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Original Research Article

Biochemical changes in leaves of important trees exposed to pollution and exhausts at roadsides of Jabalpur, India

Keywords: Pollution, Resistant trees, Automobile exhausts, *Pongamia pinnata, Dalbergia sissoo, Azadirachta indica, Ficus religiosa, Cassia siamea*

Running title: ***Automobile pollution on trees***

Abstract

Leaves from five resistant trees viz. *Pongamia pinnata, Dalbergia sissoo, Azadirachta indica, Ficus religiosa* and *Cassia siamea* were assayed for a series of pollution sensitive chemical and biochemical parameters like total carbohydrate, nitrogen, calcium, sodium, potassium, magnesium, phosphorus, ascorbic acid and phenol. The assays were done for tender leaves collected from least polluted areas (control set) as well as for samples collected from very busy road sides at Jabalpur, M.P. where the plants were being exposed to almost continuous automotive exhausts and fumes. For few parameters like sodium and potassium, there found minimum difference in values between the experimental and control samples, while for the others there found significant differences, might have been caused due to damage-for-pollution or protection-to-pollution-resistance as reflected by high or low indices in values. The findings have provided a index-guideline for many important individual elements and compounds in resistant trees and clearly demonstrated the probable sequences of end reactions of many a physiological events when the specified trees get exposed to automotive air pollution in likely environment that of Jabalpur. The findings are significant in relating plantation schedules in many crowded and polluted cities in tropical South Asian countries.

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Introduction

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Jabalpur is one of the polluted cities in M.P. of India, where perhaps no study has been conducted so far on the effect of general air pollution, dust, more specifically of automobile exhaust on the composition of road side rhizospheric soil. This study was carried out to assay the effect of road side dust and auto exhaust gases on vegetation.

Air pollution is a major part of the overall atmospheric pollution and the motor vehicle emissions usually constitute the most significant source of ultra-fine particles in an urban environment (1). Important chemical pollutants emitted by land vehicles are carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and total suspended particles (TSP). The smoke is a mixture of particles and gaseous chemicals of varying physical and chemical properties.

- **Exhaust emission pollutants**

The six major air pollutants prevailing in any polluted city are carbon monoxide (CO), hydrocarbon (HC), oxides of nitrogen (NO_x), sulfur dioxide (SO_2), particulate matter (PM-10) and lead (Pb). Out of these, carbon monoxide, hydrocarbons and oxides of nitrogen are pollutants prevalent in automobile exhaust. Ozone (O_3) is produced through a complex series of chemical reactants that result from pollutants (HC and NO_X). Mixing in the atmosphere in the presence of sunlight, ozone concentrations are highest in urban centers and downwind of urban.

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On road vehicles like vehicle miles of travel (VMT) (Like buses, trucks) auto ownership, personal trips, and fractions of single occupant vehicles (SOV) have been significantly contributed to air pollution. Today's growing population and increasing urbanization has resulted in sudden increase in the number of vehicles, which discharge lots of pollutants; more than 60 % air pollution in city is caused by automobiles only (3). Trees / rhizospheric soil were considered for investigation of effect of auto exhaust pollutant.

Jabalpur is an important city of Madhya Pradesh, India, situated almost at centre of India, latitude being N+23°10'00'', longitude E+79°56'60.00'' and altitude 393 meter from the sea level; population 1,117,200 (2001 Census), per capita income being almost Rs. 30,000/-.

- **The Mechanics of Exhaust Emissions**

In ideal combustion, oxygen and fuel (HC) are combusted and produce byproduct emissions of carbon dioxide (CO_2) and water (H_2O). Air, however, contains nitrogen (N_2) among other chemicals, and combustion is always incomplete, producing byproducts of HC, CO, Oxygen (O_2), Carbon dioxide (CO_2), water (H_2O) and NOx.. The air to fuel ratio is an important factor in determining the quantity of pollutants produced by combustion. Generally, rich fuel mixtures produce high amounts of CO and HC because combustion is incomplete. Lean fuel mixtures (high a/f ratios) will typically produce higher amounts of NOx (especially during very hot, lean conditions) and lower amount of CO and HC because combustion is more complete. When considering vehicle activity, high power demand (sharp accelerations, heavy loads etc.) creates a rich fuel mixture resulting in elevated CO and HC emission rates while NOx generally decreases. At high speeds with low accelerations rates, a lean fuel mixture develops which increases NOx emissions rates (Fig. 1).

The four, six and eight cylinder engines used in motor vehicles make certain demands on gasoline which are mostly determined by the engine construction. In the first place, the gas must form a highly homogeneous and highly flammable mixture with air it needs for combustion. This mixture of fuel and air must also have a low change of igniting spontaneously. That is, it must have a high octane rating. If the octane rating is low, the fuel and air mixture can ignite prematurely and disturb the smooth running of the engine and cause knocking. Until recently, the octane rating of gasoline was normally raised by the addition of lead in concentration of between 0.3 and 0.6 grams per liter, despite the unfavorable effects (4) it had on the environment (Fig. 2).

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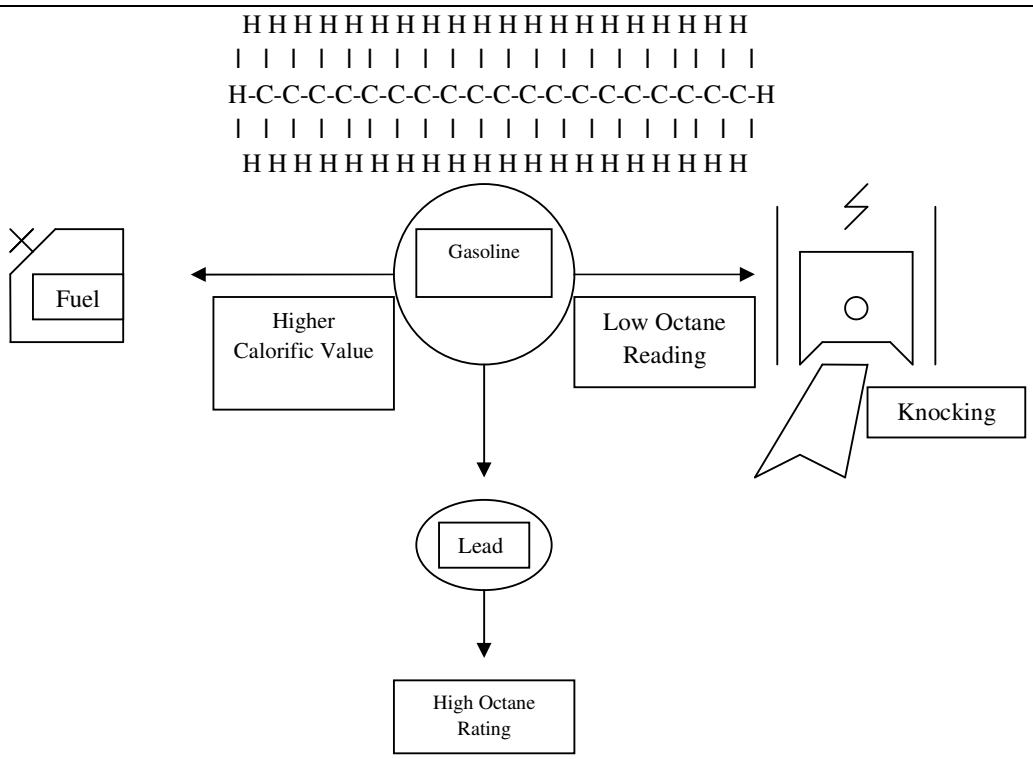

Fig. 1. Properties of Gasoline.

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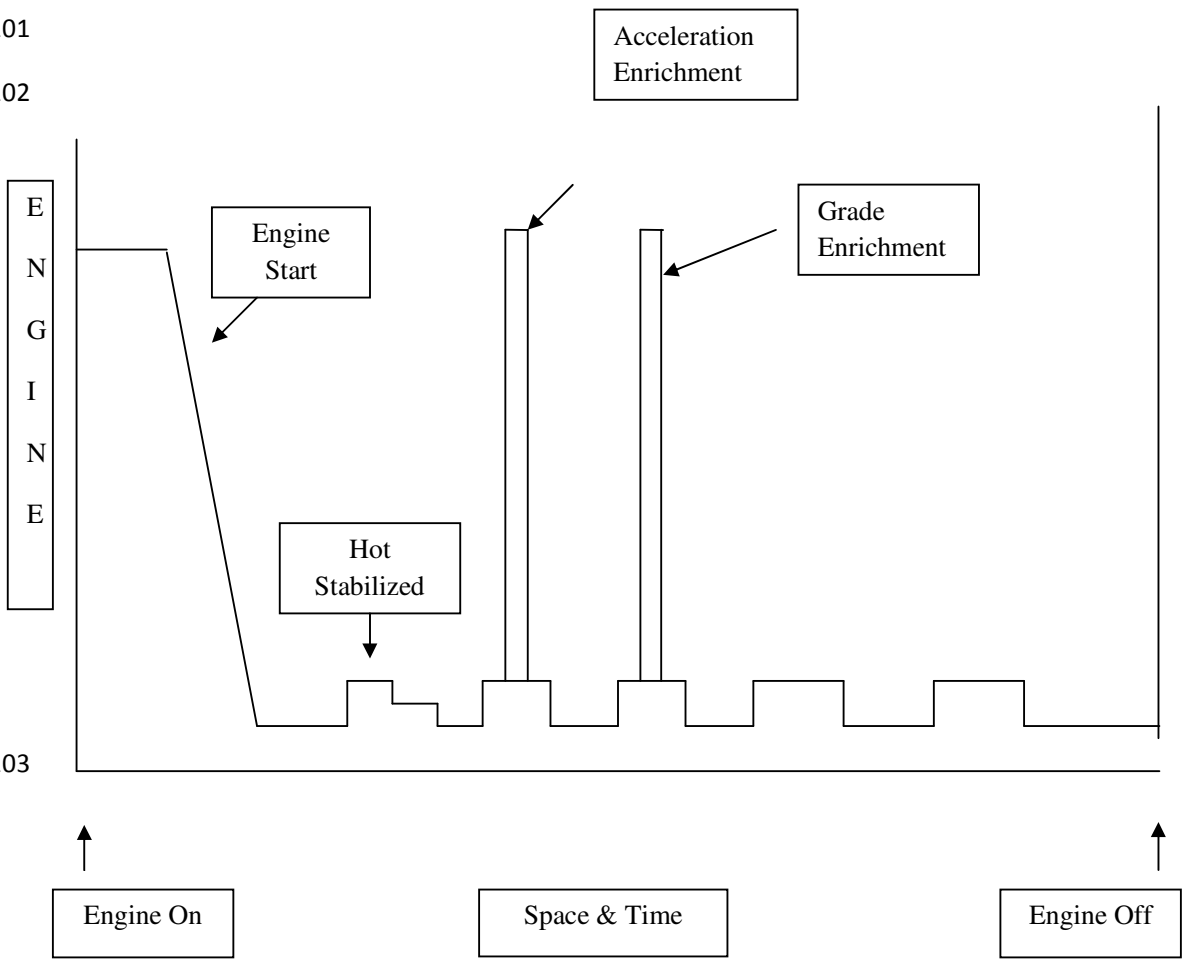

Fig. 2. CO emissions for a hypothetical vehicle trip.

