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DETERMINATION OF TOXIC METALS (Ni, Pb, Cr & Cd) IN SELECTED BRANDS OF CHOCOLATES AND CANDIES MARKETED IN BAHIR DAR CITY, ETHIOPIA

Molla Zewdie*

ABSTRACT

Chocolates and candies are among sweet food items consumed primarily by children. In the present study, four toxic metals; cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) were determined in different brands of chocolate and candy samples available in local markets of Bahir Dar city, Ethiopia. Three chocolate and three candy brands were randomly selected. The concentrations of toxic metals under study were determined by inductively coupled plasma optical emission spectrometry (ICP−OES) following the conventional wet-acid digestion on an electric hot plate. The concentrations of Cd, Ni, Cr and Pb were analyzed in different brands of chocolate and candy samples. The concentration of Ni ranged from 0.95 ± 0.022 mg/Kg to 3.56 ± 0.055 mg/Kg for chocolate samples was the highest among all other toxic metals determined. Lead was absent in two brands of chocolate and one brand of candy. Cadmium was ranged from 0.067 ± 0.001mg/Kg to 0.1 ± 0.002 mg/Kg in chocolates and from not detected to 0.15 ± 0.0005mg/Kg in candy samples. Excel computer system, one-way analysis of variance (ANOVA) and origin software were used for data analysis. Except for Cr in candy samples, the ANOVA result showed that there is no significance difference in the mean concentration of toxic metals ($P > 0.05$). The results were compared with the provisional maximum tolerable daily intake (PMTDI) which is set by joint FAO/WHO expert committee on food additives (JECFA). Except cadmium, the other toxic metals were found to be above the limit.

Keywords: Toxic metals, Chocolates, Candies, ICP−OES, wet−acid digestion

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1. INTRODUCTION

Chocolate is an ingredient usually used in the preparation of several foods (cakes, cookies, breads, ice creams, etc.), which are consumed mainly by children. This indicated that the mineral content of this ingredient has a tremendous value for nutritional control of the foods. Nevertheless, the determination of toxic metals like Ni, Cd, Pb and Cr in such samples requires a difficult step of digestion because its matrix consists of high contents of organic compounds [1].

Chocolate is a very good nutritious energy source, with a fast metabolism and good digestibility. The presence of cocoa, milk and sugar in its composition make it the proper source of proteins, carbohydrates, fats, minerals and vitamins. Other main ingredients of chocolates are cocoa butter, hydrogenated vegetable oil and vegetable fats. [2]. It is therefore necessary to monitor human exposure to toxic metals present in the diet [2, 3]. Candies and chocolates are associated with joy and pleasure, potentially being stimulant, relaxant, and antidepressant. Different types of candies and chocolates are available in the national and international market [3, 4].

Recently, there is an increasing concern about the quality of imported foods and food related products in several parts of the world [4] particularly from overseas due to the large size of shipments, the variety of food imported and the large numbers of potential contaminants. Importantly, food products such as candies that are likely to be eaten frequently by children are covered by colorful packages, which can induce heavy metals such as Pb and Cr from the printed surface to the food contact surface [5].

Some heavy metals such as Pb, Cd and Ni may be toxic for human body if excessive amounts are taken [6]. Nickel occurs naturally more in vegetables than in animal flesh. However, its toxicity in humans is not a very common occurrence because the intestinal absorption of nickel is very low [6, 7].

Toxicity of cadmium is known by toxicity symptoms like gastrointestinal pains, nausea, respiratory distress, diarrhea, impaired reproductively, kidney damage and hypertension [8]. Cadmium is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources.

Lead contamination in chocolates and candies is a very old problem that has evolved with time. Since the middle of the 19^{th} century various measures including regulations and public education were implemented to minimize the contamination of chocolates and candies from such sources [9]. Nowadays, industrial activities dominate the global flux of lead on the environment [8, 9] and have become predominant sources of lead in many food items, including candies [10].

Different complex matrices of the analytical sample required prior mineralization for most analytical methods, and this method is critical in the complete analytical procedure for the determination of metal concentration. Analysis of metals in food samples has conventionally been performed by atomic absorption spectrometry [11, 12]. Sample digestion techniques, such as microwave and conventional acid digestion

method (CDM) for toxic metals determination have been used widely for the dissolution of target elemental analysts. These digestion techniques however require the use of concentrated mineral acids and high temperatures whereas high pressures are required in the case of microwave applications [13].

Chocolates and candies are favorite food items for children who are the most susceptible age groups to toxic metal intake from such types of food items. Most of them, in Bahir Dar city, are sold at roadside shops, supermarkets, near schools, university campus shops and other small kiosks. Further, they are packaged by colorful wrappers, which might result in contamination of candies and chocolates by toxic metals.

Due to the contamination of toxic metals and other hazardous substances, candies and chocolates may pose human health risks. The current study is aimed to determine the concentration of Ni, Cd, Pb, and Cr in different brands of candies and chocolates available in the local markets of Bahir Dar city.

2. MATERIALS AND METHODS

2.1 Chemicals

All the chemicals and reagents used for the laboratory work were of analytical grade. Distilled water has been used throughout the laboratory work for sample preparation, dilution and rinsing each apparatus before working with it. HNO_3 (65%, Spectrosol, BDH, UK) and H_2O_2 (30%, Blulux, Laboratories, PLTD, India) were used for sample digestion. Stock standard solutions containing 1000 mg/L of the metals Ni, Cd, Pb and Cr were prepared from $NiCl_2.6H_2O$,$Cd(NO_3)_2$, $Pb(NO_3)_2$, and $Cr_2(SO_4)_3$, respectively of which all were from Sigma Aldrich. Working solutions were prepared from the stock standard solutions of metals by appropriate dilution of the intermediate solution (10 mg/L).

2.2 Sample preparation and digestion of samples

The samples collected from the local markets in Bahir Dar city were dried using microwave oven (GALLENKAP) for about three hours at 70^0C operating temperature and then ground into pieces. Around 20.00 g of each sample was kept until digestion. From this mass of sample, about 1.50 g of triplicate chocolate samples and 1.50 g of candy samples were measured and kept for digestion.

Conventional wet-acid digestion method was employed to digest the collected samples.10.0 ml mixture of HNO_3 (65%) /H_2O_2 (30%) (v/v) (1:1 ratio) was used by maintaining the temperature at 80 ^{0}Cfor 2−3 hours on the hot plate. After cooling the resulted clear solution for about 10 minutes, 15 ml of distilled water was added and mixed. Then, each replicate sample was transferred into 50ml volumetric flasks using a funnel and Whatman No.41 filter paper. The resulted solution was diluted to 50 ml with distilled water.

3. RESULTS AND DISCUSSION

3.1 Instrument calibration: linearity of calibration curves

The quality of data resulted from ICP−OES measurements are affected by sample digestion, calibration and standard solution preparation procedures [14]. In this study, the instrument was calibrated by using five series of working standard solutions prepared from intermediate solutions for each metal under determination. For the determination of Ni, Pb and Cr the range of concentration for standard solutions was from 0.001 mg/L – 2.401 mg/L. For Cd determination, it was in the range of 0.001 mg/L – 1.296 mg/L.

The intensity of each standard solution for each toxic metal was measured by using ICP−OES and the calibration curves were plotted. The squared correlation coefficient (R^2) values of calibration curves for Ni, Pb, Cr and Cd were 0.9973, 0.9964, 0.9992 and 0.9947 respectively. These high square correlation coefficient values indicate the linearity of the calibration curves.

3.2 Field Emission Scanning Electron Microscopy (FESEM)

To verify the precision of the method, each sample was measured three times. The percentage relative standard deviations (% RSD) of measurements were calculated. Based on this, the % RSD value of Ni, Pb, Cr and Cd for the analysis of chocolate was found to be 2.08 %, 11.68%, 5.27 % and 11.39%, respectively.

In order to evaluate the accuracy of the method, the recovery tests were studied for four toxic metals. Percentage recovery of the spiked concentrations for Ni, Pb, Cr and Cd in chocolate and candy samples was calculated by using the following equation.

$$\%RC = 100\ x\left(\frac{SPS - S}{SP}\right)$$

Where % RC = percentage recovery (in %)

SPS = amount found in spiked sample (in mg/L)

S = amount found in unspiked sample (in mg/L)

SP = amount added as a spike (in mg/L)

Percentage recovery of the spiked metals ranged from 89.6 % to 97.2 % for chocolate samples and from 87.0 % to 99.4 % for candy samples.

TABLE 1: Percentage recovery of spiked concentrations

Samples	Toxic metals	Concentration before addition of a spike (mg/L)	Amount of Spike added (mg/L)	Concentration of the spiked solution (mg/L)	% Recovery
Chocolate	Ni	0.084	0.200	0.2638	89.9
	Pb	0.072	0.200	0.2632	95.6
	Cr	0.134	0.200	0.3132	89.6
	Cd	0.001	0.200	0.1954	97.2

Candy	Ni	0.229	0.200	0.4278	99.4
	Pb	0.049	0.200	0.2230	87.0
	Cr	0.279	0.200	0.4758	98.4
	Cd	0.018	0.200	0. 2044	93.2

3.3 The limit of detection (LOD) and quantification (LOQ)

The LOD is defined as the lowest concentration of an analyte in a sample that can be detected. The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the analytical conditions of the method. The LOD values were calculated by using an equation, LOD= $\frac{3SD}{m}$, where SD is for standard deviation and m is for slope of the blank in the calibration curve of each toxic metal. Similarly, LOQ values were calculated by the equation, LOQ= $\frac{10SD}{m}$.

Based on the above equations, the calculated LOD values for Ni, Pb, Cr and Cd were 0.846, 0.077, 0.055 and 0.108 mg/L, respectively. The calculated LOQ values were 2.807, 0.256, 0.184 and 0.363 mg/L, respectively.

3.4 Concentration of toxic metals in different brands of chocolate and candy samples

The concentration of four toxic metals (Ni, Pb, Cr and Cd) was determined by ICP−OES in different brands of chocolates and candies. Replicate solutions of each sample were measured. The concentrations were calculated using the mean of each value with standardization of the instrument by using the standard solutions and blank reagent. Toxic metal concentrations were based on the samples' dry weight and the mean results are the average values of the three replicates. The results indicate that the mean concentration of Ni, and Cr in chocolate samples was greater than that of their mean concentration in candy samples. On the other hand, the mean concentration of Pb and Cd in candy samples was greater.

