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RESEARCH ARTICLE

Identification of Calcium oxalate Crystal Deposition at Different Periods, Major Crystal Adherence Sites and its Injuries in Ethylene Glycol Administered Male Albino Rat Kidneys.

Pandi Lakshmi P[1]*, C.D.Lethi[1] and P. Kokilavani[2]

[1]Department of Zoology, Holy Cross College, Tiruchirapalli-620002, Tamil Nadu, India

[2]Department of Environmental Biotechnology, Bharathidasan University, Tiruchirapalli-620024, Tamil Nadu, India

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***Address for correspondence**
Mrs.P.Pandi Lakshmi,
Asst.Professor,Department of Zoology,
Holy Cross College,Tiruchirapalli-620002.
TamilNadu, India.
E.mail: vksppandi@gmail.com.

ABSTRACT

Calcium oxalate kidney stone model in rats induced by Ethylene glycol has been used for this study. Crystal deposition at different periods of feeding, sites of crystal attachment and significant pathological changes were identified.Histological studies of kidney on the 15th, 30th and 60th days of the Ethylene glycol administration revealed increased calcium oxalate deposition.Intratubular and interstitial location of crystals and the resultant renal cell damages were apparent. No crystal deposits and major cellular injuries were found in control rat.

Key words: Kidney stone, calcium oxalate, ethylene glycol, intratubular and interstitial location, renal cell damages.

INTRODUCTION

Kidney stone is a hard mass developed from crystals that separate from urine and build up on the inner surface of the kidney.The development of kidney stones requires formation of crystals followed by their retention in the kidney[1].Stone constituent crystals may be washed off surfaces depending on the shear force applied or they may adhere to surfaces for long periods. Crystals washing off the surfaces to which they adhered temporarily will end up in the urine and are generally of little consequence clinically[2,3].Crystals adhering for prolonged periods can

presumably serve as the nidus for the developmentof clinical stones.For stone formation; crystals must form, grow and be retained in the kidneys, which is indeed a rare occurrence. Only pathological changes in the kidneys, renal cell dysfunction and injury can accomplish crystal retention and formation of stone nidus[4]. Cellular dysfunction can be intrinsic or provoked.Lethal epithelial cellular injury promotes crystal nucleation, aggregation and retention. Sub lethal injury or dysfunctional cells may produce ineffective crystallization modulators and localized areas of supersaturation in the interstitium [5]. In addition, an inflammatory response to the crystals may be necessary for the evolution of interstitial crystals into the stone nidus [4]. Crystals themselves may induce renal cellular changes; other conditions such as hyperoxaluria may induce changes that facilitate crystal adherence.It appears that crystal attachment is mediated by specific molecules that appear on the cell surface or are constituents of extracellular matrix when cell injury or tissue damage occurs[6]. Although many investigators carried out studies on crystal adherence and renal injuries, definite proof on intratubular and interstitial location of calcium oxalate crystal and its major renal damages are not yet available.The major objectives of this study were to identify calcium oxalate stone deposition at different periods, major sites of its attachment and the associated renal cell injuries in ethylene glycol administered rat kidney tissue.

MATERIALS AND METHODS

Healthy adult male Wister albino rats weighing between 150 and 200g were selected for the induction of kidney stone and they were used as per the ethical committee recommendation.Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of human [7] and also earlier studies shown that the amount of stone deposition in females rats was significantly less [8].They were provided with regular rat chow and drinking water *ad libitum.*

Ethyleneglycol induced urolithiasis model

Ethylene glycol (1%) was administered to the experimental animals in drinking water throughout the period[9]. The control group was fed on water without ethylene glycol. The kidney tissue was studied on 15th, 30th and 60th day of the experiment.

Identification of calcium oxalate stone and its deposition at different periods

Animals were sedated and killed; kidneys were extracted, sliced, fixed in 10% neutral formalin, dehydrated and embedded in paraffin. Five micrometer thick cross-sections were stained with Pizzolato staining method [10] to detect oxalate containing crystals. To identify Pizzolato positive stained substances, photomicroscopy was used.

Detection of sites of crystal attachment and renal cell damages

Renal cell injuries of Calcium oxalate crystals and their sites of location in kidney sections were evaluated by inspecting each tubule (Proximal tubule, loop of Henle, distal tubule and collecting ducts) and interstitial region. The number of crystals was quantified by direct counting.

RESULTS

In the present study, Calcium oxalate deposition at different periods, its renal cell injuries and its sites of attachment in ethylene glycol administered rat kidney tissue were identified. The control kidney section showed no crystal deposition in renal tubules and interstitial region and no renal cell damages such as glomerular and tubular degeneration (Figure 1). After 15th day of 1% ethylene glycol induction marginal amount of positive stained calcium oxalate crystals were observed (Figure 2). On the day 30 and 60, an increasing number of crystals were found

attached to the luminal membrane of tubular epithelial cells (Figure 3).Quantification of calcium oxalate deposits on kidney sections at different periods is plotted in Graph 1, which revealed a gradual increase in the number.

Figure 3 shows the retention of crystals in proximal convoluted (with loss of brush border), distal convoluted and collecting tubules. Distal tubules showed increased crystallization than proximal and collecting tubules.Attachment of crystals in thin and thick loops of Henle is apparent (Figure 4). The movement of crystals from tubule to interstitial region and sloughing of epithelial cells into tubular lumina is visible (Figure 5).

Rats treated with Ethylene glycol showed interstitial location of crystals and glomeruli degeneration (Figure 6), widened intercellular space and nidus formation in interstitium (Figure 7) and nucleation of crystals in tubular region (Figure 8).Dilation of tubules, signs of necrosis with damaged epithelial cell wall (Figure 9) and disruption of surface epithelium were also observed (Figure 10).

DISCUSSION

In the present study we have investigated the induction of kidney stone by administration of Ethylene glycol, sites of its attachment and renal injuries in Wister male albino rats. The results of this study have shown the gradual depositing of the stones in the different locations. The long period of treatment with 1% ethylene glycol results in increased calcium oxalate deposits on the day 30 and 60. Since ethylene glycol is a nephrolithiasis causing agent, it would have caused urinary calcium oxalate nucleation, precipitation and subsequent crystal growth in kidney tissue. Crystals adhering for prolonged periods can presumably serve as the nidus for the development of clinical stones [2]. Hyperoxaluria and hypercalciuria have caused increased urinary calcium oxalate supersaturation.Mild supersaturation can only produce small particle crystalluria. They do not aggregate, come in contact with the epithelial cells and get retained inside the kidneys. On the otherhand they are excreted in the urine without causing any pathological changes and urolithiasis [11].

The results of this study reveal the formation of stone constituents in extracellular and interstitial sites. Intratubular and interstitial location of calcium oxalate is apparent. One of our outstanding findings is that increased crystal deposition in distal tubule, which is the major site for the regulation of potassium, sodium, calcium and pH. According to Khan and coworkers (1999) [12], calcium oxalate can form within a tubule by adhering either to intact cells or injured areas of the tubules that have become denuded of cells. These crystals would have formed by nucleation in tubular fluid supersaturated with stone constituents.These crystals can also be engulfed by cells resulting in their movement to the interstitium. Calcification may also occur in the basement membranes of loops of Henle [13].

The results also show renal injuries caused by the kidney stones. The interaction of cells and tissues with crystals has caused tubular necrosis, glomerular degeneration, sloughing of epithelial cells into tubular lumina, loss of brush border in proximal tubule, widened intercellular space in interstitium and disruption of surface epithelium. Some of these cellular responses can make cells receptive to the adherence of crystals formed in renal tubules or could be responsible for interstitial calcification. According to Khan (1996) [4],renal injury promotes crystal retention and the development of stone nidus on renal papillary surface. Studies performed in vivo in animal models have shown that renal epithelial injury promotes crystallization of calcific crystals.