- **Diesel**

Diesel or Diesel fuel is a specific fractional distillate of fuel oil (mostly petroleum) that is used as fuel in a diesel engine invented by German engineer Rudolf Diesel. The term typically refers to fuel that has been processed from petroleum, but increasingly, alternatives such as bio-diesel or biomass to liquid (BTL) or gas to liquid (GTL) diesel that are not derived from petroleum are being developed. Petroleum derived diesel is composed of about 75% saturated hydrocarbons (primarily paraffins including *n*, *iso*, and cycloparaffins), and 25% aromatic hydrocarbons (including naphthalenes and alkylbenzenes).

Diesel is produced from petroleum, and is sometimes called petro-diesel (or, less seriously, dino-diesel) when there is a need to distinguish it from diesel obtained from other sources. As a hydrocarbon mixture, it is obtained in the fractional distillation of crude oil

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between 250 °C and 350 °C at atmospheric pressure. Petro Diesel is considered to be a fuel oil and is about 18% denser than gasoline. Diesel typically weighs about 7.1 pounds (lb) per US gallon (gal) (850 grams per liter (g/l)), whereas gasoline weighs about 6.0 lb per US gal (720 g/l), or about 15% less. When burnt diesel typically releases about 147,000 British thermal units (BTU) per US gal (40.9 megajoules (MJ) per liter), whereas gasoline releases 125,000 BTUs per US gal (34.8 MJ/l), also about 15% less. Diesel is generally simpler to refine than gasoline and often costs less (although price fluctuations often mean that the inverse is true; for example, the cost of diesel traditionally rises during colder months as demand for heating oil, which is refined much the same way, rises).

Diesel fuel, however, often contains higher quantities of sulfur. In Europe, emission standards and preferential taxation have both forced oil refineries to dramatically reduce the level of sulfur in diesel fuels. In contrast, the United States has long had "dirtier" diesel, although more stringent emission standards have been adopted with the transition to ultra-low sulfur diesel (ULSD) occurring in 2006 (see also diesel exhaust). U.S. diesel fuel typically also has a lower cetane number (a measure of ignition quality) than European diesel, resulting in worse cold weather performance and some increase in emissions.

High levels of sulfur in diesel are harmful for the environment. It prevents the use of catalytic diesel particulate filters to control diesel particulate emissions, as well as more advanced technologies, such as nitrogen oxide (NOx) adsorbers (still under development), to reduce emissions. However, lowering sulfur also reduces the lubricity of the fuel, meaning that additives must be put into the fuel to help lubricate engines. Bio-diesel is an effective lubricity additive. Diesel contains approximately 18% more energy per unit of volume than gasoline, which, along with the greater efficiency of diesel engines, contributes to fuel economy (Distance traveled per volume of fuel consumed).

- **Petrol**

Petrol is extracted from crude oil and there are many derivatives which are extracted from crude oil. Diesel, Kerosene, LPG, CNG, White kerosene (aviation fuel), petrol, ethanol, methanol to name a few. Recently the crude oil rates touched an all time record of 148$ a barrel. The rates have come down ever since but the rates are likely to go up in future. The OPEC (Organization of the Petroleum Exporting Countries) estimates that there is enough crude oil in the world to last another 80 years. The normal unleaded petrol you use has an octane rating of 87 or higher. The other octane petrol available in India are 91, 93, 97 RON octane respectively. Petrol is made up of aliphatic and aromatic hydrocarbons along with toluene and benzene. In this, toluene is an explosive and it is used as TNT (trinitrotoluene) in

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explosives. That's why petrol is highly inflammable and can explode when it comes in contact with fire. Benzene and Toluene are added to increase the octane rating.

The higher the octane rating the greater the resistance of petrol to explosion, which is why all high performance engines need high octane fuels. High performance engines have high compression ratios compared to normal engines because they produce more power and are high revving. The higher the compression ratio the more efficient the engine is. An example we have is the use of only speed 97-octane petrol for the R1, Hayabusa and racecars in India else their engines wouldn't perform optimally due to loss of power.

- **Difference between diesel and petrol**

Both Diesel and petrol are liquid fractions with different composition of hydrocarbons and difference in boiling range. Petrol, more volatile and less abundant fraction has wider applications than the less volatile larger fraction, diesel.. Further fractionated petrol is used in aero plane with higher cost. The loss on storing, transport etc too is more for the petrol than diesel.

Diesel is a cetane fuel and works in a compression ignition engine where the fuel air mixture is compressed to such an extent that it burns. Cetane fuels burn in gradual layers and produce sustained power. Running of a heavy duty truck, water pump, or tractor are the ideal jobs for a diesel engine. A Petrol or Octane engine requires an external spark to ignite the fuel air mixture which is known as a spark ignition engine .Octane fuels burn or explode with the entire fuel air mixture catching fire almost simultaneously. This provides petrol engines with much better pick up for other similar characteristics. However the designing of a diesel engine is much more complex. Cost of producing both fuels is comparable but lower taxes on diesel make it cheaper in India.

- **Emission from internal combustion of engine**

The contaminants discharged to the atmosphere from fuel burning, incineration of refuse and internal combustion engine are as follows:

- Particulate matter (Soot and fly ash)
- Sulfur dioxide
- Natural gas
- Exhausts from engine include (1) CO, (2) Oxides of nitrogen, (3) Hydrocarbons, (4) Oxygenates of hydrocarbons, (5) lead compounds, (6) carbon particles, (7) motor oil and (8) Non- volatile reaction products formed from motor oil in the

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combustion zone. High molecular weight of olefines and carbonyl compounds are produced in the reactions.

The amount of above products depends upon the compositions of fuel method of firing and other factors (5) (Tables - I, II and III).

Table I. Emission factors for uncontrolled automobile exhaust.

Type of emission	Emission Pounds per 1000 Vehicle – Miles	Pounds per Vehicle day
Particulates	0.8	0.022
Organic acids (acetic)	0.3	0.007
Oxides of sulfur (SO_2)	0.6	0.016
Oxides of Nitrogen (NOx)	8.5	0.202
Hydrocarbons (CxHv)	12.5	0.363
Carbon monoxide (CO)	165.0	4.160
Aldehydes (HCHO)	0.3	0.007

Table II. Emission factors for diesel engines (Pounds per 1000 gallons of diesel fuel).

Type of Emission	Emission factor
Particulates	110
Organic acids (acetic)	31
Oxides of sulfur (SO_2)	40
Oxides of nitrogen (NOx)	222
Hydrocarbons (CxHv)	136
Carbon monoxide (CO)	60
Aldehydes (HCHO)	10

The following study was conducted for physicochemical assessment of the effect of pollution, with special reference to automotive exhausts at busy road-sides at Jabalpur, on the-root associated or rhizospheric soil of some pollution-resistant trees and on the leaves of some pollution-resistant trees.

Materials and methods

Rhizospheric or root associated soils and fresh leaves were collected by summer time (April - May 2009, atmospheric temperature 40 - 42°C) with the help of crow-bar and khurpi from the busy roadside of Jabalpur, namely Sadar Bazar, Indira Market and Raddi Chowk.

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One tree was sampled from each of the area, and the control trees were sampled from TFRI nursery where it is expected that the effect of pollution is minimal. The tree species were carefully chosen, which were found to be resistant at Korba, Talcher and Raigarh industrial areas as described by our laboratory group earlier. The methodology followed in this paper was as described by Sadasivam and Manickam (6). The resistant tree species selected for this exhaustive study were *Pongamia pinnata*, *Dalbergia sissoo*, *Azadirachta indica*, *Ficus religiosa*, *Cassia siamea.*

The number of the passing vehicles was counted physically in between 10.30 am to 11.30 am when the frequency of fueled vehicles is found maximum on road as the first business hour. The counting was done once in a week consecutively for 3 weeks during April - May 2009 at the 3 points at Jabalpur as described above which indicates the magnitude of height of automobile pollution at the said roadside points (Table III), ~1247 diesel / petrol fueled vehicles per hour, means ~21 vehicles per minute.

Table III. Frequency of fueled vehicles per hour between 10.30 – 11.30 am at Jabalpur (data presented as mean of 3 hour counts in consecutive 3 weeks).

Car	Auto rickshaw	Truck	Bike	Bus	Pick-up van	Total
266	97	8	815	14	47	1247

Rhizospheric soil analysis

A. pH

Procedure

20 g of soil was taken in a 100 ml beaker and was added 50 ml of distilled water; it was stirred well. Using buffer solution of pH-7.0, 4.0 and 9.2 standardized the instrument. Readings of the samples were then measured.

B. Bulk Density

Calculation

1. Initial weight of the soil was taken
2. Final and sun dried weight of soil was taken subtract the initial weight and final weight of soil and calculate the water absorbed in the soil sample, whereas final weight of the soil samples was to be known as volume of concentration soil samples.

Bulk Density = Weight of concentrate soil samples / 147cc (Volume)

C. Electrical conductivity

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Calculation

Took 20 g soil in a 100 ml beaker added 50ml. distilled water kept for 1 hour; took readings by electrical conductivity meter at room temperature.

