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Preface

We would like to present, with great pleasure, the inaugural volume-3, Issue-3, March 2017, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Agricultural Sciences	
Soil Science	Plant Science
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Agricultural Chemistry	Basic biology concepts
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Agricultural Management Practices	Agricultural Technology
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Crop Production	
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Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
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Dairy Sheep	Water Buffalo
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Effect of Grazing Land Improvement Practices on Herbaceous production, Grazing Capacity and their Economics: Ejere district, Ethiopia

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Abstract— *The effects of different grazing land improvement practices on herbaceous production, grazing capacities and their economics were studied in Ejere district, west Shoa zone, Ethiopia. Four different treatments, i.e., application of Urea and Diammonium phosphate (DAP), cattle manure, wooden ash, and a control/no application) were randomly applied to the study plots in three replications for each treatment. All experimental plots were fenced throughout the study period. The application of urea and DAP significantly increased grass (3620.86 kg ha^{-1}) and total biomass production (5742.93 kg ha^{-1}). Of the 6 herbaceous species recorded in the Urea and DAP plots, four of them were grasses with Setaria verticellata having the highest percentage composition (35.54%) while the control plot was dominated by Cyperus rotundus (31.5%) and Cerastium octandrum (31.5%). Less land is required to maintain a tropical livestock unit (TLU) in Urea and DAP applied plots (0.03 ha TLU^{-1}) than in plots applied with other treatments (mean = 0.09 ha TLU^{-1}). Similar to the result of the biological data, the participants of the grassland day rated the Urea and DAP applied treatment best because of the high production of grass. Considering total biomass production, application of manure was advantageous to the farmers due to increased net benefits and the marginal rate of return is above the minimum accetable rate for this sort of treatment. On the other hand, considering grass production alone, application of Urea and DAP was more profitable for farmers as far as they store and sell it in the dry seasons. In conclusion, we recommend a long-term study to examine the effects of the different treatments on productivity of grazing lands, herbaceous species composition, grazing capacities, livestock, the environment, and their economics.*

Keywords—* *Ash, grazing land improvement, manure, Urea, DAP.

I. INTRODUCTION

Ethiopia holds the largest livestock population in Africa estimated at about 54 million heads of cattle, 25.5 million sheep, 24.06 million goats, 0.92 million camels, 4.5 million donkeys, 1.7 million horses, 0.33 million mules, 54 million chicken and 4.9 million beehives [1]. It is also among the 28 smaller countries (25 in Africa) where grazing land accounts greater than 60% of the total land area [2]. Despite these huge resources, the productivity of livestock in general is low and its contribution to the national economy is below expected. Among the major problems affecting livestock production and productivity in Ethiopia, feed shortage in terms of quantity and quality is the leading problem [1].

The major feed resources in Ethiopia are natural pasture (grasslands) and crop residues with varying proportion among the different zones of the country. Similar to the other parts of Ethiopia, the role of grazing lands as a major livestock feed resources is diminishing from time to time because of natural and human induced factors (increased conversion of grazing lands to crop land) which created heavy grazing pressure on the remaining grazing lands although the extent of degradation varies from site to site [4, 5, 6]. In addition, grazing land improvement practices are relatively less common particularly in the highlands of Ethiopia owing to the lack of awareness and appropriate training, lack of appropriate improvement methods and little attention given to grazing lands by the agricultural extension system. The pressure is likely to intensify in the coming decades creating more pressure on the remaining grazing lands justifying the need to improve the available remaining grazing lands to increase their livestock holding capacity [1]. Thus, the current study examined the effect of applying different grazing land improvement techniques on biomass production and herbaceous species composition, grazing capacities of the grazing lands and the economics of the different treatments. This paper will contribute to better understanding of grazing land rehabilitation techniques in Ethiopia and for similar ecosystem elsewhere.

II. MATERIALS AND METHODS

2.1 Description of the study area

This study was carried out in Ejere district, West Shoa zone, Ethiopia. The district was selected due to its potential for livestock production (dairy, small ruminant, poultry and apiculture) and it is one of the intervention districts for Livestock and Irrigation Value Chain for Ethiopian smallholders (LIVES) project of the International Livestock Research Institute (ILRI). The altitude ranges from 2063 to 3158 meters above sea level (m.a.s.l). The rainfall of the area is distinctly bimodal pattern, viz-a-viz. the main rainy season occurs from June to end of September and the short and small rainy season is in February and March. The mean annual rainfall ranges from 900 to 1200 mm.

The livelihood of the communities in the study district is based on mixed crop-livestock production system and the human population is about 104 709 (49 829 males and 55 057 females) [1]. The livestock population is estimated to be: 119 854 cattle, 37 423 sheep, 11 600 goats, 9436 mule, 356 donkey, 10 117 horses and 43 125 poultry. Crop residues, natural pasture, improved forage, hay, agro-industrial by-products and others contribute as livestock feed [1]. The district has upland and wetland grazing areas. In addition to grazing, the wetlands are the sources of water for livestock and irrigation for lower riparian's [6]. Particularly, the Berga wetland is one of the two known breeding sites (Weserbi-near Sululta and Berga) for the globally threatened White-winged Fluff tail Sarothrura ayresi [7]. With regard to grazing land ownership, there are two types, i.e., private and communal although the former is larger (80%) than the latter in terms of area coverage (20%) at the current moment while the opposite was true in the past. The private grazing lands (0.25 to 0.5 ha/household on average) are used for hay making and/or grazing [8].

2.2 Site selection

In site selection for the study, which was undertaken with the help of farmers, livestock experts and development agents, the representativeness of the site for grazing lands in the mid altitude (2378 m.a.s.l), and poor herbaceous production condition of the site was taken into consideration.

The treatments described in this study are mainly based on locally available resources (manure, wood ash, enclosing) except Urea and DAP which is imported and can be purchased at the service cooperative level in the villages. Fifteen plots of 4 m x 4 m were laid out to apply 4 treatments (Urea and DAP, wooden ash, cow manure, and untreated/control) randomly in 3 replications. All plots were fenced during the main growing season (June to November, 2015). The distance between plots and replications/blocks was 1 and 2 meters, respectively. The amount of urea and DAP, ash and dry manure applied on 16 m^{-2} plots were 0.24 kg and 0.16 kg, 4.8 kg and 12 kg, respectively. The plots were ripped to incorporate the treatment materials into the soil. The manure, obtained from farmers was decomposed for three months at backyards of farmers and dissolved in water and added into the soil in form of slurry. Wood Ash from farmers was scattered over the plots. Urea and DAP was over sown by broadcasting. The treatments were applied after the beginning of main rainy season. At the end of the growing season, the different plots were harvested using hand sickles and sorted into grass, and non-grass components. Furthermore, they were sorted into different species using field guide [9] and experienced technician from Adami Tulu research center, Ethiopia. The sorted materials were oven-dried at 65 ^oC for 72 hours.

2.3 Organization of grassland day and field assessment

Thirty male and nine female model farmers and 23 male and 2 female extension staff drawn from 4 districts (Ejere, AdaBerga, Meta-Robi and Dendi of west Shoa zone) and west Shoa zonal office attended the grassland day which was organized with the objective of creating awareness on the importance of improved grazing land management for the public.

2.4 Statistical and economic analyses

Analysis of variance (ANOVA) was conducted to verify the significant differences among the treatments using the STATA/SE 14 program. The formula proposed by Moore et al. [10], modified by Moore and Odendaal (1987) [11] and Moore (1989) [12], was used for grazing capacity estimation by taking in account the grass and total biomass yields. The equation is as follows:

$$Y = d/(DM \times f)r$$

where Y is the grazing capacity (ha TLU^{-1}), d the number of days in a year (365), DM the grass and total biomass DM yield (kg ha^{-1}), f is the utilization factor, r the daily grass DM required. The grazing capacity was calculated using tropical livestock unit (TLU) which is an animal weighing 250 kg and consuming 2.5% of its body weight. Thus, each TLU will consume 6.25 kg of forage dry matter daily and utilization factor of 0.5 (50%) was used [13].

The partial budget analysis (economic analysis) was done according to Upton 1979 [14] and CIMMYT 1988 [15] to determine economic benefit of the different treatments. Total variable cost, total return (TR), net benefit (NB), change in net benefit and marginal rate of return (%) were calculated for total biomass and grass production separately as grass is the most important feed resource for cattle and sheep. Furthermore, the economic analysis was undertaken considering the price of baled hay at harvest time and during the dry season.

III. RESULTS

3.1 Biomass production, herbaceous species composition and grazing capacities

Grass dry matter yield was highest (P<0.02) in Urea and DAP treated plots (Table 1). Compared with the control, application of ash or manure increased grass production although it was non-significant (P>0.05). On the other hand, the non-grass biomass was the highest in manure-treated plots although not significant (P<0.05). Application of ash produced more non-grass biomass than the control. Urea and DAP application significantly increased (P<0.02) total biomass production (TBP). While the control was the least in TBP, ash and manure applications were comparable in TBP.

TABLE 1
APPLICATION OF DIFFERENT TREATMENTS ON MEAN HERBACEOUS DRY MATTER PRODUCTION ($kg\ ha^{-1}$) AND GRAZING CAPACITIES ($ha\ TLU^{-1}$)

Treatments	Grass	Non-grass	Total biomass (TB)	GC (grass) (ha/TLU)	GC (TB) (ha/TLU)
Control (no treatment)	1042.7^{b}	1786.7^{b}	2829.3^{c}	0.11	0.04
Ash	1170.7^{b}	2773.3^{b}	3944^{bc}	0.09	0.03
Urea and DAP	3620.80^{a}	2122.13^{b}	5742.93^{a}	0.03	0.02
Manure (cow)	1716^{b}	2986.7^{b}	4702.7^{ab}	0.07	0.03
SEM	151	121.6	178		
Significance level	0.0155	0.2361	0.0168		

Means with different letters down the column are significantly different (P<0.05); SEM= standard error of the mean.

Cyperus rotundus (31.57%) and *Cerastium octandrum* (31.57%) were the highest in percentage herbaceous species composition in the control plot while *Setaria verticellata* (35.54%) in Urea and DAP applied plots (Table 2). The ash applied plots were dominated by *Cerastium octandrum* (35.16%) and *Trifolium ruppellianum* (35.16%) which are non-grass species. The most abundant herbaceous species in the manure applied plot were *Cerastium octandrum* (28.87%) and *T. ruppellianum* (28.87%). The *T. ruppellianum* is a legume palatable to livestock.

TABLE 2
PERCENTAGE COMPOSITION OF THE DIFFERENT HERBACEOUS SPECIES IN THE TREATMENT GROUPS

Herbaceous species	Control	Ash	Urea and DAP	Manure
Hyperhenia rufa	5.76	3.63	3.44	
Cyperus rotundus	31.57	0.00	28.15	5.77
Eragrostis tenuifolia	6.91	8.48	18.34	13.84
Andropogon abysincus	11.52	3.03	5.73	10.06
Cerastium octandrum	31.57	35.16	0.00	28.87
Setaria verticellata	12.67	10.90	35.54	12.58
Trifolium ruppellianum		35.16	8.80	28.87
Commulina benegalensis		3.63	0.00	
	100.00	100.00	100.00	100.00

The land required per TLU ($ha\ TLU^{-1}$) was the lowest for the Urea and DAP applied plots while it was the highest for the control plot (Table 1).

3.2 Assessment and perception of the community

The participants of the grassland day selected plot with Urea and DAP application as the most preferred one which was followed by plots treated with manure. The reasons for their choice of the Urea and DAP applied plot was that it favored the production of grass than other herbaceous plants which corresponds with the herbaceous biomass data.

3.3 Economic Analysis

The partial budget analyses undertaken considering two time periods and total biomass and grass biomass are shown in Tables 3 and 4. Considering the total biomass production, both at the time of harvest and during the dry season (Table 3), application of ash was advantageous to the farmers as the marginal rate of return was so attractive to invest more. Subsquently, it will also be interesting for farmers to invest more in manuring their land as it helps them to fetch additional income though the cost was higher. The dry season would be more encauraging since the price of hay will be better. The next treatment (DAP and Urea) did not yield better net benefits if hay is to be sold in the harvest season. For that of the dry season, the marginal return was not so convincing and it is less likely to shift to this treatment. Considering grass production (Table 4) as a result of Urea and DAP application, during the harvesting time, the marginal rate of return was 53%, while during the dry season the MRR was 113% which is more attractive for farmers to invest in fertilizing, and is the most economical option combined with enclosing the field. Therefore, the decision is sensitive to the price of hay, and hence it is advisable to store and sell in the dry season.

TABLE 3
GRAZING LAND IMPROVEMENT OPTIONS, COSTS AND RETURNS BASED ON TOTAL BIOMASS IN ETHIOPIAN BIRR (ETB) AND USD WHERE 1 USD = 22.63 ETHIOPIAN BIRR AND THE NUMBER IN BRACKETS IN THE TABLE ARE IN USD

Improvement options	Cost of fertilizer	Labor cost *	CVT	Herbaceous production (bales/ha)	Sell of hay harvest time	Sell of hay (dry season)	NB (HT)	NB (DS)	MRR1 (%) (HT)	MRR2 (%) (DS)
Enclosing	0	1381.5 (60.1)	1381.5 (60.1)	166.4	5991.5 (264.8)	8321.5 (367.7)	4610.0 (203.7)	6940.0 (306.7)		
Enclosing + Ash	0	2276.0 (100.6)	2276.0 (100.60)	232.0	8352.0 (369.1)	11600.0 (512.6)	6076.0 (268.5)	9324.0 (412.8)	164	267
Enclosing + manure	0	3213.04 (142)	3213.0 (142)	276.6	9958.7 (440.1)	13831.5 (611.2)	6745.6 (298.0)	10618.5 (469.2)	72	138
Enclosing + Urea and DAP	3065 (135.4)	2777.56 (122.70)	5842.6 (258.1)	337.8	12161.5 (537.4)	16891 (746.4)	6318.9 (279.2)	11048.4 (488.2)	-	16

**** Fencing, fertilizing, manuring, harvesting, transport and storage***

HT-price at harvest time = 36 ETB/bale (1.59 USD/bale), DS-price in dry season = 50 ETB/bale (2.21 USD/bale), CVT = Costs that vary by treatment, NB = Net benefit, MRR% Marginal rate of return in percent

TABLE 4
GRAZING LAND IMPROVEMENT OPTIONS, COSTS AND RETURNS BASED ON GRASS PRDUCTION IN ETHIOPIAN BIRR (ETB) AND USD WHERE 1 USD = 22.63 ETHIOPIAN BIRR AND THE NUMBER IN BRACKETS IN THE TABLE ARE IN USD

Management Option	Cost of fertilizer	Labor cost*	CVT	Herbaceous production (bales/ha)	Sell of Hay (HT)	Sell of Hay (DS)	NB (HT)	NB (DT)	MRR % (HT)	MRR % (DT)
Enclosing	0	1381.46 (60.1)	1381.5 (60.1)	61.3	2208.1 (97.5)	3066.7 (135.5)	826.6 (36.5)	1685.31 (74.5)		
Enclosing + Ash	0	2276.0 (100.6)	2276 (100.6)	68.9	2479.1 (109.6)	3443.2 (152.2)	203.1 (8.1)	1167.2 (51.6)		
Enclosing + Manuring	0	3213.04 (142)	3213.0 (142)	100.9	3633.9	5047.1	420.9	1834.0		71.2
Enclosing + Urea and DAP	3065 (135.4)	2777.56 (100.6)	5842.6 (258.2)	213.0	7667.6 (338.8)	10649.4 (470.6)	1825.0 (80.7)	4806.9 (212.4)	53.4	113.1

*** Fencing, fertilizing, manuring, harvesting, transport and storage**

IV. DISCUSSION

The significant improvement in grass production in response to chemical fertilizer application is document in literatures. For instance, increase in grass production with Urea application which is an excellent sources of soluble nitrogen was reported by Cameron et al. [16] and Gagnon *et al*. [17] and nitrogen is also known as the most limiting nutrient for pasture production[16]. Furthermore, the findings of other researchers like Cinar *et al*. [18]; Ayan and Acar (2008) [19] revealed increase in forage production in response to Urea/nitrogen and DAP applications. The higher non-grass biomass in manure and ash applied plots although non-significant among the treatments is possibly associated with mineral composition of the treatments though not investigated in this study [20].

Cyperus rotundus is a species of sedge (Cyperaceae) found in all tropical, subtropical and temperate regions of the world (native to Africa, southern and central Europe and Southern Asia [21] and cattle feed on it when they are forced. *Cerastium octandrum* is an annual herb which is less palatable to livestock and indicator of degraded grazing land. *Seteria verticellata* grows in disturbed (due to overgrazing, trampling or drought) and damp soil. It is also an annual and palatable grass [22]. Although long-term experiments are required to separate the effects of the different treatment on herbaceous species composition, Urea application encourages the production of grasses as evidenced in this study [16, 17]. This result was also supported by the perception of the participants of the field day which justified the findings of the biological study and acted as one means of disseminating knowledge of improved grazing land technologies among different actors. Mamusha (2007) [23] justified the importance of improved flow of information and knowledge to, from and within the extension system as the key to realize agricultural transformation.

The mean grazing capacities revealed that more land was required to sustain a TLU on the control, manure and ash plots than on the Urea and DAP applied plots which is a direct reflection of the increased production in response to Urea and DAP application than in the other treatments which is in agreement with the findings of others [16, 17].

It was shown in the results that the net benefit and minimum MRR (%) favoured manure treated plots when considering total biomass production while it was better for Urea and DAP applied plots when taking into account grass production alone. The cost incurred for the former was lower than the latter and the non-grass production was higher numerically in the former than in the latter. Previous studies in Ethiopia indicated increasing price of grass hay from time to time [24, 25] which favored the Urea and DAP applied plots when considering grass production alone. Nevertheless, as this economic analysis is based on one season data, it is essential to undertaken detailed economic analysis by conducting research for more years and in different seasons of the year.

V. CONCLUSION

The study revealed local wooden ash, cow manure, urea with DAP applications improved total biomass production. Further long-term study is required to examine the effects of the different treatments on productivity of grazing lands, livestock productivity, soil and plant nutrients and economic considerations.

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CdTe quantum dots/Poly (diallyl dimethyl ammonium chloride) multilayer films: preparation and application for gaseous sensors

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Abstract— *CdTe quantum dots (QDs)/Poly (diallyldimethylammonium chloride) (PDDA) multilayer films (QDMF) have been self-assembled by layer-by-layer (LBL) technique. CdTe quantum dots (QDs) were synthesized by using Te,* $NaBH_4$*, and* $CdCl_2$ *as precursors and mercaptopropionic acid (MPA) as stabilizer. The as-prepared composites were characterized by transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FTIR), UV-vis adsorption spectrum(UV-vis), and Fluorescence spectrum(FS), respectively. It was shown that the self-assembled QDMF in this study could be used as gaseous sensors for detecting organic gases, such as ammonia, acetone, methanol and formaldehyde. The quenching mechanism of CdTe QDs multilayer films by formaldehyde was studied in detail and The detection limit was 10-236ppm.*

Keywords— CdTe quantum dots, gaseous sensor; PDDA, QDMF.

I. INTRODUCTION

A trend in current sensor development is miniaturization to obtain inexpensive and compact gas sensors that are robust and safe, have low power consumption and enable multiplexing of sensor arrays and remote sensing [1, 2]. Gas sensors play a key role in a variety of fields , such as environmental pollution monitoring, industrial process monitoring, leak detection of explosive gases, and medical breath analysis. In order to increase sensitivity, devices with nanostructure for gas sensors has shown lots of advantages[3].

From previous literatures, we can find that there are many gas detecting methods so far. But, compared with those traditional gas detection techniques, which are often costly, low sensitivity and time-consuming, semiconductor sensors have drawn a great deal of attention in recent years for its unique optical advantages. Semiconductor luminescent nanocrystals, known as “quantum dots” (QDs), exhibit unique optical and electronic properties, including high luminescent quantum yields, tunable emission, high photostability and relatively long emission lifetime. All these advantages explain the reason of its presence [4]. Furthermore, it has been used for energy conversion and storage[5-8], fabrication of optoelectronic device[9], and particularly, sensor applications[10-12].

Recently, a number of QD-based sensors have been reported for ions, [13] biomacromolecules, [14-16] and small organic molecules. [17] And, CdTe QDs have been proved to be a promising material as elemental building blocks for the next generation of nanodevices [18, 19]. Self-assembled multilayers by layer-by-layer (LBL) method are stable, well-ordered, easy to prepare and low cost, and have been extensively applied for the constructions of chemo- and bio-sensors [20, 21]. Another important feature of LBL is recharging of the surface at every step of the adsorption self-assembly, which results in oppositely charged molecules to be adsorbed in the next step with molecular order during the films fabrication process. Therefore the stable LBL method can encapsulate the QDs efficiently into flexible nanofilms (thickness below 60 nm) [22]. It has been also successfully applied to the preparation of multiplayer films of polyelectrolytes with other materials such as proteins, graphite oxides, gold colloids, dyes and nanoparticles [23]. Many groups have developed QDs-based sensing systems for the detection of Hg^{2+} [24-26] and Cu^{2+} [27-29] based on the fluorescence quenching of QDs.

In this paper, CdTe QDs multilayer films was fabricated by the standard LBL assembly technique. The CdTe nanoparticles with negative charged in the presence of MPA can serve as the anionic entity needed in the multilayer fabrication technique.

Besides, The effects of assembling methods, concentration of PDDA and layer number of films on fluorescence intensity of QDMF have been studied.

II. MATERIALS AND METHODS

2.1 Materials

Cadmium chloride (A. R.), sodium borohydride ($NaBH_4$, A. R.), tellurium powder (H.R.), mercaptopropinic acid (MPA, >90%) and sodium hydride (A. R.) were purchased from Sinopharm Chemical Reagent Company, Tianjin Guangfu Fine Chemical Research Institute, Tianjin Delan Fine Chemical Factory, Shanghai Jifeng Biotechnology Company and Tianjin Kermel Chemical Reagent company respectively. Poly (diallyl dimethyl ammonium chloride) (PDDA, G. R.) was obtained from Sigma Chemical Co. All regents were used as received without further purification.

2.2 Synthesis of CdTe QDs coped with MPA

The method for the preparation of NaHTe was described elsewhere[30,31] with a few modifications. 40 mg of sodium borohydride was put into a small flask, followed by adding 0.5mL of secondary distilled water. After 15 mg of tellurium powder was added in the flask, the reacting flask was rapidly sealed via a rubber plug with a small long syringe pinhead inserted into the flask to discharge pressure from the resulting hydrogen. After 4-5 hours, the black tellurium powder disappeared and a white sodium tetraborate precipitate appeared at the bottom of the flask. The resulting NaHTe aqueous solution with light pink was obtained.

The CdTe QDs were prepared using a simple refluxing route in aqueous solution with some improvement [32]. The synthesized NaHTe was added into $CdCl_2$ (0.173 mmol/mL) solution at pH 9.1 in the presence of MPA (80 μL) in N_2 atmosphere. The CdTe precursor solution was heated to 96℃ at different refluxing times. The whole process was carried out in N_2 atmosphere.

2.3 Preparation of CdTe quantum dots/ Poly (diallyl dimethyl ammonium chloride) Multilayer films (QDMF)

The glass substrates were pretreated according to the literature [33]with some modifications. The glass chips were ultrasonically washed with pure water and boiled for 30 min, after being immersed in H_2SO_4 /H_2O_2 solution with the volume ratio of 7:3. Finally, the chips were washed with DI water and dried with nitrogen. QDMF were prepared using a layer-by-layer procedure[34]. The chips were dipped in a PDDA aqueous solution for 20 min to modify a monolayer of positive PDDA. Then the modified substrates were rinsed with DI water several times to remove the physically absorbed PDDA, and dried under a stream of nitrogen. Then the chips were immersed in CdTe QDs aqueous solution to charge negatively QDMF were formed by repeating these steps in a cyclic fashion. The principle of multilayers' self-assembling was based on the electrostatic interaction between negatively charged QDs and positively charged PDDA.

2.4 Characterization

The transmission electron microscope (TEM) images were taken with a Hitachi-7650 electron microscope operated at an acceleration voltage of 100kV. Fourier transform infrared spectroscopy (FTIR) was performed using a FTIR-650 spectrometer. The UV-vis adsorption spectral values were measured on a Helios-γ spectrophotometer. Fluorescence experiments were performed with the help of F-380 spectrofluorimeter.

2.5 Quenching

In this process, the prepared QDMF was taken in a bottle containing a certain amount of organic gases. After 40 minutes' standing, the change of fluorescence intensity of QDMF was detected by spectrofluorimeter to investigate the concentration of organic gases. Finally, the result of the detection reflects sensitivity of prepared samples.

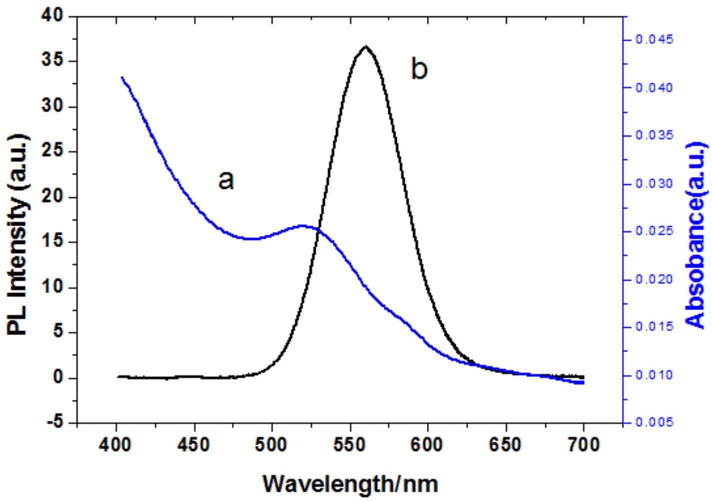

FIG. 1. UV-VIS ABSORPTION (a) and PL (b) SPECTRA OF AS-PREPARED CdTe AQUESOUS SOLUTION AT THE REFLUXING TIME OF 4H WITH EXCITATION WAVELENGTH OF 390 nm

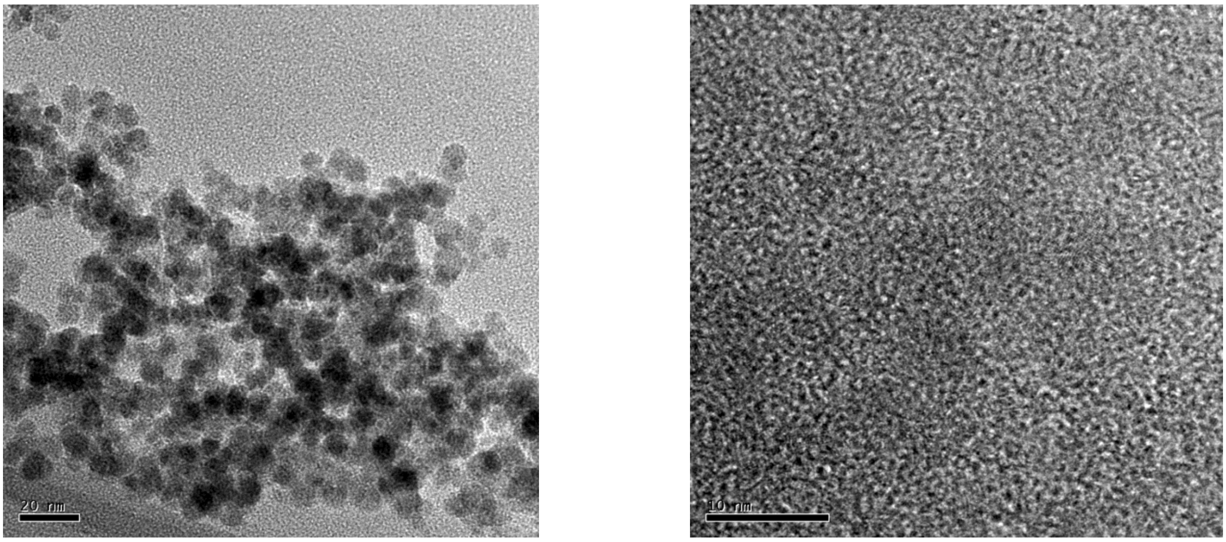

FIG. 2. TEM IMAGES OF CdTe QDS (A), CdTe LATTICE STRUCTURE (B)

III. RESULTS AND DISCUSSION

3.1 Optical feature of CdTe QDs and QCMF

Fig. 1 presents the typical absorption and PL spectra of CdTe QDs aqueous solution. It was found that the fluorescence intensity of CdTe quantum dot with 4 hours refluxing time was comparatively strong.The PL emission wavelength of QDs was 563 nm with a relatively narrow half peak width and best crystal quality, which is consistent with the literature report [35].

The distinct absorption (a) and PL (b) peaks of 0.024 a.u. and 38a.u. indicate that the QDs were highly monodispersed. The absorption shoulder of CdTe QDs was located at 525 nm, while the emission spectra display an emission maximum around 563 nm upon excitation at 390 nm. The size of CdTe QDs is estimated to be ca.3.4 nm in virtue of the following empirical formula according to previous report [31]:

$$D = \left(9.8127\times10^{-7}\right)\lambda^3 - \left(1.7147\times10^{-3}\right)\lambda^2 + \left(1.0064\right)\lambda - 194.84 \quad (1)$$

Where D (nm) is the size of CdTe QDs, and λ (nm) is the wavelength of the first excited absorption peak of the corresponding sample. The TEM image of CdTe QDs is shown in Fig. 2. The QDs was deposited with a high monodispersion without particle agglomeration, which corresponds to the Fig. 1, and the size of QDs is in line with the estimated results from equation 1.

3.2 Effect of assembling conditions on FL intensity of QDMF

3.2.1 Assembling styles

Two different assembling methods of QDs with PDDA were compared. First, aliquot of PDDA and CdTe QDs were dipped onto a glass ship by a pipette [32]. Second, the cleaned glass chip was immersed in positively charged PDDA and negatively charged QDs aqueous solution alternately [10]. It was found from Fig. 3 that the PL intensities of QDMF prepared by the two methods were 174 nm (a) and 123 nm (b), respectively, which indicates that the dip method was better than another one. In this study, the dip method was adopted for further experiment

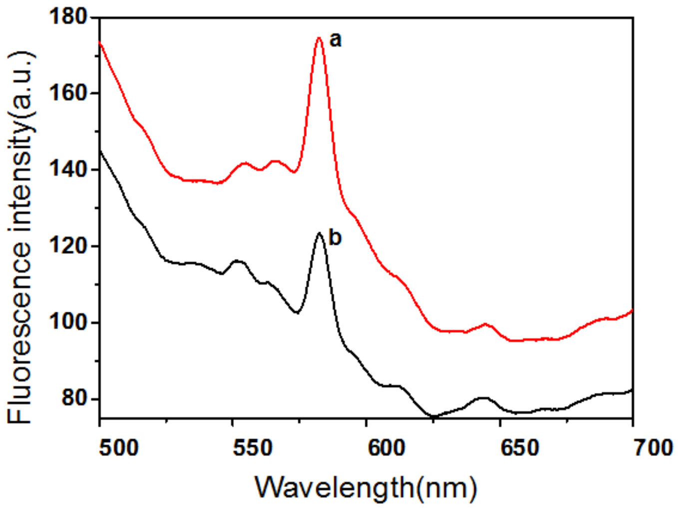

FIG. 3. FLUORESCENCE INTENSITIES OF QDMF BY DIFFERENT ASSEMBLING METHODS: (A) DIPPING QDS AND PDDA SOLUTION ONTO THE GLASS CHIPS AND (B) IMMERSING THE GLASS CHIPS INTO QDS AND PDDA SOLUTION. THE CHIPS WERE DEPOSITED 6 TIMES FOR EACH CONDITION.

3.2.2 The concentration of PDDA

The influences of the concentration of PDDA on the PL intensity of QDMF are shown in Fig. 4. Note that the PL intensity of the QDMF increased remarkably with the increasing concentration of PDDA and reached a maxiumum at 0.03% (Fig..4C), so it was chosen for the further experiment. In addition, all the CdTe QDs/ Polyeletrolyte multilayer film exhibited photoluminescent behaviors.

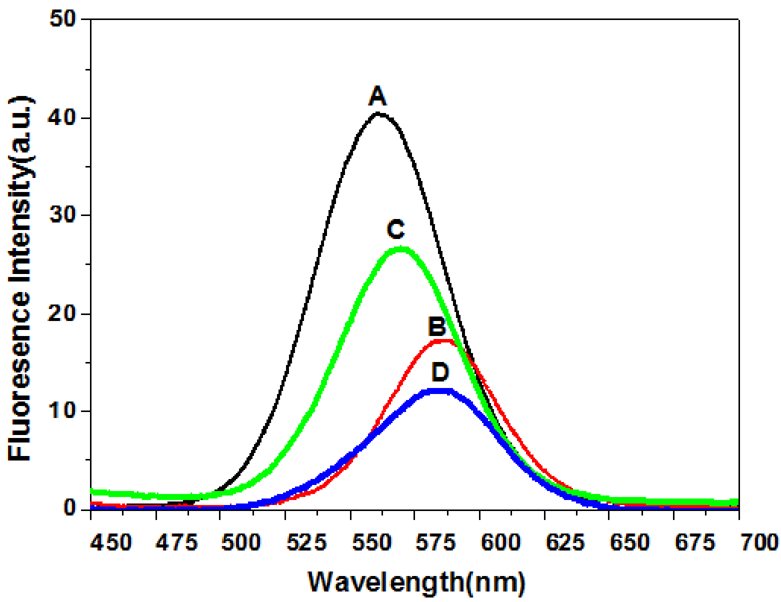

FIG. 4. THE PL INTENSITY WITH DIFFERENT CONCENTRATION OF PDDA DEPOSING ON THE QDMF. (A) 0 PDDA, (B) 0.003% PDDA,(C) 0.03% PDDA, (D) 0.3% PDDA.

3.2.3 Layer number of QDMF

The fluorescence intensity proportionately enhances along with the increasing of deposited QDs layer number as shown in Fig. 5. The PL intensity of QDMF can be adjusted by varying the number of deposited CdTe QDs layers. However, with the number of layers increased, the fluorescence emission peak at 550nm has a negative effect on the detection (detection accuracy). Therefore, in this study, six layers of QDMF is chosen for the further experiment.

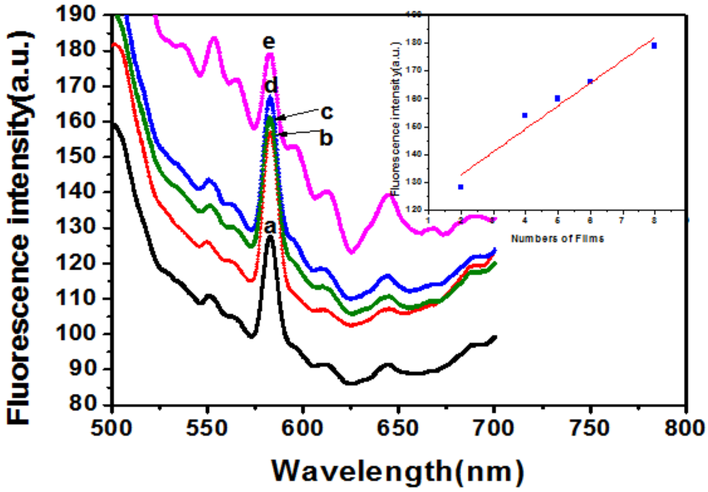

FIG. 5. FLUORESCENCE EMISSION SPECTRUM OF QDS-MULTILAYER FILMS WITH DIFFERENT QDS LAYERS. (A) 2-LAYER, (B) 4-LAYER, (C) 5-LAYER, (D) 6-LAYER, (E) 8-LAYER. INNER GRAPH IS THE RELATIONSHIP BETWEEN PL INTENSITY OF QDMF AND THE NUMBER OF QDS LAYERS. (n=5, RSD 5.47%).

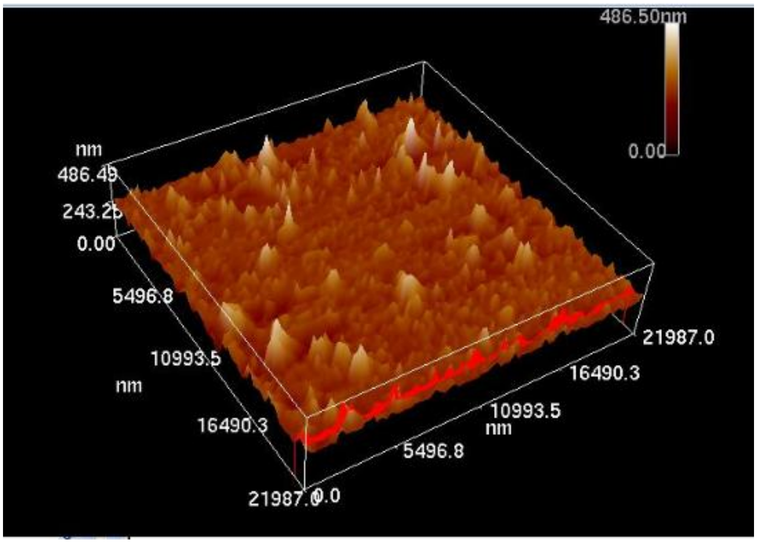

FIG. 6. AFM PICTURES OF QDMF

The AFM picture of CdTe QDs-multilayer films with 5 QDs layers are shown in Fig. 6. We can see that there are some peaks with different diameter on the surface of film, confirming that CdTe QDs were successfully grown in the reaction. The emergence of the larger peak on the surface of PDDA may be due to the aggregation of CdTe quantum dots in process of electrostatic self-assembly. However, Tanaka [33]pointed out the reason for this phenomenon. First, only one end of the Polyelectrolyte molecules was combined on the surface of the substrate, and second, many pendant groups combined with Polyelectrolyte molecules have coupled with substrate .

3.3 The quenching study of QDMF

3.3.1 Different quenching time

Quenching time was an important factor for the interaction between CdTe QDs and ammonia. QDMF was reacted with ammonia gas for different time. The corresponding fluorescence spectra of multi-layers are shown in Fig. 7. The fluorescence intensity progressively decreases along with the increasing of the quenching time .As shown in the picture, the fluorescence intensity dramatically decreases from ca.193.3 to ca.128.5 in the first 40 min, and there is no decrease of PL intensity when the quenching time exceeds 40 min. This indicates that the quenching of ammonia on the PL intensity of QDMF achieved saturation when the quenching time reached 40 min. Based on the above results, 40 min was adopted as the optimal quenching time.

3.3.2 Different organic gases

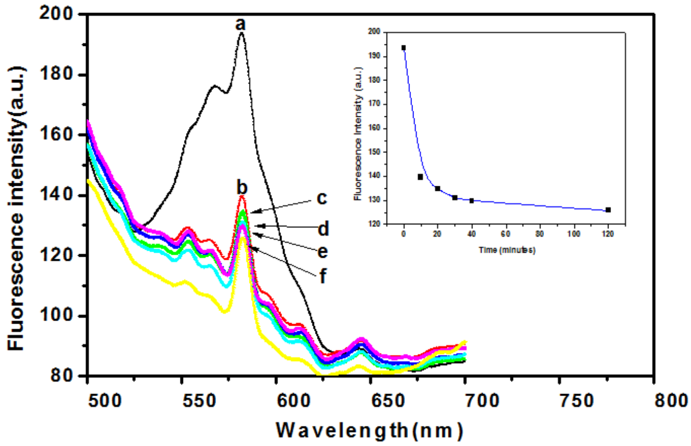

FIG. 7. QUENCHED PL OF QDS-MULTILAYER FILMS BY AMMONIA GAS AT DIFFERENT REACTION TIMES. (a) 0 min, (b) 10 min, (c) 20 min,(d) 30 min, (e)40 min, (f) 2 h.

Fig. 8 shows that QCMF was quenched by the four kinds of gases with different concentrations. All the gas molecules include the group of amidogen and carbonyl. PL intensity of QDMF (6 layer numbers for all the experiments) was decreased by all the quenching gases: (A) 65.9 a.u. by formaldehyde; (B) 27.0 a.u. by methanol; (C) 45.8 a.u. by acetone; and (D) 64.8 a.u. by ammonia. Therefore, the QDMF can be used as the sensors for certain gases with the group of amidogen and carbonyl, such as acetone, methanol, ammonia, formaldehyde, and so on.

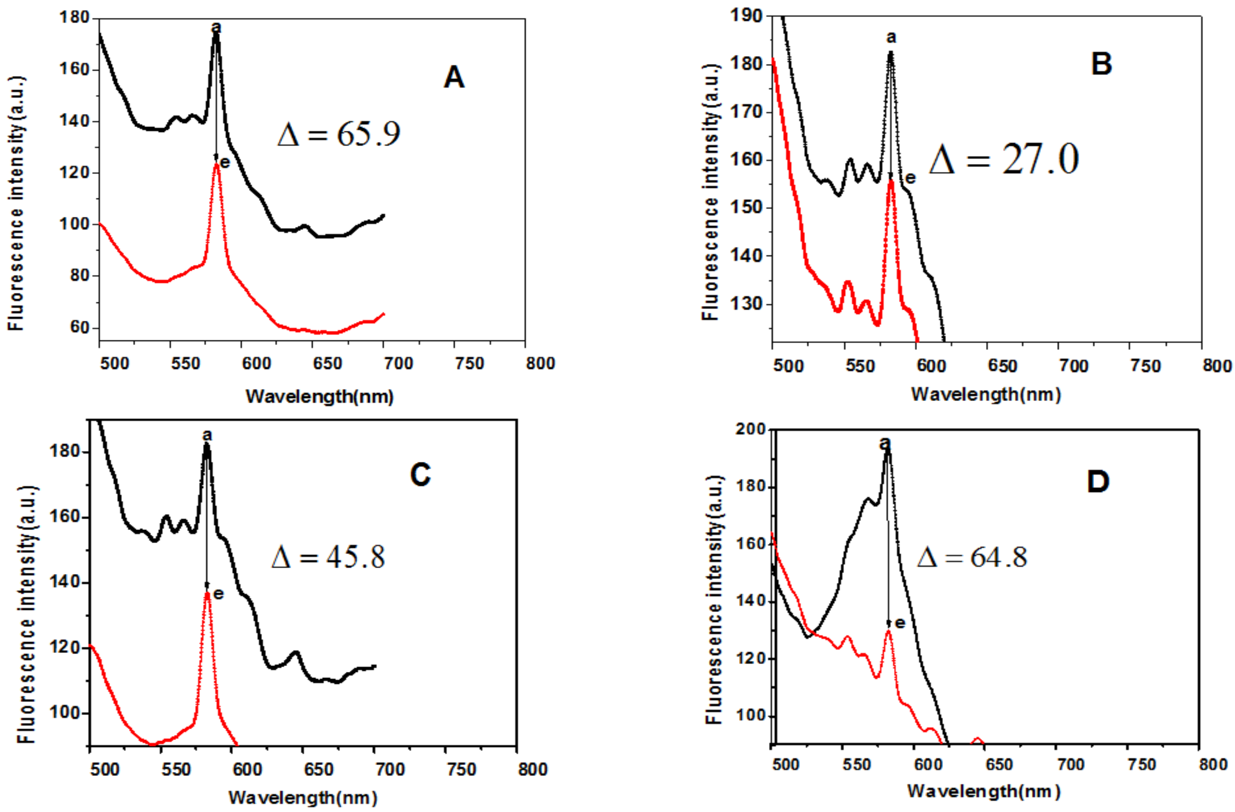

FIG. 8. QUENCHED PL SPECTRA OF QDMF BY DIFFERENT GASES AT QUENCHING TIME OF 40 MIN. (A) BY FORMALDEHYDE, (B) BY METHANOL, (C) BY ACETONE, (D) BY AMMONIA.

3.3.3 The fluorescence image of QDMF

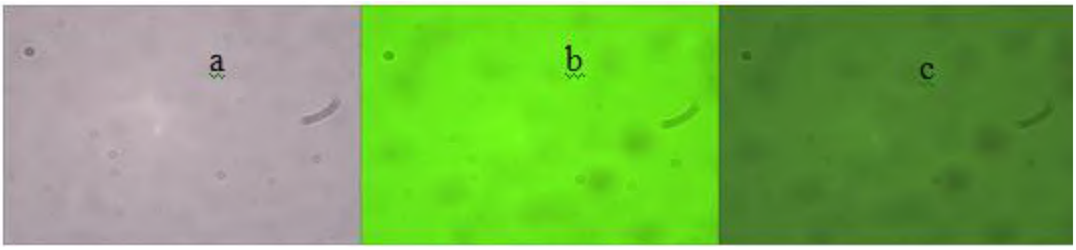

FIG. 9. THE FLUORESCENCE IMAGE OF QDMF, BEFORE EXCITATION(a),UV(365nm) excitation(b), UV(365nm) EXCITATION AFTER QUENCHED BY METHANOL(c).

In our previous work, a preliminary study on the detection effect of the current sensor has been implemented. In this process, commercial UV light is used to detect prepared samples under conventional conditions(without the aid of fluorescence spectrometer and other advanced instruments).Fig.9 shows that the prepared sensors have high detection ability for formaldehyde and other harmful gases. As a result, the family self-detection could be initially realized and the market has huge potentiality.

3.3.4 The detection limit of formaldehyde

To study the detection limit, the as-prepared QDMF and different amount of formaldehyde (10ul, 20ul, 30ul, 40ul, 50ul) were placed into a bottle for fluorescence quenching experiment. After 40 min reaction, the PL intensity of sensors was detected. Fig.10 shows the change curves of PL intensity of QDMF with different concentration of formaldehyde, and its kinetics can be expressed as: I/I_0=1.04586-0.00442*CHCHO (ppb),where I and I_0 are the PL intensity of QDFM (before quenching and after quenching, respectively). The detection limit was calculated to be 10-236ppm, which is far below the country safety standard (80ppm).