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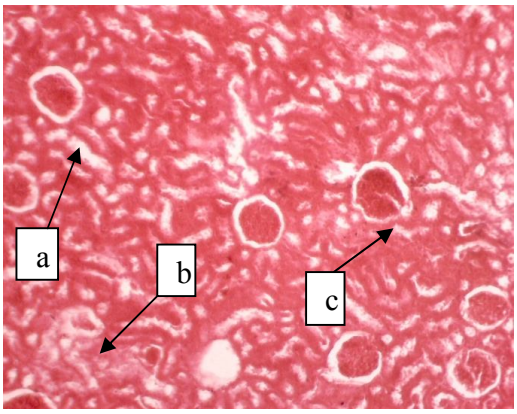
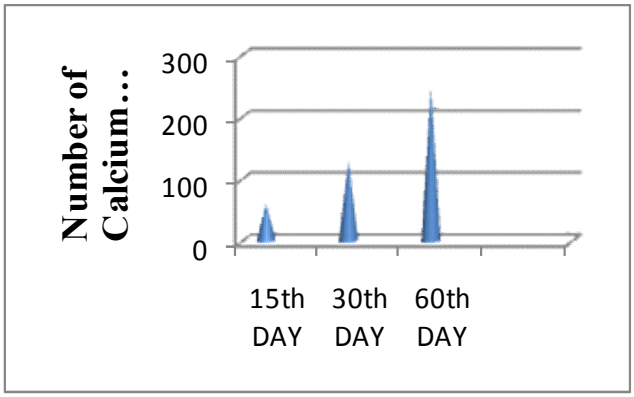


Figure 1: Kidney tissue of a control rat showing tubules (a), interstitial regions (b) and glomerulus (c).



Graph 1: Quantification of Calcium oxalate deposits in Ethylene glycol administered rat kidney tissue.

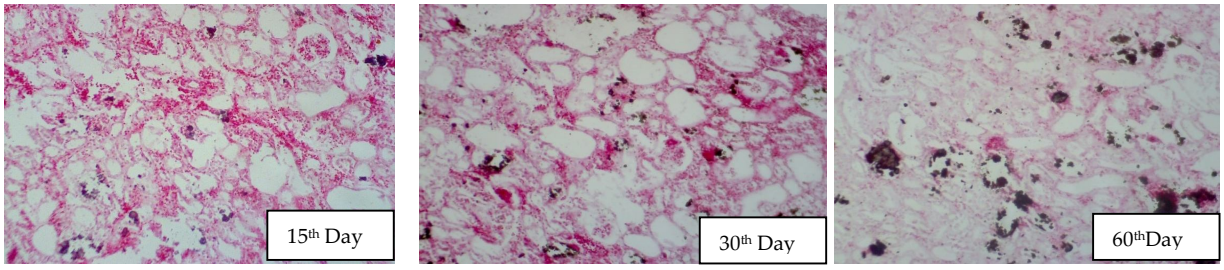


Figure 2: Calcium oxalate deposition in rat kidney tissue after different periods of Ethylene glycol administration.

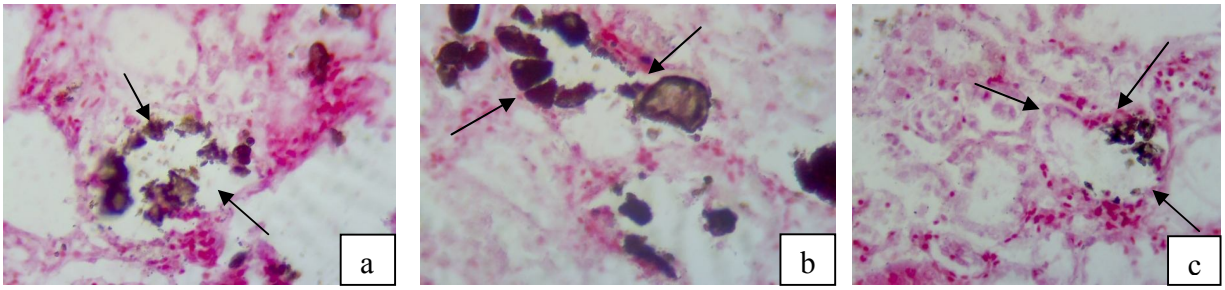


Figure 3: Intratubular location of calcium oxalate crystals in Ethylene glycol administered rat kidney tissue a) Collecting tubule b) Distal tubule c) Proximal convoluted tubule with loss of brush border.

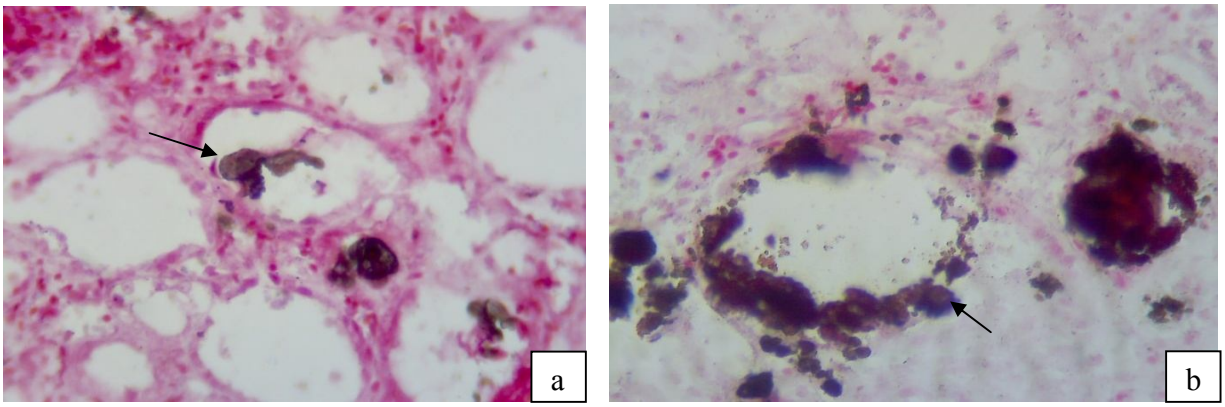


Figure 4: Intratubular location of calcium oxalate crystals in Ethylene glycol administered rat kidney tissue a) Thick loop of Henle b) Thin loop of Henle

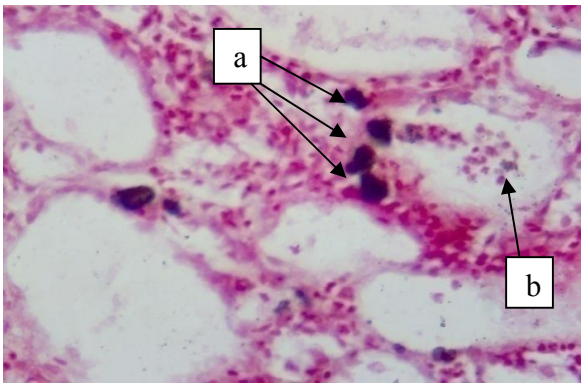


Figure 5: Translocation of calcium oxalate crystals from tubule lumen to interstitial region through tubule wall (a) and release of epithelial cell content to the tubule lumen (b) in Ethylene glycol administered rat kidney tissue.

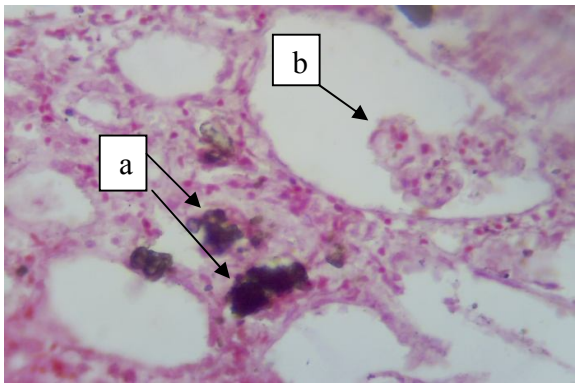


Figure 6: Interstitial location of crystals (a) and Glomerular degeneration (b) in Ethylene glycol administered rat kidney tissue.

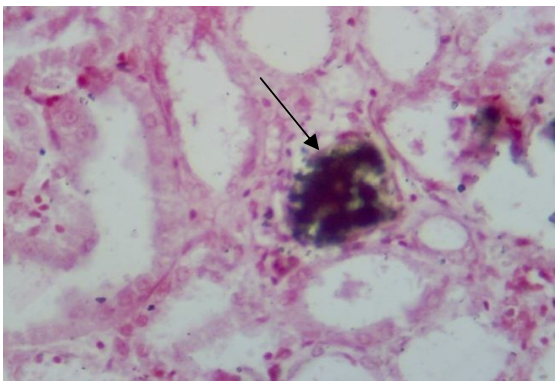


Figure 7: Widened intercellular space and nidus region formation in interstitial region of Ethylene glycol administered rat kidney tissue.