EC = m. mhos / cm

O.1N KCl – 7.459 grams KCl in / liter distill water

$$1N = \frac{\text{Eqwt x 100}}{\text{\% purity x Specific gravity}}$$

1N = Eg. Wp / liter

D. Cation Exchange Capacity

Reagents required

1. Ammonium acetate – 1 N 250 gm ammonium acetate in 3.250ml of distilled water.
2. 0.1 N H_2SO_4 - 2.77 ml concentrate H_2SO_4 in 1 liter distilled water.
3. Mixed indicator - 0.5 gm of bromocresol green + 0.1 gm methyl red in 100 ml of ethanol (95%).
4. N a_2CO_3 (0.1 N) - 1.325 gm of N a_2CO_3 in 250ml distilled water.
5. 0.01 N AgNO3 – 1.69 gm AgNO3 in 100ml distilled water.
6. Ammonium chloride - NH_4Cl (1 N) - 26.75 gm NH4Cl in 500ml distilled water. Adjust the pH 7 of the solution by NH_4OH.
7. Ammonium Oxalate (2%) -2 gm Ammonium oxalate in 100 ml distilled water.
8. Ammonium Acetate (1 N) - 250 gm Ammonium acetate in 3250 ml distilled water.

Calculation

m eq exchange per 100 gm = Titration value x N x 100 / sample weight in gm.

N = Normality of H_2SO_4

% C.E.C. (m.eq) =1x for 10 gm soil

% C.E.C. (m.eq) =2x for 5 gm soil

Method: Taken 10 gm soil sample in 500 ml beaker, add 100ml Ammonium acetate solution, stir well, keep it overnight at room temperature then filter through a Whatman no. 44 filter paper, leach with 1 N Ammonium acetate solution till the 500ml leachate is collected.

1. Did washing till the filtrate does not give any test for Ca , NH_4 and chloride, Take 5 ml filtrate, add 2 drops of each solution NH4OH, NH_4Cl, Ammonium oxalate till it is free from white ppt. (i.e. free from Ca).

2. Washed the residue soil of filter paper with alcohol, take the small quantity of filtrate and added the Nessler's reagent (Ranbaxy) till it is free from red ppt. (free from NH_4).

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Determination of CEC

Procedure: Took the residue with filter paper in round bottom flask, add 2-3 gm MgO and 200ml distilled water. Then took 10ml boric acid in conical flask, absorb NH_3 in the boric acid; tested with red litmus paper, carried out distillation till red litmus paper remained as it is titrated with 0.1N H2SO $_{4.}$

E. Organic matter

Reagents required

(a) 1N $K_2Cr_2O_7$

49 g of $K_2Cr_2O_7$ was taken and was dissolved in 1000 ml of distilled water

(b) Diphenylamine

0.5 g Diphenylamine was taken in a 100 ml volumetric flask; into this was added 30 ml of concentrated H_2SO_4 and added of distilled water to make up the volume to 100 ml.

(c) 0.5 N FAS

196.07 g of Ferrous Ammonium sulfate and 15 ml of concentrated H_2SO_4 were taken in 1000 ml of volumetric flask and the volume was made up it to the mark using distilled water.

Procedure: 1g of soil sample was taken in a 500 ml conical flask and added 10 ml of 1N $K_2Cr_2O_{7.}$ To this added 20 ml of concentrated H_2SO_4; this solution was left for 10 min. In this mixed solution 200 ml of distilled water, 5ml of orthophosphoric acid and a pinch of sodium fluoride (NaF) were added; added 3 drops of diphenylamine and the solution was titrated with 0.5 N FAS until the color changes from blackish green; the reading of point was noted.

Calculation formula

Percentage of organic carbon =

$$\left[10 - \text{Titration value} \times \frac{\text{volume of } K_2Cr_2O_7 \text{ taken}}{\text{Blank reading}} \right] \times \frac{0.003 \times 100}{\text{weight of soil}}$$

Percentage of organic matter = Percentage of organic carbon x 1.724

F. Available Nitrogen (N)

Reagents required

(a) 0.32% $KMnO_4$

(b) 2.5% NaOH

(c) Boric Acid

(d) 0.1 N H_2SO_4

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Procedure: 10 g of soil sample was in a round bottle flask, then added 100 ml of 2.5% NaOH and 100 ml of 0.32% $KMnO_4$. This solution was warmed on a burner for about 25 min. The round bottom flask was connected with a condenser and was put a conical flask having 10 ml of boric acid at the exit point of condensation. The condensed water was checked every minute. When the condenser water stopped the color change of red litmus paper then the boiling was stopped and the boric acid was titrated against 0.1N NH_2SO_4 until color changes blue to red. The reading of end point of burette was noted.

Calculation

$$N\% = T - B \times N \text{ of } H_2SO_4 \times \frac{1.4}{\text{Weight of soil}}$$

Taken 10gm of soil + 20ml distilled water + 100ml 0.32% $KMNO_4$ + 100ml of 2.5% NaOH. Carried out distillation absorb in 10 ml. Boric Acid. Titrate with 0.1N H_2SO_4.

Reagents required

1. 0.32% $KMNO_4$ – 3.2gm. $KMNO_4$ in 1 liter distilled water.
2. 2.5% NaOH – 25gm. NaOH in 1 liter distilled water.
3. Boric acid – 20gm. Boric Acid in 500ml. distilled water + 2.5 ml. mixed indicator.
4. N/10 (0.1N H_2SO_4) – 2.77ml. concentrate H_2SO_4 in 1 liter distilled water mixture solution add drop by drop NaOH to adjust pH4.5.
5. Mixed indicator - Mixed indicator is prepared by dissolving 0.5 gm. Of bromo cresol green and 0.1 gm. Methyl red in 100 ml. of 95% of ethanol.

   NaoH adjust pH (4.5)

   N/10 H_2SO_4 – 2.77 ml./ liter (0.1 N H_2SO_4) minimum 180 kg./ha.

Calculation

% of N in Soil = (T-B) x N x 1.4/ weight of soil

T- Sample Titration ml. Standard acid.

B- Blank Titration Volume.

S- Sample weight

(T-B) X 313.6 Content factor

G. Available Potassium (K)

Reagent required

Ammonium Acetate Solution (100 ppm)

250 gm of ammonium acetate was taken and was dissolved in 3.250 liter of distilled water.

Calculation

$$K\% = kg / ha = \underline{G.R \times FPR \times 2.5 \times \text{volume of Ammonium Acetate}}$$

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Weight of soil

H. Available Phosphorous (PO_4)

Reagents required

(A) 4N HCL -

33.3 ml of concentrated HCl was dissolved in a 100 ml volumetric flask.

(B) 2 - 4 dinitrophenol (0.25%) - 250 mg was taken a in 100 ml volumetric flask and the volume was made to 100 ml with distilled water.

(C) 0.5 M $NaHCO_3$ – 42 g of $NaHCO_3$ was taken a in 100 ml volumetric flask and the volume was made to 100 ml with distilled water.

(D) Ammonium molybdate (1.5%) – 15 g Ammonium molybdate + 300 ml distilled water; the solution was warmed, to that added 291 ml concentrated HCl and the volume was made up to 1000ml with distilled water.

(E) Stanous Chloride [$SnCl_2.2H_2O$] (0.5%) - 500 mg $SnCl_22H_2O$ + 1.25 ml concentrated HCl; volume was made up to 66 ml with distilled water.

Procedure (Blue color method)

2.5 g of soil sample was mixed with 50 ml $NaHCO_3$ with a pinch of charcoal; this was shaken for 1 hour and was then filtered. 5ml of the above filtrate was taken in 50 ml volumetric flask. 2-3 drops of 2-4 Dinitrophenol indicator was added (solution became yellow), added 4N HCl or 4N NH_4OH drop by drop to adjust the pH. The solution became colorless, added 10 ml of ammonium molybdate + 0.5% of $SnCl_2.H_2O$; the volume was made up to 50 ml with distilled water. The color density is normally constant for 4-20 minutes; the readings were taken photometrically after 5 minutes at 660 nm wavelength on UV-visible spectrophotometer with the use of filter paper number 6.

I. Exchangeable Potassium (K)

Method: Took 5 gm soil sample in 250 ml volumetric flask to which added less than 100 ml. of ammonium acetate keep it overnight then filtered and makeup the volume to 100 ml. with ammonium acetate.

Stock Solution: 'K' 0.0558 gm. K_2SO_4 dissolved in 250 ml of ammonium acetate.

Calculation

GR = ppm/ FPR

'K' m. eq./ 100gm. = GR x FPR x Volume / Wt. of Soil x 390

[GR – Graph Readings; FPR – Flame Photometer Reading; PPM – Parts Per Million]

J. Exchangeable Sodium (Na)

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Method - Taken 5 gm soil in 250 ml conical flask, add 100ml ammonium acetate. Kept it overnight; filtrated is used for the analysis.

Na and k and the residue are used for the analysis of CEC.

Reagent

1. Na_2CO_3 – 105.99 gm molecular weight

Atomic weight of Na = 46

As, 46 = 105.99 gm

Therefore, 1 gm = $\frac{105.99}{46}$

Therefore, 100 gm = $\frac{105.99 \times 100}{46}$ = 230.41

230.41 gm = 10^6 (ppm) ml of water

As, 10^6 (ppm) ml of water dissolved (100 ppm) = 230.41 gm

Therefore, 1 ml = $\frac{230.41}{10^6}$

Therefore, 10^3 ml = $\frac{230.41 \times 10^3}{10^6}$ = 0.2304

Therefore, 0.2304 gm Na_2CO_3 dissolved in 1 lit i.e. 100 ppm Na.

Stock solution of 100 ppm of sodium -

Took 0.0575 gm Na_2CO_3 in 250 ml volumetric flask and made the volume with ammonium acetate.

Calculation

GR = $\frac{PPM}{FPR}$

Na in m.eq /100 gm. = $\frac{FPR \times GR \times Volume}{Weight\ of\ soil \times 230}$

K. Exchangeable Calcium (Ca)

Procedure: 5 ml of soil extract was added to 15-20 ml of distilled water + 10 drops of NaCN + 10 drops of NH_2OHHCI + 1-2 drops KFe $(CN)_2$; yellow color was obtained; then added NaOHto it. The solution became colorless; added little Murexide solution, reddish pink color obtained; titrated the solution with 0.01N EDTA at the end point violet or purple color was obtained.

L. Exchangeable Calcium (Ca) + Magnesium (Mg)

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Procedure: 5 ml of extract was added to 10-20 ml of distilled water + 10-12 drops of NaCN + 10 drops of NH_2OHHCl + 1-2 drops of $K_4Fe\ (CN)_6$ solution become yellow. The solution was decolorized by buffer added 1or 2 drop of E.B.T; color became light gray. The solution was titrated with E.D.T.A. At end point blue color was obtained; the end point of burette was noted.

