International Journal of Environmental & Agriculture Research

IJOEAR

www.ijoear.com

ISSN 2454-1850

Volume-6, Issue-11, November 2020

Preface

We would like to present, with great pleasure, the inaugural volume-6, Issue-11, November 2020, 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.

Mukesh Arora
(Managing Editor)

Dr. Bhagawan Bharali
(Chief Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
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)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
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
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Dr. Goswami Tridib Kumar

Presently working as a Professor in IIT Kharagpur from year 2007, He Received PhD degree from IIT Kharagpur in the year of 1987.

Prof. Khalil Cherifi

Professor in Department of Biology at Faculty of Sciences, Agadir, Morocco.

Dr. Josiah Chidiebere Okonkwo

PhD Animal Science/ Biotech (DELSU), PGD Biotechnology (Hebrew University of Jerusalem Senior Lecturer, Department of Animal Science and Technology, Faculty of Agriculture, Nau, AWKA.

Prof. Özhan ŞİMŞEK

Agriculture Faculty, Department of Horticulture, Çukurova University, Adana, 01330 Turkey.

Dr. Anka Ozana Čavlović

Working as Professor in the department of Faculty of Forestry, University of Zagreb, Svetošimunska 25, Zagreb.

Dr. Rakesh Singh

Professor in Department of Agricultural Economics, Institute of Agricultural Sciences, Banaras Hindu University, Also Vice President of Indian Society of Agricultural Economics, Mumbai.

Dr. Sunil Wimalawansa

MD, PhD, MBA, DSc, is a former university professor, Professor of Medicine, Chief of Endocrinology, Metabolism & Nutrition, expert in endocrinology; osteoporosis and metabolic bone disease, vitamin D, and nutrition.

Dr. Ajeet singh Nain

Working as Professor in GBPUA&T, Pantnagar-263145, US Nagar, UK, India.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000- March 2002 and DBT overseas Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Dr. Mahendra Singh Pal

Presently working as Professor in the dept. of Agronomy in G. B. Pant University o Agriculture & Technology, Pantnagar-263145 (Uttarakhand).

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Prof. Salil Kumar Tewari

Presently working as Professor in College of Agriculture and Joint Director, Agroforestry Research Centre (AFRC) / Program Coordinator in G.B. Pant University of Agric. & Tech., Pantnagar - 263 145, Uttarakhand (INDIA).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Mr. Jiban Shrestha

Scientist (Plant Breeding & Genetics)

Presently working as Scientist (Plant Breeding and Genetics) at National Maize Research Programme (NMRP), Rampur, Chitwan under Nepal Agricultural Research Council (NARC), Singhdarbar Plaza, Kathmandu, Nepal.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Mr. Isaac Newton ATIVOR

MPhil. in Entomology, from University of Ghana.

He has extensive knowledge in tree fruit orchard pest management to evaluate insecticides and other control strategies such as use of pheromone traps and biological control to manage insect pests of horticultural crops. He has knowledge in agronomy, plant pathology and other areas in Agriculture which I can use to support any research from production to marketing.

Mr. Muhammad Farooq

Mphil (Food Technology) Gold Medalist

PhD Scholar, Food Science and Biological Engineering, Jiangsu University in China

Production Manager Zeal Food & Beverages Industry Aladand Road, Amandara Batkhela Malakand Agency KP. He developed the skills for Preparation of different fruit pulp preserve Orange, Peach, Guava, Strawberry, and orange squash, orange marmalade.

Table of Contents

Inoculation of (*Prosopis Laevigata*) by Arbuscular Mycorrhizal Fungi in Different Doses of Organic Matter in Two Types of Soil

Abdul Khalil Gardezi[1*], Sergio Roberto Márquez-Berber[2], Eduardo Valdés Velarde[3], Manuel Ortega Escobar[4], Miguel Jorge Escalona-Maurice[5], Gabriel Haro-Aguilar[6], Mario Ulises Larqué-Saavedra[7]

[*1,4,6] Postgraduate College, Agricultural Science, Department of Hydrosciences, Montecillo Campus, Km. 36.5 Carretera Fed. México-Texcoco, Montecillo, Texcoco, Estado de México, México, 56230.

[2]Crop Science Department, Chapingo Autonomous University, Km 38.5 Carretera Fed. México-Texcoco s/n, Col. Chapingo, Texcoco, Estado de México, Mexico, 56230.

[3]Agroforestry Center for Sustainable Development, Chapingo Autonomous University, Km 38.5 Carretera Fed. México-Texcoco s/n, Col. Chapingo, Texcoco, Estado de México,Mexico, 56230.

[5]Postgraduate College, Rural Development, Regional Geographic Analysis, Montecillo Campus, Km. 36.5 Carretera Fed. México-Texcoco, Texcoco, Estado de México, México, 56230

[7]Metropolitan Autonomous University, Azcapotzalco, Mexico

[*1]Corresponding author: Dr. Abdul Khalil Gardezi, Postgraduate College, Agricultural Sciences, Department of Hydrosciences, Montecillo Campus. Km. 36.5 Carretera Fed. México-Texcoco, Texcoco, Estado de México, México, C.P. 56230. Tel. 595-95-20200 ext. 1165, 1167 or 1171.

Abstract—*The mesquite tree (Prosopis laevigata), besides its conventional uses, has a high potential to recover agricultural areas with salinity problems. It improves the physical and chemical properties of the soil, and in the rehabilitation of degraded arid and semi-arid zones, or those tending to desertification.The aim of this research was to test the effect of organic fertilization and inoculation with Glomus sp. on mesquite trees. We did the experiment under greenhouse conditions. The effect of applying Glomus sp. and organic matter in different doses during the growth of shoots and roots was evaluated in 13 agronomic variables in mesquite seedlings grown in Lithosols and Xerosols soils. We used a complete randomized block design with three replications. After 180 days, we analyzed the data using Statistical Analysis Software (SAS) version 9.2. We observed a positive and significant effect on the growth of the agronomic variables studied under greenhouse conditions, and inoculation with Glomus sp. The organic matter factor presented significant differences (p ≤ 0.05). We determined that the ideal dose was 55 g per experimental unit. According to the soil used, positive effects (p ≤ 0.05) were observed for 50% of the agronomic variables in the Xerosols soil with relation to the Lithosols soil. We concluded that the inoculation of Prosopis laevigata with Glomus sp., and adding organic matter favors the growth of both the shots and the roots of the plant.*

Keywords— Earthworms, Glomus sp., Lithosols soil, mesquite tree, vermicompost, Xerosols soil.

I. INTRODUCTION

Mesquite [*Prosopis laevigata* (Humb. Et Bonpl. ex Willd).M.C. Johnst.], has multiple uses: as wood for fuel (firewood and coal), for construction of fences (live fences or walls of logs), for handicrafts and kitchen tools. Also, the use of its pods is for forage and food. The industry uses its resins to manufacture glues, varnishes, and other solvents. The mesquite flowers are very important for honey production because of the nectar bees collected from the trees. We can also use it as a medicinal plant to treat different diseases (Meraz *et al*., 1998; Ríos-Gómez *et al*., 2010). Mesquite trees grow in the desert and semi-desert regions of Mexico. We can find them along the central and southern Pacific coast, the arid regions of the northeast and in the central highlands of Mexico (Sauceda *et al*., 2014).

Mesquite trees and beans (*Phaseolus vulgaris* L.) are very important in the ecosystems because they can fix nitrogen and their organic matter increases the fertility of the soil when incorporated. Thus, improving the nutrition of nearby plants (Gardezi *et al*., 2016; López *et al*., 2010; Prieto-Ruiz *et al*., 2013).

Such an increase in organic matter also contributes to improving the stability and structure of the soil, reducing erosion of marginal soils, degraded soils, and tepetates soils. Tepetates are indurated earthy materials from Mexico that have been reported with different names in different countries in the USA is known as silcrete. The soil capacity for storage water increases, as does the infiltration rate. Mesquite trees have one of the highest photosynthetic rates because of their optimal use of nitrogen and water (Ruiz-Tavares, 2011).

Mesquite (*P. laevigata*.) is the only tree in the ecosystem that has a great potential to rehabilitate arid and semiarid regions prone to desertification Gardezi *et al*., (2008). Also, it can recover agricultural lands with problems of salinity in the soil and water.

Recently, there has been a growing trend to produce mesquite trees in degraded ecosystems for restoration (Prieto-Ruiz *et al*., 2013). Around nine million plants of *Prosopis laevigata* and *Prosopis glandulosa* (Torr.) were produced in 2011 for reforestation programs in Mexico per the National Forestry Commission (Comisión Nacional Forestal (CONAFOR), 2012).

On a different matter, the application of natural and biological fertilizers has received great interest by researchers because they have increased yields with a reduced ecological footprint when compared with the chemical ones (Vessey, 2003; Dadrasan *et al*., 2015). Vermicompost is compost produced when some earthworms (*Eisenia foetida, Eisenia andrei, Lumbricus rubellus,* as an example) transform organic residues into a stable sub product (Soto and Muñoz, 2002). Also, characterized by materials finely divided as peat, with high porosity, good drainage, and great moisture retention. It has a large surface able to absorb and keep essential nutrients in forms easily assimilated by plants, such as nitrates, exchangeable phosphorus, soluble potassium, calcium, and magnesium (Atiyeh *et al*., 2000a; Atiyeh *et al*., 2000b). The organic matter added to the soil improves soil properties such as density, porosity, and the capacity of water absorption (Sree Ramulu, 2001; Singh and Agrawal, 2007).

We have found that the dual inoculation with arbuscular mycorrhizal fungi (*Glomus fasciculatum*), and *Rhizobium* strains helped trees from the genus *Leucaena* and *Prosopis*to mitigate the adverse effects of sodium chloride on the growth and development of juvenile seedlings (Dixon *et al*., 1993). Therefore, the aim of this study was to test the effects of *Glomus* sp., and the application of organic matter on the shots and root growth of mesquite trees (*Prosopis laevigata*) in two different soils.

II. MATERIALS AND METHODS

2.1 Experimental site description

The study was done under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2017. We used two soil types, one red (Xerosols) and the other grey (Lithosols), with the characteristics shown in Table 1. Lithosols soils limited in depth by continuous coherent and hard rock within 10 cm of the surface. Xerosols*soils* have a weak ochric A horizon and an aridic moisture regime; lacking permafrost within 200 cm of the surface.We obtained the soil from Salinas, San Luis Potosi, Mexico (Fig. 1). The location of Salinas is 2,200 meters above sea level, its geographical coordinates are Longitude: 22º 46 '32' ', Latitude: -101º 47' 06 ".

TABLE 1
ANALYSIS OF TWO TYPES OF SOILS

Soil	SP	EC	pH	OM	N inorg	P	K	Ca	Mg	Fe	Cu	Mn	Zn
		dS m^{-1}	1:02	%	mg kg^{-1}								
Xerosol (red)	28	0.54	8.3	1.78	35.27	0.85	477.04	6413.24	110.0	12.33	0.11	19.18	1.90
Litosol (gray)	34.7	0.65	7.07	2.05	28.86	1.07	453.58	2249.79	209.6	21.96	2.81	47.96	2.28

Key: SP=Saturation point, EC=Electric conductivity, pH= Hydrogen potential, OM= Organic matter, N inorg= Inorganic nitrogen.

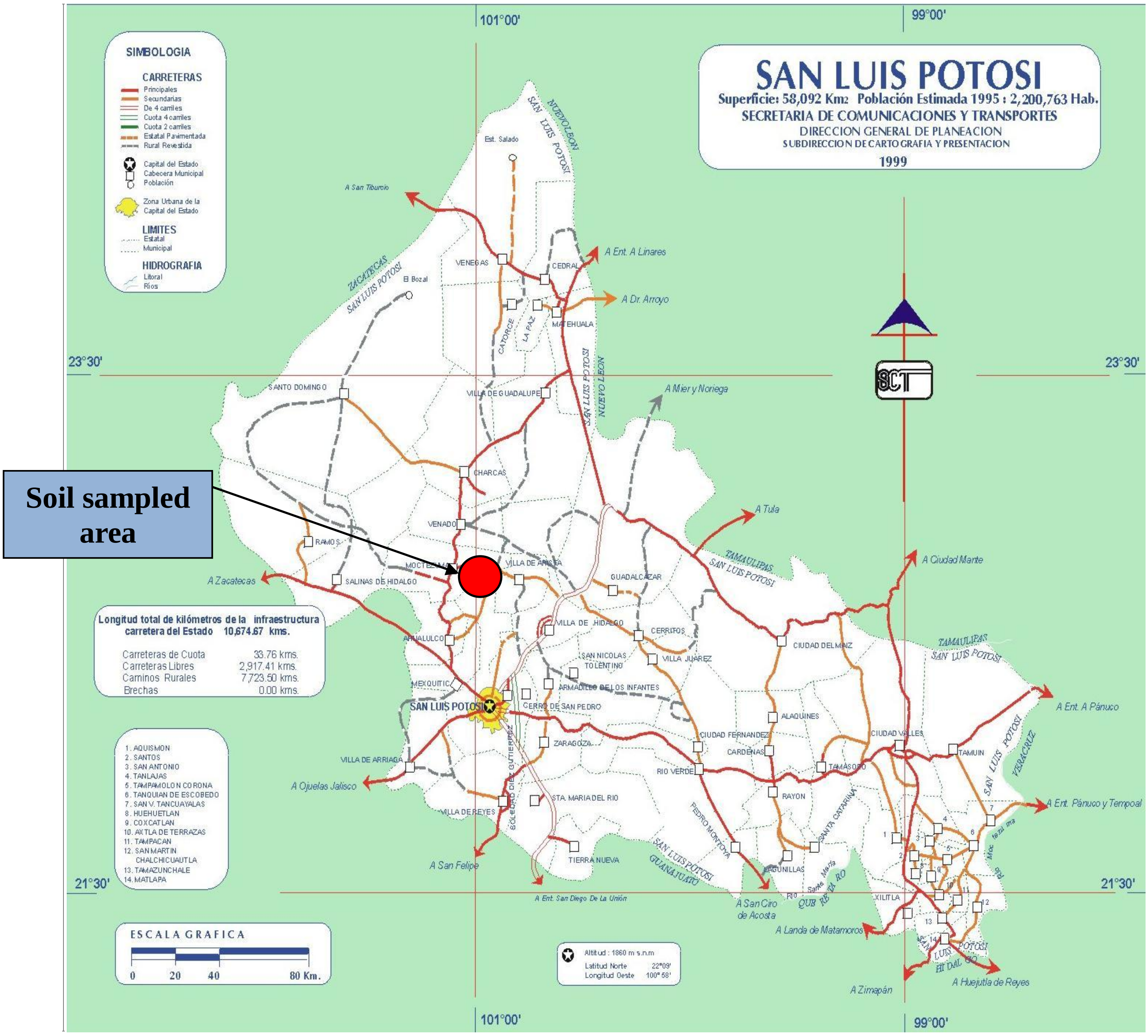

FIGURE 1: Soil sampled area at San Luis Potosi, Mexico
Source: Ministry of Communications and Transport. Mexico.

2.2 Soil analysis

We used the Walkey and Black method to determine the soil's organic matter and the Olsen method for phosphorus. We measured interchangeable bases using ammonium acetate pH 7:1 Normal (CH_3COONH_4) and micronutrients with diethylenetriamine penta-acetic acid (D.T.P.A.).

2.3 Experimental materials

The seeds of *Prosopis laevigata* were collected from Acatlán, Hidalgo State, Mexico and the inoculation were done at planting, mixing 5 g of sand with sorghum roots with 85% colonization of *Glomus sp.* and 1050 spores per 100 g of inert material. The fungus was provided from the collection of the laboratory of microbiology, Department of Agricultural Sciences, Postgraduate College. We applied two levels of mycorrhiza *Glomus* sp., (with and without *Glomus*). Then the mesquite seeds were treated with mechanical scarification (scarification drum with fine sandpaper) for five minutes. Later on, we put the seeds to germinate in foam trays with sterile red volcanic rock as a substrate.When the plants reached an average height of 5 cm, they were transplanted to black polyethylene bags with 3kg of soil and pasteurized with steam water for 4 hours for two consecutive days.

We applied organic matter as a source of vermicompost, prepared by using 60 kg of bovine manure, 25 kg of melon waste, and 15 kg of wheat straw. The mixture was subjected for four months to the action of earthworms. We put four doses of 0, 18.5 g, 37 g, and 55.5 g of vermicompost mixed in three kg bags. The equivalent of 0, 25, 50, and 75 t ha^{-1} of organic matter.

The study lasted 180 days, from planting until harvest, as recommended by Gardezi *et al*., 2009. Thirteen agronomic variables were tested: plant height (cm), the number of branches, leaf area (cm^2), the number of leaves, root length (cm), root volume (cm^3), root fresh weight (g), root dry weight (g), stem diameter (mm), leaves fresh weight (g), leaves dry weight (g), shoot fresh weight (g), shoot dry weight (g). Length measurements were taken with a ruler, diameter with a caliper, weight with an Ohaus digital scale model 605, and leaf area with a Li-Cor LI-3100C area meter.

2.4 Experimental design and treatments

A factorial arrangement with 16 treatments (4x2x2) was used with a randomized block design using three replications. An analysis of variance for all variables registered was done using SAS Computer Software version 9.2, and a Tukey means comparison test for the significant variables (Bautista-Calles *et al*., 2008).

III. RESULTS AND DISCUSSION

3.1 Primary effects

In this study, the treatments influenced all variables but root fresh weight (g). The inoculation of mycorrhiza presented the same tendency. Regarding the application of organic matter, most variables but leaf area (cm^2), the number of leaves, and root fresh weight (g) had significant differences ($p \leq 0.05$). The soils used in this experiment mainly affected the growth of the shoot part (Table 2).

The inoculation with mycorrhiza interacted with the organic matter applied in growth of the mesquite plants measured in plant height, the number of branches, root length, and root volume. Also, an interaction between the two soils used and inoculation with *Glomus sp*. was found in plant height and the number of branches. An interaction between the two soils used and the organic matter applied hada triple interaction of the organic matter, the soils, and the inoculation with *Glomus sp*. as recorded in the number of branches. Therefore, the branching of the mesquite trees was the most sensitive variable to the factors studied in this experiment (Table 2).

In a similar matter, it was found in another species of mesquite (*Prosopis articulata*), and in *Parkinsonia microphylla* a positive response to (height, the number of branches, and stem diameter) to the inoculation with plant growth-promoting microorganisms, arbuscular mycorrhizalfungi, and compost (Bashan *et al*., 2012).

TABLE 2
EFFECT OF THE TREATMENTS ON THE VARIABLES MEASURED

Source of variation	Degrees of freedom	Plant height (cm)	Number of branches	Leaf area (cm^2)	Number of leaves	Root length (cm)	Root Volume (cm^3)	Root fresh weight (g)	Root dry weight (g)	Stem diameter (mm)	Leaves fresh weight (g)	Leaves dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Treatments	15	3330.9111*	4500.1875*	5410.0764*	4040.9097*	57.2097*	80.1875*	36.9973	0.662*	2.2627*	2.9654*	0.8904*	4.205*	1.1857*
Organic matter (OM)	3	2461.8333*	2664.0208*	3677.9097	1721.6875	61.4097*	93.6875*	53.9969	1.0113*	1.9067*	4.4181*	1.6318*	5.813*	2.3528*
Soil	1	9075.0000*	12065.0208*	8295.0208*	7525.0208*	15.1875	20.0208	25.0708	0.0326	0.8008	7.5764*	1.3906*	0.6464	0.0729
OM x Soil	3	250.3889	1982.1875*	1900.5208	745.2431	8.8542	9.6319	32.1747	0.1828	0.0364	0.3116	0.3001	0.8526	0.2107
Glomus sp.	1	26226.7500*	22663.5208*	45325.5208*	33761.0208*	402.5208*	540.0208*	78.5664	4.0542*	21.3333*	19.9563*	5.542*	40.793*	9.4963*
OM x *Glomus sp.*	3	828.1389*	2895.5764*	2138.5764	2948.3542	66.7431*	106.2986*	31.3733	0.6745	1.0311	0.2668	0.0575	0.1128	0.0908
Soil x *Glomus sp.*	1	3710.0833*	4200.0208*	180.1875	42.1875	2.5208	3.5208	21.7487	0.0188	0.6075	1.0121	0.0668	0.1553	0.1863
OM x Soil x *Glomus sp.*	3	110.2500	1982.9653*	1399.7986	1013.1875	8.9653	3.4653	25.6463	0.073	0.7586	0.3154	0.1296	0.3818	0.0222
Error	32	592.8542	553.8958	1900.6458	1373.9167	15.9792	11.6875	21.7923	0.3165	0.5221	1.0169	0.3115	1.7548	0.3847
Coefficient of variation		31.7200	40.3	40.91	36.11	17.09		139.36	49.78	22.7		50.63	42.79	46.25

The numbers followed by an asterisk have significant differences ($p \leq 0.05$).

3.2 Effect of the organic matter

There was a trend to have higher values in most of the variables recorded, with the application of vermicompost. However, only the dry weight of the leaves had a significant difference between the control and the use of organic matter. Just plant height, stem diameter, shoot dry weight, and root volume showed higher values with greater application of organic matter, but the amount used did not have significant differences ($p \leq 0.05$, Table 3). Taking into account all the variables, the medium quantity of vermicompost used (37.5 g) can be recommended.

TABLE 3
EFFECT OF THE ORGANIC MATTER ON THE VARIABLES MEASURED

Organic matter *	Variables									
	X1	X2	X5	X6	X8	X9	X10	X11	X12	X13
55.5	91.667a	61.917ab	26.083a	24.75a	1.5417a	3.6333a	2.7567ab	1.2592a	3.8042a	1.8667a
37.5	84.417ab	77.083a	22.833ab	23.25a	1.0958ab	3.2667ab	3.0392a	1.3875a	3.5142ab	1.5050ab
18.5	71.75ab	52.667ab	24ab	21.5ab	1.0167ab	3.1667ab	2.7483ab	1.2008a	2.7975ab	1.1500b
0	59.167b	41.917b	20.667b	18.25b	0.8667b	2.6667b	1.665b	0.5617b	2.2667b	0.8425b

**** Grams per experimental unit.***
X1= plant height (cm), X2= number of branches X5= root length (cm), X6= root volume (cm^3), X8= root dry weight (g), X9= stem diameter, X10= leaves fresh weight (g), X11= leaves dry weight (g), X12= shoot fresh weight (g), and X13= shoot dry weight (g).
Means with the same letter within the same column are statistically equal (Tukey, $p \leq 0.05$).

It showed that the addition of organic matter to the soil increased the sustainability of the agricultural production. Organic matter has several desirable properties such as high-water retention, elevated cationic exchange capacity, it improves the availability of nutrients, and the ability to sequester contaminants (Aggelides and Londra, 2000; Weber *et al*., 2007; Asgharipour and Rafiei, 2011). Therefore, vermicompost has great potential for agriculture and horticulture as a source of nutrients (Atiyeh *et al*., 2000a; Atiyeh *et al*., 2000b).Also, the amount of compost applied improves the physical properties of the soil(Aggelides and Londra, 2000).

3.3 Effect of the soil

The Xerosol soil promoted a significantly higher growth of the shoot part of mesquite plants ($p \leq 0.05$, Table 4). The higher quantity of inorganic nitrogen, potassium, and calcium (Table 1) contributed to the better development of the plants. The quantity of nitrogen in the environment is a limiting factor for growth (Erisman, 2011).Phosphorous is another nutrient important for plants, non-available on the soil (Raghothama, 1999; Hammond and White, 2008). However, it seems in sufficient quantities in the Xerosols soils.

TABLE 4
EFFECT OF THE SOILS ON THE VARIABLES MEASURED

Soils	Variables					
	X1	X2	X3	X4	X10	X11
Xerosol	90.5000a	74.2500a	119.7100a	115.1700a	2.9496a	1.2725a
Lithosols	63.0000b	42.5420b	93.4200b	90.1300b	2.1550b	0.9321b

X1= plant height (cm), X2= number of branches, X3= leaf area (cm^2), X4= the number of leaves,X10= leaves fresh weight (g), X11= leaves dry weight (g).
Means with the same letter within the same column are statistically equal (Tukey, $p \leq 0.05$).

3.4 Effect of the mycorrhiza

The inoculation with *Glomus sp*. promoted higher root and shoot growth in the mesquite plants (Table 5). Gardezi *et al*., (2008) showed similar results with the inoculation of *Glomus intrarradices* in the same trees. They showed that high absorption of mineral nutrients caused an improvement in the growth of the plants. Adding *Glomus sp*. increase the growth of the trees in soils with lower P content, such as the case of the arboreous legume *Acacia farnesiana* (Gardezi *et al*., 1990).

Glomus sp. can stimulate the growth of plants in a better way than phosphorous fertilization (Gardezi and Ferrera-Cerrato, 1992).

TABLE 5
EFFECT OF THE INOCULATION WITH *GLOMUS SP.* ON THE VARIABLES MEASURED

Glomus sp.	**Variables**											
	X1	**X2**	**X3**	**X4**	**X5**	**X6**	**X8**	**X9**	**X10**	**X11**	**X12**	**X13**
Inoculated	100.1250a	80.1250a	137.2900a	129.1700a	26.2920a	25.2917a	1.4208a	3.8500a	3.1971a	1.4421a	4.0175a	1.7858a
Non inoculated	53.3750b	36.6670b	75.8300b	76.1300b	20.5000b	18.5833b	0.8396b	2.5167b	1.9075b	0.7625b	2.1738b	0.8963b

X1= plant height (cm), X2= number of branches, X3= leaf area (cm^2), X4= leaves number, X5= root length (cm), X6= root volume (cm^3), X8= root dry weight (g), X9= stem diameter, X10= leaves fresh weight (g), X11= leaves dry weight (g), X12= shoot fresh weight (g), and X13= shoot dry weight (g).

Means with the same letter within the same column are statistically equal (Tukey, $p \leq 0.05$).

IV. CONCLUSION

In this study, the inoculation of *Prosopis laevigata* with *Glomus* sp. favored the growth of mesquite seedlings under greenhouse conditions, shoot, and root growth increased. The Xerosols soils from the northern central highlands provided better conditions for the shoot parts. The highest quantity of organic matter applied gave the largest increase in growth. Therefore, we recommend the use of *Glomus* sp. in Xerosols soils with high quantities of organic matter for the production of mesquite plants.

REFERENCES

[1] Aggelides, S.M., and P.A. Londra. 2000. Effects of compost produced from town water and sewage sludge on the physical properties of a loamy and clay soil. *Bioresource Technology*.71 (3): (253-259). https://doi/10.1016/S0960-5824(99)00074-7.

[2] Asgharipour, M., and Rafiei, M. 2011. Effect of Different Amendments and Drought the Growth and Yield of Basil in the Greenhouse. *Advances in Environmental Biology*. 5(6): 1233-1239. ISSN 1995-0756.

[3] Atiyeh, R. M. Subler, S., Edwards, C. A., Bachman, G., Metzger, J. D., and Shuster, W. 2000a.Effects of vermicomposts and composts on plant growth in horticultural container media and soil. *Pedobiologia*.44, (5), 2000: 579-590. https://doi.org/10.1078/S0031-4056(04)70073-6.

[4] Atiyeh, R. M., Arancon, N., Edwards, C. A. and Metzger, J. D., 2000b. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. *Biores. Technol*.75 (3): 175-180. https://doi.org/10.1016/S0960-8524(00)00064-X.

[5] Bashan, Y., Salazar, B. G., Moreno, M. López, B. R., and Linderman, R.G. 2012.Restoration of eroded soil in the Sonoran Desert with native leguminous trees using plant growth-promoting microorganisms and limited amounts of compost and water. *Journal of Environmental Management*.102: 26-36. https://doi/org/10.1016/j.jenvman.2011.12.032.

[6] Bautista-Calles, F., Carrillo-Castañeda, G., y Villegas-Monter, A. 2008. Recuperaciónde la alta capacidad de germinación de la semilla de papaya mediante la tecnología de preacondicionamiento y biorreguladores. *Agrociencia* (inSpanish). 42(7): 817-826. *versión On-line* ISSN 2521-9766. *versión impresa* ISSN 1405-3195.

[7] Comisión Nacional Forestal (CONAFOR). 2012. Evaluación complementaria del PROCOREF. Ejercicio fiscal 2011. Universidad Autónoma Chapingo (in Spanish).

[8] Dadrasan M., Chaichi M.R., Pourbabaee A.A., Yazdani D., and Keshavarz-Afshar R.2015. Deficit irrigation and biological fertilizer influence on yield and trigonelline production of fenugreek. *Industrial Crops Products*.77: 145-162. https://doi/10.1016/j.indcrop.2015.08.040.

[9] Dixon, R.K.; Garg, V.K. and Rao, M.V. 1993. Inoculation of Leucaena and Prosopis seedlingswith Glomus and Rhizobium species in saline soil: Rhizosphere relations and seedling growth. *Arid Soil Research and Rehabilitation*.7(2):133-144. https://doi/10.1080/15324989309381343.

[10] Erisman, J.W. 2011. The New Global Nitrogen Cycle in Ecological Aspects ofNitrogenMetabolism in Plants (Eds. J. C. Polacco and C. D. Todd), *John Wiley & Sons, Inc., Hoboken, NJ, USA.* Pp. 5-15. https://doi/org/10.1002/9780470959404.ch1.

[11] Gardezi, A.K., J.D. Contreras D., J., Guzman-Plazola, R.A, and Ferrera-Cerrato, R., 1990.Growth of *Acacia farnesiana* associated with mycorrhizal fungi in three types of Mexican soils. *Nitrogen Fixing Tree Research Reports*.8: 99-102.

[12] Gardezi, A.K. and R. Ferrera-Cerrato. 1992. Mycorrhizal inoculation of*Caesalpinea cacalaco*.*Nitrogen Fixing Tree Research Reports*.10: 116-118.

[13] Gardezi, A. K., Gardezi, H.S., Ojeda-Trejo, E. y Márquez-Berber, S.R. 2008.Respuesta a la inoculación de *Glomus intraradix*, materia orgánica y dosis de fertilización fosfatada en el crecimiento de mezquite (*Prosopis* sp.). *Agroproductividad* (in Spanish).1(6): 24-28.

[14] Gardezi, A.K., Exebio-Garcia, A., Mejia-Saenz, E., Ojeda-Trejo, E., Tijerina-Chavez, L.,Gardezi, Habibsha, and Delgadillo-Pinon, M. 2009. Sewage Water Irrigation and Growth Response of *Leucaena leucocephala* Inoculated with*Glomus intrarradices* and Application of Organic Matter. *Journal of Applied Science*.9:1373-1377**.**URL: https://scialert.net/abstract/?doi=jas.2009.1373.1377.

[15] Gardezi, A.K., Márquez-Berber, S.R., Martínez-Menez, M., Flores-Magdaleno, H.,Escalona-Maurice, M.J., M.U. Larqué-Saavedra, and G. AlmaguerVergas, 2016. Soil Contamination and its Effects on Beans (*Phaseolus vulgaris* L.) Growth Affected by Organic Matter and Associated with *Glomus intrarradices*. *European Scientific Journal*,special edition: ESJ June 2016, Special Edition,107-118. https://dx.doi.org/10.19044/esj.2016.v12n10p%25p*On-line version* ISSN 1857-7431. *Print version* ISSN 1857-7881.

[16] Hammond, J.P., and White, P.J. 2008. Diagnosing phosphorus deficiency in cropplants. In: White, P.J., and J.P. Hammond (Eds.), The Ecophysiology of Plant-Phosphorus Interactions. *Plant Ecophysiology*, vol 7. Springer, Dordrecht. Pp. 225-246.*On-line version* ISBN 978-1-4020-8434-8. *Print version* ISBN: 978-1-4020-8435-5.

https://doi.org/10.1007/978-1-4020-8435-5_10.

[17] López H., J. A., J. C. Ríos S., J. C. Monárrez G., R. Rosales S., J. M. Mejía B. yV.Bustamante G., 2010. Tecnología disponible para la obtención de semilla de mezquite en el norte de México. *Folleto Técnico Núm. 45. Campo Experimental Valle del Guadiana. INIFAP. Durango, Dgo. México* (in Spanish).39 pp.

[18] Meraz V., S., J. Orozco V., J. A. Lechuga C., F. Cruz S. y J. Vernon C., 1998. Elmezquite,árbol de gran utilidad. *Ciencias* (in Spanish).51: 20-21.

[19] Prieto Ruiz, J. A., Rosales Mata, S., SigalaRodriguez, J. A., Madrid Aispuro, R. E.,y Mejía Bojorques, J. M. 2013. Producción de *Prosopis laevigata* (Humb, et Bonpl ex Wild) MC Johnst. con diferentes mezclas de sustrato. *Revista Mexicana de Ciencias Forestales* (in Spanish). 4(20): 50-57. *versión impresa* ISSN 2007-1132.

[20] Raghothama, K.G. 1999. Phosphate acquisition. Annual Review of *PlantPhysiology and Plant Molecular Biology*.Vol. 50: 665–693.https://doi/.org/10.1146/annurev.arplant.50.1.665.

[21] Ríos-Gómez, R., Salas-García, C.E., Monroy-Ata, A., and Solano, E. 2010. Salinityeffect on*Prosopis laevigata* seedlings. *Terra Latinoamericana*.28(2): 99-107.

[22] Ruiz Tavares, D. R. 2011. Uso potencial de la vaina de mezquite para laalimentaciónde animales domésticos del altiplano potosino. *Tesis de título facultad de maestría en ciencias ambientales* (in Spanish). Universidad Autónoma de San Luis Potosí. 60 pp.URL:http://ninive.uaslp.mx/xmlui/handle/i/3648.

[23] Sauceda, R. E.N.; Rojo, M. G. E.; Ramírez, V. B.; Martínez, R. R.; Cong, H. M. dela C.; Medina, T. S. M. y Piña, R. H. H. 2014. Análisis técnico del árbol del mezquite (*Prosopis laevigat*a. & Bonpl. Ex Wild.) en México. *Ra Ximhai* (in Spanish). vol.10, núm. 3, enero-junio, 2014, pp. 173-193.*versión impresa* ISSN 1665-0441.

[24] Singh, R.P. and Agrawal, M. 2007. Potential benefits and risks of land applicationof sewage sludge. *Science Direct*.28: 347-358. https://doi.org/10.1016/j.wasman.20016.12.010.

[25] Soto, G., and Muñoz, C. 2002. Consideraciones teóricas y prácticas sobre elcompostysu empleo en la agricultura orgánica. *Manejo Integrado de Plagas*(in Spanish). (Costa Rica). (65):123-129.

[26] Sree Ramulu, U.S., 2001. Reuse of municipal sewage and sludge in agriculture.*Scientific Publishers*, Jodhpur, India. https://books.google.com.mx>books.

[27] Vessey J.K. 2003. Plant growth-promoting rhizobacteria as biofertilizers. *Plant andSoil*.255(2): 571 586.

https://doi.org/10.1023/A:1026037216893.*Print version* ISSN 0032-079X*On-line version* ISSN 1573-5036.

[28] Weber, J., Karczewska, A. Drozd, J. Licznar, M.,Licznar S.,Jamroz, E., andKocowicz, A.2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biol. Biochem*.39(6): 1294-1302.ISSN 0038-0717.

Cattle Ticks and Risk Factors Related to Tick Infestation of Livestock in Periurban Farms in Southern Cote D'ivoire

Claude Aimée DIAHA-KOUAME[1*], Valery Edgard ADJOGOUA[2],
Marc Hermann AKAFFOU[3], Egomli Stanislas ASSOHOUN[4],
N'guessan Fidèle DIOBO[5], Konan Adjoua Rose Marie Clémence KOUASSI[6]

[*1,2,3]Institut Pasteur de Côte d'Ivoire
[4]Laboratoire de mathématiques appliquées et informatique UFR-MI (UFHB), Côte d'Ivoire
[5]Université Félix Houphouët-Boigny, UFR Biosciences 22 BP 582 Abidjan 22, Côte d'Ivoire
[6]Université Nangui Abrogoua Abidjan, Côte d'Ivoire

***Abstract*—** *Tick-borne diseases are a global public health problem, particularly in sub-Saharan Africa, where most of the disease is caused by malaria and many other diseases of viral, parasitic or bacterial origin. This study aimed to identify the bovine tick's species in cattle farms and to determine possible risk factors related to tick infestation in Abidjan district and Azaguié commune. Thus, in July 2019, thirteen (13) herds distributed in these localities were visited for tick sampling and to conduct epidemiological investigations. At each visit, ticks were harvested from 15 cattle per herd. All the farms visited were infested with ticks. 96.92% of sampled animals had ticks. A total of 1796 ticks were collected of which 89.42% (1606) were adults, 10.41% (187) were pupae and 0.17% (3) was larvae. Two species of ticks have been identified, Amblyomma variegatum with 25% of the population and Rhipicephalus (Boophilus) microplus with 75%. 96% of the cattle were infested by ticks of the species R. (B.) microplus and 56% of the cattle were infested by ticks of the species A. variegatum. The co-infestation of cattle by the two identified species was 53%. The distribution of the sexes showed that in the species A. variegatum, males were more numerous (13.44% for males and 8.76% for females). However in the species R. (B.) microplus, females were more numerous (5.08% for males and 62.3% for females).The analysis of risk factors associated with tick infestation in cattle has shown that factors such as Undefined parks, Type of pasture, Training in the use of acaricides and Presence of wild animals contribute to major ectoparasite infestations in cattle. Tick samples collected from peri-urban farms in the district of Abidjan and the locality of Azaguié as part of this study, indicate that the relatively recent introduction of the species Rhipicephalus (Boophilus) microplus presents a threat to animal and human health.*

Keywords— Ticks, Rhipicephalus (Boophilus) microplus, Risk factors, Côte d'Ivoire.

I. INTRODUCTION

Among the potentially emerging diseases, those transmitted by arthropods, particularly ticks, are very numerous. Ticks pose a number of problems in human and veterinary health by their direct nuisance following their bites, but also by the infectious agents they are likely to transmit (Aubry & Gaüzère, 2016). These ectoparasites play a major role in human and animal epidemiology. Thus, they transmit a greater variety of pathogens than any other group of arthropods and are among the most important vectors capable of infecting both humans and domestic animals (Boyard, 2007).Tick-borne diseases cause enormous economic damage to livestock farmers and according to Guerrero *et al.,* (2014), the economic impact of ticks is major, especially in areas where cattle breeding is an important source of income. Also in terms of animal and human health, the consequences can be fatal and can even lead to death if the intervention is late or even leave physical and cognitive after-effects that can be very disabling if they are not treated early enough in humans.