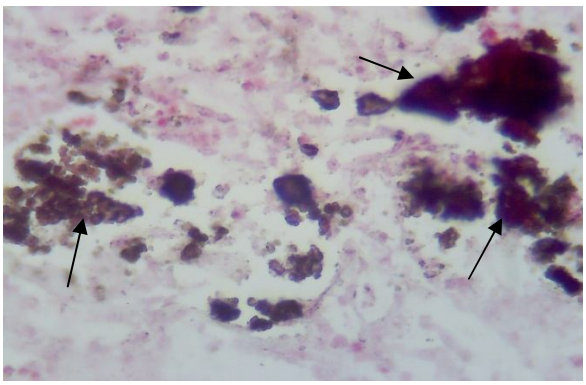


Figure 8: Nucleation of crystals in tubular region.

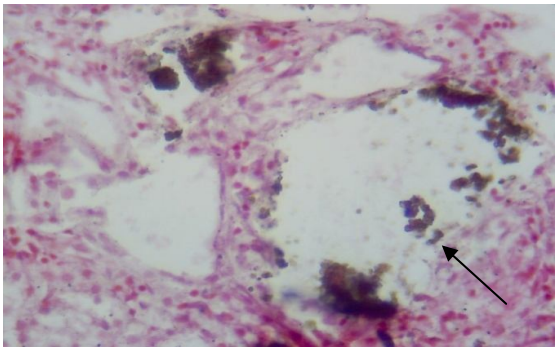


Figure 9: Dilation of tubules with damaged epithelial cell wall.

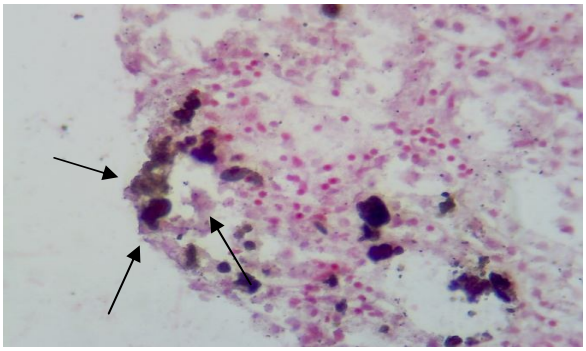


Figure 10: Stretch of surface epithelium by underlying crystals.

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RESEARCH ARTICLE

Study on the Fuel Properties of Fatty Acid Methyl Ester from *Azadirachta indica* A. JUSS. Seed Oil

Nithya G.[1]* and Litty Koria[2]

[1]Department of Zoology, Michael Job College of Arts and Science for Women, Coimbatore-641103, TamilNadu, India.

[2]Department of Zoology, LRG Govt. of Arts College for Women, Tirupur- 641 604, TamilNadu, India.

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***Address for correspondence**
Dr. G.Nithya
Assistant Professor, Department of Zoology,
Michael Job College of Arts and Science for Women,
Coimbatore-641103, TamilNadu,India.
Email : nithyagunasekeran@gmail.com

ABSTRACT

Bio-fuels produced from renewable energy sources are gaining importance in the light of rising fossil fuel prices, depleting oils reserves and increasing 'green house effect'.The known world wide reserves of petroleum are predicted to last for about 40 year; hence future availability of petroleum is uncertain. Alternative fuels have to be considered in order to undertake energy security and import substitution for diesel crude imports by 5% in 2015 and 10% in 2020. In this circumstances non-edible oil source play a vital role to meet the diesel requirement. Biodiesel is a mixture of fatty acid alkyl esters obtained by the reaction of triglycerides of vegetable or animal origin with alcohol in the presence of a catalyst. In the present study, biodiesel has been prepared from *Azadirachta indica* oil by transesterification method. The biodiesel samples isolated from the oil *Azadirachta indica* were characterized by measuring their chemical properties such as acid value, iodine value, free and total glycerol, ash content. Physical properties like, kinematic viscosity, flash point, cetane number and heat of combustion were estimated. *Azadirachta indica* biodiesel is analyzed for its fatty composition using Gas chromatography assisted with mass spectrometry. These parameters were assessed in the above feedstock and discussed about their suitability for engine.

Key words: Biodiesel, *Azadirachta indica*, Transesterification, Cetane number, Gas chromatography mass spectrometry

INTRODUCTION

The petroleum products are under the threat of inadequate supply and fluctuating prices. In addition, continuous and increasing use of petroleum products has created local air pollution, leading to global warming due to increase of CO_2. Hence, considerable interest has been shown to find out alternate energy sources especially renewable fuels [8]. India stands seventh largest net importer of oil in the world in 2006 and during 2007, net oil import was 68% of its oil consumption. The EIA (Energy Information Administration report, 2008)[4] expects India to become the fourth largest net importer of oil in the world by 2025, followed by the US, China and Japan. Hence, considerable interest has been shown to find out alternate energy sources especially renewable fuels[8].Among various renewable energy resources oil bearing crops showed greater potential in the production of biofuel, like biodiesel to meet the energy requirements [17].There are several non-edible oilseed species such as *Shorea robusta,* karanja (*Pongomia pinnate*), jatropha (*Jatropha curca*), neem (*Azadirachta indica*) etc, that could be utilized as a feedstock oil in biodiesel production.

Among these Azadirachta indica is one of the major non-edible oil bearing trees with high production potential of about 83,000 tons of oil per annum, a large portion of which can be diverted for the production of biodiesel. Neem seed contains 30% oil content [2]. The present work reports the transesterification of Azadirachta indica oil to fatty acid methyl ester and its fuel properties and GC-MS analysis. The obtained results were analyzed and compared with ASTM (American Society for Testing and Materials) standard.

MATERIALS AND METHODS

Collection of Sample

Neem seed oil was obtained commercially from Thiruchengode area, Erode district, Tamil Nadu, India.

Production of Biodiesel

Determination of Free fatty acid in the oil was estimated as detailed by Sadasivam et al. (1996)[19]. The acid value is defined as the mg of KOH necessary to neutralize the free fatty acids present in 1g of lipid.Determination of NaOH requirement was carried out in *Azadirachta indica* seed oil for transesterification process by the method followed by Gerpen *et al.* (2004)[6].

Fatty Acid Methyl Ester Preparation by Acid and Alkali Catalyzed Transesterification (Two-Stage Method)

In order to avoid the problem of saponification, the two stage method was used for the isolation of biodiesel from *Azadirachta indica* oil.Oil sample was poured in to a round bottomed flask equipped with a reflux condenser and heated to the reaction temperature. 1% (v/v) H2SO4 in methanol (8% v/v) was added to the flask. After the reaction, the mixture was allowed to settle in a separating funnel over night.To the pretreated oil 0.35% (w/v) of sodium hydroxide in 12% (v/v) methanol was added to the reaction flask. The methanol to oil molar ratio was 6:1. The reaction was allowed to proceed until completion. The lower glycerol layer was drawn off. The reaction time depended on the type of feedstock used.

Washing and Drying

After separating the unwashed biodiesel and glycerol, water was added to biodiesel and stirred well. During stirring a white cloudy substance was formed at the bottom of the separating funnel. This cloudy substance was separated carefully.The layer was heated to 100ºC to remove the remaining water [15].

Fuel Properties Measurement

The physical and chemical properties of *Azadirachta indica* oil were measured and tabulated in Table 1. Acid value was determined by the titremetric method of Pearson (1970)[16]. Iodine value was determined by the titremetric method of Pearson (1970)[16]. A.O.C.S. Ca.14 – 56 methods [5] was used to determine the total glycerol content of test sample. Free glycerol content of sample was determined using A.O.C.S Ca 14 – 56 method[5].Ash content is measured as detailed in Indian Standard Method: 1448 (1992)[9].The kinematic viscosity of the sample was determined by Redwood viscometer using American Standard Test Methods (ASTM) 445-88 (1994c)[1].The flash point, by Pensky Martens closedcup method [10]. The determination of cetane index was carried out in the present study following the method, as detailed by Krishnangkura (1986)[11]. Heat of combustion of FAME was estimated as detailed by Krishnangkura (1991)[12].