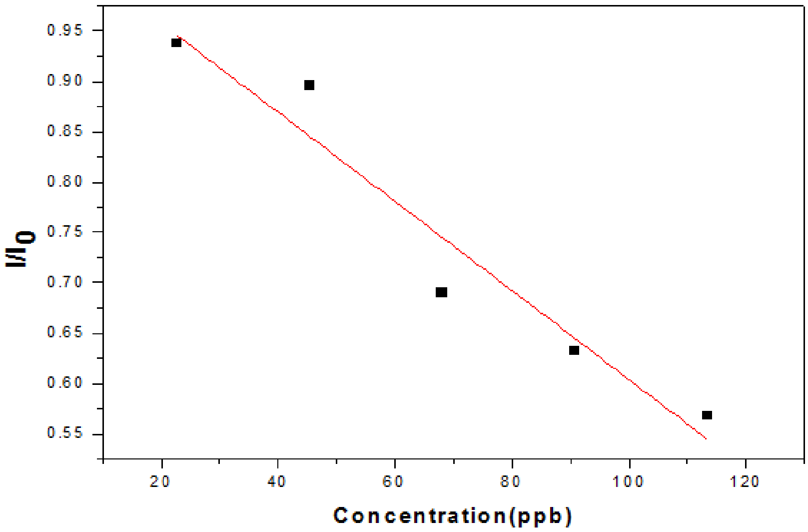

FIG.10 THE FLUORESCENCE INTENSITY OF QDMF WITH DIFFERENT FORMALDEHYDE CONCENTRATION

IV. CONCLUSION

The layer-by-layer method was adopted to prepare the QDMF, which was built up with PDDA and CdTe QDs modified by MPA. Owing to electrostatic interactions between anionic QDs and cationic PDDA, the LBL method affords more stable coating than physical adoption. The standard LBL self-assembling technique has been proven to be a rapid and convenient way to produce complex layered and hybrid structures. The multicolor CdTe quantum dots were prepared using a refluxing method in aqueous solution with high PL intensity. The PL intensity of QDMF can be quenched effectively by the groups of amidogen and carbonyl. Organic gas molecules without carbonyl and amidogen groups have no effect upon the fluorescence quenching of QDMF. Thus, the self-assembled QDMF in this study could be used as gaseous sensors for detecting organic gases, such as ammonia, acetone, methanol and formaldehyde. And for formaldehyde, The detection limit was calculated to be 10-236ppm.

ACKNOWLEDGEMENTS

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Biosorption of Malathion pesticide using *Spirogyra* sp.

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***Abstract*—** *The biosorption of Malathion from aqueous solution by green algal biomass was investigated. The green algae used were of the species Spirogyra and was collected from Neugal river near Sujanpur, Himachal Pradesh. Batch biosorption experiments were performed to examine the effect of contact time, pH, biomass concentration and initial Malathion concentration. The concentration of residual Malathion concentration after biosorption was determined using UV-Vis Spectrophotometer at a wavelength of 309 nm. The maximum adsorption was found to be at pH 7 after a contact time of 5 hours with initial Malathion concentration of 100 mg/L and biomass of weight 75 mg. The equilibrium biosorption data were analyzed using Langmuir and Freundlich isotherm. Freundlich isotherm was found to be more favorable than Langmuir isotherm.*

***Keywords*— algae, biosorption, isotherm, Malathion, pesticide.**

I. INTRODUCTION

The use of pesticide is essential for the modern agricultural practice. Pesticides not only kill unwanted pests and insects, they also increase the productivity of agriculture. In India, agricultural production increased by 100% while the cropping land increased by only 20% [1]. Pesticide residues that get released into the environment tend to stay in the environment for a very long time, and get accumulated throughout the food chain, making it hazardous to the environment. The U.S. Geological Survey conducted a study from which they reported that more than 90% of the water and fish samples that they collected from major rivers or water streams were contaminated with pesticides. The rivers and streams which were contaminated by pesticides were influenced by agriculture and urban land use [2]. Currently, India ranks 10th in the world pesticide consumption list [3] and the Indian agrochemical market is expected to reach U.S $ 6.3 billion by 2020 [4].

Pesticides are classified into many classes, out of which, organophosphates and organochlorines are deemed the most important ones. Malathion is an organophosphorus pesticide which is most commonly used in agriculture all over the world. It is used for killing insects on agricultural crops and stored products. It is also widely used for killing mosquitos in urban and residential areas. It is also used for the control of flies, household insects and head and body lice. In 2006, it was reported that approximately 15 million pounds of Malathion were used worldwide annually [5]. The Environmental Protection Agency has identified Malathion as a toxicity class III pesticide and a general use pesticide (GUP). Malathion interferes with the normal function of the nervous system and thus indirectly affects the function of other organs. The effect of exposure to Malathion on human health may include, but not limited to, difficulty in breathing, vomiting, diarrhoea, headaches, dizziness and loss of consciousness and death [6].

Several methods have been proposed for the removal/treatment of Malathion from raw water and wastewater such as electrocoagulation, advanced oxidation and coagulation/flocculation [7, 8, 9]. The limitations to these methods are that they are quiet expensive and the chemicals used for these methods require constant observation and it is preferable that they be handled by a skilled person. Adsorption with activated carbon is an easy and cost effective method for the removal of pesticides and has been extensively studied for the removal of Malathion [10- 11]. In recent years, studies about the removal of Malathion by biological materials have increased due to their easy availability, low cost and efficiency. Biosorption by activated sludge, isolated bacillus sp., *Rhizopus oryzae*, nanocellulose, algal biomass of *Chlorella vulgaris*, chesnut shells and the fungal biomass of *Phanerochaete chrysosporium* has been studied with positive results [12, 13, 14, 15, 16, 17, 18].

Either live or dead biomass can be used in biosorption process. Live biomass has been used for the removal of heavy metals in the past [19- 20]. The use of dead biomass is more desirable than the live ones as dead biomass do not require nutrients to sustain them and can be stored to be used later. While using live biomass, sorption as well as biodegradation may also occur and it is very difficult to distinguish which one of them has more contribution.

The present study investigates Malathion removal from aqueous solution by biosorption using green algal biomass of species Spirogyra, which is abundant in fresh water lakes and rivers. Laboratory batch experiments were carried out using a shaker-incubator at a constant temperature to determine the optimum contact time, pH, weight of biomass and initial Malathion concentration for maximum removal. Langmuir and Freundlich isotherms were used for determining the sorption capacity of *Spirogyra* sp.

II. MATERIALS AND METHOD

2.1 Malathion solution

Commercially available Malathion 50% E.C, Osothion, was used in this study.

2.2 Biomass

The green algae, Spirogyra sp., used in this study was collected from Neugal River near Sujanpur, located at 31.83ºN 76.50ºE, Himachal Pradesh. The algae was washed with distilled water to remove dirt and other impurities after which it was sundried for 6 hours and then it was kept in an oven for 48 hours at 130º C to make sure it was completely dead. The dead biomass was grounded using a pestle and mortar to get fine powder. The powdered biomass was kept in a crucible and stored in a desiccator until used.

2.3 Measurement of residual Malathion concentration

Pure solution of Malathion was scanned in an UV-Vis Spectrophotometer from Aligent Technology, Cary Series to determine the wavelength with maximum absorbance. Maximum absorbance was found at wavelength 309 nm. Using this wavelength, a standard curve of Malathion solution of known concentration (50 – 250 mg/L) was prepared. This data was used to determine the unknown concentration of residual Malathion concentration.

2.4 Batch Kinetic Study

Batch kinetic study was performed by taking 100 mL of Malathion solution in 250 mL Erlenmeyer flasks. Optimum removal of Malathion from the solution was determined based on contact time, pH, amount of biomass kept in contact with the pesticide solution and initial Malathion concentration. The flasks were kept in a shaker-incubator for a specific amount of time at 130rpm and the temperature of the incubator was kept at 27 ± 2º C. The pH of the solution was adjusted using 0.1M NaOH and 5% HCl. The final residual concentration of Malathion in the solution was determined using UV-Vis Spectrophotometer as mentioned before.

The amount of Malathion adsorbed by the algal biomass was calculated using the formula

$$Q_e = (C_0 - C_e)\ V/m \quad (1)$$

Where,

Q_e (mg/g) is the amount of Malathion adsorbed per unit weight of the algal biomass.

C_0 (mg/L) is the initial Malathion concentration.

C_e (mg/L) is the Malathion concentration at equilibrium

V (L) is the amount of Malathion solution.

m (g) is the amount of biomass used.

All tests were performed in triplets and their average was taken for the actual calculation.

2.5 Biosorption isotherm

To evaluate the biosorption performance, a graph between the sorbate in the solution and the amount of sorbate sorbed on the biosorbent is plotted. There are various isotherm models which have been proposed and used throughout the years. In this study, Langmuir and Freundlich isotherm are studied to evaluate the data.

The equation for Langmuir isotherm is

$$Q_e = Q_m b C_e / (1+b.C_e) \quad (2)$$

Where,

Q_e is the amount of Malathion adsorbed (mg/g)

Q_m is the maximum Malathion adsorbed per unit biomass (mg/g)

C_e is the equilibrium concentration of Malathion (mg/L)

b is the Langmuir equilibrium constant (L/mg) and it explains the affinity between the sorbent and sorbate..

The Langmuir isotherm equation can be arranged into linear form

$$C_e/Q_e = 1/b.Q_m + C_e/Q_m \quad (3)$$

Which can be further rearranged into?

$$1/Q_e = 1/b.Q_m\, C_e + 1/Q_m \quad (4)$$

A plot between $1/Q_e$ and $1/C_e$ will give intercept $1/Q_m$ and slope $1/b.Q_m$.

The Freundlich isotherm is described by the following equation

$$Q_e = k.C_e 1/n \quad (5)$$

Where,

k and n represents Freundlich constants. k indicates the adsorption/binding capacity (L/g) and n indicates the intensity of the adsorption i.e. the affinity between the sorbent and sorbate.

The linearized form of equation (4) can be written by taking logarithm on both sides

$$\ln Q_e = 1/n \ln C_e + \ln k \quad (6)$$

III. RESULTS AND DISCUSSION

3.1 Effect of contact time

After performing batch biosorption study, it was observed that with the increase in time, the percentage removal of Malathion increases. Highest Malathion removal was observed at 5 hours after which, the removal percentage remained constant. This can be explained by the fact that after 5 hours, all the active biosorption sites have been occupied by Malathion. The result of the contact time test is shown in Fig. 1.

3.2 Effect of pH

After performing biosorption test with different pH, it was found that highest amount of Malathion removal was achieved at pH 7, after which the removal percentage starts to decrease again, suggesting that at lower and higher pH, all the functional groups responsible for biosorption have bounded, thus resulting in lower biosorption. While, at neutral pH, the functional groups are free for biosorption. The result of Malathion removal with respect to pH is shown in Fig. 2.

3.3 Effect of biomass weight

Batch biosorption studies were done to determine the effect of weight of biomass made in contact with the Malathion solution for the removal of Malathion with varying weight of biomass. It was found that the removal of Malathion increased with increase in biomass concentration till 75 mg after which the removal decreased again, making 75 mg the optimum dose of biomass. This can be explained by the fact that with less amount of biomass, the amount of Malathion was much more such that there were not enough biomass surfaces where Malathion could bind on to. While at higher biomass concentration, all available sorption sites were not utilized causing agglomeration of the biomass which in turn decreases the amount of adsorption sites available. The result is shown in Fig. 3.

3.4 Effect of initial Malathion concentration

The biosorption process was found to be highly effected by the initial Malathion concentration. The result shown in Fig. 4 shows that Malathion removal decreases with increase in initial Malathion concentration. This is because with increase in Malathion concentration, there is not enough binding sites available for Malathion. And the Malathion molecules compete with each other for the available binding sites, thus reducing the biosorption efficiency.

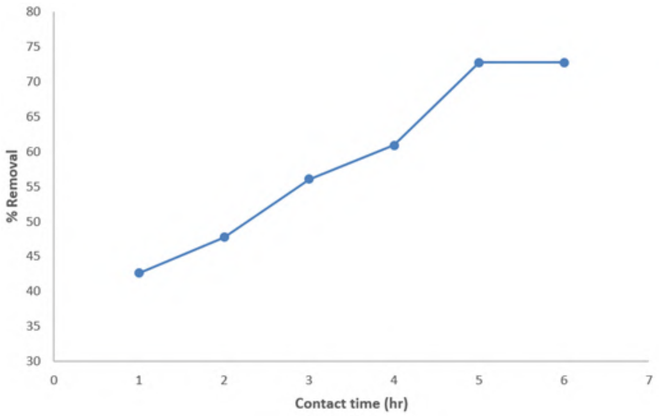

FIG. 1 EFFECT OF CONTACT TIME FOR THE REMOVAL OF MALATHION ON *SPIROGYRA* SP. BIOMASS

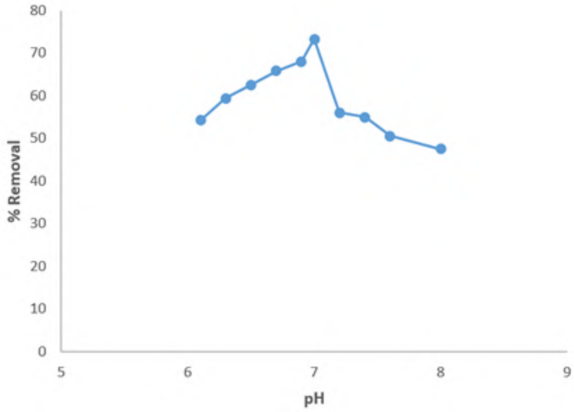

FIG. 2 EFFECT OF PH FOR THE REMOVAL OF MALATHION USING *SPIROGYRA* SP. BIOMASS

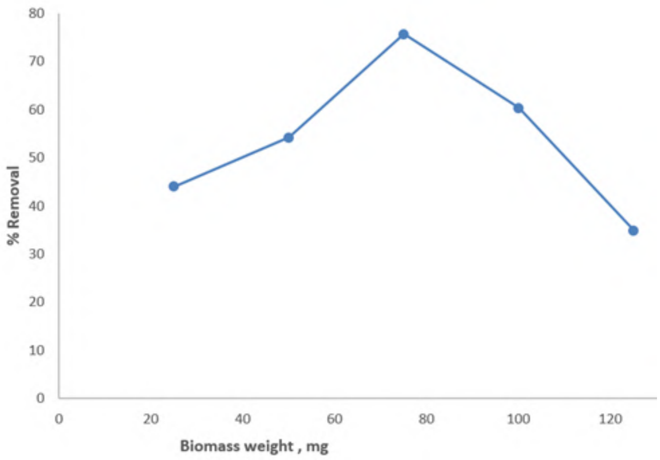

FIG. 3 EFFECT OF AMOUNT OF BIOMASS FOR THE REMOVAL OF MALATHION USING *SPIROGYRA* SP. BIOMASS

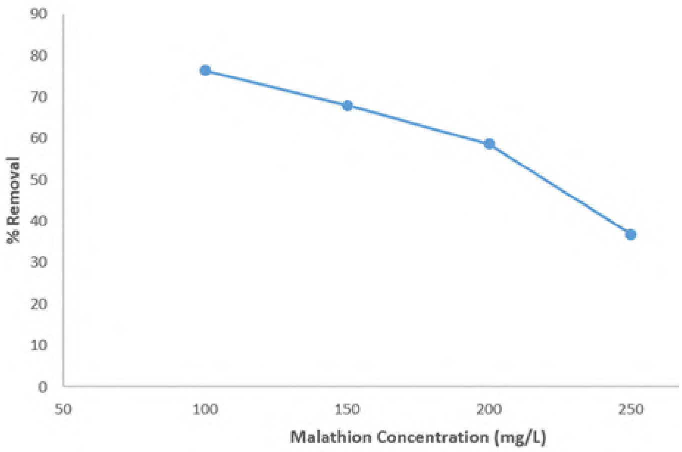

FIG. 4 EFFECT OF INITIAL MALATHION CONCENTRATION FOR THE REMOVAL OF MALATHION USING *SPIROGYRA* SP. BIOMASS

3.5 Biosorption isotherm analysis

For the isotherm study, 100ml of Malathion solution was taken in 250ml Erlenmeyer flasks with different initial Malathion concentration ranging from 50mg/L to 250mg/L. After preliminary kinetics study, it was found that equilibrium was reached at 5 hours. The solution was made in contact with constant amount of biomass (75mg) and the pH was fixed at 7. From the results, a graph between $1/C_e$ and $1/Q_e$ was plotted for Langmuir isotherm model (Fig. 5) and between ln C_e and ln Q_e for Freundlich isotherm (Fig. 6). The coefficient of correlation for Freundlich was found to be higher than that of Langmuir isotherm indicating that the biosorption follows Freundlich isotherm model. The sorption following Freundlich isotherm can mean that sorption occurred at heterogeneous surface or that the binding sites on the Spirogyra sp. have different affinities for the Malathion molecules.

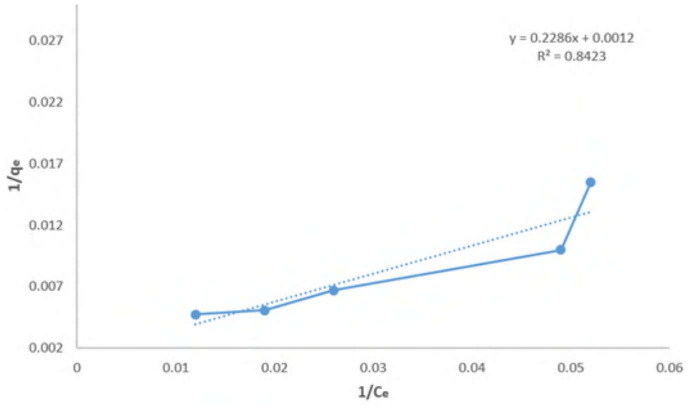

FIG.5 LANGMUIR ISOTHERM MODEL FOR THE REMOVAL OF MALATHION USING *SPIROGYRA* SP. BIOMASS

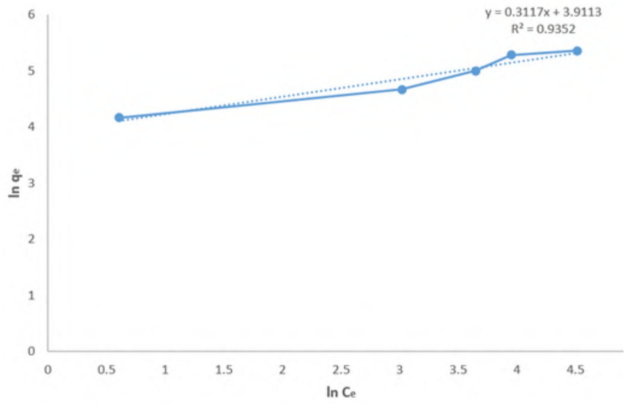

FIG. 6 FREUNDLICH ISOTHERM MODEL FOR THE REMOVAL OF MALATHION USING *SPIROGYRA* SP. BIOMASS

The values of Langmuir and Freundlich constants and their coefficient of correlation are shown in TABLE 1.

TABLE 1
R^2 AND CONSTANTS FOR LANGMUIR AND FREUNDLICH ISOTHERM

Langmuir			Freundlich		
Q_m	b	R^2	1/n	k	R^2
833.3 mg/g	0.00525 L/mg	0.8423	0.3117	49.96 $(mg/g)(L/mg)^{1/n}$	0.9352

IV. CONCLUSION

Malathion removal from aqueous solution by biosorption using Spirogyra sp. was studied. The residual Malathion concentration was determined using UV-Vis spectrophotometer. It was found that 76.34% of Malathion was removed from initial Malathion concentration of 100mg/L when the initial pH was kept at 7, using biomass concentration of 75mg and a contact time of 5 hours. Biosorption isotherm study showed that Freundlich isotherm was found to fit the experimental data more than Langmuir isotherm with R^2 value at 0.9352 indicating that sorption occurred on heterogeneous surface. Further studies can be done by pre-treating the biomass to see if Malathion removal increases with it.

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The Relationship between Soil Moisture and Temperature Vegetation on Kirklareli City Luleburgaz District A Natural Pasture Vegetation

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Abstract**—** *This study was realized in 2014 – 2015 in two different sections of Kirklareli city Luleburgaz district Sakizkoy village natural pasture in order to research the effect of soil moisture and soil temperature on area covered by vegetation, plant species and dry yield. As research area, study was conducted in two different sections defined as A and B located to the north and south of village coppice forest area located within the borders of Kirklareli city Luleburgaz district Sakizkoy village. By this study, the relation between soil moisture and temperature with plant species were evaluated by CANOCO 4.5 computer program. Accordingly, the effect of ecological values on vegetative properties was presented. According to research results, soil moisture and temperature have significant effect on vegetation. In the first year when soil moisture was high, hay yield was 2901.9 kg/ha while the yield was detected as 480.1 kg/ha after soil temperature (which is inversely correlated with soil moisture) increased in the second year. It was determined that Lolium perenne (one of the dominant species of vegetation) is common in parcels with high moisture while Chrysopogon gryllus is common in parcels where soil temperature is high.*

Keywords**— *pasture, dry yield, soil moisture, soil temperature, CANOCO 4.5.***

I. INTRODUCTION

Climate change is expected to affect agriculture very differently in different parts of the world (Parry et al., 2004). Climate change impacts on crop yield are often integrated with its effects on water productivity and soil water balance. Global warming will influence temperature and rainfall, which will directly have effects on the soil moisture status and groundwater level (Kang et al.2009). According to Valentine, (1990), livestock products provide the major economic return from most range and pasture lands and compared with harvested or purchased feeds, pastures and pasture provide a relatively inexpensive and energy-efficient feed source for livestock. Climate change is change the community structure of grasslands (Buckland et al., 2001; Lüscher et al., 2004). Grasslands will differ in their response to climate change depending on their type (species, soil type, management) (Olesen , 2006). Management and species richness of grasslands may increase their resilience to change (Duckworth et al., 2000). Particularly climatic factors, like mean annual precipitation and precipitation variability, have a huge impact on rangeland condition and fodder production (Williams and Albertson, 2006). Climate change in the form of decreasing mean annual precipitation accompanied by increasing variability has important consequences for rangeland productivity and thus pastoral livelihood security (Martin et al.2013). Soil moisture plays a key role in vegetation restoration and ecosystem stability in arid and semiarid regions. The response of soil moisture to rainfall pulses is an important hydrological process, which is strongly influenced by land use during the implementation of vegetation restoration. Soil moisture depended strongly on precipitation (Yu et al.2015). Soil moisture is a key rangeland health parameter as it is the principal limiting factor in semi-arid ecosystems (Weber and Gokhale, 2010) . Soil moisture and temperature are together referred to as "soil climate". The effects of soil climate are mainly the basic determining criteria that separate range ecosystems from other natural ecosystems. In addition, soil climate affects all soil-plant relationships in range ecosystems. Rooting depth, water potentials, nutrient intake and nutrient element distribution are affected by the amount and time of moisture availability associated with the critical temperatures at which the root activity is observed. The temperature and moisture near the soil surface affect the germination of range plants. Besides germination, settlement and continuity also depend on the temperature and moisture of the soil (Altin et al. 2011). Temperature is an important feature that has a significant effect on the biological events that take place in the soil and directs the physical and chemical processes. The root development of most plants ceases at temperatures below 5˚C. The availability of soil and air temperature data is necessary to understand plant-soil relationships and to be able to make comments on the use of the soil (Dinc and Senol, 1998) and therefore will create a basis for the projections to be drawn for range management and improvement in the future. The objective of this study is to determine the effects of I) different pasture sites and II) soil moisture and temperature on vegetation composition in a natural pasture. Fırıncıoglu et al. (2008) and Ababou et al. (2009) stated that, to perform a redundancy analysis (RDA) to determine the topographic and edaphic factors that influence plant species occurrence to understand the most important components affecting the segregation of plant species. For this purpose, RDA analyses were performed using CANOCO 4.5 computer program in this study.

II. MATERIAL AND METHOD

The study was carried out in Sakizkoy village of Luleburgaz district of Kırklareli province in 2014-2015. Although Luleburgaz is involved in the temperate climate zone by its latitudes, it has a cold and rainy character in winters and dry and hot character in summers. The region where the study area is located has a "Semi-Arid Climate" feature (Donmez, 1968). While the total annual rainfall was 788, 8 mm in Luleburgaz District of Kirklareli Province in 2014, which was the first year of the study, it was 493,0 mm in 2015, the second year. While the average relative humidity of the year 2014 was 84.4%, the average relative humidity of the year 2015 decreased to 75.2%. The average temperatures of the years 2014 and 2015 were measured as 14, 2 ^{o}C and 15, 6 ^{o}C, respectively. It was observed that the maximum temperature average of the year 2014 was 26.9 ^{o}C and the maximum temperature average of the year 2015 was 27.6 ^{o}C (Anonymous 2016). A sites,and B sites pasture of Sakizkoy village, Kirklareli (Europe Part) in Turkey; Latitude 41;46;24 N Longitude 27;48;66 E , 41;47;16N, 27;49;38 E , respectively.

FIGURE 1. GOOGLE EARTH IMAGE OF SAKIZKOY PASTURE

As research area, study was conducted in two different sections defined as A and B located to the north and south of village coppice forest area located within the borders of Kirklareli city Luleburgaz district Sakizkoy village. Regarding two separate regions designated as sites A and B located in the North and South part of the Village coppice forest which is within the borders of Sakizkoy Village where the study was carried out, Site A is closer to the Village settlement area, Site B is closer to the sheepfolds. While site A is generally exposed to cattle grazing pressure, site B is further exposed to small cattle grazing pressure although they are grazed on both sites. Presence/absence data of all vascular plant species were recorded in the sites. A total of 40 sites were sampled at two units to determine dry matter yield according to weight. For determination dry matter yield of the samples were harvested about 5 cm above the soil surface. All the plots in 0.25 m^2 were clipped from 20 sites in 2014 and 2015 year. The aboveground standing crop was measured by cutting herbaceous biomass (at ground level) and dried at hay yield was determined by drying the samples at 78ºC for 24 h. We also determined soil moisture and temperature these sites. We recorded soil temperature values using a portable electronic thermohygrometer (Mannix THPen , model PTH 8708) and soil moisture values were determined using “Economy Soil Moisture Tester”.

Statistical Analysis

Multivariate relationships between environmental variables and vegetation composition were determined Redundancy analysis ***(***RDA***)*** using Lepsˇ and S ˇ milauer, (2003) and Monte Carlo permutation test was used to prove if the results of the ordination are significant All default parameters in CANOCO(Version 4.5) were used (Ter Braak and Smilau, 2002). An analyses of variance was conducted using SPSS, (Version 18.0).

III. RESULTS AND DISCUSSION

Soil moisture measurement results in the years 2014-2015, during which the study was carried out, are presented in Table 1.

TABLE 1
ANALYSES OF VARIANCE, SOIL MOISTURE (%) AND SOIL TEMPERATURE (^{0}C)

	Soil Moisture %		Soil Temperature ^{0}C	
Sites	2014	2015	2014	2015
A Sites	8.4a	5.6b	16.8b	24.5a
B Sites	7.6a	6.4b	16.8b	26.4a
	Year:22.814**	Year * Site 4.176*	Year * Site 462.352**	Site11.027** Year x S ite11.027**

*** P< 0.05,** P<0.01 , [a] Means within rows with different superscripts differ significantly**

Soil temperature measurement results in the years 2014-2015, during which the study was carried out, are presented in Table 1. Soil temperature measurement results in the years 2014-2015, It was observed that the soil temperature increased from 16,8 ^{0}C to 24,5 ^{0}C on site A and from 16,8 ^{0}C to 26,4 ^{0}C on site B (Table 1). When these measurement results were compared, while the soil moisture was 8.4% on site A in 2014, it decreased to 5.6% in 2015. While the soil moisture was 7.6% on site B, it was decreased to 6.4% (Table 1). Deeply-rooted plants are more likely to survive extended periods of drought by accessing lower soil layers that contain higher levels of soil moisture (Chaves et al. 2003). It is generally assumed that plants respond to drought in surface layers by shifting water and nutrient uptake to deeper soil layers (Garwood and Sinclair 1979; Sharp and Davies 1985), Soil temperature, a controlling factor for soil moisture as it affects evaporation, is also affected by the amount of litter (Davidson et al. 1998). During the dry period, deep-rooted plants can well utilise the water and nutrients in the lower layers of the soil (Hoekstra et al. 2014).

TABLE 2
ANALYSES OF VARIANCE, DRY YIELD (KG/HA) AND REDUCTION OF DRY YIELD (%)

	2014	2015	Reduction of Yield %
Sites	Grasses(kg/ha)	Grasses (kg/ha)	
A Sites	1509.2a	451.0b	70
B Sites	1091.3a	154.1b	85
Average	1300.2a	302.5b	76
	Legumes(kg/ha)	Legumes (kg/ha)	
A Sites	513.4a	128.0b	76
B Sites	831.7a	17.9b	97
Average	672.5a	72.9b	89
	Others(kg/ha)	Others(kg/ha)	
A Sites	1444.0a	177.8c	87
B Sites	414.1b	31.0d	95
Average	929.0a	190.4b	79
A SitesTotal	3466.6a	757.0c	
B Sites Total	2337.2b	203.0d	
	2901.5a	480.1b	
	GrassYear:17.816**	LegumesYear:31.633**	OthersYearxSites:7.453* Others Site:13.234** OthersYear:25.997**

*** P< 0.05, ** P<0.01, [a] Means within rows with different superscripts differ significantly**

In the first year when soil moisture was high dry yield was 2901.9 kg/ha while the yield was detected as 480.1 kg/ha after soil temperature (which is inversely correlated with soil moisture) increased in the second year. As it is understood from Table 2 above, there was a significant yield loss in dry yields in the second year of the study. In both years, the measurements were performed during the same vegetation period, and there was not any difference in grazing. Therefore, rainfall regime is estimated to be the effective factor for this yield loss. Indeed, it was determined that the effect of soil moisture factors on dry yields was very significant. Drought is usually the most important environmental stress for the range plants that try to survive against the constant consumption of the animals. Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth . Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel et al., 2008). Thomas and Squires (1991) argue that soil moisture is the principal determinant of productivity and the primary driver of rangeland condition in semi-arid ecosystems. Seedling emergence rate and root and shoot growth were decreased by limiting soil water content, while root-to-shoot length ratio (43%) was increased (Gazanchian et al. 2006). Low water availability in arid and semiarid regions severely limits seed germination, seedling establishment, and persistence of perennial grasses (Bassiri et al., 1988). Johnson and Asay (1993) reported that water deficit also limits the establishment, growth, and production of cool season grasses on semiarid rangelands. There is a strong linear relationship between aboveground net primary productivity (ANPP) and annual precipitation in rangeland ecosystems (Le Houérou, 1984; Sala et al., 1988; Scholes, 1993). Annual forage pastures are seeded every spring (with the exception of fall-seeded winter cereals), have shallower root systems with lower biomass (Baron et al. 1999; Mapfumo et al. 2002), and therefore tend to use less water than perennial forages (Baron et al. 1999; Twerdoff et al. 1999). Differences in water use occur among perennial forage species. Root depth, root density, and timing of canopy closure impact procurement and evaporative demand for soil moisture among species (Bradshaw et al. 2007).

As a result of the study, according to dry yields, it was observed that the yield loss was less in grasses compared to legumes and other family members. In the results of the study, while the average yield loss in grasses between 2014-2015 was found to be 70%, this ratio was 89% in legumes and 79.4% in other families (Table 2). Olesen (2006) stated that climate variability is one of the most significant factors influencing year to year crop production, even in high-yield and high-technology agricultural areas .

Redundancy analysis (RDA) was performed to determinate which of the measured environmental variables would significantly explain the species composition.

TABLE 3
REDUNDANCY ANALYSIS (RDA) OF ENVIRONMENTAL-VEGETATION COMPOSITION

	Axis1	Axis2	Axis3	Axis4	Total
Eigenvalues	0.304	0.058	0.201	0.122	1.000
Species-environment correlations	0.926	0.602	0.000	0.000	
Cumulative percentage variance of species data	30.4	36.2	56.3	68.5	
of species-environment relation	84.1	100.0	0.0	0.0	

The RDA showed a high cumulative percentage variance of species occurrence data explained on the fi rst two axes of the RDA (34.4%) (Table3). With RDA of the vegetation data, eigen values of 0.304, 0.058, 0.201, 0.122 were found for axes one to four, respectively (Table 3). There was a strong relationship between the vegetation and the environmental factors, with species-environment correlations of 0.926 on the first axis and 0.602 on the second axis. The Monte Carlo permutation test was significant for the first axis (*P* = 0.002), the second axis (*P* = 0.002).

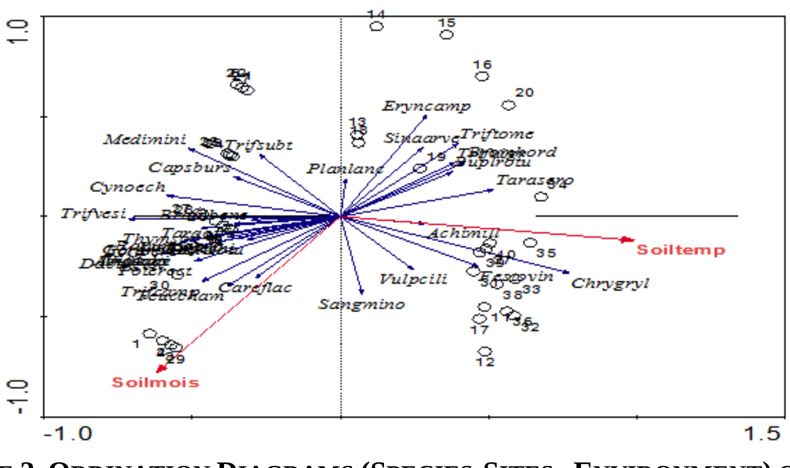

FIGURE 2. ORDINATION DIAGRAMS (SPECIES-SITES--ENVIRONMENT) OF RDA

Species:*Achillea millefolium* Achimill; *Aira caryophyllea* Airacary, *Bromus benekenii* Brombene, *Bromus hordelymus* Bromhord, *Bupleurum rotundifolium* Buplrotun, *Capsella bursa-pastoris* Capsburs,*Carex flacca* Careflac, *Cynosurus echinatus* Cynoech, *Chrysopogon gryllus* Chrygryl, *Dactylis glomerata* Dactyglom, *Eryngium campestre* Eryncamp, *Festuca ovina* Festovin, *Galium rotundifolium* Galirotu, *Geranium robertianum* Gerarobe, *Koeleria nitidula* Koelnitu, *Lamium purpureum* Lamipurp, *Lolium perenne* Lolipere, *Medicago minima* Medimini, *Plantago lanceolata* Planlanc, *Potentilla recta* Poterect, *Ranunculus neapolitanus* Ranuneap, *Sanguisorba minor* Sangmino, *Sinapis arvensis* Sinaarve, *Taraxacum officinale* Taraoffi, *Taraxacum serotinum* Tarasero, *Teucrium chamaedrys* Teuccham, *Thymus striatus* Thymstri, *Trifolium campestre* Trifcamp, *Trifolium echinatum* Trifechi, *Trifolium nigrescens* Trifnigr, *Trifolium ochroleucum* Trifochr, *Trifolium subterraneum* Trifsubt,*Trifolium tomentosum* Triftome, *Trifolium vesiculosum* Trifvesi, *Vicia sativa* Vicisati,*Vulpia ciliata* Vulpcili

Environmental variables: Soil mois Soil moisture, Soil temp Soil temperature

The distribution of the sites according to the soil temperature and moisture and species of the study area is presented in Figure 2. There is a mutual relationship between plant species and environmental factors such as soil temperature and soil moisture. Weniger (1973) stated that the excess of water in the soil leads to a decrease in soil temperature and that the soils with a higher amount of air presenting in soil pores get heated more quickly compared to water-saturated soils as the amount of heat the water needs to get heated is more with respect to the air.

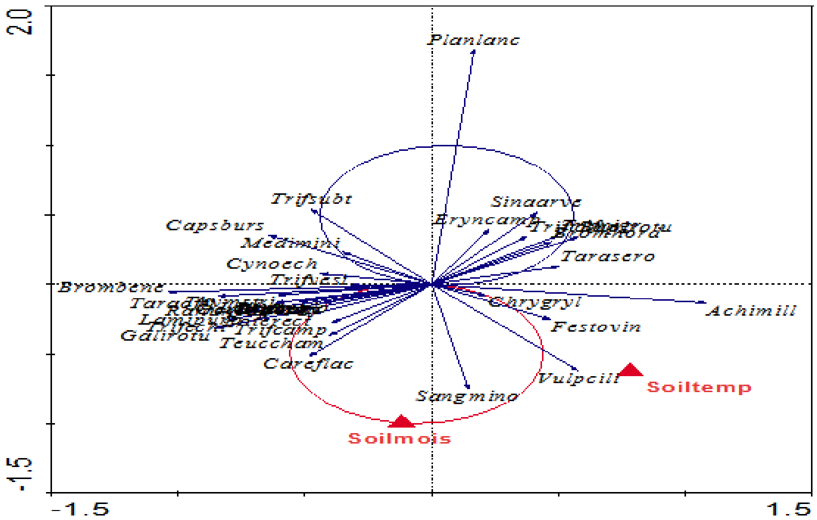

FIGURE 3. T- VALUE PLOT OF SOIL MOISTURE

Species: *Achillea millefolium* Achimill; *Aira caryophyllea* Airacary, *Bromus benekenii* Brombene, *Bromus hordelymus* Bromhord, *Bupleurum rotundifolium* Buplrotun, *Capsella bursa-pastoris* Capsburs, *Carex flacca* Careflac, *Cynosurus echinatus* Cynoech, *Chrysopogon gryllus* Chrygryl, *Dactylis glomerata* Dactyglom, *Eryngium campestre* Eryncamp, *Festuca ovina* Festovin, *Galium rotundifolium* Galirotu, *Geranium robertianum* Gerarobe, *Koeleria nitidula* Koelnitu, *Lamium purpureum* Lamipurp, *Lolium perenne* Lolipere, *Medicago minima* Medimini, *Plantago lanceolata* Planlanc, *Potentilla recta* Poterect, *Ranunculus neapolitanus* Ranuneap, *Sanguisorba minor* Sangmino, *Sinapis arvensis* Sinaarve, *Taraxacum officinale* Taraoffi, *Taraxacum serotinum* Tarasero, *Teucrium chamaedrys* Teuccham, *Thymus striatus* Thymstri, *Trifolium campestre* Trifcamp, *Trifolium echinatum* Trifechi, *Trifolium nigrescens* Trifnigr, *Trifolium ochroleucum* Trifochr, *Trifolium subterraneum* Trifsubt, *Trifolium tomentosum* Triftome, *Trifolium vesiculosum* Trifvesi, *Vicia sativa* Vicisati, *Vulpia ciliata* Vulpcili

Environmental variables: Soil mois Soil moisture, Soil temp Soil temperature

In Figure 3, the t-values of the statistical significance relation between soil moisture and plant species are presented by a Van-Dobben circle. It was found out that the plants such as *Carex flacca*, *Trifolium campestre* and *Lolium perenne* had a positive relationship with soil moisture, and *Eryngium campestre* and *Sinapis arvensis* had a negative relationship with soil moisture. In addition to this, it was observed that especially *Lolium perenne*, *Carex flacca* developed better in moist soils. These plants are dominant especially in areas where soil moisture is high. Annual legumes such as *Trifolium campestre*, *Trifolium echinatum*, *Trifolium vesiculosum*, remaining in the circle can also utilise the water in the soil with the taproot systems in the drought period.

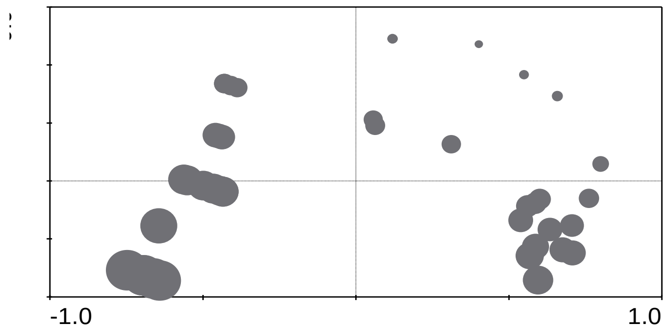

FIGURE 4. DISTRIBUTION OF SITES ACCORDING TO SOIL MOISTURE

The distribution of the parcels according to the soil moisture of the study area is presented in Figure 4. The soil moisture in the parcels displayed with dark-colored symbols in the biplot parcel refers to high values. The parcels with the high ratios of *Lolium perenne (Figure 5)* generally show parallelism with the parcels with high soil moisture (Figure 4)

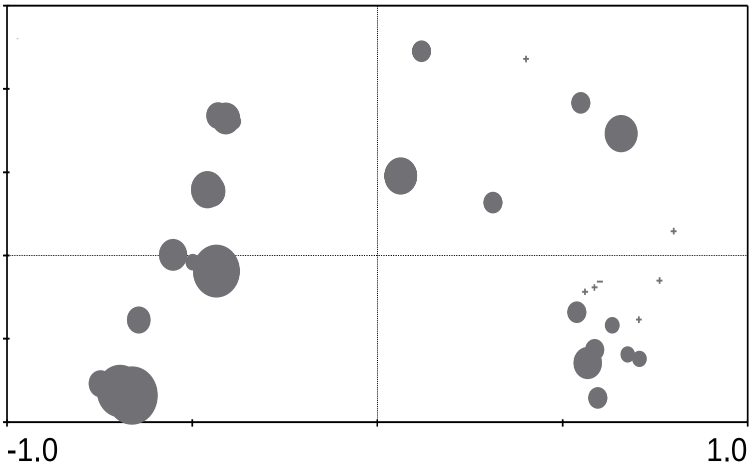

FIGURE 5. PRESENCE OF *LOLIUM PERENNE* IN SITES

Lolium perenne among common plants was selected as an example, and their statuses of presence in sites are shown in Figure 5. The *Lolium perenne* in the parcels displayed with dark-colored symbols in the biplot parcel refer to high values. *Lolium perenne, Carex flacca,* and *Trifolium repens* among the plant species in these shaped pasture sites are more common in wet soils. *Lolium perenne* is a graminae that prefers damp, fertile and heavy soils Altin (1992)**.** Dengler *et al.* (2014) reported that water limitation reduces productivity as it reduces the ability of dominants to develop sufficient growth, even under nutrient-rich conditions. At the same time, taller plants were probably favoured by deeper soils which were more frequent in abandoned grasslands (Vassilev *et al.*2011).

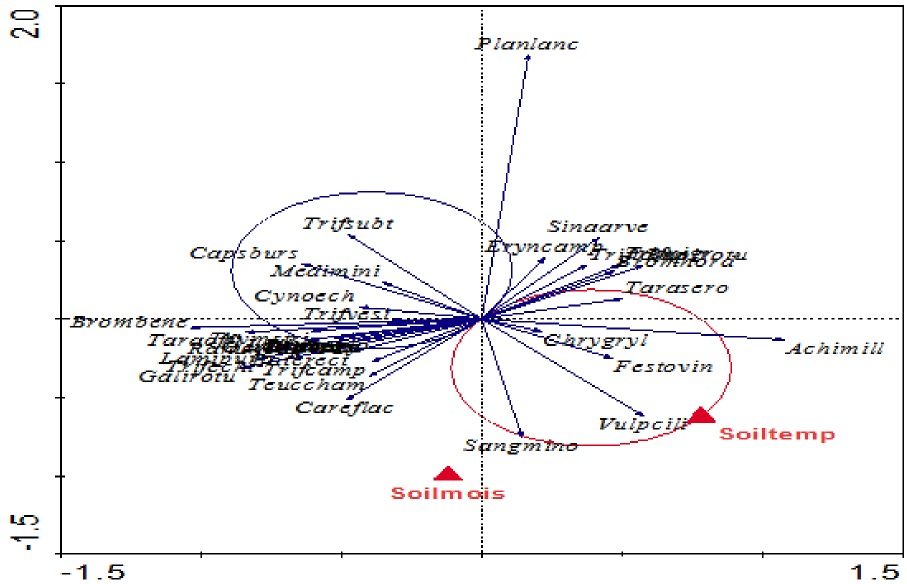

FIGURE 6. T- VALUE PLOT OF SOIL TEMPERATURE

Species:*Achillea millefolium* Achimill; *Aira caryophyllea* Airacary, *Bromus benekenii* Brombene, *Bromus hordelymus* Bromhord, *Bupleurum rotundifolium* Buplrotun, *Capsella bursa-pastoris* Capsburs,*Carex flacca* Careflac, *Cynosurus echinatus* Cynoech, *Chrysopogon gryllus* Chrygryl, *Dactylis glomerata* Dactyglom, *Eryngium campestre* Eryncamp, *Festuca ovina* Festovin, *Galium rotundifolium* Galirotu, *Geranium robertianum* Gerarobe, *Koeleria nitidula* Koelnitu, *Lamium purpureum* Lamipurp, *Lolium perenne* Lolipere, *Medicago minima* Medimini, *Plantago lanceolata* Planlanc, *Potentilla recta* Poterect, *Ranunculus neapolitanus* Ranuneap, *Sanguisorba minor* Sangmino, *Sinapis arvensis* Sinaarve, *Taraxacum officinale* Taraoffi, *Taraxacum serotinum* Tarasero, *Teucrium chamaedrys* Teuccham, *Thymus striatus* Thymstri, *Trifolium campestre* Trifcamp, *Trifolium echinatum* Trifechi, *Trifolium nigrescens* Trifnigr, *Trifolium ochroleucum* Trifochr, *Trifolium subterraneum* Trifsubt,*Trifolium tomentosum* Triftome, *Trifolium vesiculosum* Trifvesi, *Vicia sativa* Vicisati,*Vulpia ciliata* Vulpcili

Environmental variables: Soil mois Soil moisture, Soil temp Soil temperature

It was found out that there was a positive relationship between soil temperature and *Sanguisorba minor, Chrysopogon gryllus, Vulpia ciliata*. Annual legumes such as *Medicago minima, Trifolium vesiculosum, Trifolium subterraneum* have a negative relationship with soil temperature (Figure 6).

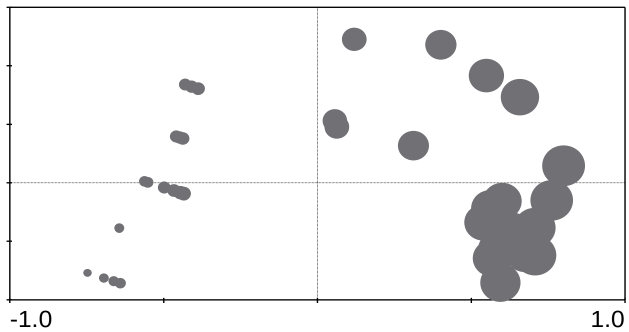

FIGURE 7. DISTRIBUTION OF SITES ACCORDING TO SOIL TEMPERATURE

The distribution of the parcels according to the soil temperature of the study area is presented in Figure 7. The soil temperatures in the parcels displayed with dark-colored symbols in the biplot parcel refer to high values. These parcels include the soil temperatures during the second year of the study. It is observed in Figures 6 and 3 that *Sanguisorba minor* efficiently benefits from the soil temperature and soil moisture. The distribution (Terri and Stowe, 1976; Tieszen et al., 1979) and seasonal activities of C3 and C4 grasses (Kemp and Williams, 1980; Hicks et al., 1990) often are highly correlated with temperature(IPCC, 1995). According to previous research Adams et al. (1986), grasses are usually dominant in pastures all over the world. Indeed, it was determined that *Chrysopogon gryllus* among these common species are found in grazed dry pasture sites (Figure 8).