Concentrations in mg/Kg of each toxic metal in the digested samples were also calculated using the following expression.

$$[M] = \frac{C}{W} \times V$$

[M] = total metal concentration (in mg/Kg)
C = concentration of the metal in the digested samples (in mg/L)
W = weight/mass/ of the digested sample (in g)
V = final volume of the digested sample solution (ml)

TABLE2: Concentrations (mean ± SD) of toxic metals determine different brands of chocolates and candies

Sample	Brand Name	Concentration (mg/Kg)			
		Ni	Pb	Cr	Cd
Chocolate	Galaxy(ChB−1)	0.95 ± *0.022*	0.23 ± *0.060*	ND	0.067 ± *0.001*
	Take (ChB−2)	3.56 ± *0.055*	ND	2.1 ± *0.050*	0.1 ± *0.002*
	Combo (ChB−3)	1.52 ± *0.010*	ND	2.13 ± *0.017*	0.1 ± *0.001*
Candy	Toppy (CB−1)	ND	ND	ND	ND
	Lollipop (CB−2)	2.94 ± 0.107	2.29 ± *0.022*	0.28 ± 0.034	0.15 ± *0.005*
	Desta (CB−3)	1.4 ± 0.030	0.91 ± 0.070	0.23 ± 0.035	0.12 ± 0.016

*ND = Not detected

3.4.1 Comparison of the average toxic metal concentrations among the different brands of chocolates and candies

From the three chocolate brands, only ChB−1 was found to be free from Cr. Pb was absent in the two brands of chocolate samples. On the other hand, Ni and Cd were found in all chocolate brands. In the case of candy samples, except the first brand of candy (CB−1), the rest were found to contain toxic metals.

Chocolate samples: From all the determined TMs, Ni was found in the greatest concentration. Its concentration ranged from 0.95 mg/Kg for ChB−1 to 3.56 mg/Kg for ChB−2. The least determined toxic metal was Cd whose concentration range is from 0.067 mg/Kg to 0.10 mg/Kg. Pb was found in a concentration of 0.23 mg/Kg only in ChB−1 chocolate brand. It was not detected in the rest of two chocolate brands. On the other hand, the content of Cr concentration ranged from ND to 2.13 mg/Kg. In general, Ni and Cd were detected in all chocolate brands whereas Pb is least detected. Figure 1 shows the comparison of concentration of TMs in different brands of chocolates.

Candy samples: Three different brands of candies were subjected to toxic metal determination. None of the toxic metals were detected in candy brand CB−1. The rest two brands of candies were found to contain toxic metals. The concentration of toxic metals in both CB−2 and CB−3 was in the order of Ni > Pb> Cr > Cd. The concentration of Ni ranged from ND (in CB−1) to 2.94 mg/Kg (in CB−2). The concentration of Pb, Cr and Cd was found in the range of ND to 2.29 mg/Kg, 0.28 mg/Kg and 0.15 mg/Kg, respectively, for the three candy samples. Figure 2 shows the comparison of concentration of toxic metals in the two candy brands.

From the toxic metals, in all chocolate and candy brands, the concentration of Cd was found to be relatively least than any other toxic metal determined. Its concentration is somewhat varied in candy samples. However, it has comparable concentrations in

chocolate samples. Its content was in the range of 0.067 mg/Kg to 0.10 mg/Kg and from ND to 0.15 mg/Kg in chocolate and candy samples respectively. In the case of Pb, it was least detected in chocolate samples even if its concentration is relatively high, i.e., 0.23 mg/Kg for chocolate samples and in the range of ND to 2.29 mg/Kg for candy samples.

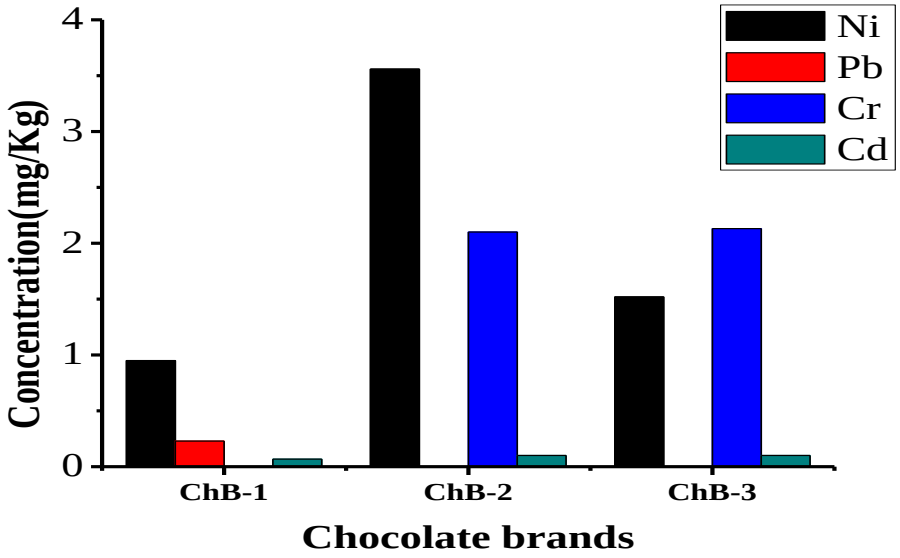


Figure1: Mean concentration of TMs in the three brands of chocolates.

In general, the concentration of toxic metals in different brands of chocolates and candies marketed in Bahir Dar city is relatively higher and its amount is significant. This high concentration of toxic metals in such types of food items is expected because of the ingredients and packaging wrappers used by the manufacturers. Relatively, chocolate samples were found to contain higher concentration of toxic metals. The high concentration of Ni may be due to its usage as a catalyst during manufacturing process of chocolate, i.e., when hardening is done by hydrogenation of unsaturated fats.

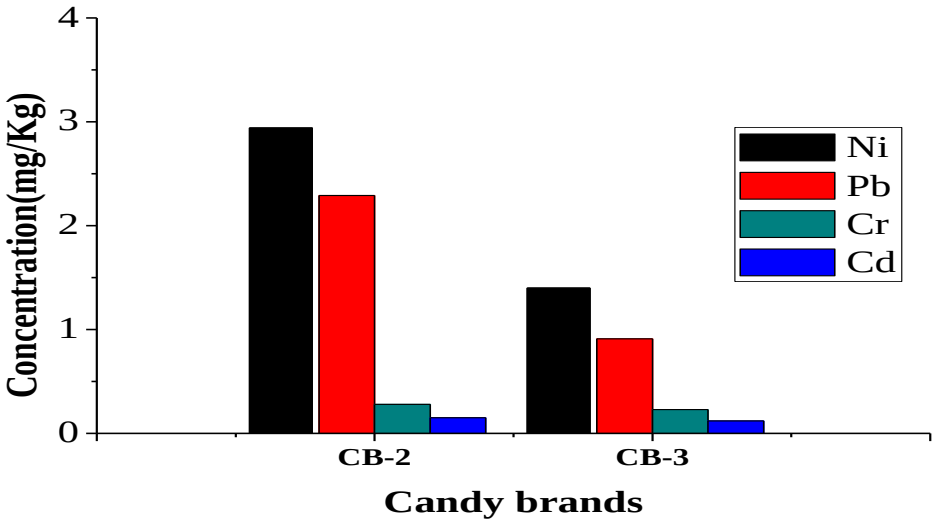


Figure 2: Mean concentration of TMs in the two brands of candies.

In previous researches, the concentration of Ni was reported in the range of 0.15 mg/Kg −3.55mg/Kg with a mean of 0.88mg/Kg for cocoa, milk and sugar-based chocolates [15]. In Pakistan, a similar research conducted to determine the concentration of toxic metals showed that the concentration of Ni ranged from 1.45 mg/Kg −4.33mg/Kg [10] of which the highest concentration was determined for cocoa-based chocolates as compared to milk and sugar-based chocolates.

The natural Ni content of various food items varies from 0 mg/Kg−10 mg/Kg [10]. The observed concentration for Ni in the present study is in agreement with this value, ranged from 0.95 −3.56 mg/Kg for chocolate samples and from ND−2.94 mg/Kg for candy samples. The highest Ni concentration (4.78 mg/Kg) was also obtained in the previous works done in Turkey [16]. Nickel's highest content in the previous works as well as in this present study is probably because of the high content of hydrogenated vegetable oil and cocoa butter which are the main ingredients used in the production of chocolates. Nielsen and Flyvholm (1983) suggested that nickel intakes in Denmark could reach over 900 μg/day by the consumption of certain foods having nickel composition, oatmeal, legumes (including soybeans), nuts, cocoa, and chocolate [17].

Research reports indicate that Pb contamination in chocolates and candies may be associated with atmospheric emission of lead gasoline as cocoa bean shells can absorb lead in a higher extent. The Industrial Revolution gave rise to an increase for lead in the environment and even bigger increase occurred around 1920 when leaded gasoline was introduced. Leaded gasoline is still not banned everywhere in the World, and still used in the developed countries. In areas where leaded gasoline is banned, the major exposure pathways of nonsmoking adults are from food [9]. Although the usage of leaded gasoline is not frequent and banned in most of the countries, there may be some

other sources of lead contamination like confectionery wrappers in chocolates and candies [16].

Another source of lead contamination could be packaging. Candy packaging has significantly higher levels of Pb (110−6394 mg/Kg) and Cr (137−1429 mg/Kg); particularly green and yellow- colored packaging has higher content of Pb which might be originated from the lead-based ink used to print the packaging [18].

Most of the metals analyzed in this study as well as in others are used in making alloys, which are used as containers to prepare candies and chocolates. Hence, utensils used during the preparation of candies and chocolates may cause metal contamination [18].

Cocoa beans can accumulate Cd naturally from the soil [19, 20]. As a result, its concentration could vary significantly in candies and chocolates. According to the FAO/WHO (2010) data, the level of Cd for chocolate brands ranges from 0.20 mg/Kg−2.00 mg/Kg [21]. The European Union also recommends the maximum of 0.30 mg/Kg Cd in chocolates containing ≥ 50% cocoa solid [22]. As compared to this, the present study showed lower level of Cd in chocolates as well as candies. However, it should be noted that the high intake of Cd could lead to distortion in calcium and the formation of kidney stones. It can also affect the skeletal and respiratory system [7, 23].