GC-MS Analysis

The fatty acid composition of the biodiesel sample was analyzed by gas chromatography coupled with mass spectrometer.The FAME was analyzed using Gas chromatography/ Mass Spectrometer (Thermo Fischer, USA), equipped with a SPB 1000 nukol capillary column (25m x 0.36mm, film thickness of 0.25µm). The detector temperature was programmed for 250°C with flow rate of 0.3 ml/min. The injector temperature was out at 250 °C. Nitrogen was used as the carrier gas. Identification of the peaks was performed by comparing retention times with those of standards analyzed [22].

RESULTS AND DISCUSSION

The transesterification process seems to be the best choice, as the physical characteristics of fatty acids methyl esters produced (biodiesel) are very close to those of the diesel fuel and the process is relatively simple.The free fatty acid is an important parameter for determining the viability of the transesterification process[13], as it could affect the chemical reaction. High acid value in the feed stock would result in soap formation when alkali chemicals are used as catalyst; hence the FFA has to be neutralized [24]. Hence, it has been proposed that a combined process of acid catalyzed pretreatment prior to alkali catalyzed transesterification is followed to overcome the problem [7,25]. The Azadirachta indica seed oil used in this present study were tested for FFA content and showed 3.7% respectively. Due to the presence of high FFA content (above 3%) in the feed stock direct conversion of the above oil via alkaline transesterification was not possible. Hence, a combined process of catalyzed pretreatment followed by alkali catalyzed transesterification was used to convert the triglycerides. The methyl ester obtained from *Azadirachta indica* oil were 80.4 % (w/w) with 27.1% of glycerol as the end product and with a catalyst concentration of 0.96 wt% of oil. Similar studies have been reported in Rubber seed oil[18]; *Nicotiana tobaccum*[23]; *Jatropha curcas* [21].

Chemical and Physical Properties

The acid number is used to quantify the amount of acid present in a sample of biodiesel. Acid value is a measure of the amount of free acids present in a given amount of oil sample. Acid value of *Azadirachta indica* oil was high ranging 7.4mg KOH/g but by using two-stage transesterification process the acid value of *Azadirachta indica* methyl

ester was reduced substantially to 0.45 mgKOH/g and it was within the limit of the ASTM standard. Iodine value is an important measure of the oxidative stability of the oil and the polymerization of glycerides. Iodine value of *Azadirachta indica* methyl ester was 97.7gm/100gm. Free glycerol is a by-product of the transesterification process and is separated from the ester. Total glycerol content (the sum of free and bound glycerol) is one of the main parameters indicating the final quality of biodiesel. The amount of total glycerol determined in the *Azadirachta indica* biodiesel was 0.302% and the free glycerol content is found to be 0.100%.The present results showed that free glycerol content was slightly deviated from the ASTM limit and total glycerol content was nearer to the test method. The ash content determined in the biodiesel sample is 0.01% which is within the maximum limit specified by ASTM and diesel value. Kinematic viscosity is an important parameter regarding fuel atomization as well as fuel distribution [14]. *Azadirachta indica* methyl ester had viscosity of 3.07 mm^2/s. The flash point is the temperature at which the fuel will ignite when exposed to an ignition source. The flash point *Azadirachta indica* of biodiesel was 108°C.Bangboye and Hansen (2008)[3] observed that a feedstock that is high in saturated fatty esters has a high CN, while feedstock predominant in unsaturated fatty acid has lower CN values (20-40). In the present study, the cetane index value of the *Azadirachta indica* biodiesel tested were found to be 52, in the medium range, reflecting the slight dominance of saturated fatty acids. The heating value of a fuel decides the ability of a fuel to be used in an engine. The heating value of *Azadirachta indica biodiesel was* about 2835.25 k-cal / mol.

Fatty Acid Analysis

It is considered that the quality of the fuel is reflected by the composition of these fatty acids. Fatty acid components of *Azadirachta indica* biodiesel were detected by GC – MS. The major component present in this methyl ester was Stearic acid (18:0) 18.07%, Myristic acid (14:0) 19.33% ,oleic acid (18:1) 24.57%, Palmitic acid (16:0) 11.93% and Capric acid (10:1) 10.21%. Some of the minor fatty acid components are Linoleic acid (18:2) 5.55%, Palmitoleic acid (16:1) 4.11%, Alpha – linolenic acid (18:3) 3.09% and Lignoceric acid 3.09% respectively (Table 2). In *Azadirachta indica* methyl ester 28.68% fatty acids were found to be monounsaturated ($C_{18:1}$) and ($C_{16:1}$). Polyunsaturated fatty acids were found to be 8.64% ($C_{18:2}$, $C_{18:3}$). The predominant of mono unsaturated fatty acid mainly oleic acid can exhibit better fuel properties. In the present study oleic acid was found to be predominant fatty acid. Knothe (2008) [10] has reported that methyl oleate can be the desirable fatty acid among the other common fatty acids that can enrich the fuel properties of biodiesel produced.Similar GC-MS analysis of fatty acid composition has been reported in the cases of *Ervatamia coronaria* [20].

CONCLUSION

The production of biodiesel from edible oil is currently much more expensive than diesel fuels due to relatively high cost of edible oil. There is a need to explore non-edible oils as alternative feed stock for the production of fatty acid methyl ester. This present study is intended to consider aspects related to the feasibility of the production of biodiesel from *Azadirachta indica* oil.The biodiesel sample prepared in the present study showed better results and not deviating from ASTM standard except the free and total glycerol content which can be rectified by further suitable methods.

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Table 1: Fuel Properties of *Azadirachta indica* Methyl Ester

Property	Units	Sample value	ASTM Specification	Standard diesel value
Acid value	mg KOH/gm	0.45± 0.155	0.50max	NA
Iodine value	g/100gm	97.7 ± 0.694	NA	NA
Total glycerol	%mass	0.302 ± 0.005	0.240 max.	NA
Free glycerol	%mass	0.100 ± 0.005	0.020 max.	NA
Sulphated ash	%mass	0.01 ± 0	0.020 max.	0.02
Kinematic viscosity	mm^2/s (cst)	3.075 ± 0.05	1.92-6.0	2-4
Flash point	ºC	108 ± 1.15	130 ºC	65-88 ºC
Cetane index		52 ± 0.96	47 min	45-50
Heat of combustion	k-cal / mol	2835.25±118.01	NA	NA

Table 2: GC-MS analysis of Fatty Acid Methyl Ester in *Azadirachta indica*

S.No.	Fatty Acid	% of Fatty Acid in FAME	Retention Time
1	Capric Acid methyl ester	10.21	4.234
2	Myristic Acid methyl ester	19.33	7.754
3	Palmitic Acid methyl ester	11.93	9.970
4	Palmitoleic Acid methyl ester	4.11	10.456
5	Stearic Acid methyl ester	18.07	13.231
6	Oleic Acid methyl ester	24.57	13.648
7	Linoleic Acid methyl ester	5.55	14.906
8	Alpha-Linolenic Acid methyl ester	3.09	16.389
9	Lignoceric Acid methyl ester	3.09	26.725

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RESEARCH ARTICLE

Green Solution to Blue Crisis – A Technique to Save Domestic Water

Mohammed Mohiuddin Asad

Department of Civil Engineering, Muffakham jah College of Engineering and Technology, Banjara Hills Road No-3, Hyderabad-500034, Andhra Pradesh, India.

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***Address for correspondence**
Abdul Samad M Kamdod,
Assistant Professor, Department of Civil Engineering,
Muffakham Jah College of Engineering and Technology,
Banjara Hills Road No-3, Hyderabad-500034, Andhra Pradesh, India.
Email: prof.asmk@gmail.com

ABSTRACT

In this paper the two pipe system is used to collect soil waste from water closet (soil pipe) and the sullage (waste pipe).The soil pipes are connected directly to the drain whereas waste pipes are connected through a trapped Gully. The wastewater from top floor is collected in a collecting tank provided in the bathroom of lower floor. The collecting tank is basically a grease trap where excess waste water comes out at the outlet and joins the waste pipe.The tank is provided at a suitable height and dimensions are decided based on the quantity of waste water to be stored. The water stored in the collecting tank is to be used for flushing. A conventional flushing cistern which has one inlet and one outlet, the flushing cistern is to be provided with two inlets, one outlet, an extra float for extra inlet and larger plan width to accommodate extra float. One inlet draws water from the waste water collecting tank and other draws water from the normal water supply system.The reason for providing two inlets is that in case the upper floor is vacant for reasons, the residents can use water from normal supply system for flushing. The inlet supply from each inlet can be controlled by control valves provided.