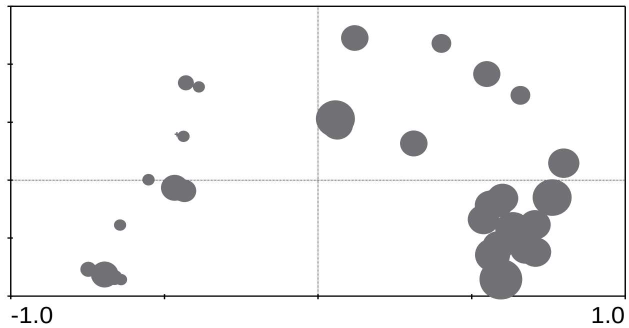

FIGURE 8. PRESENCE OF CHRYSOPOGON GRYLLUS IN SITES

The distribution of the hot climatic plant *Chrysopogon gryllus* (C4), one of the important species in the study area, is presented in figure 8. The *Chrysopogon gryllus* in the parcels displayed with dark-colored symbols in the biplot parcel refer to high values. The parcels with the high ratios of *Chrysopogon gryllus* (Figure 8) generally show parallelism with the parcels with a high soil temperature (Figure 7). *Chrysopogon gryllus,* grows on warm, dry, illuminated, sandy grassy slopes and hills as well as on dry pasture land (Djurdjevıć et al.2005, Dajıć Stevanovıć et al. 2008). The most widely spread species on Buzagici(Tekirdag) pasture was scented grass *(Chrysopogon gryllus*) (Uluocak, 1974; Davis, 1985; Tuna et al. 2011). Actually, there is a mutual relationship between plant species and environmental factors as is known.

IV. CONCLUSION

According to our study results, it was determined that the annual amount of rainfall affected the soil moisture and temperature. Indeed, during the two years of the study, rainfall differences caused significant changes in the soil moisture and temperature. Accordingly, decreases were found in dry yields along with the decreasing soil moisture and increasing temperature, and this was found to have a significant effect on plant species distribution. Such studies will create a resource for the studies to be carried out from now on for the ranges of arid and semi-arid regions. In addition, they will allow us to reach more information about climate, soil and pasture interactions by extending the study area and scope and ensuring their continuity for many years. Such studies will also allow the determination of species that can adapt to the arid conditions in the pastures.

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Comparison of Resistance to Fusarium wilts disease in Seeded and Regenerated Sesame (*Sesamum indicum* L.)

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Abstract— *Plant tissue culture has been used as a tool for crop improvement in many different ways. Such as somaclonal variation that occurred in many different crops. In this study a program for disease resistance was established in sesame using somaclonal variation. As resistance to Fos is very important so different kinds and concentrations of Plant Growth Regulators were tested for producing of plantlet regenerated from apical shoot explants.*

The results showed that the combination of BA and NAA also BA and IAA with ABA could be used for regenerating sesame plantlets from apical shoots. The difference in BA concentrations had a positive effect on shoot and root regeneration and at least plant regeneration. So with combination of high level of BA and low level of NAA shooting from explants was dominant and with low level of BA and high level of NAA rooting was progressed. Regenerated plantlets and seeded planlets were compared for examining of resistance or susceptibility to Fos. The result showed that somaclonal variant resulted from regeneration of shoot and root of sesame could lead to producing resistant plantlets.

Keywords*— *fusarium, sesame, somaclonal variation.

Abbreviations: ABA – Abscisic acid; BA – 6 benzylaminopurin; *Fos* – *Fusarium oxysporum* fsp *sesami*, IAA – Indole-3 acetic acid; MS – Murashige and Skoog's medium 1962; NAA – α-naphthalene acetic acid; PGRs – Plant Growth Regulators.

I. INTRODUCTION

Somaclonal variation, resulting from a sum of genetic and epigenetic changes can induce mutations (Wei *et al* 2016). Somaclonal variation occurs through tissue culture in plants and plant tissue culture techniques proffer a substitute method of vegetative propagation of horticultural crops (Krishna *et al*. 2005; Alizadeh *et al*. 2010). On the other hand somaclonal variation is a basic method for inducing resistance in many plants against biotic and abiotic stresses (Chae *et al*., 1987; Kim *et al*., 1987 and Kariallappa, 2003). However Larkin and Scowkraft in 1981 coined a general term "somaclonal variation" for plant variants derived from any form of cell or tissue cultures, It is important that genetic variations occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of in vitro raised plants (Bairu *et al*. 2011; Currais *et al*. 2013). So for inducing of variation in plants duo to somaclonal variation at first we need to a suitable and practical system for regeneration of plants. It means that denovo organs must to be regenerated by callusing phase. In this research we established a system for sesame regeneration and compared resistance of regenerated and seeded sesame against *Fusarium oxysporu*m fsp *sesami* that is a one of the most devastating agents for sesame in Iran. Also In Iran alike all over the world sesame is the quine of oilseed.

This important oilseed ranks third among the oilseed in production. Its oil content varies from 44 – 66 % containing two unsaturated fatty acids – oleic and linoleic together account for 85% (Maximum) with a combination of different essential amino acids and vitamins particularly β carotene (Brar, 1982 and Arslan *et al*., 2007).

II. MATERIALS AND METHODS

2.1 Preparation of plant materials

Seeds of sesame CV. Darab1 were obtained from Seed and Plant Improvement Institute, Karaj, Iran.

Mature seeds of sasame were aseptically sterilized by immersing in 100% commercial hypochlorite sodium (with 5% available chlorine) for 20, 30 and 60 min. Sterilized seeds then rinsed with sterilized distilled water for 4 – 5 times in order to remove the effects of disinfecting agent then cultured on 0.5 MS (Murashige and Skoog 1962), plus 3% sucrose and 0.7% agar in 9 cm petri dishes that were closed with parafilm. The cultures maintained in room temperature and mild light. Seven days later pieces of hypocotyl (3 – 5 mm), apical shoots (2 – 3 mm) and cotyledon (9 – 16 mm^2) from seedlings were used as explants.

2.2 Media and culture conditions

For production of regenerated plants the explants were cultured on MS media supplemented with different kinds and concentrations of PGRs such as BA, IAA, NAA, ABA and 554.94 μm^{-1} myo-Inositol, 0.3 μml^{-1} Thiamin, 24.3 μml^{-1} Pyridoxine, 4.06 μml^{-1} Nicotinic acid , and 26.64 μml^{-1} Glycine. Explants were subcultured to fresh media with the same composition every 4 weeks duo to consumption of nutrition and oxygene. All media included 3% sucrose and 4% phytagel in this phase.

pH of all media was adjusted to 5.7 prior to autoclaving. All cultures were incubated at 25 ± 2 º C under a 16-h photoperiod and 1500 Lux illumination.

2.3 Inoculum preparation

To prepare of inoculum approximately 3 – 4 discs (1 cm^2 length) from PDA including *Fos* mycelia were cut and put on autoclaved wheat grains that placed in clear plastic bag. Clear plastic bag plugged by cotton and incubated at 25º for three weeks. Seventy five grams inoculum was mixed with two kg autoclaved soil and prepared for each pot before sowing.

Plantlets inoculation and evaluation of resistance

Two kinds of plantlets including seeded plantlets (4 foliage) and regenerated plantlets (approximately 4 foliage) planted in inoculated soil for evaluating for resistance or susceptibility against *Fos*. For evaluation of resistance dead or alive of plantlets was mentioned (See Pavlou)

III. RESULTS AND DISSCUTION

Explants (Leaf, hypocotyl and apical shoot) produced callus after nearly two weeks. Leaf explants doubled in size and produced callus on the wounded edges. Hypocotyl explants produced callus on their entire surface and apical shoots produced callus a little. The calli were apparently categorised based on the potential of organogenesis or embryogenesis. The effective factore on this classification was at first the component of media and the second explant type. So that on media supplemented with BA the adventitious shoots developed from hypocotyl and apical shoot within 14 – 28 days after callusing. Shoot developed much more by increasing of BA concentration. 4.44 and 22 μml^{-1} BA produced adventitious shoots with 5.70 μml^{-1} IAA + 3.78 µml-1 ABA and 1.61 μml^{-1} NAA respectively (Fig 1). Because a cluster of shoots usually formed on explants it was difficult to count the number of shoots on these two media. It is clear that regeneration efficiency in a range of BA (4.44 – 22 μml^{-1}) increased as BA concentrations increased but dense mass of shoots formed on explants affected not only by BA concentrations but also kind and concentration of auxins and other plant growth regulators. On the other hand BA concentrations with auxin(s) concentrations had influence in shoot regenerations. In Fig 7 the effect of BA and auxin(s) (NAA) on shoot regeneration was summerized. Also this picture shows that the kind of auxin(s) that cooperats with BA on shoot regeneration has an effective role. Shoots (1 – 2 cm) were excised and cultured on suitable rooting media especially medium with 8.05 μml^{-1} NAA and 0.13 μml^{-1} BA. After 7 - 10 days 5 – 6 white, thick and semi strong roots appeared on base of regenerated shoots (Fig 2). It is interesting that BA and NAA with different balances formed both shoot and root in sesame. Successful regeneration have a key role for somaclonal variation. At BA and NAA concentrations regeneration tended to shooting by rising in BA concentration and rooting by rinsing in NAA concentration. Similar results were observed in apple cultivars or rootstocks (Welanders, 1992; Ancherani *et al*., 1990; Yepes *et al.*, 1994 and Famiani *et al*., 1994) where the low concentration of auxin in combination with height cytokinin content resulted in an increase in number of shoots per explants. Also there are many reports about the positive effect of BA in shooting (Lee *et al*., 2003; Ahmad *et al*., 2010; Rai *et al*., 2012; Kadota *et al*., 2001).

After establishment of regenerated plantlets and seeded plantlets the resistance (Fig 3 and Fig 4 respectively) of both compared against *Fos*. Two different kinds of plantlets displayed significant differences. According to Pavlou and Vakalounakis in 2005 sign of necrosis in xylem and phloem, dead or dying plantlets and wilt is indicator for susceptibility. As figures show four foliage seeded planlets after transplanting in inoculated soil displayed all of mentioned signs and dead after 20 days completely (Fig 5). So regenerated plantlets not only died but also grew and produced new leaflets after 12 – 20 days transplanting in inoculated soil (Fig 6). So we can result resistance to *Fos* altered in sesame by tissue culture. It is clear that a wide range of plant characteristics can be altered as a result of regeneration from cell and tissue culture including agronomacally important traits such as diseases resistance (Van den bulk 1991 and Sebastiani *et al*., 1994). Also Somaclonal variation was claimed to be a source of variation for crop improvement (Ching – Yan Tang, 2005). Although somaclonal variation has been reported in crop with reproduction vegetatively (Larkin and Scowcroft, 1981) more there are a few reports about selection of somaclonal resistant to alternaria blight in sesame through tissue culture (Lokesha and Naik, 2011). On the other hand Fusarium is one of the most devastating microorganism that has been combatted by somaclonal variation (Pierk, 1994 and Ching Yan – Tang, 2005). Also this study that their results demonstrated the resistance of regenerated sesame plantlels against *Fos* agreed with researches that mentioned above. As figures show clearly one of the difficult in this research was weakness and smallness of regenerated plants when they compared with seeded plantlets duo to unfavourable conditions especially oxygen deficiency and high level of humidity. We tested seeded plantlets in 4 folige stage. Long-term maintenance (regenerated plantlets) in order to complete adaptation was not our purpose. But it is interesting that regenerated plantlets resisted against pathogen (*Fos*) despite of weakness and smallness.

So included in this study that would be presented method for inducing resistance in sesame by somaclonal variation as the one of results of tissue culture.

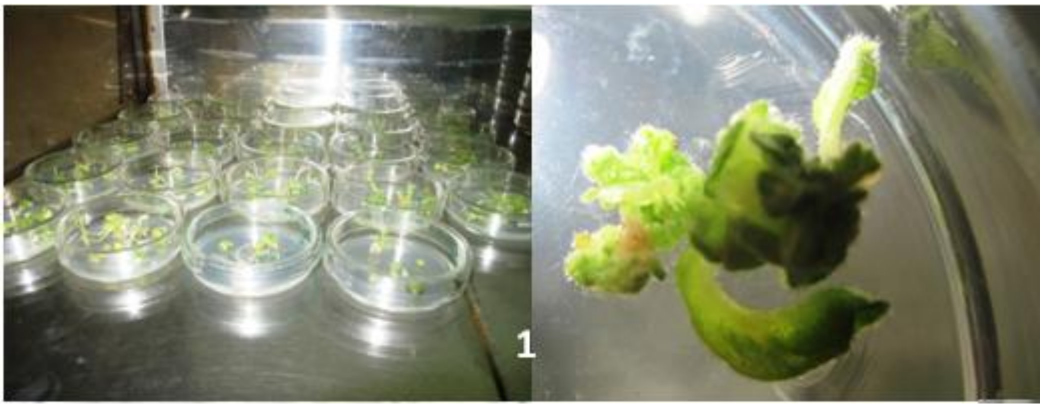

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FIGURE 1 – 6. Occurance of somaclonal variation in regenerated sesame and the effect of it on resistance or susceptibility plantlets to *Fos*. 1- Shooting on apical shoots explants on MS medium with 8.88 µml^{-1} BA and 1.61 8.88 NAA. Rooting of regenerated shoots on MS medium with 8.05µml^{-1} NAA and 0.13 µml^{-1} BA. 3- Regenerated plantlets in pot. 4- Seeded plantlets in 4 foliage phase. 5-Dead seeded plantlets 20 days after inoculating with *Fos*. 6- Resistance of regenerated plantlets 20 days after inoculating with *Fos*.

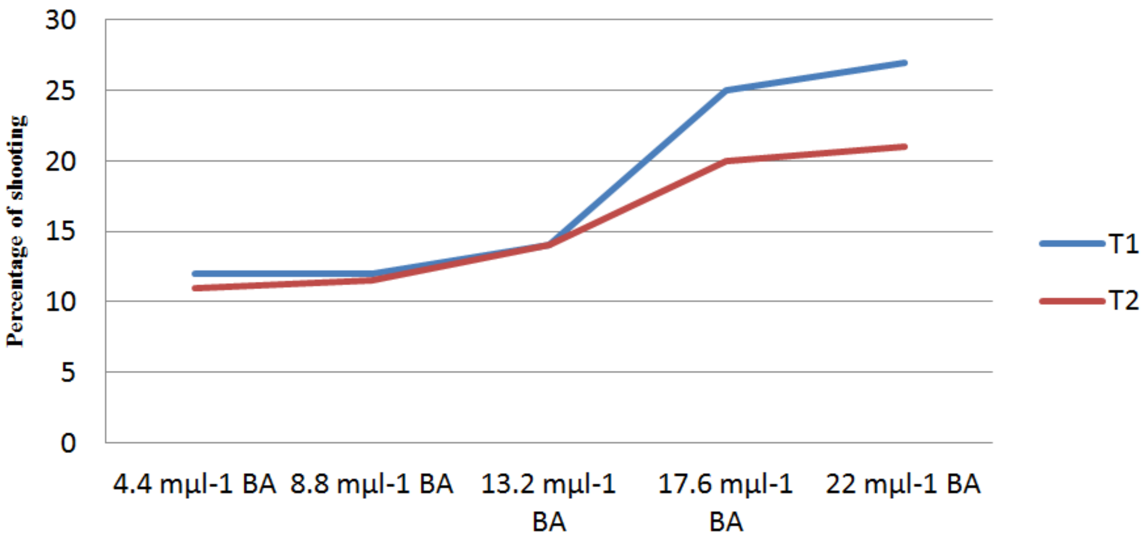

FIGURE 7. Effect of elavated BA concentrations with 5.7 mµl^{-1} IAA + 3.78 mµl^{-1} ABA (T1) and 1.61 mµl^{-1} NAA (T2)

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Radial variation in microfibril angle of *Acacia mangium*.

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Abstract**—** *Thirteen years old provenance trials of Acacia mangium from five provenances were established at five sites in the state of Sarawak, Malaysia, were sampled for this study. Fifty trees were sampled at random and cut to study radial variation in microfibril angle in the SS2 of secondary wall of the fibre using polarised microscope. Microfibril angle decreased from pith to bark with the greatest decrease occurred within the first two radial sampling near to the pith. It ranged from 5.9° to 28.8° with an overall mean and coefficient of variation of 12.6° and 45.8% respectively. It had a mean value of 21.4° at pith and 6.9° near the bark, which is a decrease of 67.8%. Highly significant different in mircrofibril angle were detected between radials of individual trees at α≥0.001. It was the major contributors to the total variance in which contributed for about 64.8%. Variations between trees were highly significant at α≥0.001 and accounted for 25.5% of the variation in microfibril angle while differences between the two orientations were not significant at α≤0.05.*

Keywords**— *Acacia mangium, microfibril angle, radial variation, pith to bark, interaction.***

I. INTRODUCTION

In future, it is expected that Malaysian and international market will be flooded with wood produced by short rotation fast growing timber species. A major concern with short rotation is the present of higher proportion of juvenile wood known as core wood (Burdon *et al.* 2004). Juvenile wood displays poor characteristics like higher microfibril angle (MFA), lower density, low stiffness, thinner cell walls, and shorter tracheids than mature wood (Cown 1992). Lower densities and reduced fibre dimensions, higher MFA, and low stiffness of juvenile woods are expected to produce a poorer quality product, often causing dimensional instability, for example in loblolly pine (Kretschmann and Bendtsen 1992) and Sitka spruce (Macdonald and Hubert 2002), resulting in poor acceptance in the market (Cown and van Wyk 2004). For these reasons, wood property traits have begun to receive more attention in the tree improvement programs as well as in forest industry (Powell *et al.* 2004).

In addition to specific gravity the other most important wood characteristic, which has direct impact on wood stiffness and strength, is microfibril angle (Butterfield 1998; Bendtsen and Senft 1986; Cave 1969). It also has an influence on shrinkage of wood (Harris and Meylan 1965; Ying *et al.* 1994). Microfibril angle (MFA) is referred to the mean helical angle that the cellulose microfibril in the S_2 layer of the cell wall makes with the longitudinal axis of the cell (Barnett & Bonham 2004). Microfibril angle is a property of the cell wall of wood fibers, which is made up of millions of strands of cellulose called microfibril (Walker & Butterfield 1995 and Butterfield 1998).

Now in Malaysia as *Acacia mangium* is gaining popularity for both timber and for pulping, understanding the wood properties of this species is particularly important to effectively utilize this timber and before any improvement program has been developed to improve its quality. This study was therefore carried out to fulfil this objective. The work described in this paper forms part of a larger study of the genetic and environmental influences, and their interaction on growth, wood properties and mechanical properties in *Acacia mangium*. The main objective of this study is to establish a radial variation in MFA and to study the extent of variations in microfibril angle between trees, orientations and radial subsamples.

II. MATERIALS AND METHODS

2.1 The trial

Thirteen years old provenance trials of *Acacia mangium*, which were established in five sites in the state of Sarawak, Malaysia, were sampled for this study. Five provenances were planted. Details of the trials were reported in Lokmal & Mohd Noor (2010). The trial was conducted using randomised complete block design and was laid in complete factorial of five sites x five blocks x five provenances x 25 trees.

2.2 Tree sampling

Three trees were sampled randomly and cut from each treatment plot making a total of 375 sampling trees. However, due to some heart-rot and termites attack, only 362 trees were managed for final process. For the purpose of this study, 50 trees were sampled at random. The number was a compromise between high variability in MFA within tree and the difficulty of measuring the MFA.

2.3 Wood sampling

A two (2) cm thick disc was cut at 1.3 m height for every tree. A strip of two (2) cm width was cut running through the centre (pith) of the disc along east west orientation of the tree. The strip was cut at the centre into two parts i.e. east and west. Each part was measured and cut into four (4) equal-length samples, hence producing 400 samples (50 trees x 2 orientations x 4 radial positions).

2.4 Measurement of the microfibril angle

The method for measuring microfibril angle was adopted from Leney (1981). It involved several stages and were briefly explained below. All selected samples were separated and placed in a 40-ml beaker and 25 ml distilled water was poured over it. The sample was then boiled in the autoclave at 100°C for a total of 10 hours. Slices were cut from the tangential face of the samples using a sliding microtome with thickness set at 10 micron (about 50% of fibre thickness) in order to produce half-cut fiber required for the measurement of MFA through this method.

2.5 Maceration process

The slices were placed in 50 ml test tubes with 2 ml of maceration solution (a mixture of 44 parts of glacial acetic acid and 56 parts 30% hydrogen peroxide). The test tubes were heated in a water bath at 90°C - 95°C for 12 hours until the samples were bleached white and easily separated into components cells or fibres when shaken gently. The maceration solution was then poured off. The remaining maceration solution was diluted by adding 20 ml distilled water into the test tube, shaking and removing the mixed distilled water and solution using a 30 ml pipette. This step was repeated three times. A wide-mouth bulb pipette (8 mm inside diameter) was put in the suspension of fibres. The bulb of the pipette was then released quickly to suck a random sample of fibres into the pipette.

2.6 Preparation of the slides

The pipette was held vertically and moved to a position over a slide on a hot plate (80-90°C). The fibre suspension was squeezed out of the pipette on to the centre of the slide.The fibres were allowed to settle on the slide, and part of the water was allowed to evaporate on the hot plate. A cover glass was then put on the slide taking special care not to trap bubbles in between the fibres. Two slides were prepared from each sample to ensure enough microfiber are captured. A microfibril angle was measured once from each of the individual 29 half-cut fibres generating a total of 11,600 MFA for this study. The high sample number of fibres was needed because of the high variability of microfibril angle between fibres within a tree.

2.7 Polarised microscope procedures

With the polariser and analyser of a polarised light microscope set in the cross position (darkest), a first order red wave plate was introduced into the beam below the analyser at an angle of 45° to give a red field. The slide was then introduced into the field of view of the polarised light microscope. It was rotated clockwise to an angle where the colour changed from yellow to red to blue, indicating that it was in the major extinction position (MEP). The red plate was then removed and slight adjustment was made for the best major extinction position in black and white (when the central part of the fibre was the darkest). The angle of the rotary stage was recorded. Then the fibre axis was aligned parallel to the vertical cross hair line in the eyepiece. Again the angle of the rotary stage was recorded. The difference between these two readings was the microfibril angle for the secondary cell wall (S2).

2.8 Data analysis

SAS (SAS 2008) was employed throughout the analysis of this study. Two types of analysis were carried out in this study as described below.

2.9 Analysis of variance

Mean over 29 microfibrils were subjected to analysis of variance using Procedure general linear model (SAS 2008) using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \eta_{ij} + \gamma_{ik} + \theta_{jk} + \varepsilon_{ijk}$$

Where

Y_{ijk} is the mean microfibril angle in *k*th radial position at *j*th orientation of *i*th tree.

μ is the overall mean.

α_i is the random effect of *i*th tree (*i*=1, 2 .... 50).

β_j is the fix effect of *j*th orientation (*j*=1,2).

δ_k is the fix effect of *k*th radial position (*k*=1,2,3,4)

η_{ij} is the interaction between *i*th tree and *j*th orientation.

γ_{ik} is the interaction between *i*th tree and *k*th radial position.

θ_{jk} is the interaction between *j*th orientation and *k*th radial position.

ε_{ijk} is the random error associated with the *k*th radial position in the *j*th orientation at the *i*th tree.

Student-Newman-Keuls multiple-range test was also performed to identify differences between samples within the radial.

2.10 Variance components

Variance components were estimated using proc varcomp via restricted maximum likelihood (REML) method (SAS 2008) with the same model as in the analysis of variance.

III. RESULTS

3.1 Radial Variation

Microfibril angle decreased from pith to bark. Its mean ranged from 5.9° to 28.8° with an overall mean and coefficient of variation of 12.6° and 45.8% respectively (Table 1). It had a mean value of 21.4° near pith and 6.9° near the bark, which involved a decrease of 67.6%, or a reduction of 14.5°. The most rapid changes occurred between the first two radial sampling i.e. SS1 and SS2 (Figure 1 and Table 1). It involved a reduction of 39.4% (reduction of 8.5°). The decrease in MFA from SS2 to SS3 and SS3 to SS4 were 28.7% and 25.0% involving a reduction of 3.7° and 2.3° respectively.Highly significant different in mircrofibril angle were detected between radials at $\alpha \geq 0.001$. Student-Newman-Keuls multiple range test have shown that all the four radials were differed significantly at $\alpha \geq 0.05$.

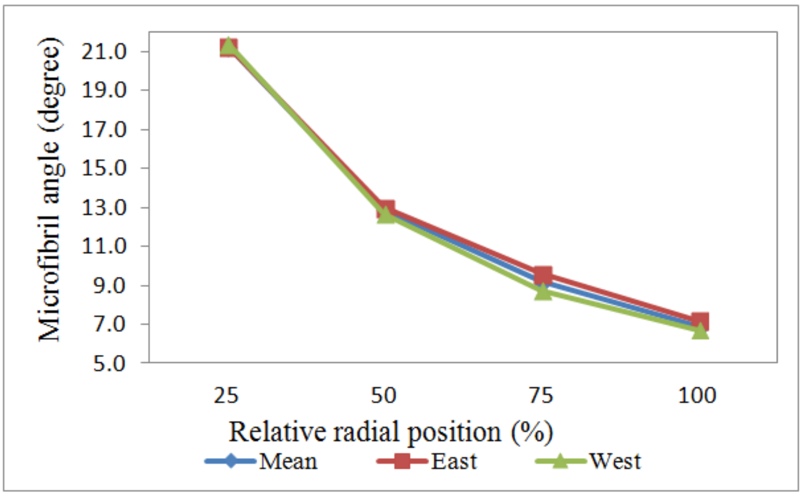

FIGURE 1: VARIATION IN MICROFIBRIL ANGLE FROM PITH TO BARK IN *ACACIA MANGIUM*.

TABLE 1
VARIATION IN MICROFIBRIL ANGLE FROM PITH TO BARK IN *ACACIA MANGIUM*.

Radial	Mean	Min	Max	CV (%)	Std error
SS1	21.4°a	16.6°	28.8°	15.1	0.32
SS2	12.9°b	11.5°	15.5°	7.2	0.09
SS3	9.2°c	7.6°	11.6°	10.4	0.10
SS4	6.9°d	5.9°	8.8°	9.10	0.06
Overall	12.6°	5.9°	28.8°	45.8	0.29

Notes: SS1 sample nearest to pith; SS4 sample closest to bark; Mean with the same letter are not significantly different at α≤0.05 via Student Newman Keuls.

3.2 Variation between trees

Mean of individual tree varied from 11.0° to 15.3° with an average and coefficient of variation of 12.6° and 9.7% respectively. Differences between trees were also highly significant at α≥0.001 (Table 2). Student-Newman-Keuls multiple-range test was performed and separated all trees in to 22 significantly distinct groups reinforced the high variation between trees. Tree accounted for 25.5% of the total variance in MFA.

3.3 Variation between orientations

Mean of MFA for east and west orientation 12.8° and 12.4° respectively. The differences between the two orientations were not significant at α≤0.05 (Table 2 and Figure 3).

TABLE 2
ANALYSIS OF VARIANCE AND VARIANCE COMPONENT FOR MICROFIBRIL ANGLE

Source	df	Mean square	Variance	Variance (%)
Tree (T)	49	12.1^{***}	11.0	25.5
Orientation (O)	1	16.8^{ns}	0.1	0.1
Radial (R)	3	4015.1^{***}	28.1	64.8
TXO	49	0.4^{ns}	0.1	0.1
TXR	147	3.6^{***}	2.6	6.0
OXR	3	4.2^{***}	1.1	2.5
error	147	0.4	0.4	0.9
Total	399		43.3	100.0

Notes: *, **, *** significant at p≥0.05, p≥0.01 and p≥0.001. ^{ns} not significant at p≤0.05

3.4 Interaction between Trees and Orientations

Interaction between trees and orientations was not significant (Table 2). Although there was highly significant different between trees, the different between both orientations for individual tree was very small (Figure 3).

3.5 Interaction between Trees and Radials

Interaction between trees and radials was highly significant (Table 2). Further examinations have shown that the interaction was due to different rate of reduction in microfibril angle within each radial of individual tree (Figure 2). This was very obvious especially within the first quarter from pith where tremendous change in MFA occurred.

3.6 Interaction between orientations and radials

Interaction between orientations and radials were highly significant (Table 2). Further examinations have shown that the interaction was due to different rate of reduction in microfibril angle within each radial of different orientations especially in the region of 50%-75% from pith.

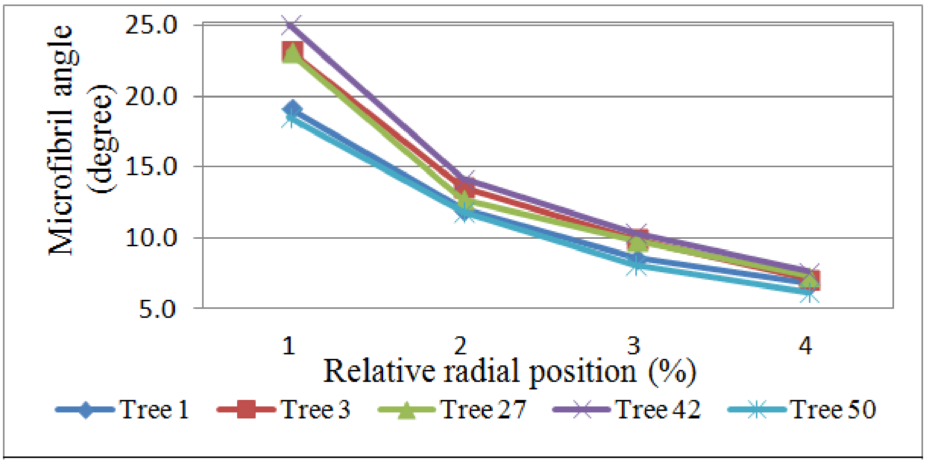

FIGURE 2: VARIATION IN MICROFIBRIL ANGLE IN FIVE SELECTED TREES FROM PITH TO BARK IN *ACACIA MANGIUM*.

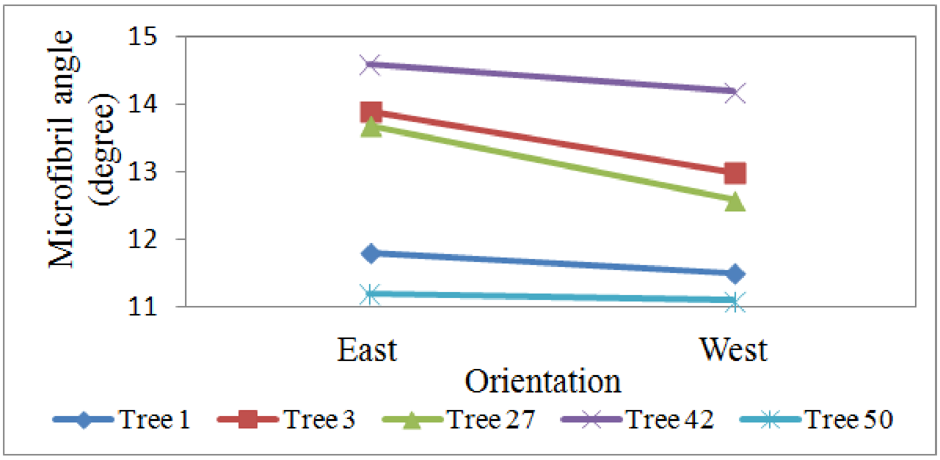

FIGURE 3: VARIATION IN MICROFIBRIL ANGLE IN FIVE SELECTED TREES IN EAST AND WEST ORIENTATIONS OF *ACACIA MANGIUM*.

IV. DISCUSSIONS

4.1 Radial Variation

A decrease in microfibrils angle from pith to bark in *Acacia mangium* is consistent with work on *Pinus radiata* by Baltunis *et al.* (2007), Donaldson & Burdon (1995), Donaldson (1997). This was also reported in other species such as loblolly pine (Megraw *et al.* 1998, and Myszewski *et al*. 2004) and in *Picea abies* (Lundgren 2004). A significantly higher value of MFA near pith was probably due to the present of juvenile wood in the region of core wood, which eventually led to the inconsistent and poor strength at the centre (Cown 1992). Bendtsen (1978), further revealed that juvenile wood displayed shorter tracheids, with thinner cell walls and consequently lower wood density, while in mature wood in which the MFA is smaller, the trachieds are longer with thicker walls and consequently higher wood density.

The most drastic reductions which occurred between SS1 and SS2 may suggest the presence of juvenile wood within this region; however, almost the same magnitude of reductions which extended between SS2 and SS3 may further suggest that the juvenile regions may presence up to SS3 or within 75% radially from pith. Microfibril angle in the S2 layer of the tracheid cell wall is the only known physical characteristic of wood that is capable of affecting large changes in the stiffness of wood (Meylan and Probine 1969). Independent studies have shown that decreasing in MFA have increased MOE and MOR from pith to bark in eastern cottonwood (Bendtsen and Senft 1986) and in quaking aspen (Roos *et al.* 1990).

Fibers with high MFA at the centre of the tree, which were produced when the tree was in the sapling stage, endow the wood with a low Young's modulus. This enables the sapling to bend during strong wind without breaking. As the tree grows, the stem has to become stiffer to support the increasing weight of the stem and crown. The lower MFA at the outer wood means the tree has higher Young's modulus which enables them to fulfill the role. The other possibility is probably due to its colonising habit (Wiemann & Williamson, 1988), which combine rapid early growth in stature with the production of a weak stem due to high microfibril angle. As the tree grows, reducing microfibril angle in inevitable as to increase stem stiffness to maintain structural stability. These changes are probably associated with the ecological habit of pioneer species of wet tropical lowland forest. Differences in mircrofibril angle between radial was highly significant at $P \leq 0.001$. This is in contrast with the work by Lima *et al.* (2004) on 11 clones of hybrid between *Eucalyptus grandis* and *E. urophylla* who found no significant different in microfibril angle between radials.

The decreasing radial variation alone contributed for 64.1% of the total variation in MFA, implied that the wood strength in the region of core wood would be low and caused further problem during wood processing. This was consistence with the finding by Butterfield (1998) who concluded that MFA was the dominant wood characteristics underlying the poor wood quality in many fast-grown and short-rotation plantation softwoods. However this situation creates an opportunity in tree improvement by reducing the MFA in the central regions. This will result in improved wood quality at the central hence improve wood strength. Evans & Ilic (2001) found that MFA accounted for 96% of the variation in modulus of elasticity of *Eucalyptus delegatensis*. The scenario was unwelcome as in future most timbers reaching the markets were most likely will be coming from fast grown short rotation with high proportion of juvenile wood.

4.2 Variation between trees

Effect of trees was significant and contributed for 25.5% toward the total variation in MFA. Though the amount was only a third of radial variance, it was substantial to ensure a successful gain in tree selection. Very high MFA variation between radials within trees implicated that attempts to improve MFA must take this into account and must make sure that considerable variation between trees exists. Similar observation was reported in *Pinus radiata* (Donaldson & Burdon 1995). Differences among trees, a large part of observed variation was probably attributed to the differences in genetic makeup of the individual tree provenance which was not taking care during sampling and microclimate surrounding the individual tree. Evans *et al*. (2000) also found very large variation in microfibril angle between hardwood trees of red alder (*Alnus rubra*) and proposed for tree selection for steeper MFA in the central region. The same strategy was suggested by Donaldson & Burdon (1995) following their work in *Pinus radiata*.

4.3 Variation between orientations

There was no effect of orientation on MFA in this study. Although there was some variation in MFA between east and west orientation, it was very small and was not significant. The small variation observed in this study was probably an inherent. It may also due to the influence of strong wind blow which came from the east direction annually during heavy-rain monsoon season which also triggered formation of new fiber. To our knowledge, there was no work been done comparing the MFA between two opposite direction to compare with this study.

4.4 Interaction between trees and orientations

Interaction between trees and orientations was not significant and only accounted for 1% of the total variance in MFA. Since there was no significant different between orientations, the interaction between trees and orientations is probably and solely due to highly significant differences in microfibril angle between trees (Table 2). To our knowledge, there was no work been done comparing the MFA between trees and orientations to compare with this study.

4.5 Interaction between trees and radials

Interaction between trees and radials was highly significant (Table 2). Further examination of the data proved that the interaction was due to differences in rate of reductions between radials of different trees (Figure 2). To our knowledge, there was no work been done comparing the MFA between trees and radials to compare with this study.

4.6 Interaction between orientations and radials

Interaction between orientations and radials was significant (Table 2). Further examination of the data proved that the interaction was due to different rate of reductions in MFA between radials of both orientations (Figure 1). To our knowledge, there was no work been done comparing the MFA between orientations and radials to compare with this study?

V. CONCLUSIONS

All trees exhibited the same decreasing pattern of microfibril angle radially from pith to bark. The largest decrease occurred in the first two sub samples from pith followed by a gradual decrease in the last two sub samples towards bark. Radial variation in microfibril angel is the most important factor affecting variation in MFA. It accounted for 61% of the total variation. Variation between trees is substantial and contributed for 22.3% of the total variation. Although this variation is only one third of the radial variation, it provides a basis for an improvement in MFA hence the wood strength in *Acacia mangium*.

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Atmospheric Deposition of Nitrogen compounds in Assam (India)

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Abstract**—** *The study comprises estimate of wet deposited nitrogenous compounds in Assam (India). Deposition has been estimated from a survey works (2010-11) at urban and peri-urban areas of Assam. Air samples were collected by clinical syringe (10 cm^3) for Oxides of Nitrogen (NO, NO_2: NOx) and reduced Nitrogen (NH_3, NH_4^+: NHy). The samples were diffused into 10 cm^3 each of distilled water and 0.1N HCl respectively for estimation of mean concentration of weighted hydrogen (μeql^{-1}), quantity of elemental nitrogen (N) or nitrate (NO_3^{-1}) and ammonium (NH_4^+) ions expressed in mg l^{-1} or kg ha^{-1}y^{-1}. The measured concentrations of the nitrogenous compounds were interpolated with a properly used Kriging Technique on a 1km x 1km grid covering districts characterised by varying congestions of population, vehicular transport and of industrial evidences. There were many fold variations of these air quality parameters among the major sites and locations of the pollutants e.g. nitrogen deposited through aerosol of its oxides ranged from 6.0-38 kg ha^{-1}yr^{-1}, whereas nitrogen accumulation from the reduced aerosol was 7-24 kg ha^{-1}yr^{-1}. Tissue nitrogen in some indicator plant species (e.g.Pinus longifolia, Ficus benjamina), collected from the square grids of polluted areas was also elevated. Thus, the hypothesis that the North-east India, especially Assam is also facing with enrichment of nitrogenous pollution due to anthropogenic activities, mass vehicular and industrial growth, was tested.*

Keywords**—** ***Oxides of nitrogen, reduced nitrogen, weighted hydrogen, aerosols, pollution.***

I. INTRODUCTION

The inputs of reactive N (termed here as NH_x:NH_3 and NH_4^+, NO_x :(NO + NO_2), and nitrate (NO_3^-) and nitrite (N_2O) are through increased biological fixation of atmospheric N_2 in certain crops, combustion of fossil fuels and release of NO_x, and production of synthetic fertilizer nitrogen (Galloway *et al*., 1994, 1995). There is an additional release of reactive N from stable soil organic matter as a result of soil disturbance for crop production that is not readily quantifiable (Edu Dorland *et al.*, 2013).

Nitrogen oxides have increased in importance in recent decades as atmospheric pollutants in rapidly growing urban and its surrounding areas of India (Bharali *et al.*, 2012; Vitousek *et. al.*, 1997). Deposition of gaseous Ammonia and particulate NH_4^+ may also contribute to the acidification of the ecosystem (Mohan and Kumar, 1998; McClean *et. al*. 2011). The wet and dry deposited oxides of N redistribute nitrogen throughout the environment. Ammonia as an atmospheric pollutant can contribute to a substantial portion of total deposition of nitrogen (Sutton, Moncrieff and Fowler, 1992). In fact, emissions of ammonia and nitrogen oxides have been strongly increased globally since the 1950s (Galloway *et. al.* 2008). The source of volatilised Ammonia is the intensive agricultural systems related to dairy farming, animal husbandry, whereas nitrogen oxides are linked to mainly anthropogenic activities viz., burning of fossil fuel by traffic, industry and households. So, many natural and semi-natural ecosystems across the world are deposited with the atmospheric nitrogen as a result of short- and long-range transport of the nitrogenous pollutants (Bobbink *et. al.* 2010). Since 1990s, areas recognised historically with high atmospheric N deposition (20–100kgNha^{-1}year^{-1}) have been the central and western Europe, eastern USA, eastern Asia and India. Estimated background inputs (pre-1900s) ranged between 1 and 3 kgNha^{-1} year^{-1} (e.g. Asman *et. al.* 1998; Dentener *et. al.* 2006; Galloway and Cowling 2002). Thus, atmospheric nitrogen deposition and climate variability are both measured as major components of global change (Gaudnik *et al.* 2011). The exploration for possibilities of current deposition of nitrogen in the province of Assam by virtue of any reason needs a strong attention.

II. MATERIALS AND METHODS

*Collection of Air (by clinical syringe,) and vegetation samples during October 2010 to January 2011 for their analyses***:** Air samples were collected for NO_2 concentrations based on diffusion tube measurement (Hyvarinen and Crittenden, 1998) by dissolving 10 cm^3 of it into equal volume of double distilled water, and NH_3 concentrations based on trapping 10 cm^3 air into a 10 ml of 0.1N HCl to analyse NH_4^+ (Harrison *et. al.*, 1989). The places for collection of air samples in Northeast India (Latitude 26^o North, Longitude 92^{o}E, Altitude 86.6 meter above the mean sea level) were from Guwahati (district of Kamrup Metro), Jagiroad (Morigaon), Jorhat-Titabor (Jorhat) and Namrup (Dibrugarh). The sites comprised of the highly populated, most traffic congested areas in the cities and towns, factories and industrial complexes (e.g.Pulp and paper industry at Jagiroad, Fertilizer manufacturing complexes at Namrup), Oil refinery at Guwahati). The Kriging Technique (Zapletal, 1998) was properly used for square grids of size about 1Km x 1Km inside the cities and crop fields nearby National Highways at certain elevation (5-10 feet above ground). The grids consisted of the locations, representative of a range of N deposition loads. We collected some vegetation samples also to examine the possibility of nitrogen deposition in the sampling sites.

The pH values of the aerosols were measured using a digital pH meter**.** Blanks for the oxides of nitrogen with distilled water, and 0.1N HCl for reduced nitrogen were also considered for net deposition of nitrogen. The pH value for blanks for NOx (distilled water only) was 6.61 (with weighted hydrogen ion: -48.2 $\mu l l^{-1}$), and pH values for blanks for NHy (0.1N HCl only) was 2.7 (with weighted hydrogen ion: 119.39$\mu l l^{-1}$). The values of the parameters obtained after subtracting the blank values from the solution values were considered as the net values. The values for calculation of weighted hydrogen from pH values in case of NOx (sample dissolved in distilled water) were referred to UKRGAR (1997). The calculation of weighted hydrogen from pH values for NHy (samples dissolved from 0.1N HCl) were done using the equation $\mu eqH^{+}l^{-1}$ = antilog(6-pH) directly. The relationship of pH with respect to weighted hydrogen and nitrogen in samples collected, and the calculations of Nitrate and Ammonium ions or Nitrogen (mgl^{-1}, or kg ha^{-1}), depending upon the quantity of rainfall in the places surveyed are as follows:

Parameters of NO_x: $mgNO_3^-l^{-1}$=weighted H^+ for pH x 0.062**, $gNO_3^-ha^{-1}yr^{-1}$ = [**$mgNO_3^-l^{-1}$ x amount of rainfall]/1000; **$gNha^{-1}yr^{-1}$** = [$mgNl^{-1}$ x amount of rainfall]/1000, 0.014$mgNl^{-1}$=0.062$mgNO_3l^{-1}$.

Parametrs for NH_y: $mgNH_4^+l^{-1}$=weighted H^+ for pH x 0.018, **$gNH_4^+ha^{-1}yr^{-1}$** = $mgNH_4^+l^{-1}$ x amount of rainfall]/1000**; $gNha^{-1}yr^{-1}$ =** [$mgNl^{-1}$ x amount of rainfall]/1000, 0.014$mgNl^{-1}$=0.018$mgNH_4^+l^{-1}$. **Wet Nitrogen Deposition:** D_M (gMm^{-2}) = [M](mg l^{-1}) x rainfall (mm) x 10 $^{-3}$

Where D_M is the deposition of ion M and [M] is the concentration, 1g m^{-2} = 10 kg ha^{-1}. In pH scale, lower is the value than the neutral pH (7.0), higher is the value of weighted hydrogen ion, and more is the Nitrogen in the sample.

Annual rainfall data were collected from the respective meteorological observatories situated nearby the air sampling areas. The total annual rainfall data considered for calculations for Guwahati & Morigaon, Jorhat & Titabor, and Namrup & Dibrugarh were 1440.3mm, 2344.10mm and 3129.90mm respectively. Nitrogen contents in the vegetation samples collected from the various polluted and relatively unpolluted sites were also analysed. The Kjeldhal method was used to determine total Nitrogen estimation, which is based on catalytic conversion of organic nitrogen into ammonia and its subsequent estimation by acid base titration (Yoshida, 1976)**.** 500 mg of oven dried (at 60-80^oC) samples were digested in a 100 cm^3 Kjeldahl flask. Added the same amount of salt mixture (K_2SO_4 or Na_2SO_4 with $CuSO_4.5H_2O$ and metallic selenium @ 50:10:1 ratio) and 3 ml of concentrated H_2SO_4. Each tube was heated gently and then at increasing intensity up to 400^oC after the initial vigorous reaction is subsided. When the digest becomes clear on continued heating for about 1-1.5 hour, allowed 30 minutes to cool it. The digested samples were diluted with 10 ml of distilled water, mixed thoroughly and allowed the sample to cool again. Blank digestions were also carried out.