Chromium is another detected toxic metal in both of the samples. Except in ChB−1 and CB−1, it was detected in the rest of chocolate and candy brands selected for this study. A study conducted in Bosnia and Herzegovina showed the concentration of Cr in the range of ND−5.37 mg/Kg for chocolate samples of which the highest concentration belongs to cocoa-based chocolates [3]. In this present study, Cr was found to have an average concentration of 1.47 mg/Kg (ranged from ND−2.13 mg/Kg) for chocolate samples. For candy samples, its average concentration was 0.17 mg/Kg (ranged from ND−0.28 mg/Kg) which indicates that this much concentration of Cr in the present study is below the value determined in Bosnia and Herzegovina.

According to the research conducted in Serbia [21], the concentration of Cr in cocoa and milk chocolate samples was in the range of 0.12 mg/Kg−0.38 mg/Kg and ND−0.38 mg/Kg, respectively.

The presence of Pb and Cr in chocolate and candy samples can be linked with the colored packages (wrappers) of which pigments such as lead chromate ($PbCrO_4$), lead sulphate ($PbSO_4$) and lead oxide (PbO) could confer these colors [15]. These harmful pigments may intentionally be used in countries during manufacturing process. Accumulation of Pb in body tissues may cause acute toxicity by causing anemia, headache, fatigue, and peripheral nervous disorders [24]. Therefore, it is advised to minimize the excessive intake of food items that can contain Pb such as chocolates and candies.

What we understand from this is, parts of samples can be contaminated due to their packages. The result illustrates that some toxic metals could migrate from the packages when chocolate and candy surfaces are sticky, allowing the surface of the samples to adhere into the inner coverage of the package.

In general, the findings of this study indicate that toxic metals like Ni, Pb, Cr and Cd are found in food items like chocolates and candies. This higher concentration of toxic metals in candies and chocolates may be due to the raw materials such as cocoa beans, cocoa solids, hydrogenated vegetable oils and cocoa butter as well as their colorful packages.

3.4.2 Statistical analysis of variance (ANOVA)

The significance of variation in mean concentration of toxic metals between chocolate and candy samples was analyzed using one-way ANOVA. Based on the results, except for Cr, the mean concentration of toxic metals has not a significant difference between both chocolate and candy brands ($P > 0.05$) at the 95 % confidence interval. The variation of Cr within the two samples might arise from measurement errors.

4. CONCLUSION

In this study, three chocolate and three candy brands marketed in the city of Bahir Dar, Ethiopia were analyzed for their toxic metal content. The analysis was done after digesting the samples using conventional wet acid digestion method. Determination of the concentration of toxic metals was performed by using ICP−OES.

The results showed that all the toxic metals under study except Cr (in candies) and Cd found in chocolate and candy samples were generally higher than the JECFA limit. However, their concentration value agrees with most of other literatures. The concentration of Ni was found in the range of $(0.95 \pm 0.0215 - 3.56 \pm 0.0545)$ mg/Kg for chocolate samples and (ND -2.94 ± 0.107) mg/Kg for candy samples. Pb was found only in ChB−1 chocolate sample with concentration of 0.23 ± 0.06 mg/Kg and in two candy brands with a concentration of 0.91 ± 0.07 mg/Kg and 2.29 ± 0.022 mg/Kg.

Another toxic metal determined was Cr whose concentration ranged from ND -2.13 ± 0.0167 mg/Kg in chocolates and ND $- 0.28 \pm 0.034$ mg/Kg in candies. The Cd content in this study was below its values reported by other literatures as well as the JECFA provisional maximum tolerable daily intake limits. Its concentration ranged from 0.067 ± 0.001mg/Kg $- 0.1 \pm 0.0017$ mg/Kg and from ND-0.15 ± 0.0005 mg/Kg for chocolate and candy samples, respectively.

The reasons for the high levels of toxic metals in the samples could arise from the unsafe storage conditions or at any point in the production chain i.e., from the raw materials, processing, and packaging. The higher concentration of toxic metals in chocolates and candies may also be due to the raw materials such as cocoa beans, cocoa solids, hydrogenated vegetable oils and cocoa butter. The daily dietary intake of chocolates and candies must be reduced to keep the toxic metals within the prescribed limits set by JECFA. Hence, to reduce the dietary exposure, raw materials having lower content of these toxic metals should be used in the manufacturing process of chocolates

and candies. Furthermore, clear awareness should be created for the society about the existence of toxic metals in chocolates and candies marketed in Bahir Dar city.

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The Effect of Rural Out-Migration on Farm Income of Migrant Sending Rural Households: The Case of Alaje *Woreda*, Tigray Regional State, Ethiopia.

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ABSTRACT

Research on the relationship between rural-out migration and agricultural production is highly hampered by empirical evidence fromthe perspective of migrant-sending rural households. This paper assesses the effect of rural-out migration on farm income of migrant- sending rural households with evidence from Alaje woreda, Southern Tigray, Ethiopia. This paper utilized both desk and field research that depends on both secondary and primary data. A mixed research approach was applied. Using a simple random sampling technique 145 sample households were used for the household survey. The quantitative data were analyzed by descriptive and inferential statistics. Cobb-Douglas production function approach and ordinary least squares regression model was used to estimate the impact of rural-out migration on farm income. The findings showed that rural out-migration has significantly reduced the available labor in migrant-sending households and remittances play a positive and significant role in boosting investment in capital stock. Migrant sending households have obtained higher income from the farm as a result of positive marginal productivity of labor and remittance incomereceived.

Keywords: Farm Income; Migrant sending households, Cobb- Douglas production function, rural out-migration

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1. INTRODUCTION

The patterns of migration in Africa are complex and its causes or drivers vary widely. In general, people may be induced or compelled to move as a result of cultural, demographic, socio-economic, environmental, and/or political factors (Zoomers, 2010).

Rural-urban migration has historically been played a significant role in the urbanization process and economic growth of several countries andcontinues to be significant in scale. Rural-urban migration accounted for urban population growth and urban growth in Africa in the past three decades. Also, Migration has been an important livelihood and survival strategy for many poor groups across the developing world, particularly in Africa (Greenwood & Hunt, 2003; Lall *et al*., 2006).

By taking into account the case of European cities in the 19th and early 20th century, empirical evidence in development economics suggest that rural-out migration results in industrialization and economic growth. However, concerning developing countries particularly Africa,some key questions have not found clear answers and did not get empirical explanations (Lall *et al*., 2006). Rural households also use rural-out migration as an alternative strategy to diversify their sourceof income and livelihood. Empirical evidence regarding the effect of rural-out migration on farm income is a highly debated and mixed issue. On one hand, it implies a loss of labor force from the rural sectorand on the other hand, the inflow of remittance from migrants promotes agricultural investment (De Brauw, 2014).

Empirical studies show how remittances increase the total income of households directly or indirectly through stimulating investment in production and how it could compensate for the lost labor. Nevertheless, it has been argued that the micro impacts of migration and remittances on agriculture production and productivity arecomplex, little explored and the topic has been a growing area of research on agricultural and development economics (Taylor and Martin, 2001).

In Africa, research has been carried out on the effects of rural-out migration on the livelihoods of households left behind. Some of these studies include those of (Ajaero and Onokala, 2013; Yamarak, 2013), and others. However, these studies have been done at the macro level and on the effect of formal international remittance flows on national economies. Other studies on internal rural-out migration have alsofocused on the livelihood of the migrants themselves based on urban centers. Therefore, this research analyzes the effect of rural out- migration from the perspective of migrant-sending rural households.

Ethiopia is experiencing a high level of population pressure, populationredistribution, and rural-out migration (Mberu, 2006). Although rural- out migration has a long history in Ethiopia; empirical evidence on the relationship between rural-out migration and socio-economic development is little explored from the context of migrant-sendingrural households. As a result migration and population policies are poorly designed (Beneberu, 2012). To the knowledge of the researcher,most of the rural-out migration studies done in Ethiopia excluded the effects of rural-out migrations on the

rural migrant-sending householdsand mainly focus on the livelihoods of migrants. Some of these studies include Belay, (2013); Birhan, (2013), Gebru and Beyene, (2014), and others.

Despite the limited number of empirical researches in the country that investigates the effect of rural-out migration from the perspective of migrant-sending rural households, there exist no previous studies that investigated the impact of rural-out migration on farm income of migrant-sending rural households in the study area. In light of the fore mentioned gaps, this paper is designed in filling the gap to the existing limited empirical research on internal migration particularly rural- urban migration by taking Alaje as the study site. Therefore, the focus of this paper was to assess the effect of rural out-migration and remittance flows on farm income of migrant-sending rural households by taking Alaje as the study site.

2. MATERIALS AND METHODS

2.1 Research Design

To understand properly the existing effect of out-migration on farm income of migrant-sending rural households a cross-sectional survey design was employed. A mixed research approach was used as they do support each other at a different level.

2.2 Sample design

This study was conducted in Alaje *Woreda*, Tigray region. The *woreda* for this particular study was purposefully selected because the migration of labor is prevalent in this particular woreda and known for sending-out labor migrants to destination locations such as to Mekelle and Humera areas.

The study area comprises 21 rural *kebeles* in total. Three sample *kebeles* were selected purposively by taking into account the magnitude of migrants. A total of two thousand and one hundred nine (2109) migrant-sending households were found. Using Yamanes' (1967) formula 145 sample households were selected using a simple lottery random sampling procedure. To have a proportional number of sample households from the study *kebeles* proportional sampling was used.

2.3 Data sources and data types

Primary data sources for this research were household heads or senior household members. The instruments of data collection from these data sources were questionnaires and focus group discussion with migrant- sending households of the study area and government officials, and local administrators. The type of data generated from household's survey is demographic characteristics, Agricultural production, use of Agricultural inputs, farming activities, expenditure pattern, landholding size, migration, and remittance received. Secondary data sources also used such as**;** books, journals, magazines, and reports from migration bureaus, agriculture

bureaus, publications ofgovernmental and non-governmental organizations, statistical and census reports, and the internet.