Keywords: soil waste, sullage, flushing cistern, one-pipe system, two-pipe system and trap.

INTRODUCTION

Rainwater harvesting (RWH) for urban sustainability gives benefits and it is emerging as a key concern in order to clip with water scarcity in cities. Due to lack of knowledge regarding the most adequate scale in financial terms for RWH infrastructures particularly in dense areas .Water is a vital element for human life and for the development of communities, without which economical and social development are not possible. To assure its sustainability, the actual tendency of increase the extraction to supply the rising demand for potable water has to be reverted. The over-exploitation of water resources has been leading to water stress and restrictions on water supply in many countries

[1]. The importance of securing water supply necessitates that all options be explored. Research has indicated that demand on water catchments can be substantially decreased when a large proportion of households reuse grey water and/or install rainwater tanks [2]. Water scarcity (Blue crisis) is not new problem in under developed or developing countries. It may be either due over exploitation of ground water due to lack of awareness, contamination of available water, poor planning and allocation of funds by local authorities, environmental effects etc. As sources of water are limited every attempt should be made to supply potable water to the farthest consumer and that to free from pathogens, because 780 million people lack access to an improved water source; approximately one in nine people and every 21 seconds, a child dies from a water related illness [5]. Rainwater utilization may be one of the best available methods for recovering natural hydrological cycles and aiding in sustainable urban development [6]. Thus is appropriate to say "No clean water, No life" than "No water, No life". Hence people should use water in effective way i.e., by proper use of available water and using waste water for less important industrial and domestic purposes.

The main purpose of providing a flushing cistern (tank) to a water closet is to push the soil waste (night soil) from one side of the trap to the other (as shown in Figure 1 & 2), "to prevent the entry of foul sewer and drain gases into the houses". The flushing cistern requires 10 – 15 liters of water per flushing [3]. But we can also do this using sullage (The term, sullage is used to indicate the waste water from bathroom, kitchens etc. It is merely waste water and does not create bad smell [4].)

MATERIALS AND METHODS

The waste water collecting tank, flushing cistern (tank) with extra float, brackets, and control valves are required. In this method the two pipe system is used where one pipe collects soil waste from water closet (soil pipe) and other collects sullage (waste pipe).The soil pipes are connected directly to the drain whereas waste pipes are connected through a trapped Gully [3]. These connections are shown in figure 3.

In this method waste water from top floor is collected in a collecting tank provided in the bathroom of lower floor as shown in Figure 4. The collecting tank is basically a grease trap (in this type of trap lighter matter like oil, soap latter, detergents etc., which floats on the surface are allowed to escape through the outlet[3]) where excess waste water flows out through the outlet and joins the waste pipe. The tank is provided at a suitable height (as shown in Figure 5) and dimensions are decided based on the quantity of waste water to be stored. The water stored in the collecting tank is to be used for flushing. Unlike a conventional flushing cistern which has one inlet and one outlet, in this method flushing cistern is to be provided with two inlets, one outlet, an extra float for extra inlet (as shown in Figure 6) and larger plan width to accommodate extra float.As shown in Figure 7 one inlet draws water from the waste water collecting tank and other draws water from the normal water supply system. The reason for providing two inlets is that in case the upper floor is vacant for any reason, the residents can use water from normal supply system for flushing. The inlet supply from each inlet can be controlled by control valves provided.

RESULTS AND CONCLUSION

By using this technique a family of five can save about 70-100 liters of water every day and if a community or society is adopting this method, the total water saved can be supplied to localities where quantity of water is less and quality is inferior.It's high time that people understand the value of water and do their bit to humanity by saving water. This is a simple cost effective technique, which is easy to adopt and has no secondary problems thus giving us a "Green solution to blue crisis".

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Fig.1: Indian type water closet

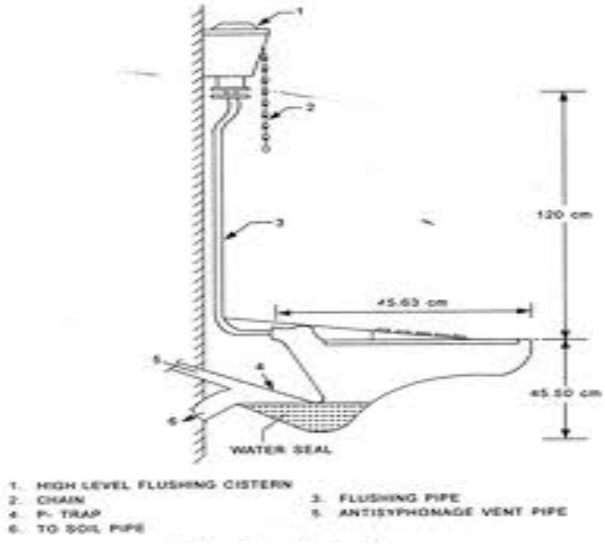

Fig.2: Structural drawing of Indian type water closet

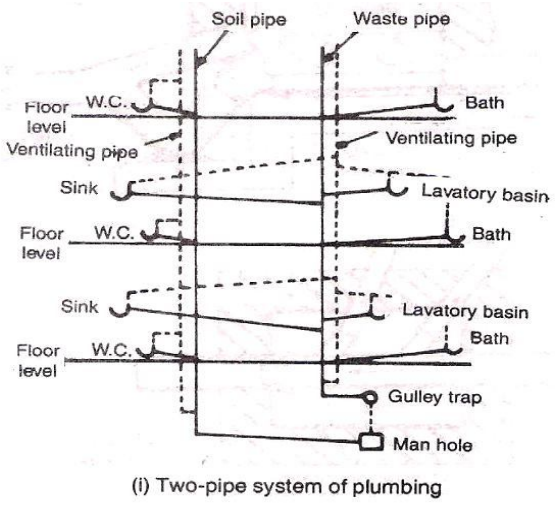

Fig. 3: Plumbing systems

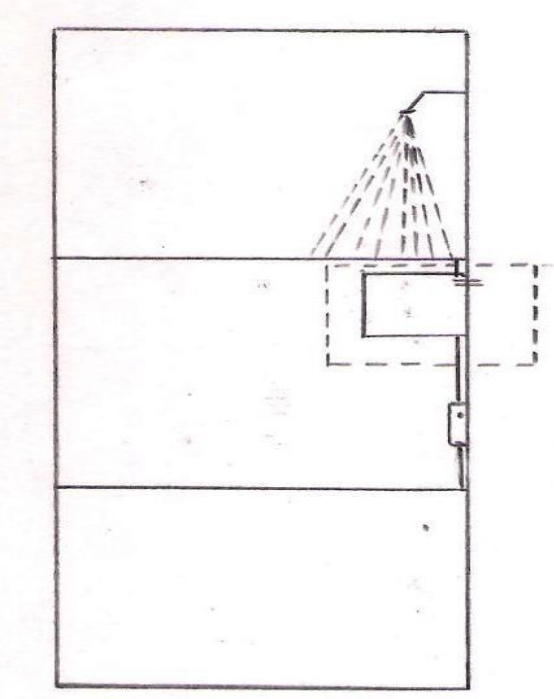

Fig.4: Collecting tank (lower floor)