Automatic analyses of nitrogen were undertaken using the Kjeltech Auto Analyzer. Transferred the digested sample and three rinses with distilled water into the micro-Kjeldahl distillation tube. Then added 10 ml of the 40 per cent NaOH to it. Prepared a 200-ml flask containing 10 ml of 4 per cent boric acid reagent and three drops of mixed indicator (0.3 g of bromo cresol green and 0.2 g methyl red in 400 ml of 90 per cent ethanol). Placed the flask under the condenser of the distillation apparatus, and made sure that the tip of the condenser outlet was beneath the surface of the solution in the flask. Allowed steam from the boiler to pass through the sample, distilling off the ammonia into the flask containing boric acid and mixed indicator solution for about 7 minutes. The tip of the condenser outlet was washed by distilled water. Then, titrated the solution of boric acid and mixed indicator containing the 'distilled off' ammonia with the standardized 0.1 N HCl. The reading was noted down each time and calculations were done as: Total N (%) in sample = [(Sample titre - Blank titre) x normality of HCl x 14 x 100]/ Sample weight (g) x 1000.

III. RESULTS AND DISCUSSION

Results on various parameters of oxidized and reduced nitrogen compounds collected from different sites of urban and peri-urban areas of Assam are presented on **Table (1-4).** There were significant differences of weighted hydrogen, nitrate, ammonia and elemental nitrogen present in the aerosols of oxidized and reduced nitrogen. The four major sites and also the specific locations of sample collections varied significantly. The concentrations of weighted hydrogen, nitrate, ammonium ions and content of elemental nitrogen increased in the aerosol samples collected near the sources of NOx & NHy emissions as compared to the samples taken away from the relatively unpolluted areas. The annual average total wet depositions of nitrogen compounds estimated in the province of Assam were NO_3^-:23.83, 72.63, 112.33, 104.79 $kgha^{-1}yr^{-1}$ and NH_4^+: 10.65, 35.78, 26.42, 97.06 $kgha^{-1}yr^{-1}$ for the districts of Kamrup, Morigaon, Jorhat and Dibrugarh respectively (Table 1-4).

TABLE 1
PARAMETERS ON AEROSOL PRODUCTS OF NO_x AND NH_y SAMPLES FROM EACH THREE MAJOR SITES (I, II & III) AT GUWAHATI CITY (KAMRUP DISTRICT OF ASSAM)

Parameters (Mean values)	Mean of ten NO_x samples (S) from each site										
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Blank (Without air sample)
PH of Air sample in distilled water	5.1	**5.3****	4.9	**4.8***	4.97	5.1	5.0	5.0	5.1	4.9	6.61 (distilled water only)
Weighjted H^+ (μeql^{-1})	120	**70***	140	**207****	173	93	103	113	93	130	< 0
mg $NO_3^- l^{-1}$	7.44	**4.34**	8.7	**12.8**	10.75	5.79	6.41	7.03	5.79	8.06	0
kg $NO_3^- ha^{-1}yr^{-1}$	17.59	**10.94**	**38.16**	32.55	19.07	20.92	29.85	22.49	20.92	26.52	0
kg N $ha^{-1}yr^{-1}$	24.19	**14.11**	28.23	**41.7**	34.95	18.82	20.83	22.85	18.8	26.21	0
Mean of ten NHy samples from each site											
PH of Air sample in 0.1N HCl	**1.12****	0.947	1.03	1.07	1.06	10.9	**0.89**	1.01	0.98	0.987	2.7 (0.1N HCl only)
Weighjted H^+(μeql^{-1})	**158.4***	162	160.2	159.5	159.6	159	**163**	160.6	161.	161.1	119.39
mg $NH_4^+ l^{-1}$	**2.85**	**2.92**	2.887	2.870	2.873	2.863	2.933	2.890	2.90	2.90	2.975
kg $NH_4^+ ha^{-1}yr^{-1}$	**41.07**	42.0	41.54	41.36	41.38	41.22	**42.26**	41.65	41.8	41.76	30.95
kg N $ha^{-1}yr^{-1}$	**37.94**	38.86	38.38	38.21	38.18	38.03	**39.06**	38.45	38.6	38.55	24.07

Site-I: Around Oil Refinery at Guwahati (S1:Noonmati refinery, (S2:Noonmati training centre, S3:Noonmati main gate, S4:Noonmati sector 3, S5:Birkuchi, S6:Patherkuwari,S7:Narengi oughuli road, S8:Narengi Junior college, S9:Narengi Tiniali, S10:New Guwahati Baminimaidan

Site-II: Around Maligaon: (S1-S5):Adabari Buses' stand-(S6-S7):Near Assam Engineering College, (S8-S9):near Sarighat Bridge at Jalukbari, S10: Towards Gopinath Bordoloi Airport

Site-III: (S1-S2):Manik Nagar, (S3-S5):Rajdhani Nursery, (S6-S8):Baisistha, (S9):Near Reginal passport office, (S10)Near Arohan , Beltola

*** The lowest pH value (highest acidity) with maximum H^+ concentration**

****Highest pH value (lowest acidity) with minimum H^+ concentration**

TABLE 2
PARAMETERS ON AEROSOL PRODUCTS OF NO_x AND NH_y AT JAGIROAD (MORIGAON DISTRICT OF ASSAM)

Parameters	Mean of sixteen NO_x samples (S) at Jagiroad																
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	Blank
PH of Air sample in distilled water	5.4	5.3	5.3	5.4	5.5**	5.2	5.3	5.3	5.2	4.9*	5.2	5.4	5.4	5.5*	5.4	5.1	6.61 (distilled water only)
Weighjted H^+(μeql^{-1})	45	50	55	35	30*	60	50	50	50	100**	55	40	40	30	40	80	< 0
mg NO_3 l^{-1}	2.79	3.10	3.41	2.17	1.86	3.72	3.10	3.10	3.10	6.20	3.41	2.48	2.48	1.86	2.48.	4.96	0
kg NO_3^- ha^{-1}yr^{-1}	40.18	44.65	49.11	31.25	26.79	53.58	44.65	44.65	44.65	89.30	49.11	35.72	35.72	26.79	35.72	71.44	0
kg N ha^{-1}yr^{-1}	9..07	10.08	1109	7.06	6.05	12.10	10.08	10.08	10.08	20.16	11.09	8.07	8.07	6.05	8.07	16.13	0
Mean of sixteen NHy samples at Jagiroad																	
PH of Air sample in 0.1N HCl	0.857	0.59*	0.857	1.07	0.97	1.07	0.97	0.97	0.88	1.14	0.97	0.93	0.95	0.78	1.18	1.75**	2.7 (0.1N HCl only)
Weighjted H^+(μeql^{-1})	163.6	168.5**	163.8	159.4	161.5	159.5	161.5	161.5	163.2	158.1	161.5	162.4	162	165.3	157.3	144.7*	119.39
mg NH_4^+ l^{-1}	2.947	3.033	2.95	2.870	2.091	2.873	2.910	2.910	2.940	2.850	2.907	2.923	2.917	2.970	2.830	2.60	2.975
kg NH_4^+ ha^{-1}yr^{-1}	42.42	43.68	42.46	41.34	41.88	41.35	41.88	41.88	42.32	40.99	41.87	42.10	42.00	42.84	40.77	37.51	30.95
kg N ha^{-1}yr^{-1}	32.99	33.97	29.36	32.15	32.57	32.16	32.57	32.57	32.91	31.88	32.57	32.74	32.66	33.32	**31.71**	25.85	24.07

In Table 2 Morigaon District: Nawgaon Paper Mill and nearby areas:
(S1) Near ASTC, (S2) Near industry, (S3) CISF Unit, (S4) Choudhury Nursery, (S5) Near Main Gate (1), (S6) Wall (Main Gate), (S7) Residential area (A), (S8) Near Main Gate (2), (S9) Near field, (S10), Kendriya Vidyalay, (S11) Near ATM, (S12) Residential area (B), (S13) Near Factory, (S14) Near Servo petrol pump, (15) near Trucks' stand, (16) Near Field. *** The lowest pH value (highest acidity) with maximum H^+ concentration, **Highest pH value (lowest acidity) with minimum H^+ concentration**

TABLE 3
PARAMETERS ON AEROSOL PRODUCTS OF NO_x AND NH_y AT TWO SITES OF JORHAT DISTRICT (SITE 1:TITABOR &SITE2:JORHAT) OF ASSAM

Parameters	Mean of ten NOx samples (S) each at Jorhat and Titabor										
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Blank (Without air sample)
PH of Air sample in distilled water	5.317	5.26	**5.66****	5.54	5.46	5.01	5.08	5.42	4.99	**4.67***	6.61 (distilled water only)
Weighjted H^+(μeql^{-1})	39.33	66.67	**26.67***	33.33	43.33	50.00	58.33	66.67	130.0	**193.33****	< 0
mg NO_3^-l^{-1}	3.93	4.13	1.65	2.07	2.69	3.10	3.62	3.24	8.54	11.99	0
kg NO_3^-ha^{-1}yr^{-1}	92.04	96.89	98.75	48.45	62.98	72.67	84.78	96.89	188.9	280.99	0
kg N ha^{-1}yr^{-1}	20.783	21.877	8.75	17.14	19.65	23.22	26.29	29.34	35.13	55.92	0
Mean of ten NHy samples each at Jorhat and Titabor											
PH of Air sample in 0.1N HCl	**1.26***	1.38	1.39	1.43	1.44	1.45	1.55	1.60	**1.68****	1.49	2.7 (0.1N HCl only)
Weighjted H^+(μeql^{-1})	**155.6****	152.9	152.7	151.9	151.4	151.3	149.3	148.1	**146.2***	150.5	119.39
mg NH_4^+ l^{-1}	2.797	2.750	2.750.	2.733	2.723	2.723	2.687	2.670	2.633	2.713	2.975
kg NH_4^+ha^{-1}yr^{-1}	66.65	64.51	64.44	64.11	63.91	63.84	62.98	62.50	61.70	63.52	30.95
kg N ha^{-1}yr^{-1}	51.06	50.18	50.12	49.86	49.70	49.65	48.98	48.61	47.99	49.41	24.07

SiteI: Rice growing areas towards Titabor**:** (S1) Namghar area , Titabor, (S2):Regional Rice research station Titabor, (S3): Titabor tiniali,, (S4):Titalbaor Chariali, (S5):Chinamora tiniali, (S6): Gatany factory, (S8-S10): ONGC area, Cinnamora;

Site II: Jorhat town: (S1):Samples from Cinnamora railgate, :Cinamora petrol pupm, (S2): Lahoti petrol pump, (S3):AT Road, Joraht town, (S4): Bhogdoi bridge, & AT Road, Borpool, (S5):samples from Garali, Dos & Co. (S6): Borbheta Tiniali,, Rowraia chariali, Civil Hospital, Jorhat, (S7):Baruah chariali, ASTC, Jorhat, (S8): Malowali Tiniali, (S9):Tarajan, Byepass, (S10): Dikha Nursery & Moubandha,

*** The lowest pH value (highest acidity) with maximum H^+ concentration, **Highest pH value (lowest acidity) with minimum H^+ concentration**

TABLE 4
PARAMETERS ON AEROSOL PRODUCTS OF NO_x AND NH_y AT NAMRUP (DIBRUGARH DISTRICT OF ASSAM)

Parameters	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	Blank (Without air sample)
NOx samples (S) around Barak Valley fertilizer Corporation of India Ltd. Namrup																
PH of Air sample in distilled water	5.45	**5.55****	5.35	5.45	**5.35***	5.50	5.45	5.50	5.50	5.45	5.50	5.45	5.50	5.45	5.40	6.61 (distilled water only)
Weighjted H^+ (μeql^{-1})	40.0	**35.0***	55.0	40.0	**45.0****	35.0	40.0	35.0	35.0	35.0	35.0	35.0	35.0	40.0	40.0	< 0
mg $NO_3^- l^{-1}$	2.480	2.170	3.410	2.480	2.790	2.170	2.480	2.170	2.170	2.170	2.170	2.170	2.170	2.480	2.480	0
kg $NO_3^-ha^{-1}yr^{-1}$	77.62	67.92	106.73	77.62	87.32	67.92	77.62	67.92	67.92	67.92	67.92	67.92	67.92	77.62	77.62	0
kg N $ha^{-1}yr^{-1}$	17.53	15.34	24.10	17.53	19.72	15.34	17.53	15.34	15.34	15.34	15.34	15.34	15.34	17.53	17.53	0
NHy samples around Barak Valley fertilizer Corporation of India Ltd. Namrup																
PH of Air sample in 0.1NHCl	1.135	1.23	1.395	1.24	1.44	1.14	**1.49****	1.14	1.17	1.09	**1.08***	1.29	1.20	1.16	1.25	2.7 (0.1N HCl only)
Weighjted H^+(μeql^{-1})	158.2	156.2	152.7	156.1	151.6	158.2	**150.4***	158.2	157.4	159.2	**159.3****	155.5	156.8	157.7	155.9	119.39
mg NH_4^+ l^{-1}	2.85	2.81	2.75	2.81	2.73	2.8/5	2.71	2.82	2.81	2.87	2.87	2.79	2.85	2.84	2.81	2.975
kg $NH_4^+ha^{-1}yr^{-1}$	89.12	87.99	86.14	87.96	45.42	89.12	84.76	89.12	88.71	89.69	89.76	87.31	88.36	88.83	87.81	30.95
kg N/$ha^{-1}yr^{-1}$	69.32	68.43	66.92	68.41	66.43	69.32	65.92	69.32	68.99	69.76	69.81	67.90	68.72	69.10	68.29	24.07

Namrup : Around Brahmaputra Valley Fertilizer corporation (BVFC) Pvt. Ltd., Duliajan and Dibrugarh: (S1): BVFC Gate, (S2):inside BVFC, (S3): Loha Gate 10km away from BVSC, (S4) Oil collecting site, Jaipur (S5):Naharkotia Market, (S6-S7)Tipling Duliajan, (S8):Chaukidinghi, (S9):Dibrugarh, (S10):Dibrugarh Chalkhowa station, (S11):): Duliajan Oil market, (S12): Duliajan Uco Bank, (S13):Duliajan Chariali, (S14):Near Oil India, (S15):Near Police Station

Conversions as per UKRGAR (1997):Weighted H^+ (μeql^{-1}) for NO_x and NH_y =ln (6-pH), Parametrs for NO_x**:** $\mathbf{mgNO_3^- l^{-1}}$=weighted H^+x 0.062**,** $\mathbf{gNO_3^- ha^{-1}yr^{-1}}$ **= [**$mgNO_3^- l^{-1}$ x amount of rainfall]/1000; $\mathbf{gNha^{-1}yr^{-1}}$ = [$mgNl^{-1}$ x amount of rainfall]/1000

Parametrs for NH_y: $\mathbf{mgNH_4^+ l^{-1}}$=weighted H^+(μeql^{-1}) x 0.018, $\mathbf{gNH_4^+ ha^{-1}yr^{-1}}$ = $mgNH_4^+ l^{-1}$ x amount of rainfall]/1000**;** $\mathbf{gNha^{-1}yr^{-1}}$ = [$mgNl^{-1}$ x amount of rainfall]/1000

*** The lowest pH value (highest acidity) with maximum H^+ concentration**

****Highest pH value (lowest acidity) with minimum H^+ concentration**

TABLE 5
GUWAHATI AND ITS PERIURBAN AREAS (KAMRUP DISTRICT)

S. No.	Samples (Local with Botanical names)	Nitrogen %
1	Forget me not (*Hydrangea macrophyla*)	2.579
2	Kanchan (*Bouhinia alba*)	2.492
3	Hasnahana (*Cestrum nocturnam*)	2.268
4	Ficus (*Ficus benzamina)*	1.344**
5	Litchi (*Litchi chinensis*)	0.924
6	Titasopa (*Michelia champaca*)	2.94
7	Gerbera (Gerberas anandria)	1.176
8	Arjun (*Terminalia arjuna*)	1.54
9	Radhasura (*Delonix regia)*	1.904
10	Bottlebrass (*Callistemon lanceolatus*)	1.148
11	Gamari (*Gmelina arborea*)	1.568
12	Aralia (*Aralia apioides*)	2.716
13	Boga chandan (*Santalum album* L)	0.728
14	Pine (*Pinus longifolia)*	1.344**
15	Thuja (*Tthuja orientalis)*	0.70

TABLE 6
ADJOINING AREAS OF NAGAON PAPER MILL, JAGIROAD (NAGAON DISTRICT)

S. No.	Samples	Nitrogen %
1	*Dahlia* (*Dahlia coccinea*)	0.784
2	Zerenium (*Pelargonium hortorum*)	1.184
3	Silverdust (*Centaurea cineraria*)	0.504
4	Dianthus *(Dianthus chinensis)*	0.28
5	Pancy (*Viola tricolour*)	0.224
6	*Gerbera (Gerbera jamesonii*)	0.168
7	Ficus (*Ficus benjamina*)	0.364*
8	Ashok (*Saraca indica*)	0.196
9	*Ixora* (*Ixora coccinea*)	0.308
10	Tomato (*Solanum lycopersicum)*	0.112
11	Brinjal (*Solanum melogana*)	0.224
12	*Cabbage* (*Brassica oleraceae*)	0.14
13	Mausombi (*Citrus raticulata*)	0.336

In Table 5-8: Lowest value (*), Highest value (**) of the same species at different locations.

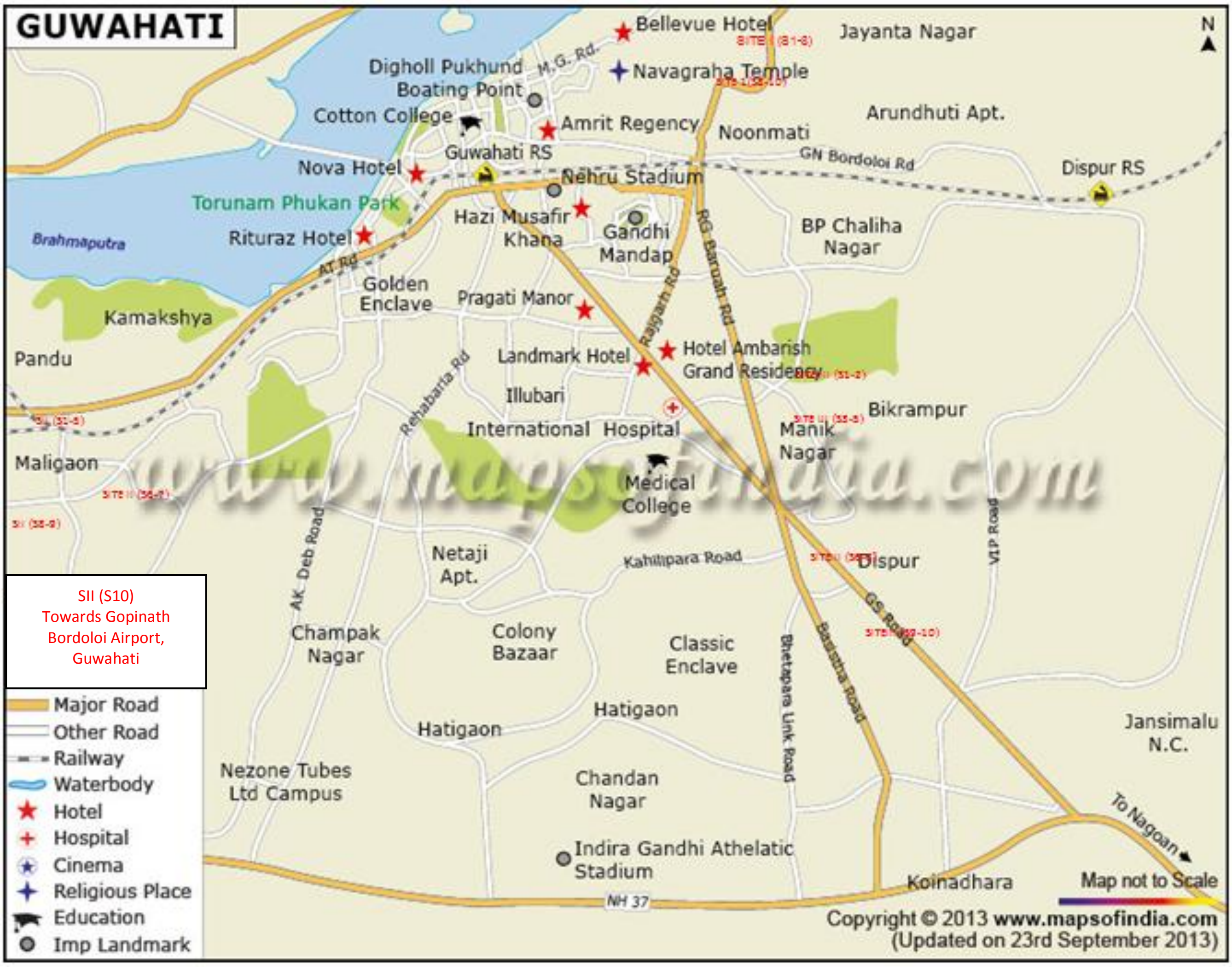
TABLE 7
JORHAT DISTRICT OF ASSAM (JORHAT & TITABOR)

S. No.	Samples	Nitrogen %
1	Rice (*Oryza sativa L.*)	0.056
2	Marygold (*Tagetis erecta*)	0.056
3	Bakul *(mimusops elengi)*	0.084
4	Hedge plant (*Ccleodendron inerme*)	0.168
5	Hibiscus (*Rosa sicencis*)	0.196
6	*Khutara (Amaranthus viridi)*	0.224
7	Thuja (*Thuja orientalis)*	0.112
8	Pine (*Pinus longifolia)*	0.140*
9	Mussanda (*Musanda philippica*)	0.168
10	Bougainvillea (*Bougainvillea spp*.)	0.364
11	Mango (*Mangiffera indica* L.)	0.168
12	Rabab Tenga (*Citrus grandis*)	0.196
13	Clove (*Syzygium aromaticum*)	0.224
14	Guava (*Psidium guajava*)	0.140
15	Peeple (Ficus religosa)	0.150
16	Tomato (*Solanum lycopersicum*)	0.168
17	Pumkin (*Cucurbita moschata)*	0.140
18	Rice (*Oryza sativa* L)	0.168

TABLE 8
BRAHAMAPUTRA FERTILIZER CORPORATION PVT. LIMITED, NAMRUP (DIBRUGARH DISTRICT)

S. No.	Samples	Nitrogen %
1	Bamboo (*Bambusa textiles*)	*0.168*
2	Carpet grass (*Axonopus fissifolius)*	*0 .196*
3	People tree (*Ficus relegiosa)*	*0.084*
4	Krishnasura (*Delonix regia)*	0.112
5	Creeper fruit (*Quisqualis indica)*	0.014
6	Papaya (*Carica papaya*)	0.112
7	Gourd (*Lagenaria siceraria)*	0.56
8	Knolkhol (*Brassica oleraceae)*	0.84
9	Potato (*Solanum tuberosum)*	0.112
10	Lai sak (*Brassica juncea)*	0.168
11	Black pepper (*Piper nigram)*	0.112
12	Tea (*Camalia sinensis)*	0.14
13	Brinjal (*Solanum melogana)*	0.196

The analyses of vegetation samples from the polluted sites (e.g. city of Guwahati) also revealed the enrichment of tissue nitrogen while compared to the nitrogen content in the same plant species from the distant relatively unpolluted places (**Table 5-8**). N is the primary limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially under oligotrophic and mesotrophic conditions in temperate and boreal regions (Bobbink *et. al.* 2010; Sala *et. al.* 2000). Many plant species in such ecosystems are adapted to nutrient-poor conditions, and can only survive or compete successfully on soils with low N availability (Aerts and Chapin 2000; Tamm 1991). The series of events which occur when N inputs increase in an area with originally low background deposition rates is complex. Many ecological processes interact and operate at different temporal and spatial scales. As a consequence, large variations in sensitivity to atmospheric N deposition have been observed between different natural and semi-natural ecosystems (e.g. Maskell *et. al.* 2010). The increased N deposition may impact on foliar toxicity, changes in structure and function of plant species by soil-mediated effects of acidification, such as stunted root growth by NHy. All these may largely linked to significant changes in the N cycle, in vegetation composition and in plant diversity in many ecosystems of high nature conservation value (Bobbink *et. al.* 2010, 1998).

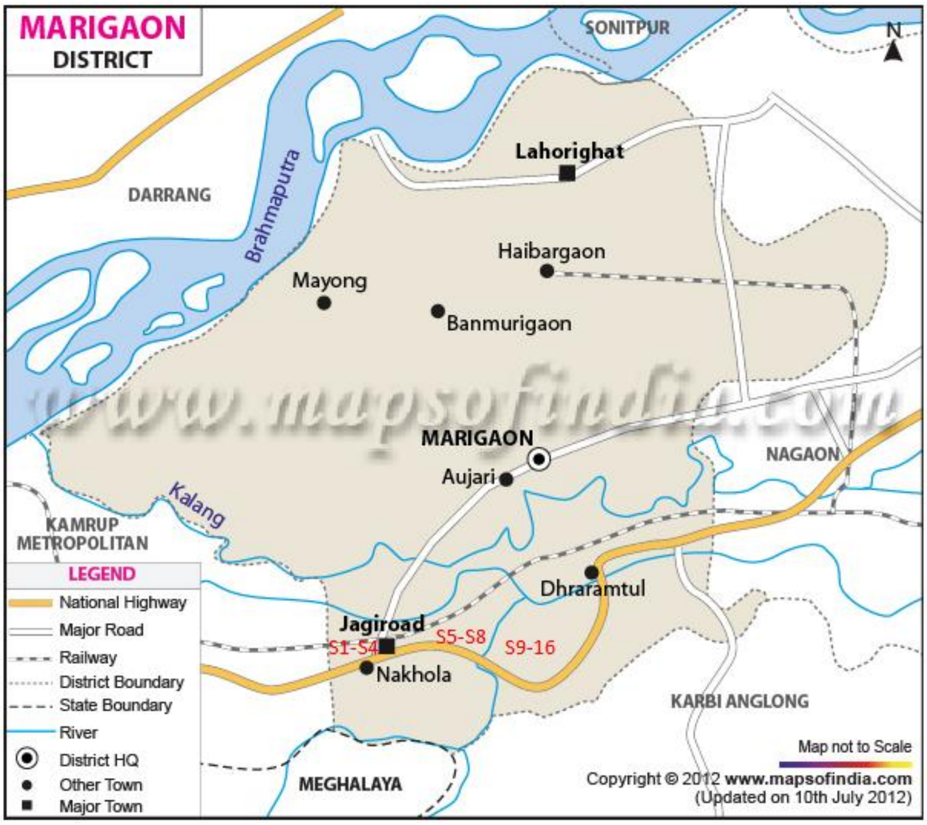


SITE MAP1: GUWAHATI CITY AND SURROUNDING AREAS

Site-I: Around Oil Refinery at Guwahati (S1:Noonmati refinery, (S2:Noonmati training centre, S3:Noonmati main gate, S4:Noonmati sector 3, S5:Birkuchi, S6:Patherkuwari,S7:Narengi oughuli road, S8:Narengi Junior college, S9:Narengi Tiniali, S10:New Guwahati Baminimaidan

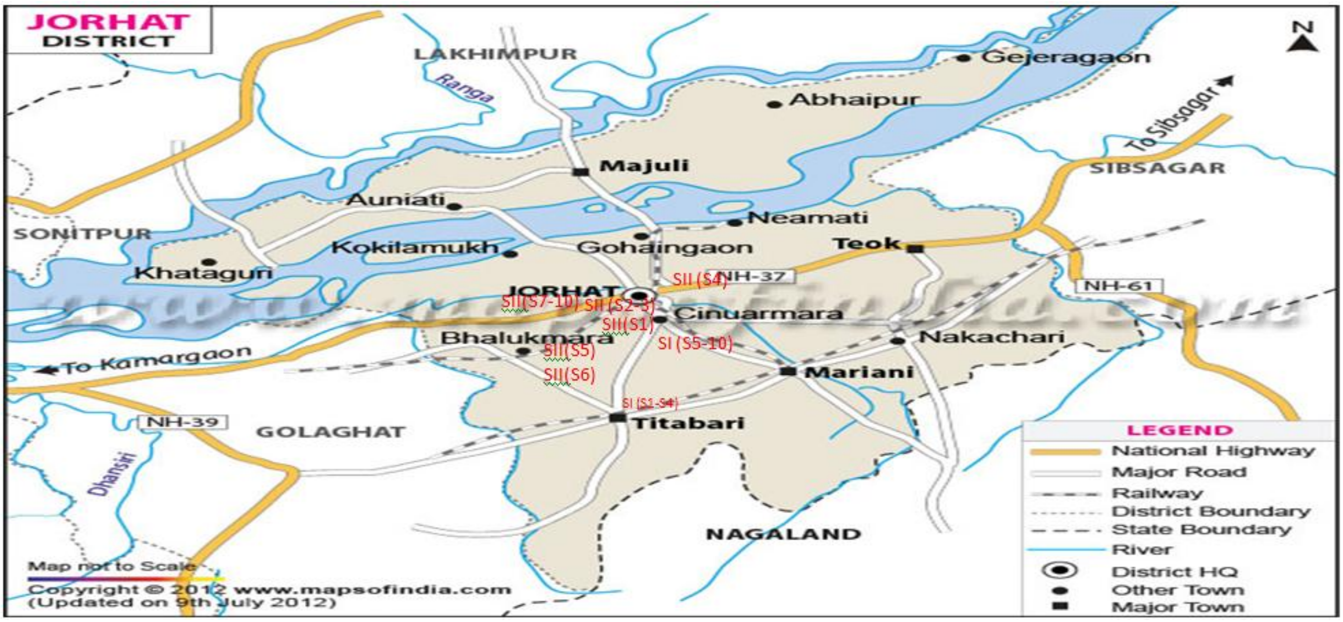
Site-II: Around Maligaon: (S1-S5):Adabari Buses' stand-(S6-S7):Near Assam Engineering College, (S8-S9):near Sarighat Bridge at Jalukbari, S10: Towards Gopinath Bordoloi Airport

Site-III: (S1-S2):Manik Nagar, (S3-S5):Rajdhani Nursery, (S6-S8):Baisistha, (S9):Near Reginal passport office, (S10)Near Arohan , Beltola



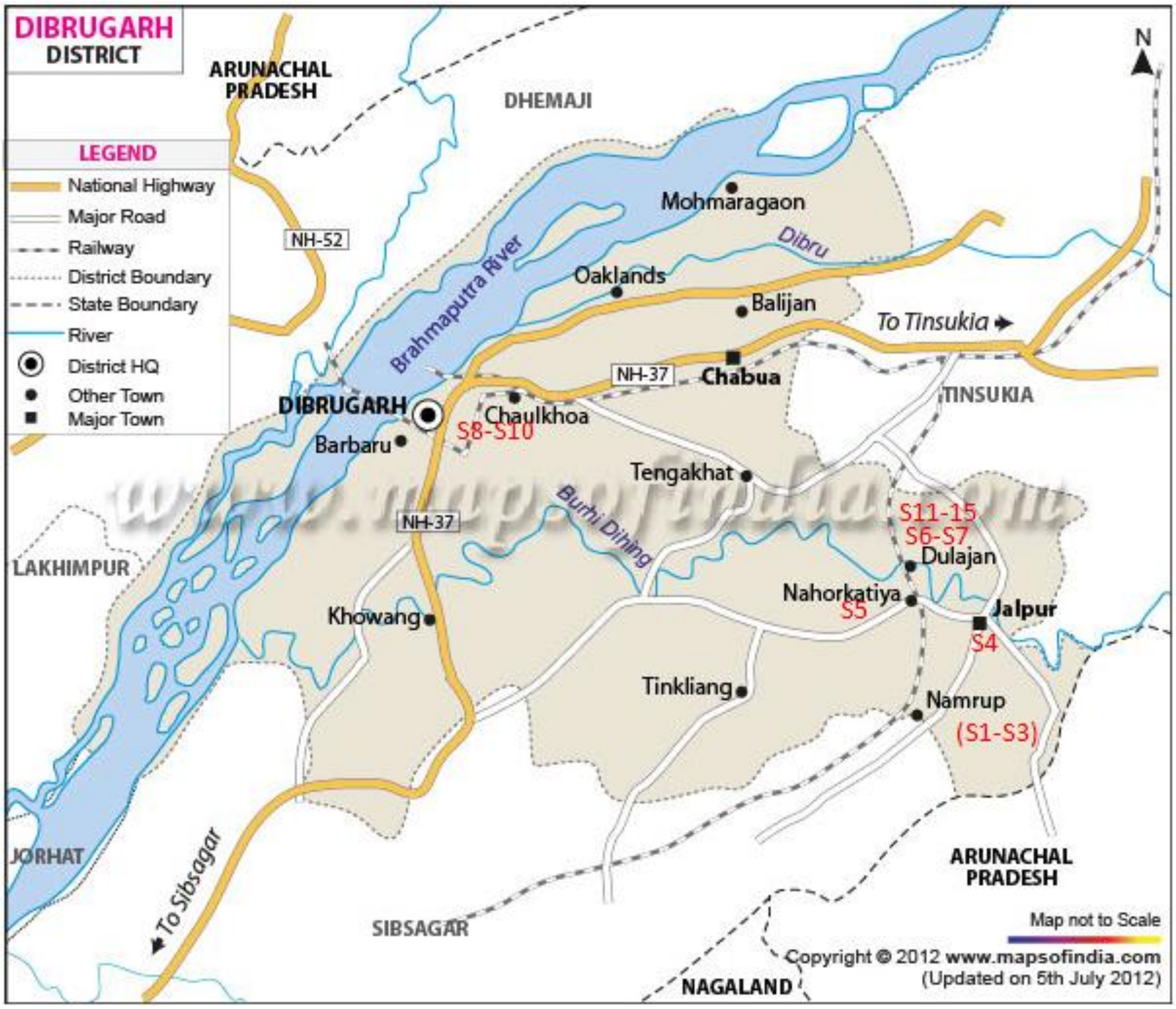
SITE MAP2: MORIGAON DISTRICT: NAWGAON PAPER MILL AND NEARBY AREAS

(S1) Near ASTC, (S2) Near industry, (S3) CISF Unit, (S4) Choudhury Nursery, (S5) Near Main Gate (1), (S6) Wall (Main Gate), (S7) Residential area (A), (S8) Near Main Gate (2), (S9) Near field, (S10), Kendriya Vidyalay, (S11) Near ATM, (S12) Residential area (B), (S13) Near Factory, (S14) Near Servo petrol pump, (15) near Trucks' stand, (16) Near Field.



SITE MAP3: JORHAT DISTRICT OF ASSAM

SiteI: Rice growing areas towards Titabor**:** (S1) Namghar area , Titabor, (S2):Regional Rice research station Titabor, (S3): Titabor tiniali,, (S4):Titalbaor Chariali, (S5):Chinamora tiniali, (S6): Gatany factory, (S8-S10): ONGC area, Cinnamora; **Site II:** Jorhat town: (S1):Samples from Cinnamora railgate, :Cinamora petrol pupm, (S2): Lahoti petrol pump, (S3):AT Road, Joraht town, (S4): Bhogdoi bridge, & AT Road, Borpool, (S5):samples from Garali, Dos & Co. (S6): Borbheta Tiniali,, Rowraia chariali, Civil Hospital, Jorhat, (S7):Baruah chariali, ASTC, Jorhat, (S8): Malowali Tiniali, (S9):Tarajan, Byepass, (S10): Dikha Nursery & Moubandha,



SITE MAP4: NAMRUP: AROUND BRAHMAPUTRA VALLEY FERTILIZER CORPORATION (BVFC) PVT. LTD., DULIAJAN AND DIBRUGARH:

(S1): BVFC Gate, (S2):inside BVFC, (S3): Loha Gate 10km away from BVSC, (S4) Oil collecting site, Jaipur (S5):Naharkotia Market, (S6-S7)Tipling Duliajan, (S8):Chaukidinghi, (S9):Dibrugarh, (S10):Dibrugarh Chalkhowa station, (S11):): Duliajan Oil market, (S12): Duliajan Uco Bank, (S13):Duliajan Chariali, (S14):Near Oil India, (S15):Near Police Station

Air samples were collected from major urban and peri-urban areas of Assam (Refer to Site Maps: 1-4). On an average, about 40 kg Nitrogen was deposited from each of oxidised and reduced forms. The vegetation samples collected from the square grids nearing the highways and locations away from the emission sources provide evidences that air is concentrated with the nitrogenous pollutants. There is higher Nitrogen content in the tissues of plants surrounding the cities than in the same or other plants abundant away from the emitters**.** Port and Thomson (1980) also reported that landscape plants closer to highways frequently grow in poor soil conditions, and have elevated levels of total nitrogen in their tissues. The changes have been brought about by emissions from mass vehicles or industrial sources. In Assam, there are increases in number of vehicles, especially in the greater Guwahati City (Kamrup district), and growth of different smaller industries in larger scale in its adjoining areas. Air enriched with Ammonia is evident around the pulp-paper industry at Jagiroad (Nagaon district), and fertilizer manufacturing factory at Namrup (Dibrugarh district). The smaller urban areas (e.g. Jorhat District) were also not getting rid of these pollutants with the presence of considerable number of vehicles, probably some of them are old and their efficiency is lower. Deposition of N-aerosols was much more dispersed than reduced ammonia aerosols generally due to the vehicular growth. The larger agricultural areas accompany the later and intensive live stocks raring units also. Sheppard *et. al*. **(**1988) pointed that incorporation of CNG in vehicles might be another cause of increasing ammonia near highways at distance up to 500 meters. Pitcairn *et. al*. (2002) reported that annual mean concentration of NH_3 close to live stock building were very large (60µgm^{-3}), and declined to 3 µgm^{-3} at a distance of 650 m from the building. The estimated total N deposition ranged from 80kg N ha^{-1} year^{-1} at a distance of 30 m to 14kgNha^{-1}year^{-1} at 650 m downward. In our studies also the mean deposition of nitrogen by NOx was 6-38kgha^{-1}yr^{-1}, whereas by NHy was 7-24kgha^{-1}yr^{-1} from all the sites. The annual average of total deposition of nitrogen compounds in state has been computed as the sum of the annual averages of wet deposited nitrogen in the representative districts of Assam. Thus, a total maximum deposition of N through oxidized and reduced forms of Nitrogen together is about 18-46 kg ha^{-1}yr^{-1}. Spatial distribution of total respective depositions of NOx and NHy on a 1km x 1 km grid in 2010-11 is shown as sites (urban and peri-urban areas) of sample collections in the district maps for better orientation. Several significantly different gradients for NOx and NHy were recognized for the sites as well as the locations of sample collections. Plausibly, the total deposition of the former was especially influenced by their emissions from populated cities and larger vehicular traffic congested areas. The reasons for the later were mostly influenced by depositions from industries (e.g. pulp & paper factory at Jagiroad, fertilizer factory at Namrup), and intensive cultivable as well as animal rearing hot spots of the urban and peri-urban areas in the state.

IV. CONCLUSION

The wet deposited nitrogen (NOx, NHy and their compounds) was estimated following a square grid (1km x1km) resolution in a few major districts of Assam. The deposition had been assessed from the measured and modeled concentrations of acidifying compounds in the atmosphere and the precipitations with reference to UKRGR (1997).Net deposition of Nitrogen through its oxides ranged from 6.0-38kgha^{-1}yr^{-1}, whereas net deposition of nitrogen by ammonia was in between 7-24 kgha^{-1}yr^{-1}. A total maximum deposition of N through oxidized and reduced forms of Nitrogen together is about 18-46 kg ha^{-1}yr^{-1}. The hypothesis that Assam (especially Guwahati and its adjoining areas) is also facing with enrichment of nitrogenous pollution due to anthropogenic activities and mass vehicular and industrial growth was tested positive.

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The Influence of Vermiculite on the Uptake of Silver Nanoparticles in a Terrestrial System

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***Abstract*— *The uptake of silver from silver nanoparticles in soil was investigated in the presence of increasing concentrations of Vermiculite, typical 2:1 clay. Two insect species, Acheta domesticus and Tenebrio molitor, and two plant species, Helianthus annuus and Sorghum vulgare, were exposed to silver nanoparticles in the presence of increasing concentrations of Vermiculite in soil. Silver nanoparticles were characterized using techniques including transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction. The levels of silver in test species exposed to silver nanoparticles were measured using an inductively coupled plasma-optical emission spectrometer. An increase in the cation exchange capacity of soil was observed with the increase in the concentration of vermiculite in soil. The results suggested a decrease in the uptake of silver from silver nanoparticles in soil by Acheta domesticus as a function of increasing concentrations of Vermiculite in soil. No apparent trend was observed in the remaining species. Both plant species were found to accumulate silver in their roots. The translocation of silver to stems and leaves was observed in the case of Helianthus annuus. Results from this study suggest that the presence of Vermiculite in soil could possibly decrease the uptake of silver from silver nanoparticles.*

***Keywords*— Silver nanoparticles, Acheta domesticus, Tenebrio molitor, Helianthus annus, Sorghum vulgare, inductively coupled plasma-optical emission spectrometer, Cation exchange capacity.**

I. INTRODUCTION

The widespread use of silver nanoparticles (Ag NPs) for a variety of applications has resulted in an increase in the concentrations of Ag NPs in terrestrial ecosystems. Typically, Ag NPs find their way into terrestrial ecosystems through the application of sewage sludge as fertilizer to land [1-3]. The environmental behavior, fate, and ecotoxicity of metal-based nanoparticles in a terrestrial ecosystem are known to be influenced by the physicochemical characteristics of both the nanoparticles themselves and the soil. Physical properties include size and shape of nanoparticles while the chemical characteristics include acid-base character, aqueous solubility and surface coatings, if any [4]. The physicochemical properties of soil can influence the mobility, bioavailability and toxicity of pollutants in a terrestrial ecosystem. These properties include pH, soil texture, organic matter, and cation exchange capacity (CEC), etc [5,6].

The texture of a soil is comprised of sand, silt, and clay. The particle size of sand, silt, and clay are > 50 μm, 2-50 μm, and < 2 μm respectively [7,8]. Clay particles belong to a group of minerals described as hydrous silicates [9]. Clay particles typically have a negative charge [10]. Additionally, clay particles considerably contribute to the CEC of soil. CEC of soil is the quantity of positively charged ions that could be held by the negatively charged surface of clay minerals [9]. The CEC of a soil provides electrostatic binding sites for cations like silver ions (Ag^+) thus rendering them electrostatically immobilized [11]. The negative charge of clay minerals, the high surface area of clay due to small particle size [12] and the contribution of clay minerals to the CEC of soil [9] play a key role in determining the fate of metal contaminants in soil. The four major groups of clay minerals include: 1) the kaolinite group; 2) the mont-morillonite/smectite group; 3) the illite group and 4) the chlorite group [9]. The mont-morillonite/smectite group of clay minerals are known to possess high surface area and CEC compared to the remaining three groups of clay minerals. The general formula of mont-morillonite/smectite group of clay minerals is $(Ca,Na,H)(Al,Mg,Fe,Zn)_2(Si,Al)_4O_{10}(OH)_2$-$XH_2O$ [9].

A member of the mont-morillonite/smectite group of clay minerals, Vermiculite, is used in the present study. The effect of increasing concentrations of Vermiculite in soil on the uptake of silver from Ag NPs in a terrestrial system was investigated. Terrestrial isopods have been used as model organisms to understand the uptake, kinetics and transformation of metal nanoparticles [13-15]. The uptake of nanoparticles in terrestrial isopods occurs exclusively through the oral route. Negligible surface uptake of nanoparticles is observed in terrestrial isopods facilitating the uptake and transformation studies of metal nanoparticles [16]. However, it is equally important to investigate the uptake of metals from metal nanoparticles by other components of terrestrial ecosystems that serve a crucial link in the metal transport chains between trophic levels in the food web.

Two species of insects, *Acheta domesticus* and *Tenebrio molitor*, and two species of plants, *Helianthus annuus* (a dicot plant) and *Sorghum vulgare* (a monocot plant) were used. The insect and plant species were exposed to Ag NPs in soil containing a range of concentrations of Vermiculite. Insects serve an important role in the metal-transport chains between trophic levels in food webs [17]. For instance, during the breeding season, insects and larvae constitute an important food source for insectivorous birds [18]. Therefore, it is important to determine if insects are able to bioaccumulate Ag from Ag NPs in soil. As seeds are another important food source for granivorous birds [19], the possibility of translocation of Ag to plant tissues was investigated. Results from this study would help understand the role of plants in bioaccumulation of metal nanoparticles.

II. MATERIAL AND METHOD

2.1 Soil collection and preparation

Soil used in the present study was collected 40 minutes south of Colorado City, Texas at an elevation of 684 m above sea level. Exact coordinates were as follows: Universal Transverse Mercator 14 S 0319752 mE 3557792 mN. All soil was collected from the top 10 cm of soil, shoveled into clean plastic containers and transported back to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX. The soil was processed for homogeneity at TIEHH. All large rocks, roots, living organisms, and other organic matter were removed first and large clumps of soil were crushed. The soil was then sifted through a 2 mm wire screen into another clean plastic storage container. Processed soil was covered and stored indoors until ready for use.

2.2 Soil analysis

The characterization of soil samples was performed at Midwest Laboratories Inc. (Omaha, NE). Soil samples were characterized by evaluating parameters such as soil texture, percent humic matter, percent organic matter, exchangeable cations (K^+, Mg^{2+}, Ca^{2+}), available phosphorus (P), soil pH, percent base saturation of cations (K^+, Mg^{2+}, Ca^{2+}, H^+), CEC, and sulfur (S) content.

Nanoparticle Characterization

Uncoated silver nanoparticles (30-50 nm) were purchased from US Research Nanomaterials, Inc. (Houston, TX). All silver nanoparticles were reported by US Research Nanomaterial, Inc (www.us-nano.com) to consist of $\geq$ 99.99% Ag.

In order to confirm the size range and shape of the nanoparticles, transmission electron microscopy (TEM) was used. Samples were prepared by dispersing the Ag NP powder in ethanol (EtOH) and sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried before analysis. TEM (Hitachi H-8100 TEM) images were taken at 200 kV using a tungsten filament side-mounted camera.

Dynamic light scattering (DLS) was used as an additional method to confirm the size Ag NPs. The sample preparation process for DLS involved placing approximately 10 mg of Ag NP powder in 10 mL of reagent grade acetone (Fisher Scientific, MA, USA). Samples were sonicated until Ag NPs remained suspended in solution. Samples were analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA) and Mictrotrac Flex Software (Version: 10.3.14).

Powder x-ray diffraction (PXRD) was used to confirm the composition of Ag NPs. A Rigaku Ultima III X-Ray Diffractometer was used for this purpose. Samples were analyzed using Cu Kα radiation as x-ray source. The Ag NPs were analyzed using the following instrument parameters: parallel-beam geometry was used with a step width of 0.03° and a count time of one second; the divergence, scattering, and receiving slits were set at one. Once completed, the diffraction patterns were compared and matched to the phases in the International Center for Diffraction Data (ICDD) powder diffraction file (PDF) database.