2.4 Methods of data collection

The structured questionnaire was designed to collect data from migrant-sending household heads. The household survey questionnaire was consists of different sections pertains to collecting the amount of necessary information on household demography, agricultural production, migration and remittance, socio-economic conditions of migrant-sending households. Focus group discussion was also conducted with selected community members for each sample village to collect complementary information for certain questions in the research topic.

2.5 Method of data analysis

The data were analyzed by descriptive and inferential statistics. The descriptive analysis includes percentages, averages, tables, and graphs, whereas the inferential statistics include regressions analysis. OLS regression model was also used to estimate the impacts of rural out- migration on farm income of migrant-sending households.

2.6 Specification of Econometric model for the effect of out-migrationon farm income

Out-migration affects the total labor force available in the household. Similarly, change in the available capital stock is expected as a result of remittances obtained from migrants.

To analyze the effect of rural-migration on-farm income via its effect on production factors, the log-linear Cobb-Douglas production function is used which is the commonest in literature. First, the effectof out-migration on production factors (labor input and capital inputs) were analyzed, including the characteristics of migration variables as determinant factors. Based on the predicted result on production factors, the effect on farm income is estimated using Ordinary Least Squares (OLS).

Migration involves the removal of labor on one hand, and the flow of remittances on the other that changes the available labor and capital input and thereby affecting farm income. Therefore, the followingregression model is specified for the determinants of available labor and capital stock for migrant-sending households.

$$\text{Labor} = \alpha_0 + \alpha_1 NM + \alpha_2 SOH + \alpha_3 NOD + \varepsilon_L \quad (1)$$

$$\text{Capital} = \beta_0 + \beta_1 RI + \beta_2 FI + \beta_3 MC + \varepsilon_K \quad (2)$$

2.7 Dependent variables

Labor: Is farm labor available in a household at a given time offarming measured in Labor-hours. Labor-hours is the product of the number of individuals in a household working on the farm and the average working hours spent per day and calculated for a given cropping season. In the research area, the average on-farm cropping season where farmers intensively engage in farm activities is six months.

Capital: Is farm capital stock allocated for agricultural productionincluding physical

capital and working capital valued in monetaryterms.

2.8 Explanatory variables for labor input

Number of migrants in a household (NM): it is expected that households who send migrants to destination region are expected to allocate less labor on-farm activities (negative relationship).

Size of a household (SOH): it is expected that households with larger family size, in general, are expected to allocate a greater number of labor on-farm activities (positive relationship).

Several dependents in a household (NOD): it is expected that households with more dependents are expected to allocate fewer labor-hours on-farm activities as working members are also responsible to provide care for the dependents in the house and to other non-farm activities (negative relationship).

2.9 Explanatory variables for capital input

Remittance income (RI): higher remittances are expected to enhance the investment in the capital stock of migrant-sending households (positive relationship).
Farm income (FI): Higher-income from the farm is expected to predict higher investment on capital stock (positive relationship).
Migration Cost (MC): Migrant sending households incur the cost of migration as part of initial investment while sending migrants.
To estimate the impact of out-migration and remittance on-farm income the predicted values of labor and capital variables from OLS regression results of Equations 1 and 2 including the variable land size (LS) are taken as explanatory variables. For estimation, the log-linear form of the Cobb-Douglass type production function is used as indicated in equation 3 below.

$$\ln FI = \eta_1 + \alpha_1 \ln PL + \beta_1 \ln PC + \gamma_1 \ln LS + \varepsilon_{YF} \quad (3)$$

Where,
lnFI is a log of the annual farm income of households (in ETB for2020).
lnPL and lnPC are a log of the predicted values of labor and capitalfrom Equations 1 and 2, respectively.
lnLS is a log of farmland size of the households measured in hectaresη_1 is constant.

3. RESULTS AND DISCUSSION

3.1 Econometric Results of the effect of rural out-migration onproduction factors

Table 1 and 2 below presents the econometric analysis or the linear regression model result, for the determinants of labor input and farm capital stock of migrant-sending rural households respectively.
Before running the regression model, the problem of multicollinearity among the explanatory variables was checked by using collinearity diagnostics or Variance

Inflation Factor (VIF). The value of VIF for each variable proved that the assumption of multicollinearity was not violated or there was no serious problem among the variables, as VIF values for each variable are less than 10. Similarly, using the Durbin-Waston authocollinearity checked and found that there was no authocollinearity problem.

TABLE 1: determinants of labor input of migrant-sending ruralhouseholds

Dependent variable labor input				
Explanatory variables	Coefficients	Std.Error	t-value	P>|t|
NM	-1480.43	149.34	-9.91	0.000***
SOH	1329.1	84.21	15.78	0.000***
NOD	-1211.54	155.14	-7.81	0.000***
Constant	475.2	487.77	0.98	0.33
No of observation = 145 F(17,236) = 114.73				
Prob> F = 0.000				
R- square = 0.71Adj.				
R- square = 0.70				

*** Significant at 1% **Significant at 5%Source: Survey result, 2020

Table 1 above depicts the regression result for the determinants of labor input among migrant-sending rural households. The Adjusted R.-square model summary value (coefficient of determination) shows that about 70% of the change in labor-hours among migrant-sending households are explained by the changes in the number of migrants, size of a household, and several dependents. The ANOVA statics of 114.73 (for 3 numerator degree of freedom and 141 denominator degree of freedom) shows that the model is different from zero and significant at 99 percent of confidence level. In other words, the model is fit at a 99 percent confidence level. It implies that the independent variables were related to the dependent variables.

The regression coefficient result shows that out-migration of a family member in households is associated with labor hour allocation for farmincome negatively and significantly (p=0.000) at 99 percent of confidence level. A unit of increase in the number of out-migrants in the household reduces the labor hour allocation for farm income by 1480.43 units. The finding confirms the NELM model which argues that the labor loss effect of out-migration. And the finding is consistentwith Lucas (1987) which out-migration reduces the available labor in the households. The household size

is also associated with the allocation of labor-hours positively and significantly (p=0.000) at 99 percent of confidence level. The result indicates that, as the member of household size increases, the labor hour allocation increases by 1329.1 units. Also, the result revealed that households with a dependent member are found to have a negative and significant (p=0.000) relationship with labor hour allocation at 99 percent of confidence level. An increase in a dependent family member in the household by one unit has reduced the allocation of labor-hours on farming activities by 1211.84 units. Households with more dependents are supposed to take care of the dependents left in the house and that hampers the time to be allocated for farm activities. From the above regression coefficient result, we can conclude that available labor in a household is negatively influenced by the number of out-migrants and dependentsin a household and positively affected by the household size.

TABLE 2: Determinants of the capital stock of migrant-sending ruralhouseholds

Dependent variable farm capital stock				
Explanatory variables	Coefficients	Std.Error	t-value	P>|t|
RI	0.41	0.142	2.87	0.005***
FI	0.26	0.025	10.48	0.000***
MC	-0.209	0.606	0.344	0.731
Constant	-506.75	974.95	-0.520	0.604
No of observation = 145F(3,141) = 43				
Prob> F = 0.000				
R- square = 0.48				
Adj. R- square = 0.47				

*** Significant at 1% **Significant at 5%Source: Survey result, 2020

Table 2 above presents the OLS estimation results regarding the determinants of the capital stock of migrant-sending rural households.
The Adjusted R-square value or the coefficient of determination result above shows, about 47% of the variation in farm capital stock ofmigrant-sending rural households is explained by the variation in remittance income, farm income, and cost of migration. The ANOVA statics of 43 (for 3 numerator degree of freedom and 141 denominator degree of freedom) shows that the model is different from zero and significant at 99 percent of confidence level. In other words, the model is fit at a 99 percent confidence

level. It implies that the predictor variables were related to the criterion variables.
The study result also indicates, remittance income has a positive and significant (p=005) effect on farm capital stock investment in migrant- sending rural households at 95 percent of confidence level. According to the result, as the remittance income increase by one unit the amount of farm capital stock in migrant-sending households increases by 0.41 units. This implies that remittances received by households may have been used to acquire capital goods (or agriculture inputs) to boost yield. The finding is consistent with the findings of Quinn (2009) in Mexico and Beneberu (2012) in Ethiopia found that remittance incomeboosts the working capital of migrant-sending rural households. This finding contradicts the finding of Raju et al. (2014) in Nepal; where found that remittance-receiving rural households did not use productivity-enhancing agricultural inputs such as chemical fertilizers even though remittances enhanced their income.
The regression coefficient result also shows that farm income has positive and significant (p= 0.000) effects on farm capital stock of migrant-sending rural households at 99 percent of confidence level. The regression result shows that, as the farm income increase by one unit the capital stock increases by 0.26 in migrant-sending rural households. This means the increase in the amount of farm income predicts increased investment in farm capital stock. In other words, higher-income from farm increases the savings and then investment on the capital stock that in turn enhance farm production and income. The regression model also shows that the effect of migration cost on-farm capital stock of migrant-sending rural households is found to be insignificant. This means that the capital stock of migrant-sendinghouseholds is almost not depleted as a result of sending migrants. The cost of migration can be sponsored from the savings from farm income, from relatives/friends in origin or destination region, or loans. From the above econometric estimation results and test statistics, we can confirm that remittances and farm income positively affect the farm capital stock whereas the effect of the cost of migration on-farm capital stock of migrant-sending households is negligible.

3.2 Econometric Results of the effect of out-migration and remittanceon farm income

Rural-out migration and inflow of remittances affect the farm income of rural households through changing the farm labor and farm capital stock. To see the effect of out-migration and remittance income on farm income of migrant-sending rural households, the predicted values of the labor variable and capital stock variable based on the OLSestimation results in table two and three including land variable would be used as determinants factors affecting farm income of migrant-sending rural households. The effects of out-migration and remittances are already embedded in labor and capital variables.
Before running the regression model, the problem of multicollinearity among the explanatory variables was checked by using collinearity diagnostics or Variance Inflation Factor (VIF). The value of VIF for each variable proved that the assumption

of multicollinearity was not violated or there was no serious problem among the variables, as VIF values for each variable are less than 10. Similarly, using the Durbin-Waston authocollinearity checked and found that there was no authocollinearity problem.