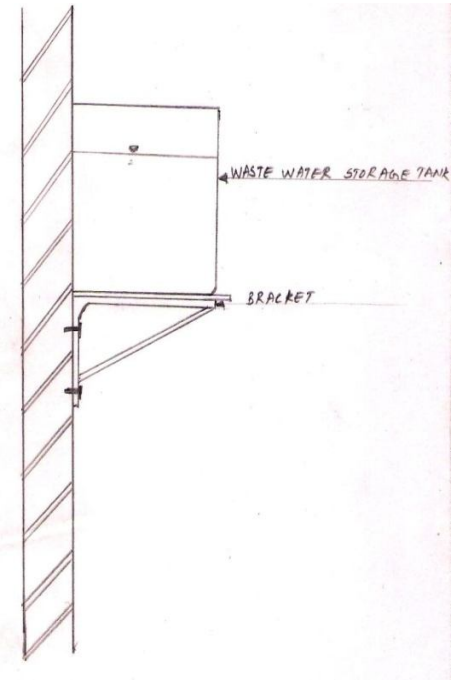

Fig.5: Waste water storage tank

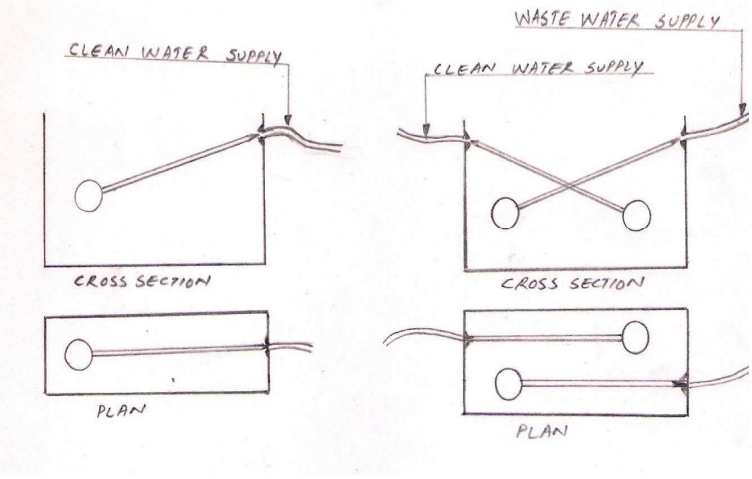

Fig.6: Extra inlet with floatplan

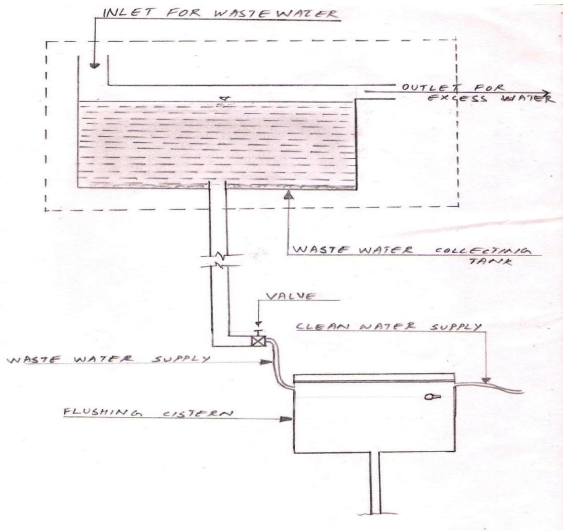

Fig.7: Inlet supply system

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RESEARCH ARTICLE

Impact of Vehicular Emission on Morphological Characteristics of NH-210 Road side Flora

Packiyaraj Rengasamy, M.Senthilkumar and Komalavalli Narayanasamy*

PG and Research Department of Botany, H.H.The Rajah's college, Pudukkottai, TamilNadu, South India.

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***Address for correspondence**
Dr. Komalavalli Narayanasamy,
Professor and Head, PG and Research Department of Botany,
H.H. The Rajah's college,
Pudukkottai, TamilNadu, India.
E.mail: nkomalavalli13@gmail.com.

ABSTRACT

The research was conducted from September to July 2011on the selected plants grown along NH-210 Road, designated as polluted site and along agricultural field, designated as control site. The study sought to ascertain plant responses to vehicular emission in terms of gross morphological changes in selected 14 most common plants indicating the presence of vehicular air pollutants in the environment and for the preliminary screening of the hyper-accumulation potentials of plants.The floristic survey of 75 km of busy road sides of a biodiversity-rich tropical zone, Pudukkottai District of TamilNadu, South India showed species differently tolerant to the stressful environment, which included exotics as well as medicinal plants.The botanical details and ecological potentials of the tolerant species found on these roadsides are discussed. Results revealed that leaf gross morphological changes were more observed in plants from the more polluted site than in the control site. Plants growing along roadsides are changed due to the stress of automobile exhaust emission with high traffic density in urban areas.These modifications can be considered as indicators of environmental stress.

Keywords: Vehicular emission, air pollution, plant morphology, roadside plants, tolerant species.

INTRODUCTION

Air pollution is one of the severe environmental problems, mainly in the developing countries due to rapid economic development, industrialization, population growth, unplanned urbanization and booming vehicular population.

Vehicular emission constitute amajor source of air pollution mostly arises from cars, buses, mini buses, wagons, rickshaws, motorcycles and trucks.The problem is much more aggravated due to narrow and congested roads, and old poorly maintained vehicles. These automobile sources introduces varieties of pollutants such as benzene, carbon monoxide, organic compounds, oxides of nitrogen and sulphur, hydrocarbon, ozone, particulate matters like ultrafine primary particles, smoke, metals (cd, co, cu, pb etc.,), hydrogen fluoride, peroxyacyl nitratesand inert dust [13] in to the environment is responsible for innumerable problems [7].

Pollutants came from the vehicular emission has a drastic impact on living and non-living components of the ecosystem, because air being an important and vital component of earth's environment and slight change in its composition can have varied effects on the growth, development and survival of different organisms on this planet [11, 13]. Vehicular emission has increased the level of metals in environmentwhich not only put adverse effect on the health of human beings and animals, but seriouslythreatening the flora of such areas. Automobile emission can directly affect the plants by entering in to the leaf, destroying individual cells, and reducing the plant ability to produce food [12].

Plant improves the quality of air in general [1,2] bycarbon dioxide sequestration and oxygen releasing through photosynthesis. Moreover it act as a sink for trapping and absorbing many gases, particulates, aerosols and airborne pollutants [4].Thus the road side vegetation are exposed to diverse pollutants play a significant role in assimilation and accumulation of pollutants and act as efficient interceptors of airborne pollutants. Road side vegetation of South India, are expected to be rich in unique pollution-tolerant species. Tolerant plants in heavy metal polluted road sides may be excluders or accumulators or hyper-accumulators of the metals. Phytosociology of communities on road sides is significant in the identification of the degree of tolerance of species, because the method in general, is considered efficient and appropriate to assess the ecological potentials of plants in natural communities.

Several authors have reported theeffects of air pollution on the morphology and anatomy of different plants species grown in different regions, but very little is known about the plants of Pudukkottai city. Researchers also reported that plants which are sensitive to air pollutants had showed changes in their morphology, anatomy, physiology and biochemistry [9,3]. Hence the present study was conducted to look into the effects of air pollution on the selected plant representatives of NH 210 road side taxa, specifically on morphological features.

MATERIALS AND METHODS

The research was conducted from September to July 2011on the selected plants grown along NH-210 high way road.This road begins in Trichy and go via Pudukkottai - Tirumayam - Karaikkudi -Devakottai -Devipattinam and ends in Ramanathapuram. This is a 160 KM (99 miles) road. The exact area taken for the study is from Trichy to Tirumayam (75 KM) and designated as polluted site. Plants were grown along agricultural field site with ideal ecological conditions was selected as the control (unpolluted) site. The floristic survey of 75 km of busy road sides was equally divided in to ten locations of 7.5 Km each.Three replicates of each plant samples were taken randomly in all the ten locations.The plant samples were taken to the laboratory for analysis. The plants studied in ten locations were 6 months to 3 years old.

Morphological measurements

The plant was carefully uprooted without damaging the root system in order to measure the height of the entire plant, root length, shoot length, root number, branch number, internode length, petiole length, leaf number, leaf length and leaf width. It was done for 3 plant samples for height of the entire plant, root length and root number and 20 plant samples for other parameters from each replicates.

Root length

Root length was taken from the point of root and stems transition to the tip of the tap root and expressed in centimeters. The lateral roots were counted and total root number was expressed per plants.

Shoot length

The length from shoot tip to the point of root-stem transition region was taken as shoot length and expressed in centimeters.The branches were counted and total branchnumberwas expressed per plants.

Root/Shoot Ratio

The root/shoot ratio was calculated by using below formula

$$\text{Root/Shoot Ratio} = \frac{\text{Root length of the plant}}{\text{Shoot length of the plant}}$$

Leaf area (mm2)

Leaf area was measured by Graph method.