To explain the spread of tick-borne diseases the study of the properties of the pathogen is therefore insufficient (Estrada-Peña*et al.,* 2015). And according to Brownstein *et al.* (2005) the notion of acarological risk must be taken into account, integrating both the notions of vector abundance in the environment and the prevalence of infection among the vector population. Given the central role of the vector in explaining cases of tick-borne diseases, knowledge of its lifestyle and distribution is an essential asset in the fight against these diseases. Tick-borne diseases pose real public health problems around the world and particularly in sub-Saharan Africa, where most diseases have very often been overlooked in favour of major viral or bacterial epizootics. In the district of Abidjan and its suburbs, there is strong demographic growth followed by galloping urbanisation. According to United Nation estimates, urban dwellers accounted for one third of the world's population in 1950, whereas they accounted for more than half in 2015 and will probably account for two thirds in 2050 (United Nations, 2014).In the face of this rapid urbanisation, previously isolated agricultural farms are now found close to the houses. This proximity can thus pose a threat to the population because the human-animal interface is much reduced. In the general context of assessing the infectious risk of tick-borne pathogen transmission, it is necessary to study the distribution of species present in an environment where human populations and farmed animals come into frequent contact. This study aims to identify bovine tick species in peri-urban cattle farms in Abidjan district and Azaguié commune and to determine possible risk factors related to ectoparasitic infestation.

II. MATERIAL AND METHODS

2.1 Study area

This study took place in the south of Côte d'Ivoire, precisely in the district of Abidjan and the locality of Azaguié. The district of Abidjan is bordered to the south by the Atlantic Ocean. It has a humid tropical climate with two dry seasons (from December to April, then in July and September) and two rainy seasons (from May to July, then in October and November) (Tapsoba, 1995).The temperature varies between 24 and 31°C. The district of Abidjan has a surface area of 2119 km², with a body of water representing about 15% of this surface. The district of Abidjan, the capital of Cote d'Ivoire, has a large part of forest vegetation in the image of its Banco National Park and protected areas. Four communes in the district of Abidjan (Cocody, Port-Bouet, Yopougon and Songon) and Azaguié commune were visited for sampling. In all these localities, thirteen (13) peri-urban cattle farms were visited. All farms were georeferenced using a Garmin Etrex 20 GPS (Figure 1).

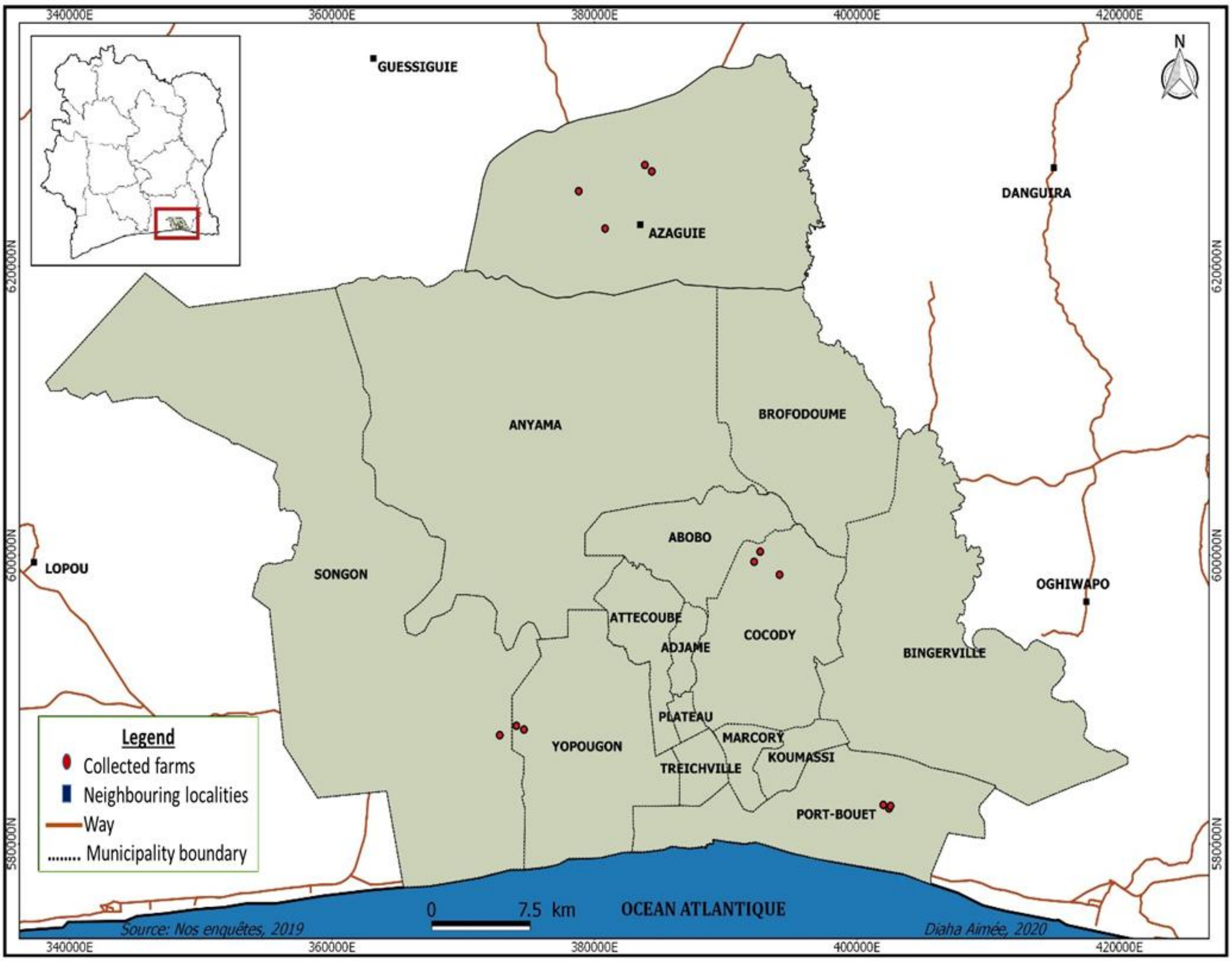

FIGURE 1: Location of farms

2.2 Sampling and conservation of ticks

In July 2019, thirteen (13) peri-urban cattle farms were visited. During each of these visits, for an average of 5 minutes per animal, ticks were collected from 15 animals over one year old. A questionnaire was also submitted to the manager of each farm in order to collect information on the farms and data that would allow us to characterize the different herds in our sample. For the collection of ticks, the animals were restrained in the restraint corridors for those farms that had them and on the ground for those that did not, with the support of farm workers. The ticks collected from each animal were placed in 10 ml collection tubes containing 70% ethyl alcohol for preservation. Each tube was subsequently identified with a tag indicating the location, date of collection, age, sex and identification number of the animal collected.

2.3 Collection of farm information

In order to describe the cattle tick population present on the farms visited and to establish the risk factors for cattle tick infestations, a questionnaire was developed. This questionnaire included information on epidemiological data on the cattle on the farm, and information on the environment. This questionnaire was completed at the time of tick harvesting and completed during interviews with each farm owner or manager. This questionnaire includes the following information: physical characteristics of cattle; overall health characteristics; habitat; feeding; geographical location by GPS; distance of farms from human habitations; type of farm construction; number of people caring for animals; level of education of the people caring for animals; health history of the farms; other domestic animals in vicinity of cattle; tick control; description of local ecology.

2.4 Ticks identification and conservation

After harvests, all samples were sent to laboratory of Institut Pasteur de Côte d'Ivoire (IPCI) for diagnosis. All adult specimens, nymphs and larvae were identified by microscopic examination to confirm species and sex using standard taxonomic keys. Thus identification was carried out according to stasis, genus and species. It was carried out using an OPTIKA binocular magnifier (G x 10 or G x 20) and identification keys (Walker *et al.*, 2003; Meddour-Bouderda & Meddour, 2006; Apanaskevich &Horak, 2007; 2009).

2.5 Statistical analysis

The data collected was entered using Microsoft Office Excel version 2013. Data processing was carried out using R software version 4.0.0 (multiplatform software: Windows, linux and Mac OS X downloadable at http://cran.r-project.org). The Student t-test was used to compare means of two sample groups.

III. RESULTS

3.1 Frequency of tick genera and stasis of collected ticks

In thirteen farms visited, ticks were sampled from 195 cattle. Of these, 189 were tick carriers, representing an infestation rate of 96.92%. The total number of ticks collected was 1796, of which 89.42% (1208) were adults, 10.41% (187) were nymphs and 0.17% (3) was larvae. The identification revealed the presence of two (2) distinct genera. These are the genus *Amblyomma* and the genus *Rhipicephalus*. All ticks of the genus *Rhipicephalus* belong to the subgenus *Rhipicephalus (Boophilus)*.

The distribution of different stasis in the genus *Amblyomma* and the subgenus *Rhipicephalus (Boophilus)* showed that in the genus *Amblyomma*, males were more numerous with 13.44% and 8.76% for females. On the other hand, in the subgenus *Rhipicephalus (Boophilus)*, females were more numerous with 62.3% and 5.08% for males (Figure 2).

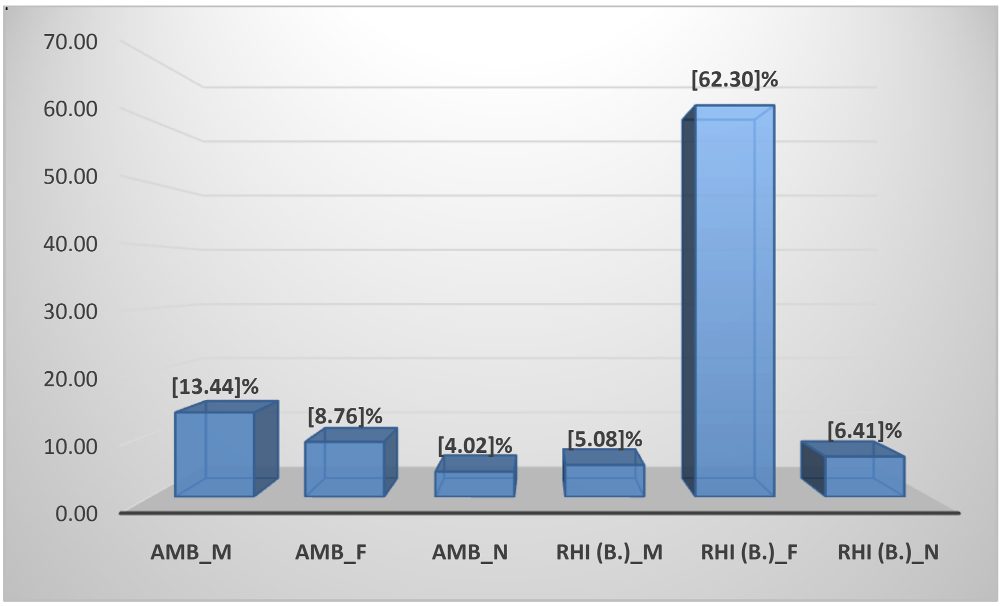

FIGURE 2: Frequency of Tick Stasis

AMB_M :male *Amblyomma*, AMB_F :female *Amblyomma*, AMB_N :nymph *Amblyomma*, RHI (B.)_M :male *Rhipicephalus (Boophilus)*, RHI (B.)_F :female *Rhipicephalus (Boophilus)*, RHI (B.)_N :nymph *Rhipicephalus (Boophilus)*

3.2 Tick species identified

After identification of ticks, the two genera obtained were represented by one species each. The infestation rates of animals by two tick species were 25% for *Amblyomma variegatum* (Fabricius, 1794) and 75% for *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) (Figure 3).

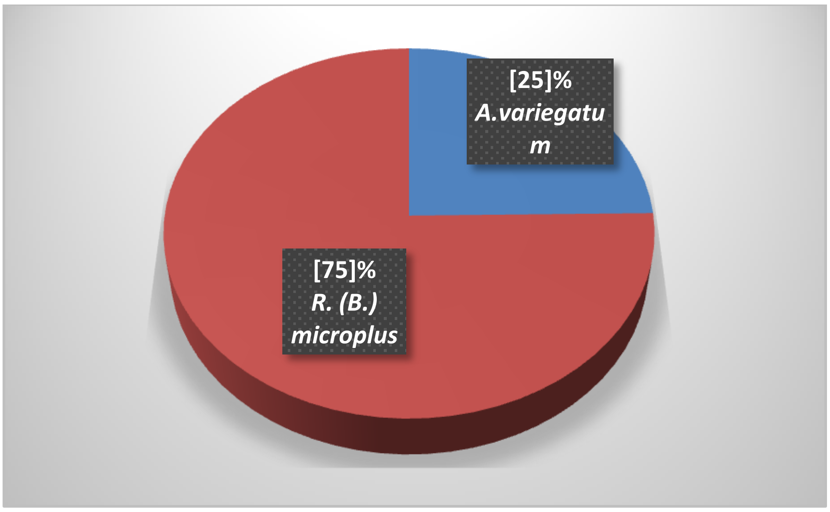

FIGURE 3: Proportion of Tick Species

3.3 Infestation of Cattle by Ticks

Of all animals sampled, 96.92% had ticks. All infested steers were parasitized by the species *Rhipicephalus (Boophilus) microplus* (96.92%) and 56% of beefs were infested by ticks of the species *Amblyomma variegatum*. The co-infestation of cattle by two identified species was 53%.

TABLE 1
PREVALENCE, INTENSITY AND ABUNDANCE OF TICK INFESTATION IN CATTLE

Ticks number	Moyenne (Ecartype)	Min	Med	Max	IQR (CV)
	138.2 (71.4)	60	127	348	51 (0.5)
Ticks species	**Prévalence %** [a]	**Mean intensity**[b]	**Mean abundance**[c]	**% of beef infested**	
Rhipicephalus (Boophilus) microplus	98,94	6,46	6,19	96,92	
Amblyomma variegatum	57,67	3,65	2,04	53	

a. Number of infested beefs by a tick species/number of infested beefs × 100
bNumber of collected tick species/number of infested buffaloes by a tick species
cTotal number of collected a tick species/total number of analyzed beefs

3.4 Analysis of the risk factors associated with cattle infestation by tick

Following the questionnaires submitted to each farm manager, a summary of the characteristics of farm, history of tick infestation and pasture management is carried out in order to determine risk factors associated with tick infestation in cattle for all farms. The student T-test applied to our data showed that four factors were significant. These were: undefined parks, type of pasture, training in the use of acaricides and presence of wild animals (Table 2).

TABLE 2
RISK FACTORS RELATED TO THE PRESENCE OF TICKS ON FARMS

Variable	Modality	Freqs (% of Valid)	Mean (sd)	P value
Location of farm	Dense vegetation	3 (23.1%)	149.3 (76.76)	0.469
	Clear vegetation	10 (76.9%)	101(36.75)	
Herd size	Large (more than 150 heads)	4 (30.8%)	97.75 (30.71)	0.079
	Small (less than 150 heads)	9 (69.2%)	156.11 (78.14)	
Type of pasture	Mixte	3 (23.1%)	93.67 (6.03)	
	Native	10 (76.9%)	151.5 (76.97)	0.049
Presence of wild animals	No	3 (23.1%)	88.67 (25.01)	0.047
	Yes	10 (76.9%)	153 (74.76)	
Training in the use of acaricides	No	12 (92.3%)	137.08 (74.42)	0.042
	Yes	1 (7.7%)	151 (NA)	
Effect of acaricides on ticks	Effective	0 (0%)	0 (NA)	NA
	Not effective	13 (100.0%)	138.15 (71.36)	
Type of speculation of breeding	Meat	9 (69.2%)	151.78 (79.53)	0.414
	Milk-meat	4 (30.8%)	107.50 (41.15)	
Delimited park	No	12 (92.3%)	144.67 (70.38)	0.042
	Yes	1 (7.7%)	60 (NA)	
Containment corridor	Absent	10 (76.9%)	149.3 (76.76)	0.174
	Present	3 (23.1%)	101.0 (36.76)	
Education	Cannot read or write	10 (76.9%)	149.3 (76.76)	0.174
	Can read and write	3 (23.1%)	101.0 (36.76)	
Tick treatment	Some of the animals	12 (92.3%)	145.00 (70.38)	0.154
	All animals	1 (7.7%)	60 (NA)	
Treatment method used	Manual sprayer	12 (92.3%)	145.00 (70.38)	0.154
	Pour-on	1 (7.7%)	60 (NA)	
Frequency of tick treatment	Upon observation of ticks	13 (100.0%)	138.15 (71.36)	NA
	Every week	0 (0%)	0 (NA)	

IV. DISCUSSION

Emerging diseases in humans are thought to be zoonoses in more than 60-70% of cases. Thus, faced with an increase in the incidence of tick-borne diseases, it would be better to know the species of ticks present in our environment in order to be able to control potential vector-borne diseases. This study on ticks in the district of Abidjan and the commune of Azaguié in the south of Côte d'Ivoire has shown the presence of two genera which are: *Amblyomma* and *Rhipicephalus*. In Côte d'Ivoire, these genera have also been highlighted in earlier work (Achi *et al.*, 2011; Diaha-Kouamé 2013; Amoia, 2015; Diaha-Kouamé 2017).In this study, the two genera obtained were represented by one species each, *Amblyomma variegatum* and *Rhipicephalus (Boophilus) microplus*. The latter was the predominant species with a proportion of 75%. The work carried out by Knopf *et al*. (1999) and Achi *et al.*, (2011) showed that *A. variegatum* was the predominant tick species in cattle farming. It should also be noted that in Côte d'Ivoire (CI), the species *R. (B.) microplus* was first identified in 2007 in Azaguié (Madder *et al.,* 2007). Since then, several studies have shown that this species is even in the majority in several large breeding areas of Côte d'Ivoire (Madder *et al.,* 2011; Touré *et al.,* 2012; Diaha-Kouamé 2013; Boka *et al.,* 2014, Amoia, 2015; Diaha-Kouamé, 2017).In this study we observed a decrease in the specific diversity of tick species. Indeed, previous studies carried out in these regions had recorded several other species. Among these species, we noted the presence of other species of subgenus *Rhipicephalus (Boophilus)* such as *Rhipicephalus (Boophilus) decoloratus* (Koch, 1844), *Rhipicephalus (Boophilus) geigyi* (Aeschlimann & Morel, 1965) and *Rhipicephalus (Boophilus) annulatus* (Say, 1821). The fact is that since the introduction of the species *R. (B.) microplus* in Côte d'Ivoire, these species have been identified in very small proportions or even non-existent in certain farms (Madder *et al.*, 2011; Touré *et al.*, 2014; Diaha-Kouamé, 2017).This has been observed in our study where we note a complete elimination of these species to the detriment of *R. (B.) microplus* which is the species newly introduced into Côte d'Ivoire. This study still shows the invasion capacity of *R. (B.) microplus*, as shown by Madder *et al.* (2011)in the Azaguié region and also by Diaha-Kouamé, (2017) on the transhumance corridor between Côte d'Ivoire and Burkina Faso where a small proportion of these species (0.02% for *R. (B.) decoloratus*, 0.49% for *R. (B.) annulatus* and 0.30% for *R. (B.) geigyi)* were obtained. The importance of ticks lies in particular in the fact that they can be vectors of disease. It is therefore important to be aware of their way of life, as well as the areas where animals are likely to become infested, in order to control and prevent diseases transmitted by these mites. Very little is known about the risk factors associated with the presence of ticks in cattle, particularly in the rapidly urbanising southern Côte d'Ivoire region.

In this study the analysis of risk factors associated with tick infestation in cattle showed that factors such as undefined parks, type of pasture, training in the use of acaricides and presence of wild animals would contribute to major ectoparasite infestations in these animals. The fact that the parks are not indefinite and the fact that the animals go to feed in the natural environment favour frequent contact between farmed and wild animals. The presence of wildlife is then very strong in most cases. And according to (Aubry & Gaüzère, 2016) the reservoir of several tick species is represented by wild rodents. According to these authors, risk areas should be avoided through the use of marked paths for leisure activities in the forest and contact with wild animals. It should also be noted that these increasing contacts between wild and domestic fauna and humans are progressively favouring exchanges of pathogens that may have harmful sanitary consequences on the three compartments. As is the case at the periphery of protected areas in southern Africa where these health risks are easily manifested in cases where livestock co-exist with wildlife species that have co-evolved with major livestock pathogens (Jori, 2017). There is therefore a growing interest in the health of populations around the world in relation to these wild animal species. According to (Wiethoelter *et al.*, 2015), this progressive interest is justified by the fact that, generally speaking, the global transformations of our planet have progressively favoured interactions between human and wild animals populations and are shaping what is known as the interface between wild and anthropised environments. It is an interface in which animals (wild and domestic) and humans interact and promote the circulation and transmission of their infectious agents. One such disease is African Swine Fever (ASF) transmitted by a double-stranded DNA virus of the Asfaviridae family. Its epidemiology and ecology includes both direct transmission between infected domestic pigs and/or wild boar and vector transmission (by soft tick bites) (Jori, 2017).Further research is therefore needed to better understand the association between the increasing environmental risk in the region and exposure to human disease and other emerging tick-borne infections (Kulkarni et *al.*, 2017). A study carried out in Brazil has shown that buffaloes reared in the municipality of Santarém have different levels of tick and lice infestation depending on the direct influence of the characteristics of the Amazonian ecosystem. Thus, the floodplain environment, which is widely used for buffalo breeding, contributed to minor ectoparasite infestations in these animals (Batista *et al.*, 2018).Measures to prevent tick-borne diseases include eliminating ticks by applying acaricide to animals and reducing the habitat of ticks in the environment. Livestock farmers generally control ticks because of the repugnance associated with heavy animal infestations but most are unaware that ticks can transmit diseases to animals and more often than not the animals are carriers of pathogens but are asymptomatic. In this study the lack of training in acaricide applications would contribute to the infestation of cattle. Indeed, the work of Furlong, (2004) showed that the appearance and then the evolution of tick resistance to acaricides are due to the inappropriate use of chemical acaricides in several regions. According to Aubry & Gaüzère, (2016) reducing and controlling tick populations is very difficult and that no single measure is therefore sufficient to completely prevent tick infestation in environments where ticks are present, whether in humans or animals. A study in livestock farms in northern Côte d'Ivoire showed that antibiotic doses and acaricide dilutions were not appropriate for the treatment of animals. Under dilution and overdoses of the drugs were therefore more observed (Yéo *et al* 2017). The lack of health monitoring in this type of livestock farming is frequent and represents a considerable and neglected health risk.

V. CONCLUSION

The identification of tick species and the knowledge of possible risk factors related to ectoparasitic infestation are very important in the framework of the fight against these parasites and the prevention of diseases that ticks can transmit. Two tick species were identified in this study, *Amblyomma variegatum* with 25% of the population and *Rhipicephalus (Boophilus) microplus* with 75%.The analysis of risk factors associated with tick infestation in cattle has shown that factors such as undefined parks, type of pasture, training in the use of acaricides, presence of wild animals contribute to major ectoparasite infestations in cattle. Entomological and microbiological monitoring is therefore necessary as it could help to anticipate an epidemic event. Vector surveillance and tick control measures should therefore be improved as part of a One Health approach. These initiatives will therefore need to be brought together in order to map the risk and distribution of ticks in Côte d'Ivoire, as well as the pathogens they carry. This knowledge will be used to guide prevention and control actions.

REFERENCES

[1] Achi YL, Koné P, Stachurski F, Betschart B. Impact des tiques sur des bovins métis dans le nord de la Côte d'Ivoire. Bulletin of Animal Health and Production in Africa, 2012, 53 (2):139-145.

[2] Amoia C. Diagnostic des pratiques de lutte anti-tique et distribution spatiale de la tique invasive *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) en Côte d'Ivoire. Mémoire de Master, Université Nangui Abrogoua Abidjan, 2015, 77 p.

[3] Aubry P, Gaüzère B A. Maladies transmises à l'homme par les tiques, Médecine vétérinaire 2016 10 p

[4] Baffi MA, De Souza GR, De Sousa CS, Ceron CR, Bonetti AM. Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of *Riphicephalus (Boophilus) microplus* (Acari, Ixodidae). Mol Biochem Parasitol, 2008 ; 16: 70-73.DOI: 10.1016/j.molbiopara.2008.03.009

[5] Batista H R, Sarturi C, Stelmachtchuk F N, Oliveira DR, Morini AC, Gennari SM, Marcili A, Bastos FAN, BarataLES, Minervino AHH.Prevalence and risk factors associated with ectoparasite infestation of buffaloes in an Amazonian ecosystem Parasites & Vectors; 2018, 11:335 https://doi.org/10.1186/s13071-018-2917-2

[6] Boka OM, Madder M, Achi YL, Kaboret YY, Berkvens D. Modélisation du remplacement de *Rhipicephalus (Boophilus) decoloratus* par *Rhipicephalus (Boophilus) microplus*, une tique émergente en Côte d'Ivoire, European Scientific Journal, 2014 ; 10: 120-132. DOI: 10.1007/s10493-017-0129-7

[7] Boyard C. Facteurs environnementaux de variation de l'abondance des tiques *Ixodes ricinus* dans des zones d'étude modèles en Auvergne. Thèse de Doctorat, Universite Blaise Pascal, Universite d'auvergne, 2007, 233 p.

[8] Brownstein JS, Holford TR, Fish D. Effect of climate change on Lyme disease risk in North America. Ecohealth, 2005, 2: 38-46. DOI: 10.1007/s10393-004-0139-x

[9] Diaha-Kouamé CA. Caractérisation des tiques le long du couloir de transhumance ivoiro-burkinabé et évaluation de l'activité acaricide de quatre (4) plantes locales sur *Rhipicephalus (Boophilus) microplus* (canestrini, 1888). Thèse de Doctorat, Université Félix Houphouet Boigny, Abidjan, 2017, 230 p.

[10] Diaha-Kouamé CA. Contribution à l'étude de la distribution des tiques infestant les bovins en Côte d'Ivoire. Mémoire de Master Ecologie Tropicale, Université Félix Houphouët Boigny, Abidjan, 2013, 73 p

[11] Estrada-Peña A, Fuente J, Ostfeld RS, Cabezas-Cruz A. Interactions between tick and transmitted pathogens evolved to minimise competition through nested and coherent networks, Scientific Reports, 2015, 5: 10361, DOi: 10.1038/srep10361

[12] Furlong J. Controle estratégico do carrapato dos bovines, A Hora Veterinaria,2004,23(137): 53-56.

[13] Guerrero FD, Andreotti R, Bendele KG, Cunha RC, Miller RJ, Yeater K, Pérez de León AA.*Rhipicephalus (Boophilus) microplus* aquaporin as an effective vaccine antigen to protect against cattle tick infestations, Parasites & Vectors, 2014, (7): 475. doi: 10.1186/s13071-014-0475-9

[14] ICTTD. Ticks of veterinary and medical importance: Latin America, 2004.

[15] Jori MF, Faune sauvage et risques sanitaires en milieu tropical, Mémoire (HDR) Université de Montpellier-École doctorale GAIA.2017, 181 p

[16] Knopf L, Komoin-Oka C, Zinsstag J. Le rôle du parasitisme dans les élevages bovins N'dama de la savane humide de la CI. Rapport d'activité Centre Suisse de Recherches Scientifique en CI,1999,114 p.

[17] Kulkarni M, Kryuchkov R, Statculescu A, Thickstun C, Dibernardo A, Lindsay L, Talbot B. Distribution géographique de la tique *Ixodes scapularis* et taux d'infection en 2017 à Ottawa (Ontario), Relevé des maladies transmissibles au Canada, 2018;44(10):269-75 https://doi.org/10.14745/ccdr.v44i10a02f

[18] Madder M, Thys E, Achi L, Toure A, De Deken R. *Rhipicephalus (Boophilus) microplus*: a most successful invasive tick species in West-Africa. Exp Appl Acarol, 2011; 10493-010-9390-8. DOI: 10.1007/s10493-010-9390-8

[19] Madder M, Thys E, Geysen D, Baudoux C, Horak I. *Boophilus microplus* ticks found in West Africa. Exp Appl Acarol, 2007; 43: 233-234. DOI: 10.4102/ojvr.v74i1.133

[20] Meddour-Bouderda K, Meddour A. Clés d'identification des Ixodina (Acarina) d'Algérie. Sciences et Technologies, 2006 ; 4: 32-42.

[21] Peter RJ, Van Den BP, Penzhorn BL & Sharp B. Tick, fly, and mosquito control lessons from the past, solutions for the future,Veterinary parasitology, 2005, 132, 205-215.

[22] Tapsoba S. Contribution à l'étude géologique et hydrogéologique de la région de Dabou (sud de la Côte d'Ivoire) : hydrochimie, isotopie, et indice cationique de vieillissement des eaux souterraines. Thèse de doctorat 3ème cycles, Université de Cocody, 1995, 201 p.

[23] Toure A, Diaha CA, Sylla I, Kouakou K, Récente recomposition des populations de tiques prévalent en Côte d'Ivoire. International Journal of Biology and Chemical Sciences, 2014 ; 284 (8): 566-578. DOI: 10.4314/ijbcs.v8i2.15

[24] Toure A, Komoin-Oka C, Sylla I. Cattle ticks population and prevalence of *Babesia spp* amongst it vector: *Rhipicephalus (Boophilus) microplus* in a zone of Ivory Coast. International Journal of Biological and. Chemical Sciences, 2012 ; 6: 1514-1581.

[25] United Nations, World Urbanization Prospects The 2014 Revision, 2014, 517p

[26] Walker AR, Bouattour A, Camicas JL, Estrada-Pena A, Horak I, Latif A, Pegram R, Preston PM. Ticks of domestic animals in Africa: a guide to identification of species, vol. 1, Bioscience Reports, Scotland, UK. 2003, 222 p.

[27] Yeo N, Karamoko Y, Soro D, Zouh Bi ZF & Traore SI.Elevages de bétail dans la région du Poro (Côte d'Ivoire) : Caractérisation et modalités de lutte contre les pathogènes transmis par les tiques. International Journal of Bioloical and Chemical Sciences, 2017, 11(1): 237-246.DOI: 10.4314/ijbcs.v11i1.19.

Rubber Tree Cultivation and Improvement: Biological Aspects and the Risk of Inbreeding Depression

Ong Chin Wei[1], Shamsul Bahri Abdul Razak[2]

[1]Rubber Research Institute of Malaysia, Sungai Buloh, Selangor, Malaysia
[2]Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia

Abstract— *Rubber trees (Hevea spp.) are among the essential plants cultivated and have contributed to Malaysia's economy growth for many decades. Latex harvested from rubber trees is an irreplaceable raw material and accounts for a wide range of uses in tires, tubes, footwear, rubber gloves, and other rubber-based products. There were many attempts to produce ideal rubber tree for increasing latex yield production through the improvement programmes since 1950s. However, the risk of inbreeding depression and the planting materials produced from the chosen parents that are closely related in the improvement programmes is fairly high. Inbreeding depression caused discouraging effects such as uneven bark surface, leaf disease infection, easily damaged by wind blows and eventually reducing the production of latex yield overall. This review highlights the important of biological aspects for latex production in rubber tree and seeing minimizing the risk of inbreeding depression with the necessity of broader genetic base in the rubber tree cultivation and improvement programmes.*

Keywords— Inbreeding depression, genetic base, rubber.

I. INTRODUCTION

Genus of *Hevea* belongs to the *Euphorbiaceae* family, mainly climber herbs, shrubs, and trees. It is among the largest families of plants, comprising over 230 genera and 5,700 species. Generally specified in the grasslands, *Euphorbiaceae* has the most vegetation types, including grain forest trees, weeds, and succulents. In South America, *Hevea* trees can be found in forests in Brazil, Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, and Venezuela (Webster and Paardekooper, 1989; Schultes, 1990; Priyadarshan, 2011). The successful transplantation and domestication of rubber trees from the Amazon forest has allowed Malaysia to play an essential role as one of the most critical natural rubber suppliers in the world for the past several decades. According to a report by the Malaysian Rubber Board (MRB) in 2019, the estimated total production of natural rubber in 2019 from smallholdings and estates in Malaysia was recorded to be 639,000 tons. The total export value of natural rubber and rubber products amounted to approximately RM 40 billion (Natural Rubber Statistics 2019, http://www.lgm.gov.my/nrstat). The primary destinations for raw rubber exports in 2019 were China, Germany, Finland, Iran, the USA, and other countries. In recent trends, the high global demand for natural rubber is a positive sign and robust progress for many rubber-producing countries such as Malaysia.

II. RUBBER TREE CULTIVATION AND IMPROVEMENT IN MALAYSIA

The most famous event in natural rubber history was the considerable amount of natural rubber produced by plantations to meet the Industrial Revolution's demand. Currently, the use of natural rubber has triumphed from diverse domestic products, to those used in industry. Natural rubber is the polymers' chain that displays high resilience and impact resistance, elasticity, and low heat swelling during production process. Due to the distinct molecular structure of rubber, it is difficult to be matched by synthetic rubber that is extracted from petroleum sources. It began in 1876 when Henry Wickham collected approximately 70,000 rubber seeds, primarily *H. brasiliensis*, near the Tapajos River in Brazil, and attempted to sow them in the UK. These small seedling populations were then transported to Ceylon (Sri Lanka) and Singapore in 1876. During the transportation journey, only 22 seedlings survived, and arrived in Kuala Kangsar in 1877. The 22 seedlings in Kuala Kangsar constitute the genetic base for rubber planting materials in The Federation of Malaya (Ong *et al.*, 1983; Baulkwill 1989; Barlow 1978; Loadman 2005, MRB 2005). In 1957, the Rubber Research Institute of Malaya, later known as the Rubber Research Institute of Malaysia (RRIM), launched its major *Hevea* improvement program. RRIM initiated with a small organization with only a few divisions, and took over the responsibility for research and support of works related to rubber from the Department of Agriculture, Malaysia. It has established several experimental stations in Selangor and Johor to provide technical support, services, and *Hevea* improvement programs in the country (MRB, 2005). Before the 1950s, seedlings that were germinated from rubber seeds were widely recognized as planting materials by either rubber plantations or smallholdings. Generally, seedling trees can produce different yields and unpredicted characteristics. However, seeds obtained from the established RRIM experimental gardens are considered useful and highly recommended for planting in quality (Mass, 1919; Cramer, 1924; Holder and Heusser, 1928; Heuser 1932; RRIM, 1957).

In the 1950s, bud grafting was tested and recognized as a useful technique in which clonal seedlings produced a high amount of latex from the planting materials, including Tjirandji 1, RRIM 501, RRIM 526, AVROS 157, and PR 107 (RRIM, 1952; Webster and Baulkwill, 1989). Subsequently, the planting materials derived from bud-grafting rapidly substituted the unselected seedlings from the experimental seed gardens (Burkill, 1959; Priyadarshan, 2011). Rubber planting guidelines began with the first introduction in 1957, and provided information and evidence on high yielding planting materials, disease resistance, and wind damage incident in Malaysia's rubber growing areas (Ong *et al.*, 2011). PB 86, Tjirandji 1, RRIM 501, RRIM 513, and GI 1 were recognized as excellent planting materials at the time, and were recommended for wide-scale planting in Malaysia. In 1967, the RRIM 500 clone series was replaced by the RRIM 600 clone series; which later emerged to be the most popular planting materials for several decades (RRIM, 1959; RRIM, 1961; RRIM, 1963; RRIM, 1967; Shepherd, 1969). Later, numerous rubber-planting materials were introduced in the 1970s, including RRIM 709, RRIM 801, IAN 873, PB, and 230, PB 243, PB 253, PB 254, PB 259, PB 262, PB 310, PB 311, and PB 312. However, all of these planting materials were derived from the parent trees of *H. brasiliensis* (RRIM, 1975; RRIM, 1977).

In 1980-1997, RRIM planting recommendations were reported to detect major leaf diseases, soil categorization, and wind damage incidents. Planting materials were classified into three primary categories and grouped into Classes I, II, and III. This planting recommendation highlighted Class I planting materials for a wide area of planting. RRIM 600, RRIM 712, GT 1, PB 217, PB 260, PR 255, and PR 261 were highly recommended for planting from 1980 to 1994 (RRIM, 1980; RRIM, 1983; RRIM, 1986; RRIM, 1989). RRIM thus tested several rootstocks with proven performance like the PB 5/51 and the RRIM 623, which produced a high survival rate when bud-grafted with commercial planting materials such as GT 1, RRIM 605, RRIM 712, RRIM 901, PB 217, and PB 235 (RRIM, 1983; RRIM, 1986; RRIM, 1992). In 1995-97, MRB planting recommendations emphasized the selection of Latex Timber Clones (LTC) as suitable planting materials for producing high latex yields and rubberwood through their breeding programs. Latex yield remains the most auspicious characteristic, while rubberwood production was another aspect of planting LTC (RRIM, 1995; Ong and Nasaruddin, 2011; Ratnasingam *et al.*, 2011). Currently, commercial planting materials typically originate from the Large Scale Clone Trials (LSCT) such as RRIM 901, RRIM 908, RRIM 911, RRIM 921, RRIM 936, RRIM 940, PB 260, PB 350, PB 355, PB 359, and PB366 (Ramli *et al.*, 1996; MRB, 2006; MRB, 2009). The typical commercial planting materials were developed from *H. brasiliensis* ever since then and were extensively cultivated in Malaysia.

III. BIOLOGICAL ASPECTS FOR YIELD PRODUCTION

3.1 Flowers and Fruits

Rubber trees and other *Hevea* species have a diploid number of chromosomes (2n = 36) (Ong, 1981; Webster and Paardekooper, 1989; Vinod and Meenattoor, 1991). The standard features of *Hevea* species include prominent trifoliate leaves with separated male and female flowers at the same inflorescence and a trilocular capsule fruit pod, containing three seeds and latex in almost all parts of the tree. The inflorescence has multiple branches and panicles with both male and female flowers. The female flowers are produced at the end of the branches, while the male counterparts are born on other panicle parts. After five years of planting, they begin to blossom, but rarely open together, encouraging cross-pollination (Barlow, 1978). Insects usually pollinate the flowers, and cross-pollination is common in rubber trees. Five months after the female flowers are pollinated and fertilized, they ripen as a trilocular capsule of fruit pods that explode and eventually disseminate the seeds (Barlow, 1978; Webster and Paardekooper, 1989).

3.2 Bark

Natural rubber or latex is harvested by cutting the outer layers of the bark structure of a rubber tree where a group of specific cells known as laticifers (commonly known as latex vessels) are located in the inner bark (Shamsul Bahri, 2000). The bark structure consists of several distinct soft and hard layers. Soft bark is the thin layer of cells that produces rows of cells as concentric cylinders of parenchymatous tissues and tube cells that eventually form the laticifers. The laticifers are arranged in regular rows that are nearly parallel to the cambium that ultimately routes up in a tree trunk. Meanwhile, hard bark is the waterproof layer with an abundance of stone cells and active phellogen, forming a cork layer that hardly produces latex (Barlow, 1978; Gomez, 1982).