Foliar analysis

A. Total Nitrogen

Kjeldal method for total nitrogen

1. Plant sample taken - 0.250 g
2. Potassium sulfate (K_2SO_4) - 4.2 g
3. Selenium power - 0.4 g
4. Copper sulfate - 0.4 g
5. Concentrated H_2SO_4 - 10 ml

Added 1+2+3+4+5 $\longrightarrow$ Heated in burner digest.

A green, later on colorless supernatant liquid appeared, cooled it.

Made the volume in 50 ml volumetric flask.

Took 10 ml extract + 10 ml NaOH (40% NaOH).

Absorbed in 10 ml of boric acid.

Titrated against 0.1 N H_2SO_4

N% = Reading x 3.248

B. H_2O_2 digestion method for assay of K, Ca, Mg, Na and P

Reagents required

1. Concentrated H_2SO_4
2. 10% Sodium Silicate
3. 10% NaOH Solution
4. H_2O_2
5. Nessler`s Reagent.

Digestion procedure: Took 0.1 g of plant sample and added 2 ml of concentrated H_2SO_4 and digested it for 2 hours. Black color appeared then added 0.5 ml H_2O_2; again kept the solution on hot plate, then added H_2O_2 drop wise till the solution became colorless. Then filtered the solution in a 100 ml volumetric flask; made up the volume with distilled water up to the mark.

Procedure: Took 5 ml of digested solution in a 50 ml volumetric flask and add 2.5 ml 10% sodium silicate solution and 2.5 ml 10% NaOH. Then made the volume to 50 ml. Took 5 ml

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of this solution in a 10 ml volumetric flask and then added 0.5 ml Nessler's reagent and 4.5 ml distilled water. Make up the volume to the mark with distilled water. Read optical density / absorbance, after 5 minutes at 525 nm with UV / Visible Spectrophotometer.

Reagents required

Concentrated HNO_3

Ternary Mixture

Concentrated HCl.

Ternary Mixture: Took 50 ml of concentrated HCl and added 5 ml of concentrated H_2SO_4 and 20 ml of $HClO_4$ (Perchloric Acid). The ratio of this reagent was kept at 10:1:4.

Procedure: Took 0.5 g. of leaf sample and added 5 ml of concentrated HNO_3. Kept the mixture for digestion up to 1 hour, till the solution becomes colorless. Added 5 ml of ternary mixture, again kept the solution on hot plate till the solution becomes colorless, cooled it and added 5 ml of concentrated HCl and filtered the solution in a 100 ml volumetric flask and made up the volume to the mark with distilled water.

Calcium and Magnesium (Ca & Mg)

Reagents required

1. Buffer solution: Took 33.75 g NH_4Cl in 100 ml of distilled water and added 285 ml of concentrated NH_3 solution and made the volume to 1 liter with distilled water.
2. E.D.T.A. (0.01 N): 2 mg of EDTA in one liter of distilled water.
3. Standard (0.01N) $CaCO_3$: Took 0.5 g of $CaCO_3$ and added 2.5 ml Conc. HCl, made the volume to 1 liter.
4. 1% KCN or NaCN: 1 g in 100 ml of water.
5. 5% $NH_2OH.HCl$: 5g-ammonium Hydroxylamine Hydrochloride in 100 ml of distilled water.
6. 4% $K_4Fe\ (CN)_6$: 4g $K_4Fe\ (CN)_6$ in 100 ml of distilled water.
7. E.B.T. (Eriochrome Black T indicator): 0.2g of E.B.T. Powder added to 50 ml methanol and 2 - 3 drops of triethyl amine.
8. NaOH (10%): 10 g of NaOH in 100 ml of distilled water.
9. Murexide (Ammonium Purpurate): 0.5 Murexide powder mixed with 100 g of K_2S0_4.

B.1. Calcium (By Titration method)

Procedure: Took 5 ml of the digested sample and added 15-20 ml of distilled water. To this added 10 drops of NaCN, 2-3 ml of $NH_2OH.HCl$ and 2-3 drops of $K_4Fe\ (CN)_6$. Light yellow color was obtained. Decolorized this solution with NaOH and added very small quantity of murexide indicator, reddish pink color was obtained. Titrated this solution with E.D.T.A. The

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end point violet or purple color is obtained.

B.2. Magnesium (By Titration method)

Took 5 ml of the digested sample and added 15-20 ml of distilled water. To this added 10 drops of NaCN, 2-3 ml of $NH_2OH.HCl$ and 2-3 drops of $K_4Fe(CN)_6$. Light yellow color was obtained. Decolorized this solution with buffer solution and added 2-3 drops of EBT indicator, light gray color is obtained. Titrated this solution with E.D.T.A. The end point light blue color was obtained.

B.3 and B.4. Sodium and Potassium

Principle: Measurement of light emitted by Na^+ K^+ when these elements are excited in a flame.

Procedure: Followed the instruction provided by the manufacturer and the sample was free of solids. Prepared a calibration curve; diluted the sample until the Na^+ content is lower than 5 mg.

B.5. Phosphorus

Yellow color method

Reagent:

Solution A: Ammonium molybdate

25 gm ammonium molybdate +400 ml of distil water

Solution B: Ammonium metavandate

1.25gm Ammonium metavandate +300ml boiled water +250ml con HNO_3

Vandomolebdate: - solution A + Solution B and dilute in 1 liter

Method: From the digested solution take 5 ml extract in 50ml volumetric flask add 10 ml vando-molybdate keep it for half an hour and make up the volume by distilled water used filter blue 470 nm wavelength.

To digest the solution, took 0.5 gm sample and 5 ml of HNO_3, kept it on hot plate; heated till the solution become colorless, add 5ml ternary mixture again keep it on hot plate till the solution became colorless, made it cool, added 5 ml concentrated HCL, filtered it and made up the volume to 100 ml with distilled water.

Calculation: ppm = SPR x Total Volume x Volume made / Extract taken x wt of sample

$\% P = ppm \times 100 / 10^6$, where SPR = Spectrophotometer reading

C. Ascorbic acid

Volumetric method: Ascorbic acid otherwise known as vitamin C is an anti-scorbutic. It is present in gooseberry bitter gourd etc. in high amount generally it is present in all fresh

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vegetable and fruits. It is a water soluble and heat labile vitamin. The method described below is easy rapid and large number of sample can be analyzed in a short time.

Principle: Ascorbic acid reduces the 2, 6-dichiorophenol indophenol dye to a colorless leuco-base. The ascorbic acid gets oxidized to dehydro-ascorbic acid. Though the dye is a blue colored compound, the end point is the appearance of pink color. The dye is pink colored in acid medium. Oxalic acid is used as the titrating medium.

Materials: 1.Oxalic acid

2. Dye solution: Weighed 0.021g sodium bicarbonate in to a small volume of distilled water.

3. Stock standard solution: Dissolved 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution in a standard flask.

4. Working standard: Diluted 10 ml of the stock solution to 100 ml with 4% oxalic acid. The concentration of working standard was 100 µg/ml.

Procedure: 1. Pipette out 5 ml of the working standard solution in to a 100 ml conical flask.

2. Added 10 ml of 4% oxalic acid and titrate against the dye end point is the appearance of pink color with persists for a few minutes.

3. Extracted the sample in 4% oxalic acid and make up to a known volume (100 ml) and centrifuged.

4. Pipette out 5ml of this supernatant add 10ml of 4% oxalic acid and titrated against the dye.

Calculation: 0.5 / V1 ml x V2 / 5 ml x 100 ml / wt. of the sample x 100

D. Chlorophyll

Chlorophylls are the essential component for photosynthesis and occur in chloroplast as green pigment in all photosynthetic plant tissue. They are bound loosely to proteins but are readily extracted in organic solvents Such as acetone or ether. Chemically, each chlorophyll molecule contains a porphyrin (tetrapyrole) nucleus with a chelated magnesium atom at the centre and a long chain hydrocarbon (phytyl) side chain attached through a carboxylic acid group. There are at least five types of chlorophyll in plant. Chlorophyll a and b occur in higher plant. Ferns and mosses chlorophyll c, d and e are only found in algae in certain bacteria.

Principle: Chlorophyll is extracted in 80% acetone and the absorption at 663nm and 645nm are read in a spectrophotometer using absorption coefficient the amount of chlorophyll is calculated.

Material: Diluted analytical grade acetone to 80% acetone

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Procedure: 1.Weighed 1 gm of finely cut and well mixed representative sample of leaf or fruit tissue in to a clean mortar.

2. Grinded the tissue to a fine pulp with the addition of 20 ml of 80% acetone.

3. Centrifuged (5,000 rpm for 5 min) and transferred the supernatant to a 100 ml volumetric flask.

4. Ground the residue with 20 ml of 80% acetone centrifuge and transferred the supernatant to the some volumetric flask.

5. Repeated this procedure until the residues became colorless. Washed the mortar and pestle thoroughly with 80% acetone and collect the clear washing in the volumetric flask.

6. Made up the volume up to 100 ml with 80% acetone.

7. Read the absorption of the sodium at 645, 663 and 652 nm against the solvent (80% acetone as blank).

Calculation: Calculated the amount of chlorophyll present in the extract mg chlorophyll per gm tissue using the following equation.

mg chlorophyll a/gm tissue =12.7 (A663) – 2.69 (A645) x V/1000 x W

mg chlorophyll b/gm tissue =22.9 (A645) – 4.68 (A663) x V/1000 x W and

mg total chlorophyll/ gm tissue = 20.2 (A645) + 8.02 (A663) x V / 1000 x W

Where, A = absorbance at specific wavelengths,

V = final volume of chlorophyll extract in 80% acetone,

W = fresh weight of tissue extracted.

E. Phenol

Phenol is the aromatic compounds with hydroxyl group, are widespread in plank kingdom. They occur in all part of the plants. Phenols are said to offer resistance to disease and pests in plant grain containing high amount of poly-phenol are resistant to bird attack. Phenol include an array of compound like tannins, flavonoids etc. Total phenol estimation can be carried out with the Folin Ciolcateatu reagent.

Principle: Phenol reacts with phosphor-molybdic acid in Folin Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue).