2.3 Insect treatment groups

Two 37.8 L terrariums were prepared for each insect treatment group, including a control group. The clay content of soil was adjusted before the terrariums were spiked with Ag NPs. The required amount of clay (Sta-Green® vermiculite) was weighed out to constitute 1, 5, 10, 15, and 20% of clay (by weight) in 2.5 kg soil for each treatment group. Each terrarium was spiked with 62.5 mg of uncoated 30-50 nm Ag NPs so that a final soil concentration of 25 µg/g Ag NPs was obtained.

Once the terrariums were prepared, insects were purchased from Reptilefood (reptilefoods.com, Ohio, USA). Each terrarium received either 300 small crickets or 400 large mealworms. Insects were provided with fresh food and water as needed for the duration of the 28-day exposure period. After 28 days, insects were carefully extracted from the terrariums and placed in glass jars. The jars were then placed in a -80°C freezer until all the insects were deceased. Insects were then freeze dried (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO) for at least 48 hours to ensure the removal of all moisture. Freeze dried insects were then crushed into a fine powder and stored in a freezer until further analysis.

2.4 Plant treatment groups

Commercially available 7.6 L plastic nursery containers were purchased and filled with approximately two inches of commercial pond pebbles to aid in proper drainage. The clay content in the soil was adjusted as was done with the insect treatment groups. This soil was then spiked to have a final soil concentration of 25 µg/g Ag NPs in soil. The spiked soil was transferred into the plastic nursery containers.

Seeds of each plant species were planted into the prepared nursery containers and were transported to the TTU greenhouse. The plants remained in the greenhouse until maturity, approximately three months for *H. annuus* and six months for *S. vulgare*. While in the greenhouse, plants received shaded sunlight and were maintained at 60°F or above. To prevent the soil from drying out, plants were misted for three minutes every eight hours in a day. Once plants reached maturity, the entire plant was harvested. The roots were separated from the remainder of the plant and rinsed using tap water for a full minute to remove all attached soil. The shoot system of the plant was separated into leaves, stems, and seeds. The plant samples were stored in a freezer until further analysis.

2.5 Sample digestions

Three identical samples were weighed out using the insect samples collected from each terrarium. For each plant treatment group, four samples were prepared from each nursery container: a root sample, a leaf sample, a stem sample, and a seed sample. For each sample, either plant or insect, approximately 1 grams were weighed into a 100 mL beaker. Wet weights (ww) were taken into account in the case of plants samples. 10 ml of 70% nitric acid (HNO_3, reagent grade) was added to each sample. This was followed by an addition of 10 ml of 30% hydrogen peroxide (H_2O_2, reagent grade). A solution containing 10 ml HNO_3 and 10 ml H_2O_2 was used as the reagent blank. All beakers were covered with a Teflon watch glass and placed on hot plates overnight for sample digestions. All samples were slowly heated in increments of 50 ˚C until the solutions began to reflux gently. Care was taken to ensure that none of the solutions boiled over resulting in a loss of sample. All samples were swirled periodically during the reflux process to aid in the digestion of the samples. The digestions were considered complete when the volume of samples in the beaker was reduced to approximately 5 ml. Once complete, beakers were removed from the hot plate and placed in an ice bath to cool. Cooling was followed by filtering the samples into 50 ml centrifuge tubes (Corning CentriStar ™, Corning, NY) using ashless filter paper (Whatman No. 41, Fisher Scientific, PA). This was to ensure all remaining solids and/or digested lipids were removed from the samples. The original sample beakers were then rinsed twice with 10 ml of 5% HNO_3 and the rinse contents were added to the centrifuge tubes. The centrifuge tubes with sample extracts were stored at room temperature until analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

2.6 ICP-OES analysis

All samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion ICP-OES. The samples were analyzed for silver at three wavelengths: 224.643, 328.068, and 338.289 nm. The three wavelengths were aligned using a 10 ppm silver standard solution (SPEX CetriPrep). The instrument was calibrated using a range of silver concentrations from 0-20 ppm.

2.7 Statistical Analysis

All data was compared using ANOVA with Rstudio software [20]. The Shapiro test was used to test the normality of data [21]. All statistical analysis was compared with 95 % confidence interval. Data was analyzed using a one-way ANOVA, followed by a multiple comparison test (Tukey HSD) to identify significant differences among the treatment groups ($\alpha<0.05$).

III. RESULTS AND DISCUSSION

3.1 Soil characterization

The control soil was characterized as sandy loam soil as it contained 54% sand, 36% silt, and 10% clay. The soil was also found to contain 0.01% humic matter, 1.7% organic matter, and 9 ppm S. The pH of the soil was slightly basic, 8.1. The CEC of the soil was calculated to be 18.0 meq/100g. However, the CEC of soil was affected by the presence of increasing concentrations of Vermiculite in soil and is summarized in Table 1.

TABLE 1
EFFECT OF VERMICULITE ON THE CEC OF SOIL

Treatment Group	CEC (meq/100 g)
Control	18.0
1% Vermiculite	20.7
5% Vermiculite	22.4
10% Vermiculite	25.4
15% Vermiculite	25.1
20% Vermiculite	26.3

As is evident from Table 1, an increase in CEC of soil was observed as a function of increasing concentration of Vermiculite in soil.

3.2 Transmission electron microscopy

The 30-50 nm uncoated silver nanoparticles were found to be heavily aggregated after being dispersed in EtOH. However, the TEM was able to confirm the spherical shape of the nanoparticles (Fig. 1). And most of the particles were found to be within the in 30-50 nm range, although there were outliers on either side of the range.

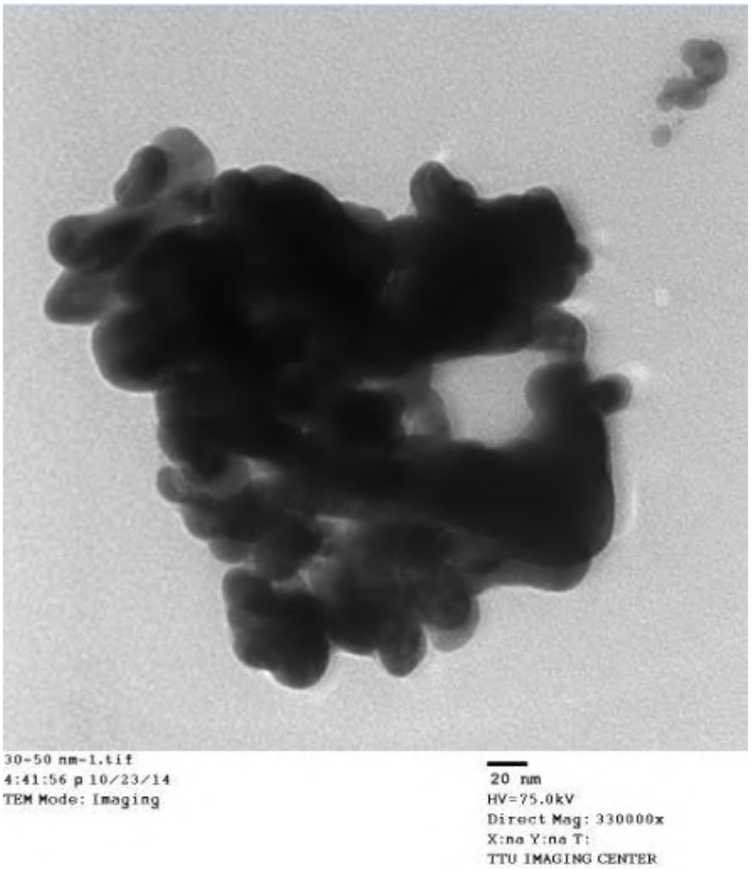

FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE FOR 30-50 nm UNCOATED SILVER NANOPARTICLES.

3.3 Dynamic light scattering

Approximately 95% of the 30-50 nm had a size between 30.70 to 52.90 nm (Fig. 2). The average size of the particles was found to be 41.80 nm, well within the parameters set by the manufacturers.

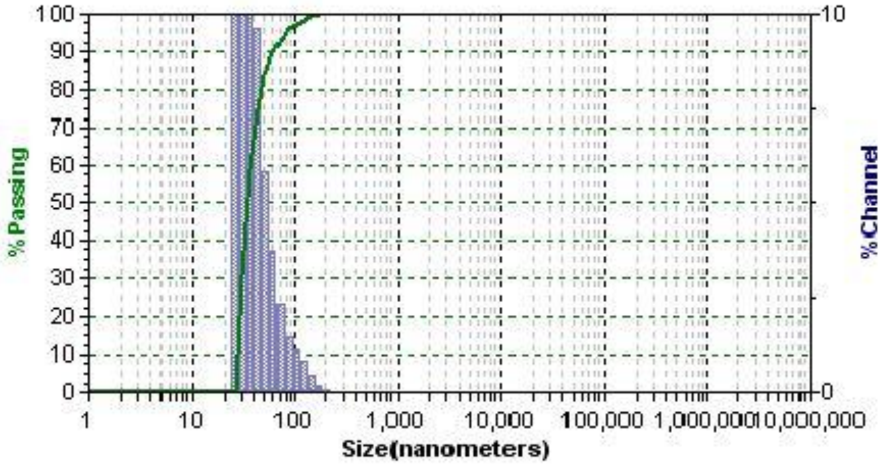

FIG. 2: SIZE DISTRIBUTION OF 30-50 nm UNCOATED SILVER NANOPARTICLES DETERMINED BY DYNAMIC LIGHT SCATTERING

3.4 Powder X-ray diffraction

The PXRD analysis of the silver nanoparticles confirmed their composition. The diffraction patterns matched both those in the ICDD and those provided by the manufacturer. A typical diffraction pattern can be seen below (Fig. 3).

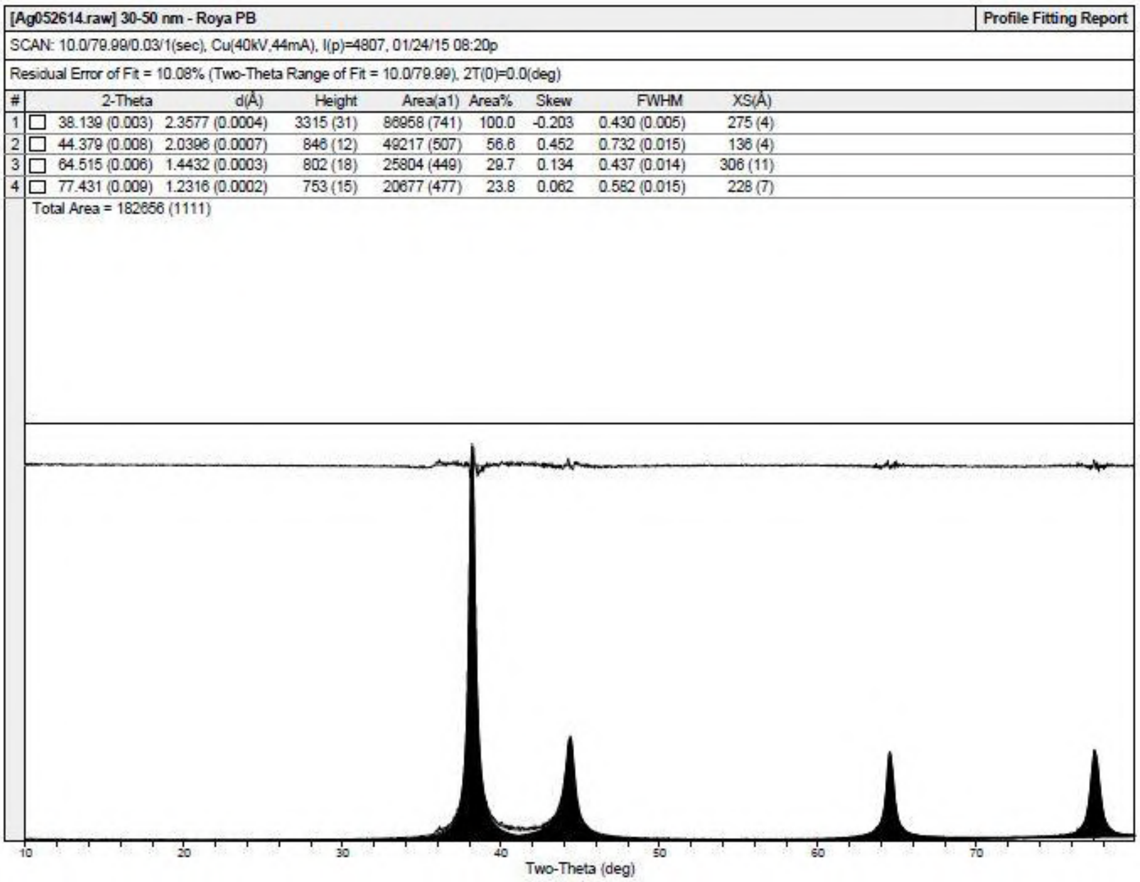

FIG.3: DIFFRACTION PATTERN OF 30-50 nm UNCOATED SILVER NANOPARTICLES DETERMINED BY POWDER X-RAY DIFFRACTION

3.5 Uptake of Ag from Ag NPs in soil by insects

Fig. 4 summarizes the results of the uptake of Ag from Ag NPs in soil by insect species in the presence of increasing concentrations of Vermiculite.

In the case of *A. domesticus*, a decrease in the uptake of Ag from Ag NPs in soil was observed as a function of increasing concentrations of Vermiculite in the soil. No uptake of Ag from Ag NPs in soil was observed at the highest concentrations of Vermiculite employed in this study, i.e. 20%. More importantly, the concentration of silver in the control group (0% Vermiculite in soil) is found to be significantly higher than the concentration of Ag in the insects from the treated group that had various concentrations of Vermiculite. No specific trend in the uptake of Ag from Ag NPs as a function of increasing concentrations of Vermiculite in soil was observed in the case of *T. molitor*. However, a decrease in the uptake of Ag from Ag NPs in soil was observed in the presence of Vermiculite. The decrease in the uptake of Ag by insects in the presence of Vermiculite could be attributed to the increase in CEC of soil due to Vermiculite [22,23].

Despite the levels of Ag found in both insect species, it is important to consider the type of soil used in the present study is Sandy loam. Some of the important characteristics of sandy loam soil include low organic matter content, low organic carbon content, low clay content and low CEC. All these properties of sandy loam soil usually facilitate the uptake of metals [6]. The discrepancies in the levels of Ag uptake observed in both the species may be attributed to the inherent differences in the ability of soil-dwelling invertebrates to uptake metals in contaminated soils. Due to the difference in habitats, diet and physiological responses, some invertebrates are known to accumulate metals preferentially [24].

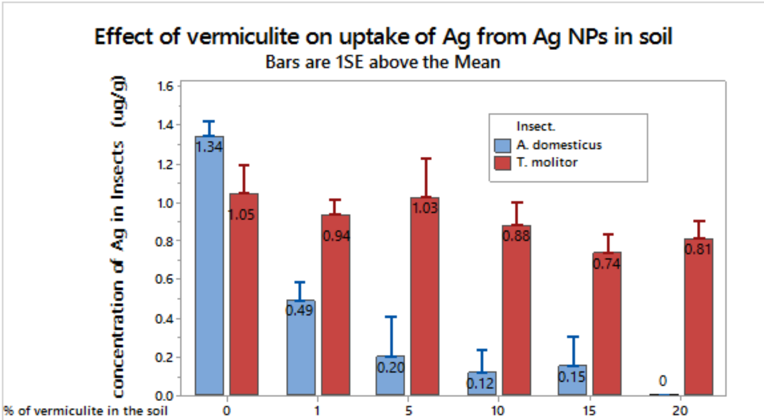

FIG. 4: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL (n=2).

3.6 Uptake of Ag from Ag NPs in soil by plants

Fig. 5 and 6 summarize the uptake of Ag from Ag NPs in soil by the plant species *S. vulgare* and *H. annuus*, respectively) used in the study as a function of increasing concentrations of Vermiculite.

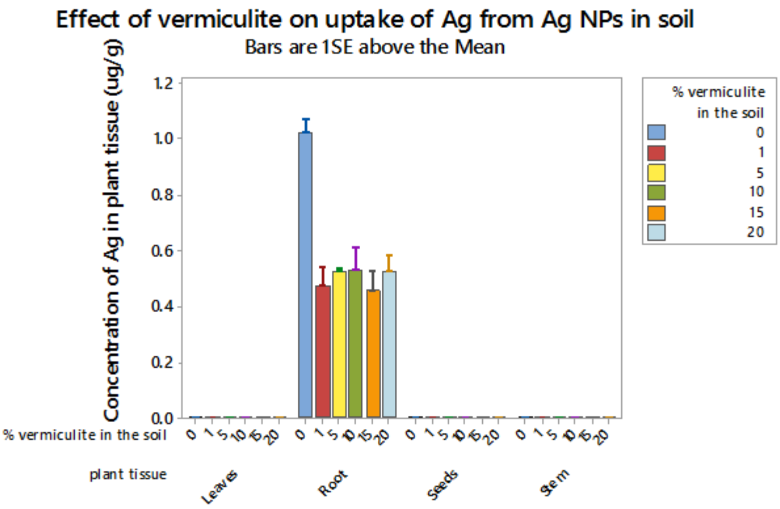

FIG. 5: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY *S. vulgare* (n=2).

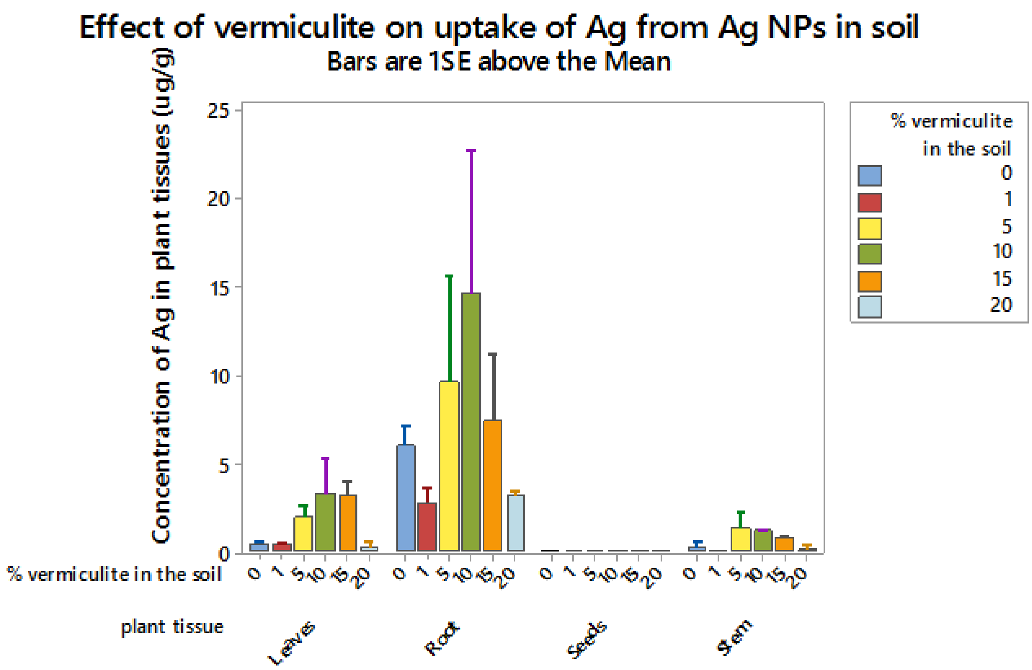

FIG. 6: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY *H. annuus* (n=2).

Fig. 5 suggests an accumulation of Ag in the roots of *S. vulgare*. Although no translocation of Ag to other parts of the plant tissues was observed, a decrease in the uptake of Ag from Ag NPs in soil by roots was observed in the presence of Vermiculite. This could be attributed to the increased CEC of soil in the presence of Vermiculite. The increase in CEC of soil due to the presence of Vermiculite would facilitate electrostatic binding of Ag to the soil [22,23] thereby inhibiting its uptake by the roots of *S. vulgare*.

The observed uptake of metals by monocot plants like *S. vulgare* is predominantly attributed to their root morphology. The presence of numerous thin roots in monocot plants presents a very high surface area for the penetration and accumulation of nanoparticles [25]. However, the lack of translocation of heavy metals has been observed in other monocot plants like maize, rice, and wheat [26,27,28]. This retention of metals at the roots could be partially explained by the insolubilization of metals at the root surface and in the root apoplast [26,27].

Fig. 6 suggests an accumulation of Ag in the roots of *H. annuus* and subsequent translocation of Ag to other parts of the plant tissues such as leaves and stems. The concentration of Ag in the roots were found to be significantly higher than the levels of Ag in other tissues ($p<0.05$). The translocation of heavy metals like Ag to other plant tissues occurs by the phenomenon of pholem transport. Phloem-mobile metals are known to translocate to other plant tissues like leaves, stem, seeds, etc [29].

It is also evident from Figure 6 that the roots of *H. annuus* have accumulated higher concentrations of Ag than those of *S. vulgare*. Dicot plants have been known to accumulate metals better than monocots [30]. The root exudates of dicot plants contain organic acids such as citric acid, maleic acid, ascorbic acid, and oxalic acid [31,32]. These organic acids lower the pH near the vicinity of roots thereby solubilizing metals. This results in an increased uptake of metals by dicot plants compared to monocots [33,34,35]. Additionally, the decrease in pH of the soil also decreases the CEC of soil. The decrease in CEC of soil also facilitates an increase in the uptake of Ag from Ag NPs in soil [36].

No apparent trend in the uptake of Ag form Ag NPs in soil was observed as a function of increasing concentrations of Vermiculite in soil. This could be attributed to the limited sample size (n=2) used for the study. Despite starting with three replicates for each insect and plant species used in the study, the data from only two of the replicates could be used for data analysis. Contamination from the glassware and during the process of sample digestions limited our ability to consider all three replicates for analysis.

Nevertheless, it could be inferred that the uptake of Ag from Ag NPs in soil will decrease as a function of increasing concentrations of Vermiculite in soil. Clay minerals such as Vermiculite possess permanent negative surface charges. These permanent negative charges are an inherent property due to non-stoichiometric/isomorphous substitution of cations within the structure of Vermiculite. These substitutions include Al^{3+} instead of Si^{4+} in the tetrahedral sheet, etc [37,38,39]. As the concentrations of Vermiculite in the soil increases, the presence of these negative charges become independent of the pH of soils. Additionally, the phenomenon of cation adsorption in expandable clay minerals such as Vermiculite occurs predominantly in inter-layer spaces, not on the relatively inactive planar surfaces [38].

IV. CONCLUSION

The influence of a clay mineral, Vermiculite, on the uptake of Ag from Ag NPs in soil by insect and plant species was investigated. The presence of Vermiculite resulted in an increase in the cation exchange capacity of soil. The presence of Vermiculite has also potentially resulted in a decrease in the uptake of Ag from Ag NPs in soil by insect and plant species. The increase in CEC of soil due to the presence of clay minerals, the increased surface area of clay minerals due to their small particle size, and the negative charge of clay minerals may cumulatively result in a decrease in the uptake of Ag from Ag NPs in soil. Consequently, clay minerals may be used as sorbents for heavy metals in soil thereby decreasing the possibility of their entrance into food webs.

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Analysis of Trend and Variability of Temperature in Ebonyi State, South-eastern Nigeria, 1984-2015

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Abstract**—** *Temperature being one of the indicators of climate change has become one of the most important discussions of recent times. Changes in temperature influence a variety of processes directly or indirectly which cut across every aspect of man existences. This paper therefore examined the trend in temperature as tools of climate change over Ebonyi State, South-Eastern Nigeria, which is an area well known for crop agriculture. Temperature data covering a period of 31 years (1984-2015) were collected and analyzed using mean, moving average, standard deviation coefficient of variation and linear regression. Result revealed that there is a positive trend in temperature over the study period and that the area is getting warmer by 0.0037^{o}c annually, which is an Indication that Ebonyi State is experiencing a rise in air surface temperature. Since most of the inhabitants are dependent on economic activities that are temperature sensitive like farming, the study therefore recommends that measures should be taken by all stakeholders including the government, individuals and cooperate bodies to take the issue of climate variability serious in the study area in order to mitigate its impact in the long run.*

Keywords**— *Climate Change, Ebonyi, Trend, Temperature, Agriculture.***

I. INTRODUCTION

Issues related to climate change and global warming arising from anthropogenic emission of greenhouse gases have emerge as one of the most important environmental issues in the past few decades (Singh et al., 2013). Human activities at present are altering the carbon cycle by adding more CO2 to the atmosphere and also by influencing the ability of natural sinks, like forests, to remove CO2 from the atmosphere. The emission of carbon dioxide comes from a variety of natural sources; but human-related emissions are responsible for the increase that has occurred in the atmosphere since the industrial revolution. Evidence has shown that the level of carbon dioxide in earth's atmosphere presently is higher than at any time in the last 800,000 years. For instance in 2014, global CO2 emissions were projected to increase by an additional 2.5% over the 2013 level (USNCDC, 2013). Other sources of these emissions include water vapour, chlorofluorocarbons (CFCs), methane, tropospheric ozone and nitrous oxide. These gases are released into the atmosphere due to human activities such as burning of fossil fuel, gas flaring and deforestation amongst others .These gases are termed greenhouse gases, because they act as the glass of a greenhouse or sunroom which is relatively transparent to solar radiation which is in short wavelength but absorbs and emits terrestrial radiation which is in long wavelength, and thereby increasing the temperature within the glass house or room. The continuous built up of greenhouse gases may induce changes in climatic system including increases in mean global temperatures. The global mean surface air temperature has risen by about 0.74°C from 1906 to 2005 and this rise has been attributed mostly to a rise in greenhouse gases (IPCC, 2007). Changes in temperature influence a variety of processes directly or indirectly for example the hydrological process. Temperature increases causes intensification of the hydrological cycle due to increase in evaporation and precipitation(Jain and Kumar, 2012; Tshiala et al., 2011) Temperature changes can also lead to changing patterns of precipitation, the spatial and temporal distribution of runoff, soil moisture, and groundwater reserves as well as increased frequency of drought and flood occurrences (Tshiala et al., 2011). Changing temperature patterns could also have effects on soil and plant growth characteristics since temperature and water content are important physical factors for plant growth especially in Ebonyi State were majority are engaged in one form of agriculture or another as their source of livelihood. Non-optimum levels of water and temperature conditions can strongly perturb plant development, especially at the early stages of growth such as seed germination and emergence (Tshiala et al., 2011). Changes in climate may also impact the water availability and water needs for agriculture. If temperature increases and more sporadic rainfall events result from global warming, this will increase the demand irrigation needs in the future. The increasing number of heat-related deaths worldwide over the last few decades have portrayed an alarming picture of the extreme weather conditions and devastating impacts on human health to come if this warming continues unmitigated (CENR, 2008).Developing countries however have a challenge of inadequate information to tackle the inherent consequences that might result from the changes in temperature. Some of the measures put in place to tackle climate change such as mitigation, adaptation and vulnerability assessment may not yield valuable result if the extent to which these variables have varied is not known. This study therefore examines the trend and variability in temperature over Ebonyi State South- eastern Nigeria.

Area Description

The study area Ebonyi State, shown in fig. 1, is located in South-eastern part of Nigeria which lies approximately within latitudes 5° 40’ and 6° 45’ North and longitudes 7°30' and 8°30'East. The mean temperature range within the study area is usually between 27° to 30^0c over the year (Ogbuene, 2010). Temperature is highest from February to April and it is about 31^0c (Ogbodo, 2013). The soil is texturally clay loam, fairly to poorly drain with gravely subsoil in some locations especially the upland adjacent to lowland areas (Ekpe, Okpone, Ogbodo, & Nwite, 2005). Agriculture is a major industry in Ebonyi State, an estimated eighty-five per cent of the population earn their living from one form of agricultural activity (Ogbodo, 2013). The presence of large arable land, rivers and streams has made farming very attractive. Crops grown in the area include; rice, yam, cassava, cocoyam, groundnut, cowpea and vegetables. Livestock farming, especially the extensive system of rearing sheep, goats and native cattle, is also practiced by the people. Fishing activities are predominant in the southern zone of the State.

FIGURE 1: THE STUDY AREA: EBONYI STATE, SOUTH- EASTERN NIGERIA.

II. MATERIAL AND METHOD

2.1 Data Used

Mean monthly maximum and minimum temperature covering 31 years period for the study area were obtained from the Nigerian Meteorological Agency (NIMET), Oshodi, Lagos, The temperature data was transformed to mean monthly temperature and furthermore to annual.

2.2 Data Analysis

Data analysis was carried out from the temperature data obtained and analyzed for trend and fluctuation using mean, moving averages, Standard deviation, coefficient of variation and linear regression. Their expressions are as follows

The mean statistic is used in the study to determine the differences in the decadal means temperature as a way of showing decadal variation between 1984 and 2015 and also for the calculation of temperature anomaly i.e. deviation ($d = x - \bar{x}$)

$$x = \frac{\sum_{i=1}^{n} xi}{n} \quad (1)$$

Where i runs from 1 to n; x, the temperature or rainfall values, and n, the number of years.

Moving average is a smoothing method that is needed to check out some up and down i.e. in finding trend that might exit in data as trends tend to be obscured by the random errors. The simplest way of smoothing a time series data is to use a moving average. An average value is computed by using only a specified set of values. In this study, a 10-year moving average is used.

The 10- years moving average is written as:

$$\frac{y_1 + y_2 + y_3 + y_4 \ldots . y_{10}}{n} \quad (2)$$

Where n is 10 years order and y the variable in this case temperature

Standard deviation is one of the simplest ways of measuring climate variability by using the standard deviation estimator in measuring dispersion. It is used in this study to show the absolute variability in temperature from 1984 to 2015. Sample standard deviation S_x is given as

$$sx = \sqrt{\sum \frac{xi - \overline{x)2}}{n-1}} \quad (3)$$

where Sx= the estimator of the standard deviation σx of a climate variable X

It is not easy to interpret the standard deviation as a measure of dispersion on its own. This is because a small value for standard deviation shows that the dispersion of the dataset is low. Nevertheless, the magnitude of these values depends on what is being analysed. Therefore, a method to overcome this difficulty of interpreting the standard deviation is to take into account the value of the mean of the data and employ the use of coefficient of variation. It is used in this study to show the relative variability of temperature over time. The coefficient of variation, Vx, is a relative measure of variability and is defined as follows (Waller, 2008):

$$Vx \frac{Sx}{\overline{x}} \quad (4)$$

The least squares regression is used to model the trends in temperature data over the 31 years period. The result helps to determine the overall average rates of change in trends of annual temperature in the study area.

The equation for least square regression is

$$y \quad = a \quad + \quad bx \quad (5)$$

Where

$$b \quad = \quad \frac{n \sum xy - \quad (\sum x)(\sum y)}{n \sum x^2 \quad - \quad (\sum x)^2} \quad (6)$$

and

$$a \quad = \quad \frac{\sum y}{n} - \frac{b \sum x}{n} \quad = \quad \bar{y} - b\bar{x} \quad (7)$$

a is the intercept; b the regression coefficient or slope;
y =the temperature values (dependent variable);
x=the time in years ;(independent variable)
$\bar{x}$ = the mean time; and
$\bar{y}$= the mean temperature value.

III. RESULT AND DISCUSSION

The results obtained for the study as well as its discussion are presented under this section. 4.1 Descriptive statistics and Variation in Temperature

Fig. 2 shows the relative variability of average temperature over time measured by standard deviation. It exhibits a steady ups and down indicating variability within the study period. Absolute variability of average temperature depicted in fig. 3 also exhibits a similar behavior with relative variability.

Three decades are considered to observe variability over time in this study as shown in Table 1.Which indicates significant variability in mean temperature. There was a significant increase between 1984-1993 and 1994-2003; while the decade between 2004 and 2013 experienced a slight increase over the period between 1984-1993 .Both absolute variability and relative variability increased steadily throughout the three decades over the study period.

TABLE 1
TEMPERATURE VARIABILITY FROM SIMPLE STATISTIC METHODS

climate variable	Statistical tool	1984-1993	1994-2003	2004-2013	2014-2015
Average temperature (°C)	Mean Standard Deviation Coefficient of variation (%)	27.32 5.08 18.61	27.50 5.14 18.69	27.40 5.16 18.82	27.30 4.98 18.25

Fig. 4 represents the anomalies of air temperature experienced in Ebonyi State using 1984-2015 mean. Here mean temperature ranged between 0.5°C colder and 1.0°C warmer than the baseline temperature of 27.4°C .Fig. 4 shows that more areas are getting warmer at a higher rate than it is cooling. The highest positive anomaly recorded was in 2010 which gave an indication of the most probable warmest year in the period under study with surface air temperature of 1.0°C in and is followed by 0.8°C in 1998. Generally there is an indication of increasing warmer years which agrees with global temperature trend (IPCC, 2007) as well as Nigeria trend (Odjugo, 2010).

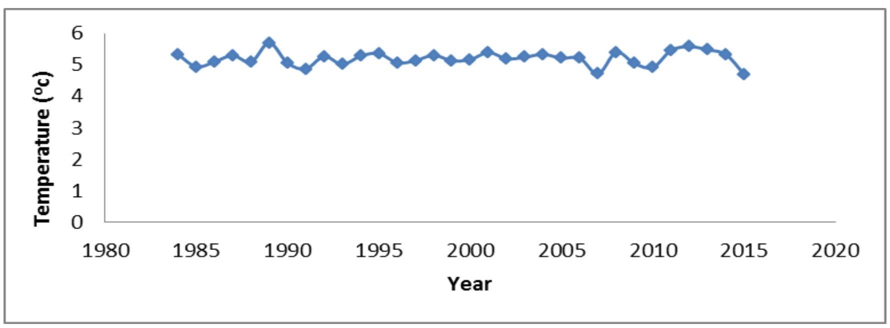

FIG. 2: STANDARD DEVIATION OF AVERAGE TEMPERATURE

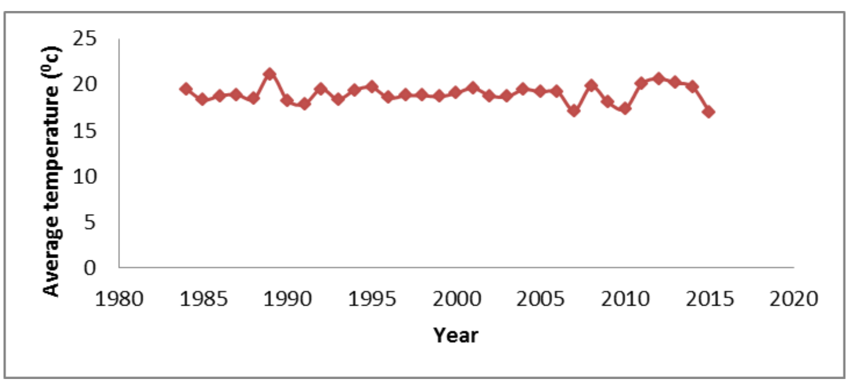

FIG. 3: COEFFICIENT OF VARIATION OF AVERAGE TEMPERATURE

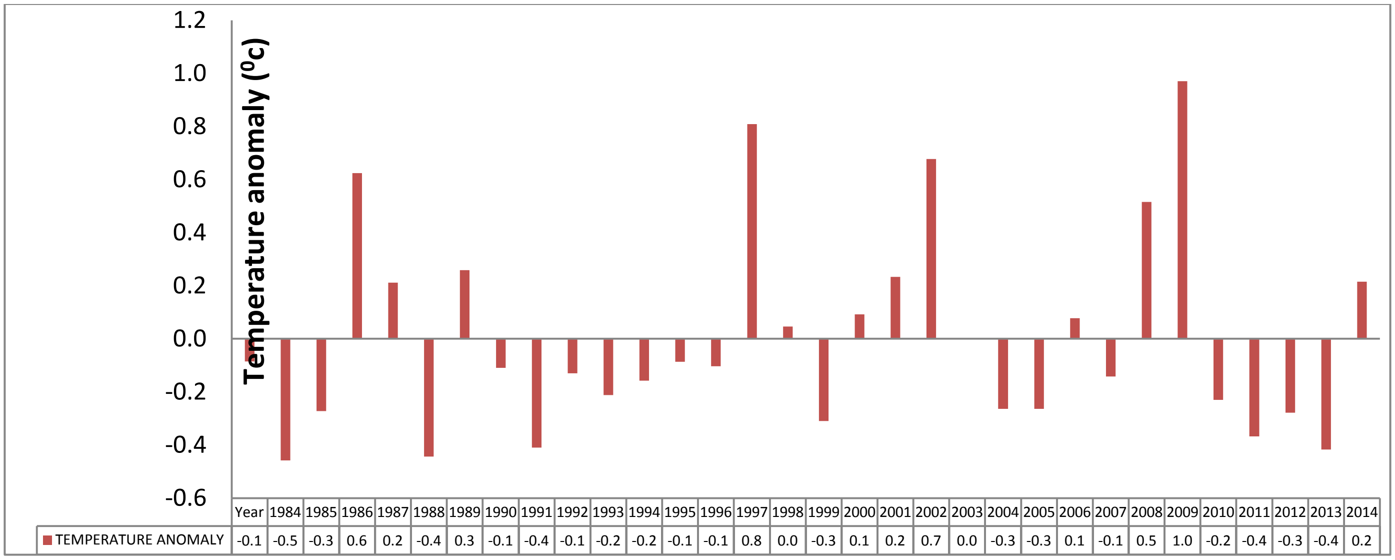

FIG. 4: TEMPERATURE ANOMALY (1984-2015)

Trend Analysis of Annual Temperature

The temporal air temperature trend exhibits a steady increase with some fluctuations over the period. Generally, the overall trend is upwards. The highest temperature recorded over this period was in 2010. After the application of the 10-years moving averages to filter out the erratic fluctuations for temperature observation, with few peaks and depression left annual temperature trend lines were fitted (fig. 5).

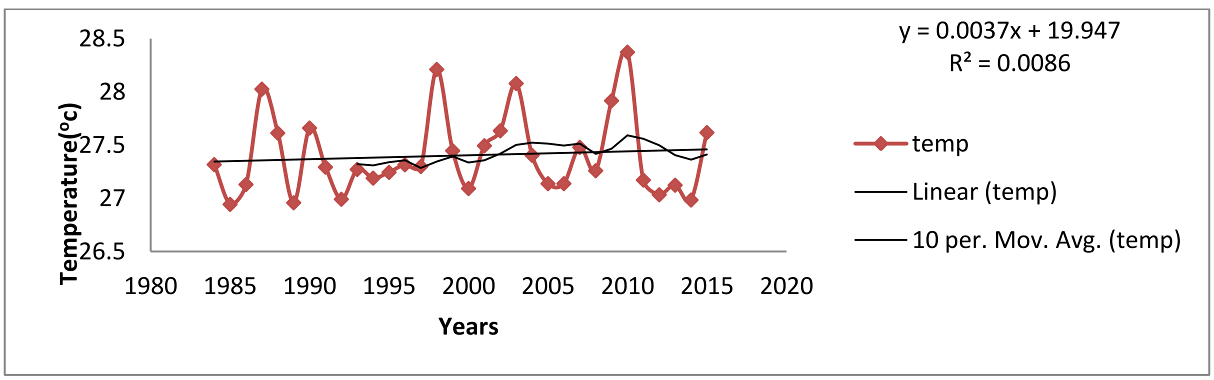

FIG. 5: TRENDS OF MEAN ANNUAL TEMPERATURE FOR EBONYI STATE

Thereafter, the linear regression was applied to highlight the general tendency, intercepts, slopes and regression lines were generated. The result generated is shown in Table two and as graphs in fig. 5. Results revealed that temperature possess an upward trend. This means that the area is warming by 0.0037^{o}c per annum. Though the annual rate of increase in mean temperature (0.0037^{o}c) appears insignificant but has implication for other element of weather and climate for instance rainfall.

TABLE 2
TRENDS IN MEAN TEMPERATURE FOR EBONYI STATE

Climate variable	period	Regression line equation
Mean temperature	1984-2015	Y= 19.947+0.0037x

This increase in mean annual temperatures will make the area even hotter than before. This would bring about increase in evapotranspiration if wet surfaces exist (and by extension other component of the ecosystem such as vegetation, agriculture, water-bodies. This is because as climate is caused to change, other components of the earth atmosphere system might respond to influences exerted on them by these changes. NEST (2003) provided indicators that can be used to assess the evidence of climate change in a region to include increasing temperature. Others includes increasing evapotranspiration, decreasing rainfall amount in the continental interiors, increasing rainfall in the coastal areas, increasing disruption in climate patterns and increasing frequency and intensity of unusual or extreme weather related events such as; thunderstorms, lightning, landslides, floods, droughts, bush fires, unpredictable rainfall patterns, sea level rise, increase desertification and land degradation, drying up of rivers and lakes and constant loss of forest cover and biodiversity. This study however reveals that an indicator is already present in Ebonyi State by way of increasing temperature, as this study agrees with the studies of Amadi, Udo and Ewona (2014) and Abiodun et al. (2011) on the rising trend in temperature over Southeast Nigeria.

Potential impact of temperature variability on crop agriculture

Climate change through increasing temperature can impact on agricultural sector directly or indirectly by impacting on crop productivity and production which can either enhance crop availability or a decrease in crop production. The most significant factors in climate are temperature and rainfall this is because of their role not only as elements of weather and climate but also as factors of climate with the implication that any change in them will likely cause change in the other elements. A continuous rise in temperature beyond a certain threshold will affect productivity in crop and could cause growing season to become shorter, thereby causing a reduction in yield. Climate change being experienced at present has resulted in extreme events such as flooding, droughts, heat/cold waves, changes in weather patterns which have posed serious challenges to the sustainability of crop production especially in regions were agriculture is dependent on weather. Ebonyi State being an agricultural State, that is experiencing climate change by way of increasing temperature, may experience a situation whereby crops will be smothered by excessive heat thereby reducing crop production in the State. Khanal (2009) stated that heat stress might affect the whole physiological development, maturation and finally reduces the yield of cultivated crop. As crop water requirement is directly linked to evaporative demand of the atmosphere in which the crop is grown. Increasing temperature will further increase the water demand of crops and if there is no corresponding moisture could lead to a drought situation. However, increasing temperature could be beneficial to some crops but detrimental to others this is because various crops require certain optimum temperature to survive. That is to say for any particular crop, the effect of increased temperature will depend on the crop's optimal temperature for growth and reproduction (USGCRP, 2014). In some areas, warming may benefit the types of crops that are typically planted there, or may give opportunity to farmers to shift to crops that are currently grown in warmer areas. However, if the higher temperature exceeds a crop's optimum temperature, yields will decline. On the other hand, Increase in temperature could reduce the length of the effective growing season, particularly where more than one crop per year is grown .Increased temperature could also affect the physiological processes necessary for crop growth and development thereby resulting to a drop in yield. Consequently, a drop in yield will lead to increased dependence on importation of crop to feed the population which could result to food insecurity.

IV. CONCLUSION

This study reveals that Ebonyi State is experiencing a rise in air surface temperature which by implication means that the State is susceptible to the attendant consequences of global warming. As a result, the inhabitant who are mostly farmers who sources of livelihood is dependent on returns from agriculture that is temperature sensitive are vulnerable to the risk pose by increasing temperature. As changes in temperature may have impact on water availability and water needs for agriculture, thereby increasing the need for alternative source of water such as irrigation for crop survival in Ebonyi State. Plant and animal also respond to temperature variability which could have economic consequences on agricultural productivities and thereby increasing food scarcity The implication of the rising trend in temperature will also have impact on the environment as well as the wellbeing of the inhabitant of Ebonyi State for example, the human body responds to thermal stress by forcing blood into peripheral areas to promote heat loss through the skin, therefore health disorders are expected at higher temperature. The inhabitants of Ebonyi State, Southeastern Nigeria could be vulnerable to medical disorder which can include heat stroke, heat rash, heat cramp, heat exhaustion and heat syncope. Therefore, proactive steps should be taken by all stakeholders ranging from government, individuals and cooperate bodies to take the issue of climate variability serious and put measures in place to mitigate its effect in the study area and Nigeria in general.

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Analyzing Marketing Margins and the Direction of Price Flow in the Tomato Value Chain of Limpopo Province, South Africa

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Abstract— *The Limpopo Province is home to South Africa's tomato farming giants, some of whom also occupy the position of the largest producers of the commodity in the Southern Hemisphere. Regardless of its importance in the tomato industry of the country, there are few studies analysing the mechanism through which prices of tomatoes are determined and transmitted from the farm gate in Limpopo to the various provincial, local and international markets. This study attempts to fill the knowledge gap on the performance of Limpopo Province's tomato markets by examining the marketing margins and the direction of price flow amongst the successive tomato marketing levels. By means of the Concurrent Marketing Margin Analysis approach, it was established that the farmers' portion of the consumer's Rand is low. About 85.1% of the consumer's Rand goes to pay for marketing margins. Granger causality tests show that both the wholesale and retail prices are caused by farm gate prices. The farm level is therefore key to tomato price determination in Limpopo Province of South Africa and pertinent policies that improve the commodity's primary sector may potentially enhance the other sectors in value chain that depend on tomato production.*

Keywords— Limpopo Province, market dominance, marketing margins, price flow, tomato markets, vertical price linkage.

I. INTRODUCTION

Agricultural productivity has improved throughout history because of economic progression, innovation, specialization, research and development (Fuglie et al., 2007; Fuglie and Nin-Pratt, 2012; Nin-Pratt, 2013). This has increased the productive capacities of farmers who in time have been able to produce in excess of what is needed for home consumption and subsistence. Such developments augmented the significance and complexity of marketing and have seen the establishment of arenas to facilitate the exchange of marketable surplus between producers and consumers.

In the context of the tomato industry of South Africa, it is highlighted in Department of Agriculture, Forestry and Fisheries (DAFF) (2011) that production of tomato occurs in all the nine provinces of South Africa by both commercial and emerging farmers. However, different provinces produce varied volumes due to environmental disparities amongst other factors. The Limpopo Province with its warmer climate plays the most vital role in the country's tomato production. According to DAFF (2011) the province contributes about 3 590 ha to the country's total area planted to tomatoes. Limpopo Tourism and Parks Board (2011), suggests that almost 60% of the country's tomatoes are produced in Limpopo Province which is responsible for about 45% of the Johannesburg Fresh Produce Market's annual turnover. However, the Limpopo Province's tomato producing industry's concentration is high which in economics sense means that a large share of total production is dominated by a few large producers. Limpopo Tourism and Parks Board (2011), indicates that only one farming company is responsible for about 40% of the province's 60% contribution to South Africa's total tomato production.