3.3 Leaves

The leaves are typically arranged into three leaflets on a rubber tree, sometimes with prominent leaf stories and canopies, and often experience leaf defoliation once a year (Webster and Paardekooper, 1989; Schultes, 1990). Features such as the leaf shape and orientation allows the identification of different planting materials observed in *H. brasiliensis* (Md.Zain *et al.,* 1997; Mercykutty *et al.,* 2002). Leaf characteristics in the plant kingdom usually show higher dissimilarities in morphological appearance than those of the stems and roots; leaf shape and venation architecture are particularly useful identification features (Dale *et al.,* 1971; Hickey, 1973; Dickinson *et al.,* 1987). Hickey (1979) and Mercykutty *et al.* (2002) found the essential morphological features for the classification of specific plant species in a type like a leaf shape, leaf size, leaf venation pattern, leaf margin, and the shape of the leaf's tip and base. In budwood nurseries, the leaf's presence for clonal identification is particularly important to ensure the propagation of correctly identified planting materials. More than 200 rubber budwood nurseries currently exist in the country country, and the private and public sectors annually produce nearly 1 million clonal polybag plants (Shamsul Bahri *et al.,* 2013).

3.4 Stomata

The guard cells are specialized epidermal cells assisted by subsidiary cells in a normal condition, where the contraction and expansion of the stomata for gas exchange occurs during photosynthesis. Stomata actively open and close during the daytime, due to a response to light intensity, carbon dioxide concentration, and humidity, with the aid of surrounding cells. Several stomata classifications are proposed for *Hevea*; primarily based on the evolution of related ontogeny structures (Metcalfe and Chalk, 1950; Esau, 1977; Hickey, 1979; Patel, 1979; Baranova, 1992; Das, 2002; Carpenter, 2005). However, the most commonly accepted classification of stomata is based on the mature stomata and subsidiary cells proposed by Metcalfe and Chalk (1950). In general, several types of stomata in dicotyledonous plants are classified based on the number and arrangement of the subsidiary cells into anisocytic, anomocytic, actinocytic, cyclocytic, diacytic, and paracytic groups. Anisocytic type stomata are surrounded by only three subsidiary cells that vary in their position, shape and size, and are distinctly smaller than the other two. Stomata of the anomocytic type are surrounded by many subsidiary cells that are irregular in shape and size. Moreover, actinocytic type stomata are surrounded by at least four or more subsidiary cells. On the other hand, the diacytic type stomata are surrounded by a couple of distinct or indistinct subsidiary cells that are at right angles to the guard cells. The cyclocytic type stomata are surrounded by several subsidiary cells encircling the guard cells. In contrast, in the paracytic type of stomata, stomata are surrounded by two subsidiary cells that are arranged parallel to them.

3.5 Laticifers

Latex is harvested by systematically removing the external bark layers of rubber trees. Gomez (1981) and MRB (2005) stated that the anatomical organization of the bark structure of *Hevea* could be divided into two distinct layers of the internal soft zone (closer to the cambium) and the external hard zone (stone cells and cork). In *Hevea*, the laticifer is developed mainly in the soft bark layer in which latex is produced, and the laticifers are successively produced in the cambium region. However, in previous research, the rubber plant breeders paid little attention to describing the bark surface and several laticifers in *Hevea* species (Frey-Wyssling, 1930, RRIM, 1957; RRIM, 1963, RRIM, 1994). The laticifers' functions are speculated as latex production, physiological activities, cellulose content production, protection against insect attacks, and contribution in a transportation pathway (Pakianathan *et al.,* 1989; Kutchan, 2005; Pickard, 2008; Konno, 2011). Esau (1965) and Shamsul Bahri (2000) distinct that rubber tree laticifers as a series of articulated fused cells that sustain latex and form systems that penetrate the plant body via various tissues. There exist other plant species that also comprise similar laticifer systems, including *Papaver* and *Lactuca* (lettuce) species. Meanwhile, non-articulated laticifers are produced from individual cells and develop into branched or unbranched tube-like structures where these type of laticifers are not fused (de Fay and Jacob, 1989; Shamsul Bahri, 2000; Hagel *et al.,* 2008).

3.6 Rubber Particles

Rubber particles derived from *H. brasiliensis* are described as the hydrophobic center of cis-1, 4-polyisoprene surrounded by complex lipoprotein layers with two types of rubber particle proteins known as Rubber Elongation Factor (REF) and Small Rubber Particle Protein (SRPP) (Siler *et al.,* 1997; Cornish *et al.,* 1999; Nawamawat *et al.,* 2001). In *Hevea* latex, REF and SRPP range from 117 to 298 amino acids with molecular weights of 14.5 and 24-kiloDaltons (kDa) respectively. REF and SRPP are vital proteins taking part in latex production initiation in rubber trees (Oh *et al.,* 1999; Wood and Cornish, 2000;

Cornish, 2001; Wititsuwannakul *et al.,* 2008). The variations in the surface protein contained in *Hevea* plant were indicated by studies on Large Rubber Particles (LRPs) and Small Rubber Particles (SRPPs), mainly for commercial planting materials, but no direct links were made between rubber-formed particles size and molecular weight suspended in latex, and surface proteins (Cornish, 1993; Wood and Cornish, 2000; Sight *et al.* 2003). According to Paardekooper (1989), latex collected from *Hevea* is a cytoplasm that is rich of mainly rubber particles and other cell organelle such as plutoids in which rubber particles are made of a group of polymer chains that are bound together by a glycoprotein membrane. Additionally, lipids membranes, proteins, and other substances surround rubber particles in *Hevea* (Cornish *et al.,* 1993). The rubber particles' surface comprises various types of enzymes, binding undertaking sites for latex biosynthesis (Oh *et al.,* 1999; Kang *et al.,* 2000; Singh *et al.,* 2003). Previous studies on latex and rubber particles were primarily focused on the consistency aspects such as dry rubber content percentage, mechanical stability, Wallace plasticity, Mooney viscosity, and plasticity retention index relevant to rubber growers and smallholdings in Malaysia (Yip, 1990; Bonfils *et al.,* 2000; Ong, 2000).

3.7 Seeds

Rubber seeds are vital for producing seedling planting materials and as rootstock for bud-grafting of high-yield clones in natural rubber-producing countries. There is one or sometimes two seed fall seasons a year in many rubber-producing countries such as Malaysia. A mature rubber tree can be identified by patterns on the seed coat that display noticeable differences in size and color. The rubber seed coat characteristics are inherited from the female parent. The rubber seeds originating from the same female parent are identical, irrespective of the pollen source, although seed size may vary because of the growth environment (Lacey *et al.,* 1997; Mercykutty *et al.,* 2002). Thus, a visual description of the variations on the seeds' morphological characteristics can be utilized to verify the authenticity of the planting material (Mercykutty *et al.,* 2002; Md.Zain *et al.,* 2003). Each rubber seed has its dorsal and ventral sides. The dorsal side is convex with a central ridge, while the ventral side is nearly flat with lateral cheeks. The seed coat color among planting materials may vary from grey to light brown, brown, and dark brown. The presence of mottling of deep brown or greyish brown shades on the seed coat is evident in different planting materials of *H. brasiliensis* (Md.Zain *et al.,* 2003).

3.8 Rootstock-Scion Compatibility

The observation of planting materials' latex yield with specific rootstock resulted in several findings to evaluate the rootstock effects. Several attempts were made to select elite rootstocks that enhanced scion growth and overall yield performance (Djikman, 1951; Buttery, 1961). Several seedling families and commercial planting materials were tested as rootstock. The majority exhibited strong evidence of high latex yield and diverse rootstock dynamic form (Buttery 1961). More recent experiments tested several commercial planting materials such as PB 5/51, RRIM 600, RRIM 628, SG 170, RRIC 52, and 48 E/130, grafted on the rootstocks derived from PB 5/51, RRIM 623, RRIM 600, RRIM 501, Tjir 1 and unselected seedling as an initial screening for elite rootstock (Ng *et al.,* 1982; Ng, 1983). Ng *et al.* (1983) demonstrated that rootstocks derived from *H. brasiliensis* could significantly influence the scions' growth and production through rootstock-scion interaction. Although plant breeders emphasized the selection of rootstock capacity before bud grafting from RRIM since the 1980s (Leong and Yoon, 1979; Ng *et al.,* 1982; Ng, 1983), there exist no specific standardized criteria for rootstock selection, particularly after the replacement of PB5/51, RRIM 501 and RRIM 603 by newly planting rootstock (RRIM 1992; RRIM 1994). Nowadays, it is common for seeds to be collected from various sources without knowledge about their origin or rootstock-scion compatibility.

IV. GERMPLASM RESOURCES

Rubber plant breeders from the Rubber Research Institute of Malaysia have been attempting to resolve the narrow genetic basis of rubber plants in the country, including the conservation of germplasm and planting material exchange exercises with other countries. There might be several attempts to apply the rubber genetic resources by private plantations and smallholdings over the past few decades, but there remains a lack documentation and clarity in records of the improvement programmes in germplasm availability and utilization.

4.1 Seedling Trees Exchanged Programmes in the 1950s

In 1951-52, RRIM introduced several *Hevea* seedlings from Brazil, including *H. brasiliensis*, *H. spruceana* and *H. benthamiana*. These seedling trees displayed strong resistance against the South American Leaf Blight (SALB) disease that attacked rubber trees in South America. However, the main objective was to employ these seedlings for high latex production in the RRIMM breeding programs. The progress of identifying potential planting materials from *Hevea* species was halted for several years, attributable to their low yields and poor growth, compared to commercial planting materials such as RRIM Clone Series (RRIM, 1952; RRIM 1957; Ong and Tan, 1987).

4.2 *Hevea* Germplasm 1967

About 100 rubber trees originated from the Amazon and were brought to Malaysia, including species of *H. brasiliensis*, *H. benthamiana*, *H. camargoana*, *H. guianensis*, *H nitida*, *H. pauciflora*, *H. spruceana*, and *H. rigidifolia*. These trees were planted and tested in a small plot area at the RRIM Research Station, Sungai Buloh. Ong (1977) concluded that these *Hevea* species were inferior in latex production and morphological characteristics, including bark renewal and commercial planting materials. However, the reporting and improvement programs on these *Hevea* species have remained unclear, with few publications and limited information publicly available to rubber planters and growers in Malaysia.

4.3 *Hevea* Germplasm 1981

In 1981, the International Rubber Research and Development Board (IRRDB) and the Brazilian Government initiated a joint expedition to collect *Hevea* seeds and budwood in South America. The expedition explored the Amazon forest in three leading states of Brazil, namely, Acre, Mato Grosso, and Rondonia, where the *Hevea* species were distributed. The expedition collected 64,736 rubber seeds and 1,522 meters of budwood. Malaysia received about 24,000 seeds for preservation and conservation, while the remaining seeds were retained in Brazil. The materials collected from the expedition focused on *H. brasiliensis,* and approximately 13,000 seedlings were successfully germinated and transplanted to the conservation site at RRIM Research Station, Sungai Buloh (Ramli *et al.*, 1996). Over the years, progenies derived from the hand-pollination program have been evaluated in several experimental sites. These progenies were primarily assessed for latex yield potential and growth, two and a half years after planting (Ong and Tan, 1987; Ong and Nasaruddin, 2011). According to Ramli *et al.* (1996), the potential yield of the trees planted in 1981 was lower than the commercial planting materials. The most vigorous trees were from the state of Acre, and the growth vigor between the trees was considerably different from the same state. The selected tree was explored as parents and incorporated into the improvement programs.

4.4 *Hevea* Germplasm 1995

The Rubber Research Institute Malaysia (RRIM), Forest Research Institute Malaysia (FRIM), and Forestry Department Peninsular Malaysia (FDPM) have initiated the Amazon forest expedition in 1995 to collect Hevea seeds. The expedition aimed to collect *Hevea* seeds that would produce high rubberwood and latex yields, and return to Malaysia. The expedition took place across 13 locations in Brazil, including Acre, Atalia de Norte, Belem, Borba, Benjamin Constant, CPAA Manaus, Iranduba, Manaus, Manicore, Sao Gabriel, Sao Paulo de Olivencia, Tapajos, and Tabatinga (Ong and Nasaruddin, 2011). Seeds of eight *Hevea* species, including *H. brasiliensis*, *H. benthamiana*, *H. camargoana*, *H. guianensis*, *H. nitida*, *H. pauciflora*, *H. rigidifolia*, and *H. spruceana* were identified and brought back to Malaysia. However, two *Hevea* species were not brought back from this expedition, namely, *H. camporum* and *H. microphylla*. These germinated seedlings were subsequently assigned to the planting site according to seedling batches. Moreover, the unsatisfactory low germination percentage of less than 20% of the total seeds' sowing and survival percentage forced the seedlings to be planted without properly assigned accession numbers (RRIM, 1995; Ong and Mohd Nasaruddin, 2011). Ong and Nasaruddin (2011) reported that two selections were made to select a new planting source. However, both selections were focused on a high rubberwood potential rather than a latex yield. The capacity of these *Hevea* species as planting materials was established by sorting the highest truncated wood volume (Ong and Nasaruddin, 2011). The circumference of these *Hevea* species was measured at the 5th and 10th year after planting.

V. THE RISK OF INBREEDING DEPRESSION

From the beginning of the improvement programs, parent trees were chosen for the crossing, where the pollen of selected male parents was collected and introduced into female parents through the hand-pollination program. Seedlings resulting from the hand-pollination program were selected into Hand Pollinated Seedling Trials and further evaluated in Small Scale Clone Trials. The trees that presented promising characteristics such as high latex yield and healthy leaves were selected for Large Scale Clone Trials. Lastly, the trees that indicated potential in high latex production and other favorable characteristics were recommended as new commercial planting materials for wide-area planting. Plant breeders found it challenging to improve the rubber tree in yield and growth vigor, due to the inbreeding decline complemented by the narrow genetic base. This effect was seen in the frequently used classical breeding system of "Generation Wise Assortative Mating" (GAM) that involves the sibling mating of rubber planting materials derived from *H. brasiliensis* (Tan, 1987; Ramli *et al.*, 1996). In such a breeding system, the finest genotypes in one generation of planted rubber planting materials were selected as parents for the next cycle of breeding, subsequently continuing the same procedure in the coming cycles (Simmond, 1989; Ramli *et al.*, 1996). Homozygosity and the occurrence of lethal alleles in the *Hevea* plant lead to general weaknesses such as low latex yield, loss of general vigor and adverse morphology affecting bark smoothness; the biochemical quality of rubber particles from inbred progenies are the critical consequences of the inbreeding method. Such developments would severely affect the performance of the rubber tree as a crop plant. Inbreeding depression caused by the narrow genetic base in *Hevea* affects not only future breeding programs for yield improvement, but also the availability of gene sources for useful morphological and biochemical characteristics.

Hereafter, attempts must be made to improve the genetic base by adding new genetic materials from germplasm to overcome these issues. Once a new pool of genetic resources is introduced, efforts can then be made to utilize them for complete crossing. Subsequently, close consideration and caution must be taken concerning the use of novel materials in breeding to improve yields and other essential characteristics. Broadening the *Hevea* genetic base is an essential strategy to produce high latex yielding planting materials in future breeding programs. In hybridization programs, the common practice is to produce progenies with suitable characteristics that would contribute to a more productive plant. There is an additional gain in widening the genetic base for crop improvement, where inter-specific crosses are involved. In other natural rubber producing countries, crop improvement programs often incorporate attempts to produce a hybrid between *H. brasiliensis* and *Hevea* species, to develop immunity towards leaf diseases (Schultes, 1990). However, the interspecific hybrids developed between commercial planting materials and other *Hevea* species are discouraging due to the low latex production and severe infection by the South American Leaf Blight (SALB). Although SALB is not present in Asia, Malaysia also embarked on several trials involving inter-specific hybrids in the 1970s (Gilbert *et al.*, 1973; Tan and Subramaniam, 1976; Tan, 1987).

VI. CONCLUSION

The selection of productive planting materials to produce vigorous trees capable of producing higher latex quantities has become crucial for Malaysian smallholdings, plantations, and rubber growers. For commercial and large-scale planting, the preferable planting materials should generate high latex yield, healthy leaves and canopies, smooth and soft bark surface for tapping, fast bark renewal rate after tapping, straight stem and trunk, vigorous girth growth and high survival rate of rootstock-scion compatibility. Besides, characteristics such as bark thickness, number of laticifers, number of stomata, leaflet position, seeds, and photosynthetic rates were the primary concerns in *Hevea* improvement. The characteristics that should be found in suitable rubber planting materials and approved by the rubber growers are as follows: (1) High latex yield production; (2) Smooth and soft bark surface; (3) Low susceptibility to leaf diseases; and (4) High tolerance to wind damage. Currently, rubber plant breeders are still trying to improve rubber-planting materials with all the right characteristics into a single model or "Ideotype", while at the same time considering minimizing the effect of “Inbreeding depression” when breeding for a new rubber planting material for commercial planting. Inbreeding depression ought to reduce the vigorous growth and latex yield production with a narrow genetic base. Therefore, *Hevea* improvement programs necessitate to incorporate broader genetic resources with a selection of materials that includes commercial rubber planting materials derived from *H. brasiliensis* and other *Hevea* species that are compatible with crossing or hybridization. The highlights from this research aim to provide opportunities to employ the potential rubber species and genetic resources to select desirable characteristics with the urge to utilize a new source of rootstocks in bud-grafting and multiplication of planting materials in the future.

REFERENCES

[1] Baranova M (1992) Principles of comparative stomatographic studies of flowering plants. *The Botanical Review* 58(1):49-99.

[2] Barlow C (1978) *The Natural Rubber Industry*. Oxford University Press, London.

[3] Baulkwill WJ (1989) The history of natural rubber production. In: Webster CC and Baulkwill (Eds.) *Rubber*, Longman Scientific and Technical, UK and John Wiley and Sons, Inc, New York.

[4] Blaser H (1945) Anatomy of *Cryptotesgia grandiflora* with Special Reference to the Latex System. *American Journal of Botany* 32:135-141.

[5] Bonfils F, Char C, Garnier H, Sanago A, Sainte-Beuve J (2000) Inherent molar mass distribution of clones and properties of crumb natural rubber. *Journal of Rubber Research* 3(3): 164-184.

[6] Bouncing-balls. Accessed on 12th January 2015. URL: http://*Hevea*.bouncing-balls.com/ chemistry tech conservation / vulcanization.htm

[7] Burkill HM (1959) Large Scale Variety Trials of *Hevea brasiliensis* Mull-Arg. on Malayan Estate 1934-53. *Journal of The Rubber Research Institute of Malaya* 16(1): 1-37.

[8] Buttery BR (1961) Investigation into the relationship between stock and scion in *Hevea*. *Journal of The Rubber Research Institute of Malaya*. 17: 46-76.

[9] Carpenter KJ (2005) Stomatal architecture and evolution in basal angiosperms. *American Journal of Botany* 92(10): 1595-1615.

[10] Cornish K, Siler DJ, Grosjean O, Goodman N (1993) Fundamental similarities in rubber particles architecture and function in three evolutionarily divergent plant species. *Journal of Natural Rubber Research* 8: 275-187.

[11] Cornish K, Wood DF, Windle JJ (1999) Rubber particles from four different species, examined by transmission electron microscopy and electron-paramagnetic-resonance spin labelling, are found to consist of a homogeneous rubber core enclosed by a contiguous monolayer biomembrane. *Planta* 210(1): 85–96.

[12] Cramer PJS (1924) Our inoculated *Hevea* trees in their first test year; their growth and production. *Archief Voor De Rubbercultuur* 8:458-493.

[13] Dale MB, Groves RH, Hull VJ, O'Callaghan JF (1971) A new method for describing leaf shape. *New Phytologist* 70: 437-442.

[14] Das S (2002) On the ontogeny of stomata and glandular hairs in some Indian mangroves. *Acta Botanica Croatica* 61(2): 199-205.

[15] de Fay E and Jacob JL (1989) Anatomical organization of the laticiferous system in the bark. In: d'Auzac J, Jacob JL, Chrestin H (Eds.), *Physiology of rubber tree latex*, 4-14. CRC Press, Boca Raton, Florida.

[16] Dickinson TA, Parker WH, Strauss RE (1987) Another approach to leaf shape comparisons. *Taxon* 36:1-20.

[17] Dijkman MJ (1951) *Hevea*: *thirty years of research in the Far East*. Florida: University of Miami Press.

[18] Dunlop Tires. Our 124-Year Journey. Accessed on 18th December 2014. URL: http://Hevea.dunloptires.com/en-US/company/tire-history

[19] Esau K (1965) *Plant Anatomy*. Wiley. New York.

[20] Esau K (1977) *Anatomy of Seed Plants*. Wiley. New York.

[21] Frey-Wyssling A (1930) Investigation into the relation between the diameter of the latex tubes and the rubber production of *Hevea brasiliensis*. *Archief Voor De Rubbercultuur* 14:135-166.

[22] Fry C (2009) *The Plant Hunters*. Andre Deutsch. London.

[23] Gilbert NE, Dodds KS, Subramaniam S (1973) Progress of breeding investigations with *Hevea brasiliensis*. Analysis of data from earlier crosses. *Journal of Rubber Research Institute of Malaysia* 23(5); 365-380.

[24] Goulding M (1980*) Fishes and the Forest: Explorations in Amazonian Natural History*. University of California Press.

[25] Gomez JB (1981) Anatomy and ultracytology related to breeding of *Hevea*. In: *Hevea Breeding Course*. Rubber Research Institute of Malaysia.

[26] Gomez JB (1982) Anatomy of *Hevea* and its influence on latex production. MRRDB Monograph No.7, Malaysian Rubber Research and Development Board.

[27] Gomez JB and Samsidar H (1980) Variations in leaf morphology and anatomy between clones of *Hevea*. *Journal of Rubber Research Institute of Malaysia* 28(3): 157-172.

[28] Hagel JM, Yeung EC, Facchini PJ (2008) Got milk? The secret life of laticifers. *Trends in Plant Science*. 13(12):631-639.

[29] Heusser C (1932) Yields figures from legitimate *Hevea* seedlings in 1930 in the Soengei Pantjoer Experimental Station. *Archief Voor De Rubbercultuur*. Rubber Research Institute of Malaya 16:129-156.

[30] Hickey LJ (1973) Classification of the architecture of dicotyledonous leaves. *American Journal of Botany* 60:17-33.

[31] Hickey LJ (1979) A revised classification of the architecture of dicotyledonous leaves. In: Metcalfe CR and Chalk L, *Anatomy of the dicotyledons Volume 1. Systematic anatomy of leaf and stem, with a brief history for the subject*, 25-39. Clarendon Press, Oxford.

[32] Holder HJVS and Heusser C (1928) Experimental tapping on *Hevea* buddings and seedlings on Boekit Maradja Estatae. *Archief Voor De Rubbercultuur* 12(58):37-49.

[33] Kang H, Kang MY, Han KH (2000) Identification of natural rubber and characterization of rubber biosynthetic activity in fig tree. *Plant Physiology* 123(3):1133-1142.

[34] Konno K (2011) Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein. *Phytochemistry* 72(13): 1510-1530.

[35] Kutchan TM (2005) A role for intra- and intercellular translocation in natural product biosynthesis. *Current Opinion in Plant Biology* 8(3):292-300.

[36] Kew. *Hevea brasiliensis* (rubber tree). Accessed on 9th January 2014. URL: http: // Hevea.kew.org / plants-fungi / Hevea-brasiliensis.htm.

[37] Kew. Neotropical Euphorbiaceae. Accessed on 9th January 2014. URL: kew.org /science /tropamerica /neotropikey/families/ Euphorbiaceae.htm

[38] Lacey EP, Smith S, Case AL (1997) Parental effects on seed mass: Seed coat but not embryo/endosperm effect. *American Journal of Botany* 84(11):1617-1620.

[39] Loadman J (2005) *Tears of the Tree: The Story of Rubber - A Modern Marvel*. Oxford University Press, UK.

[40] Leong SK and Yoon PK (1979) Effects of bud eyes on early scion growth of *Hevea*. *Journal of Rubber Research Institute of Malaysia* 27(1): 1-7.

[41] Maas JGJA (1919) The vegetative propagation of *Hevea Brasiliensis*. *Archief Voor De Rubbercultuur*. Rubber Research Institute of Malaya 3:279-287.

[42] Martins MBG and Zieri R (2003) Leaf anatomy of rubber-tree clones. *Scientia Agricola* 60(4):709-713.

[43] Md.Zain AA (1997) *RRIM 2000 Series Clones: Characteristics and Description*. Rubber Research Institute of Malaysia.

[44] Md.Zain AA (2003) *Prestasi awal klon lateks balak Siri RRIM 2000 di Projek Pembangunan Terkawalselia*. Malaysian Rubber Board.

[45] Metcalfe CR and Chalk L (1950). *Anatomy of the Dicotyledons Volume I*. Oxford University Press, Oxford UK.

[46] Mercykutty VC, Marattukalam JG, Saraswathyamma CK, Meenakumari T (2002) *Identification of Hevea clones: a manual*. Rubber Research Institute of India, Kottayam, Kerala, India.

[47] Michelin. A brief history of the Michelin brand in the cycle tire field. Accessed on 18th December 2014. URL: http: // bike.michelinman.com /advice /miscellanous/a-brief-history-of-the-michelin-brand-in-the-cycle-tire-field

[48] MRB (2005) *The evolution of the rubber industry in Malaysia: Navigating and re-inventing the industry*. Malaysian Rubber Board.

[49] MRB (2009) *Rubber Plantation and Processing Technologies*. Malaysian Rubber Board, Kuala Lumpur.

[50] Natural Rubber Statistics 2019. Accessed on 1st August 2020. URL: http://www.lgm.gov.my/nrstat.

[51] Nawamawat K, Sakdapipanich JT, Ho CC, Ma Y, Song J, Vancso JG (2011) Surface nanostructure of *Hevea brasiliensis* natural rubber latex particles.*Colloids and Surfaces A: Physicochemical and Engineering Aspects* 390(1): 157–166.

[52] Nelson T and Dengler N (1997) Leaf vascular pattern formation. *The Plant Cell*9:1121-1135.

[53] Ng AP, Ho CY, Sultan MO, Ooi CB, Lew HL, Yoon PK (1982) Influence of six rootstocks on growth and yield of six scion clones of *Hevea brasiliensis*. *Proceedings of the Rubber Research Institute of Malaysia Planters' Conference*, Kuala Lumpur, 134-151.

[54] Ng AP (1983) Performance of rootstocks. *Planters' Bulletin of Rubber Research Institute of Malaysia* 175: 56-63.

[55] Oh SK, Kang H, Shin DH, Yang J, Chow KS, Yeang HY, Wagner B, Breiteneder H, Han KH (1999) Isolation, characterization, and functional analysis of a novel cDNA clone encoding a small rubber particle protein from *Hevea brasiliensis*. *The Journal of Biological Chemistry* 274 (24): 17132–17138.

[56] Ong SH (1977) Investigations on clones derived from seedlings of various*Hevea*species introduced in 1966 by the Rubber Research Institute of Malaya. Workshop on International collaboration in*Hevea* breeding and the collection and establishment of materials from the neo-tropics, Kuala Lumpur, 12-16 April 1977.

[57] Ong SH (1981) Correlations between yield, girth and bark thickness of RRIM Clone trials. *Journal of Rubber Research Institute of Malaysia* 29(1): 1-14.

[58] Ong CW, Nasaruddin MA, Shamsul Bahri AR, Zarawi AG, Nurmi Rohayu AH, Rasyidah MR, Abdul Aziz AK (2011) Malaysia experience on Rubber Planting Recommendations since 1925. IRRDB Workshop on Improving productivity Through Good Agricultural Practices. 26th-28th September 2011, Mawlamyine, Myanmar.

[59] Ong SH and Tan H (1987) Utilization of *Hevea* genetic resources in RRIM. *Malaysian Applied Biology Journal* 16(1): 145-155.

[60] Ong CW and Nasaruddin MA (2011). The potential timber yielding genotypes identified from the mother tree selection (second selection) in 1995*Hevea* Germplasm. Report: Malaysian Timber Council, 2011.

[61] Pakianathan SW, Harridas G, d'Auzac J (1989) Water relation and latex flow. In: d'Auzac J, Jacob JL, Chrestin H (Eds). *Physiology of rubber tree latex*, 233-256. CRC Press, Boca Raton, USA.

[62] Patel JD (1979) New morphological classification of stomatal complexes. *Phytomorphology*29:218-229.

[63] Pickard WF (2008) Laticifers and secretory ducts: two other tube systems in plants. *New Phytologist* 177:877-888.

[64] Priyadashan PM (2011) *Biology of Hevea rubber*. CABI, Wallingford, UK.

[65] Ramli O, Masahuling B, Ong SH (1996) Genetic enhancement for *Hevea* improvement. Second National Congress on Genetics, 13th-l5th November 1996, Kepong, Malaysia.

[66] Ratnasingam J, Ioras F, Wenming L (2011) Sustainability of the rubberwood sector in Malaysia. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39(2): 305-311.

[67] RRIM (1952) Seed gardens for estates. *Planters' Bulletin.* Rubber Research Institute of Malaya.

[68] RRIM (1957) Planting Recommendations 1957-58. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[69] RRIM (1959) Planting Recommendations 1959-60. *Planters' Bulletin*. Rubber Research Institute of Malaysia.

[70] RRIM (1961) Planting Recommendations 1961-62. *Planters' Bulletin*. Rubber Research Institute of Malaysia.

[71] RRIM (1963) Planting Recommendations 1963-64. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[72] RRIM (1964) Planting materials used on estate in Malaysia. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[73] RRIM (1965) Planting Recommendations 1965-66. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[74] RRIM (1967) Planting Recommendations 1967-68. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[75] RRIM (1975) Planting materials used in Peninsular Malaysia for 1971 and 1972. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[76] RRIM (1977) Environmax Planting Recommendations 1977-79. *Planters' Bulletin. Rubber Research Institute of Malaysia.*

[77] RRIM (1983) RRIM Planting Recommendations 1983-85. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[78] RRIM (1986) RRIM Planting Recommendations 1986-88. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[79] RRIM (1989) RRIM Planting Recommendations 1989-91. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[80] RRIM (1992) RRIM Planting Recommendations 1992-94. *Planters' Bulletin.* Rubber Research Institute of Malaysia.

[81] RRIM (1994) Planting materials used in Peninsular Malaysia, 1989-1992. *Planters' Bulletin.* Rubber Research Institute of Malaysia 3-11.

[82] RRIM (1995) *Hevea*wood availability in Peninsular Malaysia. *Planters' Bulletin.* Rubber Research Institute of Malaysia 73-83.

[83] Schultes RE (1990) *A brief taxonomic view of the genus Hevea*. MRRDB Monograph No.14, Malaysian Rubber Research and Development Board.

[84] Shamsul Bahri AR (2000) Development and distribution of laticifers in plants (Doctoral dissertation). Durham University.

[85] Shamsul Bahri AR, Ong CW, Jamilah MS, Ariffin MM (2013) Transmission electron microscopy (TEM) study on rubber particles in laticifers of native *Hevea* species. *Malaysian Journal of Microscopy* 9:140-144.

[86] Shepherd R (1969) Aspects of *Hevea* breeding and selection. Investigations undertaken on Prang Besar Estate. *Planters' BulletinBulletin of Rubber Research Institute of Malaysia* 10: 206-216.

[87] Siler DJ, Goodrich-Tanrikulu M, Cornish K, Stafford AE, McKeon TA (1997) Composition of rubber particles of *Hevea brasiliensis, Parthenium argentatum, Ficus elastica, and Euphorbia lactiflua* indicates unconventional surface structure. *Plant Physiology and Biochemistry* 35(11):881–889.

[88] Singh AP, Wi SG, Chung GC, Kim YS, Kang H (2003) The micromorphology and protein characterization of rubber particles in *Ficus carica*, *Ficus benghalensis* and *Hevea brasiliensis*. *Journal of Experimental Botany* 54(384): 985-992.

[89] Tan H and Subramanian S (1976) A five-parent diallel cross analysis for certain characters of young *Hevea* seedlings. *Proceedings of International Rubber Conference RRIM*, Kuala Lumpur, 13-16.

[90] Tan H (1987) Current status of *Hevea* breeding. In: Campbell AI, Abbott AJ, Atkin RK (Eds.), *Improvement of vegetatively propagated plants*. Academic Press, London.

[91] Useful Tropical Plants. *Hevea*. Accessed on 20th January 2019. URL: http://tropical. theferns.info/letter-index.php

[92] Vertrees GL and Mahlberg PG (1978) Structure and ontogeny of laticifers in *Cichorium intybus* (Compositae). *American Journal of Botany* 65(7):764-771.

[93] Vinod KK and Meenattoor JR (1991) Genetic complexity in *Hevea brasiliensis* - Some theoretical considerations. *Rubber Board Bulletin* 26(3):13-17.

[94] Webster CC and Baulkwill WJ (1989) *Rubber*. Longman Scientific and Technical. UK.

[95] Webster CC and Paardekooper EC (1989) The botany of the rubber tree. In: Webster CC and Baulkwill (Eds.), *Rubber*, 57-84. John Wiley and Sons, New York.

[96] Wititsuwannakul R, Rukseree K, Kanokwiroon K, Wititsuwannakul D (2008) A rubber particle protein specific for *Hevea* latex lectin binding involved in latex coagulation. *Phytochemistry* 69: 1111–1118.

[97] Wood DF and Cornish K (2000) Microstructure of purified rubber particles. *International Journal of Plant Sciences* 161(3):435-445.

[98] Wycherley PR (1992) The genus *Hevea*- botanical aspects. In: Sethuraj MR and Mathew NM (Eds.), *Natural Rubber: Biology, Cultivation and Technology*, 50-66. Elsevier Science Publishers, The Netherlands.

[99] Yip E (1990) Clonal characterisation of latex and rubber properties. *Journal Natural Rubber Research* 5(1):52–80.

Malting Sludges as Soil Amendment

Campaña, D.H.[1*], Airasca A.[2]

Universidad Tecnológica Nacional – Facultad Regional Bahia Blanca-GEAQB Grupo de estúdio Ambiente Química y Biología – Argentina

Abstract**—** *Residual sludge that results from the treatment of malt house effluents produced during the malting of barley in malt house (Argentina) was evaluated to determine its fertilising potential and capability for improving soil as a way to reuse or recycle this material. The Cabildo soil (Southwest of Buenos Aires Province), of the typic Argiustoll subgroup had a sandy clay-loam texture and was tested in this field experiments. This soil plots (4 m^2) were amended with equivalent malting sludges doses of 5, 15, and 25 Mg/ha. When biosolids, like malting sludges, are incorporated as an amendment, many micronutrients are provided. Chemical properties of the soil, particularly the pH, EC (Electrical Conductivity) and Cation Exchange Capacity (CEC), were improved. Dactylis glomerata L. were utilised as growing crop (forage) in this field test, productivity (Dry matter) and quality (protein %) gave results following 0,205 kg/m^2 and 11.94 % at control in comparison with 0,4 kg/m^2 and 15.15 % of amended soil. Calcium concentration in grasses was significantly different for control (4651 mg/kg^{-1}) in comparison with high sludge dose (8907 mg/kg^{-1}), the same trend was found with micronutrients like Copper and Molybdenum. This result from field test indicate that this residual sludge constitutes a suitable amendment for agricultural soils, increasing quality and productivity of Dactylis glomerata L and improving several soil properties*.

Keywords**—** ***Malting Sludge, Soil Amendment, Forage.***

I. INTRODUCTION

Nutrient depletion is a major form of soil degradation. The sludge from effluent treatment plants in agro-industrial processes is a potential source of organic fertilisers (Roy et al., 2003) that can be used to restore the fertility of agricultural soils with better prospects than even inorganic or conventional fertilisation. When biosolids are incorporated as an amendment, many micronutrients that are not incorporated with conventional (synthetic) fertilisation are provided. This incorporation is an advantage, given that the design of fertiliser dosages at the micro-level would be notably costly. The bioavailability of trace elements, such as micronutrients or toxic elements, is not determined by the total concentrations of the elements in question; rather, it depends directly on the chemical properties of the soil, particularly the pH and cation exchange capacity. The application of biosolids, organic (plant/animal) waste to the soil should be reconsidered as an economic practice, both from the standpoint of operating costs and from the standpoint of the environment, given the facts that matter is recycled and it can effectively compete with chemical fertilisers at lower environmental costs. All of these factors support the pursuit of an effectively sustainable agricultural-livestock production method. The use of organic waste would also be an advantage for countries with relatively low industrialisation that could more easily "close" the cycle of nutrient recycling in contrast to highly industrialised countries. Ideally, sustainable agricultural-livestock production would be stable when organic waste arising from the study area is reused within the same area (Schulz et al., 1997). This stability is possible when the surface areas of agricultural land are large, and the generation of agribusiness and domestic waste is not excessive, as is the Argentina situation. One way to improve or restore long-term soil quality is to intervene in the complex processes of agro-ecosystem biocycles.

Taking into account the limiting soil factors for growing crops in marginal zones of the Argentine Pampas, such as the southwestern regions of the province of Buenos Aires (sub-humid – semi-arid zones), a typical Mollysol soil (Argiudol suborder) was tested with the objective to propose practices, such as amendment with biosolids (organic waste), like malting sludges that would improve the soil quality and increase the sustainable productivity of cereals and fodder to prevent deterioration of the ecosystem. Sludge from waste biomass (activated sludge treatment of malting effluents), mainly organic matter had a chemical composition about 50% cellulose, hemicellulose 20%, 18% of lignin, 6% protein, 5% amino acids and sugars and other pectin, waxes, pigments, etc.

II. MATERIALS AND METHODS

Soil plots were established in Rucalen-Cabildo (Buenos Aires, Province) as soil C (Torres Carbonell et al., 2012). Malting sludges were from a malt house nearby Bahia Blanca- Argentina (Campaña D.H. et al., 2014). 15 plots were laid out square, 4 m2 each, with ploughing and raking up soil, and, after 8 days, biosolids was applied superficial. 4 trials were conducted with three replicates each, adding doses equivalent to 5, 15 and 25 Mg. per há. Control plots (soil without sludge), also was tested. Once added, sludge was mixed with the topsoil (15 - 20 cm), then raking to prepare the seedbed. Five weeks later (36 days), it was take the first soil sample from each plot and was seeded, orchard grass (*Dactylis glomerata L*). The seeding rate was 1.25 grams of seed per m2 each plot (equivalent to the rate typically used forage cropping). Any irrigation was not performed, so that the water intake was only by rainfall during the experimental period. After fourteen weeks, the grass was cutted, immediately was make the second sampling of soil and finally the third sample was collected after completion of the final collection of grass, which was made by hand with scissors cutting leaving a minimum of grass remaining in the plot (less than 2 cm in height). The harvest included, orchard grass and the other species grow together. The pH, electrical conductivity, exchange cations and the effective exchange capacity, were measured for soil samples. For vegetables, after performing acid digestion (nitric/perchloric acid) samples were determined total contents of P, Ca, Mg, K, Cu, Zn, Mo atomic emission spectrometer inductively coupled plasma (ICP-AES). To estimate the crude protein, the determination was made on a dry sample of plant total N measured by the Kjeldahl method, multiplied by the factor 6.25, assuming that the average content of N is 16% crude protein. Statistical validation was by analysis of variance (ANOVA) to test significant differences of measured variables; all cases were determined properties by triplicate samples. As an alternative nonparametric analysis of variance (ANOVA) were used: in some cases the Kruskal-Wallis, or the Wilcoxon test.

