of hexose sugar to ascorbic acid and confers tolerance (Ogagaoghene 2017). The content of ascorbic acid in different plant samples collected was estimated by titration method. Standardization of DCPIP was done by standard ascorbic acid. Calculations were performed by taking the standard values i.e. 1ml of DCPIP is equivalent to the 1.5625×10^{-4} g ascorbic acid (Ugbe et al. 2017).

In *F. racemosa*, the highest ascorbic acid content of 0.5 mg/gm was observed in leaves collected from site-3 with pH 6.7 and lowest of 0.09 mg/gm at site-1 (control) with pH 6.1. Thus, with the increase in pH, the ascorbic acid content also increased. At site 2 though the pH was high (7.7) but the ascorbic acid content was low, this could be due to experimental or handling error. In *R. communis*, as no significant change was observed in pH, thus ascorbic acid content also showed almost similar values.

RWC is an important indicator of plant's water balance status. It is the absolute quantity of water that plant needs to attain full saturation. Plants with higher RWC exhibit better drought tolerance. In plants, higher RWC helps in preserving the physiological stability of plants under stressed conditions. Significant variations were found in the RWC of the leaves of plants growing at different sites. Maximum RWC of 60.60% and 87.5% was recorded in *R. communis* and *F. racemosa* plants collected from site 2 and site 3 respectively. This could be because of their stress tolerant nature. In order to maintain the protoplasmic permeability required to survive the adverse effects of air pollution, an increase in RWC is required (Jyothi and Jaya 2010). Hence higher RWC in the polluted population was observed.

pH of a solution is the measurement of the concentration of hydrogen ions. Solutions having lower hydrogen ions have a higher pH and vice versa. It is also an important parameter to be studied for calculating APTI. Acidic pollutants lead to decline in the pH of leaves and decrease was more evident in the sensitive plants species when compared with the tolerant ones (Bi et al. 2015). Plants having lower pH were found to be more sensitive (Kumar and Nandini 2013). In our study, both *F. racemosa* and *R. communis* plant leaves exhibited pH values higher than 6 suggesting their tolerant nature.

Table 2: APTI analysis in ***Ricinus communis***

Area	Ascorbic acid content (mg/gm)	Chlorophyll content (mg/gm FW)	pH	Relative moisture content (%)	APTI
Site 1	2.656	0.725	6.4	37.5	5.642
Site 2	2.3436	0.3364	6.2	57.57	7.288
Site 3	1.75	0.917	6.3	60.60	7.322

Table 3: APTI analysis in ***Ficus racemosa***

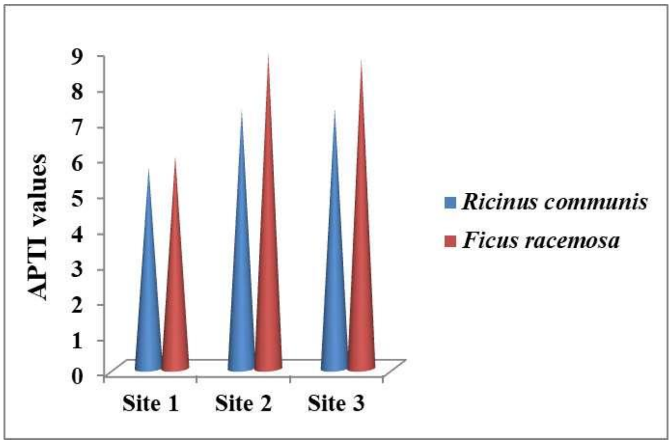
Area	Ascorbic acid content (mg/gm)	Chlorophyll content (mg/gm FW)	pH	Relative moisture content (%)	APTI
Site 1	0.093	0.402	6.1	59.01	5.9618
Site 2	0.156	0.491	7.7	87.5	8.877
Site 3	0.5	0.254	6.7	83.67	8.714

APTI is a species dependent, plant measurement which states the native capability of plants to overcome environmental stress associated with pollution (Spedding and Thomas 1973; Jyothi and Jaya 2010). The current investigation reveals that both the plant species have APTI value < 9 (Table 2 and 3 and graph 4). The value of APTI increased with the increase in air pollution in both the plants specifying that the population of *F. racemosa* and *R. communis* collected from more polluted site are tolerant to pollution and can be employed as a way to mitigate pollution (Jyothi and Jaya 2010). The results suggest that species in the polluted environment improve their capability to encounter stress and become tolerant to air pollution (Begum and Harikrishna 2010). This might be due to continuous exposure of the plants to emanations of gaseous and particulate matter from industries as well as emissions from vehicular exhaust (Lohe et al. 2015; Gholami et al. 2016). Therefore APTI estimation gives an authentic method to identify plants tolerant to air pollution and which further can be used as indicator species for landscaping near roadside for trapping air pollutants.

Conclusions

Plant species having higher APTI values

can be recommended to be planted along roadside to control vehicular pollution (Mahecha et al. 2013). On the basis of biochemical and physiological parameters studied it was found that *F. racemosa* was more tolerant to air pollution as compared to *R. communis*. APTI values for both the plants were calculated and it came out to be approximately 8 and 7 at the polluted sites as compared to the control site. It was observed that both the studied plant species have higher APTI at sites with higher AQI, hence these species can be used for development of green belt in the city around road sides as well as near the industrial zones as they can tolerate air pollution. The current investigation clearly demonstrates that air pollutants significantly affect the biochemical and physiological aspects of plant. The present study also recommends that both *F. racemosa* and *R. communis* can be used for controlling air borne pollution in urban areas.



Graph 4: Air Pollution Tolerance Index in *Ricinus communis* and *Ficus racemosa*

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