Even though the majority of tomato is produced in the Limpopo Province, the commodity is consumed in all parts of the country and is also exported thus; there is some degree of spatial separation between production and consumption. Such spatial distribution calls for the need for an efficient and effective marketing system to facilitate the movement of tomatoes from the point of production to the end user. Bringing the agricultural product from the agricultural enterprise to the consumer according to Saccomandi (1998), involves a series of functions and productive activities that interconnect. The process also depends on the current state of technology, the organization of productive activities and the spatial distribution of production and consumption.

II. PROBLEM STATEMENT

There is high industry concentration in the tomato producing industry of the Limpopo Province. Such a situation where the industry is dominated by a few large firms paves way to major producers potentially using their market power to influence pricing strategies from time to time. As to which of the participants in the chain will consequently suffer or benefit from impending price shocks depends on the degree to which market players adjust to price signals. It also depends on the timing in response and the extent to which their adjustment to price shocks is asymmetric as shown in Vavra and Goodwin (2005).

Particular aspects of participants' response to price movements may also have important implications on marketing margins of several players in the marketing chain of tomato in Limpopo Province. While farmers may view their proportion of the consumer's Rand as low, tomato consumers on the other hand experience continual rising prices of this commodity. It may be questioned therefore, as to whom in the marketing chain sits at point(s) of price determination. It may also be of interest to determine the direction of price causality along the marketing chain for tomato in Limpopo Province so as to guide any pricing policy in the respective food market.

III. AIM AND OBJECTIVES

This study seeks to analyze marketing margins and the direction of price flow in the tomato value chain of Limpopo Province South Africa so as to determine the performance of tomato markets in the region. It's is imperative to estimate the point(s) of price determination and direction of causality along the marketing chain for tomato in Limpopo Province so as to guide food marketing and pricing policies.

IV. RESEARCH HYPOTHESES

1. Price and direction of causality along the marketing chain for tomato in Limpopo Province are determined beyond the farm gate.
2. The proportion of the consumer's dollar that goes to pay for marketing margins is uncertain.

V. LITERATURE REVIEW

According to FAO (2002), the percentage share of the final price, which is taken up by the marketing functions, is what is known as the marketing margin. Guvheya et al. (1998) treated the concept of marketing margins as differences between prices at different levels in the marketing channel which capture the proportion of the final selling price that a particular agent in the marketing chain adds. Elitzak (1996) characterize marketing margins or the farm-to-retail price spread as the difference between the farm value and retail price that represents payments for all assembling, processing, transporting and retailing charges added to farm products. Cox (2009) noted that producers often wonder about the large difference between the prices that consumers pay for food and the prices that farmers receive. However, high marketing margins can be justified by costs involved in distributing the product from point of production to the final consumer. It can be argued though, that too high a percentage reflects some exploitation of either farmers or consumers. Wohlgenant (2001) investigated marketing margins and identified some of the questions that are frequently asked about the issue. These questions, which have attracted considerable interest across literature and amongst researchers and policy makers, include; Are marketing margins too large? What is the incidence of marketing costs on retail prices and farm prices? How quickly are farm prices transmitted to the retail level and how quickly are retail price changes transmitted to farmers? In order to have a thorough and exact investigation, Mojtaba et al. (2010) recommended that the marketing margin be divided into two smaller portions namely Retailer Margin (RM) and Wholesaler Margin (WM). RM refers to the difference between the price paid by consumers and the price that retailers pay to the wholesalers. WM refers to the difference between the price at which wholesalers sell their product and the price that they pay to the farmers.

While price represents the equilibrium point where demand and supply meet in the market place, Schnepf (2006) emphasizes that the general price level of an agricultural commodity, at any stage, is influenced by a variety of market forces that can alter the current or expected balance between supply and demand. According to Holland (1998) price determination for many consumer products is in most cases a function of the cost of production and a desired level of mark-up. Price determination by this desired level of mark-up is what is referred to as cost-plus pricing, mark-up pricing or full-cost pricing which in Salvatore (1993) is related to three rules of thumb. The first is termed; mark-up percent, which is the proportion of profit to total cost. The second is the gross margin percent, which is the proportion of profit to the selling price, and lastly, profit margin, which is the difference in selling price and total cost.

VI. STUDY AREA, DATA COLLECTION AND SAMPLING TECHNIQUE

This study is based mainly on the Limpopo Province which is the northernmost province of South Africa covering a total surface area of 123,910 km2 making it the fifth largest province amongst the country's nine provinces. Limpopo Province can be described as the garden of South Africa due to its rich fruit and vegetable production. Apart from being South Africa's main tomato producer Limpopo Province is responsible for about 75% of the country's mangoes, 65% of its papayas, 36% of its tea, 25% of its citrus, bananas, and litchis, 60% of its avocados, 285 000 tons of potatoes, and 35% of its oranges. Most of tomato production in the province is done by a private corporation, who is also the largest tomato farmer in South Africa. The province is also involved in the production of coffee, nuts, guavas, sisal, cotton, tobacco, sunflower, maize, wheat, grapes and timber. Livestock production and game ranching is also prominent particularly in most of the higher-lying areas (vanNiekerk, 2012; Limpopo Tourism and Parks Board, 2011).

This study used both primary and secondary data of time series in nature. Daily tomato prices were collected synchronously at farm gate, wholesale and retail levels for mixed grades of cooking tomatoes to achieve a sample size of 50 observations. Semi structured interviews and desk studies were also conducted to explore the procurement and marketing procedures followed by various participants in tomato marketing in the Limpopo Province.

VII. MARKETING MARGIN ANALYSIS

In analysing the marketing margins, the Concurrent Margin Method was used. As described by Singh (1998) the method is a static analysis of the distributive margin usually adopted to calculate the price spread in one market town by considering differences between prices prevailing at successive stages of marketing at a given point of time.

The model is defined as thus;

$$M_t = P_{t,L} - P_{t,L-1} \quad \text{where;} \tag{1}$$

M_t= Marketing margin between market level (L) and its preceding level (L-1) at time (t)

$P_{t,L}$ = Price at market level (L) at time (t)

$P_{t,L-1}$ = Price at market level (L-1) at time (t)

Where marketing margins at different levels of the marketing chain are compared, Guvheya et al. (1998) emphasizes the use of consumer price as the common denominator for all margins.

The two indices that were used in this study are Total Gross Marketing Margin (TGMM) and Producers' Gross Marketing Margin (GMMp) as given in Scott (1995) where Gross Marketing Margin is the difference between consumer's price and farmer's price. TGMM and GMMp were calculated as;

$$\text{TGMM} = \frac{Consumer\ Price - Farmer's\ Price}{Consumer\ Price} \text{ x } 100, \tag{2}$$

and

$$\text{GMMp} = \frac{Price\ paid\ by\ the\ consumer - Gross\ Marketing\ Margin}{Price\ paid\ by\ the\ Consumer} \text{ x } 100 \tag{3}$$

VIII. TESTING FOR UNIT ROOT NON-STATIONARITY

The Augmented Dickey Fuller test was performed on each of the logarithmic series of farm prices (FP), wholesale prices (WP) and retail prices (RP) to formally ascertain whether they contained a unit root.

IX. PRICE DETERMINATION AND DIRECTION OF CAUSALITY

In assessing the points of price determination and the direction of causality along the major marketing channels for tomato in Limpopo Province, Granger causality tests (Granger, 1969) were performed.

Three sets of Vector Autoregressive (VAR) models were formulated and estimated;

(a) Testing causality between farm gate and wholesale levels:

$$ln\text{FP}_t = \alpha_1 + \sum_{i=1}^{n} a_i ln\text{WP}_{t-i} + \sum_{i=1}^{n} b_i ln\text{FP}_{t-i} + U_{1t}$$

$$ln\text{WP}_t = \alpha_2 + \sum_{i=1}^{n} c_i ln\text{WP}_{t-i} + \sum_{i=1}^{n} d_i ln\text{FP}_{t-i} + U_{2t} \tag{5}$$

(b) Testing causality between wholesale and retail levels:

$$lnWP_t = \alpha_1 + \sum_{i=1}^{n} a_i lnRP_{t-i} + \sum_{i=1}^{n} b_i lnWP_{t-i} + U_{1t}$$
$$lnRP_t = \alpha_2 + \sum_{i=1}^{n} c_i lnRP_{t-i} + \sum_{i=1}^{n} d_i lnWP_{t-i} + U_{2t} \quad (6)$$

(c) Testing causality between farm and retail levels:

$$lnFP_t = \alpha_1 + \sum_{i=1}^{n} a_i lnRP_{t-i} + \sum_{i=1}^{n} b_i lnFP_{t-i} + U_{1t}$$
$$lnRP_t = \alpha_2 + \sum_{i=1}^{n} c_i lnRP_{t-i} + \sum_{i=1}^{n} d_i lnFP_{t-i} + U_{2t} \quad (7)$$

**where*; *ln* is the natural logarithm of each respective price series

FP_t is the farm price at time (t)

WP_t is the wholesale price at time (t)

RP_t is the retail price at time (t)

FP_{t-i} is lagged farm gate price

WP_{t-i} is lagged wholesale price

RP_{t-i} is lagged retail price

n is the upper limit set at the optimal lag length

a_i, b_i, $ci_,$ and d_i are coefficients to be estimated using the ordinary Least squares method and α_1 and α_2 are intercepts

U_{1t} and U_{2t} are error terms that are assumed uncorrelated and white noise

The inference in the first mathematical statement of equation 5 is that current farm prices are dependent on past farm prices and past and present wholesale prices.

Likewise, the second mathematical statement postulates that current wholesale prices are dependent on past farm prices and past and present wholesale prices.

The inference in the first mathematical statement of equation 6 is that current wholesale prices are dependent on past wholesale prices and past and present retail prices.

Likewise, the second mathematical statement postulates that current retail prices are dependent on past wholesale prices and past and present retail prices.

The inference in the first mathematical statement of equation 7 is that current farm gate prices are dependent on past farm prices and past and present retail prices.

Likewise, the second mathematical statement postulates that current retail prices are dependent on past farm gate prices and past and present retail prices.

In each of the cases (a), (b) and (c), four causality relationships were tested by placing the appropriate restrictions on each model. P-values and F-tests were used to confirm statistical significance of the causality relationships.

For instance, in case (a) the following were the causality relationships tested between farm gate and wholesale prices;

(i) A unidirectional causality from wholesale to farm gate levels would be concluded if;

$\sum_{i=1}^{n} \boldsymbol{a}_i \neq 0$ and $\sum_{i=1}^{n} \boldsymbol{d}_i = 0$

(ii) A unidirectional causality from farm gate to wholesale levels would be concluded if,

$\sum_{i=1}^{n} \boldsymbol{a}_i = 0$ and $\sum_{i=1}^{n} \boldsymbol{d}_i \neq 0$

(iii) An absence of a causal relationship between the variables that is independence would be concluded if both

$\sum_{i=1}^{n} \boldsymbol{a}_i = 0$ and $\sum_{i=1}^{n} \boldsymbol{d}_i = 0$

This would imply that both sets of the lagged exogenous variables were not statistically different from zero.

(iv) a bilateral causality or feedback would exist if both

$\sum_{i=1}^{n} \boldsymbol{a}_i \neq 0$ and $\sum_{i=1}^{n} \boldsymbol{d}_i \neq 0$

This would imply that both sets of the lagged exogenous variables were, as a group, statistically significantly different from zero.

In formulating the above sets of VAR models, the VAR Lag Order Selection Criteria (VLOSC) was used to determine the optimal lag length.

X. RESULTS OF THE STUDY

10.1 Marketing Margin Analysis

Prices of the tomatoes varied across the marketing chain over time. For the ten week period the highest weekly average farm gate price was ZAR1.85/kg, while the lowest was ZAR1/kg and the average farm gate price for the whole period was ZAR1.37/kg. The highest wholesale price was ZAR5.45/kg; while the lowest was ZAR3.10/kg and the whole period average was ZAR4.73/kg. The highest retail price was ZAR9.60/kg, while the lowest was ZAR8.79/kg and the average was ZAR9.20/kg.

Table 1 shows the marketing margin structure of Limpopo Province’s tomato marketing chain on five day weekly basis for the period of study.

TABLE 1
FIVE DAY WEEKLY MARKETING MARGINS FOR LIMPOPO PROVINCE’S TOMATO MARKET CHAIN

	South African Rands (ZAR)/ kg						%	
Week	FP	WP	RP	F-to-W GMM	W-to-R GMM	F-to-R GMM	Total GMM	Producer GMM
1	1.33	4.17	8.79	2.84	4.61	7.45	84.84	15.16
2	1.34	4.93	9.28	3.60	4.34	7.94	85.58	14.42
3	1.85	4.80	9.37	2.95	4.57	7.52	80.27	19.73
4	1.76	5.23	8.95	3.48	3.71	7.19	80.36	19.64
5	1.14	4.42	9.08	3.28	4.66	7.94	87.44	12.56
6	1.00	3.10	9.60	2.10	6.50	8.60	89.58	10.42
7	1.46	5.45	9.49	3.98	4.04	8.03	84.56	15.44
8	1.29	5.19	9.15	3.90	3.97	7.86	85.94	14.06
9	1.18	4.98	9.09	3.81	4.11	7.92	87.07	12.93
10	1.36	5.02	9.20	3.66	4.18	7.83	85.19	14.81
Average	1.37	4.73	9.20	3.36	4.47	7.83	85.08	14.92

The average farm-to-wholesale gross marketing margin (FWGMM) of R3.36 and the average wholesale-to-retail gross marketing margin (WRGMM) of R4.47 in sum give us the average farm-to-retail gross marketing margin (FRGMM) of R7.83. With the average retail price of R9.20, it follows that consumers typically had to part with R9.20 for every kilogram of tomato they purchased from retailers. However, comparing the FRGMM of R7.83 with the average retail price we can conclude that the total gross marketing margins constituted about 85.1% of the consumer’s Rand. With such a high marketing margin, the common hypothesis that the farmers’ portion of the consumer’s Rand is low holds true. During the period of analysis, tomato farmers were getting only 14.9% from every Rand spent by consumers on tomatoes at retail level.

10.2 Unit Root Tests

The information on margins, given in the previous section, prompts the need to undertake further explorations on vertical price linkages amongst different levels in the marketing chain of tomatoes in Limpopo Province. The statistical properties of the price series are analysed before carrying out causality tests. Table 2 presents an abstract of results of the unit root tests performed on lnFP, lnWP, and lnRP according to the Augmented Dickey-Fuller (ADF) criteria.

TABLE 2
AUGMENTED DICKEY FULLER TEST RESULTS ON lnFP, lnWP, lnRP IN LEVELS

Null Hypotheses: each of lnFP, lnWP, and lnRP contain a unit root			
lnFP	ADF test statistic Test Critical Values	t-Statistic Prob. -2.083252 0.5419 1% level -4.156734 5% level -3.504330 10% level -3.181826	Lag Length: 0 (Automatic - based on SIC, maxlag=5) DW stat: 1.799676
lnWP	ADF test statistic Test Critical Values	t-Statistic Prob -0.073823 0.6531 1% level -2.614029 5% level -1.947816 10% level -1.612492	Lag length: 1 (Automatic - based on SIC, maxlag=5) DW stat: 2.089489
lnRP	ADF test statistic Test Critical Values	t-Statistic Prob 0.126524 0.7180 1% level -2.613010 5% level -1.947665 10% level -1.612573	Lag length: 0 (Automatic - based on SIC, maxlag=5) DW stat: 2.548604

The results in Table 2 shows that ADF test-statistic values of the three price series are greater than the MacKinnon critical values for rejecting the hypotheses of a unit root. As a result, we cannot reject the null hypothesis of a unit root at 1%, 5% and 10% levels of significance. This regression result can be trusted since the Durbin-Watson statistics are all significant enough to reject the presence of serial correlation in each of the three series. With the statistical evidence generated, we can therefore conclude that the farm gate, wholesale and retail price series of Limpopo produced tomatoes for the period of analysis is non-stationary.

Vavra and Goodwin (2005) noted that most economic time series being non-stationary in nature need some transformation through differencing or de-trending, otherwise the regression will be spurious. Spurious regressions occur when the mean, variance and covariance of a time series vary with time. The classic results of a usual regression cannot be legitimate if non-stationary series of data is used for analysis.

As such, first differences were taken on each of the three series lnFP, lnWP, lnRP to come up with a differenced set of data, DlnFP, DlnWP, and DlnRP that is unit root free. The summary of the ADF test on the first differenced series are shown in Table 3.

TABLE 3
AUGMENTED DICKEY FULLER TEST ON DlnFP, DlnWP, and DlnRP (FIRST DIFFERENCES)

Null Hypotheses: each of DlnFP, DlnWP and DlnRP contain a unit root			
DlnFP	ADF test statistic Test Critical Values	t-Statistic Prob -6.644587 0.0000 1% level -4.161144 5% level -3.506374 10% level -3.183002	Lag length: 0 (Automatic - based on SIC, maxlag=3) DW stat: 2.004682
DlnWP	ADF test statistic Test Critical Values	t-Statistic Prob -11.33005 0.0000 1% level -4.161144 5% level -3.506374 10% level -3.183002	Lag length: 0 (Automatic - based on SIC, maxlag=5) DW stat: 2.097217
DlnRP	ADF test statistic Test Critical Values	t-Statistic Prob -9.150489 0.0000 1% level -2.614029 5% level -1.947816 10% level -1.612492	Lag length: : 0 (Automatic - based on SIC, maxlag=5) DW stat: 2.030275

Table 3 shows that ADF test-statistic values of the three differenced price series are less than the MacKinnon critical values for rejecting the hypotheses of a unit root. As a result, the null hypothesis of a unit root in first differences of lnFP, lnWP and lnRP can be rejected at 1%, 5% and 10% levels of significance. Again, the regression result can be trusted since it passed the Durbin-Watson test. It can therefore be concluded with 99% confidence that the DFP, DWP and DRP series of Limpopo produced tomatoes for the period of analysis are stationary since there is enough statistical evidence to support this. The implication of the ADF tests results is that the series generating the three price variables are all integrated of order one, that is, I(1).

10.3 Lag Order Selection Criteria

The next step was to carry out the lag-order-selection criteria for correct specification of the VAR model to use in Granger Causality tests. Results showing the optimal lag lengths to use in the causality tests are summarized in Table 4. For the sample with 50 observations, lnFP and lnRP were the endogenous variables, while the constant C was the only exogenous variable.

TABLE 4
VECTOR AUTO REGRESSIVE LAG ORDER SELECTION CRITERIA (lnFP and lnRP)

Lag	LR	FPE	AIC	SC	HQ
0	NA	6.40e-05	-3.980930	-3.900634	-3.950997
1	70.25371	1.44e-05	-5.475860	-5.234972*	-5.386059*
2	6.503296	1.46e-05	-5.460664	-5.059184	-5.310997
3	4.282047	1.56e-05	-5.395572	-4.833499	-5.186037
4	9.614758*	1.44e-05	-5.484871	-4.762206	-5.215469
5	7.539553	1.39e-05*	-5.528845*	-4.645588	-5.199575

In Table 4, the asterisk (*) indicates lag order selected by each criterion, LR is the sequential modified LR test statistic (each tested at 5% level), FPE is Final Prediction Error, AIC is the Akaike Information Criterion, SC is the Schwarz Information Criterion and HQ stands for Hannan-Quinn Information Criterion. The VAR lag order selection criteria results summarized in Table 4 show that AIC and FPE chose 5 lags, SC and HQ 1 lag and only LR chose 4 lags. However, retailers indicated that they in most cases alter their tomato prices on weekly basis. It is therefore reasonable to accept the AIC and FPE choices given the available knowledge of the tomato retail markets in Limpopo Province. As a result, five lags were used as the optimal lag length for testing Granger Causality and cointegration relationships between farm gate prices and retail prices.

A similar procedure was done to determine the optimal lag length between farm gate and wholesale prices and results are shown in Table 5. For the sample with 50 observations, lnFP and lnWP were the endogenous variables, while the constant C was the only exogenous variable.

TABLE 5
VECTOR AUTO REGRESSIVE LAG ORDER SELECTION CRITERIA (lnFP and lnWP)

Lag	LR	FPE	AIC	SC	HQ
0	NA	0.001598	-0.763229	-0.682933	-0.733296
1	59.44108*	0.000464*	-2.000715*	-1.759827*	-1.910915*
2	3.811524	0.000504	-1.918226	-1.516745	-1.768558
3	2.279414	0.000569	-1.800433	-1.238360	-1.590898
4	7.039860	0.000563	-1.818206	-1.095541	-1.548804
5	4.482782	0.000594	-1.772275	-0.889018	-1.443006

Table 5 shows that the optimal lag length between the farm and wholesale is one. In the same way the VAR Lag Order Selection Criteria results for wholesale and retail prices are shown in Table 6.

TABLE 6
VECTOR AUTO REGRESSIVE LAG ORDER SELECTION CRITERIA (lnWP and lnRP)

Lag	LR	FPE	AIC	SC	HQ
0	NA	3.93e-05	-4.467427	-4.387131	-4.437494
1	26.85320*	2.48e-05*	-4.929011*	-4.688123*	-4.839211*
2	7.009408	2.49e-05	-4.926469	-4.524988	-4.776801
3	2.730931	2.78e-05	-4.820558	-4.258485	-4.611023
4	3.816023	3.00e-05	-4.748781	-4.026116	-4.479378
5	7.354435	2.92e-05	-4.787310	-3.904052	-4.458040

Table 6 shows that the optimal lag length between the wholesale and retail is one. lnWP and lnRP were the endogenous variables.

10.4 Granger Causality Tests

The results for the pair wise Granger causality test on the series lnFP, lnWP and lnRP are presented in Table 7.

TABLE 7
RESULTS OF PAIR WISE GRANGER CAUSALITY TESTS FOR lnFP, lnWP, and lnRP

Null Hypothesis	Lags	Obs.	F-stat.	Prob.	Decision
lnWP does not Granger Cause lnFP	1	49	9.1E-06	0.9976	Do not reject
lnFP does not Granger Cause lnWP			8.19633	0.0063	Reject
lnWP does not Granger Cause lnRP	1	49	0.77784	0.3824	Do not reject
lnRP does not Granger Cause lnWP			0.28772	0.5943	Do not reject
lnFP does not Granger Cause lnRP	5	45	3.93156	0.0064	Reject
lnRP does not Granger Cause lnFP			1.12593	0.3654	Do not reject

10.5 Price Causality between Farm and Wholesale

Table 7 shows that the p-value (0.0063) is significant at 1% level. As a result the hypothesis that lnFP does not cause lnWP can be rejected. In support of this assertion, the p-value (0.9976) on the other hand is insignificant which means we cannot reject the hypothesis that lnWP does not cause lnFP. With such statistical evidence, it can be concluded that there exists a unidirectional causality from the farm level to the wholesale level. This implies that farm prices cause wholesale prices. A high industry concentration that is present at the farm level can be the reason for the direction of price flow from the farm gate (who has market power) to the wholesale (who use the commission system based on the prices set by farmers).

10.6 Price Causality between Wholesale and Retail

Table 7 also shows that both p- values (0.3824 and 0.5943) are insignificant at 5% level, as such we do not reject the null hypotheses of lnWP not Granger causing lnRP and lnRP not Granger causing lnWP. However, the F-statistic probabilities indicate a slight possibility of the hypothesis of lnWP not causing lnRP more likely to be rejected at a higher significance level. In this sense, it is more likely that wholesale prices may perhaps cause the retail prices than the opposite even though the chances are trivial. This weak causal relationship is represented by the dotted line in Figure 1. The empirical evidence available at 5% level even so, still suggests that there is no extrapolative power between wholesale prices and retail prices. It therefore follows that an independent causal relationship exists between the wholesale and the retail levels. Possible explanations for such a causal relationship is the growing outsourcing behaviour of, and direct purchasing from the farms by some tomato retailers in the Limpopo Province. For instance during the exploratory phase of this study, it was established that some retailers consider the National Fresh Produce Markets as a competitor than a supplier.

10.7 Price Causality between Farm and Retail

The p-value (0.0064) is significant at 1% level while p-value (0.3654) is insignificant at 1%, 5% and 10% levels. The hypothesis that lnFP does not cause lnRP can therefore be rejected while the one for lnRP not causing lnFP cannot. It can be concluded that prices stream unidirectional from the farm level to the retail level. In other words, farm gate prices have a predictive power on retail prices. The market structure of the tomato industry in Limpopo Province suggests a high concentration at the farm level than at the retail. Such can be the reason for the direction of price flow from the farm (who has more market power) to the retail level.

The causality relationships that exist amongst the three series are demonstrated in Figure 1. Arrows represent the direction of price flow, where solid and dotted lines symbolize a strong and a weak causal relationship respectively.

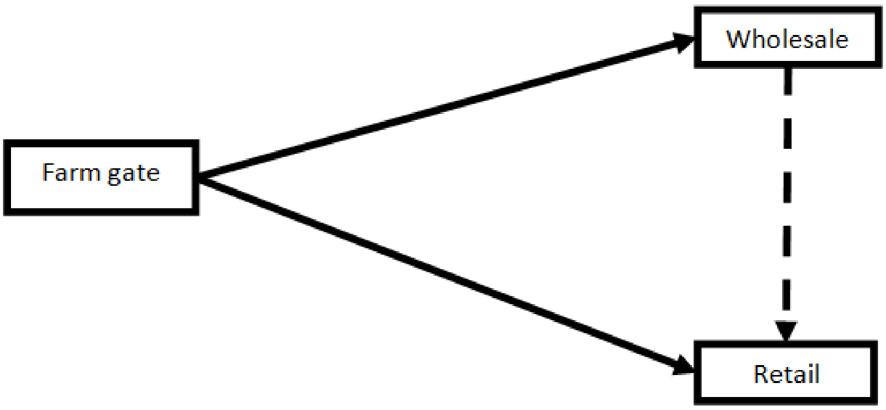

FIGURE 1: POINTS OF PRICE DETERMINATION AND DIRECTION OF PRICE CAUSALITY

As shown in Figure 1, the farm gate plays a major role in the price formation process of tomato markets in Limpopo Province. Therefore, the farm gate's current and past price information is useful in improving the forecasts of both the wholesale and retail prices it causes. Figure 1 also indicates that there is a weak causal relationship between the wholesale and retail level prices. The weak dependence of Limpopo retail prices on wholesale prices can be attributed to the proximity of retailers to tomato farms than they are to the National Fresh Produce Markets. As a result it is sensible that Johannesburg wholesale prices may be seldom useful in predicting tomato retail prices in Polokwane considering the increasing direct sourcing by Limpopo retailers from farmers.

XI. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The study attempted to fill the knowledge gap on the performance of Limpopo Province's tomato markets by examining prices at successive marketing levels. The gross marketing margins constitute about 85.1% of the consumer's South African Rand. There exists a large gap between what consumers pay for each unit of tomatoes purchased from retailers, and the amount farmers receive for the same quantity from retailers in Limpopo Province. It therefore follows that the producers' portion of the consumers' purchasing power is low since according to the findings, a major part of tomato retail prices is taken up by the marketing functions.

The VAR lag order selection criteria showed that the optimal lag lengths between the levels; farm gate and wholesale, farm gate and retail, wholesale and retail are 1, 5 and 1 respectively. Since the reaction to a price change at the point of determination does not reflect instantaneously at the other levels in a marketing chain, these optimal lag lengths indicate that wholesale prices are faster in adjusting to farm gate price changes than do retail prices.

The results of the pair-wise Granger Causality tests suggested a unidirectional causality from farm to wholesale and also from farm to retail. However, a weak causal relationship was found between wholesale and retail levels. It therefore follows that tomato prices are determined at the farm gate in Limpopo Province. This finding may be consistent with the expectation that whenever an industry is highly concentrated, the most dominant participant may also act as the price leader as is the case in the tomato producing industry of Limpopo Province, South Africa.

An important conclusion drawn from the causal relationships between the farm gate, wholesale and retail prices is that the farm level is key to tomato price determination in Limpopo Province. It therefore means that current and past information on farm gate prices is useful in improving estimations of both wholesale and retail prices it causes. According to Guvheya et al. (1998), information on causality shows the direction of price flow between levels and thus helps in the identification of points of price determination along the marketing chain. In the light of this assertion, it can be concluded that tomato prices in Limpopo Province are determined at the farm level. With the farm level being key to tomato price determination in Limpopo Province of South Africa, pertinent policies that improve the commodity's primary sector may by the ripple effect, potentially benefit the other sectors in the value chain who are also dependent on tomato production.

This this study has shown the importance of the farm level at deciding the market price for food. Governments in the developing economies such as South Africa are encouraged to intensify small scale farmers support programs so as to reduce industry concentrations as well as encourage fairer competition in the food producing sectors of their respective economies. This will ensure that agricultural produce prices will not be determined by a few major producers. A scenario where only one producer dominates a market potentially leads to situation where all price movements are influenced by this single player while the other farmers are simply price takers regardless of their cost structures.

XII. AREAS FOR FURTHER STUDY

The study established that the gross marketing margins of tomato in Limpopo Province are high. However the source of such huge marketing margins has not been ascertained between marketing costs and retailers' profits. This study only managed to alert on the existence of high marketing margins that constitute the major portion of the consumer's Rand. It is therefore recommended that further research be undertaken to establish whether the retailers' profits are unfairly high or peradventure whether the marketing margins are as a result of high marketing costs. Such a complementary study will assist in identifying the actual basis of the high marketing margins so that corrective policy measures can be directed appropriately and more specifically.

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Genome–Wide Analysis and Expression Pattern of the AP2/ERF Gene Family in Kiwifruit under Waterlogging Stress Treatment

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***Abstract*—*APETALA2/ethylene response factor (AP2/ERF) transcription factors play important roles in the response to abiotic stresses. It is now possible to identify all of the AP2/ERF genes in the kiwifruit genome because the kiwifruit genome project has been completed. 183 AP2/ERF genes were identified and compared with AP2/ERF genes from Arabidopsis in this study. The 183 AP2/ERF kiwifruit genes were classified into four subfamilies: DREB (64), ERF (94), AP2 (19) and RAV (5), as well as one soloist. RNA-sequence and Quantitative RT-PCR (qRT-PCR) analysis results showed that 20 genes were responsive to waterlogging stress, suggesting that AP2/ERF transcription factors play important roles in the response to waterlogging stress in kiwifruit*

Keywords*—*AP2/ERF, kiwifruit, waterlogging stress.

I. INTRODUCTION

The APETALA2/ethylene response factor (AP2/ERF) superfamily is defined by the AP2/ERF domain which consists of about 60 to 70 amino acids, and can be classified into at least five subfamilies: AP2 (containing two repeated AP2/ERF domains), DREB (dehydration responsive element binding, containing a single AP2/ERF domain), ERF (containing a single AP2/ERF domain), RAV (containing a B3domain and a single AP2/ERF domain), and others(Zhang et al. 2012b). The ERF and DREB subfamilies can be further divided into six subfamilies: the B1–6 subfamilies and A1–6 subfamilies, respectively(Zhang et al. 2012b). It has been demonstrated that AP2/ERF play important roles in the plant cell cycle, growth and development, as well as the response to biotic and abiotic stresses (Zhang et al. 2012b).

Waterlogging is one of the most common stresses affecting plant growth and development. Many important crop plants are sensitive to waterlogging conditions caused by heavy rain. Waterlogging and submergence conditions impose a variety of challenges on the plants(Hinz et al. 2010). Previous research has shown that the AP2/ERF genes play an important role in the regulation of gene expression during waterlogging stress. RAP2.2 is induced in shoots by ethylene and functions in an ethylene-controlled signal transduction pathway and the overexpression of RAP2.2 resulted in improved plant survival under hypoxia (low-oxygen) stress, whereas lines containing T-DNA knockouts of the gene had poorer survival rates than the wild type(Hinz et al. 2010). The RAP2.2 gene plays a significant role in the metabolic adaptation to flooding stress in Arabidopsis(Hinz et al. 2010). The SUB1A-1 allele could reduce elongation growth and carbohydrate consumption, and to confer submergence tolerance (Fukao 2006; Xu et al. 2006; Jung et al. 2010). Flooding sensitive japonica cultivar over-expressing SUB1A -1 could increase ADH1 expression and flooding tolerance(Fukao 2006; Xu et al. 2006). Four ERF subfamily genes in Arabidopsis, namely RAP2.2 (At3g14230), RAP2.12 (At1g53910), HRE1 (At1g72360), and HRE2 (At2g47520) have been documented that play important role in the response to hypoxia (Hinz et al. 2010; Licausi et al. 2010). HRE1 over-expressing plants showed an increased activity in the fermentative enzymes pyruvate decarboxylase and alcohol dehydrogenase together with increased ethanol production under hypoxia, showed an improved tolerance of anoxia (Licausi et al. 2010). RAP2.2 was induced in shoots by ethylene and functions in an ethylene-controlled signal transduction pathway(Hinz et al. 2010). Overexpression of RAP2.2 resulted in improved plant survival under hypoxia stress, whereas lines containing T-DNA knockouts of the gene had poorer survival rates than the wild type(Hinz et al. 2010). Deepwater rice requires SNORKEL1 (SK1) and SK2 ERF transcription factors to elongate stem internodes and extend the hollow stems to the water surface for survival (Hattori et al. 2009). Du et al. reported (Du et al. 2014) that 38 of 184 AP2/ERF transcript factor genes were responsive to waterlogging stress and 25 genes were ERF subfamily.

Kiwifruit is a major fruit worldwide. However, the majority of currently growing kiwifruit cultivars, like ‘Hongyang’, are susceptible to waterlogging stress in East China. A thorough knowledge of kiwifruit resistance mechanisms will help to limit crop loss due to waterlogging stress, and to decrease the economic losses. It’s known to us that AP2/ERF transcript factor play important roles in the response to waterlogging stresses. It is possible to identify the AP2/ERF genes in many species as these plant genome projects have been completed. Previous studies showed that there are 147, 184, 132, 200, and 116 AP2/ERF genes in the Arabidopsis (Nakano 2006) , maize(Du et al. 2014), grapevine(Zhuang et al. 2009), poplar(Zhuang et al. 2008), and Chinese plum (Du et al. 2013), respectively. However, few reports of the AP2/ERF superfamily are available in kiwifruit. Kiwifruit genome projects have been completed(Huang and al. 2013). In this article, AP2-like genes from kiwifruit were surveyed and comparatively analyzed. Here, 183 AP2/ERF transcription factors were identified from the kiwifruit genome database and the transcriptome sequencing database (Zhang et al., 2015). The expressions of kiwifruit AP2/ERF genes under waterlogged stress were performed. These analyses will be valuable to isolate and understand the molecular mechanism of AP2/ERF genes responded to waterlogging stress in kiwifruit.

II. MATERIAL AND METHOD

2.1 Plant Material and Treatment

The kiwifruit cultivars 'Jinkui' were obtained from the Institute of Botany, Jiangsu Province & Chinese Academy of Sciences, China. Plants were grown in pots containing a 2:1 mixture of garden soil and vermiculite without any added fertilizer, and were maintained in a plant growth chamber. The growth chamber conditions were: relative humidity of ~ 60%, 160μmol m^{-2} s^{-1} PAR, photoperiod of 12 h light/12 h dark for 24 h, and 25°C average temperature. Seedlings, already grown to the 8–10 node stage were selected for uniformity. The waterlogging treatment was performed as described previously (Yin et al. 2009a). The pots were flooded by standing in a 28 cm×14 cm×14 cm container filled with tap water to 2.5 cm above the level of the soil surface. The tap water had a pH of 7.3. The water temperature was held at ~25°C. Roots were sampled at 0, 24, 48, and 96 h after treatment, frozen in liquid nitrogen and stored at -80°C, which were later used for quantitative real time-PCR (qRT–PCR) for studying the expression profile of AP2 TF genes. Each treatment was repeated three times and there were 10 plants per treatment in every biological replication.

2.2 Database

Kiwifruit Genome Database (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi) and transcriptome sequencing database (NCBI, Accession number: SRR2048539) were mined to identify members of the AP2/ERF. The amino acid sequence of the AP2/ERF domain (IYRGVRQRNSGKWVSEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNF) from Arabidopsis AtCBF1 (also named AtDREB1B; accession number AT4G25490) was used as queries to search against the databases from Kiwifruit Genome Database Web site (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi) using the BLASTP program at the e-value of 1e-3 to avoid false positives.

2.3 Phylogenetic Tree Construction

Phylogenetic trees of the aligned kiwifruit AP2/ERF protein sequences were constructed using MEGA version 5.0 (Tamura et al. 2011)(http://www.megasoftware.net) via the neighborjoining (NJ) method with the following parameters: Poisson correction, pairwise deletion, and bootstrap (1,000 replicates; randomseed).

2.4 Differentially expressed analysis of AP2/ERF family genes under waterlogging stress

We have previously conducted the transcriptome sequencing of kiwifruit under waterlogging. The number of reads per kilobase per exon region in a given gene per million mapped fragments (RPKM) was performed to identify AP2/ERF family genes are regulated by waterlogging stress(Chen et al. 2014). Differentially expressed AP2/ERF family genes of 0h and 96h were identified by the R program(Chen et al. 2014). We applied the Pearson's chi-squared test to assess the lane effect. The p-value was computed for each gene. The Benjamini–Hochberg false discovery rate (FDR) was then applied to correct the results for the q-value. The FDR method is generally used in deep-sequencing studies to identify over-representative AP2/ERF family genes (Junttila et al. 2013). If the FDR-adjusted q-value was ≤ 0.05, the AP2/ERF family genes were considered to be differentially expressed. Heatmap were performed using the software of MEV (Multi Experiment Viewer). Color scale represents reads per kilobase per million normalized log2 transformed counts where blue indicates low level and red indicate high level.

2.5 Quantitative RT-PCR (qRT-PCR) Analysis

Quantitative RT-PCR (qRT-PCR) was used to determine the expression of AP2/ERF family genes in kiwifruit under waterlogging. Total RNA isolation, DNase I treatment, First strand cDNA synthesise, and qRT-PCR assay were performed as described by Zhang et al (Zhang et al. 2012a). The relative levels of genes to control *AdActin* (Yin et al. 2009b; Yin et al. 2012) mRNAs were analysed using the ABI-7300 system software and the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Gene-specific primers used in the qRT-PCR were listed in Table 1. Data analyses were conducted using SPSS version 17.0 statistical software. For all analyses, the level of significance between different time points was set at P<0.05. Heatmap were performed using the software of MEV (Multi Experiment Viewer). Color scale represents $\log2^{-\Delta\Delta Ct}$ counts where blue indicates low level and red indicate high level.

TABLE 1
PRIMERS FOR THIS PAPER

Gene Name	Forward primer	Reverse primer
RAV1	GCTTTTCCCGTTCAGGTCCAG	ACACCCAAATCCCAACATCTCC
RAV2	TCGGCGGGAAGAAACAATGC	GGTATCACCAGCCTGTTCAGC
RAV3	TTTGAAGGCGGGCGATGTC	ACTACTATCAACACCACCACTCAC
ERF1	CACCCCAACTTTGCCCCTAG	CCTCCTCTTCCGTGCTGAAC
ERF2	CTGGCTCGGAACATTTGATTCTG	CTCCTCACACCCATACTTCATCTC
ERF3	TGAAGGTGCCGAGCCAAAC	GCAGCGGAAGAATCAGTACAGG
ERF4	CGTTACAGAGGCGTGAGGAAG	TGGCGGTGGCAATGAGTTC
ERF5	GCCAGAGCAGCACCATCG	CGGAGGAAGAATCGGAGTCG
ERF6	GGAAAGTATGCGGCGGAAATC	GTACGCTCTCGCTGCTTCG
ERF7	GGGGTGACTTGCCGTTAAATTAC	GAGCCGTTGTCGTGGATGG
ERF8	AGGAAGCAGCAGGGGAAGAG	CAGCACCACAGCCGATGAC
ERF9	CGACCTCATCCGCCAACAC	GCAAGAACCGATTGATTCAGAGC
ERF10	CTACGATAGAGCCGCCTTCAAG	TCACCTAACCACACCTTCTTCAC
ERF11	CGTTTGATACGGCGGAGGAG	TCGGGCTCTGATTACAATGACTC
ERF12	TGGGAGATGGGTGGCTGAG	ATGAAATTCGTGCGAGTGTTGG
ERF13	GTTCTCGGCTTCTCTCTACGC	ATTCTCCTGTTTGTCTCCCTTCG
DREB1	AAGTGGGTTTGCGAGGTAAGAG	TCATTGGAATCCGTGGAAGCC
DREB2	GCTCCAACTCACTCTCGTCAAC	TCTGGGCTCTGGGTCTTGC
DREB3	CTGGCAAAATGGGGCAATCTG	TCAAGAAATCAAGACCGCAATCG
DREB4	ACGGAGGAAGGCGATAGAGG	ATGGCTTGAACCCAGAAGAAGG
AdActin	TGCATGAGCGATCAAGTTTCAAG	TGTCCCATGTCTGGTTGATGACT

III. RESULTS AND DISCUSSION

3.1 Identification and Prediction of Kiwifruit AP2/ERF Transcription Factors

A total 180 kiwifruit AP2/ERF genes were downloaded from Kiwifruit Genome Database, and 91 kiwifruit AP2/ERF genes were obtained from the results of transcriptome sequencing. However, 88 of the 91 AP2/ERF genes were the same with those from kiwifruit genome database. So, a total of 183 kiwifruit AP2/ERF genes were obtained.

183 kiwifruit AP2/ERF genes were classified into the DREB, ERF, AP2 and RAV subfamilies, and one soloist based on alignment of the AP2/ERF domain from kiwifruit and *Arabidopsis* (Table 2). We compared the AP2/ERF genes from kiwifruit, grapevine, *Arabidopsis*, rice and maize (Table 2). The number of AP2/ERF genes of kiwifruit, grapevine, *Arabidopsis*, maize and rice were 183, 132, 147, 184, and 164, respectively. In kiwifruit, 64 AP2/ERF genes were classified into the DREB subfamily, compared with 36, 57, 51 and 52 in grapevine, *Arabidopsis*, rice and maize, respectively; 94 kiwifruit AP2/ERF genes were classified into the ERF subfamily, compared with 73, 65, 107 and 79 in grapevine, *Arabidopsis*, rice and maize respectively. 19 kiwifruit AP2/ERF genes were predicted to encode proteins with two AP2/ERF domains, and were classified into the AP2 subfamily. In comparison, grapevine, Arabidopsis, rice and maize contain 18, 18, 22 and 26, respectively. Five kiwifruit AP2/ERF genes were predicted to encode proteins with an AP2/ERF domain and a B3 domain, and were classified into the RAV subfamily, compared to 4, 6, 3 and 7 in grapevine, *Arabidopsis*, rice and maize,

respectively. The remaining kiwifruit AP2/ERF gene (Achn205451) has a low homology with other AP2/ERF genes. Therefore, this gene was designated as a soloist.

TABLE 2
SUMMARY OF THE AP2/ERF FAMILY AMONG KIWIFRUIT, GRAPEVINE, ARABIDOPSIS, RICE AND MAIZE

AP2/ERF family	Group	Eudicot			Monocot	
Classification		Kiwifruit	Grapevine	Arabidopsis	maize	Rice
DREB subfamily	A1	10	7	6	10	10
	A2	9	4	8	5	4
	A3	0	0	1	1	1
	A4	27	13	16	11	15
	A5	14	7	16	12	13
	A6	4	5	10	12	9
	Total	64	36	57	51	52
ERF subfamily	B1	19	7	15	31	16
	B2	7	3	5	20	16
	B3	27	37	18	18	18
	B4	4	4	7	10	9
	B5	12	4	8	6	6
	B6	25	18	12	22	14
	Total	94	73	65	107	79
AP2 subfamily		19	18	18	22	26
RAV subfamily		5	4	6	3	7
Solosist		1	1	1	1	0
Total AP2/ERF genes		183	132	147	184	164
Total putative genes		39040	30434	26819	39656	38000
The percentage of AP2/ERF family genes (%)		0.46875	0.4337	0.5481	0.4640	0.4315
Genome size (Mb)		616	487	125	2500	430
The average number of AP2/ERF family genes per Mb		0.297	0.271	1.176	0.0736	0.3814

At the whole-genome level, the average number of AP2/ERF genes per Mb in kiwifruit is 0.297, which is more than in grapevine (0.271) and maize (0.0736), but less than in rice (0.3814) and Arabidopsis (1.176). The percentage of the AP2/ERF gene family in kiwifruit is 0.46875 %, which is more than in grapevine (0.4337 %), rice (0.4315 %) and maize (0.4640%), but less than in Arabidopsis (0.581 %, Table 2).

3.2 Phylogenetic Relationships Analysis

Phylogenetic trees of the DREB (Fig. 1) and ERF (Fig. 2) subfamilies in kiwifruit were constructed. A total of 64 DREB subfamily genes distributed into the A1–A2 and A4-A6 groups in kiwifruit; A1, A2, A4, A5 and A6 contain 10, 9, 27, 14 and 4 genes, respectively (Table 1). 94 genes belonging to the ERF subfamily in kiwifruit distributed into the B1–B6 groups; B1, B2, B3, B4, B5 and B6 contain 19, 7, 27, 4, 12, 25 genes (Table 1), respectively. Lastly, 19 genes were classified into the AP2 subfamily (Fig. 3) and five genes into the RAV subfamily (Fig. 4).

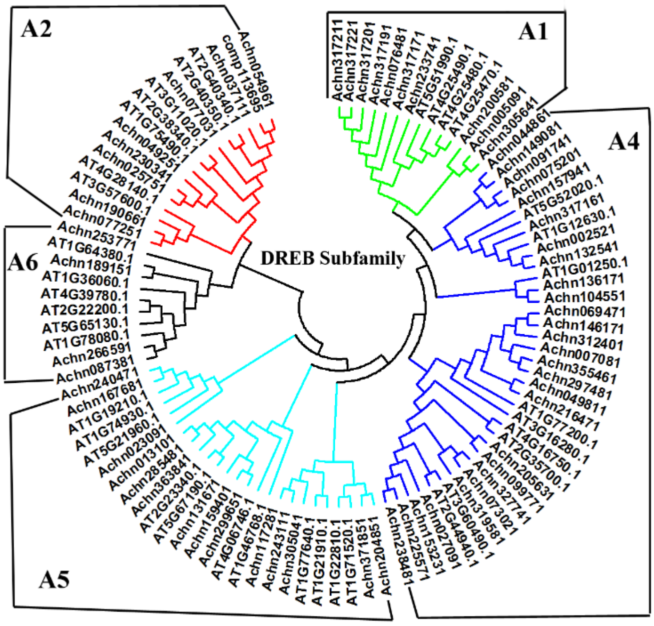

FIG. 1. THE PHYLOGENETIC TREE OF DREB SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS.