TABLE 3: OLS result of the effect of out-migration and remittanceon farm income

Dependent variable farm income				
Explanatory variables	Coefficients	Std.Error	t-value	P>|t|
lnPL	0.048	0.023	2.06	0.041**
lnPC	1.26	0.041	30.38	0.000***
lnLS	0.09	0.029	3.42	0.001***
Constant	-1.612	0.411	-3.92	0.000***
No of observation =145 F(3,141) = 551.41				
Prob> F = 0.000				
R- square = 0.821				
Adj. R- square = 0.820				

*** Significant at 1% **Significant at 5%Source: Survey result, 2020

In table 3 above the Adjusted R-square results indicate that about 82% of the variations in farm income are explained by the changes in farm labor, farm capital stock, and farm landholding of migrant-sendingrural households. The ANOVA statics of 551.41 (for 3 numeratordegree of freedom and 141 denominator degree of freedom) shows thatthe model is different from zero and significant at 99 percent of confidence level. In other words, the model is fit at a 99 percent confidence level. It implies that the predictor variables were related to the criterion variables.

Labor has a positive and significant (p=0.041) effect on farm incomeof migrant-sending rural households at 95 percent of confidence level. The result indicates that a one percent increase in the labor-hours leads to a 0.048% increase in farm income of migrant-sending households. This implies that the marginal product of labor is still positive and out- migration has made labor productive for migrant-sending rural households. This finding is inconsistent with the finding of Raju et al (2014) found that decline in agriculture production due to the shortageof labor from migration and similarly, Rozelle et al (1999) found that the migration of an additional family member led to a maize yield loss in rural China.

The farm capital stock variable also affects the farm income of migrant-sending rural

households positively and significantly (p=000) at 99 percent of confidence level. The model result indicates that a one percent increase in farm capital stock leads to a 1.26% increase in farmincome of migrant-sending rural households which is invested as a result of savings from remittances and farm income. But, the cost of migration has an insignificant effect on farm capital stock and thus having a negligible effect on farm income. The farm capital stock is more predictor than the rest of the production factors. Therefore, the change observed in farm income of migrant-sending rural households is mainly as a result of changes in farm capital stock, which is in turn influenced by the investment made from remittances and farm income. The finding is consistent with De Baruw (2014) which found thatremittance as a result of migration increases agricultural production.

Also, the size of farmland by migrant-sending households affects the farm income positively and significantly (p=001) at 95 percent of confidence level. The result indicates that a one percent increase in the size of farmland increases the farm income of migrant-sending households by 0.09%. In general, the regression estimation results haveproduced a positive and significant effect of production factors on farmincome. Out-migration and remittances affect the farm income of rural households through their effects on labor and capital inputs.

4. CONCLUSION

The central purpose of the study was to assess the effect of rural out- migration and remittance income on farm income of migrant-sending rural households of Alaje *woreda*, Tigray region, Ethiopia.

Using the Econometric model the effect of rural out-migration on farm income has been discussed. To estimate the effect of out-migration on- farm income first, the determinants of labor and capital input includingout-migration and remittances have been estimated and then the effect on farm income was estimated. The finding shows that out-migration has been found to reduce significantly the available labor of migrant- sending households and remittance income has played a positive and significant role in boosting investment in capital stock. The effect on farm income based on the predicted value of labor and capital results has shown that migrant-sending households have obtained higher income from farms even though out-migration reduces the available labor in migrant-sending households. The increment in farm income of migrant-sending households has been predicted as a result of positive marginal product of labor and higher amount of remittances received that facilitates investment in capital stock. Therefore, out-migration rather makes the remaining rural labor productive (depending on the size of working family members in a household and the size of land owned) and boosts the capital stock of households that in turn enhances farm income.

In general, rural-out migration plays an important role both in meeting the labor demands of industries and facilitating the process of rural transformation. Remittances sent to families of migrants residing of rural origin can contribute to rural development not only by facilitating investment but also by enhancing the living standard of

households. Out-migration can be taken as a strategy particularly for the poorest groups of rural households where they can supplement their farm income and then diversify risks.

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Effect of Seed and Nitrogen Rates on Some Phenological and Growth Parameters of Bread Wheat (*Triticum aestivum L.*) under Semi-arid condition in Northeast Ethiopia

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ABSTRACT

Wheat is well adapted to the mid and high land agro-ecologies of northeastern Ethiopia. The use of appropriate seed and nitrogen rates are among the most important agronomic practices that determine the phenological and growth parameters of crops. Field experiment was conducted during 2018/2019 crop season to identify the optimum seed and nitrogen rates that maximize phenological and growth parameters of bread wheat .The experiment was laid out in randomized complete block design (RCBD) with three replications. Treatments consisted of factorial combinations of three seed rates (100, 150 and 200 kg ha^{-1}) and four nitrogen rates (0, 53, 88 and 122kg N ha^{-1}).The result showed that days to heading, grain filling, 90% physiological maturity and harvest maturity were significantly affected by seed and nitrogen rates only but growth like plant height, spike length, numbers of tillers per m^{-2}were significantly ($p \leq 0.05$) affected by interaction effects of seed and nitrogen. Among the main effects and their interaction, 150 kg ha^{-1} seed rate and 122kg ha^{-1} nitrogen rate into the highest phenological and growth parameters. It is concluded that application of 122 kg N ha^{-1} with 150 kg ha^{-1} seed rate may be recommended to obtain optimum phenological and growth parameters.

Keywords: Seed and nitrogen rate, phenological and growth, wheat.

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1. INTRODUCTION

Wheat (*Triticum aestivum* L) is one of the most important cereal crops grown globally and serves as a stable food for about one third of the world population (Hussain and Sheh, 2002). It belongs to the grass family Poaceae and the tribe Hordea.Wheat is one of the most important cereals cultivated in Ethiopia and ranks fourth after tef (*Eragrostis tef*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) in area coverage, while fourth in total production after maize, sorghum and tef (CSA., 2012). Despite the large area under wheat, the national average yield of wheat in Ethiopia is about 2 t ha^{-1} (CSA., 2012).This is definitely below the world's average which is about 3 t ha^{-1} (Hawkesford *et al.,* 2013). In Ethiopia, wheat is cultivated in wide range of altitudes. Most wheat producing areas in Ethiopia lie between 6^{o} and 16^{o} N latitude and 35^{o} and 42^{o}E longitudes of an altitude range from 1500 to 3000 meters above sea level (masl). The most suitable agro-ecological zones, however, fall between 1900 to 2700 meters above sea level (Bekele *et al.,* 2000). Cultivation of local varieties, inadequate and erratic rainfall, poor agronomic practices, diseases and insect pest are among the principal limitations to wheat production in Ethiopia (Gorfu and Hiskias, 2000).Nitrogen (N) and seed rate are important management inputs, which influence phenological and growth parameters of wheat. Nitrogen plays a vital role in all living tissues of the plant (Haftom, 2009). Nitrogen is a constituent of proteins, enzymes, coenzymes, nucleic acids, phytochromes and chlorophyll. Besides, a good supply of nitrogen to the plant stimulates root growth and development as well as uptake of other nutrients (Brady and Weil, 2002).

Although, a number of researches have been conducted on the effect of Nitrogen (N) fertilizer application and seed rate on bread wheat production, such researches are scanty which determine the effect of these two factors on phenological and growth parameters of bread wheat (*Triticum aestivum L.*) in this study area. Hence, this experiment was initiated to identify the optimum seed and nitrogen rates that maximize phenological and growth parameters of bread wheat in Dessie Zuria District of the semi-arid region of northeast Ethiopia.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study area was conducted in Dessie Zuria District of the semi-arid region of northeast Ethiopia under rainfed condition during 2018/2019 crop season. It is one of the 106 districts in the Amhara National Regional State, Ethiopia. The altitude ranges from 1600 meter above seas level (m.a.s) to 3800 m.a.s.l. The experimental site is

located at $039^0 30' 22.7''$ E and $11^0 05' 30.8''$ N at an elevation of 2270 m.a.s.l. The study area receives mean annual rain fall of about 1170 mm. The mean annual minimum and maximum temperature are 8.7ºC and 23.8ºC, respectively.

2.2 Treatments and Experimental Design

The experiment consisted of treatments made from factorial combinations of three seed rates (S_1=100, S_2=150 and S_3=200) kg ha^{-1} and four nitrogen rates (N_0= 0, N_1= 53, N_2= 88 and N_3=122) kg N ha^{-1}.The experimental design was Randomized Complete Block Design (RCBD) with three replications. The gross plot size was 2m x 2m (4 m^2) containing 10 rows with 0.2 m inter row spacing. Spacing between blocks was 1m while spacing between plots was 0.5 m. The net plot size was 1.6 m x 1.8 m (2.88 m^2) excluding two outer rows and 0.1 m length from both ends of each plot.

2.3 Experimental Materials and Procedures

The experimental field was prepared using oxen driven local plow (*Maresha*) in accordance with the local farming practices. The land was leveled manually and treatments were assigned randomly. Seed were drilled in rows manually. Blended fertilizer (18.9%N, 37.7% P_2O_5, 6.95%S and 0.18% B) was used as sources of N, P, S and B, respectively. Nitrogen fertilizer was applied according to the treatments. The full dose of the NPSB fertilizer (100 kg ha^{-1}) and one-third of the nitrogen fertilizer were applied as basal at sowing. The remaining nitrogen fertilizer was applied as side-dressing at mid tillering stage of the crop (35 days after emergence).

2.4 Soil Sampling and Analysis

A total of five surface soil (0-30 cm) samples were collected diagonally from the experimental site and one composite soil sample was prepared. Soil sample was analyzed for texture, pH, EC, organic carbon, total nitrogen and available phosphorus (P). Soil texture was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962). The pH of the soil was measured in the supernatant suspension of a 1:2.5 soil to water ratio using a pH meter by potentiometer method (Rhoades, 1982). Organic carbon was determined by wet oxidation method as described by (Walkely and Black, 1934). Available P was determined by (Olsen *et al*., 1954) method. Total nitrogen was determined using Kjedahl method as described by (Bremner and Mulvaney, 1982).

2.5 Determinations

Phenological data

Days to 50% crop emergency: It was recorded when 50% of the plants in each plot emerges.

Days to heading (DH): It was determined as the number of days from sowing to the dates of 50% of the spike emerged from the flag leaf ligules.