Material: 80% Ethanol

Folin Ciolcateatu reagent

Na_2CO_3 20%

Standard (100 mg Catechol in 100 ml water)

Diluted 10 times for a working standard

Procedure:

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1. Weighed exactly 0.5 to 1.0g of the sample and grinded it in mortar and pestle in 10 times volume of 80% alcohol.
2. Centrifuged the homogenate at 10,000 rpm for 20 min.; saved the supernatant; re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants.
3. Evaporated the supernatants to dryness.
4. Dissolved the residue in 5 ml distilled water.
5. Pipette out different aliquots (0.2 to 2 ml) in to test tubes.
6. Made up the volume in each test tube to 3 ml with water.
7. Added 0.5 ml of Folin - Ciocalteau reagent.
8. After 3 min, added 2 ml of 20% NA_2CO_3 solution to each tube.
9. Mixed thoroughly and placed the tubes in boiling water for exactly 1 min, cooled down and measured the absorbance at 650 nm against a reagent blank.
10. Prepared a standard curve using different concentration of Catechol.

Calculation: Phenol mg / 100 gm = $\frac{\text{GR value x abs x 100}}{\text{Weight of plant sample}}$

Where GR = Graph reading taken from the standard graph of phenol.

F. Carbohydrate

Principle: Carbohydrate is first hydrolyzed in to simple sugars using dilute glucose is dehydrated to hydroxyl-methyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm.

Material: 2.5 N HCL, anthrone reagent, dissolved 200 mg anthrone in 100 ml of ice, made it cold. 95% H_2SO_4 prepared fresh before use.

Standard glucose: Stock: Dissolved 100 mg in 100 ml water working stand and 10 ml of stock diluted to 100 ml with distilled water. Stored refrigerated after adding a few drops of toluene.

Procedure: 1. Weighed 100 mg of the sample into a boiling tube.

2. Hydrolyzed by keeping it is a boiling water bath for three hours with 5 ml of 2.5 N HCL and cool to room temp.

3. Neutralized it with solid sodium carbonate until the effervescence ceases.

4. Made up the volume to 100 ml and centrifuge.

5. Collected the supernatant and took 0.5 and 1 ml aliquots for analysis.

6. Prepared like standards by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard; 0 served as blank.

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7. Made up the volume to 1 ml in the entire tubes including the sample tubes by adding distilled water.

8. Then added 4 ml of anthrone reagent.

9. Heated for 8 min in a boiling water bath.

10. Cooled rapidly and read the green to dark green colors at 630 nm.

11. Drew a standard graph by plotting concentration if the standard on the x-axis versus absorbance on the y-axis.

12. From the graph calculated the amount of carbohydrate present in the sample tube.

Calculation: Amount of Carbohydrate present in 100 mg of the sample

= mg of glucose x 100 / volume of test sample

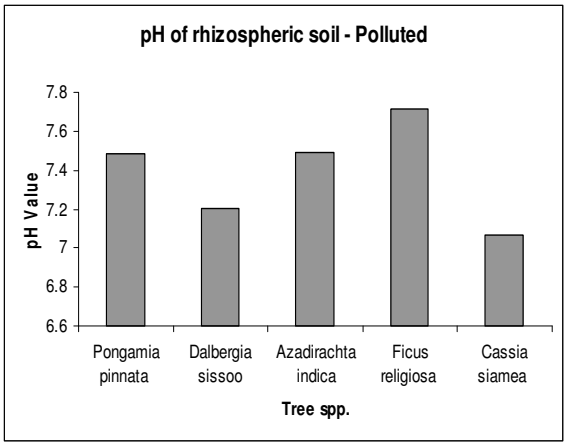
Results

The results of different parameters for rhizospheric soil analysis are shown below from Figures 3a to 14b, where 'Control' and 'Polluted' have been assigned as C and P, Bulk Density as 'BD', Electrical Conductivity as 'EC', Organic Carbon as 'OC', Available Nitrogen as 'Av. N.', Available Potassium as 'Av. K', Available Phosphorus as 'Av. P.', Exchangeable Potassium as 'Ex. K', Exchangeable Sodium as 'Ex. Na' and Exchangeable Calcium as 'Ex. Ca' in the figures. Data presented here are mean of 3 replicates for each parameter. The results of different parameters for foliar analysis are shown below from Tables IVa to XVb. The resistant tree species selected for this exhaustive study were *Pongamia pinnata*, *Dalbergia sissoo*, *Azadirachta indica*, *Ficus religiosa*, *Cassia siamea*.

UNDER PEER REVIEW

643

644 **Fig. 3a. pH of rhizospheric soil (P).** **Fig. 3b. pH of rhizospheric soil (C).**



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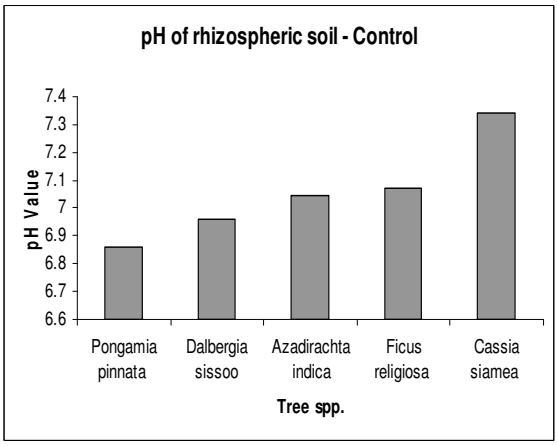

646

647 **Fig. 4a. BD of rhizospheric soil (P).** **Fig. 4b. BD of rhizospheric soil (C).**

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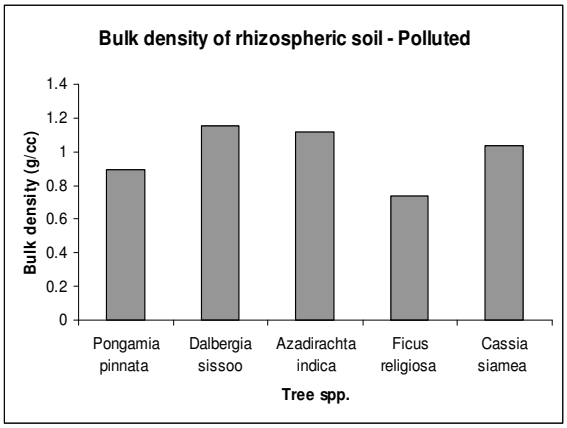

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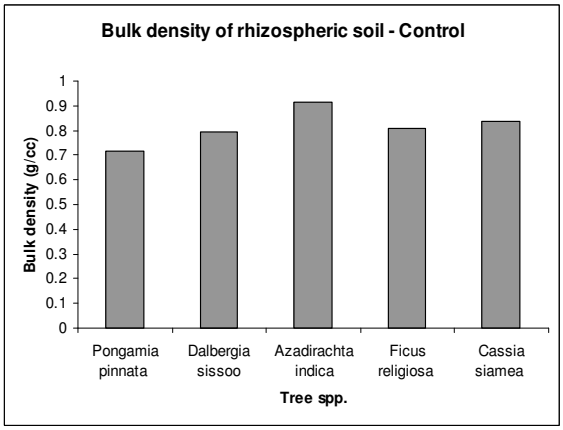

649

650 **Fig. 5a. EC of rhizospheric soil (P).** **Fig. 5b. EC of rhizospheric soil (C).**

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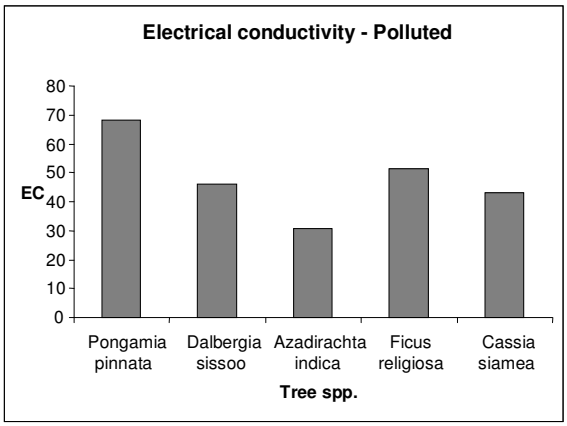

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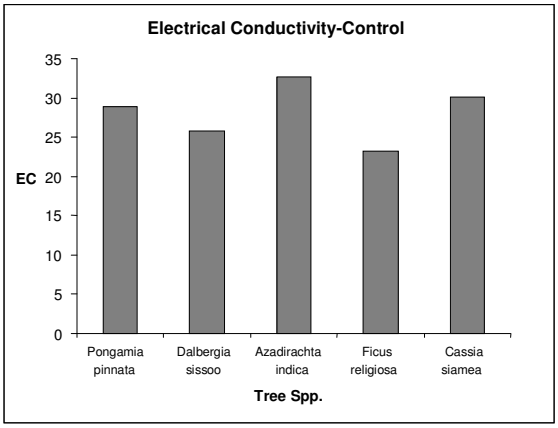

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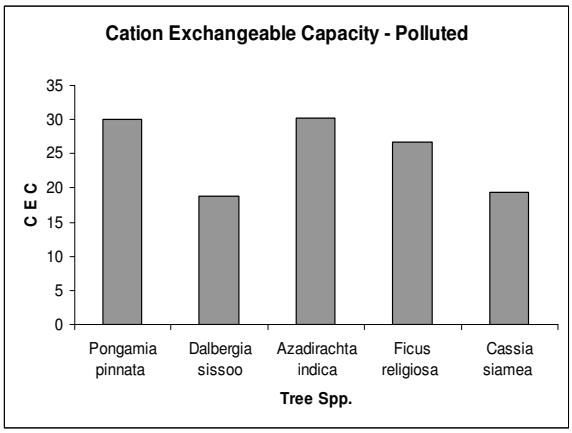
UNDER PEER REVIEW

655 **Fig. 6a. CEC of rhizospheric soil (P).** **Fig. 6b. CEC of rhizospheric soil (C).**



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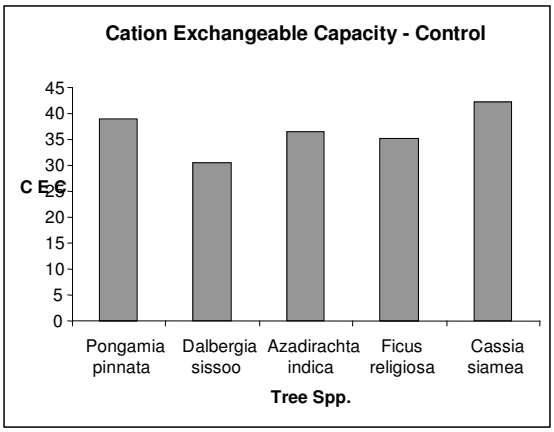