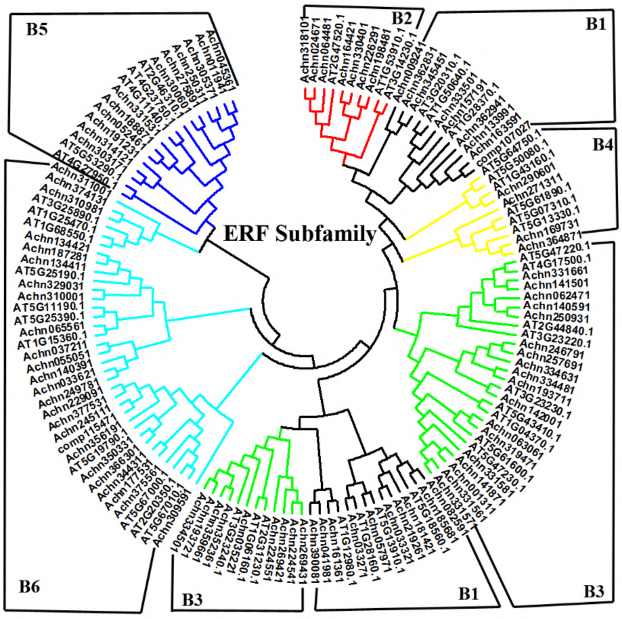

FIG. 2. THE PHYLOGENETIC TREE OF ERF SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

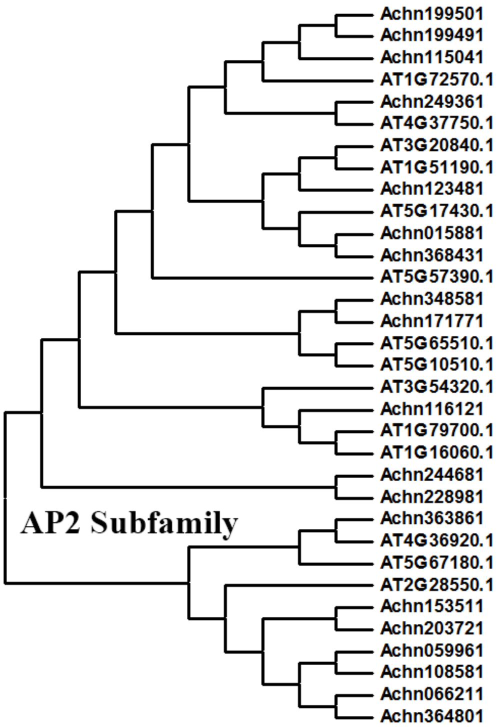

FIG. 3. THE PHYLOGENETIC TREE OF AP2 SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

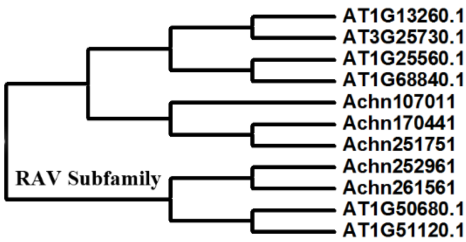

FIG. 4. THE PHYLOGENETIC TREE OF RAV SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

3.3 Expression Analysis of AP2/ERF Family Genes under Waterlogging Stress

RNA-sequencing was conducted previously (Data not show) and the results showed that the expression levels of 20 AP2/ERF genes were changed (Table 3, Fig. 5A). RAV subfamily were 3, named RAV1, RAV2 and RAV3. 13 AP2/ERF genes (named ERF1 to ERF13) in ERF subfamily were responsive to waterlogging stress. B1, B2, B3, and B6 subgroups were 4, 2, 4, and 3, respectively. DREB subfamily was 4 (named DREB1 to DREB4). A1, A2, A3, and A4 subgroups had 1, respective (Table 3, Fig. 5A). The expression levels of 20 AP2/ERF waterlogging stress responsive genes are diverse. There are 9 genes and their expression levels are decreased under 96 h waterlogging condition. There are 11 genes and their expression levels are increased under 96 h waterlogging treatment.

TABLE 3
EXPRESSION OF AP2/ERF TRANSCRIPT FACTOR GENES IN KIWIFRUIT UNDER WATER LOGGING TREATMENT

Name id for transcription	Name id for genome*	Sample CK	Sample T4	Fold_change (log2(T4/ck))	p_value	q_value	Identity (%)	Category	Name id
comp100639_c0_seq2	achn251751	11.16	73.01	2.710	7.70E-11	5.47E-09	98	RAV	RAV1
comp100786_c0_seq3	achn170441	17.79	40.54	1.188	6.10E-03	9.18E-02	100	RAV	RAV2
comp104656_c0_seq4	achn107011	26.91	2.99	-3.170	1.98E-05	6.22E-04	99	RAV	RAV3
comp81900_c0_seq1	achn219261	5.87	10.68	0.863	3.79E-01	1.00E+00	99	ERF-B1	ERF1
comp1127686_c0_seq1	achn352361	0.76	0.26	-1.547	1.00E+00	1.00E+00	96	ERF-B3	ERF2
comp249351_c0_seq1	achn064481	1.7	33.2	4.288	3.74E-07	1.64E-05	100	ERF-B2	ERF3
comp107027_c0_seq1	/	345.07	80.48	-2.100	5.41E-39	1.70E-36	/	ERF-B1	ERF4
comp110156_c0_seq1	achn133991	249.75	102.14	-1.290	6.77E-16	7.40E-14	99	ERF-B1	ERF5
comp79023_c0_seq1	achn140391	15.57	65	2.062	1.35E-07	6.38E-06	96	ERF-B6	ERF6
comp102502_c0_seq1	achn140591	14.61	34.05	1.221	1.09E-02	1.47E-01	96	ERF-B3	ERF7
comp67160_c0_seq1	achn318101	2.62	762.96	8.186	3.01E-161	4.26E-158	99	ERF-B2	ERF8
comp110147_c0_seq3	achn319471	130.03	18.1	-2.845	1.65E-20	2.45E-18	97	ERF-B3	ERF9
comp104372_c0_seq3	achn331571	14.07	2.09	-2.751	5.32E-03	8.21E-02	96	ERF-B3	ERF10
comp87832_c0_seq1	achn362941	29.67	206.84	2.801	2.50E-29	5.65E-27	96	ERF-B1	ERF11
comp117167_c1_seq1	achn366301	31.84	1.08	-4.882	1.39E-07	6.55E-06	97	ERF-B6	ERF12
comp115471_c0_seq1	/	13.14	0.32	-5.360	1.07E-03	2.15E-02	/	ERF-B6	ERF13
comp48535_c0_seq1	achn317191	3.4	12.49	1.877	4.83E-02	4.61E-01	90	DREB-A1	DREB1
comp95477_c0_seq1	achn007081	8.38	1.03	-3.024	3.46E-02	3.60E-01	99	DREB-A4	DREB2
comp113695_c0_seq3	/	13.35	111.17	3.058	1.56E-17	1.88E-15	/	DREB-A2	DREB3
comp95463_c0_seq2	achn363841	4.52	18.98	2.070	6.73E-03	9.97E-02	92	DREB-A5	DREB4

http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi

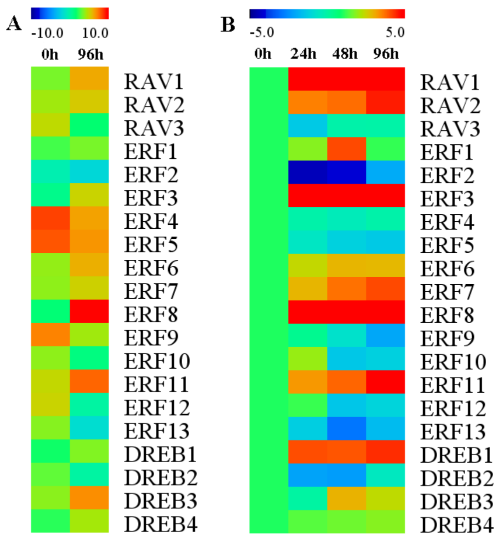

FIG. 5. Heatmap show expression level of 20 AP2/ERF genes in the root of kiwifruit after waterlogging stress. A: The expression was measured by RNA-seq analysis under 0 and 96 h waterlogging stress, respectively. Color scale represents reads per kilobase per million normalized log2 transformed counts where blue indicates low level and red indicate high level. B: The expression was measured by qRT-PCR. Color scale represents log2$^{-\Delta\Delta Ct}$ counts where blue indicates low level and red indicate high level.

At the same time, qRT-PCR analyses of 20 AP2/ERF genes were performed in kiwifruit roots treated by waterlogging at 0h, 24h, 48h and 96h. The results indicated that all of 20 AP2/ERF genes exhibited similar expression kinetics to those obtained from the RNA sequencing analysis (Fig. 5B) at 96 h after waterlogging treatment. During the first 96 h after waterlogging treatment, the expression levels of RAV2, ERF7, ERF8, ERF11, DREB1 and DREB2 were strongly induced (Fig. 5B). Expression levels of RAV3, ERF2, ERF4, ERF5, ERF13, and DREB2 were decreased during the first 96 h after waterlogging treatment. ERF9, ERF10 and ERF12 genes expression were induced at 24h after treatment and then decreased, and the expression levels of those genes were lower than those base levels between 48 h and 96 h after waterlogging treatment (Fig. 5B). Expression of RAV1, ERF1, ERF3, ERF6, and DREB3 genes rapid increased, peaking 48h after treatment with waterlogging in roots of kiwifruit, and then decreased, and the expression levels of those genes were higher than those base levels (Fig. 5B).

IV. DISCUSSION

AP2/ERF transcript factor is a superfamily (Zhuang et al. 2008; Zhuang et al. 2009), and participate in plant developmental processes (Nilsson et al. 2006; El Ouakfaoui et al. 2010; Wang et al. 2008) as well as biotic and abiotic stress signaling (Krishnaswamy et al. 2010; Hong et al. 2009; Abogadallah et al. 2011; Zhang et al. 2009; Licausi et al. 2010; Hinz et al. 2010). The growth and productivity of kiwifruit are greatly affected by abiotic stresses, especially flooding. Previous studied showed that the AP2/ERF transcript factor involved in waterlogging stress response (Du et al. 2014; Hinz et al. 2010; Licausi et al. 2010). Du *et al.* reported (Du et al. 2014) that 38 of 184 AP2/ERF transcript factor genes were responsive to waterlogging stress and 25 genes were ERF subfamily. In present study, 183 kiwifruit AP2/ERF genes were identified, classified. The results of qRT-PCR analysis have confirmed that 20 of 183 genes were responsive to waterlogging stress and 13 genes were ERF subfamily. 25 maize waterlogging responsive ERF subfamily genes belonged to B1, B2, B3, and B6 subgroups(Du et al. 2014). 13 kiwifruit waterlogging responsive ERF subfamily genes were also belonged to B1, B2, B3, and B6 subgroups. These results showed that those of AP2/ERF family genes might play a key role in waterlogging stress.

Previous studies showed that genes in the ERF-B2 subfamily play a key role in waterlogging tolerance in Arabidopsis and rice. There are five ERF-B2 subfamily genes in Arabidopsis, namely RAP2.2 (At3g14230), RAP2.12 (At1g53910), RAP2.3 (At3g16770), HRE1 (At1g72360), and HRE2 (At2g47520). RAP2.2, RAP2.12, HRE1, and HRE2 genes have been reported that play important role in the response to hypoxia (Licausi et al. 2010; Hinz et al. 2010). SubA1, which was a water tolerance gene, restricts rice elongation at the rice seedling stage during flash floods(Fukao 2006; Xu et al. 2006). Du *et al.* reported (Du et al. 2014) that There are 20 ERF-B2 subfamily genes in maize genome, and 9 of 20 are response to waterlogging stress. In our study, there are 7 ERF-B2 subfamily genes in kiwifruit genome (Table 2), and 2 of 7 are responsive to waterlogging stress (Fig. 5). Further studies about ERF subfamily responsive genes are ongoing, and will be reported in future.

V. CONCLUSION

In our study, 183 AP2/ERF genes were identified and compared with AP2/ERF genes from *Arabidopsis*. The 183 AP2/ERF kiwifruit genes were classified into five subfamilies: DREB (64), ERF (94), AP2 (19) and RAV (5), as well as one soloist. RNA-sequence and Quantitative RT-PCR (qRT-PCR) analysis results showed that 20 genes were responsive to waterlogging stress, suggesting that AP2/ERF transcription factor play important roles in the response to waterlogging stress in kiwifruit.

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New banana genotypes and cultivars more productive for southern Minas, Brazil

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Abstract— *Southern of Minas state, is a important producer of banana, especially the cultivars Prata-Anã, Nanicão and Maçã. These cultivars present low productivity, great plant height and are susceptible to major banana diseases. The objective of this study was to evaluate the vegetative and productive behavior of banana cultivars as Prata, Nanicão and Maçã, in Lavras, MG, Brazil to select those with the best features, of bunch and fruit size, lower production cycle and disease resistance in high land conditions. Were evaluated the following materials: type: 'Prata': 'Prata-Anã' (control), 'BRS Maravilha', 'BRS Vitoria', PA 94-01; type 'Nanicão': 'Grande Naine' (control) and FHIA 17 and type 'Maçã': 'Maçã' (control) and YB 42-03. The experiment was conducted in a completely randomized block design with three replications and 16 plants per plot. Regarding the type 'Prata-Anã', 'BRS Maravilha' and PA 94-01 are recommended by their greater productivity, plant height, production cycle, flavor and fruit appearance in relation to cv 'Prata-Anã' traditionally grown in region. PV 94-01 and 'Vitoria', despite the greats plant height, are recommended due to the greater productivity. The YB 42-03 genotype is an alternative to 'Maçã' because it is similar to productivity, size and production cycle.*

Keywords— Musa, Banana, Production cycle, Yield.

I. INTRODUCTION

There are more than 125 countries that are dedicated to the cultivation of bananas in the world. This fruit stands out in the first position of the world ranking around 106.5 million tons in 2011, according to Vieira (2015).

The banana is the second most consumed fruit in the world with 11.4 kg/hab/year, losing only to the orange with 12.2 kg/hab/year (FAO, 2014). The expansion of banana farming in most countries from 35 million tonnes in 1978 to 106.5 million tonnes in 2011 was made possible thanks to the intensive use of technologies (VIEIRA, 2015).

The productivity of banana growing in Minas Gerais increased from 14.103 t ha-1 in 2001 to 16.936 t ha-1 in 2012 (IBGE, 2013). This increase in productivity is mainly due to the adoption of new technologies such as fertirrigation, adequate crop management, and the introduction of new, more productive cultivars.

Several banana cultivars have been evaluated and recommended in all regions of Brazil. Among these, there are those of the type 'Prata': 'Pioneira', 'Prata Graúda', 'BRS Pacovan Ken', 'BRS Maravilha', 'BRS Platina', 'BRS Conquista', 'BRS Vitória'; tipo 'Maçã': 'Thap Maeo', 'Mysore' e 'Caipira'; type 'Ouro': 'Prata Baby' or 'Nam' and type 'Nanicão': 'Grande Naine' (SILVA; PEREIRA; RODRIGUES, 2008).

However, obtaining new, more productive banana cultivars is not enough to determine success in terms of adoption by producers. Besides the agronomic aspects, the new cultivars must present fruits with good market characteristics (Silva, et al., 2013). An improved cultivar should increase productivity, reduce production costs due to the decrease in the use of pesticides, thus increasing the income of the producer (Amorim et al., 2011).

The cultivar BRS Maravilha in dry conditions was more productive than Prata-Anã, its mother in the first two cycles in Jataí (GO) (SANTOS; CARNEIRO, 2012), in Goiânia (GO) (MENDONÇA et al., 2013), in Aquidauana (GO) (VIEIRA, 2011), in Botucatu (SP) (RAMOS et al., 2009) and in Lavras (MG) (PEREIRA et al., 2003).

Borges et al. (2011) report that the genotype FHIA 17 was highlighted in rainy conditions in production among the 14 genotypes evaluated in northern Paraná and that the cultivars BRS Maravilha, PV 94-01 and YB 42-03 presented similar behavior to the cultivars of the Same type Prata-Anã, Pacovan and Maçã, respectively.

According to the Capixaba Institute for Research, Technical Assistance and Rural Extension - INCAPER (2010); SILVA et al. (2008) and PEREIRA et al. (2005) the BRS Vitória cultivar was more productive than Prata and Pacovan in the states of Espírito Santo, Bahia and Amazonas, respectively. In irrigated conditions the 'BRS Maravilha' presented superior yield twice as much as the cultivar Prata-Anã in the Jaíba projects (MG) (RODRIGUES et al., 2006) and Gorutuba (MG) (SOUTO et al., 2001).

The influence of the environment, mainly temperature, on the banana production cycle, can be observed in experiments conducted in Lavras (MG), Maria da Fé (MG) and Jaíba (MG). In Lavras, with average annual minimum temperatures of 15.4ºC and altitude of 900 m, the 'Prata-Anã' cycle was 510 days (PEREIRA et al., 2002). In Maria da Fé, with an average annual minimum temperature of 10.7ºC and altitude of 1270 m, the production cycle of Prata-Anã was 620 days (PEREIRA et al., 2002). On the other hand, in Jaíba with a minimum annual average temperature of 18.5ºC and altitude of 500 m, the production cycle of this cultivar was 320 days (RODRIGUES; SOUTO; SILVA, 2006).

This work was conducted with the objective of evaluating new cultivars and genotypes of bananas of the types Prata, Nanicão and Maçã in the southern region of the state of Minas Gerais, Brazil, regarding the increase of productivity and fruit quality.

II. MATERIAL AND METHOD

The experiment was conducted at the Experimental Farm of AGROTESTE in Lavras (MG), Brazil, from January 2013 to December 2015, and the first two cycles were evaluated. The altitude of the place is 918 m.

According to the climatic classification of Koppen (DANTAS; CARVALHO; FERREIRA, 2007), the climate of Lavras - MG is cwa, that is, temperate rainy season (mesothermic) with dry winter and subtropical rainy summer. The average temperature of the coldest month is below 18ºC and the hottest month is over 22ºC, with the average annual temperature of 19.4ºC and relative humidity of 76.2%. The average annual rainfall is 1,529 mm, with the driest months being from June to September.

Soil of the experimental area was classified as type 3, Red Latosol Ferric 62, 15 and 23 dag kg-1 of clay, silt and sand, respectively.

The experiment was installed in randomized blocks with three replications and plots with 16 plants per cultivar or genotype planted in the 3.0 x 3.0 m spacing.

The following genotypes and cultivars were evaluated: Prata type: Prata Anã (control), BRS Maravilha and PA 94-01 (genitor Prata Anã), BRS Vitória and PV 94-01 (Pacovan); Nanicão type: Grande Naine (control, Cavendish type), FHIA 17 (Hybrid of Gros Michel), and Type Maçã: Maçã (control) and YB 42-03.

The cycle of production of the mother and daughter plants and the accumulated one (from the planting of the mother plant to the harvest of the daughter plant) were evaluated, the periods being expressed in months. Production characteristics were as follows: fresh mass of the bunch (kg), number of fruits per bunch, length and diameter of the fruit (cm) and fresh mass of the fruit (g) of the medium bunch. The bunches were harvested when the fruits presented without pistils or floral remains, sharp corners, that is, ideal point of cut for the local market.

Plant development data were taken by measuring the diameter of the pseudocaule (cm) at 50 cm of the soil level and height of the plant (m), at the time of flowering.

It was also evaluated the incidence of yellow Sigatoka in the mother plant in flowering and harvest, through the number of leaves attacked, leaves without symptoms and leaves functional or alive. It was considered as functional leaf that presented in the flowering and harvest at least 2/3 of leaf area totally green.

Statistical analysis was performed using the Variance Analysis System software for the SISVAR balanced data (FERREIRA, 2011) and the comparison of means of treatments was performed using the Scott-Knott test at 5% probability.

III. RESULTS AND DISCUSSION

The results concerning the bunch and the fruit weight, number of fruits per bunch, length and diameter of these in the two cycles of the evaluated cultivars and genotypes are in Table 1.

TABLE 1
MEAN VALUES OF BUNCH AND FRUIT WEIGHT, NUMBER OF FRUITS PER BUNCH, LENGTH AND DIAMETER OF THE FRUIT OF THE FIRST AND SECOND CYCLES OF BANANA CULTIVARS AND GENOTYPES IN THE SOUTHERN REGION OF MINAS GERAIS, BRAZIL, 2016.

Cultivars / Genotypes	Bunch weight (kg)		Fruit weight (g)		Fruits by bunch		Length of fruit (cm)		Diameter of fruit (cm)	
	Mother plant	Daughter plant	Mother plant	Daughter plant	Mother plant	Daughter plant	Mother plant	Daughter plant	Mother plant	Daughter plant
FHIA 17 (N)	19,1 a	20,2 a	142,3 b	147,0 b	135,6 a	137,4 a	21,3 a	21,4a	4,0 a	4,0 a
Grande Naine (N)	17,2 b	17,8 b	146,3 b	144,0 b	118,2 b	125,0 b	20,2 b	20,2 b	3,9 b	3,9 b
PA 94-01 (P)	16,1 bc	17,8 b	120,3 c	126,1 c	134,3 a	141,5 a	19,5 b	19,8 d	3,9 b	3,9 a
Maravilha (P)	15,3 c	17,8 b	171,1 a	187,6 a	89,3 d	95,1 d	21,6 a	21,6 a	4,0 a	4,1 a
Vitória (P)	14,6 cd	16,1 b	155,0 b	158,6 b	95,0 c	101,5 c	20,3 b	20,5 b	3,8 b	3,9 b
PV 94-01 (P)	13,5 d	17,6 b	153,8 b	187,1 a	87,3 d	94,2 d	21,1 a	21,8 a	4,0 a	4,1 a
Prata Anã (P)	10,5 e	12,2 c	103,0 de	111,5 ed	104,0 b	110,2 c	15,2 c	15,6 c	3,5 c	3,5 c
YB – 42-03 (M)	8,8 f	9,5 d	98,8 ef	102,8 de	89,7 d	90,2 de	13,2 d	13,5 d	3,3 c	3,3 d
Maçã (M)	7 ,0 e	7,9 e	91,8 f	95,7 e	76,0 d	82,6 e	13,1 d	13,4 d	2,8 d	2,8 e
CV (%)	4,29	3,96	2,40	2,72	3,30	3,58	2,82	2,35	3,10	2,85

In the column, the averages followed by the same letter do not differ from each other to 5% by the Scott-Knott test.
N: Type Nanicão; P: Type Prata; M: Type Maçã

The genotype FHIA 17 produced higher fresh weight, number of fruits per bunch, length and diameter of the fruit, being overcome in fresh weight only by PV 94-01 and 'BRS Maravilha', in the second cycle.

The length and diameter of the fruits of the genotypes of FHIA 17, PV 94-01 and cultivar BRS Maravilha were significantly higher than the other evaluated materials.

The values of bunch and fruit weight, number of fruits per bunch, length and diameter of fruits observed in this study were significantly lower than those reported by Borges et al. (2011). These differences can be attributed to management, soil fertility and annual distribution and amount of rainfall (ALVES, 1999), since the trials were conducted in fairly distinct edaphoclimatic regions.

The cultivar Grande Naine, unique of the Cavendish type, produced bunches with superior weight to the genotypes of the 'Prata' type, only in the first cycle and number of fruits per cluster inferior to the one of PA 94-01, in the two cycles. The weight of the fruit was also lower than that of PV 94-01 and cultivar BRS Maravilha. The values of bunch and fruit weight and number of fruits per bunch of 'Grande Naine' obtained in this study are lower than those obtained by Donato et al. (2006) in Guanambi (BA), under irrigation and those reported by Silva, Pereira and Rodrigues (2008) in Cruz das Almas (BA) and by Borges et al. (2011) in Andirá (PR) under rainfed conditions. It is noteworthy that in Lavras (MG) and in much of the state of Minas Gerais, rainfall has been very low, even in the rainy season (from October to March). This causes water deficit in the soil and, consequently, lower utilization of fertilizers by plants.

Regarding the production of 'Prata' type materials, genotypes PV 94-01 and PA 94-01 and cultivars BRS Maravilha and BRS Vitória produced larger bunches and fruits in terms of weight than the cultivar Prata-Anã in the two cycles evaluated. The PV 94-01 along with the cultivar BRS Maravilha had the largest increases in bunch weight and fruit size from the first to the second cycle.

Among the materials of the 'Prata' type, PA 94-01 was the one that produced clusters with the highest number of fruits being these, similar in appearance and taste to the cultivar Prata-Anã.

Analyzing plant height, pseudocaule diameter and PA 94-01 production cycle (Table 2), it can be observed that this genotype was similar to the Prata-Anã cultivar. Although the plant height, pseudocaule diameter and number of live leaves in flowering PA 94-01 were considered adequate. However, Nomura et al. (2013) report that the production cycle of this genotype was later, resulting in lower productivity. The BRS Maravilha cultivar in terms of bunch weight, together with PA 94-01, were superior to BRS Vitória and PV 94-01 only in the first production cycle.

As regards weight, length and diameter, BRS Maravilha and PV 94-01, cultivar BRS Maravilha presented the highest weight in both cycles, and in the second cycle the fruits of PV 94-01 produced larger fruits in weight, length and weight. Diameter in the two cycles evaluated. The highest weight of the bunch and size of the BRS Maravilha fruits in relation to the Prata-Anã obtained in this work in dry conditions are confirmed by the reports of Silva et al. (2003) in Lavras (MG), by Ramos et al. (2009) in Botucatu (SP), by Vieira (2011) in Aquidauana (MS), by Santos and Carneiro (2012) in Jataí (GO) and by Mendonça et al. (2013) in Goiânia (GO). BRS Maravilha also surpassed Prata-Anã in the region of Jaíba (MG) (RODRIGUES; SOUTO; SILVA, 2006) and in southwestern Bahia (DONATO et al., 2009).

The genotype PV 94-01 and cultivar BRS Maravilha had the largest increases in bunch and fruit weight and number of fruits per bunch in the second cycle. This increase is also reported by Rodrigues, Souto e Silva (2006) for BRS Maravilha, SH 36-40 (Prata Graúda MG).

The genotype YB 42-03 and the cultivar Maçã produced the smallest clusters and fruits in terms of size, number of fruits per cluster. The YB 42-03 was slightly higher in these characteristics than the cultivar Maçã, its similar. These results corroborate with those reported by Silva, Pereira and Rodrigues (2008).

The cultivars Grande Naine and Prata Anã showed the lowest heights, inferior to 2.3 m in the mother plant and 2.7 m in the daughter plant (Table 2), results similar to those reported by Nomura et al. (2013). The largest size of these BRS Vitória cultivars was 3.5 m and 4.1 m followed by the PV 94-01 genotype with 3.1 m and 3.8 m, respectively, in the 1st and 2nd cycle. The larger size of the cultivars Vitória and PV 94-01 were also reported by Nomura et al. (2013). These results are already expected, since this characteristic is inherited from her mother-in-law Pacovan.

TABLE 2
MEAN VALUES OF PLANT HEIGHT, PSEUDOCAULE DIAMETER AND CYCLE OF PRODUCTION OF MOTHER AND DAUGHTER PLANTS OF BANANA CULTIVARS AND GENOTYPES IN SOUTHERN MINAS GERAIS. LAVRAS, BRAZIL, 2016.

Cultivars / Genotypes	Height (m)		Pseudocaule diameter (cm)		Production cycle (months)		
	Mother plant	Daughter plant	Mother plant	Daughter plant	Mother plant	Daughter plant *	Accumulated**
Grande Naine (N)	2,0 a	2,2 a	18,0 b	19,2 b	19,4 c	18,5 c	25,6 c
Prata Anã (P)	2,2 b	2,6 b	17,2 a	17,5 a	18,4 b	17,3 b	24,0 b
Maravilha (P)	2,5 c	2,7 b	22,0 d	22,7 d	18,2 b	17,2 b	24,3 b
Maçã (M)	2,6 c	2,9 c	16,9 a	17,6 a	16,6 a	15,7 a	21,7 a
FHIA 17 (N)	2,6 c	2,9 c	23,0 e	23,1 d	19,1 c	18,3 c	25,5 c
PA 94-01 (P)	2,6 c	2,8 c	22,1 d	22,9 d	18,1 b	17,1 b	23,8 b
YB 42-03 (M)	2,7 c	3,1 d	17,6 b	17,7 a	16,3 a	15,2 a	22,0 a
PV94-01 (P)	3,1 d	3,8 e	22,3 d	26,9 e	18,7 b	17,8 b	24,7 b
Vitória (P)	3,5 e	4,1 f	19,8 c	21,0 c	18,4 b	17,5 b	24,7 b
CV (%)	3,22	3,16	2,27	2,79	1,24	1,41	2,44

In the column, the averages followed by the same letter do not differ from each other to 5% by the Scott-Knott test.
**** Elapsed period of the appearance of the daughter plant until the harvest of the bunch***
***** Elapsed time from the planting of mother plant to the harvest of the daughter plant***
N: Type Nanicão; P: Type Prata; M: Type Maçã

Despite the larger size of the plant and the longer production cycle of BRS Vitória and PV 94-01 in relation to Prata-Anã, they may be a good option for producers because they are more productive and resistant to yellow and black Sigatoka Mal from Panama, as reported by Pereira et al. (2005) and Silva, Pereira and Rodrigues (2008).

Among the genotypes and cultivars of the Prata type, PA 94-01 and BRS Maravilha presented plant height and pseudocaule diameter superior to those of their Prata-Anã genitora. Rodrigues, Souto e Silva (2006) also report higher height of BRS Maravilha in relation to Prata-Anã.

The genotypes FHIA 17, PA 94-01 and PV 94-01 and cultivar BRS Maravilha presented the highest diameters of pseudocaule in both cycles, results similar to those reported by Nomura et al. (2013). The largest diameter increase from the first to the second cycle was observed in genotype PV 94-01.

The cycle of the Grande Naine cultivar in this work was significantly higher than that observed in the Ribeira Valley by Nomura et al. (2000) and lower than in Viçosa-MG, Cruz das Almas-BA, Guanambi-BA (SILVA et al., 2003) presented the lowest cycles, between 16.3 and 16.7 months in the first cycle and 15.2 a 15.7 months to 30.46 months in the second, with the daughter plant cycle being about one month shorter than that of the parent plant.

The largest production cycle of the mother plant in relation to the daughter plant is due to the period of readaptation and emission of new roots, since the seedlings from spontaneous shoots undergo the descortication or elimination of the adherent roots (ALVES, 1999).

The production cycle of PA 94-01 and the 'BRS Maravilha' in the first two cycles did not differ from their 'Prata-Anã' genitor, corroborating the results reported by Donato et al. (2009).

Comparing the production cycle of cultivars and genotypes produced in different regions, a great variation can be observed in the period between planting and harvesting. Thus, it is observed that the production cycle of the cultivars Prata Anã, Vitória, Grande Naine and genotypes PA 94-01, PV 94-01 in Lavras was between 0.5 and 3.0 months higher than in Goiânia - GO (MENDONÇA et al., 2013), in Botucatu (SP) (RAMOS et al., 2009) and in Jaíba (MG) (RODRIGUES; SOUTO; SILVA, 2006) and in Vale do Ribeira (SP) (NOMURA et al., 2013). However, it should be considered that in Jaiba and Botucatu, the crop was irrigated, a fact that favors the development of the plants and, consequently, anticipating the harvest, in addition to the inherent edaphoclimatic conditions of each place.

It is important to point out that the precocity of shoots and the time of selection of the follower together with their vigor are factors that influence the time of the bunch issue of the following generations. In the banana cultivars with good tillering, such as those of the types Prata, Nanicão and Maçã, the lateral shoots (daughters) begin to appear 30 to 45 days after planting. In cultivars of the Plantain subgroup (Terra, Terrinha and D'angola), shoots usually occur at the time of the bunch (ALVES, 1999).

The production cycle of the cultivar Prata-Anã in Botucatu-SP was 15.5 months (RAMOS et al., 2009), in Lavras-MG, 17.0 months (PEREIRA et al., 2002; PEREIRA et al., 2003) and in Maria da Fé-MG, 22.6 months (PEREIRA et al., 2002). It can be observed that in Lavras, the cycle was 1.5 months larger than in Botucatu, a difference that can be attributed to irrigation, management and planting time, and bunch emission and climate. However, in Maria da Fé, this cycle was about 5.5 months higher than in Lavras and 7.0 months higher than in Botucatu. This longer cycle in Maria da Fé can be attributed to the colder climate with average minimum temperatures of 10.7º C and altitude above 1,250 m (PEREIRA et al., 2002).

IV. CONCLUSION

The cultivars BRS Maravilha and PA 94-01 are recommended because they present greater productivity and similarity in the size, production cycle, flavor and appearance of the fruit in relation to Prata-Anã, traditionally cultivated in the region.

The genotype PV 94-01 and the cultivar Vitória, despite the greater size, can be recommended because it presents good productivity.

The genotype YB 42-03 is an alternative in relation to 'Maçã', being similar in productivity, size and production cycle.

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Agricultural Waste Management in order to sustainable agriculture in Karnataka

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Abstract— *Renewable energy as an alternative of fossil fuel for minimizing pollution and related issues, has got significant role in recent years. Agricultural residues as a source of biomass can be used to produce biogas. Every year while production of agriculture product generates lots of agro-residues and in many cases either left behind with no use or burn. Whereas can be prevented losing this source of energy by converting to the other types of energy. Nevertheless in present study assessing the potential of producing biogas out of biomass (agricultural residue) during one year in Karnataka state has conducted. For this purpose, production of agricultural crop residues data during one year depends on volatile solid (VS), extracted and according to the related coefficient, potential of biogas (methane) production computed. Results showed Maximum extracted methane respectively belonged to the rice, jowar, ragi, peanut and cotton residues. The total potential of methane production during the one year, 6391403732 cubic meters estimated.*

Keywords— agriculture residues, waste management, biomass, biogas.

I. INTRODUCTION

Limitation of fossil resources, their non-renewability, increasing petroleum fuel prices, emissions from combustion of fossil fuels all cause energy policy makers and planners to focus on structural studies, to change the energy carriers, and move towards cleaner fuels (Rahi, Garshasbi,2011;shaygan et al., 1997). Burning fossil fuels results in the emission of carbon dioxide (CO2), nitrous oxide (N2O) and methane (CH4) that act as barriers to thermal radiation and prevent it from leaving the Earth's atmosphere, the so-called greenhouse' effect (IPCC, 1997).The current policy within agriculture seeks to develop crop production systems that minimize fossil energy input for a high level of output(Dalgaard, 2000). Biomass has the largest potential land can only be considered as the best option form meeting the demand and insurance of future energy/fuel supply in a sustainable manner. The modernization of biomass technologies leading to more efficient biomass production and conversion is one possible direction for efficient utilization of biomass resources .Agricultural biomass is a relatively broad category of biomass that includes the food based portion(oil and simple carbohydrates)of crops (such as corn, sugarcane ,beets)and the non- food based portion(complex carbohydrates)of crops(such as the leaves ,stalks ,and cobs of corn Stover, or chard trimmings ,rice husk, straw),perennial grasses, and animal waste (Chandra, Takeuchi, & Hasegawa, 2012). Energy can be obtained from biomass in five ways: production of crops which yield starch, sugar, cellulose and oil; solid waste which can be burnt; an aerobic digesters which produce biogas which can be used to generate heat/electricity; landfill production for methane ;and biofuel production which includes ethanol, methanol, biodiesel and their derivatives (Demirbaş, 2001). Direct combustion of biomass for heat or combined heat and power generation is the most common way of using biomass for energy today, but a large, unutilized energy potential lies in semi-solid agricultural residues, with dry matter contents below 25%, making the energy potential difficult to exploit in its current form (Chynoweth, Owens, & Legrand, 2001; Gunaseelan, 1997).Conversion to a useful energy carrier must first be performed .recently Converting biomass to biogas is one the reliable methods. Biogas is currently produced by anaerobic degradation of millions of tons of organic solid waste arising from municipal, industrial and agricultural sources(Chynoweth et al., 2001; Gunaseelan, 1997) .Biogas as one of the major sources of energy can be used directly to provide heating and electricity energy and is a good option to be used in internal combustion generators, micro-turbines, fuel cells and other power producing facilities as well (Rahi, Garshasbi,2011;shaygan et al., 1997). Biogas contains 50–70% methane and 30–50% carbon dioxide, as well as small amounts of other gases (Sasse, 1988). And typically has a calorific value of 21–24 MJ/m3. Biogas burns with a clean, blue flame and stoves have been considered as the best means of exploiting biogas in rural areas of developing countries (Kossmann et al., 1999). In 2012, about 194.8 million ton of renewable energy was consumed in the world and about 0.1 million ton was consumed in Iran (Dudley, 2012). To increase the biogas yield (Mohseni, Magnusson, Görling, & Alvfors, 2012) .Methane derived via anaerobic digestion has proved to be competitive with heat (via burning), steam and ethanol production in efficiency, cost and environmental impact of the conversion of waste streams to energy forms (Chynoweth et al., 2001; Edelmann, Schleiss, & Joss, 2000).

II. MATERIAL AND METHOD

This study evaluates agro-residue biomass production in Karnataka state located in Sothern of India. In the present study, all agriculture residue data, brought up from the Indian biomass atlas (2000-2004). Depends on the data for years of 1998-1999, assumed production ratio have not change in 6 years period and it would be considered as a consistence production, Accordingly different production ratio correlation between in two these period of time examined and significant correlation data confirmed, so production ratio experienced a slightly variation. Hence slightly variation for the next years considered too. Biomass production through agricultural residues data monitored and evaluated then methane production potential coefficient of each and every major crops residue according to cubic meters per ton Unit for volatile solid (m3/ton Vs) extracted from different sources (table 1) . According to the calculation of biomass potential of agro-residues depends on volatile solid measurement, content of biomass production (VS) for each and every product computed and used for estimating of methane potential of agro-residue.

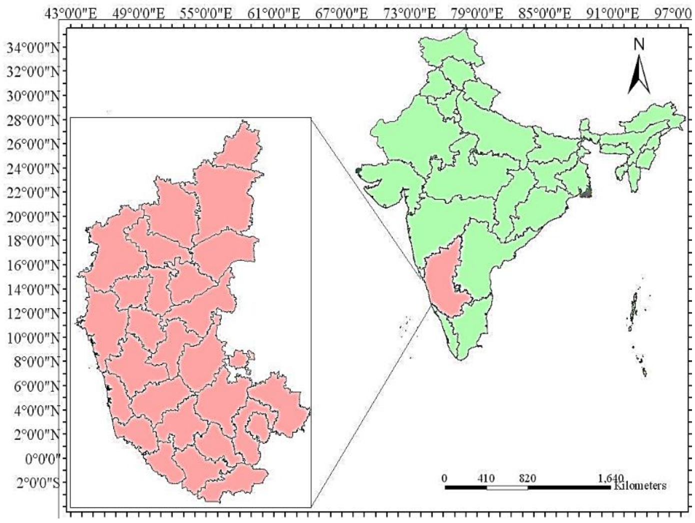

FIG 1: KARNATAKA GEOGRAPHICAL LOCATION

Methane coefficient of Finger millet residue, was not found, so in this case methane coefficient of millet residue was used (Table 1). In addition methane coefficient of peanut, gram and sugarcane residues were not found, so to obtain methane content biogas production ratio was used to calculate methane potential content of the mentioned agricultural residues (Methane content in biogas extracted from the agricultural residue is between 60 to 75 %) (Anonymous, 2016).

TABLE 1
MAJOR CROPS RESIDUE METHANE YIELDED, KARNATAKA

Agro-residue	Methane yield (m3/ton vs*)	References
Bajra	390	(Lehtomäki, 2006)
Cotton	170	(Oosterkamp, 2014)
Gram	273	(Braun *et al.*, 2008)
Groundnut	256	(Anonymus, 2016b)
Jowar	415	(Lehtomäki, 2006)
Maize	166	(Sawatdeenarunat *et al.*, 2015)
Millet	390	(Nallathambi Gunaseelan, 1997)
Paddy	303	(Nallathambi Gunaseelan, 1997; Lehtomäki, 2006; Sawatdeenarunat *et al.*, 2015)
Ragi	390	(Nallathambi Gunaseelan, 1997)
Sugarcane	140	(Karimi Alavijeh and Yaghmaei, 2016)
Wheat	390	(Menardo and Balsari, 2012; Oosterkamp, 2014)

III. RESULTS AND DISCUSSION:

Evaluating the agro-residue production revealed, Overall Karnataka by 6% producing of total production of agro-residue biomass Annually got 9th rank after, Uttar Pradesh, Punjab, West Bengal, Maharashtra, Madhya Pradesh , Rajasthan and Haryana states (Fig 2). So it seems so using agro-residue (biomass) as a source of energy can contribute significant role in economics of Karnataka. Importance of using biomass as a source of biogas highlighted in the other researcher studies. (Karimi et al., 2016) studied on feasibility of different type of renewable energies production, including agro-residues in Iran. (Lehthomaki et al., 2006) investigated feasibility of biogas production through energy crops and agricultural residues.

3.1 Karnataka biomass Production

Yearly biomass production in Belgum, Golbarga, Tomkur, Raichor, Bijapour & Belari taluks with the maximum production are 8.8, 7.3, 6.2, 5.8, 5.8, 5.4 percent respectively (Fig 3). The results revealed each taluk of Karnataka has got significant differences to the other taluks in terms of biomass production, in terms of geographical location, except of Belgum which is located in northeastern of Karnataka, the rest of high potential biomass production taluks located in north, east and center. Knowing the geographical area in high potential production biomass taluks is vital. For instance region or season with an average temperature less than 10° C and in residential area it's not convenient for biogas production **(Chen et al., 2013).** Therefore, the geographical area of biomass production areas play a vital role in further plans and agro-residues management in order to optimal productivity.

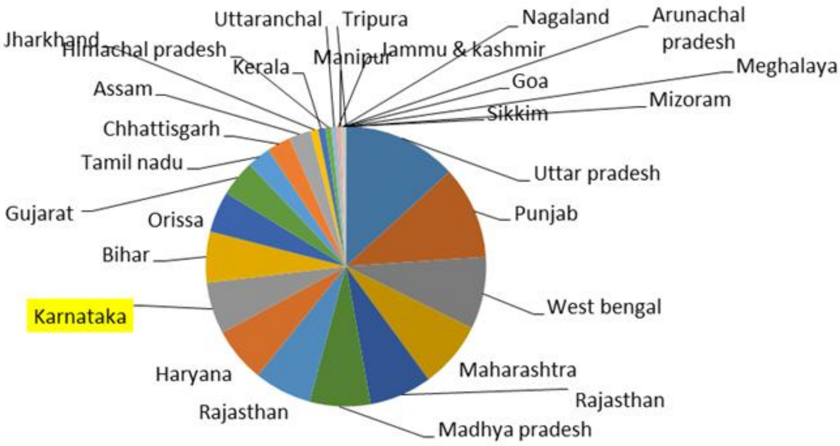

FIG 2: ANNUALLY AGRO-RESIDUE BIOMASS PRODUCTION

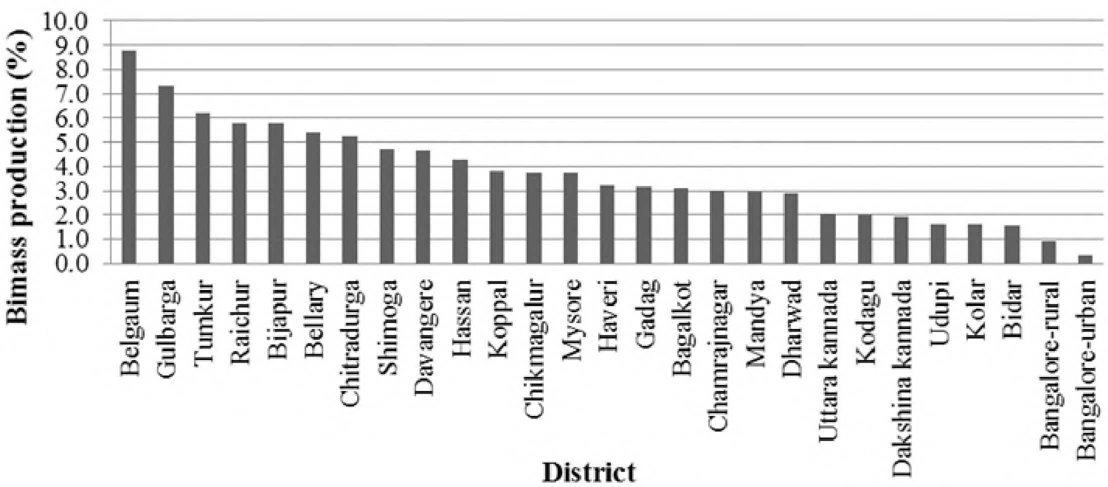

FIG 3: ANNUALLY AGRO-RESIDUE BIOMASS PRODUCTION IN DIFFERENT TALUKS OF KARNATAKA STATE

Study on agricultural production in Karnataka state indicates the main important crops in terms of production of agro-residues biomass by respectively share of 30. 2, 20. 2, 13.2, 11.7, 9.5, 7.4, 4.8, 4.1, 1 percentage belonged to the rice, jowar, cotton, corn, peanut, ragi, sugarcane, bajera and wheat. As it shows in figure 4, biomass production to cultivation area ratio of different major crops significantly is different. For instance this ratio in rice, cotton and corn is more than 1 and in case of sorghum, peanut, bajira, ragi, gram and wheat is less than 1.so the largest area under cultivation does not mean the greatest amount of biomass production and vice versa.in case of rice with low infield produce high yield product.so in terms of producing main product and by-product, rice is important.