III. RESULTS AND DISCUSSION

Eight months crop growing (forage) using orchard grass (*Dactylis glomerata L.*) in soil C, sub-humid climatic conditions (Cabildo- Buenos Aires-Argentina) was performed. At the experimental plots was determined soil pH, electrical conductivity (EC), cation exchange capacity (CEC). For *Dacylis glomerata L.* (Orchard grass), dry matter production, and quality, including protein, Ca, Mg, K, P, Zn, Cu and Mo were measured. The micronutrients evolution and forage production were evaluated, beginning March and ending mid-November. The maximum and minimum average temperatures were similar initial and finally (26º C and 13.2° C in March and 25.8° C and 10.6° C in November). The lowest temperature happend at July, with an average maximum of 11.6 Tº C and average minimum of 1.5° C. The rainfall for the period was 469 mm, the distribution was: 132 mm at 1st sampling, 124 mm at second one and 213 mm at final sampling. Intermediate sampling results were not showed (space limitation), pH, electrical conductivity, cation exchange capacity and exchangeable cátions are presented in Table 1. Finally plant production (dry matter), protein and micronutrients contents were determined and showed in Table 2.

TABLE 1
pH EC AND CEC OF SOIL AMENDED WITH MALTING SLUDGE (0, 120 AND 240 DAYS) *

Soil Properties	Low Dose	Medium Dose	High Dose	Control
Initial pH	7.26a	6.73a	7.12 B	7.59^{a}
Initial EC (Ms/Cm^{-1})	0.48b	0.47b	1.04b	0.23 A
Initial CEC (Cmol(+)Kg^{-1})	24.58a	25.17a	29.48b	23.65^{a}
Mid pH)	7.78a	7.12a	7.16a	7.90^{a}
Mid EC (Ms/Cm^{-1})	7.26 A	6.73 A	7.12 B	7.59^{a}
Mid CEC (Cmol(+)Kg^{-1})	19.98a	19.35a	23.3a	17.91^{a}
Final pH	7.58a	6.93 A	7.50a	7.77^{a}
Final EC (Ms/Cm^{-1})	0.19b	0.21b	0.23b	0.09^{a}
Final CEC (Cmol(+)Kg^{-1})	22.08a	18.10a	23.27a	18.52^{a}

Average values of three plots same dose
In each row values followed by the same letter are not significantly different (p>0.05).

TABLE 2
DACTYLISGLOMERATA L. PRODUCTION (DM -DRY MATTER), PROTEIN CONTENT (%), MACRO AND MICRONUTRIENTS FROM SOIL AMENDED WITH MALTING SLUDGE *

	Low Dose	Medium Dose	High Dose	Control
Grass Dm (kg/m^2)	0.4^a	0.346^a	0.261^a	0.205^a
Proteins (%)	12.59^a	13.58^a	15.15^a	11.94^a
K (mg/kg^{-1})	26512^a	26729^a	31080^a	27628^a
Ca (mg/kg^{-1})	5666^b	6601^b	8907^b	4651^a
Mg (mg/kg^{-1})	2018^a	2187^a	2469^a	2461^a
P (mg/kg^{-1})	2792^a	2767^a	2917^a	2558^a
Zn (mg/kg^{-1})	18.30^a	21.47^a	21.37^a	20.87^a
Cu (mg/kg^{-1})	3.07^a	3.93^b	4.37^b	2.87^a
Mo (mg/kg^{-1})	2.13^b	0.93^a	2.33^b	0.93^a

Average values of three plots same dose
In each row values followed by the same letter are not significantly different (p>0.05).

The soil pH of plots with biosolids amendment, remained at the upper limit of ideal 7 to 7.5 since in the soils of pH> 7 , 5 the enzimatic activity decreases (Schomberg et al., 1994). The initial EC of soil amended was significantly increased all doses, due to the surface application of sludge , which have increased salt concentration. Anyway was of lesser magnitude than that found by other researchers using different biosolids (dairy) (López Mosquera et al., 2000) or from poultry industry (Punshon et al., 2002). The final EC was greater with all doses of malting sludge, similar to soil amended with organic residues (Jiménez et al., 2004). The small variation of the Cation Exchange Capacity, of the soil amended with high doses of malting sludge could be due to a decrease in negative charges (organic matter). The final values of the CEC all plots were slightly lower than the initials, coinciding with the results obtained by other researchers in soil amendments with organic wastes (Rivero, 1998).

Dry matter productions obtained in this study were slightly higher than those reported by other authors in field trials where biosolids were applied with similar doses (Zebarth et al., 2000). The protein content of harvested grass was not significantly different. As other investigators found, the lack of water can limit the absorption of N by the crop (Cáceres et al., 2005). However, the concentration of N in plants is slightly higher than the range suggested by other researchers (Zebarth et al., 2000). The contents of P, Ca, Mg and K in the harvested plants were similar to those obtained in other species such as ryegrass (*Lolium perenne* subsp. Multiflorum (Lam.) Husn). P concentrations in orchard grass (*Dactylis glomerata* L.) .The increase in Ca content might be related to prevailing pH in the root zone, but mainly due to the increased availability of this element (Ca / Mg in the exchange complex greater than 6). Mg which is part of the chlorophyll molecule and is intimately involved in photosynthesis was measured at normal levels. The concentrations founded were at the limit mentioned by some researchers to prevent the development of diseases such as tetanus grass in ruminants. The increase in Cu content, agrees with that shown by other researchers found significantly increases of Cu in stems and leaves of wheat, using sewage sludge as organic amendment (Frost et al., 2000).

IV. CONCLUSION

The soil C slightly alkaline, amended with biosolids (malting sludge) have decreased pH, which is a positive change in quality (at the beginning) and have showed an slow increasing trend throughout the test period because growing of grasses and nitrate absorption of those.

Malting Sludge amendment have increased significantly available P, Zn and Cu.

The nutricional quality of *Dactylis glomerata* L. was significantly increased with the malting sludges amendment (Ca and Cu contents).

Finally this results from field test indicate that this biosolids constitutes a suitable amendment for agricultural soils, increasing quality and productivity of forage.

REFERENCES

[1] Campaña, D.H., Uribe Etchevarria M., Airasca A.O., Andrade Couce M.L. 2014. Physicochemical and Phytotoxic Characterisation of Residual Sludge from the Malting of Barley. *Pollution Effects and Control.* Volume 2 – Issue 2 pg 1-6.

[2] Cáseres, L, Bast, R., Méndez, M., Currie,H. 2005. Fertilización de trigo em condiciones óptimas de disponibilidad hídrica. *Resumen A039.,* UNNE: comunicaciones científicas y tecnológicas. Fac. Agronomía.

[3] Frost, H.L., Ketchum, L.H., 2000. Trace metal concentration in durum wheat from applicatiopn of sewage sludge and commercial fertilizer. *Adv. Envirom.Res*. 4:347-355.

[4] Jimenez, L., Larrea, M., Noguera, N., 2004. Efectos del estiércol bovino sobre algunas propiedades químicas de un ultisol degradado en el área de Machiques Colón, estado de Zulia. *Rev. Fac. Agron*., 21 (4): 236-245, Caracas.

[5] Lopez Mosquera , M.E., 2000. Evaluación Del efecto salino en suelo fertilizado con lodos de industria láctea . *Edafología* – Tomo 7 – Universidad Santiago de Compostela – España.

[6] Punshon, T., Adriano D.C., Weber, J.T. 2002 . Restoration of drastically eroded land using coal fly ash and poultry biosolid. *Sci.Total Environ*. 296: 209-225.

[7] Rivero, C. 1998. Efecto de La incorporación de residuos orgânicos sobre algunas propiedades de um alfisol degradado. *Rev.Venesuelos*. 3 (2): 55-61.

[8] Roy, R.N., Misra, R.V., Lesschen, J.P.,Smaling, E.M. 2003. Assesment of soil nutrient balance. FAO. *Fertlizer and Plant Nutrition Bulletin 14.*

[9] Schomberg, H.H., Ford, P.B., Hardgrove, W.L., 1994 . Influence of crop residues on nutrient cycling and soil chemical properties. *Managing Agricultural Residues* . Lewis Pub.

[10] Schulz, R., Romheld, V. 1997.Recycling of municipal and industrial organic wastes in agriculture: benefits lilitations and means of improvements. *Plant nutrition for sustainable food production and environment.* Kluwer academic Pub (Japan) 581-586.

[11] Torres Carbonell C.A., Marinissen A., Lauric, A., Campaña D.H., Loewy T. 2012. *XIX Congreso Latinoamericano de la Ciencia del Suelo*- Abril 2012 – Mar del Plata – Argentina.

[12] Zebarth, B.J., McDougall, N., Neilsen, G., Neilsen D., 2000. Availability of Nitrogen from municipal biosolids for dryland forage grass. *Canadian J. Plant Sci* 80:575-582.

In-Vitro Studies of Different Culture Media and Biocontrol Agents on Growth and Sporulation of *Alternaria Alternata* (Fr.) Keissler an Incitant of Broad bean (*Vicia Faba L.*) Leaf Blight Disease

David Kamei[1] and Archana U Singh[2]

[1]KVK, ICAR, Senapati District, Manipur
[2]Division of Nematology, ICAR- IARI, New Delhi

***Abstract*—** *Studies on different culture media and biocontrol agents on growth and sporulation of Alternaria alternata (fr.) keissler causal organism of Broad bean (Vicia faba L.) leaf blight disease was conducted in vitro. Six culture media was tested for growth and sporulation of A. Alternata. It was found in that in liquid broth media, Potato dextrose broth (607 mg, dry weight of mycelium) and in solid media, maximum linear growth recorded in Potato dextrose agar (90 mm) with maximum number of spores 6.2 x 103 spores/ml. The antagonistic studies of 5 fungal biocontrol agents in dual culture technique found that all biocontrol agents statistically produced a significant antagonistic effect on linear growth and sporulation of A. Alternata. Maximum linear growth inhibition of Alternaria alternata was found in Trichoderma harzianum (17.5mm) with 89.6% inhibition and at par with Trichoderma viride (19.5mm) with 84.4% inhibition followed by T. hamatum (22.3 mm) with 74.1% inhibition and Gliocladium virens (21.0 mm) with 70.6% and minimum significant inhibition effect in T. koningii (22.8 mm) with per cent inhibition of 67.2 over the check. Highest spore inhibition of was found in T. harzianum (89.6%) and T. viride (84.4%) followed by T. hamatum (74.1%) and G. virens (70.6%) while minimum inhibition was found in T. koningii (67.25). It was further observed that T. harzianum could overgrown the growth of pathogenic fungus within 2 days and for T. viride within 3 days where as T. hamatum and G. virens took 5 days.*

Keywords*— *Alternaria alternata, blight disease, broth media, solid media, efficacy, linear growth, sporulation, mycelium, biocontrol agents.

I. INTRODUCTION

Broad bean (*Vicia faba L.*) is an important leguminous crop cultivated in different parts of mild sub-tropical and temperate regions of the world. In Manipur and north eastern state of India it is mostly grown as vegetable crop, where tender green pods were used as culinary purposes and mature seeds used as dals, snacks preparation and seed purposes. This important winter vegetable crop was often attacked by various diseases and out of eight major diseases recorded, blight disease caused by *Alternaria alternata* fr keissler is most serious (Gupta, 1985). The disease first appeared as small circular, brownish colour at the margin or tip of the leaf and progressed towards the midrib or the spots coalesce together to form into an elongated, irregular necrotic dark brown lesions. The affected young plants turn pale and leaf get drooping and wilted and die later. At present there is little information on physiology of the fungus associated with the blight disease and on management practices against this disease. Therefore the present investigation was taken up to study the effect of different types of culture media on the growth and sporuration of the pathogen and also to study the antagonistic effect of various bioagents against this pathogenic fungus.(Goswami and Mittal, 2002; Mittal et al., 2005 and Goswami and Mittal, 2004)

II. MATERIALS AND METHODS

The research works was carried out in the laboratory of Department of Plant Pathology, Krishi Vigyan Kendra-Senapati district, Manipur in the year 2018-19. The details of experimental approaches and procedures adopted during the course of investigations are described as follows:

2.1 Collection of diseased specimen

Broad bean plant showing typical leaf blight symptoms were collected from different location of Senapati district of Manipur during rabi season (2018-19) and brought in the laboratory of KVK-Senapati, Hengbung, Manipur for isolation purpose and use further for study.

2.2 Isolation and purification of fungus associated with Broad bean leaf blight

The infected leaves were surface sterilized with 1% sodium hypochlorite solution for about 1 minutes and then wash by 2-3 change of sterile water and with the help of sterilized blade cut into small bits of 2-3 mm including half live and half diseased death tissue and then by using sterilized inoculating needle, 2-3 bits is inoculated aseptically in a Petri plates containing sterilized fresh potato dextrose agar (PDA). The inoculated plates were incubated at 25 ± 1^0C for 2-3 days. Purification of the isolated fungal pathogen was done by single hyphal tip culture method. The mycelium tip from the Petri plates was aseptically transferred it into PDA slants and stored at room temperature and periodically subculturing done for further investigation work.

2.3 Studies on effect of different culture media on the growth and sporulation of *Alternaria alternata* incitant of blight disease of broad bean

Six different culture media namely, Corn meal agar, Czapek's dox agar, Elliot's agar, Richard's agar, Potato dextrose agar and Host extract agar were tested on growth and sporulation of fungus both in solid and liquid broth form of the media.

Different culture media, their composition and preparation methods:

2.3.1 Corn meal agar

Ingredients composition	Quantity
(i). Corn meal	50.00 g
(ii). Dextrose	20.00 g
(iii). Agar	15.00 g
(iv). Distilled water	1000.00 ml

Preparation: 1000 ml of distilled water was heat to boiling in a pan, add 50 g of corn meal powder and dissolve 20 g dextrose and add 15 g agar, bit by bit with constant stirring by glass rod and make up the volume upto correct volume (1000ml) by adding distilled water and then dispense in conical flasks and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. For broth culture media agar is devoid.

2.3.2 Czapek's dox agar

Ingredients composition	Quantity
(i). Sodium nitrate	2.00 g
(ii). Magnesium sulphate	0.50 g
(iii). Potassium chloride	0.50 g
(iv). Ferrous sulphate	0.05 g
(v). Agar	15.00 g
(vi). Dextrose	20.00 g
(vii). Water	1000.00 ml

Preparation: Suspend the whole chemical ingredients in 1000 ml distilled water. Heat to boiling to dissolve the medium completely makes up the deficit volume by adding distilled water. Dispense the media in conical flasks, plugged with non absorbent cotton and sterilized by autoclaving at 15 lbs pressure for 15 minutes (121^0C). For broth culture media agar is devoid.

2.3.3 Elliot's agar

Ingredients composition	Quantity
(i). Sodium carbonate	1.00 g
(ii). $MgSO_47H_2O$	0.50 g
(iii). KH_2PO_4	1.36 g
(iv). Asperagine	1.00 g
(v). Dextrose	5.00 g
(vi). Agar	15.00 g
(vii). Distilled Water	1000.00 ml

Preparation: Suspend the whole chemical ingredients in 1000 ml distilled water. Heat to boiling to dissolve the medium completely makes up the deficit volume (upto 1000ml) by adding distilled water. Dispense the media in conical flasks, plugged with non absorbent cotton and sterilized by autoclaving at 15 lbs pressure for 15 minutes (121^0C). For broth culture media agar is devoid.

2.3.4 Richard's agar

Ingredients composition	Quantity
(i). Potassium nitrate	10.00 g
(ii). Monopotassium dehydrogen phosphate	5.00 g
(iii). Magnessium sulphate	2.50 g
(iv). Ferric chloride	0.02 g
(v). Sucrose	50.00 g
(vi). Agar	15.00 g
(vii). Distilled	1000.00 ml

Preparation: Suspend the whole chemical ingredients in 1000 ml distilled water. Heat to boiling to dissolve the medium completely makes up correct volume (1000ml) by adding distilled water. Dispense the media in conical flasks plugged with non absorbent cotton and sterilized by autoclaving at 15 lbs pressure for 15 minutes (121^0C). For broth culture media agar is devoid.

2.3.5 Potato dextrose agar

Ingredients composition	Quantity
(i). Potato	200.00 g
(ii). Dextrose	20.00 g
(iii). Agar	15.00 g
(iv). Distilled	1000.00 ml

Preparation: 200 g sliced potato were boiled in 500 ml of distilled water and half cook or till soften, collect the decoction by straining in a cheese cloth and add 20 gm of dextrose. In another beaker 500 ml of water is heated and 20 gm of agar is added bit by bit till it completely gets dissolved. Now agar solution and potato extracts dextrose solution were mixed together and the volume is brought upto correct volume (1000 ml) by adding more distilled water. Dispense the media in conical flasks and plugged with non absorbent cotton and sterilized by Autoclaving at 15 lbs pressure (121^0C) for 15 minutes. For potato dextrose broth media agar is devoid.

2.3.6 Host extract agar

Ingredients composition	Quantity
(i). Broad bean leaves	100.00 g
(ii). Dextrose	20.00 g
(iii). Agar	15.00 g
(iv). Distilled	1000.00 ml

Preparation: 100 gm fresh broad bean leaves were boiled in 500 ml of distilled water till soften. Collect the decoction by straining in a cheese cloth and to it add 20 g dextrose. In another beaker 500 ml of water is heated and 15 gm of agar is added bit by bit till it completely gets dissolved. Now agar solution and broad bean leaf extracts dextrose solution were mixed together and the volume is brought upto correct volume (1000 ml) by adding more distilled water, dispensed the media in conical flasks and plugged with non absorbent cotton and sterilized by Autoclaving at 15 lbs pressure (121^0C) for 15 minutes. For host leaf extract broth media agar is devoid.

1) Liquid Broth media test

50 ml each of the broth media to be tested was dispensed in 100 ml capacity conical flasks and autoclaved at 15 lb pressure (121^0C) for 15 minutes. Each treatment was replicated into four times. Mycelial discs of 3 days old test fungus mycelium was cut by using sterilize 5 mm cork borer and with the help of sterilized inoculating needle a mycelial disc was aseptically inoculate into those conical flasks containing the test media. The inoculated conical flasks was plugged again and incubated at 25 ± 1^0C for 10 days. The mycelial mats was harvested through pre-weighed Qualigens No. 651 A (11.0 cm diameter) filter paper and dried at 60^0C in a hot air oven for 72 hours and then cooled in desiccator for 24 hours and re-weighed in an electronic balance. The mycelial dry weight for each treatment was recorded and actual growth was calculated.

2) Solid media test:

50 ml each of different solid medium to be tested was autoclaved and then dispensed 20 ml of it in the sterilized Petri plates of 9 cm diameter. Each treatment was replicated into four. Mycelial discs were cut from 3 days old actively growing colony of fungal culture by using sterilized 5 mm diameter cork borer. One mycelial disc was inoculated at the centre of each test plates containing sterilized media and then incubated at 25±1^0C. The radial growth was recorded in 24 hours intervals till the fungus covered the whole plate. The differences in the growth rate were calculated for all the treatments by using the formula Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100 \quad (1)$$

Where, I=percent inhibition

C=growth in control

T=growth in treatment

For estimation of fungal spores the method described by Devi (1991) was followed. For this, 1 sq.cm mycelial block was cut from each plate and the mycelial mats were removed from the medium. It was then transferred into a test tube containing 5ml distilled water and shaken it for 5 minutes. The spore density/ml of this homogemous spores solution was estimated by using a Haemocytometer.

2.4 Effect of biocontrol agents on the growth and sporulation of *Alternaria alternate*

The efficacy of 5 biocontrol agents namely *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum, Trichoderma koningii* and *Gliocladium virens* was tested on growth and sporulation of *Alternaria alternata* by dual culture technique. The biocontrol agents were collected from ITCC, IARI, New Delhi vide Dept. of Plant Pathology, College of Agriculture, CAU, Imphal.

Biocontrol agents and pathogen was multiplied in sterilized potato dextrose agar (PDA) plates separately. Mycelial discs were cut with a sterilized cork borer of 5 mm diameter from actively growing 3 days old fungal colony. Each mycelial discs of both pathogen and bio-agents was placed in opposite pole 3cm apart in fresh sterilized potato dextrose agar (PDA) plates. The control plate was inoculated only with pathogenic fungal mycelial disc. Each treatment was replicated into four times. The seeded plates were then incubated at 25 ± 1^0C. The observation was taken at 24 hours interval till the pathogen and biocontrol agent comes in contact with each other. The per cent inhibition on the fungal radial growth was calculated.

For estimation of spores, 1 sq. cm. mycelial blocks were cut from each plate at the point of contact between pathogen and biocontrol agent. The mycelial mat was removed from the medium and then transferred it to different test tubes containing 5 ml sterile distilled water and shaken for 5 minutes, the spore density per ml from each treatment was estimated by using haemocytometer.

III. RESULT AND DISCUSSION

TABLE 1
EFFICIENCY OF DIFFERENT TYPES OF BROTH CULTURE MEDIA ON MYCELIUM GROWTH OF *ALTERNARIA ALTERNATA*

S. No.	Types of culture media	Growth (mg)*
1.	Corn meal	195
2.	Czapek's dox	230
3.	Elliot's broth	255
4.	Richard's medium	125
5	Potato dextrose	607
6.	Host extract	465
	C.D. $_{5\%}$	4.2

**** Mean of four replication***

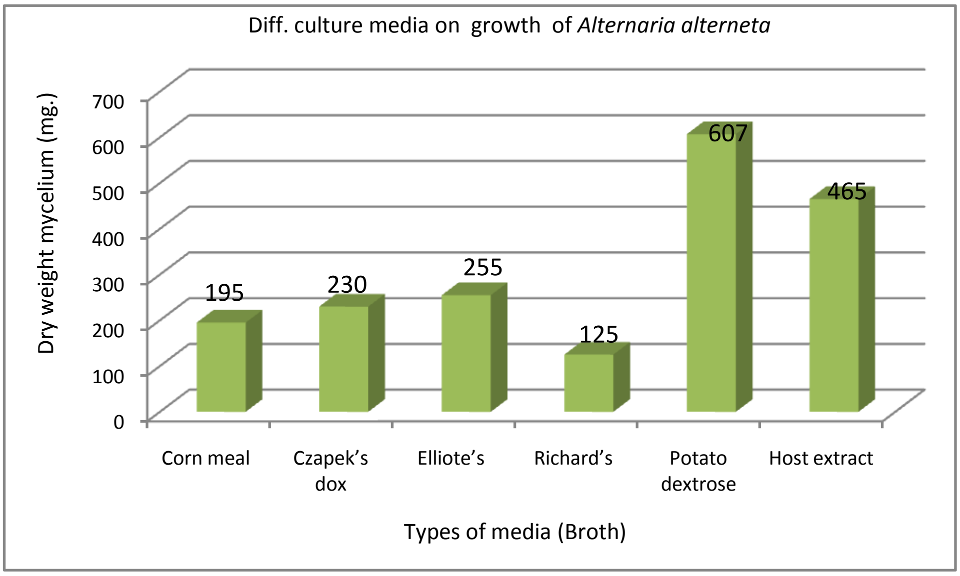

FIGURE 1: Performance of different culture media on growth of *Alternaria alternata*

The data presented in the above Table 1, fig.1, is the results of different types of broth culture media test on the growth of fungi. Among the media statistically significant and maximum growth of *Alternaria alternata* (dry wt. mycelium) was found in Potato dextrose (607mg) followed by Host extract (467mg) and Elliote's media (255mg) while minimum growth was found in corn meal (195) and Richard's medium (125). Our present investigation was in corroborate with that of Lipps and Herr (1980) who reported that maximum growth of *Alternaria helianthi* was observed on potato dextrose broth and V-8 juice, semi synthethic medium. Similarly, Singh (1994) also reported that *Alternaria alternata*, an incitant of stalk rot of sunflower could grow well on Potato dextrose broth among various media under trial.

TABLE 2
EFFECT OF SOLID CULTURE MEDIA ON GROWTH AND SPORULATION OF *ALTERNARIA ALTERNATA*

S. No.	Media	Growth (mm)*	No. of spores/ml*
1.	Corn meal agar	77	2.6×10^3
2.	Czapek's dox agar	78	2.8×10^3
3.	Elliot's agar	81	2.9×10^3
4.	Richard's agar	62	1.9×10^3
5.	Potato dextrose agar	90	6.2×10^3
6.	Host extract agar	83	3.5×10^3
	C.D. $_{5\%}$	0.6	-

**** Mean of four replication***

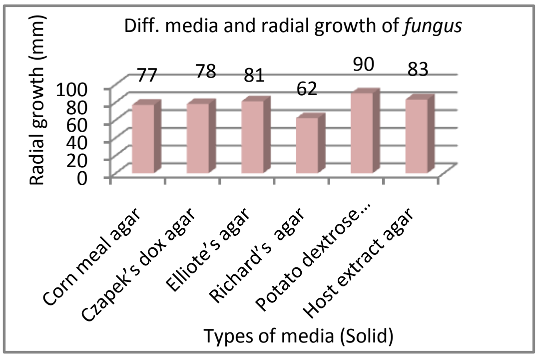

FIGURE 2: Different culture media and its effect on radial growth of *Alternaria alternata*

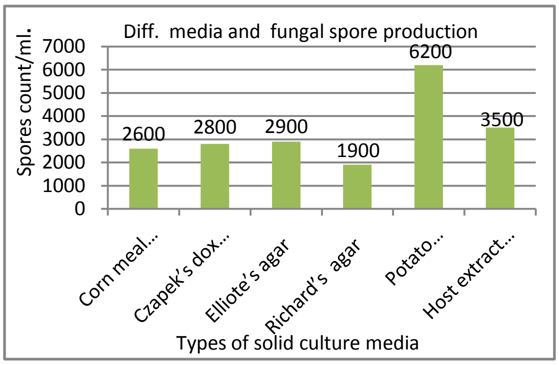

FIGURE 3: Different culture media and its effect on sporulation of *Alternaria alternata*

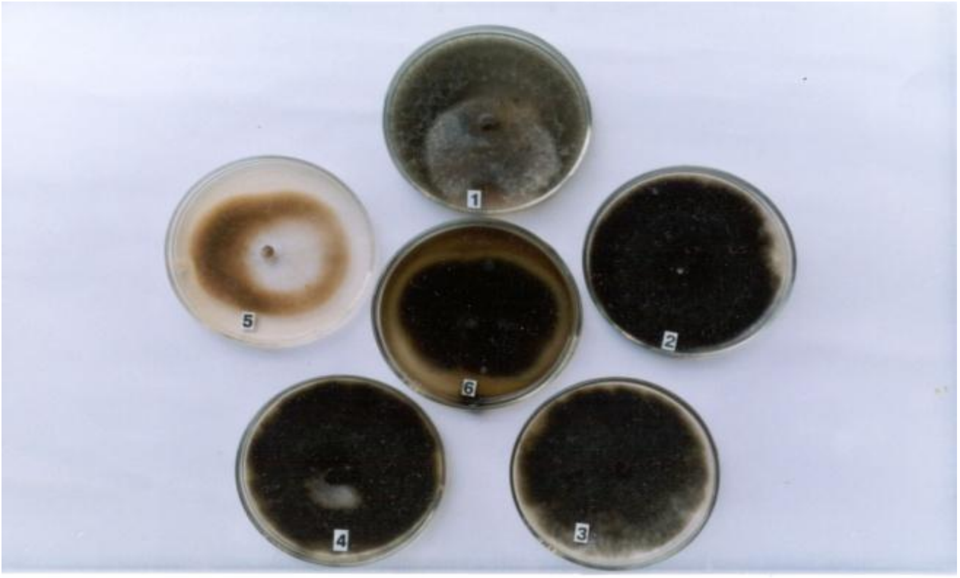

PHOTO 1: Different culture media on linear growth of *A. alternate*
(1).Elliot's agar (2).Host extracts agar (3). Potato dextrose agar (4).Czapek's dox agar (5).Richard's agar, (6).Corn meal agar

The data presented in the above Table 2. Fig. 2 & 3 were the results of different culture media and its effect on radial growth of fungus and production of spores. The results found among the tested media that maximum linear growth was found in Potato dextrose agar (90 mm) followed by Host extract agar (83 mm), and Elliot's agar (81 mm) whereas mimimum radial growth was recorded in Richard's agar (62 mm) and corn meal agar (77 mm). Our finding was in agreement with Allen et al. (1993) who reported best colony growth of *Alternaria helianthi* on Potato dextrose agar (PDA), whereas Pandey and Vishwakarma (1998) reported that maximum radial growth of *Alternaria alternata* was recorded in Capsicum dextrose agar and Radish dextrose agar, they also found that synthetic media like Czapek's dox agar and Richard's agar supported poor linear growth of fungus. This might be due to higher nutrient content of the vegetable base media to support growth of the fungus than those of synthetic media.

It was further observed that all media induce sporulation however, among the tested media maximum sporulation was found in Potato dextrose agar (6.2×10^3) and Host extract agar (3.5×10^3) followed by Elliote's agar (2.9×10^3) and Czapek's dox agar (2.8 x 103) and minimum sporulation was found in Richard's agar (1.9×10^3) and Corn meal agar (2.6×10^3). Our present investigation agreed with that of Shane et al (1981) who asserted that conidia of Alternaria helianthi per colony was maximum on Potato dextrose agar (PDA). Singh (1994) also reported that maximum sporulation of Alternaria alternata was found in Potato dextrose broth. Potato dextrose agar supported best mycelia growth and sporulation of Alternaria alternata (fr.) keissler a causal organism of chickpea blight disease, Singh Virendra et al (2001).

TABLE 3
EFFECT OF BIOCONTROL AGENTS ON GROWTH AND SPORULATION OF *ALTERNARIA ALTERNATA*

S. No.	Treatment	Time taken for meeting of bioagent and pathogenic fungi (days)	Linear growth of *A.alternata* (mm)*	Spores/ml*	% inhibition over (check)	
					Linear growth	Spores
1.	*Trichoderma koningii*	5	22.8	1.9×10^3	29.4	67.2
2.	*Trichoderma hamatum*	4	22.3	1.5×10^3	31.0	74.1
3.	*Trichoderma harzianum*	2	17.5	0.6×10^3	45.7	89.6
4.	*Trichoderma viride*	3	19.5	0.9×10^3	39.5	84.4
5.	*Gliocladium virens*	4	21.0	1.7×10^3	34.9	70.6
6	A. *Alteriaria alternata* (check)	-	32.3	5.8×10^3	0	0
	B. C.D.$_{5\%}$	-	2.6	-	-	-

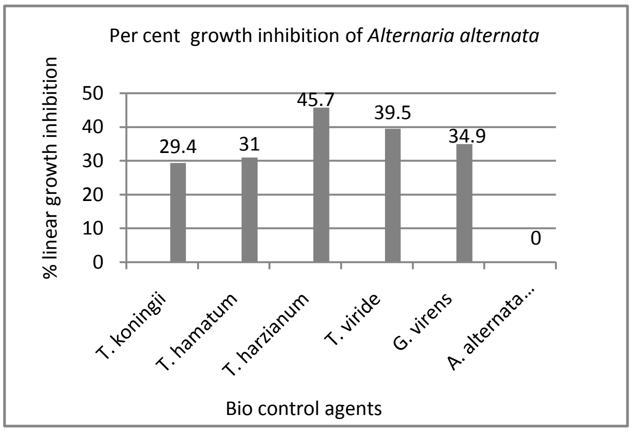

FIGURE 5: Per cent linear growth inhibition of A. alternata by different bioagents

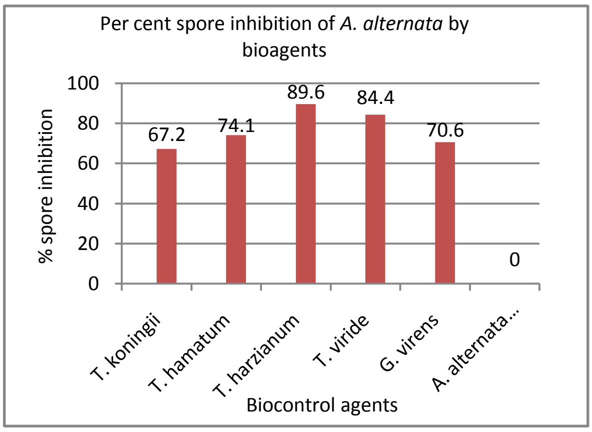

FIGURE 6: Per cent spore inhibition of A.alternata by antagonistic effect of different bioagents

PHOTO 2: Antagonistic effect of biocontrol agent on linear growth of pathogenic fungus (*Alternaria alternata*) in dual culture test (1). *T. koningii* (2). *T. hamatum* (3). *T. harzianum* (4). *T. viride* (5). *G. virence* (6). *A. alternata* (check)

IV. CONCLUSION

The data presented in the above Table 3, Fig. 4 & 5 is the antagonistic effect of biocontrol agents on the growth and sporulation of *Alternaria alternata* during dual culture test. It was found all biocontrol agents statistically produced a significant antagonistic effect on linear growth and sporulation of *A. alternata*. However, among the bioagents, maximum linear growth inhibition of the pathogenic fungi (*A. alternata*) was found in *Trichoderma harzianum* (17.5mm) with 89.6% inhibition over the check which was found at par with *Trichoderma viride* (19.5mm) with 84.4% inhibition followed by *Trichoderma hamatum* (22.3 mm) with 74.1% inhibition and *Gliocladium virens* (21.0 mm) with 70.6% and minimum significant inhibition effect was recorded in *Trichoderma koningii* (22.8 mm) with per cent inhibition of 67.2 over the check. It was also observed that highest per cent inhibition of sporulation of *A. alternata* was found in *T. harzianum* (89.6) and T. viride (84.4) followed by T. hamatum (74.1) and G. virens (70.6) while minimum per cent inhibition was found in T. koningii (67.2). It was observed that *T. harzianum* could over grown the pathogenic fungus within 2 days and T. viride within 3 days whereas *T. hamatum* and *G. virens* took 5 days. Our finding was in agreement with Basim and Katircioglu (1990) reported that in dual culture technique, bioagent Bacillus subtilis isolates could produce antagonistic activity against 7 (seven) fungal pathogens including *Alternaria alternata*. Similarly, Rajeshwari et al. (1998) reported that in dual culture test maximum inhibition of Macrophomina phaseolina was found in *Trichoderma harzianum, Trichoderma viride* and *Gliocladium virens* but least in *Trichoderma koningii*. Sudhakar et al. (1998) also reported that in dual culture technique *Trichoderma viride* was found superior in inhibiting the growth of *Rhizoctonia solani*, causal agent of rice sheath blight to *Trichoderma harzianum*. They also found lysis of the pathogen with the biocontrol agents.

REFERENCES

[1] Allen, S.J., Brown, J.F and Kochman, J.K (1983) The infection process, sporulation and survival of *Alternaria helianthi* on sunflower. *Annals of Applied Biology*. 102: 413–419

[2] Basim, H and Katircioglu, Y. Z (1990) Studies of *in vitro* antagonistic effects of some *Bacillus subtilis* isolates against important plant pathogenic fungi. *Proceeding of the second Turkish national congress of Biological control.*

[3] Devi, R.K and Tombisana (1991) Studies on fungi associated with rice sheath rot. Ph. D. Thesis submitted to Manipur University, Canchipur 117 pp

[4] Goswami, B. K and Mittal, A (2004) Management of root-knot nematode infecting tomato by *Trichoderma viride* and *Paecilomyces lilacinus. Indian Phytopath*. 57 (2): 235-236.

[5] Goswami, B. K and Mittal, A (2002) Effect of some fungal bioagents against root-knot nematode, *Meloidogyne incognita* infecting brinjal. *Pak J. Nematol.* 20 (1): 55-59.

[6] Gupta, D.K (1985) Studies on diseases of Broad bean in Manipur. *Indian J. Mycol. Pl. Pathol.* 15 (3): 297

[7] Lipps, P.E and Herr, L.J (1980) *Alternaria helianthi* in sunflower in Ohio. *Plant. Dis.* 66 (6): 509 – 512

[8] Mittal, A., Goswami, B. K and Chowdhry, P. N (2005) Hyphomycetous fungi from rhizosphere and rhizoplane of root-knot nematode affected cucurbitaceous crops from trans-Yamuna River Belt of Delhi. *Indian Phytopath* 58 (4): 480-482.

[9] Pandey, K.K and Vishwakarma, S.N (1998) Growth, sporulation and colony characters of *Alternaria alternata* on different vegetable-based media. *J. Mycol. Pl. Pathol.* 28 (3): 346–347

[10] Rajeshwari, B.J., Chandrasekhara, K and Pramod Chandra Kumar, C (1998) Antagonism of *Trichoderma* sp. and *Gliocladium* sp. against *Macrophomina phaseolina*, the incitant of dry root rot of mungbean. *Indian J. Plant Prot.* 26 (1): 9 – 11

[11] Shane, W.W., Baumer, J.S and Sederstrom, S.G (1981) *Altenaria helianthi*, a pathogen of sunflower new to Minnesota. *Plant Disease* 65(3): 269–271

[12] Singh, M.G (1994) Studies on stalk rot of sunflower caused by *Alternaria alternata* (Fr.) Keissler in Manipur. M.Sc. (Agri.) thesis submitted to CAU, Imphal. 49 pp

[13] Singh, V., Kumar, P and Sinha, A.P (2001) Influence of different media, pH and temperature on growth and sporulation of *Alternaria alternata* (fr.) keissler causing *Alternaria* blight of Chickpea. Legume Research- An International Journal. 24(4): 238-242

[14] Sudhakar, R., Rao, K.L and Reddy, C.S (1998) Studies on antagonism of *Trichoderma* and *Gliocladium* sp. against *Rhizoctonia solani* of Rice. *Indian J. Plant Prot.* 26 (1): 25 – 29

[15] Vincent, J.M (1927) Distortion of fungal hyphae in Presence of certain inhibitors. *Nature* 159 : 850.

The Impact of Population Growth on Natural Forests in Rwanda

Christine UWIRAGIYE[1], Abias MANIRAGABA[2]

[1]Initiative for Development and Environmental Awareness in Rwanda (IDEAR)
[2]Department of Environmental Economics and Natural Resources Management, University of Lay Adventist of Kigali (UNILAK)

Abstract— *Deforestation is a growing problem in many parts of the tropical world and one of the affected countries is Rwanda. The general objective of this study is to assess the effect of population growth on natural forest resource in Rwanda in general. Thus, this research focused on assessing the impact of population growth on natural forest of Rwanda. It critically examines how the population growth can impact natural forest. To achieve the set objectives, a cross sectional research design was combined with qualitative and quantitative approach. We collected secondary data from National Statistics Institute of Rwanda (NSIR), Ministry of Environment (MOE), Rwanda Environmental Management Authority (REMA), etc. The study adopted descriptive approaches in processing data. The findings show that above 45.27% of natural forests have been lost from 1984 to 2015 due to the high rate of population growth in Rwanda. As a recommendation, faced with a dense and rapidly increasing population on a fragile land resource, Rwanda must take steps towards transforming the economy and eliminating poverty through a Green Growth program. Family planning must be also adopted in reducing the impact of population growth on natural forestry preservation*.