657

658 **Fig. 7a. OC of rhizospheric soil (P).** **Fig. 7b. OC of rhizospheric soil (C).**

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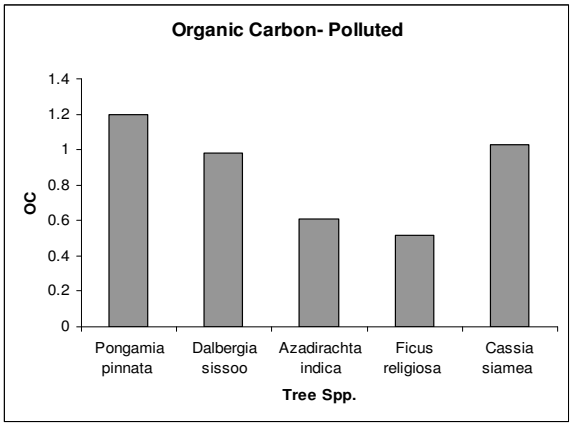
659



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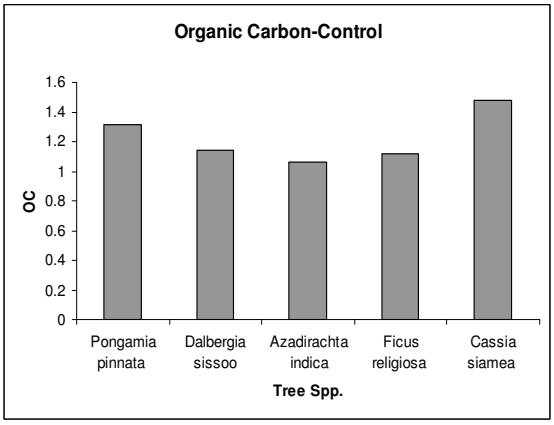
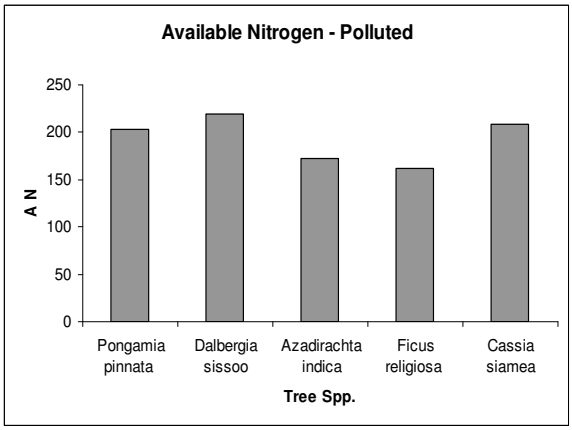


661 **Fig. 8a. Av. N of rhizospheric soil (P).** **Fig. 8b. Av. N of rhizospheric soil (C).**

UNDER PEER REVIEW



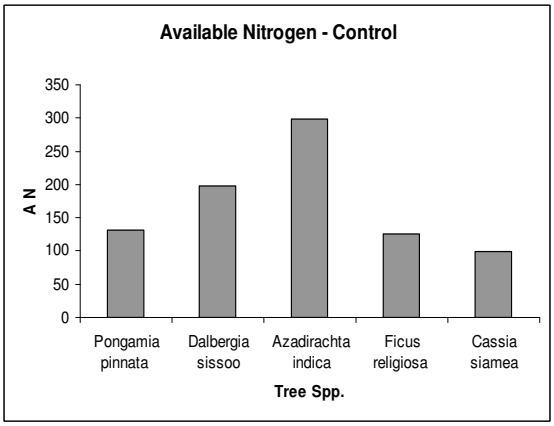
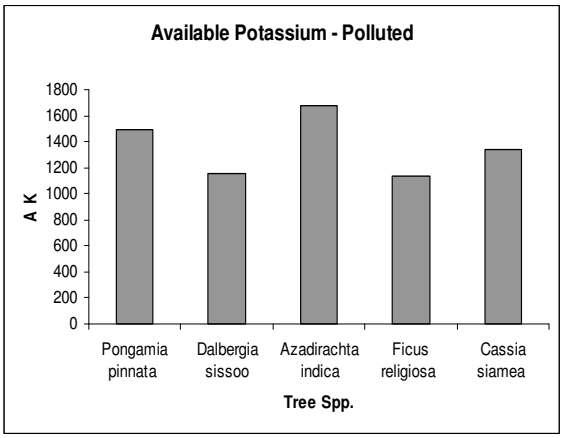


Fig. 9a. Av. K of rhizospheric soil (P). **Fig. 9b. Av. K of rhizospheric soil (C).**

UNDER PEER REVIEW

666



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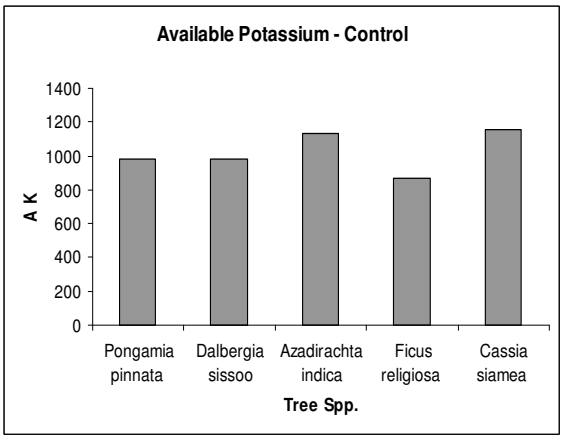

668 **Fig. 10a. Av. P of rhizospheric soil (P).** **Fig. 10b. Av. P of rhizospheric soil (C).**

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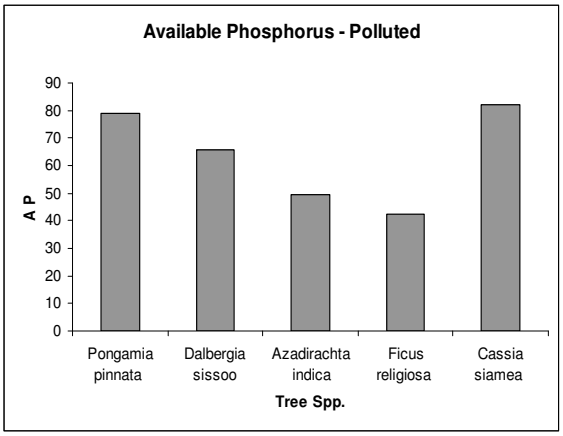

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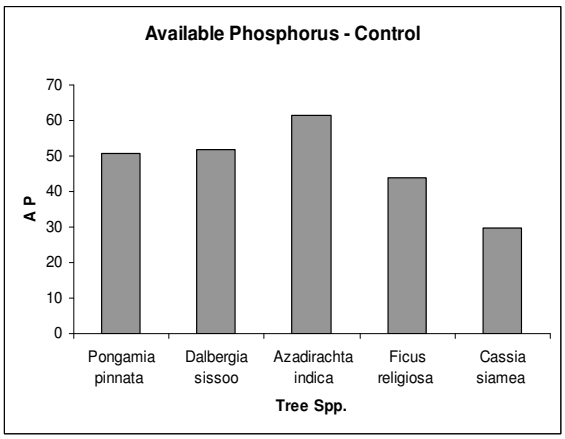
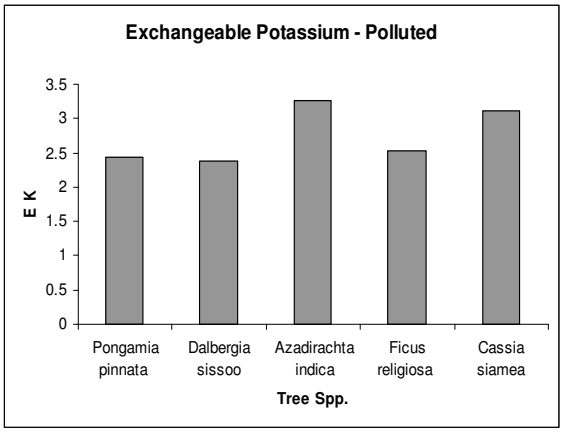


671 **Fig. 11a. Ex. K of rhizospheric soil (P).** **Fig. 11b. Ex. K of rhizospheric soil (C).**

UNDER PEER REVIEW



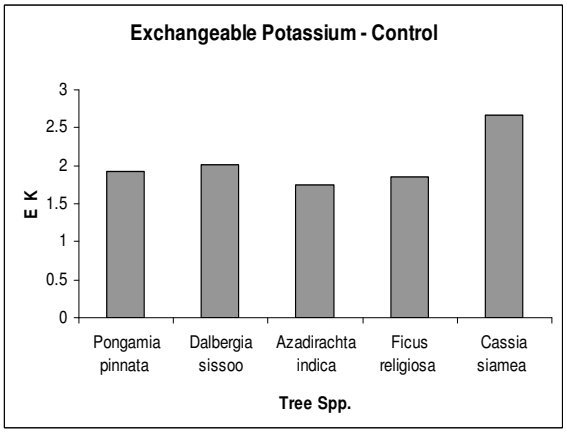


Fig. 12a. Ex. Na of rhizospheric soil (P). **Fig. 12b. Ex. Na of rhizospheric soil (C).**

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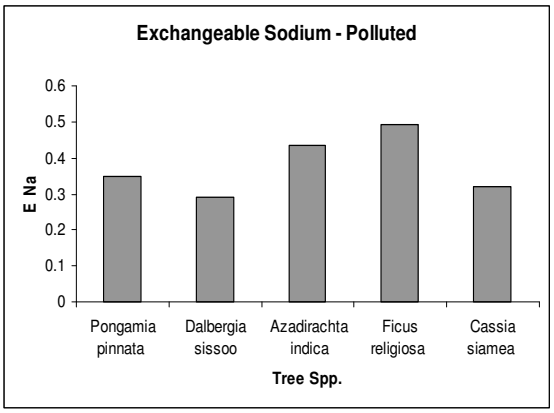