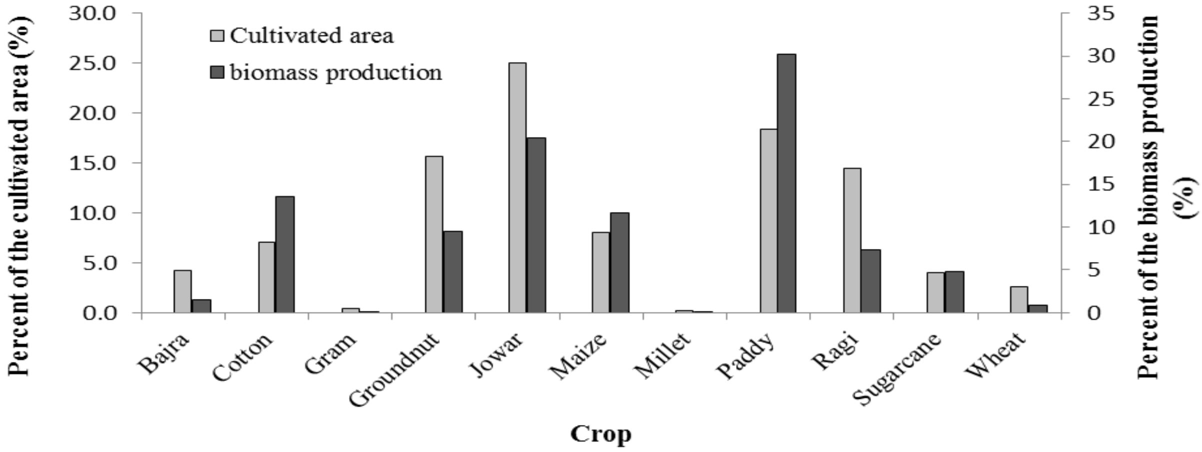

FIGURE 4: CULTIVATED AREA AND BIOMASS PRODUCTION OF MAJOR CROPS IN KARNATAKA

Over all maximum methane extraction in one year it's about **6391403732 m^3** through the rice ,jowar, ragi, peanut , cotton and corn by the respectively production share of 31.9, 29.6, 10.1, 8.4, 8 and 6.7.(table 2). Biogas production feasibility through different sources examined by researchers *(Gamal-El-Din et al., 1986; Isci and Demirer, 2007; Braun et al., 2008; Dinuccio et al., 2010; Anjum et al., 2012; Chen et al., 2013; Kiran et al., 2014)*. Different researchers expressed biomass agro-residues would be a suitable source for biogas production *(Lehtomäki, 2006; Parawira et al., 2008; Garcia-Peña et al., 2011; Khalid et al., 2011; Menardo and Balsari, 2012; Durruty et al., 2013; Janke et al., 2015; Sawatdeenarunat et al., 2015; Karimi Alavijeh and Yaghmaei, 2016; Li et al., 2016)*. Biomass residues and weeds can be source of biogas production *(Bond and Templeton, 2011)*.biogas potential production studied through livestock and slaughterhouse waste in Iran and biogas considered as renewable and sustainable energy***(Afazeli et al., 2014)***.

TABLE 2
METHANE POTENTIAL PRODUCTION THROUGH AGRO-RESIDUES DURING ONE YEAR IN KARNATAKA

Agro-residue	Cultivated area (kha)	Biomass (t/yr)	Methane production (m^3)	%
Bajra	316.1	414000	103334400	2
Cotton	532	3894100	542837540	8
Gram	32.7	33600	7521696	0.1
Groundnut	1179.7	2720500	493512302.5	8.4
Jowar	1882.2	5871400	1681275390	29.6
Maize	603.7	3345000	477532200	6.7
Millet	11.4	9600	2396160	0
Paddy	1379.7	8677300	2287423053	31.9
Ragi	1090.8	2127500	572510250	10.1
Sugarcane	298.2	1367100	164598840	2.3
Wheat	192.5	242500	58461900	0.8
Total	7519	28702600	6391403732	

IV. CONCLUSION

Besides of Limited fusil fuels and energy crisis, extensively non-renewable energy using results to the environmental issues and global warming. So one of the initial actions for the saving our environmental from global warming and related issues is replacing of non-renewable energy to the renewable form of energy. Biomass as a source of renewable energy because of variety of advantages highly suggested by researchers. One of the biomass sources is agricultural residues that left largely unused on the agriculture field and can be consider as a main important sources for renewable energies. Out of biomass, bio-methane/biogas would be produced and its valuable as natural gas or other type of fossil fuel are. Biogas can use to produce electricity or even heating, cooling, pumping, or used as vehicle fuel.

According to the results, agricultural crop residues considered as reliable source for renewable energy production in form of biogas in Karnataka. Results shows 91.6 % of total methane production obtain from rice, jowar, ragi, peanut, cotton, corn residues. Factors such a climate, (temperature above 20 ° C), solar energy and biomass resources have a key role in optimum methane extraction. ***(chen et al ., 2013)*** . According to diversified climate of Karnataka and high production of agro-residue biomass, it seems to biomass as a renewable energy can be considered for supplying some part of demanded energy in Karnataka.

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Uptake of Silver from Polyvinylpyrrolidine Coated Silver Nanoparticles in a Terrestrial System

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Abstract— *The widespread use of silver nanoparticles (Ag NPs) has facilitated their uninterrupted entry into various ecosystems. Nanoparticles are stabilized using a variety of approaches for various applications. The present study has investigated the uptake of polyvinylpyrrolidine (PVP) coated Ag NPs in a terrestrial system. Two insect (Acheta domesticus and Tenebrio molitor) and two plant species (Sorghum vulgare and Helianthus annuus) were used in the study. The effect of concentration and size of PVP-coated Ag NPs was investigated. The test species were maintained in soil spiked with 0, 1, 5, 25, 125, and 625 mg/kg PVP-coated 30-50 nm Ag NPs to test the effect of concentration of Ag NPs on uptake. Similarly, the test species were maintained in soil spiked with 25 mg/kg of 20, 30-50, and 50-80 nm PVP-coated Ag NPs to study the effect of size of nanoparticles on uptake. The PVP-coated Ag NPs were characterized using transmission electron microscopy, dynamic light scattering and powder X-ray diffraction techniques. The levels of silver in test samples were measured using inductively coupled plasma-optical emission spectroscopy. A concentration dependent increase in the levels of Ag in both the insect species was observed as a function of increasing concentrations of coated Ag NPs in soil. An increase in the levels of Ag as a function of increasing size of coated Ag NPs was observed with Acheta domesticus. No apparent trend was observed with Tenebrio molitor species. A concentration dependent increase in the levels of Ag in the roots of both the plants was observed as a function of increasing concentrations of coated Ag NPs in soil. Additionally, the translocation of Ag to other plant tissues was observed in Helianthus annuus, a dicot plant.*

Keywords*— *Silver nanoparticles, polyvinylpyrrolidine, Acheta domesticus, Tenebrio molitor, Sorghum vulgare, Helianthus annuus, inductively coupled plasma-optical emission spectroscopy.

I. INTRODUCTION

The widespread use of silver nanoparticles (Ag NPs) for a variety of consumer, industrial and medical applications has resulted in an increase in the anthropogenic release of silver in to the environment [1,2]. Silver from many Ag NP containing products is predicted to enter into the wastewater streams and eventually wastewater treatment plants [3]. Much of the silver at the treatment plants is partitioned in to sewage sludge [2,4,5]. Ag NPs eventually find their way in to the terrestrial ecosystems through the application of sewage sludge to agricultural lands [4].

The colloidal stability of nanoparticles significantly affects their mobility, uptake/bioavailability, and toxicity in a given ecosystem [6]. Environmental conditions present in an ecosystem such as pH, ionic strength, background electrolyte composition, etc. affect the colloidal stability of nanoparticles. Additionally, the presence of capping agents/coatings on nanoparticles also influences their colloidal stability [7,8]. Ag NPs are highly reactive due to their high surface area-to-volume ratio. This leads to phenomenon such as particle aggregation and settling in Ag NPs, unless stabilized/protected by suitable coatings [9,10]. Coatings prevent the aggregation of nanoparticles through electrostatic repulsions, steric repulsions, and a combination of both [9,11]. Coatings that are commonly used during the synthesis of Ag NPs include chemicals such as citrate, sodium borohydride ($NaBH_4$), and polyvinylpyrrolidine (PVP) [9,11].

PVP coatings are known to sterically stabilize Ag NPs. The mechanism of steric stabilization of nanoparticles involves the adsorption of uncharged polymer on the surface of nanoparticles [6,12,13]. Another mechanism involves the formation of weak coordinative bonds between PVP and Ag^+ ions [14,15]. The effect of surface coatings on the stability, transformation, uptake, and toxicity of nanoparticles has been discussed in literature [13, 16-21]. The present study investigates the uptake of PVP-coated Ag NPs (henceforth referred to as coated Ag NPs) in a terrestrial system by two insect (*Acheta domesticus* and *Tenebrio molitor*) and plant species (*Sorghum vulgare* and *Helianthus annuus*). PVP-coated Ag NPs were chosen as they demonstrated excellent shape and size stability characteristics in an ecotoxicological medium compared to other charge stabilized (citrate coated) Ag NPs [13]. The insect and plant species selected for the study were found to be native to the region where the soil was collected. Therefore, investigating the uptake of Ag NPs by these species would help us understand the role of these species in the transport of metal contaminants along the food chain of native insectivorous and granivorous species.

II. MATERIAL AND METHOD

2.1 Soil collection and preparation

All soil used during the insect and plant exposure experiments were collected in Mitchell County, Texas at an elevation of 684 m above sea level. The soil was collected from the top 10 cm of soil and is shoveled into clean plastic containers. Once transported to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX, the soil was processed for homogeneity. Large clumps of soil were crushed. Large rocks, roots, living organisms, and other organic matter were removed. This was followed by sieving of the soil through a 2 mm wire screen into another clean plastic storage container. Once processed, the soil was covered and stored indoors until ready for use.

2.2 Soil analysis

The analysis of various characteristics of soil samples was performed at the Midwest Laboratories Inc. (Omaha, NE). Soil texture, percent humic matter, percent organic matter, exchangeable cations (K^+, Mg^{2+}, Ca^{2+}), available phosphorus (P), soil pH, percent base saturation of cations (K^+, Mg^{2+}, Ca^{2+}, H^+), cation exchange capacity (CEC), and sulfur (S) content were all analyzed in order to fully characterize the soil.

2.3 Nanoparticle Characterization

Silver nanoparticles coated with 0.2% PVP (20, 30-50, and 50-80 nm) were purchased from US Research Nanomaterials, Inc. (www.us-nano.com, Houston, TX). The US Research Nanomaterial, Inc reported that all Ag NPs consisted of $\geq$ 99.99% Ag.

Transmission electron microscopy (TEM) was used to confirm the size range and shape of the coated and uncoated Ag NPs. The preparation of samples involved the dispersion of Ag NP powder in ethanol (EtOH). Samples were sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried prior to analysis and TEM (Hitachi H-8100 TEM) images were taken at 200 kV using a tungsten filament side-mounted camera.

The size of Ag NPs was also confirmed using Dynamic light scattering (DLS). A mixture of 10 mg of Ag NP powder in 10 ml of reagent grade acetone (Fisher Scientific, MA, USA) was sonicated until Ag NPs remained suspended in solution. Samples were analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA) and Mictrotrac Flex Software (Version: 10.3.14).

The composition of Ag NPs was confirmed using Powder x-ray diffraction (PXRD). A Rigaku Ultima III X-Ray Diffractometer was used. Ag NPs were analyzed using a Cu Kα radiation as x-ray source. The following instrument parameters were used to analyze the Ag NPs: parallel-beam geometry was used with a step width of 0.03° and a count time of one second; the divergence, scattering, and receiving slits were set at one. Once completed, the diffraction patterns were compared and matched to the phases in the International Center for Diffraction Data (ICDD) powder diffraction file (PDF) database.

2.4 Exposure of insects to coated Ag NPs

Two 37.8 L terrariums were prepared for each insect treatment group. Exactly 2.5 kg of prepared soil was weighed out into each clean terrarium. The insect species were maintained in soil spiked with a range of concentrations of 30-50 nm coated Ag NPs (0, 1, 5, 25, 125, and 625 mg/kg) to understand the effect of concentration of Ag NPs on uptake. Additionally, the effect of size of nanoparticles (20, 30-50, and 50-80 nm) on their uptake by the test species was investigated by spiking the soil with 25 mg/kg coated Ag NPs. Once the terrariums were prepared, insects were purchased from Reptilefood (reptilefoods.com, Ohio, USA). Either 300 small crickets or 400 large mealworms were placed in each of the terratiums and were provided with fresh food and water as needed for the duration of the 28 day exposure period. After the 28 day exposure had run to completion, insects were carefully extracted from the terrariums and placed in glass jars. The jars were then placed in a -80°C freezer until all the insects were deceased. Freeze drying of the insects (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO) for at least 48 hours was performed to ensure the removal of all moisture. Finally, the freeze dried insects were then crushed into a fine powder and stored in a freezer until further use.

2.5 Exposure of plants to coated Ag NPs

This set of experiments was performed in commercially available 7.6 L plastic nursery containers. The containers were filled with approximately two inches of commercial pond pebbles to aid in proper drainage. As detailed in the insect sample section above, the plant species were maintained in soil spiked with a range of concentrations of 30-50 nm coated Ag NPs (0, 1, 5, 25, 125, and 625 mg/kg) to understand the effect of concentration of Ag NPs on uptake. Additionally, the effect of size of nanoparticles (20, 30-50, and 50-80 nm) on their uptake by the test species was investigated by spiking the soil with 25 mg/kg coated Ag NPs.

Seeds for the two plant species were planted into their own completed nursery containers and were transported to the TTU greenhouse. The plants were maintained in the TTU greenhouse until maturity, approximately three months for *H. annuus* and six months for *S. vulgare*. The plants received shaded sunlight and were maintained at 60°F or above in the TTU greenhouse. The entire plant was harvested after it reached maturity. The roots of the plants were separated rinsed using tap water for one minute to remove all attached soil. The shoot system of the plant was separated into leaves, stems, and seeds. The plant samples were stored in a freezer until further use.

2.6 Sample digestions

Three identical samples were weighed out using the insect samples collected from each terrarium. For each plant treatment group, four samples were prepared from each nursery container: a root sample, a leaf sample, a stem sample, and a seed sample, if possible. Dry weights were used in the case of insects and wet weights were used in the case of plants. 10 ml of 70% nitric acid (HNO_3, reagent grade) was added to 1 gm of insect or plant samples in a 100 ml beaker. 10 ml of 30% hydrogen peroxide (H_2O_2, reagent grade) was added to this mixture. The beakers were covered with a Telfon watch glass and the samples were heated in increments of 50 ˚C until the solutions begin to reflux gently. To aid in the effective digestion of all samples, the beakers were periodically swirled. The digestions were stopped when the volume of sample in each of the beakers reduced to approximately 5 ml. This was followed by placing the beakers in an ice bath to cool. The samples were then filtered into 50 ml centrifuge tubes (Corning CentriStar ™, Corning, NY) using ashless filter paper (Whatman No. 41, Fisher Scientific, PA). Filtering at this step ensures all lipids and other solids were removed from the samples. The original sample beakers were additionally rinsed twice with 10 ml of 5% HNO_3 and the rinse contents were added to the 50 ml centrifuge tube. The extracts were stored at room temperature until further analysis. A solution containing 10 ml HNO_3 and 10 ml H_2O_2 was used as the reagent blank during the analysis of samples.

2.7 ICP-OES analysis

The samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The analysis of samples was performed at three wavelengths: 224.643, 328.068, and 338.289 nm. A 10 ppm silver standard solution (SPEX CetriPrep) was used to align all the three wavelengths of interest. Ultimately, the data from 338.289 nm was chosen for analysis.

2.8 Statistical analysis

A duplicate analysis of samples was performed in the present study (n=2). A normality check was performed and the data was normalized using Johnson transformation approach (p=0.05). ANOVA was performed on Ag concentrations more than 0.005 μg/g. Samples that contained silver concentrations below instrument detection limits (<0.005 μg/g) were treated as zeroes. All statistical analysis was performed with 95% confidence interval. The ANOVA test was followed by a multiple comparison test (Tukey) to identify the significant difference among the treatment groups. Statistical analyses were performed using MINITAB 17 [Minitab 17 Statistical Software (2010). Computer software, State College, PA: Minitab, Inc. www.minitab.com].

III. RESULTS AND DISCUSSION

3.1 Soil characterization

The control soil was found to contain 54% sand, 36% silt, and 10% clay. This type of soil is classified as a sandy loam. The additional tests found the soil to contain 0.01% humic matter, 1.7% organic matter, and 9 ppm S. The pH of the control soil was slightly basic, 8.1. And the CEC of the soil was calculated to be 18.0 meq/100g. Other data from soil analysis is summarized in Table 1.

3.2 Transmission electron microscopy

A representative TEM image of coated 20 nm Ag NPs is presented in Fig. 1. As expected with the presence of PVP coatings, the phenomenon of aggregation is observed to be minimal. The presence of bulk organic materials like PVP on the surface of Ag NPs prevents the phenomenon of aggregation of nanoparticles through steric repulsions [6,12,13]. A reduction in aggregation of coated Ag NPs may likely facilitate their uptake in the test species. Additionally, the TEM image confirmed the spherical shape of coated Ag NPs.

3.3 Dynamic light scattering

The DLS analysis of coated Ag NPs revealed the average size to be much higher than expected. The size of coated 20 nm Ag NPs ranged from 39.20-154.3 nm, with an average particle size of 48.80 nm. A representative size distribution for the coated 20 nm Ag NPs is presented in Fig. 2. The size of coated 30-50 nm Ag NPs ranged from 40.50-121.5 nm with an average size of 62.40 nm. And size of coated 50-80 nm samples ranged from 78.90- 171.5 nm with an average size of 101.7 nm. The presence of an organic layer (PVP) may have possibly resulted in the observed increase in size of coated Ag NPs. Larger particles are thought to be less likely to aggregate, but also have lower dissolution rates. Large particles and large aggregates are thought to be less mobile in porous materials, including soils [22-24].

TABLE 1
CHARACTERISTICS OF SOIL

Analysis	Results
Organic Matter	1.7%
Exchangeable Potassium	263 ppm
Exchangeable Magnesium	114 ppm
Exchangeable Calcium	3273 ppm
pH	8.1
Cation Exchange Capacity	18.0 meq/100g
Base Saturation, Potassium	3.7%
Base Saturation, Magnesium	5.3%
Base Saturation, Calcium	91.0%
Base Saturation, Hydrogen	0.0%
Sulfur Content	9 ppm
Humic Matter	0.01%
Sand Content	54%
Slit Content	36%
Clay Content	10%

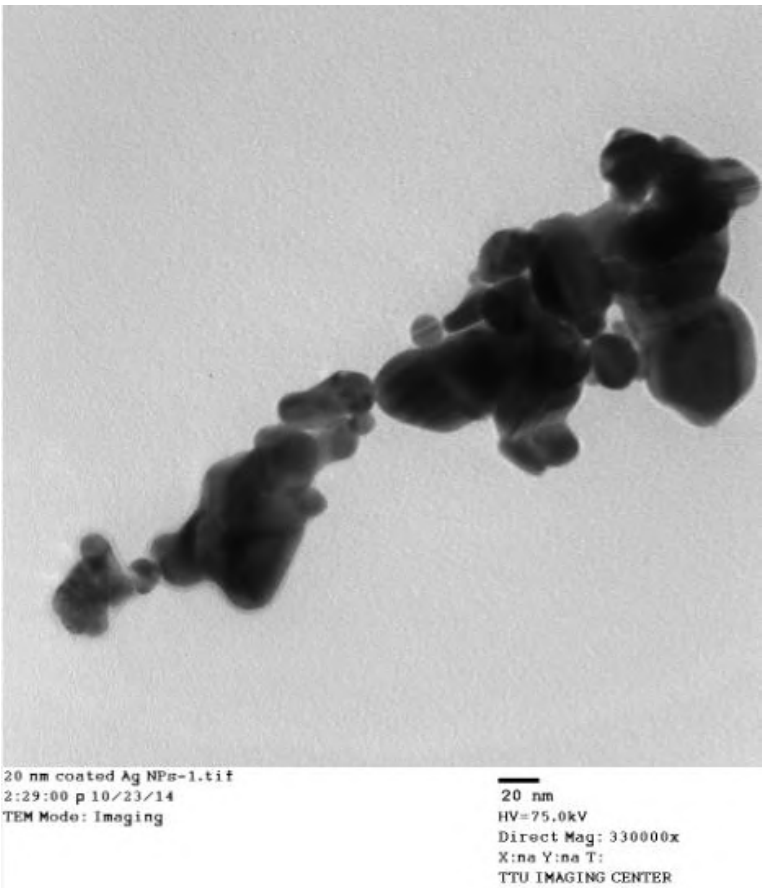

FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE OF 20 nm COATED Ag NPs.

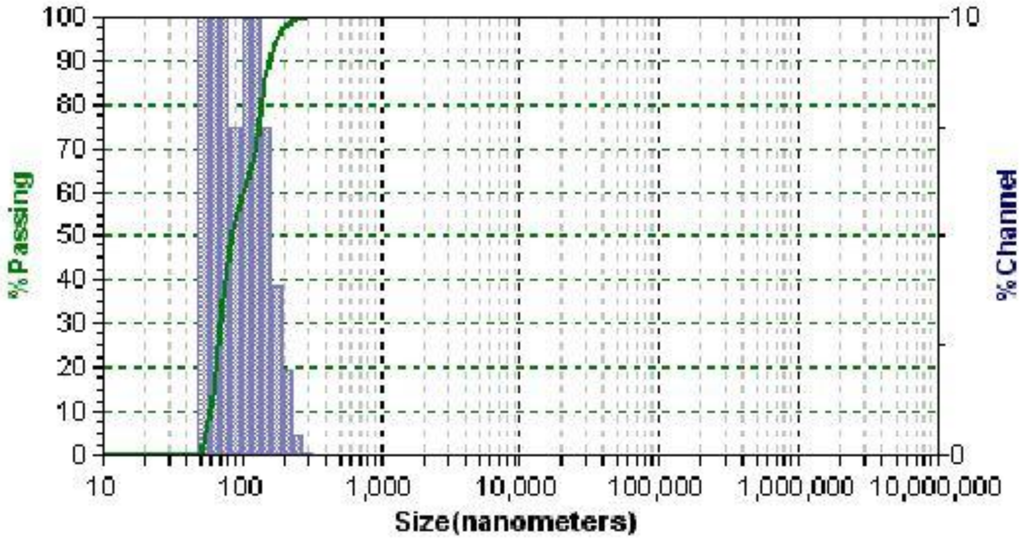

FIG. 2: SIZE DISTRIBUTION OF COATED 20 nm Ag NPs AS DETERMINED BY DYNAMIC LIGHT SCATTERING.

3.4 Powder X-ray diffraction

The PXRD analysis of the silver nanoparticles confirmed their composition. The measured crystalline structure was slightly larger most likely due to the 0.2% PVP coating. The diffraction patterns still matched both those in the ICDD and those provided by the manufacturer. A typical diffraction pattern can be seen in Fig. 3.

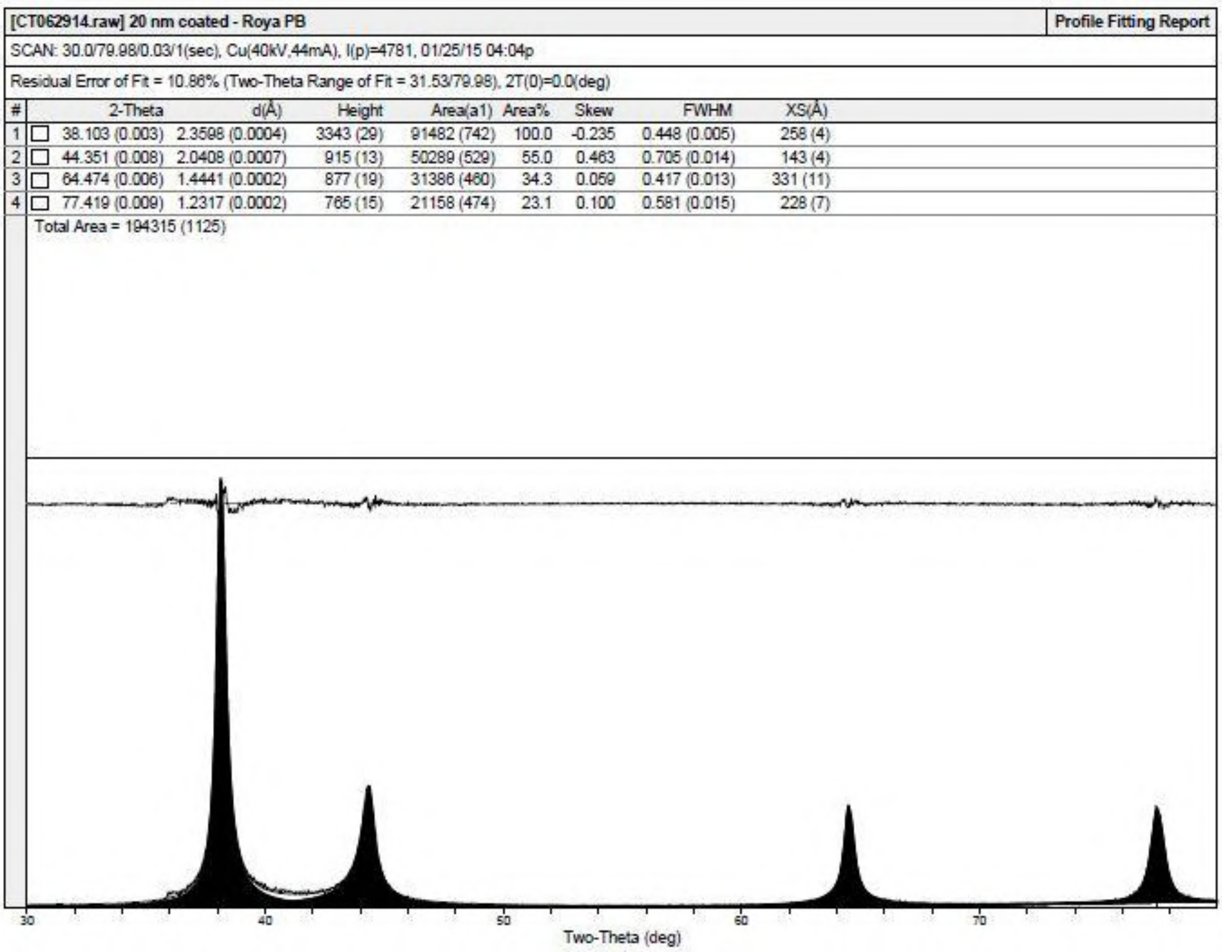

FIG. 3: DIFFRACTION PATTERN FOR 20 nm COATED Ag NPs AS DETERMIEND BY POWDER X-RAY DIFFRACTION.

3.5 Uptake of Ag from coated Ag NPs in soil by insects

No detectable amount of silver was observed in the control soil used for the insect experiments. However, increased levels of Ag were observed in *A. domesticus* and *T. molitor* samples that were maintained in soil spiked with 1 mg/kg coated Ag NPs. An error in any of the sample processing steps would have accounted for the observed increase in the levels of Ag in the insects maintained in soil spiked with 1 ppm coated Ag NPs.

With the experiments involving *A. domesticus*, no silver was observed in insects maintained in soil spiked with 1 ppm coated Ag NPs. Trace amounts of Ag (>0.02 mg/kg) were observed in insects maintained in soil spiked with 25 mg/kg coated Ag NPs. Quantifiable amounts of Ag (>0.1 mg/kg) was observed in *A. domesticus* maintained in soil spiked with 125 and 625 mg/kg coated Ag NPs. In general, an increase in the levels of Ag in *A. domesticus* was observed as a function of increasing concentrations of coated Ag NPs in soil (Fig. 4). Similar results were obtained in the case of *T. molitor* samples (Fig. 4). Additionally, the levels of Ag in both the insect species maintained in soil spiked with 625 mg/kg coated Ag NPs was found to be significantly higher than the levels of Ag in insect species maintained in soils spiked with 1, 5, and 25 mg/kg coated Ag NPs ($p<0.05$).

The uptake of Ag from coated Ag NPs by both *A. domesticus* and *T. molitor* in soil was first observed in soil spiked with 25 mg/kg coated Ag NPs. Hence, spiking with 25 mg/kg coated Ag NPs was considered to investigate the effect of size of nanoparticles on uptake of Ag from coated Ag NPs in soil (Fig. 5). An increase in the levels of Ag as a function of increasing size of coated Ag NPs was observed with *A. domesticus*. No apparent trend was observed with *T. molitor* species. Nevertheless, *T.molitor* was found to have a significantly higher level of Ag compared to *A. domesticus* ($P<0.05$). This suggests that *T.molitor* is accumulating higher levels of Ag from coated Ag NPs in soil compared to *A. domesticus*.

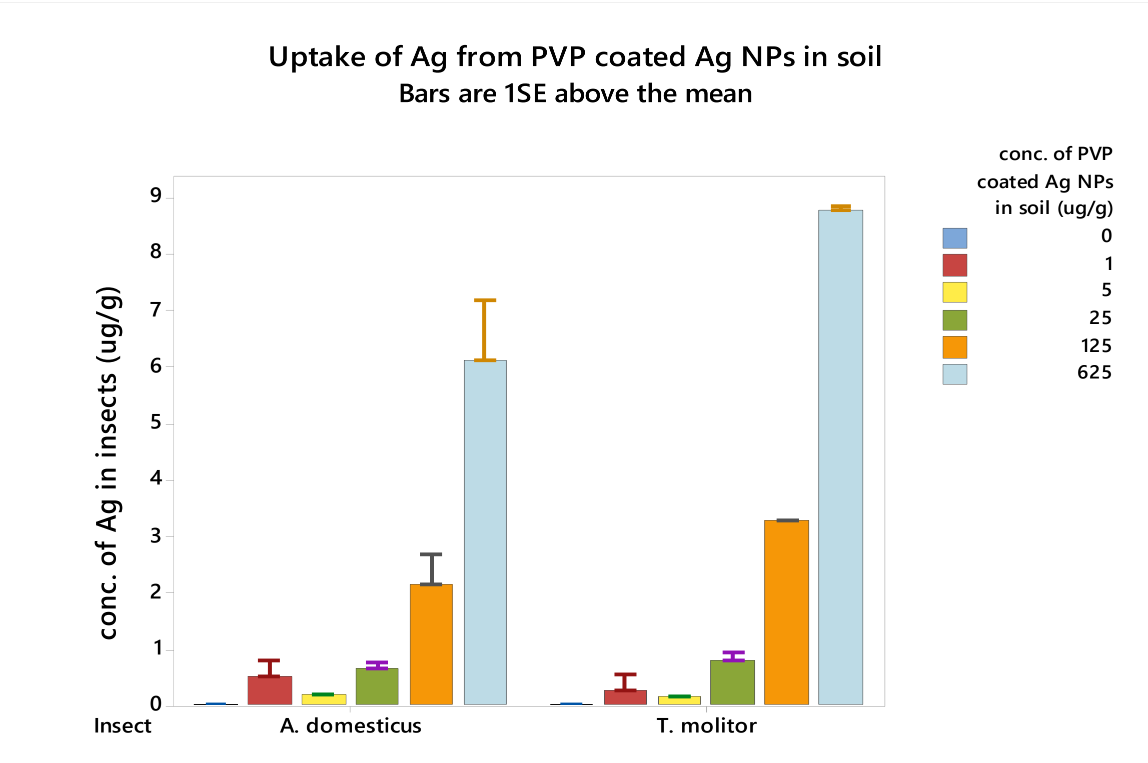

FIG. 4: LEVELS OF Ag IN *A. domesticus* AND *T. molitor* SAMPLES MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRAITONS OF COATED Ag NPs.

The difference in the uptake of Ag from coated Ag NPs in soil by *A. domesticus* and *T. molitor* could be inherent. The mechanism of uptake by either of these two species is not yet clearly established. However, the level of uptake of metals by soil-dwelling organism is found to be dependent on their habitat, diet, and physiological responses [25]. Another explanation could be the phenomenon of avoidance. Certain species like earthworms (*Eisena fetida*) are better able to sense the presence of nanoparticles in soil and avoid. This way, they tend to avoid their dwelling in such soils and the uptake of contaminants [26]. The phenomenon of avoidance by one species over another in the present study cannot be discounted.

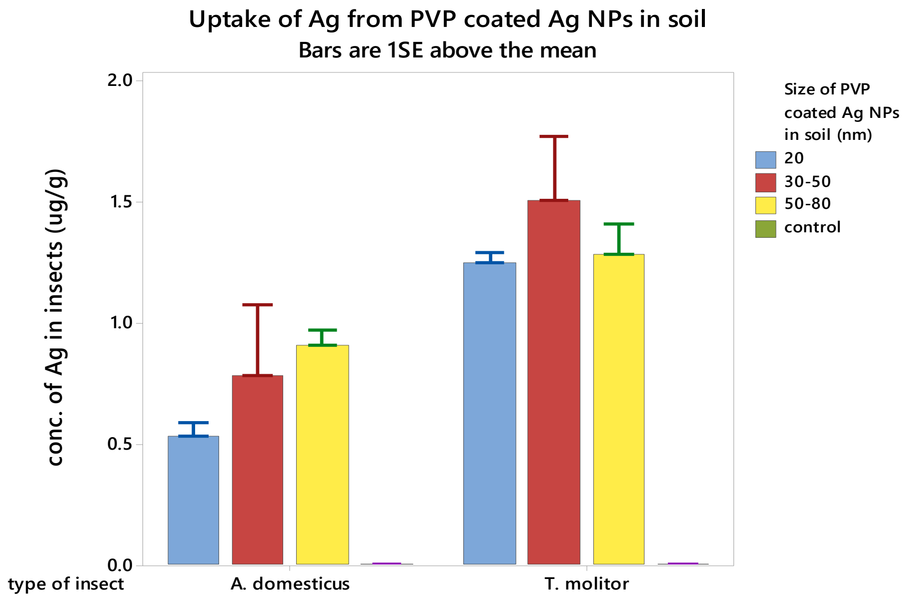

FIG. 5: LEVELS OF Ag IN *A. domesticus* AND *T. molitor* SAMPLES MAINTAINED IN SOIL SPIKED WITH DIFFERENT SIZED COATED Ag NPs.

3.6 Uptake of Ag from coated Ag NPs in soil by plants

The present study considered a monocot (*S. vulgare*) and a dicot (*H. annuus*) plant species to investigate the difference in the accumulation of Ag from coated Ag NPs in soil. In both plants, the roots have accumulated significantly higher levels ($p<0.05$) of Ag from coated Ag NPs in soil compared to other plant tissues (stem, leaves, seeds, etc.). In the roots of both the plants, a concentration dependent increase in the levels of Ag is observed as a function of increasing concentrations of coated Ag NPs in soil. Additionally, the levels of Ag in roots of plants maintained in soil spiked with 625 mg/kg coated Ag NPs is found to be significantly higher than the levels of Ag in roots of plants maintained in soil spiked with 1, 5, 25, and 125 mg/kg coated Ag NPs. Finally, the dicot plant (*H. annuus*) has accumulated more levels of Ag in its system compared to the monocot plant (*S. vulgare*). The results are summarized in Fig. 6 and 7.

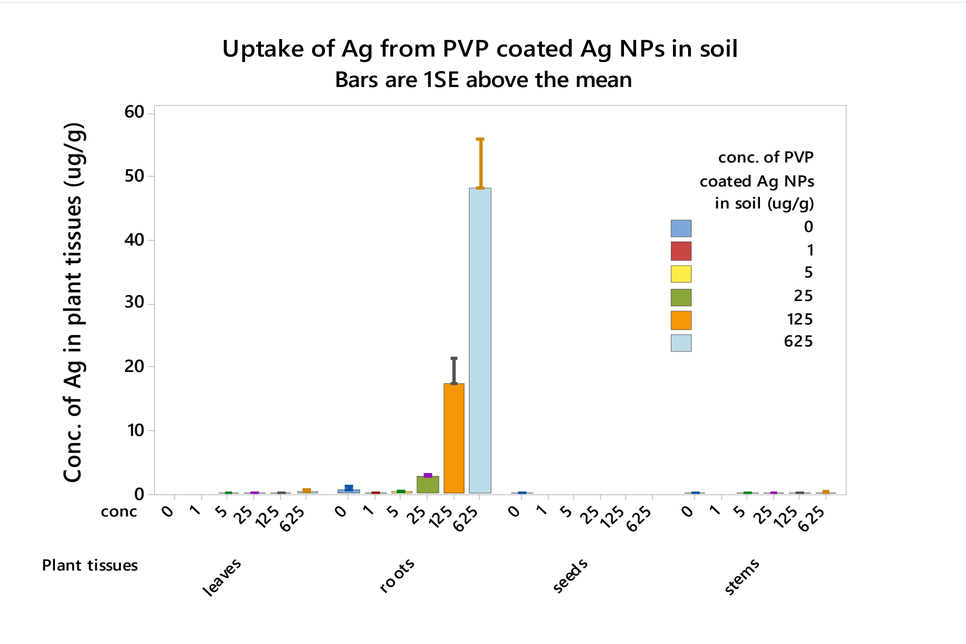

FIG. 6: LEVELS OF Ag IN *S. vulgare* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRATIONS OF COATED Ag NPs.

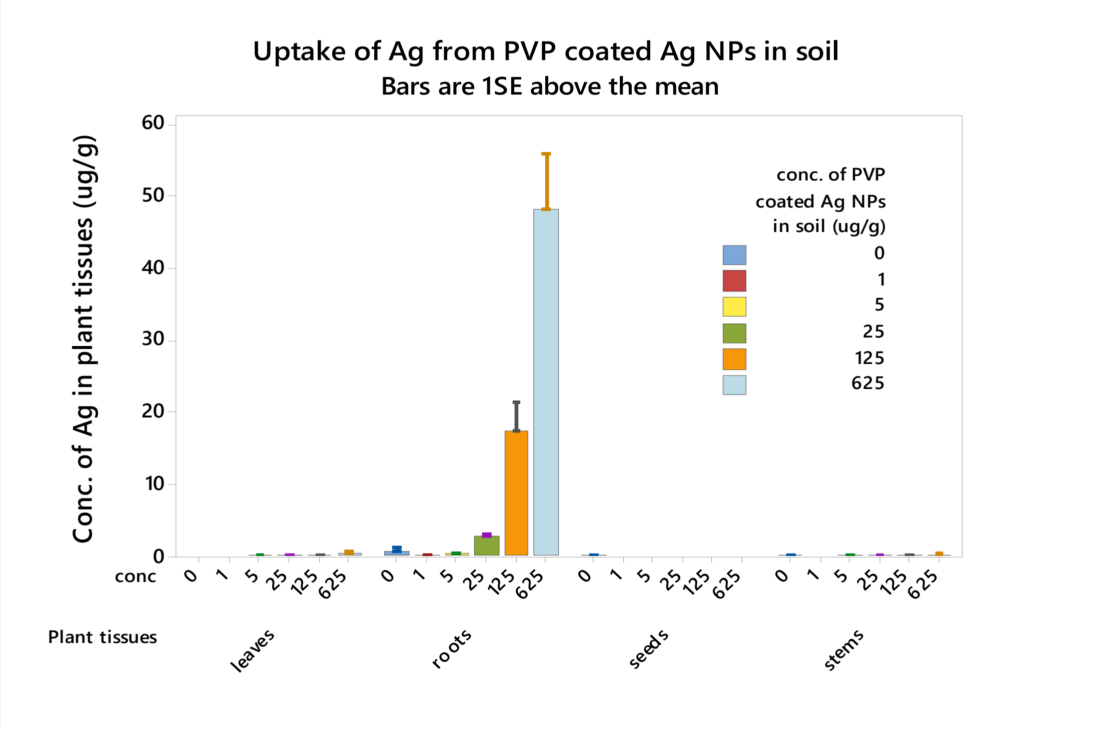

FIG. 7: LEVELS OF Ag IN *H. annuus* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRTATIONS OF COATED Ag NPs.

Fig. 8 and 9 represent the levels of Ag in tissues of *S. vulgare* and *H. annuus*, respectively, when maintained in soil spiked with different sized coated Ag NPs. Both the plants accumulated significantly higher concentrations of Ag in their roots ($p<0.05$) compared to the remaining plant tissues (stems, seeds, and leaves). Additionally, the translocation of Ag from roots to other plant tissues is observed in the case of *H. annuus*, a dicot plant (Fig. 9). No translocation phenomenon is observed in the case of *S. vulgare*, a monocot plant (Fig. 8).

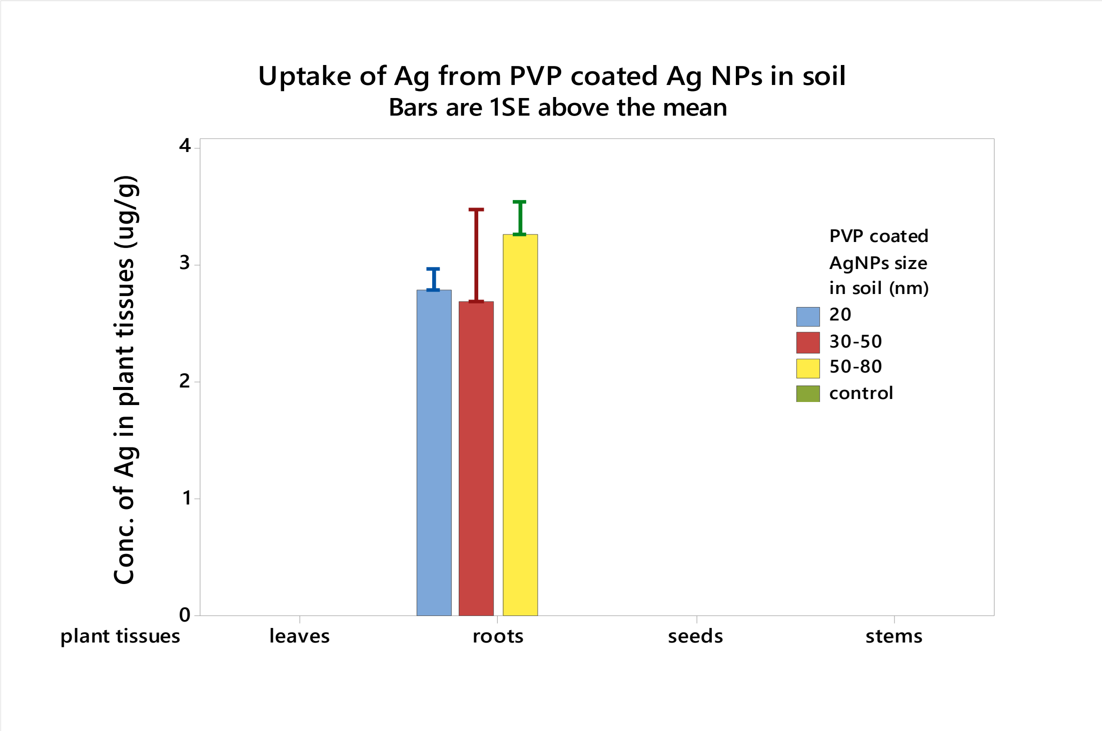

FIG. 8: LEVELS OF Ag IN *S. vulgare* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE SIZED COATED Ag NPs.

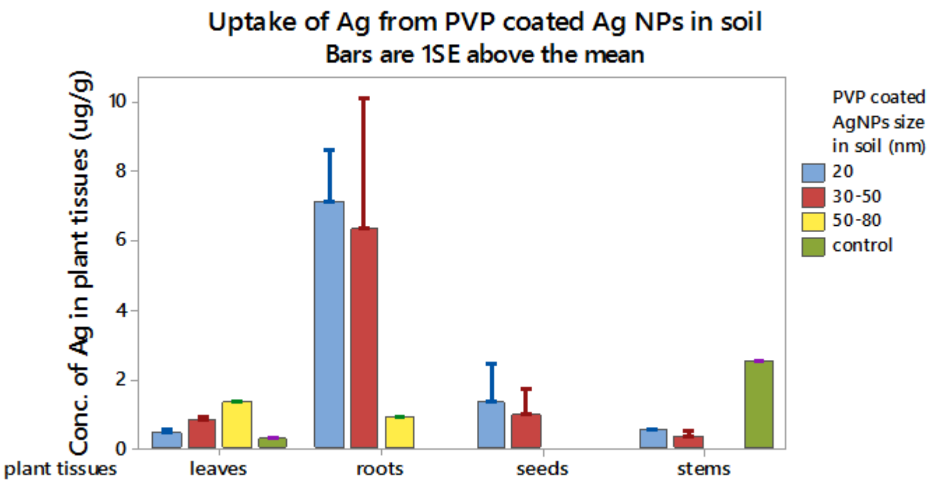

FIG. 9: LEVELS OF Ag IN *H. annuus* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE SIZED COATED Ag NPs.

Considerable accumulation of nanoparticles in the roots was observed in the case of both monocot and dicot plant species used in the study. This observation is consistent with results from other studies [27-30]. The uptake of Ag from coated Ag NPs in soil by monocots (*S. vulgare*) could be attributed to their root morphology. The presence of thin and numerous roots in monocot plants provide a very high surface area for the penetration and accumulation of nanoparticles [31]. No translocation of Ag into other plant tissues was observed in *S. vulgare*. Studies have suggested that monocot plants usually are very resistant to metal oxide and nanoparticle uptake [32-34]. On the other hand, translocation of coated Ag NPs was observed in the case of *H. annuus*, a dicot plant. The mechanism of translocation of nanoparticles to tissues such as leaves involves the accumulation of nanoparticles in plant vascular system, i.e., xylem [21]. Similarly, phloem transport explains the presence of Ag NPs in the seeds of *H. annuus* [35]. Yan et al. 2009 [36] have reported that PVP coating confers hydrophilicity to Ag NPs [36]. The hydrophilicity of coated Ag NPs could enable their translocation into various plant tissues through xylem and phloem transport mechanisms.

The differences in uptake, accumulation, and translocation of coated Ag NPs between the two plant species used in the study could also be attributed to the inherent physicochemical variations such as difference in hydraulic conductivity, pore size of the cell walls, etc [37].

Finally, PVP coating is known to prevent the oxidation of PVP-coated Ag NPs thereby affecting the chemical state of Ag NPs [6,17]. Three different Ag species, Ag^0 (Ag NPs), Ag-PVP complexes, and free Ag^+ could be present in a given suspension of PVP-stabilized Ag NPs [38]. ICP-OES measures total Ag in a sample. Hence, the effect of PVP coating on dissolution of Ag ions could not be validated in the present study. It is difficult to know for certain whether the samples contained silver ions or silver nanoparticles.

IV. CONCLUSION

The uptake of PVP-coated Ag NPs in a terrestrial system by insect and plant species was investigated. The phenomenon of aggregation of Ag NPs in the presence of a PVP coating was found to be minimal. Also, the size of PVP-coated Ag NPs was found to be larger than pristine Ag NPs. The composition of PVP-coated Ag NPs was confirmed by powder X-ray diffraction. A concentration dependent increase in the uptake of Ag from PVP-coated Ag NPs in soil was observed. No effect of size of PVP-coated Ag NPs on their uptake by the test species was observed. Considering the increasing use of Ag NPs for a variety of applications, the results from this study would be helpful in elucidating the role of coatings on Ag NPs in determining their uptake by plant and insect species. Eventually, the results from this study would also help understand the role of coatings in affecting the bioaccumulation and biomagnification of Ag NPs along the food webs.

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