RESULTS

One seventy five (175) species of 27 families were recorded from the floristic survey of 75 km of busy road sides from Trichy to Tirumayam. Most of the plants were invasive plants. According to the distribution and relative abundance 14 plants were identified as the most dominant or very common plants.The most common plants distributed throughout the NH 210 roads (Table-1) were *Acalypha indica*, *Boerhavia diffusa*, *Calotropis gigantea*, *Croton sparsiflorus*, *Pergularia daemia*, *Datura metel*, *Cleome viscosa*, *Heliotropium indicum*, *Leucas aspera*, *Ocimum sanctum*, *Parthenium hysterophorus*, *Tephrosia purpurea*, *Tridax procumbens* and *Wattakaka volubilis*.

Morphological measurements

The results of morphological measurements were given in Table 2-4.

Height of the Plants

Only three species (*Acalypha indica*, *Pergularia daemia* and *Wattakaka volubilis)* out of top 14 species shows significant difference in the plant height between road side and agricultural field grown plants. Remaining 11 species doesn't show any significant difference since these plants were adapted to the existing environmental conditions. More over these plants reaches maturity rapidly and produces large quantities of seed that are easily transported by vehicles, machinery, animals, fodder, pasture seed, stock feed and water and comes under the category of weeds. Thus significant change was not observed.

Root length, Shoot length and Root/Shoot Ratio

All the top 14 species show significant differences in Root length, Shoot length and Root/Shoot Ratio (Table 2). The root lengths in road side plants were high when compared to controlled plants. This may be due to the adaptation of the roadside plant to absorb food and water effectively under stress conditions. In contrast, the shoot lengths were high in controlled plants when compared to road side plants. The shoot length reduction may be due to the

deposition of dust and other vehicular emission particles. Thus the Root/Shoot Ratio was high in road side plants when compared to controlled plants. These traits were directly related to plant photosynthetic capacity or carbon gain. Specifically, higher carbon gain (both in biomass and carbon content), larger proportion of photosynthetic tissues, thicker leaves, larger stomatal size, higher stomatal density, and larger leaf vascular tissues were associated with the exotic species. Larger specific leaf area and higher nitrogen content on mass basis were found in the native species.

Root and Branch number and Internode and Petiole length

Our results revealed that there is a significant difference among the polluted and control plants (Table-3).The root and branch number was high in road side plants when compared to controlled plants. High number of root helps in the effective utilization of minerals and water during drought and rainy period there by increasing the number of branches which in turn maximize the biomass productivity.In contrast; we observed maximum internode and petiole length in controlled plants when compared to road side plants. In general, the availability of nutrients and water were higher for controlled plants than roadside plants. This may be the reason behind this.

Leaf number, Leaf length, Leaf width and Leaf area

The Leaf No. per 15 cm of twig were higher for road side plants when compared to controlled plants whereas leaf length, leaf width and leaf area were higher for controlled plants than roadside plants (Table-4).These traits were directly correlated to stress environment of road side plants.

DISCUSSION

In recently more attention is being focused on the global environment problems [5]. Environmental condition are becoming increasingly worse in India and it's major cities are plagued with environmental problems due to vehicular emission.The growth of plants could be affected by several reasons and one of them is the presence of toxic pollutants derived from the auto vehicular exhaust emission.The road side plants are significant reduction in different leaf variables in the polluted environment in comparison with agricultural site. Similar report was found on Jahan S., and Iqbal [6].The polluted plants in leaf area and leaf number may be due to decreased at the roadside plants. This similar result reported by Woodbury [16].The growth of road side plants shoot length, internode length and the total linear growth was reducing by vehicle emission pollutants. The same results were finding on Tiwari [15].The road side plants root growth and root length, root branches were reduction by vehicle pollutants. The similar report was observed by Jahan and Iqbal [10].The presence of toxic pollutants derived from the auto vehicular exhaust emission were depositedin shoot systemand also on the surface of leaves causing clogging of stomata and reduction in photosynthetic rate as reported by Muhammad Shafiq and Muhammad Zafar [8].

CONCLUSION

Vehicular emissions are one of the major sources of air pollutants and have a powerful negative effect on germination and growth of plants.These pollutants may induce plant responses leading to observable changes in their morophology.Plant responses may vary depending on the amount of pollutants to which they are exposed. Although this has been an intense area of research need further studies. More plants at young stage are encouraged to be planted along roadsides as potential species since they apparently indicate the symptoms of pollutant intake in terms of stomatal index and biochemical traits to improve local air quality.

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Table 1: List of very common NH 210 Road side flora.

S.No.	Taxa	Family	Life form	Status	Ecological Impact
1	*Acalypha indica*	Euphorbiaceae	Herb	Local weed	Adapted to a wide variety of environments and will out-compete most native plants for nutrients and moisture, reducing both native wildlife and plant diversity.
2	*Boerhavia diffusa*	Nyctaginaceae	Herb	Local weed	It is an exceptionally competitive weed and a few uncontrolled plants can cause minimal ecological losses.
3	*Calotropis gigantea*	Asclepiadaceae	Shrub	State weed	Seedlings are relatively poor competitors with grass species, but once established, the weed can become extremely invasive, especially on dry land sites, disturbed areas and roadsides.
4	*Croton sparsiflorus*	Euphorbiaceae	Herb	State weed	Spread and become established rapidly. This common milkweed is extremely difficult to control.
5	*Pergularia daemia*	Asclepiadaceae	Climber	Local weed	The most destructive impact is invasions on the road side shrubs or trees. It forms dense monotypic stands as it displaces native plants. Under optimum conditions, one plant can spread to cover entire sites in just one growing season. The plant's growth is generally too compact to offer cover, and cover may be as crucial to wildlife as food.
6	*Datura metel*	Solanaceae	Herb	State weed	The species can become competitive in cropland, but otherwise are more nuisance species than invasive. They can be an indicator that land is abused when these species start to appear in land.
7	*Cleome viscosa*	Capparaceae	Herb	Local weed	An aggressive species that quickly can develop into large, dense patches, thus reducing native plant communities. The plant colonizes rapidly, forming hedges. It may have allelopathic effects on neighboring plants.
8	*Heliotropium indicum*	Boraginaceae	Herb	Local weed	Become very weedy and form dense colonies, especially along waterways, ditches and roadsides in summers following wet falls. The numerous branches make flow of water very diffi cult.
9	*Leucas aspera*	Lamiaceae	Herb	State weed	Displace native plant communities by invading disturbed areas and undisturbed natural habitats. The medicinal value of the plant makes it quite noticeable, but it has little economic or ecological consequence.
10	*Ocimum sanctum*	Lamiaceae	Herb	Local weed	Grow singularly or in small patches in the northern and eastern road sides of NH210. The medicinal value of the plant makes it quite noticeable.

11	*Parthenium hysterophorus*	Asteraceae	Herb	Global noxious weed	Causes economic losses by reducing available forage, tainting the milk of cattle that graze it, and medically as a pollen source for allergies and asthma.
12	*Tephrosia purpurea*	Fabaceae	Un-shrub	State weed	Invade roadsides and waste areas. It spreads rapidly and can form very dense stands that crowd out desirable native species.
13	*Tridax procumbens*	Asteraceae	Herb	State weed	More competitive than most other native species and has the potential to infest large areas. It is tolerant to high salt concentration in soil. Although it grows best under moist conditions as most plants do, it can survive under drought conditions, which gives it a competitive advantage on semiarid rangeland.
14	*Wattakaka volubilis*	Asclepiadaceae	Climber	Local weed	The ability to produce millions of seeds in a small area, this plant is difficult to remove once it has established in an area.

Table 2: Plant height, Root length, Shoot length and Root/Shoot ratio of very common NH 210 roadside flora and Agricultural field grown flora.