Days to grain filling period: It was obtained by the number of days to maturity minus the number of days to heading.

Days to 90% physiological maturity (DPM): It was determined as the number of days from sowing to the date when 50% of the peduncle turned to yellow, that is, when no green color remained on glumes and peduncles of the tagged plants.
Days to harvest maturity: It was determined as the number of days from sowing to the date when 100% of the peduncle turned to yellow and whitish color.
Plant height (PH): It was recorded as the average height of main tillers (cm) of twelve randomly selected plants from each plot at maturity. It was measured from ground level to the tip of the spike excluding the awns.
Spike length (SL): It was measured from twelve randomly selected plants of the inner rows and the mean length (cm) was recorded on each plot by measuring from the base to the upper most part of the spike excluding awns at maturity.
Numbers of total tillers per m^2 (NT): It was determined as the capacity of tillering m^{-2} was demarcated before tillering and the number of plants existed in that area was counted at the period of statistical.

Analysis

All the collected data were subjected to Analysis of Variance (ANOVA) and the analysis was made using R-Software (V 3.5.1) appropriate to the design of the experiment. Means of all the treatments were compared using Tukey test.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Properties of the Soil

TABLE 1.Physico-chemical properties of soils at the experimental site in 2018

Soil parameters	
Textural Class	Sandy loam
Bulk density (g/cm^3)	1.36
pH(1:2.5 Water)	6.8
TN (%)	0.1143
OM (%)	2.286
OC (%)	1.326
Av. P($mg\ kg^{-1}$)	10.18
EC(ds/m)	2.45

The result showed that the texture of the experimental site was sandy loam with bulk density of 1.36 g/cm^3. The soil reaction was nearly neutral (pH=6.8). According to FAO (2008), suitable pH for most crops is between 6.5 and 7.5 where nitrogen (N) availability is optimum. The organic matter content was about 2.29%. According to Roy *et al.* (2006), soil with organic matter less than 3.74% is low potential to supply N to plants. The result also showed that low level of total N (0.11%) indicating that the nutrient may be a limiting factor for wheat production in the study area. Total Nitrogen

(%) was rated by Havlin *et al*. (1999) as very low when less than 0.1, low when the value is between (0.1 to 0.15), medium when the value is between (0.15 to 0.25), and high when is greater than 0.25. The available phosphorus content was 10.18mg kg^{-1}.

3.2 Effects of Nitrogen and Seed Rates on Wheat Phenology

3.2.1 Days to 50% emergence: The analysis of variance indicated that the main factor of seed rate and nitrogen level did not show significant ($p \leq 0.05$) difference on days to 50% crop emergence. Similarly, the interaction effect of seed rate and nitrogen level did not significantly influence days to 50% crop emergence.

3.2.2 Days to heading

Days to heading were significantly ($P \leq 0.05$) affected only by the main effects of seed and nitrogen rates. The earliest days to heading was recorded due to the application of the highest seed rate (200 kg ha^{-1}). With regards to the effect of nitrogen, days to heading was significantly delayed with the application of122kg ha^{-1} (Table 2).

TABLE 2. Mean days to heading and physiological maturity of bread wheat as influenced by seed rate and nitrogen rates

Treatments(kg ha^{-1})	DH	DPM	DGF
Seed rates			
100	67^{b}	127^{c}	72.46^{b}
150	64^{a}	126^{b}	$71.57a^{b}$
200	63^{a}	124^{a}	69.92^{a}
Nitrogen rates			
0	61^{a}	123^{a}	69.29^{a}
53	64^{b}	125^{b}	70.04^{a}
88	66^{c}	126^{c}	73.27^{b}
122	68^{d}	129^{d}	73.72^{b}
CV (%)	2.28	0.56	1.28

Note: Means within seed rate, nitrogen rate and their interactions followed by the same letter (s) are not significantly different at 5% probability level.

The result showed that as the seed rate increased, days to heading showed a decreasing trend. This might be due to the fact that increase in seed rate could encourage regular main stem which normally flower earlier than do the secondary tillers. This finding agrees with the report of Abiot (2017) that days to 50% heading were delayed when lower seed rate (100 kg ha^{-1}) were used. Days to heading were also significantly delayed when nitrogen rate was increased. This might be due to the fact that high nitrogen rate enhanced the vegetative growth and photosynthesis rate which prolonged the days to heading. Gheith *et al*. (2013) and Kandil *et al*. (2016) found that the increment in nitrogen concentrations increased the vegetative period of wheat and finally resulted in delayed heading.

3.2.3 Days to grain filling period

Days to grain filling period were significantly ($p \leq 0.05$) influenced only by the main effects of N and seed rate. The longest days to grain filling period were at higher N rates, 88 and 122 kg $ha^{-1}$and for seed rate 100kg ha^{-1}(Table 2). Despite small variability, factors showed a consistent increasing trend in days to grain filling with increasing nitrogen fertilizer and decrease seed rates. The results are in line with that of Sofonyas (2016) who stated that a steady increasing trend in grain filling period with rates of N in wheat was observed.

3.2.3 Days to 90% physiological maturity

The result of analysis of variance showed that days to physiological maturity were significantly ($P \leq 0.05$) affected only by the main effects of nitrogen and seed rates. According to the result, days to physiological maturity was significantly reduced with increasing seed rate. The shortest days to maturity was observed with the highest seed rate (200 kg ha^{-1}) (Table 2). This might be due to high plant population from the high seed rate increased competition for nutrients, soil moisture and light that influence the crop to mature early. The result of this study is in agreement with Alemayehu (2015) and Abiot (2017) who reported that increasing seed rate decreased days to physiological maturity of wheat. In contrast, increasing nitrogen rate delayed physiological maturity of wheat. High nitrogen rate could delay leaves senescence and encourage the crop growth. This condition increased the days required to attained physiological maturity of the crop. Application of low nitrogen rate in wheat shortens the growth phases by accelerating the physiological activities of the crop due to the shortage of plant growth resources (El Hag, 2011).

3.2.4 Days to harvest maturity

The statistical analysis of data has shown that days to harvest maturity were significantly ($P \leq 0.05$) affected by both the main and interaction effects of nitrogen and seed rates. Based on the result of interaction effects, the longest days to harvest maturity were recorded at seed rate of 100 kg ha^{-1} and 122 kg ha^{-1} nitrogen rate (Table 3).

TABLE 3. Mean days to harvest maturity of bread wheat as influenced by seed and nitrogen rates

	Nitrogen rate(N) (kg ha^{-1})				
Seed rate(S) (kg ha^{-1})	0	53	88	122	Mean(cm)
100	132.28^{bc}	139.40^{ef}	142.48^{fg}	144.14^{g}	139.54^{c}
150	129.00^{ab}	133.10^{cd}	139.24^{ef}	142.01^{fg}	135.79^{b}
200	126.61^{a}	128.70^{ab}	136.80^{de}	140.02^{efg}	132.98^{a}
Mean(cm)	129.29^{a}	133.70^{b}	139.50^{c}	142.06^{d}	

Note: CV (%) =1.28. Means within seed rate, nitrogen rate and their interactions followed by the same letter (s) are not significantly different at 5% probability level

According to the results, increasing nitrogen rate delayed harvest maturity of wheat. High nitrogen rate could delay leaves senescence and encourage the crop growth. Application of low nitrogen rate in wheat shortens the growth phases by accelerating the physiological activities of the crop due to the shortage of plant growth resources (El Hag, 2011). Decrease seed rates and increasing in nitrogen rates delayed day to harvest maturity of wheat. This might be due to high level of N application with low seed rates promotes leaf area index while prolongs leaf area duration i.e. delay leaf senescence and the potential photosynthetic capacity of crop is raised.

3.2.5 Effects of Seed and Nitrogen Rates on Bread Wheat Growth Parameters

Plant height

The analysis of variance revealed that both the main and the interaction effects of seed and nitrogen rates significantly ($P \leq 0.05$) affected plant height. Based on the result interaction effects, the highest plant height (99.85cm) was recorded at seed rate of 150 kg ha^{-1} and 122 kg ha^{-1} nitrogen rate whereas the lowest plant height (46.04 cm) was recorded at zero rate of nitrogen with 100 kgha^{-1} of seed rate (Table 4).

TABLE 4. Mean plant height of bread wheat as influenced by seed and nitrogen rates

Seed rate(S) (kg ha^{-1})	Nitrogen rate(N) (kg ha^{-1})				
	0	53	88	122	Mean(cm)
100	46.04^{a}	59.15^{ab}	80.43^{de}	94.74^{ef}	70.09^{a}
150	51.10^{ab}	77.26^{cd}	88.74^{def}	99.85^{f}	79.17^{b}
200	62.82^{bc}	81.42^{de}	89.58^{def}	92.68^{def}	81.63^{b}
Mean(cm)	53.32^{a}	72.61^{b}	86.25^{c}	95.66^{d}	

Note: CV (%) =7.21. Means within seed rate, nitrogen rate and their interactions followed by the same letter (s) are not significantly different at 5% probability level

Increasing seed rates and decrease in nitrogen levels increase plant height. This might be due to strong competition among the plants for light, nutrient and mutual shading. Saad (2010) also stated that the seed rate at 150 kg ha^{-1} showed significantly increased plant height up to 140 kg ha^{-1} nitrogen rate.

Spike length

The result showed that both the main and the interaction effects of seed and nitrogen rates significantly ($P \leq 0.05$) affected spike length of wheat. The highest spike length (8.41 cm) was recorded from the application of 150 kg ha^{-1}seed and 122 kg ha^{-1} nitrogen application (Table 5).

TABLE 5. Mean spike length of bread wheat as influenced by seed and nitrogen rates

Seed rate(S) (kg ha^{-1})	Nitrogen rate(N) (kg ha^{-1})				
	0	53	88	122	Mean(cm)
100	5.41^{abc}	6.36^{bc}	7.63^{de}	8.28^{e}	6.92^{b}
150	4.56^{a}	6.57^{cd}	7.54^{de}	8.41^{e}	6.77^{b}
200	4.42^{a}	5.23^{ab}	6.11^{bc}	7.82^{e}	5.90^{a}
Mean(cm)	4.80^{a}	6.10^{b}	7.10^{c}	8.17^{d}	

Note: CV (%) = 6.06%. Means within seed rate, nitrogen rate and their interactions followed by the same letter (s) are not significantly different at 5% probability level.