Keywords— Population Growth, Natural forests, Deforestation.

I. INTRODUCTION

Deforestation is a growing problem in many parts of the tropical world and one of the affected countries is Rwanda. The population of Rwanda is 13,056,337 as of Saturday, October 31, 2020, based on Worldometer elaboration of the latest United Nations data. Rwanda has the most densely populated mainland African Country. In addition, the spread of poverty, the environmental degradation especially deforestation is due to local clearing of forest for their personal needs, such as for fuel and agriculture. Deforestation is indiscriminate cutting or overharvesting of trees for lumber or pulp, or to clear the land for agriculture, ranching, construction, or other human activities.

Forest is the resource that provides many benefits to the society. In addition to providing wood, forests provide a habitat for wild life, site for recreation, wildness, watershed protection and many other benefits. Forests worldwide are absorbing more CO_2 from the air but they come up short when it comes to sucking up the vast amounts of CO_2 emitted by humans into the atmosphere (Cross, 2019).

The impact of demographic changes on forests and the environment is often discussed in terms of biological carrying capacity, i.e. the maximum number of individuals that a resource can sustain. However, many factors influence carrying capacity, such as economic development, sociopolitical processes, and trade, technology, and consumption preferences (Bijendra, 2009).

Faced with a dense and rapidly increasing population on a fragile land resource, Rwanda had a steady increase in the population growth rate since 1960sdue to changes in different socioeconomic conditions in the world. So, where there is rapid population growth, environmental degradation cannot miss. For instance, according to Forest Investment Program for Rwanda**,** the natural forest cover of Rwanda was 429,728.47 Hain 1984 but has declined to 235,192.27 Ha in 2015. This means that over 45.27%of Natural forests in Rwanda have been lost.

Rapid population growth and the low economic standard of living in Rwanda have brought in their awake numerous consequences to land cover and use changes, change in climate and hydrological status in the country. In Rwanda studies have indicated that as there is agricultural land expansion at the expense of other land uses. Rwandan lands are fragile and forest, water and its biodiversity is climate dependent, which are still under stress due to population pressure and mismanagement of natural resources. The poverty stricken economy of Rwanda needed scientific management of its natural resources and balancing the population to cope up with the climate change and the challenges of the globalization of economy. Thus, there is a need to review the population and environment interrelation at the country level in order to suggest the means to minimize adverse effect of population pressure on environment.

The general objective of this study is to assess the effect of population growth on natural forests resource in Rwanda in general.

II. MATERIALS AND METHODS

2.1 Description of Study area

Rwanda has a population of over 12.6 million, living on 26,338 km^2 (10,169 mi^2) of land, and is the most densely populated mainland African Country (United Nations, 2019). According to official data from the World Bank and projections from Trading Economics 2019, the Gross Domestic Product (GDP) in Rwanda was worth USD 10.12 billion, 24.07 % of which is contributed by Agriculture, upon which around 72% of the working population employed in agriculture (FAO, 2015).

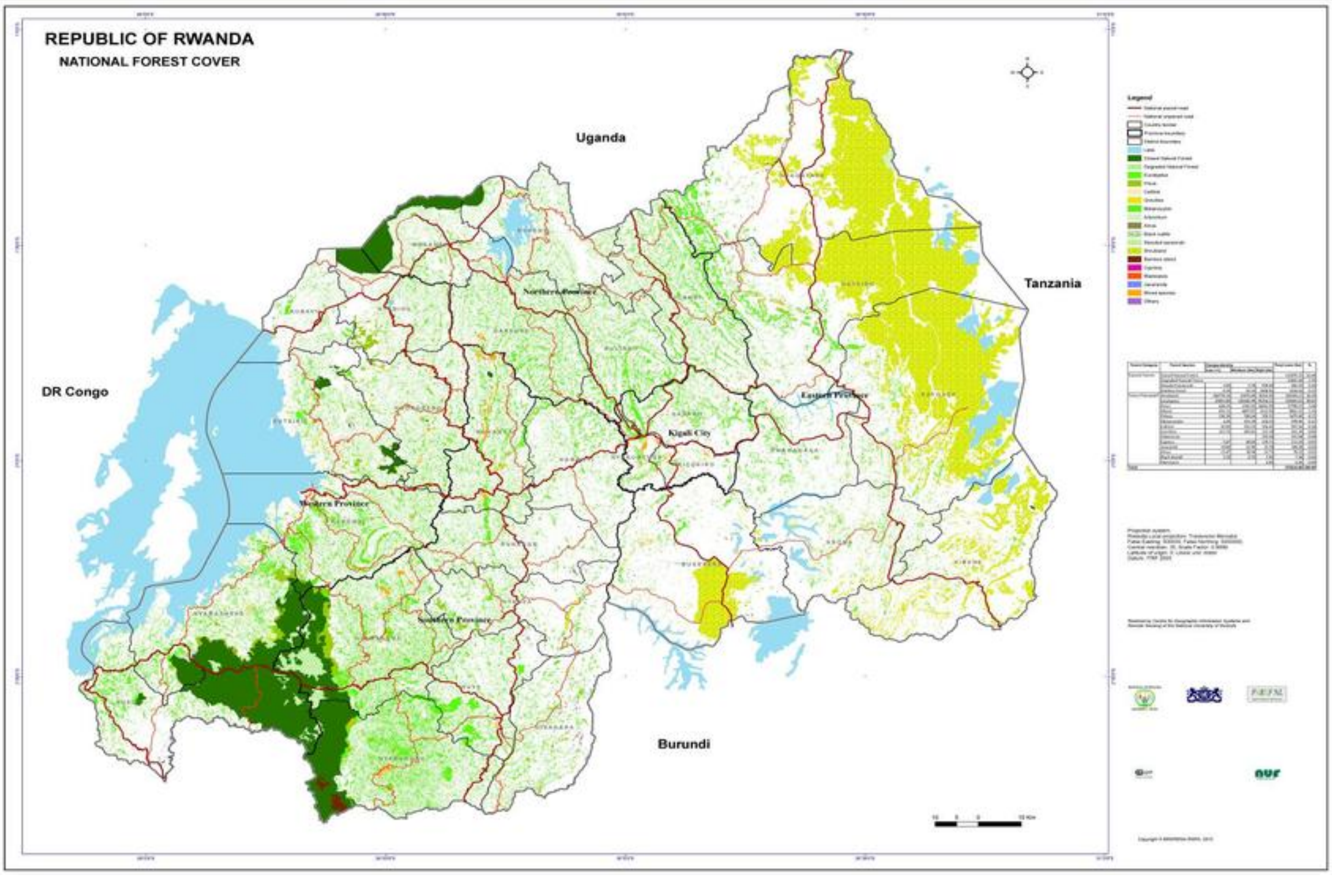

FIGURE 1: Map of Republic of Rwanda National Forests Cover

2.2 Data Collection

From 1960 to 2015, secondary data was collected from National Statistics Institute of Rwanda (NSIR), Ministry of Environment (MOE), Rwanda Environmental Management Authority (REMA), etc. We collected data on different projects related to natural forests and population growth in Rwanda. Secondary data involved different reports at the park levels, especially those from community partnership and ranger based monitoring programs.

2.3 Data analysis

We put all data in our computer and for data analysis we used SPSS and presented our data with tables and histograms.

III. RESULTS

3.1 Population Growth

Population growth trends in Rwanda from 1955 to 2017 is shown in below figure 2.

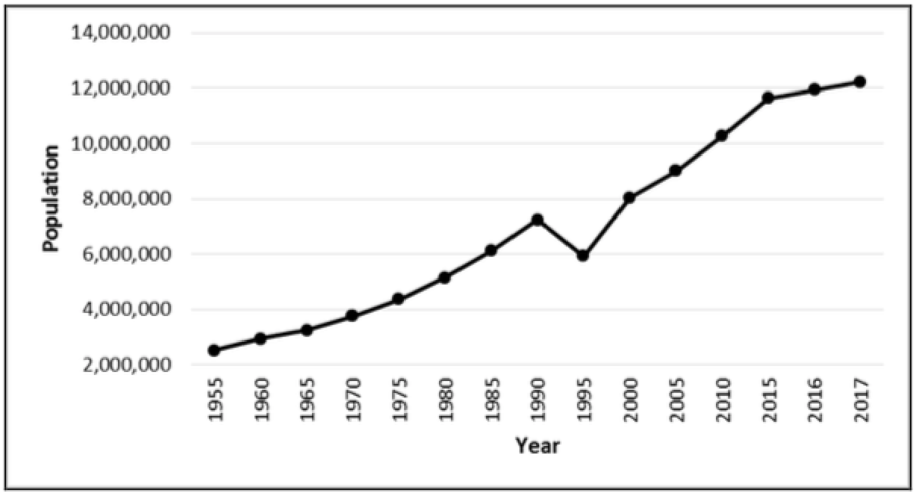

FIGURE 1. Population growth trends in Rwanda from 1955 to 2017

3.2 Natural Forest cover

Change over time from 1984 to 2015 of key natural forests in Rwanda is shown in below table 1.

TABLE 1
CHANGE OVER TIME FROM 1984 TO 2015 OF KEY NATURAL FORESTS IN RWANDA

Name of the Forest	Area (ha) 1984	Area (ha) 2015	% Loss
Buhanda Natural Forest	1116	18	98.40%
Gishwati Natural Forest	21213	1440	93.20%
Mashyuza Natural Forest	85	6	92.70%
Ibanda-Makera Natural Forest	1425	169	88.10%
Karama Natural Forest	3235	1061	67.20%
Dutake Natural Forest	31	11	65.70%
Karehe-Gatuntu Natural Forest Complex	48	19	60.30%
Nyagasenyi Natural Forest	45	19	58.20%
Akagera Natural Forest	267,741	112,185	58.10%
Mukura Natural Forest	4376	1988	54.60%
Sanza Natural Forest	49	24	51.00%
Mashoza Natural Forest	36	18	51.00%
Muvumba Natural Forest	1286	688	46.50%
Ndoha Natural Forest	39	29	26.00%
Kibirizi-Muyira Natural Forest	454	352	22.40%
Busaga Natural Forest	191	159	16.90%
Nyungwe National Park	112,230	101,005	10.00%
Volcanoes National Park	16,128	16,004	0.80%
Total	429728	235195	45.27%

IV. DISCUSSION

As the figure above shows between 1960 and 2007, natural forests declined considerably by about 45.27% due to different anthropogenic activities and resettlement of refugees. According to Forest investment Plan for Rwanda, the main drivers of deforestation and forest degradation in Rwanda are: (i) Agriculture, with 95% of households practicing traditional subsistence agriculture on small plots that have degraded soil structure and fertility due to continuous cultivation, (ii) Infrastructure development, (iii) Urbanization including the growing of built-up area, which increased by over 300% in the period from 1990 to 2016, (iv) artisanal mining practices, with a high increase in issued mining permits but no restoration of abandoned mining sites, (v) Forest product extraction, mostly firewood, charcoal and timber and (vi) Limited forestry extension services

These drivers derive from different socio-economic factors including: (i) high population growth, with 83.5% living in rural areas and 16.5% in cities, the increasing pressure on forests from agriculture, urbanization and exploitation of forest resources are linked to the high population with limited land to sustain their livelihoods; (ii) Lack of awareness and alternatives, which has led to the failure of different projects aiming to promote sustainable forest management and full engagement of local communities. A brief summary of the impact of the agricultural, livestock, oil and mining sectors and their impact on the forestry sector and forest management is presented in Table 2 below.

TABLE 2
ANALYSIS OF LIVESTOCK, AGRICULTURAL, OIL AND MINING SECTORS AND THEIR IMPACT ON THE FORESTRY SECTOR AND FOREST MANAGEMENT

Analysis of sectoral problems related to forestry	Impact on the forestry sector and forest management
Agriculture	
✓ Population pressure coupled with farming land scarcity leading to unsustainable farming practices (no fallowing of land, limited use of fertiliser inputs and in many cases poor or inadequate soil conservation practices; hence over-cultivation, erosion and low yields). ✓ Some farmers are still practicing traditional farming practices.	✓ Deforestation within farming areas ✓ Expansion onto fragile ecosystems, including shrub lands (especially in the Eastern Province); marshlands and lands on steep and very steep slopes
Livestock	
✓ Despite the zero grazing policy, some livestock keepers are still grazing out in ranches or other pastures	✓ Overgrazing of available pastures leading to grazing even on forest lands
Oil and Mining	
✓ Some mining sites are located in forests (*e.g.* Mukura Forest) ✓ Artisanal mining is most dominant throughout the country and in most cases mined areas are not effectively restored. ✓ Mining causes scarring of the landscape and often leaves behind waste rock and tailings heaps. ✓ There is also pollution of water streams and severe contamination of fields downstream of mines.	✓ Deforestation and forest degradation due to mining activities in forests ✓ Environmental degradation, pollution of water supplies, negative social impact in communities as mainly practised by young men

V. CONCLUSION AND RECOMMENDATION

According to the Ministry of Environment (MOH), forests currently cover around 30 % (10.3 % natural and 19.7 % plantations) of the dry land area of the national territory. Forests are the major source of domestic cooking energy, with 86.3% of the Rwanda population using bio-fuels in different forms, wood and charcoal being the dominant ones (MOH, 2017).

Forests also provide the foundation for Rwanda's tourism opportunities, and also protect watersheds, downstream wetlands and support agriculture. However, due to dense and rapidly increasing population on a fragile land resource, forests have been threatened by deforestation and continuous degradation.

Forests in Rwanda continue to occupy a high-table position as a major resource providing multiple functions to the population's livelihoods, supporting the equilibrium of the ecosystem and contributing to national socio-economic development.

Thus, sustainable management and use of natural resources and ecosystem services is well recognized as an important ingredient for sustained economic development and improvements in human welfare, and therefore is necessary for achieving the Sustainable Development Goals (SDGs). Environmental assets, such as soil, water and biodiversity, yield income, offer safety nets for the poor, maintain public health and drive economic growth. Yet environmental sustainability goals are often seen as distinct from, and sometime in conflict with, development goals(KTH, 2016).

The direct causes of land degradation are mainly deforestation, overgrazing and overcutting, shifting cultivation and agricultural mismanagement of soil and water resources: such as non-adoption of soil and water conservation practices, improper crop rotation, use of marginal land, insufficient and/or excessive use of fertilizers, mismanagement of irrigation schemes and over pumping of groundwater. The indirect causes of land degradation are mainly population increase, land shortage, short term or insecure land tenure and poverty and economic pressure (FAO, 2015).

The economic contribution of forest are associated with the production, consumption of goods and services, the supply of fuel wood, construction materials and timber come mainly from forest.

The rapid deforestation or the declining of forest accompanied by variety of other environmental problems is caused by agricultural expansion and wood gathering. The major factors contributing to deforestation are poverty, low level of income and population growth.

For this reason households have to depend on clouding for their fuel consumption and this is hampering the farmers from using organic manure as fertilizer for their already degraded farmlands. The wide range of topography has contributed to the presence of enormous biological and cultural diversity.

One of the solutions proposed to arrest forest degradation in certain area is to introduce the practices of participatory forest management by involving the local people. The result of this study reveals that population growth huge impact on forestry development in the ways of expanding agricultural land, using wood as energy sources and satisfying the input requirements in agricultural activity. We also recommend Rwandans to use family planning services in reducing the impact of population growth on the forestry development.

REFERENCES

[1] United Nations. (2019, December 1). *List of countries by population (United Nations)*. Retrieved October 20, 2020, from www.en.wikipedia.org: https://en.wikipedia.org/wiki/List_of_countries_by_population_(United_Nations).

[2] Bijendra, B. (2009). *Impacts of Demographic Changes on Forests and Forestry in Asia and the Pacific.* Bangkok: FAO.

[3] Cross, D. T. (2019, March 25). Forests absorb more of our CO2 emissions but hardly enough. *Sustainability times* , pp. 1-3.

[4] FAO. (2015). Retrieved October 20, 2020, from http://www.fao.org/rwanda/our-office-in-rwanda/rwanda-at-a-glance/en/.

[5] KTH. (2016). *Sustainability goals combining social and environmental aspects.* Stockholm: Eléonore Fauré.

[6] MOH. (2017). *Rwanda National Forest Policy 2017.* Kigali: Ministry of Natural Resources.

Factors Influencing Adoption of New Irrigation Technologies on Farms in Morocco: Application of Logit Model

Taoufik YATRIBI

National School of Agriculture in Meknès, Rural Economy Department, BP S/40 - 50 000 Meknès - Morocco

Abstract— *The objective of this article is to identify the determinants of the adoption of new irrigation technologies in Moroccan agriculture. The research is carried out on a sample of 82 farmers from the Souss-Massa region (Morocco). The results from the estimation of a binary logit model show that the variables: Age, level of education, non-farm income, perceived utility, possession of a computer, farm size, access to credit, ease of use, influence the probability of adoption of new irrigation technologies among farmers. The theoretical and managerial implications of this research are discussed.*

Keywords— Precision agriculture, technology adoption, irrigation, logit model, Souss-Massa region.

I. INTRODUCTION

In Morocco, the digitization of agriculture has started to gain more interest in recent years from various public and private actors. The last edition of the International Exhibition of Agriculture in Morocco (SIAM 2020) which was scheduled for April 2020 devoted its theme to technological innovations in agriculture, with the slogan "The future of agriculture lies in technological innovations ". The COVID-19 health crisis has reinforced this need to move to a digital economy, especially when this pandemic has crippled many sectors, threatening food security. The agricultural sector is then one of the most affected sectors. Empirical studies suggest that new technologies have positive agronomic, economic and environmental effects [1]. Experts now agree that technological innovation is an asset to save time and precision for the good of the farmer, the operation and the environment.

Today, Moroccan agriculture sector seems by far a sector where technological innovations are omnipresent. Unfortunately, we haven't the figures on the use of new technologies in the agricultural sector, but according to the World Economic Forum (WEF) in 2019, relating to information and communication technologies (ICT) at the global level, Morocco is ranked 100 out of 176 in the ICT Development Index, and 78 out of 139 in the NRI Index ranking (Networked Readiness Index), which gives us an idea of the level adoption of new technologies at the national level. At this level, precision agriculture (PA) appears to be a major asset for making agriculture an innovative and responsible sector. The literature indicates that PA technologies have one thing in common: optimizing agricultural production. PA technologies now allow farmers to apply the right dose of input at the right time, reduce the use of phytosanitary products, predict the outbreak of diseases in a plot, reduce energy consumption fossil fuel and accurately determine water requirements.

The Souss-Massa region, the Kingdom's main agricultural area, and one of the main centers of economic growth thanks to projects initiated under the Green Morocco Plan. However, agricultural activities are threatened by their dependence on water resources. With intensive use of irrigation gradually and dangerously depleting groundwater resources, the region's dam fill rate has fallen from 41.73 percent last year to 24 percent this year. The introduction of new precision farming technologies has become a necessity for a more rational use of natural resources.

The adoption of technologies by farmers is the starting point for agricultural development, of which farmers are the actors, and technologies are the tools of this development. Adoption in itself is a complex part of this subject. Previous studies show that the adoption of technology depends on many factors (socio-demographic, economic, institutional, ..), and the farmer is at the center of the decision to adopt and accept the technology. The reality is that Moroccan farmers are so attached to their habits and practices, and therefore they are used to the use of traditional techniques.

In this perspective, this work is positioned downstream of the decision to adopt new irrigation technologies (NT), it is intended to explain the adoption of NT from PA, by putting focus on three irrigation technologies, namely: Capacitive probes, weather stations, and mobile applications. Questions arise: On what can the adoption decision of the Moroccan farmer depend? Depending on the determinants of adoption, what may be the implications and challenges for the Moroccan agricultural sector? The interest of this research lies in the understanding of the factors which lead the farmer to the adoption of the NT which are essential at the same time for the decision makers, the diffusers of these technologies (companies), and for the researchers who study the determinants of growth. Interest in digitization, especially with the arrival of the COVID-19 pandemic, has only increased in recent times. The public authorities therefore need to understand the mechanisms of adoption by the farmer in order to be able to put in place a strategy for the development of digital agriculture.

II. THEORETICAL FRAMEWORK AND RESEARCH HYPOTHESES

The literature review allowed us to identify several variables that may influence the adoption of precision agriculture technologies. We have grouped these variables into four groups of explanatory variables: individual factors, organizational factors, environmental factors, and technological factors.

2.1 Individual factors

Age is identified as a determining factor in adoption. Older farmers tend to be conservative and reluctant to change and adopt technologies due to risk aversion [2] [3] explains that older farmers are more risk averse, less motivated to experiment with NT, less likely to be influenced by the benefits of NT. [4] explains that young farmers are more open to innovations and more likely to adopt new technologies. In the same way, [5] add that sometimes the adoption of technologies by older farmers is explained by the high investment costs, and that these farmers already have the means for their acquisition unlike the younger ones.

Hypothesis 1: Age has a negative effect on the adoption of new technologies.

The education level is highlighted by many authors as a determinant of the adoption of NT [5]–[7]. For example, [2] find that the adoption rate increases with the level of a farmer's education, especially if it’s advanced technology, and that learning is necessary for its use. The education level makes it easier for the farmer to use it, but also becomes someone more open to innovation.

Hypothesis 2: The education level has a positive effect on the adoption of new technologies.

On the other hand, the cost of acquisition has been closely linked in the literature to farm and non-farm income. Research results show that the higher the farm income, the more likely the farmer is to adopt new technologies [8]. The existence of off-farm income also increases the chances of adoption [9] [10] explains that for farmers with moderate income, non-farm income provides cash that can help the farmer learn NT. Access to off-farm income generating activities is generally associated with technology adoption [11] found that adoption has high costs, and that higher-income farmers are more likely to adopt them [12] showed that a 10% increase in income from agriculture was associated with a 9.2% increase in the chances of a producer adopting these technologies.

Hypothesis 3a: High agricultural income has a positive effect on the adoption of new technologies.

Hypothesis 3b: The existence of non-farm income has a positive effect on the adoption of new technologies.

On the other hand, farmers are more likely to adopt NT after seeing their usefulness in the field [3], [9]–[11], [13], [14]. For example, [15], in a study on the adoption of technologies in the dairy industry in Italy, he indicates that the demonstration of NT is practically done within the networks of farmers, which then pushes them to adopt the same technology [16] adds that cleaner production techniques (CPT) will only be adopted when farmers perceive their usefulness and the satisfaction of other farmers who have already adopted this technology.

Hypothesis 4: Perceived utility has a positive effect on the adoption of new technologies.

Another important factor that affects adoption is the attitude to risk. Some farmers prefer to work with the means at their disposal rather than invest in NT [8]. One of the reasons for this reluctance is uncertainty, first about the use of technology, and second about the economic return. For example, when the market price of agricultural products falls, farmers tend to invest less in capital (machine, innovation, etc.). For example, [10] points out that farmers who have less uncertainty about the economic return of NRVs are more likely to invest in these technologies. Complex technologies require additional investment in learning [9] adds that a modest level of skills and know-how negatively influence adoption. Its results show that technologies that require a lot of skill and technical know-how decrease the likelihood of their adoption.

Hypothesis 5: Risk attitude has a negative effect on the adoption of NT.

Computer use in farm management has been associated with the adoption of certain technologies such as the personal digital assistant (PDA) [17], or the Autosteer GPS guidance system [13] [17] explains that farmers who use computers in administrative management (for mail, invoicing, accounting, inventory and human resource management, etc.) may be more likely to adopt PA technology [5] postulate that farmers who are open-minded about technological advancement are also more supportive of technology adoption on their farms.

Hypothesis 6: Possession of a computer has a positive effect on the adoption of NT.

2.2 Organizational factors

Farm size is identified as a determinant of adoption. [6] explain that large farms are more complex to manage, NT have been shown to be effective in optimizing production and reducing costs [18] explain that the farm size is linked to the adoption of NT, as the farmer tends to devote some of his land to trying a NT first, unlike small farms. These findings align with several research studies on the adoption of PA technologies [4]–[6], [15], [19].

Hypothesis 7: The size of the farm has a positive effect on the adoption of new technologies.

On the other hand, [10] show that the lack of a title deed significantly decreases the likelihood of farmers adopting the technologies. This is because farmers with title deed are more likely to adopt practices that improve the soil compared to those who are in rental. Studies [12], [20] indicated that farmers who had obtained certificates of ownership felt more secure, which enabled them to make long-term investments. This is the case for example with drip irrigation; its non-adoption is most often linked to the rental status which does not benefit from subsidies.

Hypothesis 8: The absence of a title deed has a negative effect on the adoption of NT.

2.3 Institutional factors

Our exam of the literature showed the importance of the role by public services for encouraging the adoption of new technologies. It has been shown that awareness [21], [22], access to agricultural extension services [22], credit facilities and aggregation practice [7], [10], [18], [23] are essential to encourage the adoption of technological innovations [22] find that training and extension are means of raising awareness and supporting the adoption of watershed management practices [5] stress the importance of good advice, and offer the necessary information. The author points out that the lack of counseling and training is a barrier to adoption, and that internet availability alone is not a sufficient factor for farmers.

Hypothesis 9: The presence of a consulting service has a positive effect on the adoption of NT.

Farmers' access to credit services is identified as a determinant of the adoption, because bank credit allows farmers to have other financial resources, and therefore invest in new technologies. Several studies [7], [18], [23] conclude that farmers' access to credit services increases the likelihood of technology adoption [10] have shown that subsidies and taxation are tools for adopting new technologies [21] indicated that the adoption of green manure is strongly linked to certain government measures, such as subsidies for maintenance, reduction of tax for adopters, reduction of interest rate [23] show that the variable amount of subsidy is strongly correlated with the probability of adoption, the higher this amount, the greater the probability of adoption. In other words, the difficulty in accessing subsidies was associated with a relatively low adoption rate. The more difficult it is for farmers to access subsidies, the lower the likelihood of them adopting drip.

Hypothesis 10: Access to credit has a positive effect on the adoption of NT.

2.4 Technological factors

The cost of acquiring technology is often identified as a determinant of adoption [6], [7], [24]–[26] [5] point out that the majority of farmers are reluctant to introduce precision farming techniques mainly because of their high costs. Large farms are more likely to adopt precision farming technologies compared to small farms due to their financial capacity [10].

Hypothesis 11: The more the farmer perceives the cost of the technology, the lower the probability of adoption.

Ease of use is identified as an important factor in the adoption of PA technologies [21] find that farmers' intention to adopt EWS is influenced by perceived ease of use, subjective norms and perceived behavioral control. The authors show that farmers' perception of the ease of use of PA technology has a significant impact on its adoption. A farmer who perceives PA technology as complex or difficult to use is therefore less likely to adopt it [27].

Hypothesis 12: The perception of ease of use has a positive effect on the adoption of new technologies.

The figure below summarizes the variables that may explain the adoption of NT.

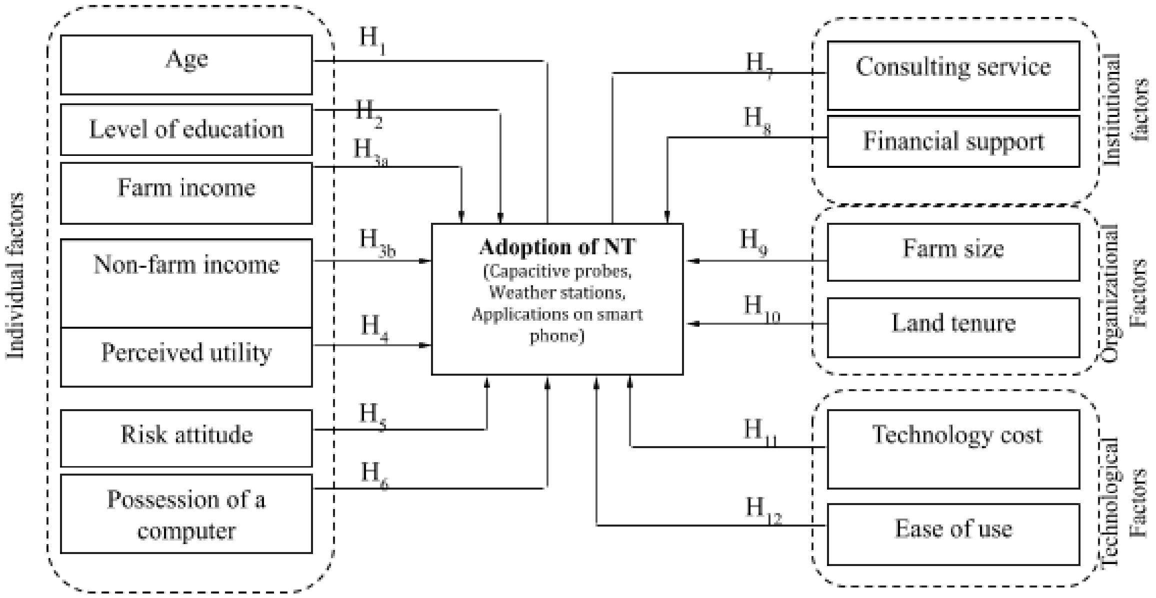

FIGURE 1: Proposed model to explain adoption of NT.

III. METHODOLOGY

In this section, we detail the methodology of the empirical study. First, we specify the research approach (3.1), the nature of the sample and the data collection method, then (3.2) we present the data analysis procedure (3.3).

3.1 Justification of the research approach

The already significant literature on the variables used in this research invited us to have a hypothetico-deductive approach. Indeed, [28] recall that when theoretical work provides enough knowledge on the phenomenon studied and lays intellectually consistent and empirically relevant theoretical bases, the deductive approach is desirable.

3.2 Sample and data collection

Our data collection was carried out in the region of Souss-Massa (Morocco). To collect the data, we opted for remote surveys during the month of April 2020. This choice is justified because of the health crisis (COVID-19), and the obligation of social distancing. A questionnaire was developed based on our theoretical framework. It is composed of four parts: Individual factors, organizational factors, institutional factors and technological factors. Table 1 gives an overview of all the variables, and the response methods.

We contacted the public services and companies supplying new technologies in the region to obtain the telephone directories of the farmers. A telephone directory of 130 farmers has been established. A pre-test was carried out on a sample of 10 farmers to test and adapt the questionnaire.

In order to be able to effectively explain the phenomenon studied, a large sample is needed. Otherwise, the results will be of limited scope, which could constitute a limit to the model used. Concretely, the rule is that for each explanatory variable, it will take at least between five to ten observations. In our case, we have retained thirteen variables, that is to say a minimum of total observations ranging between [65; 130[. And since we have made 82 observations, the minimum number required for logistic regression modeling is reached. While such a sample doesn't make it possible to ensure the representativeness of the results obtained and to seek external validity, it already leaves the possibility of improving our knowledge of the process of adoption of NT by farmers.

TABLE 1
BRIEF DESCRIPTION OF VARIABLES USED IN THE LOGIT REGRESSION MODEL

Variable	Type	Description
Adoption of NT	Binary	1 = Yes ; 0 = No
Age	Categorical	1 = <45 ; 2 = 45-60 ; 3 = Plus 60
Level of education	Categorical	1 = Illiterate ; 2 = Primarylevel ; 3 = Secondarylevel ;4 = ≥ Bac+3
Farmincome	Continuous	-
Non-Farmincome	Binary	1 = Yes ; 0 = No
Perceived utility	Scale	1 = Not at all useful; 2 = Not useful 3 = Useful; 4 = Very useful
Risk attitude	Scale	1 = Not at all risky; 2 = Not risky; 3 = Risky; 5 = Very risky
Possession of a computer	Binary	1 = Yes ; 0 = No
Farm size	Categorical	1= <20 ; 2= 20 – 50 ; 3= 50 – 100 ; 4= >100
Land tenure	Binary	1 = Property ; 0 = Location
Financial support	Binary	1 = Yes ; 0 = No
Consulting survice	Binary	1 = Yes ; 0 = No
Technologycoast	Scale	1 = Not at all high; 2 = Not high 3 = High; 4 = Very high
Easy of use	Scale	1 = Very easy; 2 = Easy; 3 = Difficult; 4 = Very difficult

3.3 Data analysis procedure

The adoption of new technologies is a YES or NO type choice, which refers to the Probit and Logit models which have been widely developed and used to study choice problems with binary dependent variables [29], [30].

The logit model is written as follows: $(\boldsymbol{Y}|\ \boldsymbol{X})$ = P (Y=1| X) =L($\mathbf{X'\beta}$)

Estimation in such a nonlinear model is more often based on the principle of maximum likelihood than on the principle of least squares. By the principle of maximum likelihood, we seek to calculate the value of the parameter β which maximizes the function L($\beta|Xn$). It is equivalent to find the parameter which maximizes the log-likelihood: l|Xn=ln L(β|Xn)

$$= \sum_{i=1}^{n} [Y_i \ln G(X'_i\beta) + (1 - Y_i) \ln\{1 - G(X'_i\beta\}]$$

IV. RESULTS AND DISCUSSIONS

In this part, we present a descriptive overview of our sample (4.1), and then we present the results of the research hypothesis tests (4.2).

4.1 Descriptive overview of the sample

All the agricultures in our sample are managed by men, with an average age of 45 years.

The distribution of farmers in our sample by educational level shows that 35% of farmers have a higher education than BAC + 3. 42% are illiterate, and 23% have primary and secondary levels.

Regarding the size of the farms surveyed, our sample is made up of farms with an area ranging from 2 ha to 800 ha. Almost 40% of the farms in our sample have a size between 5-20ha, 15% have a size between 20 and 50ha; 15% are between 50 and 100ha, and 30% are over 100ha.

For the type of crop, market gardening is identified as the main crop in 62% of the farms in the sample, against 38% for citrus.

For land tenure, rental is dominant; it represents 57% of the farms in our sample. The rest are private property.

Finally, concerning the direction of production, 43% of the production of farms is intended for the local market, 40% is reserved for export, and 17% of production is intended for processing.

4.2 Estimate of the Logit model of adoption of new technologies

Our dependent variable is NT adoption, which is a binary variable taking the value 0 if the farmer doesn't adopt any technology, versus the value 1 if the farmer adopts the three technologies pack aforementioned. The results of the estimation of the binary logistic model are presented in Table 2.

The model fits well, having fulfilled the conditions of a predictive model. Based on the forward selection method, the best fit model was created in six steps with the likelihood ratio test. Nagelkerke's R^2 of 0.52 indicates a satisfactory relationship between the independent variables and the dependent variable. R^2 logit is 0.39, which means that almost 40% of the variability in the probability of adoption is explained by the variables of the final model. We can then look at the Hosmer-Lemeshow test. This indicates whether there is a significant difference between the predicted and observed values. The ranking table shows that our model is able to correctly predict 73.4% of adoption cases, indicating a good fit of the model. Following the ascending method (Forward selection), and the likelihood ratio test (LR), only significant variables appear in the final model. The influence of the variables is expressed by the values of the logistic coefficients (B), and the odds-ratios are expressed by E [B].

TABLE 2
RESULTS FOR NT ADOPTION TESTED BY BINARY LOGISTIC REGRESSION

Variable	Coefficient B	Std, Error	Wald X²	Prob.	E[B]
Age	-0,221	0,091	5,90	0,007***	0,802
Level of education (≥ Bac+3)	0,68	0,027	634,29	0,0012***	1,974
Non-Farmincome(1 : Yes)	0,1823	0,054	11,40	0,012*	1,200
Perceived utility	0,1118	0,068	2,70	0,015*	1,118
Possession of a computer(1 : Yes)	0,096	0,047	4,17	0,0501*	1,101
Farm size (>100ha)	0,177	0,051	12,04	0,000***	1,194
Farm size ([50 – 100ha])	0,089	0,021	17,96	0,028*	1,093
Financial support(1 : Yes)	-0,118	0,045	6,88	0,031*	0,889
Easy of use	0,168	0,021	64,00	0,000***	1,183
C	5,501	1,124	23,95	0,021*	244,937

Note : Method: forward selection (likelihood ratio), dependent variable: NT Adoption (0 = no adoption; 1 = adoption of NT); original model (-2Log-Likelihood): 530,107; Full model (-2 Log-Likelihood):324,710; Chi squared: 205,389; R² (Cox and Snell) = 0.355; R² (Nagelkerke) = 0.523; Hosmer–Lemeshow test: Chi squared = 11,489, df = 5, Sign. 0,176 (n.s); R²logit = 0.399 Classification (model): 74.5%; N = 82; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.***

The age of the farmer negatively affects the adoption of new technologies ($\beta1 = -0.221$, $p < 0.01$). A farmer who belongs to the age group of 60 and over decreases the probability of adoption by 22.1%. This result is consistent with the literature. For example, [4] find that the age of the farm manager has a negative effect on the likelihood of adopting irrigation technologies. For his part, [3] explain that older farmers are more reluctant to change, less motivated to experiment with new technologies, less likely to be influenced by the benefits of new technologies. Hypothesis H_1 is validated.

The level of education of the farm manager has a positive impact on adoption ($\beta2 = 0.68$, $p < 0.01$). According to the results obtained, a farmer with a level of education of Bac + 3 or more, increases the probability of adoption by 68% compared to an illiterate. The latter is half as likely as the former to adopt NT. This result is consistent with the results of several studies [5]–[7], [31]. For example, [2] find that the adoption rate increases with the level of education of the farmer, especially if the technology is advanced, and learning is necessary for its use. The H_2 hypothesis is validated.

On the other hand, we didn't find a significant link between farm income and adoption. This result can be explained by the fact that the farmers in our sample haven't sufficient income to invest in or experiment with new technologies. Hypothesis H_{3a} is rejected. On the other hand, the existence of a non-agricultural income (or a secondary activity) increases the probability of adoption by 18.23% compared to a farmer who doesn't have a non-agricultural income ($\beta 5 = 0.1823$, $p < 0.10$). This result is similar to that of [9] who point out that the existence of a non-agricultural income increases the chances of adopting NT. Indeed, [10] explain that for farmers with moderate income, off-farm income provides cash that can help the farmer to acquire new technologies. The H_{3b} hypothesis is validated.

For perceived utility, the results of the estimate show that it has a positive effect on adoption ($\beta 5 = 0.1118$, $p < 0.05$). This result is consistent with several studies, the authors agree that farmers are more likely to adopt new technologies after seeing their usefulness [8], [13]–[15]. Hypothesis H_4 is validated.

As for Risk Attitude, we didn't find a significant link with adoption. In the literature, it's stated that risk aversion is often linked to the cost of investing in NT. Indeed, an investment in drip or in the use of drones requires a significant budget. In our study on the adoption of NT (capacitive probes, weather station, applications on smart phone), their costs aren't high, moreover there is the possibility of reselling them on the second-hand market. Hypothesis H_5 is rejected.

On the other hand, having a computer slightly affects adoption ($\beta 5 = 0.0122$, $p < 0.05$). The probability of adoption increases by 1.22% if the farmer already has a computer before the adoption. This result is similar to that of [13], who find that computer use in farm management is correlated with the adoption of certain technology (such as the autosteer GPS guidance system). Computer use is one indicator that shows farmers are open to technology. As pointed out [5], farmers who are open-minded about technological progress are also more favorable towards technology adoption. Hypothesis H_6 is validated.