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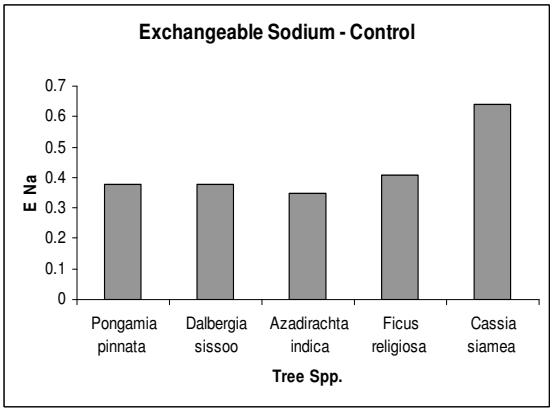
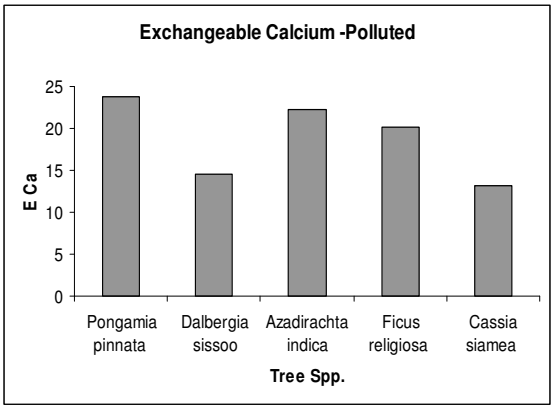


678 **Fig. 13a. Ex. Ca of rhizospheric soil (P).** **Fig. 13b. Ex. Ca of rhizospheric soil (C).**

UNDER PEER REVIEW

679



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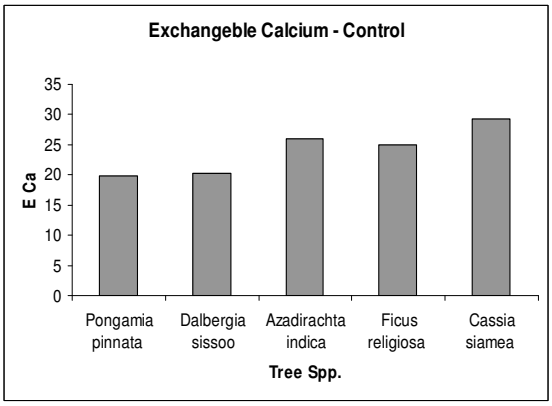

681 **Fig. 14a. Ex. Ca+M of rhizospheric soil (P).** **Fig. 14b. Ex. Ca+M of rhizospheric soil**
682 **(C).**

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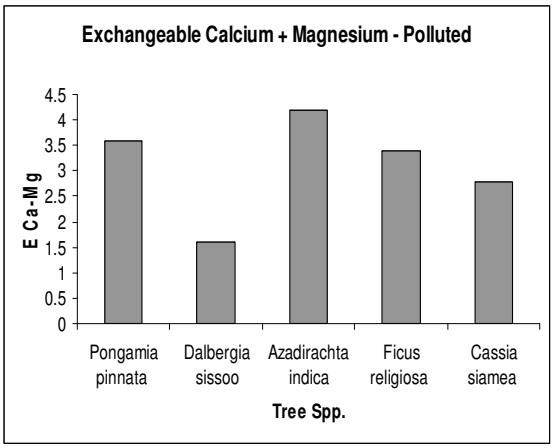

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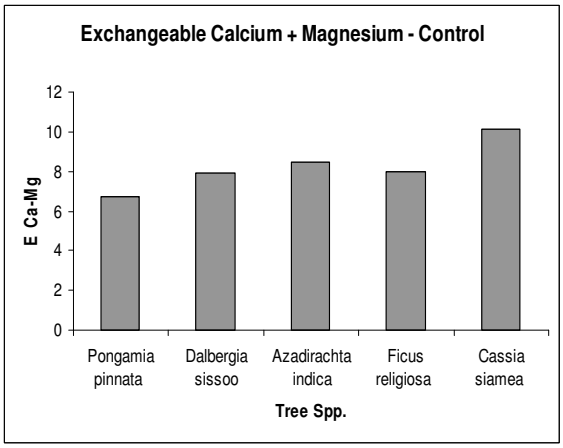

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686 **Table IVa. Levels of ascorbic acid in leaves of polluted samples.**
687

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.0193 ± 0.0023
2.	*Dalbergia sissoo*	0.0193 ± 0.0023
3.	*Azadirachta indica*	0.0270 ± 8.9964
4.	*Ficus religiosa*	0.0173 ± 0
5.	*Cassia siamea*	0.2265 ± 0.1847

688
689 **Table IVb. Levels of ascorbic acid in leaves of control samples.**
690

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.03098± 0.004242
2.	*Dalbergia sissoo*	0.02517± 0.001477
3.	*Azadirachta indica*	0.10650 ± 0.005877
4.	*Ficus religiosa*	0.02905 ± 0.00821
5.	*Cassia siamea*	0.0600± 0.002094

691
692 **Table Va. Level of carbohydrate in leaves of control samples.**
693

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	9.800 ± 8.7517
2.	*Dalbergia sissoo*	17.693± 4.3248
3.	*Azadirachta indica*	6.4163± 2.32151
4.	*Ficus religiosa*	7.2912 ± 4.2068
5.	*Cassia siamea*	11.8599± 6.0976

694
695 **Table Vb. Level of carbohydrate in leaves of polluted samples.**
696

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	10.3438 ± 6.2675
2.	*Dalbergia sissoo*	26.7151 ± 5.3657
3.	*Azadirachta indica*	14.1158 ± 8.7479
4.	*Ficus religiosa*	20.1238 ± 3.8770
5.	*Cassia siamea*	9.8383 ± 6.2675

697
698

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699 **Table VIa. Level of phenol in leaves of control samples.**
700

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	6.14 ± 2.9263
2.	*Dalbergia sissoo*	6.443 ± 1.2237
3.	*Azadirachta indica*	5.57 ± 2.1729
4.	*Ficus religiosa*	7.96 ± 4.7093
5.	*Cassia siamea*	5.75 ± 1.3851

701
702 **Table VIb. Level of phenol in leaves of polluted samples.**
703

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	5.259 ± 1.6339
2.	*Dalbergia sissoo*	6.039 ± 1.7823
3.	*Azadirachta indica*	4.636 ± 11.082
4.	*Ficus religiosa*	3.489 ± 0.7778
5.	*Cassia siamea*	7.75 ± 1.5989

704
705 **Table VIIa. Level of total Mg in leaves of control samples.**
706

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.16 ± 0.06928
2.	*Dalbergia sissoo*	0.385 ± 0.42
3.	*Azadirachta indica*	0.48 ± 0.4326
4.	*Ficus religiosa*	0.42 ± 0.2683
5.	*Cassia siamea*	0.68 ± 0.5670

707
708 **Table VIIb. Level of total Mg in leaves of polluted samples.**
709

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.312 ± 0.3412
2.	*Dalbergia sissoo*	0.568 ± 0.1091
3.	*Azadirachta indica*	0.56 ± 0.3666
4.	*Ficus religiosa*	1.26 ± 0.0848
5.	*Cassia siamea*	7.75 ± 0.3857

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Table VIIIa. Level of total Ca in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.63 ± 0.4041
2.	*Dalbergia sissoo*	1.33 ± 0.13241
3.	*Azadirachta indica*	1.2 ± 0.5291
4.	*Ficus religiosa*	4.7 ± 0.7071
5.	*Cassia siamea*	0.866 ± 0.4147

Table VIIIb. Level of total Ca in leaves of polluted samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	2.066 ± 0.6077
2.	*Dalbergia sissoo*	1.27 ± 0.6429
3.	*Azadirachta indica*	1.04 ± 0.2083
4.	*Ficus religiosa*	2.6 ± 1.9798
5.	*Cassia siamea*	1.07 ± 0.1164

Table IXa. Level of total nitrogen in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	2.2089± 0.10129
2.	*Dalbergia sissoo*	1.7205± 5.9624
3.	*Azadirachta indica*	3.2079 ± 1.1582
4.	*Ficus religiosa*	3.3966 ± 1.4127
5.	*Cassia siamea*	10.6449 ± 9.7282

Table IXb. Level of total nitrogen in leaves of polluted samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.4541 ± 0.2827
2.	*Dalbergia sissoo*	1.6206 ± 0.6231
3.	*Azadirachta indica*	18.7368 ± 9.8338
4.	*Ficus religiosa*	12.7705 ± 15.5642
5.	*Cassia siamea*	8.0808 ± 11.6909

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730 **Table Xa. Level of total phosphorus in leaves of control samples.**
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Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.4541 ± 0.7269
2.	*Dalbergia sissoo*	1.6206 ± 5.6862
3.	*Azadirachta indica*	18.7368 ± 88.9535
4.	*Ficus religiosa*	12.7705 ± 0.4997
5.	*Cassia siamea*	1.0927 ±0.6837

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733 **Table Xb. Level of total phosphorus in leaves of polluted samples.**
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Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.0298 ± 1.0298
2.	*Dalbergia sissoo*	4.3414 ± 1.1477
3.	*Azadirachta indica*	13.2701 ± 13.2701
4.	*Ficus religiosa*	9.0446 ± 11.02336
5.	*Cassia siamea*	5.7252 ±8.279861

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736 **Table XIa. Level of total K in leaves of control samples.**
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Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.366 ± 0.3214
2.	*Dalbergia sissoo*	1.133 ± 1.2148
3.	*Azadirachta indica*	2.066 ± 0.5686
4.	*Ficus religiosa*	1.5 ± 0.1414
5.	*Cassia siamea*	1.4 ±0.2645

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739 **Total XIb. Level of total K in leaves of polluted samples.**
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Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.2 ± 0.3605
2.	*Dalbergia sissoo*	2.23 ± 0.5033
3.	*Azadirachta indica*	2.133 ± 0.4016
4.	*Ficus religiosa*	2.25 ± 0.7778
5.	*Cassia siamea*	1.4 ±0.3872

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Table XIIa. Level of chlorophyll-a in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.9455± 0.39051
2.	*Dalbergia sissoo*	1.1918 ± 0.6606
3.	*Azadirachta indica*	1.1395± 0.6375
4.	*Ficus religiosa*	0.7626± 0.2091
5.	*Cassia siamea*	1.5542±0.4469