According to the result, an increase in seed rate decreased spike length of wheat. The high spike length due to low seed rate could be due to less competition among the plants for growth resources. The result agrees with Gafaar (2007) who reported that increasing seeding rates decreased the spike length in wheat. Regarding the effect of nitrogen, spike length increased with increasing nitrogen rate. Availability of high nitrogen could encourage the crop growth that leads to greater spike length .This finding agreed with Muhammad Yousaf *et al.* (2015) who observed similar effect on wheat. Based on this result, the increased in spike length of wheat at higher nitrogen rate and at lower seed rate might be associated to better utilization of growth resources.

Number of total tillers per m^2

Both the main and the interaction effects of seed and nitrogen rates were significantly ($P \leq 0.05$) affect the number of tillers per m^{2}of wheat. The highest number of tillers (801.33 m^{-2}) was recorded at seed rate of 150 kg ha^{-1} with 122 kg ha^{-1} nitrogen application (Table 6).

TABLE 6. Mean number of total tillers m^{-2}of bread wheat as influenced by seed and nitrogen rates

Seed rate(S) (kg ha^{-1})	Nitrogen rate(N) (kg ha^{-1})				
	0	53	88	122	Mean(m^{-2})
100	272.00^{a}	517.33^{bc}	563.67^{bc}	593.33^{c}	486.58^{a}
150	268.00^{a}	511.00^{bc}	610.67^{cd}	801.33^{e}	547.75^{b}
200	435.33^{b}	559.00^{bc}	741.33^{de}	753.33^{e}	622.25^{c}
Mean(m^{-2})	325.11^{a}	529.11^{b}	638.56^{c}	716.00^{d}	

Note: CV (%) =8.31. Means within seed rate, nitrogen rate and their interactions followed by the same letter (s) are not significantly different at 5% probability level.

Similar results were reported by Khalid *et al*. (2014) who that wheat was quite responsive to increased seed rate. The results of this study also showed that all nitrogen application rates significantly affected vegetative and reproductive growth of the crop depending upon the availability of needed nutrition (Iqbal *et al.*,2010). Based on interaction the result showed that combined application of 150 kg ha^{-1} seed rate with 103.5 kg N ha^{-1} produced more number of tillers per m^{-2}of wheat. At optimum seed

rate, the inter plant competition for space, nutrient, moisture and sun light decrease which results in increases of tillers m^{-2}. Similar results were reported by Shah and Hayat (2016).

4. CONCLUSION

Wheat is one of the major crops selected for food security in Ethiopia. The optimum rate of nitrogen fertilizer and plant density depends on numerous factors such as environmental conditions, management practices and genotypes. This was the primary drivers to conduct this study and identify optimum seeding rate and nitrogen fertilizer to maximum phenology and growth parameters of bread wheat in this study area. Results showed that treatment with seed rate of (150 kg ha^{-1}) has good impact most phonological and growth parameters. Effect of nitrogen rates have also showed highly significant ($P \leq 0.05$) effects on DH, GF, DPM, HM, PH, SL and NT. The highest values of DH, GF, DPM, HM, PH, SL and NT were obtained from the application of 122 kg N ha^{-1}. Most phenology and growth parameters of bread wheat exhibited increasing trend with increasing nitrogen rates. The interaction effects of nitrogen and seed rates significantly ($P \leq 0.05$) affected PH, SL and NT. The highest DH, GF, DPM, HM, PH, SL and NT were obtained from the application of 122 kg N ha^{-1} and seed rate of 150 kg ha^{-1}. From these results, it can be recommended that the, application of the highest rate of nitrogen (122 kg N ha^{-1}) and seed rate of 150 kg $ha^{-1}$are more promising obtain values of phonological and growth parameters.

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STUDY ON CHARACTERISATION FOR THE REMOVAL OF CRYSTAL VIOLET DYE FROM INDUSTRIAL WASTEWATER USING SPATHODEA CAMPANULATA LEAVES--SEM, FTIR, XRD ANALYSES.

Ravi Kumar M[1*], King P[2], Zinabu Wolde[3], Melkamu Mulu[4], Anwar Fole[5]

ABSTRACT

The objective of the present study is to remove crystal violet (CV) dye from their industrial waste water using *Spathodea campanulata* leaves powder as low cost biosorbent in a batch study. The effects of parameters were contact time, solution pH, initial CV dye concentration, biosorbent dosage, average particle size of the biosorbent and temperature. Scanning electron microscope (SEM) analysis is used to investigate the surface morphology of the biosorbent. The morphological characterization of the *Spathodea Campanulata* leaves biosorbent before and after biosorption of crystal violet was studied. The Fourier transform infrared spectroscopy (FTIR) provides structural and compositional information on the functional groups present in the biosorbent. The functional groups present in the *Spathodea Campanulata* leaves biosorbent before and after biosorption of crystal violet was investigated within the range of 400 – 4000cm^{-1} wave number. The analysis of Fourier transform infrared spectrum shows the presence of large number of functional groups present on the surface of the *Spathodea Campanulata* leaves biosorbent. XRD patterns for crystal violet treated powder exhibit more amorphous nature and increase in surface area and porosity. The maximum removal efficiency was predicted to be 89% for crystal violet, which was obtained at a temperature of 303.28 K, solution pH of 6.06, initial dye concentration of 20.09 mg/L and biosorbent dosage of 1.50g, on to *spathodea campanulata* leaves.

Keywords: Crystal violet, *Spathodea Campanulata*, SEM, FTIR, XRD.

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1. INTRODUCTION

Water is an irreplaceable natural resource on this earth on which all life depends. As a valuable natural resource, it comprises marine, estuarine, fresh water (river and lake) and ground water environments, across costal and inlands areas. Even though there is a huge water resource in this world, about 97% of water is salt water (marine) and only 3% is fresh water, and in this small fraction of fresh water most of it is locked up in polar ice caps and just 0.003% is readily available to us in the form of ground water and surface water. Fresh water is a limited resource in many parts of the world. And this will become more dearths due to increasing in population, urbanization and climate change. The major fact for this paucity of fresh water is not only due to demand for water but also due to pollution in fresh water ecosystem. Dyes are also used in industries such as rubber, paper and pulp dye and dye intermediate industries, pharmaceutical, tannery, food technology, hair colouring, plastic, cosmetic, etc. There are more than 10,000 commercially available dyes with over 7 lakh times of dyestuff being produced annually across the world **[1]**. The textile industry consumes more than 107 kg of die per year of which 90% ending upon fabrics **[2]**. Of this total usage 10 to 15% of the die is lost during the dying process and released with effluent. Colour is contributed by phenolic compounds such as tannins, lignans (two to three percent) and organic colorants (three to four percent) and with a maximum contributions from dye and dye intermediates which could be sulphur or mordant / reactive / cationic / disperse / acid / azo vat die **[3]**. Crystal violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic. The medical use of the dye has been largely superseded by more modern drugs, although it is still listed by the World Health Organization. Crystal violet when dissolved in water has a blue-violet colour with an absorbance maximum at 590 nm. The functional groups on the surface of the biosorbents that contribute to the biosorption are characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD) analyses.

2. MATERIALS AND METHODS

2.1. Preparation of dye solution

Stock of crystal violet concentration 1000 mg/L was prepared by dissolving 1 g of 100% crystal violet in 1000 ml of distilled water. The solution was prepared using standard flasks. The range of concentration of the prepared dye solutions varied between 20 and 200 mg/L was prepared using the stock solution of individual dye.

2.2. Preparation of bio sorbent

The green colored *Spathodea campanulata* leaves used in the present study were collected from the college of Engineering, Andhra University, Visakhapatnam, India.

The collected leaves were washed with deionised water several times to remove dirt particles. The washing process was continued till the wash water contains no dirt. The washed leaves were then completely dried in sunlight for 20 days. The dried leaves were then cut into small pieces and powdered using domestic mixie. In the present study the powdered materials in the range of 53–152 μm particle size were directly used as biosorbents without any pretreatment.

3. RESULTS AND DISCUSSION

3.1. Effect of contact time

The percentage of biosorption was determined at different contact times and the results are shown in Fig1. The figure reveals that the percentage of biosorption of crystal violet was steeply increased with an increase in contact time from 5 to 30 min and there after reached plateau after attaining equilibrium 30 min for crystal violet. Therefore, contact time of 30 min is sufficient for the removal of crystal violet under the experimental conditions used in this study. The percentage of biosorption of *Spathodea campanulata* leaves as biosorbent for crystal violet dye removal was increased from 12 to 66%, with an increase in contact time from 5 to 30 min for an initial dye concentration of 20 mg/L. For 200 mg/L, the percentage of biosorption increased from 7.5 to 59 %, respectively with an increase in contact time from 5 to 30 min**.**

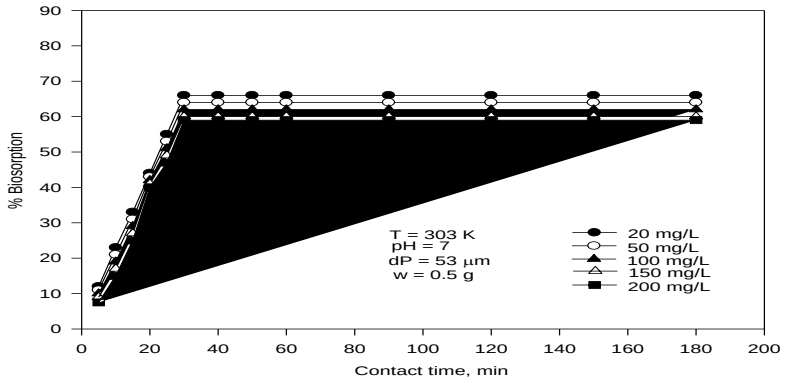


Figure.1. Effect of contact time on % biosorption of Crystal violet using *Spathodea Campanulata* leaves as biosorbent.