As for the size of the farm, the results from the model estimate show that this has a positive impact on the adoption of technologies. However, this positive effect is not felt in the same way depending on the area class. Indeed, farms whose size is between 50 and 100 hectares, increase the probability of adoption by 8.9% ($\beta 5 = 0.089$, $p < 0.01$), while farms whose size is more than 100 hectares increase the probability adoption rate of 17.7% ($\beta 5 = 0.17$, $p < 0.01$) compared to the area of less than 20 ha. This result is consistent with several studies [10], [13], [15]. This is mainly due to the fact that large farms are more complex to manage, hence the need to use new technologies to optimize production and reduce costs. Hypothesis H_7 is validated.

The results of our estimations didn't establish a significant association between the land tenure variable (property title) and adoption. This can be explained by the type of NT in question. In fact, almost half of the farmers in our sample own their land. Studies [20], [26] have shown that farmers who have ownership certificates felt more secure to invest in NT, but it also depends on the nature of this investment, particularly if it's a long-term investment. As in the case of drip, which requires significant investment, which often farmer owners who adopt NT. Hypothesis H_8 is rejected.

Although agricultural advice also has a positive impact on the adoption of NT [5], [18], [32], our results didn't lead to a meaningful relationship. This can be explained by the council's goals in relation to digitization and the introduction of NT. We questioned the farmers whether they had participated in training or awareness-raising workshops on NT. It turned out that the themes discussed during these meetings are far from NT, they often relate to other agricultural activities other than NT. Hypothesis H_9 is rejected.

Although farmers' access to bank credit services is identified as a determinant of adoption [7], [18], [23]. This finding isn't validated in the context of our study. Indeed, our results show rather that farmers who have benefited from a bank loan are less likely to adopt NT ($\beta 10 = -0.118$, $p < 0.05$). Having a bank loan lowers the probability of adoption by 11.8%. This can be explained by the fact that the farmers who applied for credit, other things being equal, is a sign that they aren't already in good financial health. Hypothesis H_{10} is validated. For grants, they are usually intended for specific activities, other than purchasing NT from precision agriculture. The farmers who declared that they had received financial support in the form of a subsidy specified that the financial support was intended for basic equipment (tractors, plows, fertilizer spreaders, seed drills, combine harvesters, mower binders).

Regarding the Cost of the technology, the results of the estimates show that it hasn't significant effect on adoption. According to our interviews with the suppliers of NT in the Souss-Massa region, they testify that the main obstacle to the non-adoption of new technologies by Moroccan farmers isn't the cost, but rather it is due to attitudes and practices with which farmers are familiar. Although in many studies on the adoption of new technologies, the cost of the technology is often linked to the decision to adopt. However, in our opinion, it depends on the cost of the technology. A technology, the cost of which is high, will make the farmer hesitate to acquire it. Hypothesis H_{11} is rejected.

Finally, Ease of use has a positive effect on adoption (β9 = 0.168, p <0.001). A farmer who perceives a technology as difficult to use has less chance of adopting it compared to a farmer who perceives use as easy. This result can be explained by the fact that a third of the farmers in our sample have a BAC+3 and above. The level of education plays an important role in the use of NT, it would be easier for educated farmers to use technology compared to illiterate farmers. Hypothesis H_{12} is validated.

V. CONCLUSION

The objective of this article was to study the determinants of the adoption of NT. Based on the existing literature on the subject, a theoretical framework was developed. To test this theoretical framework, we relied on a sample of 82 farmers from the region of Souss-Massa (Morocco). The results from the estimation of a binary logit model show that the variables : Age, education level (≥ Bac+3), non-agricultural income, perceived utility, possession of a computer, farm size, credit access, ease of use, influence the probability of adopting NT among the farmers. To our knowledge, there's no empirical work on NT adoption in Morocco. The results of this study provide some answers to the adoption of NT by Moroccan farmers. They can be useful for public authorities and companies providing NT to set up strategies to promote the digitalization of the Moroccan agricultural sector.

The low education level among farmers requires efforts in terms of training, supervision and monitoring. Indeed, the technologies that fall within the scope of PA require learning which would only be possible if the farmer is able to understand their operation (reading a user manual or a display screen, entering data, settings, etc.). As our results show, the education level can make it easier or harder to use a technology. As the literature states, a technology could be adopted even if the farmer perceives the difficulty of using it. Studies show that farmers' perception of the ease of use of PA technologies has a significant impact on its adoption. A farmer who perceives PA technology as complex or difficult to use would therefore be less likely to adopt it. In the short term, it isn't possible to increase the education level of farmers so that they can fully understand their benefits, however, the public authorities can, therefore, act on building skills and knowledge- do this through training workshops and the provision of extension services that could equip farmers with the skills needed for NT acceptance and adoption.

Risk aversion can be overcome by improving the perceived usefulness of new technologies by farmers. This is important because farmers tend to ignore the benefits of using precision technologies [31]. Governments can then encourage farmers to use NT by demonstrating their usefulness. This can be done in collaboration with private companies, suppliers of these technologies, through the organization of demonstration workshops.

However, based on our knowledge of the field, certain variables whose effects turned out to be insignificant. These results should be interpreted with caution. For example, we didn't establish a significant link between land tenure and the likelihood of adoption. In Morocco, agricultural development faces problems of various kinds, but land in rural areas is often taken to be the main cause. Indeed, the land structures, the land status and the mode of access to land are taken to be the major constraint and the most important blocking factor to private agricultural investment, a real engine of agricultural development. The collective lands in their current forms, are considered on the basis of assumptions, as major obstacles to agricultural development, because they don't provide the conditions of security and stability necessary for agricultural intensification and development. private investment. Nationally, 70% of farms have less than 5 ha, and only accumulate 24% of the utilised agricultural area (UAA). This specificity of the size of farms can pose a serious problem for the implementation of an agricultural innovation strategy.

This study isn't without its limitations. As with all cross-sectional analyzes, it's difficult to test causalities even if they remain theoretically justified. Indeed, the expression "cross section" is used when the dependent and independent variables are measured on the same date. In this case, the results obtained do not always allow the identification of causal relationships. Also, the data collection was done remotely during the health crisis (COVID-19), which didn't allow us to collect more information.

In order to broaden our understanding of the NT adoption process among farmers, it would be interesting to integrate other variables into the predictive model, such as the orientation of production (local market vs export), the type of culture. Interactive effects are also necessary to better understand adoption. For example, studying the joint effects of educational level with ease of use, and farm size with type of crop on adoption.

Finally, digitization has now become an imperative in all sectors of the economy, including agriculture. COVID-19 will certainly accelerate this drive for modernization, but its success begins with an understanding of the mechanisms that lead the farmer to accept and adopt new technologies.

REFERENCES

[1] T. Koutsos et G. Menexes, « Economic, agronomic, and environmental benefits from the adoption of precision agriculture technologies: A systematic review », *Int. J. Agric. Environ. Inf. Syst.*, vol. 10, n° 1, p. 40-56, 2019, doi: 10.4018/IJAEIS.2019010103.

[2] A. R. Kaliba, R. J. Mushi, A. G. Gongwe, et K. Mazvimavi, « A typology of adopters and nonadopters of improved sorghum seeds in Tanzania: A deep learning neural network approach », *World Dev.*, vol. 127, 2020, doi: 10.1016/j.worlddev.2019.104839.

[3] P. Brown, A. Daigneault, et J. Dawson, « Age, values, farming objectives, past management decisions, and future intentions in New Zealand agriculture », *J. Environ. Manage.*, vol. 231, p. 110-120, 2019, doi: 10.1016/j.jenvman.2018.10.018.

[4] M. Paustian et L. Theuvsen, « Adoption of precision agriculture technologies by German crop farmers », *Precis. Agric.*, vol. 18, n° 5, p. 701-716, 2017, doi: 10.1007/s11119-016-9482-5.

[5] M. Reichardt et C. Jürgens, « Adoption and future perspective of precision farming in Germany: Results of several surveys among different agricultural target groups », *Precis. Agric.*, vol. 10, n° 1, p. 73-94, 2009, doi: 10.1007/s11119-008-9101-1.

[6] M. J. Carrer, H. M. de Souza Filho, et M. O. Batalha, « Factors influencing the adoption of Farm Management Information Systems (FMIS) by Brazilian citrus farmers », *Comput. Electron. Agric.*, vol. 138, p. 11-19, juin 2017, doi: 10.1016/j.compag.2017.04.004.

[7] B. A. Gyata, « Comparative assessment of adoption determinants of electronic wallet system by rice farmers in Benue and Taraba states, Nigeria », *Food Res.*, vol. 3, n° 2, p. 117-122, 2019, doi: 10.26656/fr.2017.3(2).132.

[8] N. J. Miller, T. W. Griffin, I. A. Ciampitti, et A. Sharda, « Farm adoption of embodied knowledge and information intensive precision agriculture technology bundles », *Precis. Agric.*, vol. 20, n° 2, p. 348-361, 2019, doi: 10.1007/s11119-018-9611-4.

[9] S. K. Ng'ang'a, D. A. Jalang'o, et E. H. Girvetz, « Adoption of technologies that enhance soil carbon sequestration in East Africa. What influence farmers' decision? », *Int. Soil Water Conserv. Res.*, nov. 2019, doi: 10.1016/j.iswcr.2019.11.001.

[10] A. P. Barnes *et al.*, « Exploring the adoption of precision agricultural technologies: A cross regional study of EU farmers », *Land Use Policy*, vol. 80, p. 163-174, janv. 2019, doi: 10.1016/j.landusepol.2018.10.004.

[11] T. W. Griffin, N. J. Miller, J. Bergtold, A. Shanoyan, A. Sharda, et I. A. Ciampitti, « Farm's sequence of adoption of information-intensive precision agricultural technology », *Appl. Eng. Agric.*, vol. 33, n° 4, p. 521-527, 2017, doi: 10.13031/aea.12228.

[12] D. M. Lambert, K. P. Paudel, et J. A. Larson, « Bundled adoption of precision agriculture technologies by cotton producers », *J. Agric. Resour. Econ.*, vol. 40, n° 2, p. 325-345, 2015.

[13] J. M. D'Antoni, A. K. Mishra, et H. Joo, « Farmers' perception of precision technology: The case of autosteer adoption by cotton farmers », *Comput. Electron. Agric.*, vol. 87, p. 121-128, 2012, doi: 10.1016/j.compag.2012.05.017.

[14] B. T. Dela Rue et C. R. Eastwood, « Individualised feeding of concentrate supplement in pasture-based dairy systems: Practices and perceptions of New Zealand dairy farmers and their advisors », *Anim. Prod. Sci.*, vol. 57, n° 7, p. 1543-1549, 2017, doi: 10.1071/AN16471.

[15] P. Brown, G. Hart, B. Small, et O. M. de Oca Munguia, « Agents for diffusion of agricultural innovations for environmental outcomes », *Land Use Policy*, vol. 55, p. 318-326, 2016, doi: 10.1016/j.landusepol.2016.04.017.

[16] T. Zhang, Y. Yang, J. Ni, et D. Xie, « Adoption behavior of cleaner production techniques to control agricultural non-point source pollution: A case study in the Three Gorges Reservoir Area », *J. Clean. Prod.*, vol. 223, p. 897-906, juin 2019, doi: 10.1016/j.jclepro.2019.03.194.

[17] J. C. Walton *et al.*, « Grid soil sampling adoption and abandonment in cotton production », *Precis. Agric.*, vol. 11, n° 2, p. 135-147, 2010, doi: 10.1007/s11119-009-9144-y.

[18] G. Danso-Abbeam, G. Dagunga, et D. S. Ehiakpor, « Adoption of Zai technology for soil fertility management: evidence from Upper East region, Ghana », *J. Econ. Struct.*, vol. 8, n° 1, 2019, doi: 10.1186/s40008-019-0163-1.

[19] R. Welsh, S. Grimberg, G. W. Gillespie, et M. Swindal, « Technoscience, anaerobic digester technology and the dairy industry: Factors influencing north country new york dairy farmer views on alternative energy technology », *Renew. Agric. Food Syst.*, vol. 25, n° 2, p. 170-180, 2010, doi: 10.1017/S174217051000013X.

[20] W. Séogo et P. Zahonogo, « Land tenure system innovation and agricultural technology adoption in Burkina Faso: Comparing empirical evidence to the worsening situation of both rural people vulnerability and vulnerable groups' access to land », *Afr. J. Sci. Technol. Innov. Dev.*, vol. 11, n° 7, p. 833-842, 2019, doi: 10.1080/20421338.2019.1587257.

[21] N. Adnan, S. M. Nordin, et Z. bin Abu Bakar, « Understanding and facilitating sustainable agricultural practice: A comprehensive analysis of adoption behaviour among Malaysian paddy farmers », *Land Use Policy*, vol. 68, p. 372-382, nov. 2017, doi: 10.1016/j.landusepol.2017.07.046.

[22] F. Mengistu et E. Assefa, « Farmers' decision to adopt watershed management practices in Gibe basin, southwest Ethiopia », *Int. Soil Water Conserv. Res.*, vol. 7, n° 4, p. 376-387, 2019, doi: 10.1016/j.iswcr.2019.08.006.

[23] G. M. Kaarthikeyan et A. Suresh, « A study on understanding the adoption of water saving technology: A case study of drip irrigation », *Int. J. Recent Technol. Eng.*, vol. 7, n° 6, p. 1123-1130, 2019.

[24] S. C. Chang et C.-H. Tsai, « The adoption of new technology by the farmers in Taiwan », *Appl. Econ.*, vol. 47, n° 36, p. 3817-3824, 2015, doi: 10.1080/00036846.2015.1019035.

[25] M. Keskin et Y. E. Sekerli, « Awareness and adoption of precision agriculture in the Cukurova region of Turkey », *Agron. Res.*, vol. 14, n° 4, p. 1307-1320, 2016.

[26] A. R. Khanal, A. K. Mishra, D. M. Lambert, et K. K. Paudel, « Modeling post adoption decision in precision agriculture: A Bayesian approach », *Comput. Electron. Agric.*, vol. 162, p. 466-474, 2019, doi: 10.1016/j.compag.2019.04.025.

[27] B. A. Aubert, A. Schroeder, et J. Grimaudo, « IT as enabler of sustainable farming: An empirical analysis of farmers' adoption decision of precision agriculture technology », *Decis. Support Syst.*, vol. 54, n° 1, p. 510-520, déc. 2012, doi: 10.1016/j.dss.2012.07.002.

[28] P. Roussel et F. Wacheux, *Management des ressources humaines. Méthodes de recherche en sciences humaines et sociales*. Louvain-la-Neuve: De Boeck Supérieur, 2005.

[29] T. Amemiya, « Qualitative Response Models: A Survey », *J. Econ. Lit.*, vol. 19, n° 4, p. 1483-1536, 1981.

[30] D. McFadden, « The Revealed Preferences of a Government Bureaucracy: Empirical Evidence », *Bell J. Econ.*, vol. 7, n° 1, p. 55-72, 1976, doi: 10.2307/3003190.

[31] G. Bucci, D. Bentivoglio, et A. Finco, « Factors affecting ict adoption in agriculture: A case study in italy », *Qual. - Access Success*, vol. 20, n° S2, p. 122-129, 2019.

[32] A. Knierim, M. Kernecker, K. Erdle, T. Kraus, F. Borges, et A. Wurbs, « Smart farming technology innovations – Insights and reflections from the German Smart-AKIS hub », *NJAS - Wagening. J. Life Sci.*, vol. 90-91, 2019, doi: 10.1016/j.njas.2019.100314.

Evaluation of the Development of Roselle *(Hibiscus Sabdariffa* L.) in Two Soil Types with Interaction of Bacterial Cells and Vermicompost

Abdul Khalil Gardezi[1*], Oscar Eduardo Hernández Torres[2], Guillermo Carrillo Castañeda[3], Sergio Roberto Márquez-Berber[4], Héctor Flores Magdaleno[5], Eduardo Valdés Velarde[6], Mario Ulises Larqué Saavedra[7], Gabriel Haro Aguilar[8]

[*1,2,5,8]Postgraduate College, Agricultural Science, Department of Hydrosciences, Montecillo Campus, Km. 36.5 Carretera Fed. México-Texcoco, Montecillo, Texcoco, Estado de México, México, 56230.
[3]Postgraduate College, Genetic Resources and Productivity Area of Molecular Genetics, Montecillo Campus, Km. 36.5 Carretera Fed. México-Texcoco, Texcoco, Estado de México, México, 56230.
[4]Crop Science Department, Chapingo Autonomous University, Km 38.5 Carretera Fed. México-Texcoco s/n, Col. Chapingo, Texcoco, Estado de México, Mexico, 56230.
[6]Agroforestry Center for Sustainable Development, Autonomous University Chapingo, Km 38.5 Carretera federal México-Texcoco s/n., Col. Chapingo, Texcoco, Estado de México, México, C.P. 56230.
[7]Metropolitan Autonomous University, Azcapotzalco, Mexico.
[*1]Corresponding author: Dr. Abdul Khalil Gardezi, Postgraduate College, Agricultural Sciences, Department of Hydrosciences, Montecillo Campus, Km. 36.5 Carretera Fed. México-Texcoco, Texcoco, Estado de México, México, 56230. Tel. 595-95-20200 ext. 1165, 1167 or 1171.

Abstract— *In this study, we tested the growth of two varieties of Roselle (Hibiscus sabdariffa) cultivated in two types of soils and the effects of the inoculation of seeds with cells of bacterial strains and vermicompost. (H. sabdariffa) are a member of the Malvaceae family and an important plant because its calyxes are used to produce beverages, prepare food, in the textile industry, cosmetology, perfumery, medicine, etc.*

This research was carried out at the Postgraduate College, Montecillo Campus, Mexico. The experimental design was completely randomized blocks with a factorial arrangement (4x3x2): four levels of organic matter factor, three levels of bacterial strains factor, and two levels of soil factor with three repetitions. The variables tested were: germination percentage (GP), plant height (PH), stem diameter (SD), the number of leaves (NL), foliar area (FA cm^2), radical volume (RV cm^3), dry aerial part biomass weight (DAPBW), dry root biomass weight (DRBW), dry calyx biomass weight (DCBW) and chlorophyll content (CC). The effects of soil and bacterial strains showed significant differences ($p \leq 0.05$) on the agronomic characteristics of (H. sabdariffa) plants. The organic matter factor only presented significant differences ($p \leq 0.05$) in the germination percentage and growth variables of the vegetative phase.

To increase crop production, the use of wastewater and microorganisms is proposed. Vermicompost exploded the percentage of germination, height, the number of leaves and stem diameter during the first weeks of growth, presenting important results in Creole variety (farmers' local variety). By inoculating seeds with cells of bacterial strains, A9m and A7 helped significantly to stimulate plant growth, finding a better response in A9m strain. The effects of soil factor showed significant differences ($p \leq 0.05$) in soil irrigated with residual water over agronomic characteristics satisfying the nutritional requirements of the crop.

Keywords— Germination percentage, Malvaceae, Soil microorganisms, Organic matter, Wastewater.

I. INTRODUCTION

Roselle or Roselle rose *(Hibiscus sabdariffa* L.) is a plant that has medicinal properties backed by scientific studies; however, it is also used to dye food, soft drinks, syrups, among other products. It is of great importance to the State of Guerrero, Mexico, with a first-place nationally, in harvest area and calyx production (SIAP, 2016).

In this water-scarce world, wastewater has been used for people to grow crops because it is free, it contains a lot of plant nutrients that also are free but, all these benefits do not consider the cost of the health care of the goods consumers. The Mezquital Valley in central Mexico is an example of this issue. In sum, crops will continue to be irrigated with wastewater due to growing water shortages, but this situation is a question of management and socioeconomic costs.

Plant Growth-Promoting Bacteria (PGPB) has been used primarily in agriculture to promote seed germination, vigor, plant growth, and improve crop yield (Kloepper, *et al.,* 1989), and for the control of plant pathogen microorganisms (Beneduzi, *et al.,* 2012). Plant growth-promoting bacteria, in addition to their agricultural applications, have been used successfully to mitigate the damage of plant growth caused by wastewater discharges into crop soils, so that crops thrive and they produce satisfactorily because bacteria have the ability to improve wastewater bioremediation processes. Some other rhizobacteria promote crop plants and the plants used in bioremediation processes to allow rapid extraction of hazardous materials from soils (Bashan *et al.,* 2008; McGrath *et al.,* 1993).

Another cost-effective solution is the use of vermicompost. Studies show that organic fertilizers protect and develop the life of microorganisms by improving soil structure and allowing the recycling of organic matter (Ruíz, 2011). The vermicompost stores mainly nitrogen, phosphorus, potassium, sulfur and prolonged-release micronutrients (García, 2011; Varela and Martínez, 2013), used as a growth medium in plant species under greenhouse conditions (Gardezi *et al.*, 2008), increasing microbial load and plant growth hormones, highlighting a sustainable agriculture that prevents degradation of soils with inorganic fertilizers.

There is little information on the use of biofertilizers and organic fertilizers in the cultivation of Roselle, and the accumulation in edible parts of the plant by toxic elements present in the soil, therefore, the information that is required to get in this investigation consists of determining the practical, economic, and safe culture medium for the cultivation of *H. sabdariffa* testing two types of soil interacting with bacterial strains and vermicompost. This will give farmers options to use organic fertilization, reduce pollution, and gain new ecosystem-friendly technologies.

II. MATERIALS AND METHODS

2.1 Experimental site

The experiment was carried out under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2016. At 19° 27´ N and 98° 54´ W with an altitude of 2245 meters above sea level. With a mild climate, and an average annual temperature of 16.4° C and a rainfall of 762.7 mm per year, National Meteorological (Service, Servicio Meteorológico Nacional) (SMN, 2010). The greenhouse comprises symmetrical triangular flat roofs. The roof is glass with a 70% luminosity, and metal structure. Ventilation is through the front and side windows (Fig 1).

FIGURE 1. Greenhouse in the Postgraduate College, Montecillo Campus.

2.2 Plant material and substrate

Two varieties of Roselle, commercial (H1038) and Creole (farmers' local variety) from the state of Guerrero were used. We used two types of soils: one irrigated with sewage water that had been watered for decades with water from the Emiliano Zapata dam between the boundaries of the states of Guerrero and Morelos, which houses urban wastewater from surrounding towns. The other soil was irrigated with clean water from a well. Soil samples were obtained at 0-5 cm, 5-10 cm, 10-40 cm deep, the characteristics and method are shown in Table 1.

TABLE 1
ANALYSIS OF TWO TYPES OF SOIL IRRIGATED WITH CLEAN AND RESIDUAL WATER

	Soil sample						
	Irrigated with clean water			Irrigated with the residual water			
	0-5 cm	5-10 cm	10-40 cm	0-5 cm	5-10 cm	10-40 cm	Method
pH	7.21	7.35	7.36	7.36	7.39	7.45	Potentiometric
EC dSm^{-1}	0.46	0.55	0.4	0.71	0.46	0.43	
OM %	0.31	0.27	0.4	0.67	0.34	1.88	Walkley and Black
N $mgKg^{-1}$	13	9.3	8.4	13	16.7	9.9	Extracted with potassium chloride
CEC Cmol+/Kg	53	52	48	58	57	54	Olsen
P $mgKg^{-1}$	7.39	6.55	3.72	8.1	18.18	5.9	Flame Emission Spectrophotometry
K $mgKg^{-1}$	204	384	382	470	398	226	Atomic absorption spectrophotometry
Ca $mgKg^{-1}$	4085	4111	2810	4222	4376	4140	
Mg $mgKg^{-1}$	494	464	387	926	822	846	
Fe $mgKg^{-1}$	2.02	1.3	1.82	2.74	1.87	1.38	
Cu $mgKg^{-1}$	0.77	0.74	0.71	1.59	1.7	1.51	
Zn $mgKg^{-1}$	0.82	0.47	0.38	0.74	0.58	0.52	
Pb mgKg-1	ND	0.009	ND	ND	ND	0.011	
Cr mgKg-1	ND	ND	ND	ND	0.020	ND	
Cd mgKg-1	ND	0.004	ND	ND	0.007	ND	

pH: hydrogen potential, EC: electrical conductivity, OM: organic matter, CEC: cation exchange capacity, ND not detected.

Roselle varieties used were commercial H1038 (Hybrid variety) and Creole (Farmers' local variety), planted in 2.5 kg polythene bags filled with two types of soil.

Seeds inoculation with cells of microbial strains A9m and A7 (provided by the Molecular Genetics Laboratory, Faculty of Genetics, Postgraduate College, Montecillo Campus) was done thirty minutes before sowing by adding 200 µl of bacterial suspension per batch of 100 seeds.

Vermicompost was used as organic matter, made with 60 kg of bovine manure, 25 kg of melon residues, and 15 kg of wheat. The mixture interacted with earthworms for four months. The Vermicompost in doses of 0, 24.05g, 48.08g, 72.05g, equivalent to 0, 25, 50, 75 t ha-1 of organic matter, was added to four 2.5kg polyethylene bags containing soil. Irrigation was every third day, applying the same amount of water with a container during the development of the investigation.

2.3 Variables tested

The variables tested were: germination percentage (GP), plant height (PH), stem diameter (SD), the number of leaves (NL), foliar area (FA, cm^2), radical volume (RV cm^3), dry aerial part biomass weight (DAPBW), dry root biomass weight (DRBW), dry calyx biomass weight (DCBW), and chlorophyll content (CC).

The experimental block design was handled completely randomly with 24 treatments (4x3x2) in a factorial arrangement with three repetitions. The factors and levels studied were: (1) soil with two levels, irrigated with wastewater and irrigated with clean water; (2) vermicompost, with four levels, 0, 24.05 g, 48.08 g, 72.05 g; (3) bacterial strains, with three levels, without strains, with strains A9m and A7. Analysis of variance analyzed the response variables with a level of significance of $p \leq 0.05$ and means separation tests with Tukey ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

3.1 Physical and chemical properties of soil

The soil used had a clayey texture, with a moderately alkaline pH. The soil irrigated with clean water showed a pH of 7.36 in comparison with the soil irrigated with residual water that had a pH of 7.45 in sample 10-40 cm deep. The results of electrical conductivity (EC), organic matter (OM), cation exchange capacity (CEC), nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn) and copper (Cu) were higher in soils irrigated with sewage water. The highest concentration of N was in upper layers (0-5 cm to 5-10 cm), with a slight increase in soils irrigated with wastewater because of the contribution of organic matter (Ramón-Zamora*et al*., 2009). Likewise, the content of available P presented average concentrations and content of K showed high levels, according to the norm NOM-021-RECNAT-2000. The levels of Cr, Cd, and lead (Pb) were below the limit of concentration to be considered contaminants.

3.2 Germination percentage

Table 2 shows the germination percentage associated with organic matter at different doses and seeds inoculated with cells of bacterial strains in two different soils. There were no significant differences in soil and bacterial strains factors tested ($p \leq 0.05$), however, there was a significant effect on levels of organic matter factor on the germination percentage. The 50% dose of the organic matter showed a greater positive effect on the germination percentage for H1038 variety, while a 75% dose of the organic matter showed a greater effective impact on Creole variety (Fig 2). This result shows that the addition of the organic matter of culture media consistently improves the germination percentage of seedlings (Domínguez *et al*., 2010; Atiyeh *et al*., 2002).

TABLE 2
GERMINATION PERCENTAGE OF ROSELLE VARIETIES H1038 AND CREOLE IN TWO TYPES OF SOIL ASSOCIATED WITH ORGANIC MATTER AND CELLS OF BACTERIAL STRAINS

Factors and levels	H1038	Creole
	Germination (%)	
Soil (S)		
S. irrigated with clean water	68.33 a	51.11 a
S. irrigated with wastewater	72.78 a	52.78 a
Organic matter		
0 g	60.00 c	39.89 c
24.05 g	66.67 bc	50.00 b
48.08 g	81.11 a	48.89 bc
72.05 g	74.44 ab	70.00 a
Bacterial strain		
Without strain	67.50 a	48.33 a
With A9m strain	75.83 a	52.50 a
With A7 strain	73.33 a	56.67 a

Means with the same letter in the same column are statistically equal (Tukey, p≤ 0.05).

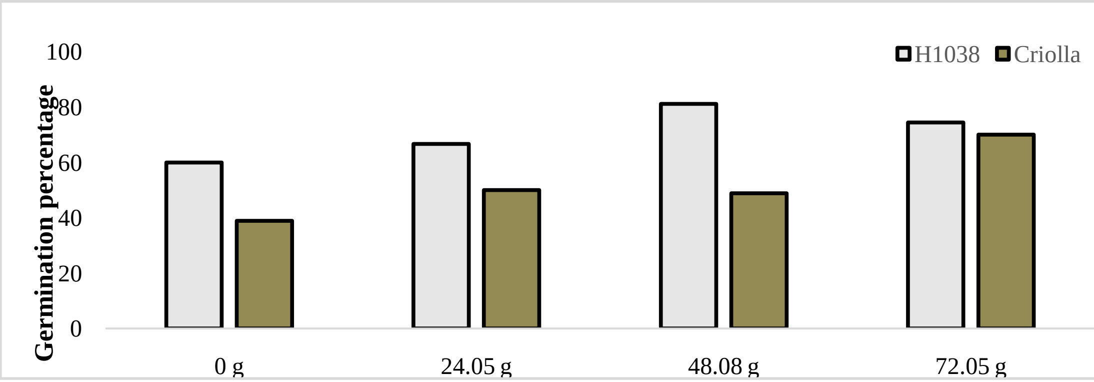

FIGURE 2:Effect of Organic Matter Added in Two Varieties of (H. Sabdariffa) on Germination Percentage

3.3 Agronomic characteristics

Table 3 presents the results of the agronomic characteristics of two varieties of *(H. sabdariffa)* associated with organic matter and bacterial strains in two types of soil.

TABLE 3
AGRONOMIC CHARACTERISTICS OF (*H. SABDARIFFA*) ASSOCIATED WITH ORGANIC MATTER AND BACTERIAL STRAINS IN TWO TYPES OF SOIL

Factors and levels	Height cm		Stem diametermm		Number of leaves		Foliar area cm^2		Radical volume cm^3	
Soil (S)	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole
S. irrigated with clean water	130.72 b	110.97 b	7.18 b	6.11 b	41.25 b	45.31 b	1202.28 b	758.55 b	5.11 b	4.65 b
S. irrigated with wastewater	147.71 a	129.41 a	8.36 a	7.08 a	49.83 a	59.47 a	1741.86 a	1070.95 a	7.45 a	5.55 a
Organic matter										
0 g	139.11^{NS}	119.17^{NS}	7.43^{NS}	6.61^{NS}	46.00^{NS}	53.83^{NS}	1365.17^{NS}	914.82^{NS}	5.67^{NS}	4.31 b
24.05 g	148.88	112.73	7.96	6.36	48.5	50.28	1671.39	818.78	6.59	4.68 ab
48.08 g	131.38	127.28	7.76	6.88	42.44	54.61	1329.89	1035.61	5.58	6.05 a
72.05 g	137.5	121.57	7.94	6.53	45.22	50.83	1521.83	889.79	7.27	5.37 ab
Bacterial strain										
Without strain	126.08 b	98.13 b	7.33 b	5.85 b	40.00 b	37.79 b	1100.58 b	576.22 b	5.99^{NS}	4.34 b
With A9mstrain	148.32 a	130.98 a	8.06 a	7.01 a	50.08 a	59.21 a	1728.17 a	1075.72 a	6.58	5.43 ab
With A7strain	143.25 a	131.46 a	7.93 ab	6.93 a	46.54 ab	60.17 a	1587.46 a	1092.30 a	6.27	5.53 a

Hybrid variety = H1038 and Creole variety = Farmers' local variety
NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p≤ 0.05)

TABLE 3
CONTINUATION

Factors and levels	Aerial dry weight (g)		Root dry weight(g)		Calyx dry weight(g)		Chlorophyll content SPAD unit	
Soil (S)	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole
S. irrigated with clean water	9.17 b	7.93 b	1.01 b	0.83 b	8.74 b	2.91 b	27.10 b	32.45 b
S. irrigated with wastewater	15.79 a	10.60 a	2.01 a	1.03 a	13.79 a	4.57 a	29.64 a	41.00 a
Organic matter								
0 g	11.40^{NS}	9.40^{NS}	1.40^{NS}	0.80 b	10.24^{NS}	5.02^{NS}	28.39^{NS}	36.24^{NS}
24.05 g	13.49	8.16	1.40	0.88 ab	12.84	3.26	27.24	35.46
48.08 g	13.49	10.29	1.55	1.07 a	10.48	3.69	28.46	38.87
72.05 g	11.53	9.21	1.70	0.98 ab	11.52	2.99	29.39	36.34
Bacterial strain								
Without strain	10.06 b	5.90 b	1.64^{NS}	0.82 b	6.06 b	1.54 b	27.70 a	30.35 b
With A9m strain	13.83 a	11.23 a	1.43	1.03 a	14.75 a	4.69 a	28.50 a	39.12 a
With A7 strain	13.55 a	10.66 a	1.47	0.95 ab	12.99 a	4.98 a	28.91 a	40.71 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤ 0.05). g=gram, SPAD=Soil-Plant Analyses Development.

3.4 Soil analysis

The analysis shows that there were significant differences in all agronomic variables tested ($p \leq 0.05$) in the levels studied in the soil factors in both varieties (Table 3). This result reflected by a slight difference in the concentration of elements such as N, P, K, Mg in both soils, because soils irrigated with wastewater increase the content of nutrients and organic matter, favoring the development of crop and improvement of soil (Veliz*et al*., 2009). Plevich *et al*., (2012) observed similar results in alfalfa cultivation that presented an increase in nutritional value and production of plants, determining that incorporation of wastewater to soil exceeds the values, compared to soil irrigated with clean water.

However, for the calyx number, there were no significant differences ($p \leq 0.05$) in any variety (Table 4). This is explained by the morphology of the plant, because the transition from flowering to fruiting is gradual, because the anthesis of the young flowers continues after the old flowers have already formed the capsules (Acosta, 1999), in addition, floral induction occurs when days are shorter (Arbex de Castro *et al*., 2004). However, an increase in elements such as nitrogen, phosphorus, and potassium have a positive response of the plant in variables such as weight of fresh and dry matter from the calyx (El-Sherif and Sarwat, 2007; Haruna *et al*., 2009) (Table 5).

TABLE 4
THE NUMBER OF CALYXES IN TWO VARIETIES OF THE ROSELLE CROP WITH DIFFERENT FACTORS AND LEVELS

Factors and levels	Number of Calyxes	
	number	
Soil (S)	H1038	Creole
S. irrigated with clean water	3.61^{NS}	13.28^{NS}
S. irrigated with wastewater	4.14	15.75
Organic Matter		
0 g	4.17^{NS}	15.06^{NS}
24.05 g	4.44	12.94
48.08 g	3.11	15.44
72.05 g	3.78	14.61
Bacterial strain		
Without strain	3.21^{NS}	7.67 b
With A9m strain	4.08	17.96 a
With A7 strain	4.33	17.92 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤0.05).

TABLE 5
WEIGHT OF FRESH AND DRIED CALYX MATTER IN TWO VARIETIES OF THE ROSELLE CROP WITH DIFFERENT FACTORS AND LEVELS

Factors and levels	Calyx fresh matter weight (g)		Calyx dry matter weight (g)	
Soil (S)	H1038	Creole	H1038	Creole
S. irrigated with clean water	26.99 b	18.73 b	8.74 b	2.91 b
S. irrigated with wastewater	40.10 a	26.65a	13.79 a	4.57 a
Organic Matter				
0 g	29.62^{NS}	27.81^{NS}	10.24^{NS}	5.02^{NS}
24.05 g	39.15	20.17	12.84	3.26
48.08 g	31.01	24.95	10.48	3.69
72.05 g	34.41	17.84	11.52	2.99
Bacterial strain				
Without strain	19.73 b	9.30 b	6.06 b	1.54 b
With A9m strain	42.83 a	28.39 a	14.75 a	4.69 a
With A7 strain	38.08 a	30.38 a	12.99 a	4.98 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤0.05).

3.5 Bacterial strains results

The results show significant differences ($p \leq 0.05$) in agronomic characteristics tested in Roselle plants from seeds inoculated with bacterial cells than in non-inoculated plants, except for the variables radical volume, dry root biomass weight and chlorophyll content in H1038 (Table 3). In Creole variety (farmers' local variety), the effect of strain A7 was numerically better in six agronomic characteristics tested, compared to three characteristics where strain A9m was superior. Higher values were found in six agronomic characteristics in plants generated from seeds inoculated with cells of strain A9m compared with those of strain A7 in variety H1038. Hassan, 2009 used (*Azospirillum lipoferum*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*), getting that inoculation of bacteria along or combined with chemical fertilizers significantly improve the growth and increase characteristics of the calyx product of (*H. sabdariffa*) plants compared to control.

Regarding variables radical volume and dry root biomass weight in H1038 variety, there was no significant difference between inoculation and non-inoculation, probably because strains only acted in plant-microorganism interaction solubilizing minerals usable for the plant and not as phytostimulators that increase the number of radical hairs and lateral roots (Hernández *et al*., 2010). Likewise, the difference in chlorophyll content between both varieties marked by physiological maturity of the plant, when the basal leaves change color and dry, happening after flowering. The harvest period for H1038 variety is 120-180 days in contrast, Creole variety is of 160-180 days (Ariza *et al*., 2014), so there is a greater deficiency of nutrients in leaves of H1038 variety in all treatments (Fig 3).

(A) (B) (C)

FIGURE 3: Calyxes In Variety H1038 at 180 Days after Sowing (Das). (A: Clean Soil without Inoculation, B: Soil Irrigated with Residual Water and C: Inoculated with the Bacterial Strain

In relation to the number of calyxes and days to flowering, no significant differences were observed ($p \leq 0.05$). This is explained by the factors that can affect the performance and effectiveness of the inoculation, among the most prominent are the competition with the native microorganisms, physical and chemical characteristics of the soil, genotype and age of the plant to be inoculated, type of radical exudates and agricultural management (Castro *et al*., 2007).

Although there are huge information gaps in the evolution of calyxes during their fruiting (Ramírez *et al*., 2011), it is necessary to generate information on the agricultural characteristics of interest.

3.6 Organic matter factor

There was a significant effect of the levels of the organic matter factor in the germination percentage ($p \leq 0.05$). The dose with 48.08 g of the organic matter showed a greater positive effect on the germination percentage for the H1038 variety, while the dose with 72.05 g of the organic matter showed a greater effective impact on the Creole variety (Fig 2). This result shows that the addition of organic matter to the culture media consistently improves the germination percentage of seedlings (Domínguez *et al*., 2010; Atiyeh *et al*., 2002).

At the end of the experiment, 180 days after sowing (Fig 4-5), on the levels studied of organic matter factor, no significant differences were found in any of the agronomic characteristics ($p \leq 0.05$), except for variables of dry root biomass weight and radical volume in Creole variety, the dose of 48.08 g of organic matter showed greater effect. The in-depth distribution of organic matter and roots follow the same trend. These results differ with those obtained by Haruna *et al*., (2011) in the effect produced by *(H. sabdariffa)* in the field because of incorporating poultry manure as organic matter, and as reported by Anyinkeng and Mih, (2011).