Taxa	Height of the plant (Cm)		Root length (Cm)		Shoot length (Cm)		Root/ Shoot ratio	
	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field
A.indica	26.3±0.75	40.9±4.45	10.5±0.33	7.6±0.44	15.8±0.42	33.3±2.55	0.67±0.045	0.23±0.0022
B. diffusa	38.7±1.22	70.9± 6.21	10.5±0.41	8.2±0.52	28.2±0.97	62.7±5.46	0.37±0.023	0.13±0.0021
C.gigantea	182.9±3.89	220.5±11.12	57.3±2.45	30.2±2.75	125.6±1.87	190.3±7.84	0.46±0.021	0.16±0.0009
C.sparsiflorus	21.6±0.95	21.6±2.14	8.3±0.57	8.3±0.97	13.3±0.21	24.5±1.07	0.62±0.061	0.34±0.0010
C.viscosa	27.2±0.92	17.5±4.82	6.7±0.28	5.2±0.43	20.5±0.66	42.3±4.32	0.33±0.082	0.13±0.0014
D. metel	60.9±1.28	80.5±6.35	20.6±0.54	16.2±1.87	40.2±0.25	54.3±4.52	0.51±0.024	0.30±0.0034
H.indicum	34.9±1.52	53.5±12.18	12.4±0.64	9.9±0.75	22.5±0.96	43.6±3.76	0.55±0.011	0.23±0.0006
L. aspera	24.4±1.33	31.1±2.28	8.1±0.52	6.3±0.58	16.3±0.58	24.8±1.85	0.50±0.032	0.25±0.0058
O. sanctum	33.1±1.26	70.5±6.45	10.5±0.46	10.2±1.47	22.6±0.85	60.3±4.91	0.47±0.017	0.17±0.0015
P. daemia	890.5±9.05	1661.6±16.15	90.2±3.83	60.9±4.98	800.3±5.46	1600.7±10.81	0.11±0.027	0.04±0.0001
P.hysterophorus	31.2±0.14	52.0±4.55	13.4±0.44	8.6±0.98	17.8±0.54	44.4±3.64	0.75±0.028	0.19±0.0037
T. procumbens	23.7±0. 42	36.4±2.45	8.2±0.32	4.3±0.24	15.5±0.43	32.1±2.11	0.53±0.022	0.13±0.0036
T.purpurea	32.1±0.36	48.3±4.87	11.2±0.45	10.5±1.28	21.6±0.88	37.8±3.47	0.52±0.052	0.28±0.0077
W.volubilis	226.7 ±0.48	1491.2±17.36	40.5±2.94	98.7±7.25	186.2±2.84	1392.5±10.6	0.22±0.008	0.071±0.0001

Table 3: Root number, Branch number, Internode length and Petiole length of very common NH 210 road side flora and Agricultural field grown flora.

Taxa	Root No. per plant		Branch No.per plant		Internode length (Cm)		Petiole length (Cm)	
	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field
A.indica	9.5±0.23	3.7±0.22	15.3±0.86	9.2±0.82	0.4±0.006	1.7±0.03	0.73 ± 0.15	2.65 ± 0.52
B. diffusa	10.2±1.22	5.4±0.25	12.4±0.74	10.6±0.62	1.8±0.041	9.5±0.46	2.26 ± 0.38	5.51 ± 0.84
C.gigantea	27.7±3.40	18.2±0.66	18.2±0.92	22.8±1.84	6.2±0.057	10.2±0.98	2.4 ± 0.20	2.64 ± 0.18
C.sparsiflorus	35.2±2.47	20.2±0.90	7.5±0.56	20.5±1.23	1.0±0.006	2.1±0.32	0.5 ± 0.08	1.52 ± 0.26
C.viscosa	38.8±3.94	15.8±0.82	4.2±0.57	3.3±0.14	0.5±0.002	2.5±0.22	1.9 ± 0.12	3.73 ± 0.53
D. metel	22.6±2.08	15.1±0.79	4.2±0.45	6.3±0.43	8.2±0.075	12.5±0.62	5.42 ± 0.57	5.85 ± 0.67
H.indicum	23.2±2.64	16.8±0.62	4.3±0.51	7.4±0.95	1.3±0.025	4.1±0.22	2.71 ± 0.20	4.65 ± 0.42
L. aspera	41.3±3.55	25.3±1.20	16.8±0.24	25.2±1.32	2.5±0.041	5.2±0.42	0.52 ± 0.06	1.22 ± 0.03
O. sanctum	27.8±2.47	16.5±0.85	8.2±0.22	20.3±0.95	0.8±0.002	2.3±0.11	0.45 ± 0.01	2.16 ± 0.42
P. daemia	12.6±1.93	7.1±0.56	15.2±0.87	30.8±1.89	9.7±0.082	15.7±0.75	2.92 ± 0.62	5.21 ± 0.58
P.hysterophorus	31.4±3.12	18.8±0.67	12.1±0.48	18.6±0.72	1.3±0.004	5.6±0.31	3.5 ± 0.88	6.71 ± 0.56
T. procumbens	13.5±1.42	7.6±0.36	7.5±0.11	15.8±0.88	1.2±0.009	7.5±0.47	2.0 ± 0.18	2.56 ± 0.32
T.purpurea	40.8±3.81	23.7±1.11	10.6±0.59	22.7±1.12	2.7±0.084	4.9±0.25	1.7 ± 0.27	2.72 ± 0.55
W.volubilis	12.6±1.35	7.1±0.86	12.6±0.38	16.8±0.86	5.2±0.027	10.3±0.74	2.8 ± 0.25	2.92 ± 0.47

Table 4: Leaf number, Leaf length, Leaf width and Leaf area of very common NH 210 road side flora and Agricultural field grown flora.

Taxa	Leaf No. per 15 cm of twig		Leaf length(Cm)		Leaf width (Cm)		Leaf area (Cm^2)	
	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field	Roadside	Agri-field
A.indica	37.5±2.41	9.2 ± 0.64	2.3±0.012	5.6±0.36	3.7±0.06	4.1 ± 0.12	2.82±0.45	19.1±0.96
B. diffusa	8.3± 0.45	1.6 ± 0.35	2.1±0.016	5.2±0.40	3.5±0.45	4.0 ± 0.22	2.66±0.56	12.3±0.84
C.gigantea	2.4± 0.79	1.5 ± 0.20	6.0±0.054	17.6±0.63	9.9 ±0.91	9.5 ± 0.67	14.43±0.82	82.8±2.45
C.sparsiflorus	16.5± 0.61	7.1 ± 0.33	1.3±0.021	3.4±0.12	1.1±0.32	2.0 ± 0.35	1.05±0.28	5.4±0.25
C.viscosa	30.8± 1.06	6.1 ± 0.23	1.4±0.013	2.5±0.42	0.5±0.20	0.9 ± 0.04	2.68±0.11	2.8±0.14
D. metel	1.8± 0.56	1.2 ± 0.42	6.0±0.047	16.2±0.86	5.5±0.65	14.9 ± 0.92	17.80±0.91	63.6±2.150
H.indicum	11.5± 0.96	3.7 ± 0.28	2.7±0.046	9.5±0.59	3.2 ±0.22	4.6 ± 0.26	3.20±0.10	42.2±1.58
L. aspera	7.8± 0.31	2.9 ± 0.26	2.0±0.025	3.9±0.22	0.5 ±0.42	1.0 ± 0.01	0.84±0.005	4.3±0.172
O. sanctum	19.8± 1.16	6.5 ± 0.42	0.9±0.002	5.5±0.38	0.8 ±0.11	2.0 ± 0.13	0.39±0.002	4.2±0.12
P. daemia	1.6±0.36	1.0 ± 0.56	2.6±0.085	4.5±0.54	4.7±0.74	5.6 ± 0.47	6.22±0.62	14.0±0.88
P.hysterophorus	12.5± 0.67	2.7 ± 0.22	1.8±0.004	8.5±0.69	0.2 ±0.30	1.0 ± 0.04	0.38±0.001	4.0±0.16
T. procumbens	13.5± 0.74	2.0 ± 0.32	1.3±0.052	4.7±0.28	2.0 ±0.43	2.6 ± 0.25	1.12±0.25	7.2±0.68
T.purpurea	5.6± 0.66	3.1 ± 0.21	2.5±0.594	2.9±0.42	0.5 ±0.21	0.7 ± 0.02	3.95±0.52	8.0±0.58
W.volubilis	1.2± 0.25	1.5 ± 0.35	4.5±0.072	6.9±0.62	7.3±0.72	9.8 ± 0.75	8.50±0.89	22.3±0.94

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2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
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