Table XIIb. Level of chlorophyll-a in leaves of pollutant samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	1.1854 ± 0.6075
2.	*Dalbergia sissoo*	0.5520 ± 0.2745
3.	*Azadirachta indica*	1.2123 ± 0.6582
4.	*Ficus religiosa*	0.6979 ± 1.0423
5.	*Cassia siamea*	1.4446 ±0.3503

Table XIIIa. Level of chlorophyll-b in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.3773 ± 0.1466
2.	*Dalbergia sissoo*	0.7595 ± 0.4288
3.	*Azadirachta indica*	1.5006 ± 1.0562
4.	*Ficus religiosa*	1.2093 ± 0.08300
5.	*Cassia siamea*	3.7831±5.0558

Table XIIIb. Level of chlorophyll-b in leaves of polluted samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.796 ± 0.4287
2.	*Dalbergia sissoo*	0.267 ± 0.1769
3.	*Azadirachta indica*	1.8329 ± 0.8779
4.	*Ficus religiosa*	0.2932 ± 0.1483
5.	*Cassia siamea*	0.7225 ±0.2903

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Table XIVa. Level of total chlorophyll in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	5.5166± 7.3459
2.	*Dalbergia sissoo*	0.5985 ± 2.4156
3.	*Azadirachta indica*	0.8928± 0.51210
4.	*Ficus religiosa*	0.3353± 0.2524
5.	*Cassia siamea*	2.3157 ±0.3827

Table XIVb. Level of total chlorophyll in leaves of polluted samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.174 ± 0.08712
2.	*Dalbergia sissoo*	0.5744 ± 0.3293
3.	*Azadirachta indica*	1.2490± 0.6788
4.	*Ficus religiosa*	0.9893± 0.5902
5.	*Cassia siamea*	2.1671±0.5786

Table XVa. Level of Na^+ in leaves of control samples.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.09 ± 0.036
2.	*Dalbergia sissoo*	0.076 ± 0.065
3.	*Azadirachta indica*	0.09 ± 0.036
4.	*Ficus religiosa*	0.115 ± 0.49
5.	*Cassia siamea*	0.10 ±0.041

Table XVb. Level of Na^+ in leaves of pollutant sample.

Sl. No.	Tree spp.	Mean ± S.D.
1.	*Pongamia pinnata*	0.086 ± 0.0229
2.	*Dalbergia sissoo*	0.076 ± 0.0206
3.	*Azadirachta indica*	0.093 ± 0.0057
4.	*Ficus religiosa*	0.095± 0.02121
5.	*Cassia siamea*	0.073 ± 0.0228

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Discussion

A summary of automotive exhaust levels has been furnished in Table XVI. Generally, it is expected that the pH of soil of highly polluted and disturb area may be found more acidic than the undisturbed one but surprisingly, it was evident from result that, except that of *Cassia siamea,* the pH of all the rhizospheric soil of the 3 tree species in the polluted area was higher than the controls, rather in the slightly alkaline range from 7.2 to 7.6, which may indicate some root associated unknown reaction which may enhance the pH of the soil; perhaps this mechanism is unique for the resistant trees to enable them to thrive.

Regarding the estimation of bulk density, no significant difference was noticed. Regarding the measurement of electrical conductivity, it was found that the EC of the rhizospheric soil in the polluted area was higher than the control as expected at par with the results of other workers. The cation exchange capacity or CEC was found little lower in the rhizospheric soil of the resistant tree species which does not match the normal phenomenon and indicate, that like that of reversing the pH phenomenon, the cation exchange capacity in the rhizospheric soil of the resistant tree species was somehow modified / rectified by the tree root-associated micro-system towards normality for enhanced absorption of nutrients molecules.

The level of organic carbon didn't show much difference between the polluted and control samples, and so was for available nitrogen. But the level of available potassium was found higher in the polluted samples of rhizospheric soil, which perfectly matches with the result of simulated acid-rain experiments at the TFRI nursery, cause of which is still to be investigated.

Levels of available phosphorous, exchangeable potassium, exchangeable sodium, and exchangeable calcium didn't show much difference between polluted and control samples, when the level of exchangeable calcium + magnesium was found significantly lower in the polluted samples then the normal one and that is at par with the normal trend of pollution reactions in the environment as reported by other workers.

From the above study, it is evident that in the summertime and in the soil composition of Jabalpur, India (where forests are tropical dry deciduous in nature), there seems existent and functional some unknown underlying mechanism which helps to bring the values of pH and cation exchange capacity of the rhizospheric soil of resistant tree species viz. *Pongamia pinnata, Dalbergia sissoo, Azadirachta indica, Ficus religiosa* and *Cassia siamea* towards normality.

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809 **Table – XVI. Summary of automotive exhaust levels.**

Gases and particles	Gasoline exhaust	Diesel exhaust	Urban polluted air	Biological activity threshold	Air quality standards
Oxygen %	1-14	1-20	20.9	16-12	
Nitrogen %	76-90		78.06		
Hydrogen %	2-6	0.05-8			
Carbon dioxide %	5-15	1-14	0.03-0.04	5	0.5
Carbon monoxide %	2-6	0-0.1	2-50 ppm	200-300 ppm	30-120 ppm
NOx ppm	30-4000	30-2000	0.001-0.15		3-10
NO_2 ppm		0.5-40	0.02-0.08	0.5-5	0.2-0.5
Photochemical oxidants ppm				0.13	0.015
Ozone ppm			0-0.2	0.1-0.5	
PAN ppm (peroxyacetyl nitrate)			0.01	0.1	0.03
SO_2 ppm	0-80	100-300	0.2-1.3	1-5	0.03-0.1
Total aldehydes ppm	40-300	10-120	0.2-1.2	0.06-0.1	
Formaldehydes ppm	10-300	5-30	0.05- 0.12	0.5-16	
Acrolein ppm			0.01	0.2-5	0.01
Total hydrocarbons	0.03-1.5%	0.01-0.10%	2-15 ppm	1-20 %	0.24 ppm
Methane ppm	200-800		1.0-1.5		1000
Benzopyrene	1-10 µg/ m³			0.01-100 µg/ 1000 m³	
Lead	70-80% of the lead in gasoline	0	0.4-10 µg/m³		15.50 µg/m³
Oils mg/m³		200-900		1200	
Particles	0.2-3 mg/g of burned gasoline	150-450 mg/m³	50-100 µg/m³		60-75 µg/m³
Cadmium µg/m³			0.0004-0.26	200	
Nickel µg/m³			0.001-0.12	1000	
Platinum µg/m³				2	

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This mechanism certainly helps in the absorption of nutrient molecules, and thus in the survival of the tree, and thus giving it the status of "resistant" in a totally adverse environment. The specific causes might be the tree root physiological reactions, might be some root associated microbe / micro-flora / micro-fauna reactions, or something else, or an admixture of all these. Anyway, these findings leave milestone for an in-depth research in the very important area in these days of extreme urban pollution.

It is already reported by many workers that in the polluted environment, the level of ascorbic acid gets reduced to a significant level, and surprisingly the phenomenon is parallel in the line of animal or human sickness, where the ascorbic acid level is found to get reduced in many cases. However, in our exhaustive study, it was found that this level was highest in case of *Azadirachta indica*, next for *Cassia siamea* in case of control samples. And interestingly, the level was found to be significantly high in case of *Cassia siamea*, when for all the other trees viz. *Pongamia pinnata, Dalbergia sissoo, Azadirachta indica, Ficus religiosa*, the levels were significantly low. This finding strongly indicates the suitability of *Cassia siamea* in the environmental region of Jabalpur, Madhya Pradesh, where the tree could develop resistance against the dreaded effects of air pollution emitted by the automotives, when the others showed physiological damage in terms of levels of ascorbic acid.

The level of total carbohydrate was found to be highest in *Dalbergia sissoo* in control area, when the same picture was reflected in the polluted samples. The increased level of carbohydrate generally reflects the damaged physiology of a tree, but in this case, as the level was found higher in both control and polluted, it may be inferred that this level is normal in case of *Dalbergia sissoo* in the environment of Jabalpur. However, the level was found to be lowest in *Pongamia pinnata* in the polluted samples, indicating its resistance and suitability in the region.

The level of phenol was found to be highest in case of *Cassia siamea* and lowest in *Ficus religiosa* in the polluted samples, where in the control samples the level was found highest in *Ficus religiosa*. This indicates damage caused by pollution in *Ficus religiosa*, and resistance offered by nature to *Cassia siamea*.

Magnesium metabolism is directly linked to the synthesis of chlorophyll molecule in plants. In the level of total magnesium in the leaves, it was found to be highest in case of *Cassia siamea* in both control and polluted samples, but the level was significantly lower in all other 4 species; this indicates that the automotive released air pollutants highly affects the

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metabolism of magnesium ions in the trees, which somehow *Cassia siamea* could counterbalance with some unknown physiological mechanism.

The metabolism and assimilation of calcium is very important in any living organism; total calcium was found to be highest in case of *Ficus religiosa* in both the control and polluted samples, indicating the resistance and suitability of the tree in the region; remarkably, this level was found much lower in all the other 4 species.

The level of total nitrogen indicates the synthesis of protein in an organism. This was found highest as a burst in case of *Azadirachta indica* and *Ficus religiosa* in the polluted samples, indicating the ability of the trees to generate the necessary resistant proteins to combat a polluted environment.

The level of total phosphorus indicates the synthesis of nucleic acids (DNA and RNA) in an organism; interestingly the level was found higher in both the cases of *Azadirachta indica* and *Ficus religiosa*, when for the other samples there found a trend of increase in the amount of phosphorus in the polluted area, which indicates that probably the formation of nucleic acids gets a boost due to the effect of air pollutants which might had switched on some gene expression to synthesize some proteins to combat the pollution effects.

No significant difference was noticed in case of total sodium and total potassium between the control and the polluted samples, which is expected in terms of the nature of the soil and regional effects.

We have already noticed that the level of total magnesium was found significantly higher in case *Cassia siamea* and magnesium is directly related to chlorophyll synthesis in plant body. Our results perfectly matched with this finding when of total chlorophyll the level was found maximum in *Cassia siamea* in polluted area.

The above described data has provided a index-guideline for many important individual elements and compounds in resistant trees and has clearly demonstrated the probable sequences of end reactions of many a physiological events when the specified trees get exposed to automotive air pollution in likely environment that of Jabalpur.

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