FIGURE 4. Variety H1038 (Hybrid variety) at 180 days after sowing (DAS).

FIGURE 5. Creole variety (farmers' local variety) at 180 days after sowing (DAS).

However, significant differences were found in growth variables at 80 days after sowing ($p \leq 0.05$). For H1038 variety, it showed only a positive increase in stem diameter in doses of organic matter of 48.08 g and 72.05g (Table 6). In the Creole variety, the dose of 48.08g expressed a greater effect on the height, the number of leaves and the diameter of the plant stem. This may show that improved varieties, unlike Creole, have high development potential in the vegetative phase and greater tolerance to biological stress (SAGARPA, 2014, 2015; Hidalgo-Villatoro *et al*., 2009), even in poor soils.

TABLE 6
GROWTH VARIABLES OF TWO VARIETIES OF ROSELLE 80 DAYS AFTER PLANTING

Factor and levels	Height(cm)		Number of leaves		Stem diameter(mm)	
Organic matter	H1038	Creole	H1038	Creole	H1038	Creole
0 g	36.94^{NS}	34.39 b	14.44^{NS}	12.50 c	4.24 b	3.16 b
24.05 g	40.26	36.48 b	14.00	14.06 bc	4.52 ab	3.34 b
48.08 g	40.64	41.74 a	14.33	18.11 a	4.54 ab	3.96 a
72.05 g	40.74	42.79 a	16.78	17.94 ab	5.04 a	4.03 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, $p \leq 0.05$).

Finally, the behavior of plants observed in the organic matter factor reflected by mineralization existing in soil, which can contain up to 45% of minerals (Labrador, 2001), influenced by climate and mineralogy of clays (Vogt *et al*., 1995; Geissen and Brümer, 1999) and by a vegetative phase of the genus (*Hibiscus*), in which amount of minerals and water absorbed is greater, during the first weeks of growth plant absorbs almost all nitrogen, phosphorus, and potassium(NPK) that will be necessary for the rest of the growing period, so a mixture of organic matter and soil will be necessary weeks before planting, to increase the use of nutrients provided by organic components.

IV. CONCLUSION

The four components: improved varieties, vermicompost, treated wastewater, and bacterial cells, play an important role in the quality, quantity, and health of plants for agricultural production with a suitable formula according to soil and water analyzes. The aggregation of organic matter shows positive effects on the percentage of the germination of both varieties and during the vegetative phase in Creole variety. Likewise, the vermicompost increased the emergency percentage of each variety studied and increased the radical volume. Plants with better agronomic characteristics were found in soils that were irrigated with sewage and inoculated with some bacterial strains in both varieties of Roselle. However, the use of microbial consortia that acts together in the crop system is suggested, having a more efficient and effective flow of nutrients in the plant's growth.

ACKNOWLEDGEMENTS

I, Oscar Eduardo Hernández Torres, express gratitude to institutions and people who made it possible to develop this research. I would like to thank my particular council, especially Dr. Abdul Khalil Gardezi, for his valuable scientific collaboration in carrying out this research project completed satisfactorily. Likewise, to Dr. Guillermo Carrillo-Castañeda, Dr. Héctor Flores-Magdaleno, Dr. Sergio Roberto Marquez-Berber, Eduardo Valdés Velarde, and Gabriel Haro Aguilar, for their support in my Master's Degree thesis, and Mario Ulises Larqué Saavedra, for his statistical contribution to this research. Finally, this research project was supported by the National Council of Science and Technology (CONACYT) and the Postgraduate College. Thank you. Also, this research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

REFERENCES

[1] Acosta, A. G. 1999. Aspectos generales del kenaf (*Hibiscus cannabinus* L.), en Cuba. Temas de Ciencia y Tecnología 7: 3-31.

[2] Anyinkeng, N and Mih, A. 2011. Soil nutrient supplementation on growth and biomass production of Roselle under tropical conditions. Agric. Biol. J. N. Am. 2(4): 603-609, https://doi/10.5251/abjna.2011.2.4.603.609.

[3] Arbex de Castro, N. E., Pereira Pinto, J. E. B., das Graças Cardoso, M., Ramalho de Morais, A., Bertolucci, S. K. V., Guimarães da Silva, F. and DelúFilho, N. 2004. Planting time for maximization of yield of vinegar plant calyx (*Hibiscus sabdariffa* L.). Ciência e Agrotecnología 38(3): 542—551.http://dx.**doi**.org/10.1590/S1413-70542004000300009.

[4] *Ariza, R., Serrano, V., Navarro, S., Ovando, M. E., Vázquez, E., Barrios, A., Michel, A., Guzman, S., y Otero, M., 2014. Variedades Mexicanas de Roselle (Hibiscus sabdariffa) "Alma Blanca" y "Rosaliz" de color claro, y "Cotzaltzin" y "Tecoanapa" de color rojo. Rev. Fitotec. Mex. Vol. 37 (2): 181–185.*printversion *ISSN 0187-7380.*

[5] Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., Metzger, J. D. 2002. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Biores. Technol. 84: 7-14. https://doi/10.1016/S0960-8524(02)00017-2.

[6] Bashan, Yoav, Puente, M. Esther, de-Bashan, Luz E. and Hernandez, Juan-Pablo, 2008.Environmental uses of plant growth-promoting bacteria.Plant-Microbe Interactions. Editors: E. AitBarka and C. Clément. Jan 2008: pp. 69-93 ISBN: 978-81-308-0212-1.

[7] *Beneduzi, A., Ambrosini, A. and Passaglia, Luciane M.P. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics andMolecular Biology, V.35(4 Suppl): pp. 1044-1051.* print version *ISSN 1415-4757 http://dx.doi.org/10.1590/S1415-47572012000600020.*

[8] Castro, S., Y. Herschkovitz, Y. Okony and E. Jurkevitch. 2007. Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiology Letters 276: 1-11.

[9] *Domínguez, J., Lazcano, Cristina., Gómez, María. 2010. Influencia del vermicompost en el crecimiento de las plantas. Aporte para la elaboración de un concepto objetivo.Acta Zool. Mex vol. 26 spe 2: 359-371, 2010.*On-line *ISSN 2448-8445* print version *ISSN 0065-1737.*

[10] *El-Sherif, M. H. and M. I. Sarwat. 2007. Physiological and chemical variations in producing roselle plant (Hibiscus sabdariffa L.) by using some organic farmyard manure. World Journal of Agricultural Sciences 3(5): 609-616.*

[11] García, P.R.E. 2011. La lombriz de tierra como una biotecnología en agricultura.Universidad Autónoma de Chapingo, ISBN 978-968-02-0299-7, 177 P, Texcoco, Estado de México, México.

[12] Gardezi, A., E. Ojeda, H. Gardezi y S. R. Márquez. 2008. Respuesta a la inoculación de *Glomus intraradix*, materia orgánica y dosis de fertilización fosfatada en el crecimiento de mezquite (*Prosopis* sp.). Agroproductividad 1(1): 24-28.

[13] Geissen, V. and Brümer, G. W. 1999. Decomposition rates and feeding activities of soilFauna in deciduous forest soils in relation to chemical parameters following liming andfertilization. Biology and Fertility of Soils Vol 29, Issue 4, pp 335-342.https://doi.org/10.1007/s003740050562 ISSN 1432-0789.

[14] Haruna, I. M., H. Ibrahim and S. A. Rahman. 2009. The yield and profitability of Roselle (*Hibiscus sabdariffa* L.) at varying poultry manure and nitrogen fertilizer rates in the Southern Guinea Savanna of Nigeria. Electronic Journal of Environmental, Agricultural and Food Chemistry 8(11): 1136-1139.**ISSN:** 1579-4377.

[15] Haruna, I. M., Maunde, S. M., Yahuza, S. 2011. Growth and Calyx Yield of Roselle(Hibiscus Sabdariffa L.) As Affected by Poultry Manure and Nitrogen Fertilizer Rates in The Southern Guinea Savanna of Nigeria, Canadian Journal of Pure and Applied Sciences Vol. 5 (1): 1345-1348. On-line version ISSN 1920-3853 Print ISSN 1715-9997.

[16] Hassan, F.A.S.2009.Response of *(H. sabdariffa* L.) plant to some biofertilizationtreatments. Annals of Agricultural Science, 54 (2): 437-446. **ISSN** 0570-1783.

[17] Hernández, A., Heydrich, M., Diallo, B., Jazini, M., Vandaputte, M. 2010. Cell-freeCulture medium of *Burkholderiacepacia* improves seed germination and seedling growth in maize (*Zea mays*) and rice (*Oryza sativa*). Plant Growth Regul. Vol. 60, Issue 3, pp 191-197. https://doi.org/10.1007/s10725-009-9433-5.

[18] Hidalgo-Villatoro, S.G., Cifuentes-Reyes, W.A.L., Ruano-Solís, H. H., Cano-Castillo,L.E. 2009. Caracterización de trece genotipos de rosa de Roselle*(H. sabdariffa)* en Guatemala. *Agronomía Mesoamericana* 20(1): 101-109. https://doi/10.15517/am.v20i1.4985.

[19] Kloepper, JW,Lifshitz, R and Zablotowicz, RM, 1989. Free-living bacterial inocula for enhancing crop productity. Trends Biotechnol Vol7, Issue 2, Feb 1989, pp. 39-43.

[20] Labrador, M. 2001. La Materia Orgánica en los Agroecosistemas. Ediciones Mundi-Prensa. Pp. 152-160.

[21] McGrath, S.P., Sidoli, C.M.D., Baker, A.J.M. and Reeves, R.D. (1993) The Potential for the Use of Metal-Accumulating Plants for the *In-Situ* Decontamination of Metal-Polluted Soils. In: Eijsackers H.J.P., Hamers T. (eds) Integrated Soil and Sediment Research: A Basis for Proper Protection. Soil & Environment. https://doi.org/10.1007/978-94-011-2008-1_145. vol 1, pp. 673-676. Springer, Dordrecht.

[22] Plevich, J., Delagado, A., Saroff, C., Tarico, J., Crespi, R, J., Barotto, O. 2012. Elcultivo de alfalfa utilizando agua de perforación, agua residual urbana y precipitaciones. Revista Brasileira de Engenharia Agrícola e Ambiental 16 (12) p 1353-1358.ISSN 1415-4366. http://dx.doi.org/10.1590/S1415-43662012001200013.

[23] Ramírez, B., F. Caro, M. G. Valdivia, M. H. Ramírez y M. L. Machuca. 2011. Cambiosen tamaño y características químicas de cálices de Jamaica *(Hibiscus Sabdariffa* L.) durante su maduración. Revista Chapingo Serie Horticultura 17 (2): 19-31.

[24] Ramón-Zamora, F., Rodríguez-Guevara N. J, Torres-Rodríguez, D. G., Yendis-Colinas,H. J. 2009. Uso de agua residual y contenido de materia orgánica y biomasa microbiana en suelos de la llanura de Coro, Venezuela. Agric. Téc. Méx. Vol. 35, núm. 2: Pp. 211-218. *Printversion* ISSN 0568-2517.

[25] Ruíz F. J., 2011. Ingeniería del compostaje, primera reimpresión. UniversidadAutónoma de Chapingo, ISBN-978-607-12-0049-5, 237 P, Texcoco, Estado de México,México.

[26] SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca yAlimentación). 2014. Servicio de Información Agroalimentaria y Pesquera, Anuario Estadístico de la Producción Agrícola. http://www.siap.gob.mx. (Consulta: junio 19, 2016).

[27] SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca yAlimentación). 2015. Agenda técnica agrícola de Guerrero. Segunda edición. ISBN volumen: 978-607-7668-42-8.

[28] SIAP (Servicio de Información Agroalimentaria y Pesquera). Agrifood and Fisheries Information Service. Date of query: 17 of August 2016.Available athttp://infosiap.siap.gob.mx.

[29] SMN, 2010 (Servicio Meteorológico Nacional). Fecha de consulta: 09 de octubre de2016.Disponible en: http://smn.cna.gob.mx

[30] Varela, S.and Martínez, B.A. 2013. Uso del compost de biosólidos en la formulación desustratos para la producción industrial de plantas de Nothofagus alpina. Revista Bosque 34 (3): 281-289.http://doi/10.4067/S0717-92002013000300004. ISSN- 0717-9200.

[31] Veliz, E., Llanes, J., Asela, L., Bataller, M. 2009. Reúso de aguas residuales domésticaspara riego agrícola. Valoración crítica. Revista CENIC Ciencias Biológicas vol. 40, núm. 1: Pp. 35-44. ISSN 0253-5688.

[32] Vogt, C., Vogt, D., Brown, S., Tilley, J. P., Edmonds, R. L., Silver, W. L., Siccama, T.G. 1995. Dynamics of forest floor and soil organic matter accumulation in boreal, temperate, and tropical forests. En: Lal R, Kimble J, Stewart BA (eds) Soil management and the greenhouse effect. Advances in Soil Science. CRC. Boca Raton, Florida USA. Pp. 159-178. http://doi.org/10.1201/9780203739310-14.

Heavy Metal Contamination in Soil of Industrial Area, Dewas, Madhya Pradesh, India

Reeta Kori[1], Alok Saxena[2], Harish Wankhade[3], Asad Baig[4], Ankita Kulshreshtha[5*], Saket Mishra[6], Smriti Sen[7]

Central Laboratory, Madhya Pradesh Pollution Control Board, Paryavaran Parisar, E-5, Arera Colony, Bhopal, India

Abstract— *A study has been conducted to assess the heavy metal contamination in soil of Dewas industrial area of Madhya Pradesh, India. Total eight locations and one control location were selected in Dewas industrial area for soil quality monitoring w.r.t. heavy metals. The nine soil samples were monitored for heavy metals such as Chromium (Cr), Manganese(Mn), Nickel (Ni), Copper (Cu), Zinc (Zn), Iron (Fe), Cadmium (Cd), Lead (Pb) and Cobalt (Co) analysis during different four quarters from April 2019 to March 2020. The heavy metal contamination with w.r.t. Contamination Index (CI), Pollution Load Index (PLI) study in selected locations in Dewas industrial area has been done. Over all Pollution Load Index of soil was found greater than 1 which shows polluted soil w.r.t. heavy metals at all selected monitoring locations in Dewas industrial area of Madhya Pradesh, India during this study*.

Keywords— Industrial Area, Soil, Heavy Metals, Contamination Index (CI), Pollution Load Index (PLI).

I. INTRODUCTION

Heavy metals and trace elements are also a matter of concern due to their non biodegradable nature and long biological half-lives [1]. Soil pollution due to heavy metals, such as cadmium, lead, chromium, copper, and iron is a problem of concern. Although heavy metals are naturally present in soil, contamination comes from local sources: mostly industry, agriculture, waste incineration, combustion of fossil fuels, and road traffic. The most important sources of heavy metals in soil are the anthropogenic activities such as mining, smelting procedures, steel & iron industry, chemical industry, traffic, and agriculture as well as domestic activities [2–11]. Soil is a dynamic medium made up of many minerals, organic matter, water, air, living creatures including bacteria and earthworms. It was formed by changing due to physical factors; the parent material, time, the climate and organisms present in composition. Generally polluted water also pollutes soil as it percolate in it. Solid waste is a mixture of plastics, cloth, glass, metal and organic matter, sewage, sewage sludge, building debris, generated from households, commercial and industries establishments add to soil pollution. Fly ash, iron & steel slag, medical & industrial wastes disposed on land are important sources of soil pollution. Chemical and metallurgical industries are the most important sources of heavy metals in soil [12–14]. The problem of soil pollution due to toxic metals has begun to cause concern now in most of the major cities. Soil pollution in the environment with toxic metals has increased dramatically since the onset of the industrial revolution [15-16]. Heavy metals in soil plays important role in biological system but it can cause harm if it in higher concentration in any system of environment. Therefore concentration of Contamination Index (CI), Pollution Load Index (PLI) w.r.t. heavy metals in Dewas Industrial area in Madhya Pradesh, India is important.

II. METHODOLOGY

2.1 Study Area

Dewas District in Ujjain Revenue Division, is situated on the Malwa plateau in the West-central part of Madhya Pradesh, India and lies between 20°17´ and 23°20´ North latitude and 75°54´ and 77°08´ East longitude. The district is bounded by Ujjain district in the north, Indore district in the west, West-Nimar district in the south-west, East Nimar district in the south, Hoshangabad district in the South East, Sehore district in the east and Shajapur district in the North-East.

2.2 Monitoring Locations

Dewas industrial area is consist of four industrial area i.e. Industrial Area 1, Industrial Area 2 & 3, Sia Industrial Area, Ujjain Road Industrial Area. Total eight locations and one control location in different industrial area in Dewas were selected for soil is depicted in table no 1 and figure no 1.

TABLE 1
MONITORING LOCATIONS AT DEWAS INDUSTRIAL AREA

S.N	Code	Industrial Area	Monitoring Locations	Latitude & Longitude
1.	S1	Industrial Area 1	Near M/S White star milk and milk products , Dewas	22.5754 & 76.2453
2.	S2	Industrial Area 1	Near M/S Tata International Ltd, Dewas	23.1064 & 77.52432
3.	S3	Industrial Area 1	Near M/S Raj Pioneer Laboratories (India), Dewas	23.07689 & 77.55652
4.	S4	Industrial Area 2 & 3	Near M/S Roca Bathroom Products Pvt Ltd (Parryware Industry) Dewas	23.11448 & 77.51583
5.	S5	Industrial Area 2 & 3	Near M/S VE Commercial Vehicle Ltd unit 2 (Eicher), Dewas	23.10886 & 77.51757
6.	S6	Industrial Area 2 & 3	Near M/S Navin Fluorine International Ltd, Dewas	23.09844 & 77.52922
7.	S7	Sia Industrial Area	Near M/S Krishna Food Products Ltd, Dewas	23.08073 & 77.53493
8.	S8	Ujjain Road Industrial Area	Near M/S Kriloskar Brother's Ltd, Dewas	23.07719 & 77.54176
9.	CS (Control Site)	Ujjain Road Industrial Area	Near M/S Bank Note Press , Dewas	23.07449 & 77.53204

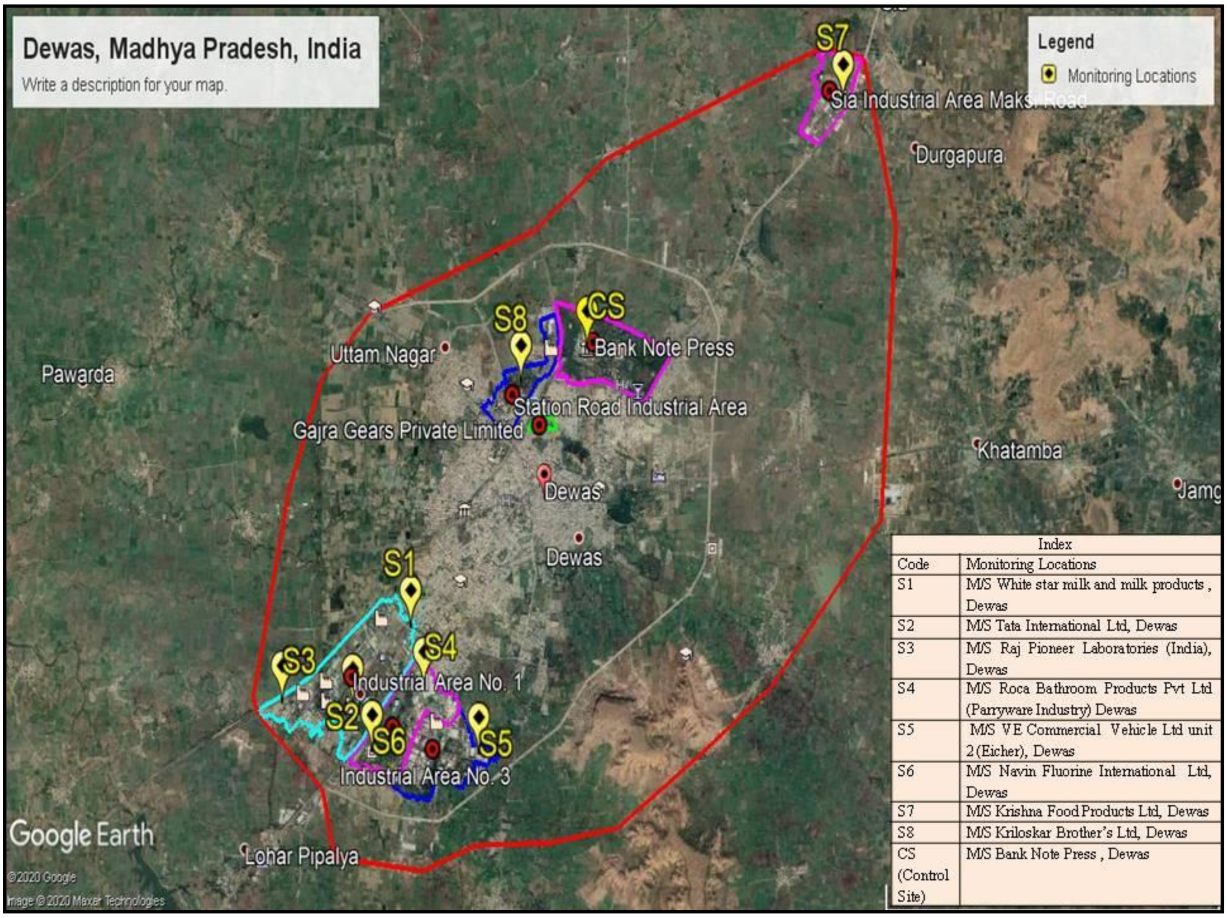

FIGURE 1: Monitoring Locations at Dewas industrial area

2.3 Monitoring

All soil samples were collected as per standard guidelines followed by Central Pollution Control Board. The aliquots of soil mixed together (unwanted matters to be separated manually before mixing of samples) during collection from the each selected sampling area. Out of this mixture, approx. 500 gm sample to be taken into polypropylene zip pouch, duly coded, labeled at field as per guidelines of Central Pollution Control Board. Soil samples were collected from the outer surface, i.e. 05–15 cm depth, after removing surface contamination. Sampling was carried out using a plastic spatula and the use of metal tools was avoided. The samples were collected in self-locking polythene bags and were sealed in double bags.

2.4 Digestion and Analysis:

Soil samples were dried for two days at room temperature. The dry soil sample was disaggregated with mortar and pestle. The sample was finely powdered to –250 mesh size (US Standard) using a swing grinding mill. All soil samples were digested by EPA Method 3050B (Acid Digestion of Sediments, Sludges and Soils) and analyzed by atomic absorption spectrophotometer (Perkin Elmer Pinnacle 900H) [17].

2.5 Soil Pollution Indices

Pollution assessment models are indicators used to assess the presence and intensity of anthropogenic contaminant deposition on soils. In this study, the following pollution assessment models were employed: Contamination Index (CI), Pollution Load Index (PLI) w.r.t heavy metals concentration present in soil.

2.5.1 Contamination Index (CI):

The contamination factors were derived by using the CI equation as defined [18]:

$$CF = Cn/Bn \tag{1}$$

Where Cn = measured metal concentration and Bn = background concentration from control site. The concentration factor observe as; CF < 1 low; 1 < CF < 3 moderate; 3 < CF < 6 considerable, and CF > 6 as high contamination [19].

2.5.2 Pollution Load Index (PLI):

The PLI gives a generalized assessment on the level of soil contamination. The PLI is obtained using approach as follows [20]:

$$PLI = [CF1 \times CF2 \times CF3 \times \ldots\ldots \times C\,n]\ 1/n \tag{2}$$

Where, CF= contamination factor; and n = number of metals. PLI > 1 indicates pollution exists; PLI < 1 indicates no metal pollution [21]; and PLI = 1 indicates heavy metal loads close to the background level [22].

III. RESULT & DISCUSSION

The observed concentration of heavy metals in soil is depicted in Table 2. Contamination index (CI) and Pollution load index (PLI) w.r.t. heavy metals in soil of all selected locations are presented in Table 3. All results are shown in figure no. 2 to figure no 11.

TABLE 2
HEAVY METALS CONCENTRATION IN SOIL

S.N	Analytes	Unit	CS	S1	S2	S3	S4	S5	S6	S7	S8
1	Cr	mg/kg	89	113	149	127	230	545	120	417	129
2	Mn	mg/kg	524	921	1301	927	771	1566	1105	891	1274
3	Ni	mg/kg	42	43	73	65	43	90	61	52	47
4	Cu	mg/kg	81	172	192	153	129	146	117	110	179
5	Zn	mg/kg	89	140	114	110	124	116	99	119	173
6	Co	mg/kg	30	36	42	38	34	39	38	32	42
7	Cd	mg/kg	1	2	1	1	1	1	2	2	2
8	Fe	mg/kg	15323	46456	56801	48573	33429	52437	43657	27668	45858
9	Pb	mg/kg	59	63	118	93	89	93	102	72	116

TABLE 3
CONTAMINATION INDEX (CI) AND POLLUTION LOAD INDEX (PLI) OF SOIL

Heavy Metal	D1	D2	D3	D4	D5	D6	D7	D8
Cr	1.269	1.674	1.426	2.584	6.123	1.348	4.685	1.449
Mn	1.757	2.482	1.769	1.471	2.988	2.108	1.7	2.431
Ni	1.023	1.738	1.547	1.023	2.142	1.452	1.238	1.119
Cu	2.123	2.37	1.888	1.592	1.802	1.444	1.358	2.209
Zn	1.573	1.28	1.235	1.393	1.3	1.112	1.337	1.943
Co	1.2	1.4	1.266	1.133	1.3	1.266	1.066	1.4
Cd	2	1	1	1	1	2	2	2
Fe	3.031	3.706	3.169	2.181	3.422	2.849	1.805	2.992
Pb	1.067	2	1.576	1.508	1.576	1.728	1.22	1.966
Pollution Load Index (PLI)	**1.573**	**1.827**	**1.568**	**1.47**	**2.051**	**1.633**	**1.636**	**1.869**

Figure no 2 is showing that average concentration of Chromium (Cr) was found 89 mg/kg at control site (CS). Minimum average concentration was found 120 (S6) mg/kg and maximum average concentration was found 545 (S5) mg/kg during this study.

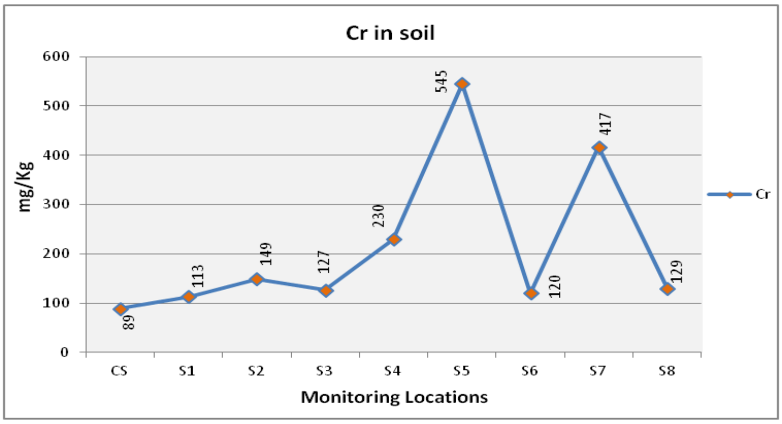

FIGURE 2: Chromium (Cr) concentration in Soil

Figure no 3 is showing that average concentration of Manganese (Mn) was found 524 mg/kg at control site (CS). Minimum average concentration was found 771 (S4) mg/kg and maximum average concentration was found 1566 (S5) mg/kg during this study.

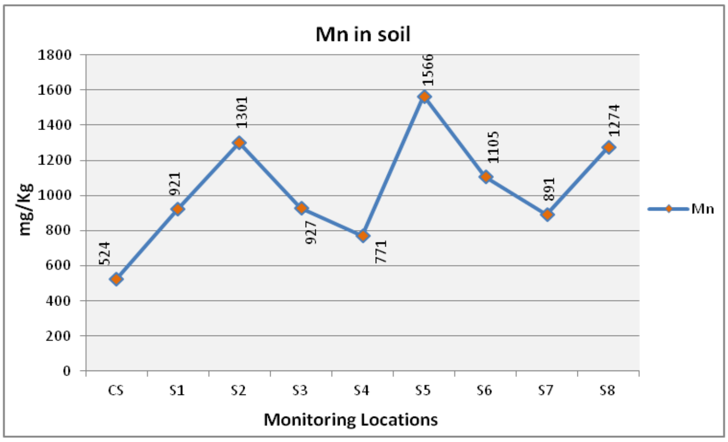

FIGURE 3: Manganese (Mn) concentration in Soil

Figure no 4 is showing that average concentration of Nickel (Ni) was found 42 mg/kg at control site (CS). Minimum average concentration was found 43 (S1) mg/kg and maximum average concentration was found 90 (S5) mg/kg during this study.

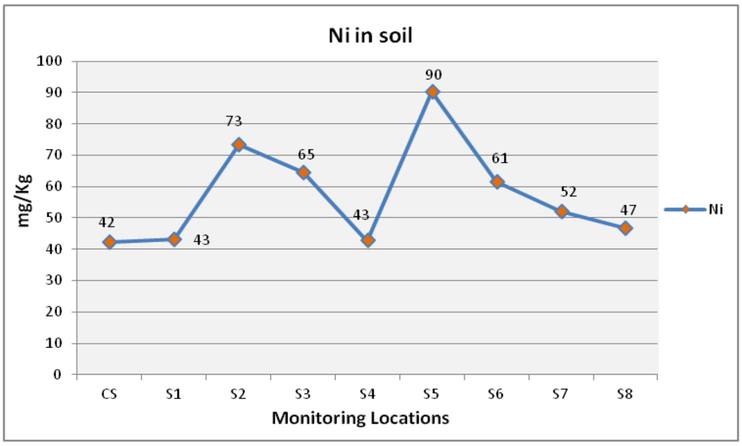

FIGURE 4: Nickel (Ni) concentration in Soil

Figure no 5 is showing that average concentration of copper (Cu) was found 81 mg/kg at control site (CS). Minimum average concentration was found 110 (S7) mg/kg and maximum average concentration was found 192 (S2) mg/kg during this study.

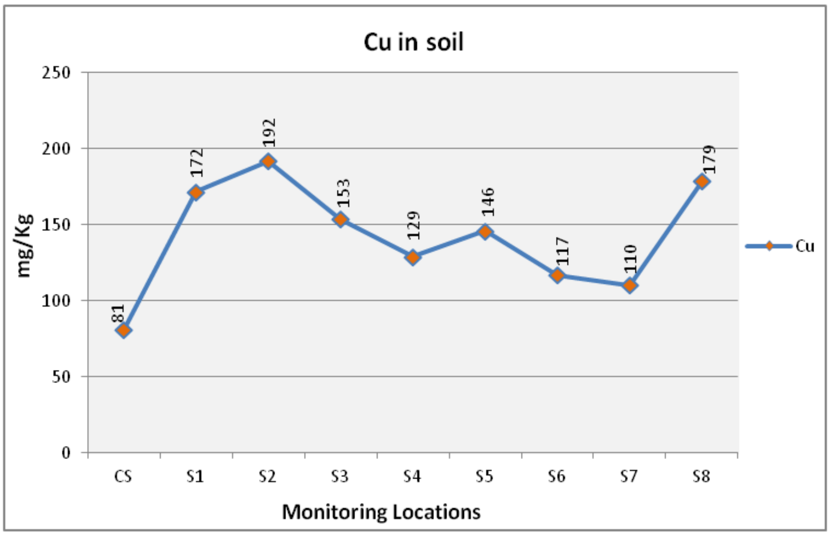

FIGURE 5: Copper (Cu) concentration in Soil

Figure no 6 is showing that average concentration of zinc (Zn) was found 89 mg/kg at control site (CS). Minimum average concentration was found 99 (S6) mg/kg and maximum average concentration was found 173 (S8) mg/kg during this study.

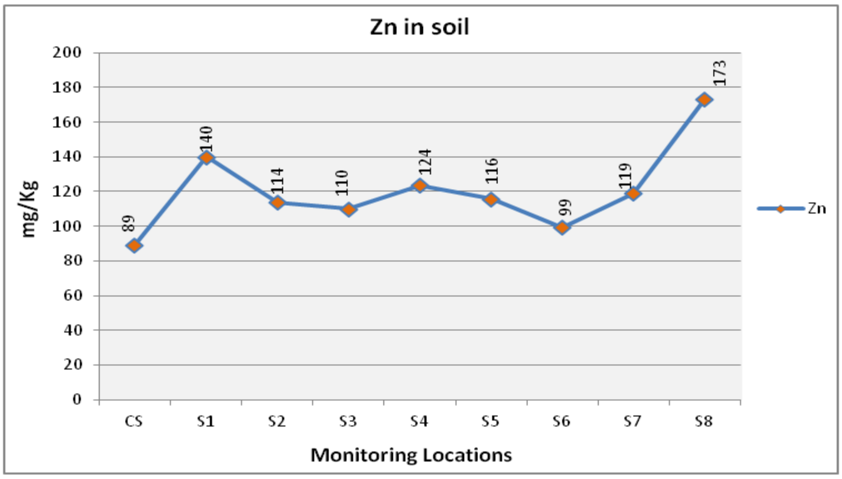

FIGURE 6: Zinc (Zn) concentration in Soil

Figure no 7 is showing that average concentration of Cobalt (Co) was found 30 mg/kg at control site (CS). Minimum average concentration was found 32 (S7) mg/kg and maximum average concentration was found 42 (S2, S8) mg/kg during this study.

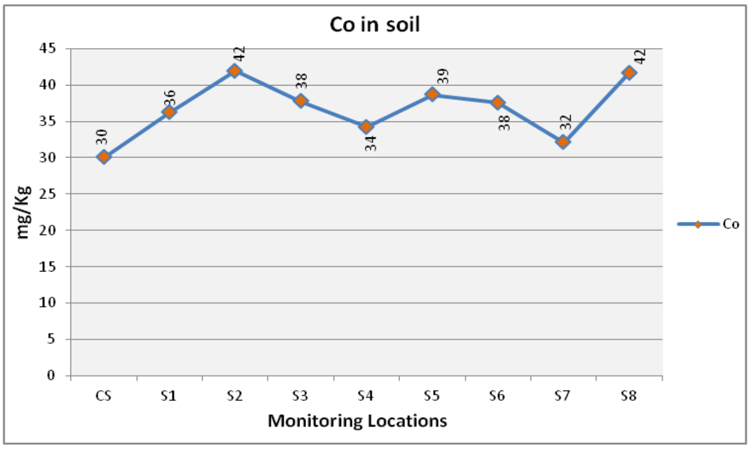

FIGURE 7: Cobalt (Co) concentration in Soil

Figure no 8 is showing that average concentration of cadmium (Cd) was found 1 mg/kg at control site (CS). Minimum average concentration was found 1 (S2, S3, S4, S5) mg/kg and maximum average concentration was found 2 (S1, S6, S7, S8) mg/kg during this study.

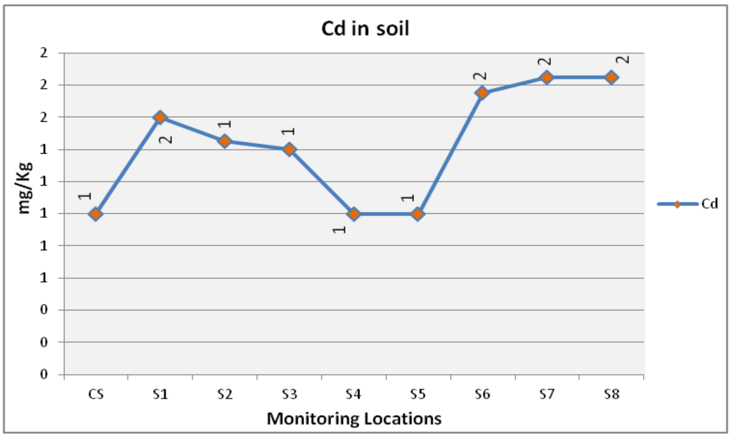

FIGURE 8: Cadmium (Cd) concentration in Soil

Figure no 9 is showing that average concentration of iron (Fe) was found 15323 mg/kg at control site (CS). Minimum average concentration was found 27668 (S7) mg/kg and maximum average concentration was found 56801 (S2) mg/kg during this study.

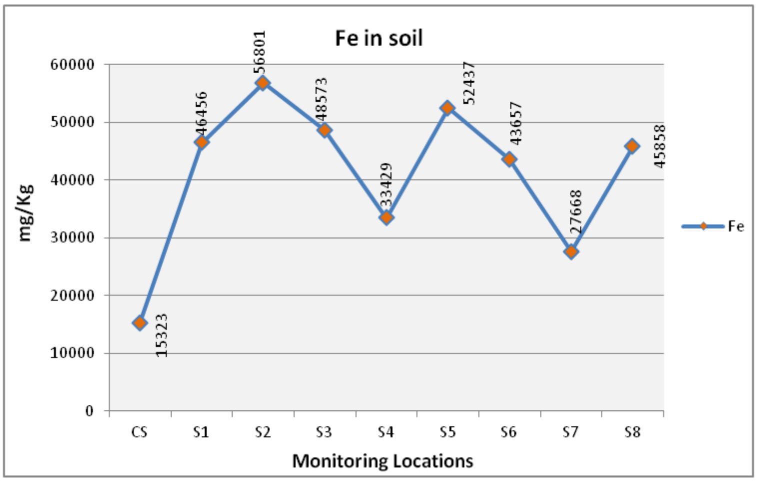

FIGURE 9: Iron (Fe) concentration in Soil

Figure no 10 is showing that average concentration of lead (Pb) was found 59 mg/kg at control site (CS). Minimum average concentration was found 63 (S1) mg/kg and maximum average concentration was found 118 (S2) mg/kg during this study.

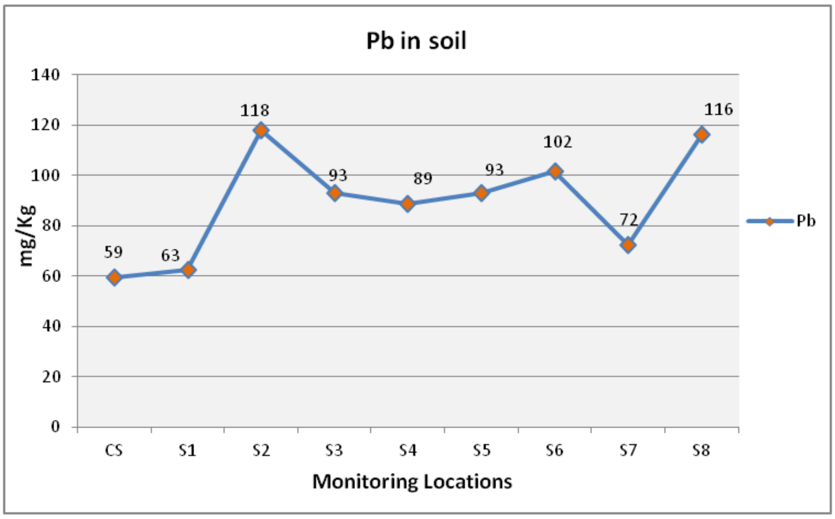

FIGURE 10: Lead (Pb) concentration in Soil

Figure no 11 is showing that Pollution Load Index (PLI) was found 1.47 (S4) and maximum Pollution Load Index was found 2.051 (S5). Over all Pollution Load Index was found greater than 1 which shows polluted soil w.r.t. heavy metals at all selected monitoring locations during this study.

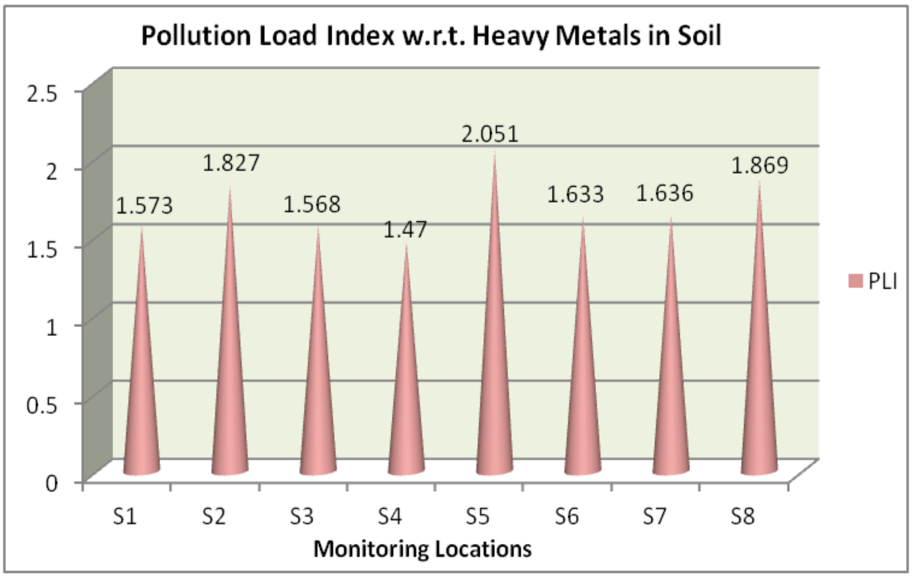

FIGURE 11: Pollution Load Index w.r.t. Heavy Metals in Soil

IV. CONCLUSION

Almost three fourths of the area in the Dewas district is covered by black cotton soils, which is occupied by Deccan Basalts. The southern part has red yellow mixed soils derived from sandstone, shale, gneiss. The heavy metal contamination with w.r.t. Contamination Index (CI), Pollution Load Index (PLI) study in selected locations in Dewas industrial area has been done. Over all Pollution Load Index of soil was found greater than 1 which shows polluted soil w.r.t. heavy metals at all selected monitoring locations in Dewas industrial area of Madhya Pradesh, India during this study.

ACKNOWLEDGEMENTS

The authors acknowledge the help received from authorities of monitoring locations of Dewas industrial area. We all authors acknowledge to the Chairman and Member Secretary, Madhya Pradesh Pollution Control Board, for encouragement of study work and kind permission to publish this paper.

REFERENCES

[1] Rahman S H, Khanam D, Adyel T M, Islam M S, Ahsan M A and Akbor M A. (2012) Assessment of Heavy Metal Contamination of Agricultural Soil around Dhaka Export Processing Zone (DEPZ), Bangladesh:Implication of Seasonal Variation and Indices. Appl. Sci. 2, 584-601.

[2] Suciu C I, Cosma M, Todicˇa S D, Bolboacˇa and Jantschi L. (2008) Analysis of soil heavy metal pollution and pattern in central Transylvania. Int J of Mole Sci.9, 434–453.

[3] Chopin E I B and Alloway B J. (2007) Distribution and mobility of trace elements in soils and vegetation around the mining and smelting areas of Tharsis, R´ıotinto and Huelva, Iberian Pyrite Belt, SW Spain. Water, Air, and Soil Pollution. 182, 1–4, 245–261.

[4] Garcia R and Mill´an E. (1998). Assessment of Cd, Pb and Zn contamination in roadside soils and grasses from Gipuzkoa (Spain). Chemosphere. 37, 8, 1615–1625.

[5] Li X, Poon C S, and Liu P S. (2001) Heavy metal contamination of urban soils and street dusts in Hong Kong. Applied Geochemistry. 16, 11-12, 1361–1368.

[6] Sezgin N, Ozcan H K, Demir G, Nemlioglu S, and Bayat C. (2004) Determination of heavy metal concentrations in street dusts in Istanbul E-5 highway. Environment International, 29, 7, 979–985.

[7] Viard B, Pihan F, Promeyrat S, and Pihan J C. (2004) Integrated assessment of heavy metal (Pb, Zn, Cd) highway pollution: bioaccumulation in soil, graminaceae and land snails. Chemosphere.55 , 10, 1349–1359.

[8] Nabulo G, Oryem-Origa H, and Diamond M. (2006) Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. Environmental Research. 101, 42–52, 2006.

[9] Oliva S R and Espinosa A J F. (2007) Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to identify possible contamination sources. Microchemical Journal. 86,1; 131–139.

[10] Kampa M and Castanas E. (2008) Human health effects of air pollution. Environmental Pollution. 151, 2, 362–367.

[11] Guo-li L, Da-xue, L and Quan-ming L. (2008) Heavy metals contamination characteristics in soil of different mining activity zone Trans, Transactions of NonferrousMetals Society of China. 18, 1, 207–211.

[12] Pantelica A, Cercasov V, Steinnes E, Bode P and Wolterbeek B. (2008) in Proceedings of the 4th National Conference of Applied Physics (NCAP '08), 25–26, Galati, Romania, September.

[13] De Vries W, R¨omkens P F and Sch¨utze G (2007). Critical soil concentrations of cadmium, lead, and mercury in viewof health effects on humans and animals. Reviews of Environmental Contamination and Toxicology. 191, 91–130.

[14] Cojocaru V, Pantelicˇa A, Pincovschi E and Georgescu I . (2006) EDXRF versus INAA in a pollution control of soil. Journal of Radioanalytical and Nuclear Chemistry. 268, 1, 71–78.

[15] BangaloreMetropolitan Rapid Transport Limited, Environmental Impact Analysis, 2006.

[16] Nriagu J O (1979) "Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. Nature, 279, 409–411.

[17] Acid Digestion of Sediments, Sludges and Soils, EPA Method 3050B, Revision 2 December 1996.

[18] Lacutusu R. Appraising levels of soil contamination and pollution with heavy metals In Heinike HJ, Eckselman W, Thomasson AJ, et al. (2000). Land information systems for planning the sustainable use of land resources. European Soil Bureau Research Report No. 4. Office of Official Publication of the European Communities, Luxembourg. 393-402.

[19] Hakanson L. (1980) An ecological risk index for aquatic pollution control: a sedimentological approach. Water Res. 14:975–1001.

[20] Thomilson DC, Wilson DJ, Harris CR, et al. (1980). Problem in heavy metals in estuaries and the formation of pollution index. Helgol Mar Res. 33: 566-575.

[21] Chakravarty M and Patgiri AD (2009). Metal pollution assessment in sediments of the Dikrong river, N.E. India. J Hum Ecol. 27(1):63–67.

[22] Cabrera F, Clemente L and Barrientos DE. (1999). Heavy metal pollution of soils affected by the Guadiamar toxic flood. Sci Total Environ . 242(1–3):117–129.

Bacteriological Characteristics of Spring Water in Ambo Town, West Shoa Zone, Oromia Region, Ethiopia

P. Sekhar

Environmental science division, Biology department, Bule Hora University, Bule Hora, Ethiopia

Abstract**—** *Present study was carried out to assess the quality of spring waters in terms of microbiological and chemical characters from Ambo. The results of the study revealed that chemical parameters such as pH (6.36-7.94),TDS(407-1041mg/L), DO(1.5-5.85mg/L),TS (1170-495mg/L), total alkalinity (313-1277mg/L), total hardness (38-1274 mg/L),COD (70.5-9mg/L)in the "Hora" water were higher than the maximum permissible levels of WHO standards for drinking waters. Total aerobic mesophilic bacterial counts tested were found to be as*0.977×10^4 *cfu/ml for SFWS,* $2.35\ \times10^4$*cuf/ml for CDSTRM,*1.14×10^4*cfu/ml for HB,*0.553×10^4 *cfu/ml for HD and*2.72×10^4*for Huluka streams samples. The "Hora" water contained different coli forms when tested by most probable number (MPN) method found to be in the order of* 0.66×10^2 *cfu/ml for SFWS,* $39\ x\ 10^2$ *cfu/ml for CDSTRM,* 0×10^2 *for HB,* 0×10^2*forHDand*28×10^2*cfu/ml for Huluka stream. The water samples from the different "Hora" water sources showed significant variations with respect to bacteriological and chemical characteristics during study period. Statistical analysis showed significant difference (*$p<0.05$*) in the distribution of total coli form, and aerobic mesophilic heterotrophic bacteria at various sampling locations. The study concluded poor water quality in terms of bacteriological and chemical characteristics of "Hora"water sources as all the parameters were well above WHO prescribed standards.*

Keywords**— Ambo town, bacteriological & chemical characteristics, Hora spring water, Huluka river, water quality, WHO standards.**

I. INTRODUCTION

Water is very essential to life and it is undoubtedly the most precious natural resource that exists on our planet. Several forces have been continued to seriously affect the quality of water resources. Many of these are result of human activities and natural processes and include ecosystem and landscape changes, sedimentation, pollution, over- abstraction and climate change. Besides the following natural and human induced factors also affects the quality of water including geology, hydrology, natural hazards, sedimentation/ erosion, agricultural activities, industrial, mining, fishing, sewage discharging/ disposal, deforestation, and other commercial activities. These activities aggravate the pollution of water body and greatly influence the quality of water [1]. Physicochemical and biological water quality indicators will be affected by various ways [2]. The health of aquatic ecosystem is depended on the physico- chemical and biological characteristics [3].The presence of certain microorganisms in water is used as an indicator of possible contamination and an index of water quality [4]. Natural hot and mineral springs water can be defined as water that, while circulating underground, undergoes changes in its composition through heat, pressure and time caused by interaction with the surrounding rock [5].Natural mineral water is characterized by its chemical and microbiological compositions which distinguishes it from drinking water and may not be treated in any way that alters these properties [6]. Besides, the temperature and other essential characteristics of natural mineral water must remain stable over time. Constituents may be present in the natural state in certain natural mineral waters because of their hydro geological origin may present risk to public health above a certain concentration. It is therefore deemed necessary to establish concentration limits for these constituents in natural mineral water [6].

Horas are used as a source of mineral supplement for livestock (cattle, sheep and goats). Water from the "Hora" is perceived to enhance fat, fertility and resistance to diseases [7].In every country that has been investigated natural hot springs mineral water have historically been attributed with therapeutically benefits due to their individual mineral compositions. Ambo "Hora" water (mineral water) is one of water sources which have been used by the city of Ambo, Ambo district and others for drinking purpose (both for humans and animals). It has been considered as having medicinal, bathing recreational, cleaning, cultural values, economic importance (income generation), and other related purpose. People believe that the Ambo "Hora" water has the medicinal value to protect the animals from different diseases, very important for their health and growth. The Ambo "Hora" water has been used throughout the history of human population, but (still no one knows the composition of the water) there was no study carried to determine the bacteriological and physico-chemical characteristics of this "Hora" water. So, study was aimed at assessing the bacteriological and chemical characteristics of Ambo Hora water. This study will also help concerned authorities to understand the status of these spring waters and take appropriate measures to protect these spring waters from being polluted from anthropogenic activities as these are important in terms of its tourism value.

II. METHODOLOGY

2.1 Description of the Study Area

This study was carried out in Ambo town; West Showa located 115km west of Addis Ababa, the capital city of Ethiopia. The town has a latitude and longitude of 8°59′N 37°51′E and an elevation of 2101 meters above sea level. Ambo town is known for its mineral water from natural springs, which is bottled and the most popular brand in Ethiopia.

2.2 Sampling Methods

Samples were collected from five different locations of natural springs which include sites for human bathing (HB), cattle drinking stream (CDSTRM), human drinking (HD), spring filewuha (SFW) and Huluka stream (HUSTR).

2.3 Sampling Size

Total 30 samples were collected from all the five different "Hora"water sources taking two representative samples from each site.

2.4 Sampling Collection procedure

The water samples were collected by using sterilized bottles in order to avoid contamination of the bottles following standard protocols of sample collection. All samples were collected from different locations of upstream and downstream of confluences or point sources with at least 1m intervals. After collection of samples they were brought to microbiology and chemistry laboratories of biology department, Ambo University.

2.5 Chemical Analysis of the Ambo "Hora" Water

Measurement of selected chemical parameters were carried out according standard procedures [8].

2.6 Bacteriological Analysis of the Ambo "Hora" Water

Bacteriological analysis of the water samples was carried out using multiple tube fermentation method for enumeration of total coliform count. Decimal dilution series up to $10^{-7}$from the original sample were prepared and inoculated into five lactose broth tubes. Durham's tubes were introduced in all tubes and sterilized them at a time. 1ml, 0.1mland 0.01ml water samples were added to each of 5 lactose broth tubes and labeled correspondingly. Lactose broth tubes were incubated at37^{o}C and examined the gas formation in Durham tubes at 24hour and 48-hour intervals. Numbers of positive tubes in each dilution were counted and a loop full of cultured from the lactose broth tube from the highest dilution that still showed positive test was taken and streaked it on EMB and ENDO agar plates. The Petri dishes were incubated upside down at the appropriate temperatures 37^{o}C for total coli forms for 24 hours. After incubation, typical colonies were identified and observed for the typical coli form colonies showed a greenish metallic sheen [9].

2.6.1 Plate Count Method

Surface plate count method was used to quantify the bacteria in the samples. Serial dilutions were prepared with sterilized water. Seven tenfold serial dilutions of water samples were prepared and 1ml of each of the dilution was evenly spread on nutrient agar (PCA) by using a sterilized bacterial spreader under laminar flow hood. This was repeated for each sample. The inoculated plates were then incubated at 28^{o}C for three days. Colonies were counted visually after three days of incubation and then stored in the refrigerator for further analysis.

Plates with less than 300 colonies and more than 30 colonies were utilized for data analysis. Results were recorded in units of CFU/1 ml of initial sample water. The concentration of bacteria in the original sample was determined as follows:

CFU = total colonies counted x dilution factor / volume plated

2.6.2 Motility Test [10].

A straight needle was slightly touched to a young colony of 24 hrs and stabbed to a depth of only 1/3 to 1/2 inch slant culture growing on agar medium. The slants were incubated at 37°C and examined daily for up to 7 days. A positive result indicates diffuse, hazy growths that spread throughout the medium rendering it slightly opaque whereas negative result indicates growth that is confined to the stab-line.

2.6.3 Biochemical characterization

2.6.3.1 Oxidase test [9].

Oxidase test was conducted according to the following procedure. A fresh culture (18 to 24 hours) of bacteria was grown in 4.5mL of nutrient broth and kept for overnight. A 0.2mL of 1% α-naphthol, and 0.3mL of 1% p-amino dimethylaniline oxalate (Gaby and Hadley reagents) to the overnight broth culture and then mixed vigorously. The culture was observed for color changes within 10s to 30s. It was interpreted an oxidase positive when the color changed to dark purple within 5 to 10 seconds and oxidase negative if the color did not change or it was taken longer than 2 minutes.

2.6.3.2 KliglirsIron Agar Test

A 57.52g of Kliglirs iron agar medium was take in one liter of purified water and heated with frequent agitation and boiled for one minute to completely dissolve the medium. And then distributed into test tubes and autoclaved for 15 minutes at 121^{o}C. After autoclaving, medium was allowed to solidify in a slanted position. Culture response in Kligler Iron Agar media incubated aerobically at 35°C was examined for 24 hours. Results were interpreted as: an alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermented). Cracks, splits, or bubbles in the medium indicates gas production [10].

2.6.3.3 Catalase test

A 24-hour old culture was used to make a homogenous suspension on the slide. A one drop of hydrogen peroxide (3%) was added to the suspension of pure culture isolates and the observed gas formation (effervescence) [11].

2.6.3.4 Simmon citrate agar Test

Simmon citrate agar was used to test citrate utilization. Using a sterile straight wire loop, a saline suspension of the test organism was first streaked on the slant and then stabbed (to create anaerobic condition). All the slants were incubated for 4 days at 37^{o}C in an aerobic atmosphere to detect the production of a positive reaction as indicated by growth with an intense blue cooler in the slant [9].

2.6.3.5 Urea agar test

Urea agar test was used for detection of urease production by proteus species and other members of Enterobacteriaceae. A 24 gm of urea powder was added in distilled water and brought the volume to 950 ml and mixed thoroughly. Slants were prepared with urea broth and slants were inoculated with isolates and incubated at 37^{0}C for 24 hours. The development of pink color colonies indicates a positive result [12].

2.7 Data Analysis

Data analysis was carried out using SPSS 21 version. Parameters like descriptive statistics and 2-tailed correlation analysis was done to know any significant relation exists among various biological and chemical parameters.

III. RESULT & DISCUSSION

Results of Physico-chemical parameters of water samples from "Hora" waters and Huluka stream were analyzed and results were represented in the following table.

TABLE 1
PHYSICO-CHEMICAL PARAMETERS OF HORA WATER SOURCES

S.No.	Parameter	Sample sites				
		SFWS*	CDSTRM*	HB*	HD*	HULSTRM*
1	Temp.	40^0C±0.50	35^0±0.50	40.83^0C±O.28.	39.76^oC±1.07	27.33^0C±1.00
2	P^H	6.37±0.00	6.68±0.01	6.34±0.01	6.38±0.00	7.93±0.01
3	Electrical conductivity (µS/cm)	1791 ±3.0	1933.33±2.08	1913±5.13	1910±8.0	403.66±2.08
4	TDS (ppm)	972.5±2.50	1039±6.55	812.5±2.51	986.53±2.62	402±2.64
5	DO (ppm)	1.6±0.10	1.2±0.04	1.6±0.95	1.8± 0.8	5.8±0.09
6	TS (ppm)	977.66±2.51	1167.00±2.00	926.83±2.36	1045.00±2.00	495.33±3.01
7	TA (ppm)	1260.66±3.05	1253.33±7.63	1256.60±7.63	1261±11.53	312±10.58
8	TH (ppm)	1269.50±10.75	1255±5.00	1277±2.00	1270±17.32	35.33±2.30
9	COD (ppm)	69.5±0.76	80.33±1.04	84.33±0.76	72.93±0.51	92±0.70

SFWS - Spring file wuha sources, HB-human drinking, HULSTRM - Huluka stream, CDSTMR - cattle drinking stream, HD–human drinking

The table 1 shows mean values of the temperature between 27.33^{o}C to 40.83^{o}C. The lowest value (27.33^{o}C) was found in sample site Huluka stream and the highest temperature value (40 .83^{O}C) at sample site of human bathing. During the present investigation, there was no great difference among the temperatures of Hora waters and all of them were above WHO standards of 15^{o}C. The temperature range during the present study was 27.33 to 40.83^{o}C. This range was also considered as above the maximum limit of temperature for drinking water specified by WHO which is 15^{o}C. During the present investigation, there was no great difference among the temperatures of Hora waters and all of them were above WHO standards of 15^{o}C. but temperatures are higher than the study conducted in Bahir Dar town (15–20 °C) spring waters [13]. Higher water temperatures promote the growth of microorganisms in the water, which may increase the taste, odor, turbidity and cause corrosion problems and also it decreases the solubility of gas in the water [14]. The temperature range during the present study was 27.33 to 40.83^{o}C. This range was also considered as above the maximum limit of temperature for drinking water specified by WHO which is 15^{o}C.

Mean pH values of water samples obtained from five sources were shown in table 1 and they are in the range of 7.93 to 6.34. Human bathing recorded the lowest pH value of 6.34 followed by spring File wuha 6.37. Other sources also shown more or less same range of pH as evident from the table no 1. But Huluka stream has registered highest pH value of 7.93. The Huluka stream, CDSTRM and other samples recorded pH within the permissible value of 6.5 to 8.5 for drinking water [15]. The high value of pH may be the result of waste discharge, microbial decomposition of organic matter in the water body [16]. The lower pH values obtained in the study from the SFWS, HB and HD samples could be attributed to mineral salts dissolved in the water. The low pH values observed in most wells and springs could be associated with carbon dioxide saturation in the ground water [17]. The relatively higher pH of the streams could be attributable to the large surface area of the stream which exposes it to sunlight thus increasing the temperature and photosynthetic activities which in turn increases alkalinity of the water [18].

Data in Table 1 shows that the mean of the conductivity of water samples from the five water sources ranged from 403.66 to1933.33µs/cm. The least conductivity was observed in Huluka stream (403.66). Conductivity is a measure of the ability of aqueous solution to carry an electric current that depends on the presence and total concentration of ions their mobility and valance and on the temperature [19]. There is a very good positive correlation exists among conductivity, total solids and total dissolved solids which indicates higher chemical pollutant character of the waters in the study sites.

The mean TDS content values ranged from 402 to 1039 mg/l as depicted in table 1. Huluka stream had the lowest TDS value of 402 mg/L followed by HD (986.53 mg/l), SFW(972.5mg/l), HB (812.5 mg/l) and the CDSTRM (1039 mg/l). The highest level was observed in CDSTRM (1039 mg/l). High values of TDS indicate that the "Hora "water is unfit for human consumption. In the present study, it is found that almost all samples have TDS values more than the prescribed standards and is unfit for drinking. The Total Dissolved Solids (TDS) represents the amount of inorganic substances present in water. It shows the general nature of water quality or salinity [19]. High values of TDS indicate that the "Hora "water is unfit for human consumption. In the present study, it is found that almost all samples have TDS values more than the prescribed standards and is unfit for drinking.

The data in table 1 indicated that the minimum DO values of 1.2 mg/L, 1.6mg/L,5.8mg/L, 1.6mg/L. 1.8mg/L were observed at sites CDSTRM, SFWS, Huluka STRM, HB and HD in respectively. The lowest values observed may be as a result of the increased mineral solution that place high demand on the DO. The maximum DO values 5.8mg/L and 1.8mg/L were seen at sites Huluka STRM and HD. The overall result shows that the DO of all "Hora" water samples and Huluka water stream were below the water quality standards. The low values of dissolve oxygen observed in the study sites may be as a result of the increased mineral solution that place high demand on the DO. The maximum DO values (5.8mg/L and 1.8mg/L) were seen at sites Huluka STRM and HD. With the progress in summer, dissolved oxygen decreases due to increase in temperature and also due to increased microbial activity [20]. The overall result shows that the DO of all "Hora" water samples and Huluka water stream were below the water quality standard so this water was not suitable for drinking. The low values observed in the present study may be as a result of the increased run off agricultural wastes and industrial effluents discharged into the drains that place high demand on the DO.

Table 1 indicated that high TS of 1167.00ppm was observed in CDSTRM water samples while relatively lowest level (495.33ppm) was registered in Huluka stream water. Significant variations were noted among the five different water sample sources ($P < 0.05$) and variation within sample was significant. The concentration of TS was well above the WHO standards of 500ppm.

All the samples from Hora water have shown high levels of total hardness exceeding WHO standard of 500mg/L but Huluka stream hardness was found to be well below the standard limit as shown in Table 1. The least level was found in Huluka stream (35.33 mg/l), followed by cattle drinking "Hora" water (1255mg/l), spring file wuha (1269.5mg/l), human bathing "Hora" water (1277 mg/l) and human drinking "Hora" water (1770 mg/l) in their increasing order. Hard water is the water that contains high levels of dissolved calcium, magnesium, and other minerals salts such as iron and the greater the amount of dissolved minerals in the water, the harder it is [21]. All the samples from Hora water have shown high levels of total hardness exceeding WHO standard of 500mg/L but Huluka stream hardness was found to be well below the standard limit as shown in table 2. The variations in total hardness may be due to decomposition and mineralization of organic materials [21].

COD is the amount of oxygen that is required to oxidize organic compounds in the water and indicated in table 1. The COD value of the Ambo "Hora" water sources as shown in table 1 were in between 92-69.33mg/L which are above the standard limit of 10mg/L. COD is the amount of oxygen that is required to oxidize organic compounds in the water. The COD value of the Ambo "Hora" water sources as shown in table 2 were in between 92-69.33mg/L which are above the standard limit of 10mg/L and these high values can be attributed to anthropogenic pollution related activities in the study sites. Based on the high values of COD well above the standards, this Ambo "Hora" water is not recommendable for drinking purpose [22].

TABLE 2
THE MEAN COUNTS OF TOTAL HETEROTROPHIC BACTERIA, AND COLI FORM BACTERIA FROMDIFFERENT HORA WATER SOURCES

Sample location /site code	Total heterotrophic bacteria count (CFU/ml)	TC (MPN/100ml)
SFWS	0.977×10^4	0.66×10^2
CDSTRM	2.35×10^4	$39x10^2$
HB	1.14×10^4	0×10^2
HD	0.553×10^4	0×10^2
HULUSTRM	2.72×10^4	$28x10^2$

SFWS - Spring file wuha sources HB - human drinking HULSTRM - Huluka stream CDSTMR - cattle drinking stream HD – human drinking

The total heterotrophic counts as showed in table 2 ranged from 0.977×10^4 to 2.72×10^4 cfu/ml. The Huluka stream samples recorded the highest count of 2.72×10^4 cfu while HD had the least bacterial count of $0.553\times10^{4.}$ The CDSTRM, HB and SFW samples had bacterial count of $2.35\times10^{4,}$ 1.14×10^4 and 0.977×10^4 cfu/ml respectively. Out of all samples of "Hora" water, CDSTRM water and Huluka stream water samples contained higher total heterotrophic bacterial counts than the others. Heterotrophic bacterial count of some samples in the present study was high exceeding standard permissible limit for drinking water. The most probable number (MPN/100ml) values were recorded (Table 2) as 0.66×10^2 MPN/100ml in SFWS, 39 x 10^2 MPN/100ml in CDSTRM and 28 x 10^2 MPN/100ml in Huluka stream water samples. Except HD and HB "Hora" water all samples such as CDSTRM, Huluka stream, and SFW water samples were not within the standards of WHO for coli forms. From all samples of "Hora" water, CDSTRM water and Huluka stream water samples contained higher total heterotrophic bacterial counts than the others. Heterotrophic bacterial count of some samples in the present study was high exceeding standard permissible limit for drinking water. This finding was in good agreement with similar studies by other workers who reported that the sources of heterotrophic bacteria in water are human and animal wastes, run off pasture, natural soil or plant bacteria, sewage, and other unsanitary practices [23]. Runoffs, sewage, agricultural waste are usually high inorganic matter and nutrients and could cause increase in the bacteriological load of the water bodies thereby resulting in high heterotrophic bacteria counts [24]. The higher number of bacterial count recorded in Huluka stream water samples could probably be as a result of the increased surface area of the stream which exposes the water to contaminants as well as human activities like swimming, washing, dipping of dirty legs or hands and inside the stream while fetching water [25].

Except HD and HB "Hora" water all samples such as CDSTRM, Huluka stream, and SFW water samples were not within the standards of WHO for coli forms. The high coli forms obtained may be an indication that the water samples were contaminated by fecal matter [26]. The presence of bacteria was not only making the water unsuitable for human consumption, but also poses serious health concerns [15]. Similar studies reported the presence of these bacteria in drinking water sources [27] and attributed it to indiscriminate human and animal defecation and general poor sanitation.

TABLE 3
DESCRIPTIVE STATISTICS

	Mean	Std. Deviation	N
TEMP	36.5840	5.65531	5
PH	6.7400	.67937	5
EC	1590.1320	665.61654	5
TDS	842.5060	260.38296	5
TS	922.3640	255.10880	5
TA	1068.5860	422.95521	5
TH	1021.3660	551.26897	5
COD	79.8180	8.98509	5
DO	2.4000	1.91311	5

Table 3 shows descriptive statistics such as mean and standard deviations. From the above table mean DO was found to be 2.4 and COD has a mean value of 79.818. Mean values of total dissolved and suspended solids were found to be 842.50 and 922.36 ppm respectively. Significant correlation among various chemical parameters was evaluated using 2-tailed Pearson correlation matrix at different sites and depicted in table 4.

TABLE 4
CORRELATION MATRIX OF CHEMICAL PARAMETERS

		TEMP	PH	EC	TDS	TS	TA	TH	COD	DO
TEMP	Pearson Correlation	1	-.978**	.897*	.771	.721	.917*	.920*	-.745	-.869
	Sig. (2-tailed)		.004	.039	.127	.169	.028	.027	.148	.056
	N	5	5	5	5	5	5	5	5	5
PH	Pearson Correlation	-.978**	1	-.968**	-.882*	-.850	-.980**	-.982**	.776	.952*
	Sig. (2-tailed)	.004		.007	.048	.068	.003	.003	.123	.012
	N	5	5	5	5	5	5	5	5	5
EC	Pearson Correlation	.897*	-.968**	1	.941*	.946*	.996**	.996**	-.715	-.993**
	Sig. (2-tailed)	.039	.007		.017	.015	.000	.000	.175	.001
	N	5	5	5	5	5	5	5	5	5
TDS	Pearson Correlation	.771	-.882*	.941*	1	.982**	.946*	.942*	-.826	-.954*
	Sig. (2-tailed)	.127	.048	.017		.003	.015	.017	.084	.012
	N	5	5	5	5	5	5	5	5	5
TS	Pearson Correlation	.721	-.850	.946*	.982**	1	.934*	.931*	-.706	-.956*
	Sig. (2-tailed)	.169	.068	.015	.003		.020	.022	.183	.011
	N	5	5	5	5	5	5	5	5	5
TA	Pearson Correlation	.917*	-.980**	.996**	.946*	.934*	1	1.000**	-.761	-.993**
	Sig. (2-tailed)	.028	.003	.000	.015	.020		.000	.135	.001
	N	5	5	5	5	5	5	5	5	5
TH	Pearson Correlation	.920*	-.982**	.996**	.942*	.931*	1.000**	1	-.758	-.992**
	Sig. (2-tailed)	.027	.003	.000	.017	.022	.000		.138	.001
	N	5	5	5	5	5	5	5	5	5
COD	Pearson Correlation	-.745	.776	-.715	-.826	-.706	-.761	-.758	1	.721
	Sig. (2-tailed)	.148	.123	.175	.084	.183	.135	.138		.169
	N	5	5	5	5	5	5	5	5	5
DO	Pearson Correlation	-.869	.952*	-.993**	-.954*	-.956*	-.993**	-.992**	.721	1
	Sig. (2-tailed)	.056	.012	.001	.012	.011	.001	.001	.169	
	N	5	5	5	5	5	5	5	5	5

*****. Correlation is significant at the 0.01 level (2-tailed)***
****. Correlation is significant at the 0.05 level (2-tailed)***

Table 4 shows correlation between different pollutants in the sites. Dissolved oxygen shows a positive correlation with pH (significant at 0.01 level) and COD. A strong correlation exists between total dissolved solids and parameters like electrical conductivity, total solids and total hardness as evident from table 4. Hardness has a very strong positive correlation with parameters like EC, TDS and TS.

TABLE 5
CORRELATION MATRIX BETWEEN BIOLOGICAL AND CHEMICAL PARAMETERS

		THB	TC	TEMP	DO	PH
THB	Pearson Correlation	1	.914*	-.896*	.615	.818
	Sig. (2-tailed)		.030	.040	.269	.091
	N	5	5	5	5	5
TC	Pearson Correlation	.914*	1	-.756	.336	.607
	Sig. (2-tailed)	.030		.140	.581	.278
	N	5	5	5	5	5
TEMP	Pearson Correlation	-.896*	-.756	1	-.869	-.978**
	Sig. (2-tailed)	.040	.140		.056	.004
	N	5	5	5	5	5
DO	Pearson Correlation	.615	.336	-.869	1	.952*
	Sig. (2-tailed)	.269	.581	.056		.012
	N	5	5	5	5	5
PH	Pearson Correlation	.818	.607	-.978**	.952*	1
	Sig. (2-tailed)	.091	.278	.004	.012	
	N	5	5	5	5	5

. Correlation is significant at the 0.05 level (2-tailed)
****. Correlation is significant at the 0.01 level (2-tailed)***

Table 5 shows correlation between heterotrophic, total coliform bacteria and some selected chemical parameters. Results revealed that there exists a positive correlation between total heterotrophic bacteria and dissolved oxygen as well as with pH. This is true in case of total coliforms which showed a positive relation with both dissolved oxygen and pH. But both bacterial counts (THB & TC) exhibited a negative trend with temperature as evident from the table 5.

IV. CONCLUSIONS

Based on the foregoing analysis, it was concluded that bacteriological and physico-chemical quality of "Hora" and Huluka water samples in the current study did not meet the standards set for drinking water. Some of the physicochemical parameters such as temperature (26.91- 0.160°C), pH (6.34-7.94), EC (404.66-1934.89 µS/cm), TDS (402-1067.50 mg/L), DO (1.2-5.8mg/L), TS (1167-1168mg/L) in the "Hora" water was found to be higher than the maximum permissible levels set for drinking water. Bacteriological parameters like total coliform and total heterotrophic bacteria were also much above the recommended standard values of WHO. In the wake of high pollution status of these waters, it was highly recommended that concern authorities who are responsible for maintaining the water quality of Hora waters in Ambo town should implement stringent rules to protect these spring water sources from being polluted further.

REFERENCES

[1] Zinabu G. The Ethiopian Rift Valley Lakes: Human interactions and water quality changes as a possible threat to the biodiversity, Awassa, Ethiopia. 2002

[2] Chatterjee C. and Raziuddin M. Determination of water quality index of a degraded river in Asansol Industrial area, Rangiganj, Burdwan, West Bengal. Environment and pollution Technology. 2002, 1 (20): 181-189.

[3] Venkatesharaju K, Ravi Kumar P, Somashekar R.K, and Prakash. K.L. Physic- chemical and bacteriological Investigation on the river Cauvery of Kollegal stretch in Karnataka, Journal of science Engineering and technology. 2001, 6 (1):123-160.

[4] Hurst C. J, Mclnerney M.J, Stezenbach. L.D, and Walter M.V. Manual of Environmental microbiology. American society for Microbiology. American Society of microbiology press, Washington, DC, 2002, 265-284.

[5] Erfurt Patricia J. An assessment of the role of natural hot and mineral springs in health, wellness and recreational tourism. PhD thesis. James Cook University. 2011

[6] Commission of the European communities (CEC). Establishing the list of concentration limits and labeling requirements for the constituents of natural waters. Journal of the Europe and Union 2003, 6 (2) : 146-150.

[7] Kabaija E. Non- conventional indigenous mineral supplements used for cattle feeding in the pastoral rangelands of Ethiopia. Trop, Anita. Hlth Prod. 2008, 21: 256- 262.

[8] USEPA. Methods for Chemical Analysis of Water and Wastes, USEPA Publication No. EPA-600/4-79- 020, United States Environmental Protection Agency. 2009

[9] American Public Health Association (APHA). Standard methods for examination of water and wastewater 20th Ed. American Public Health. New York. 1998, 81-85.

[10] Murray, Baron, Jorgensen, Landry and P faller.ed. Manual of clinical microbiology, 9th ed. American Society for Microbiology. Washington D.C., 2007

[11] Fawole M.O. and Oso B.A. *Laboratory Manual of Microbiology* Spectrum Books Limited. Ibadan, Nigeria. 2001, 71-80.

[12] Brock T.D, Madigan M.T, Martinko J.M. and Parker. *Biology of Microorganisms*. 7th edition. Prentice-Hall. New Jersey. 1994, 192-194.

[13] Milkiyas T, Mulugeta K, Bayeh A. Bacteriological and physico-chemical quality of drinking water and hygiene-sanitation practices of the consumers in Bahir Dar city. Ethiopian Health Sci. 2001, 22:19–32.

[14] Gupta B.K. and Gupta R.R. Physicochemical and bacteriological study of drinking water in Satna Maheya prade. 1999, 523–552.

[15] World health organization (WHO) Guidelines for Drinking Water. Quality. 4thEdition. WHO press. 2011; 564.

[16] Patil S.G, Chonde S.G, Jadhav A.S, and Raut P.D. Impact of Physico-Chemical Characteristics of Shivaji University lakes on Phytoplankton Communities, Kolhapur, India, *Research Journal of Recent Sciences.* 2009, 1 (2): 56- 60

[17] Amukama D, Kansime F, Mach R, Farnleitner A. Determination of *Escherichia coli* contamination with Chromo cult coliform agar showed a high level of discrimination efficiency for differing pollution levels in tropical waters of Kampala, Uganda. Appl Environ Microbiol. 1999, 66: 864-846.

[18] John KN, Orish EO, and Linus OE. Some physicochemical parameters of potable water supply in Warri, Niger Delta area of Nigeria. Sci. Res. And Essay. 2008, 3(11): 547-551.

[19] Orama.B. Sources of total Dissolved Solids.Minerals.in Drinking Water, htt://WWW.water-research.net/total dissolved solids. htm, accessed on Oct 18, 2010.

[20] Kataria H. C, Quershi H. A, Iqbal S. A, and Shandilya A. K. Assessment of water quality of Kolar reservoir in Bhopal. M.P. Pollution Research. 1996, 15 (2): 191-193.

[21] Hart. A. Total hardness and water quality, 2007 htt://ezinearticles.com/Total hardness and water quality and & id=516135, accessed on oct 18, 2010.

[22] Pakistan council of Research in water Resources (PCRWR). National Water Quality Monitoring Program. 2001, 1-20

[23] Edema M.O, Omemu A. M, and Fapet O. M. Microbiology and Physicochemical Analysis of different sources of drinking water in Abeokuta. Nigeria. Niger. J. Microbial. 2001, 57

[24] Obire O, Aguda M. Bacterial community of Leachate from a waste dump and an adjacent stream. Appl. Sci. Environ Mgt. 2004, 6 (2): 71-76.

[25] Welch P, David J, Clarke TAPD, Berston S, McDougall L, Adesiyin AA. Microbial sanitation of water in rural communities of Trinidad. Pan Ameri. J. Public Hlth. 2000, 8 (3): 172-180.

[26] Ajayi AO, Akonai KA. Distribution pattern of enteric organisms in the Lagos Lagoon. Afr. J. Biomed.Res. 2005, 8 (3): 163-168.

[27] Okonko I. O, Adejoye O. D, Ogunnusi T.A, Fajobi E. A, and Shittu O. B. Micobiological and Physicochemical Analysis of Different Water Samples Used for Domestic Purposes in Abeokuta and jota, Lagos State, Nigeria. African journal of Biotechnology. 2008, 75: 617-621.

AD Publications

Sector-3, MP Nagar, Rajasthan, India

www.adpublications.org, www.ijoear.com, info@ijoear.com