3.2. Effect of solution pH

The solution pH is one of the important controlling parameters of the biosorption process. The effect of solution pH on % biosorption for the removal of crystal violet dye was shown in the Fig.2. It was observed that the increase in solution pH from 2 to 6 for Crystal violet resulted in an increase in percentage of biosorption from 39 to 75% for Crystal violet for an initial concentration of 20 mg/L. For an initial concentration of 200 mg/L, the percentage of biosorption was increased from 33 to 68 % for Crystal violet with an increase in solution pH from 2 to 6**.**

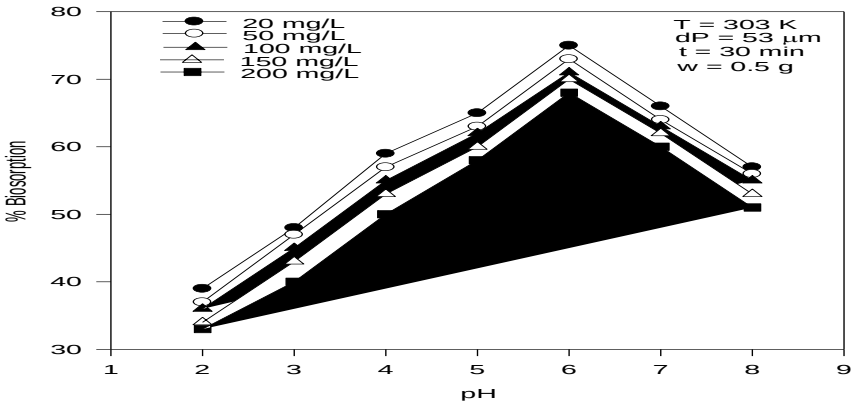


Figure.2. Effect of solution pH on % biosorption of Crystal violet using *Spathodea Campanulata* leaves as biosorbent

3.3. Effect of initial concentration of dye

The effect of initial dye concentration on the percentage of biosorption is shown in Fig.3. It is evident from the figure that the percentage of biosorption decreased with an increase in initial concentration of dye from 20 to 200 mg/L at all temperatures. The percentage of biosorption of Crystal violet decreased from 71 to 42 % and 76 to 47 for Crystal violet with an increase in initial concentration of Crystal violet from 20 to 200 mg/L at the temperature of 283 K and 323 K, respectively**.**

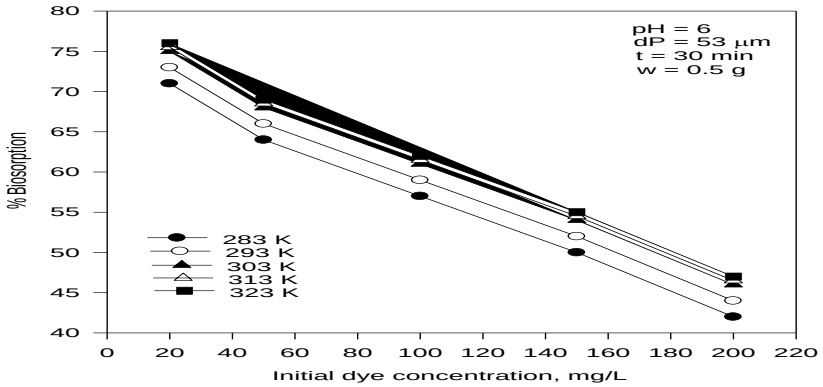


Figure.3. Effect of initial concentration of dye on % biosorption of Crystal violet using *Spathodea Campanulata* leaves as biosorbent.

3.4. Effect of biosorbent dosage

The result obtained is shown in Fig.4, illustrate that the percentage of biosorption was increased with an increase in biosorbent dosage. The percentage of biosorption

increased from 75 to 86 % from biosorbent dosage 0.5 to 4 g for an initial concentration of crystal violet 20 mg/L. For an initial concentration of crystal violet 200 mg/L, the percentage of biosorption increased from 67 to 79% from biosorbent dosage 0.5 to 4g**.**

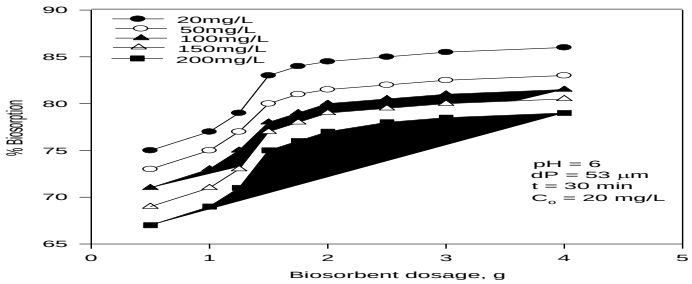


Figure.4. Effect of biosorbent dosage on % biosorption of Crystal violet using *Spathodea Campanulata* leaves as biosorbent.

3.5. Effect of particle size of biosorbent

The result obtained is shown in Fig.5. The result indicated that the percentage of biosorption was decreased with an increase in average particle size of biosorbent. The percentage of biosorption decreased from 83 to 60 % and 75 to 52% with an increase in average particle size of biosorbent from 53 to 152 μm for an initial concentration of crystal violet 20 mg /L and 200 mg/L, respectively**.**

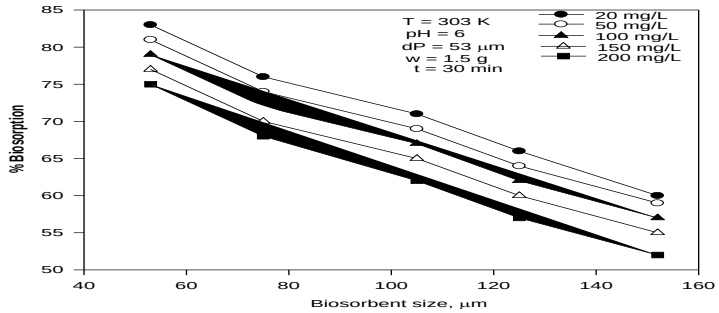


Figure.5. Effect of average particle size on % biosorption of Crystal violet onto *Spathodea Campanulata* leaves biosorbent.

3.6. Effect of Temperature

The result obtained is shown in Fig.6: The figure indicated that the percentage of biosorption was increased with an increase in temperature of the solution. This suggests the endothermic nature of the biosorption process. The percentage of biosorption increased from 80 to 85% and 72 to 77% with an increase in solution temperature from 283 K to 323 K for an initial concentration of Crystal violet 20 mg /L and 200 mg/L, respectively**.**

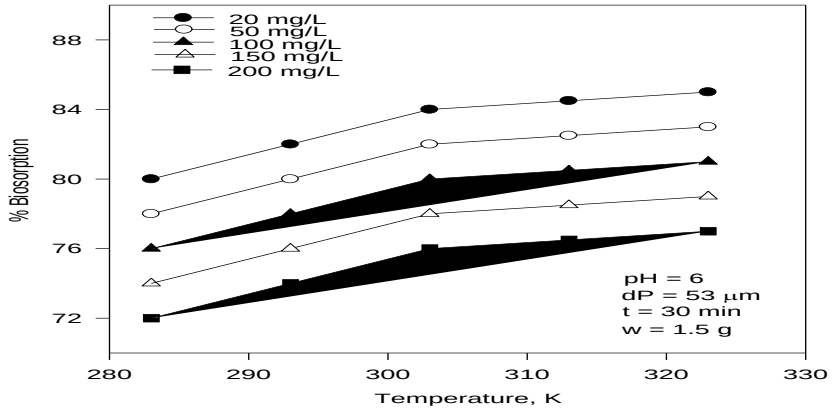


Figure.6. Effect of temperature on % biosorption of Crystal violet onto *Spathodea Campanulata* leaves biosorbent

3.7. Characterization of Spathodea Campanulata leaves biosorbent using SEM, FTIR and XRD analyses

3.7.1. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) analysis is used to investigate the surface morphology of a biosorbent. The morphological characterization of the *Spathodea Campanulata* leaves biosorbent before and after biosorption of crystal violet was studied by SEM and micrographs are shown in Figs. 7 - 8. Raw biosorbent i.e before biosorption of dye shown in Fig.7. represents a highly porous and heterogeneous morphology, which indicates that the *Spathodea Campanulata* leaves biosorbent has good biosorption capacity of dye molecules on the surface of the biosorbent. It is evident from this figure that the surface of the biosorbent is rough. SEM micrographs of the biosorbent after biosorption of crystal violet dye is shown in Fig.8. These figures showed a significant difference with that of micrograph of raw biosorbent. It is evident from these figures that the surface texture of Spathodea Campanulata leaves biosorbent is completely changed before and after biosorption of dyes. It is clear from these figures that the pores of the biosorbent were completely filled with dye molecules. Therefore, the surface of the *Spathodea Campanulata* leaves biosorbent after biosorption of crystal violet is appeared to be smooth**.**

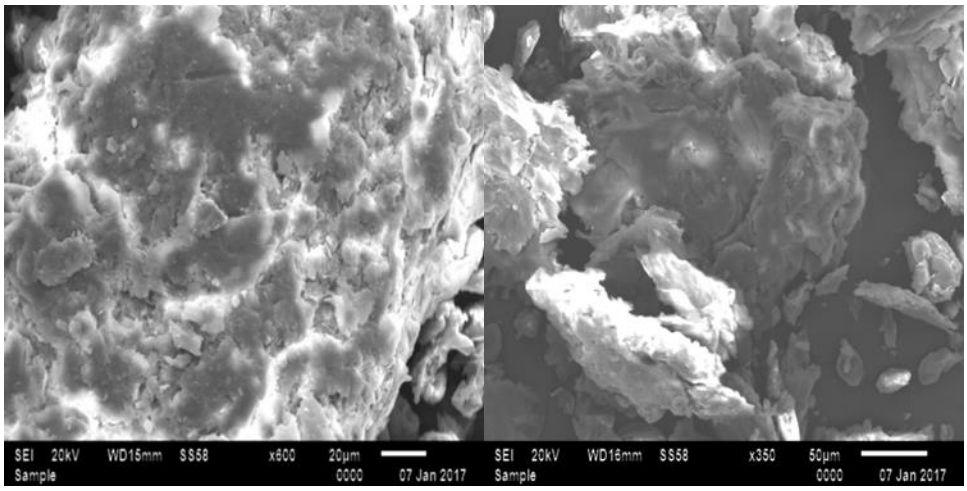


Figure.7. SEM micrographs of *Spathodea Campanulata* leaves biosorbent before biosorption.

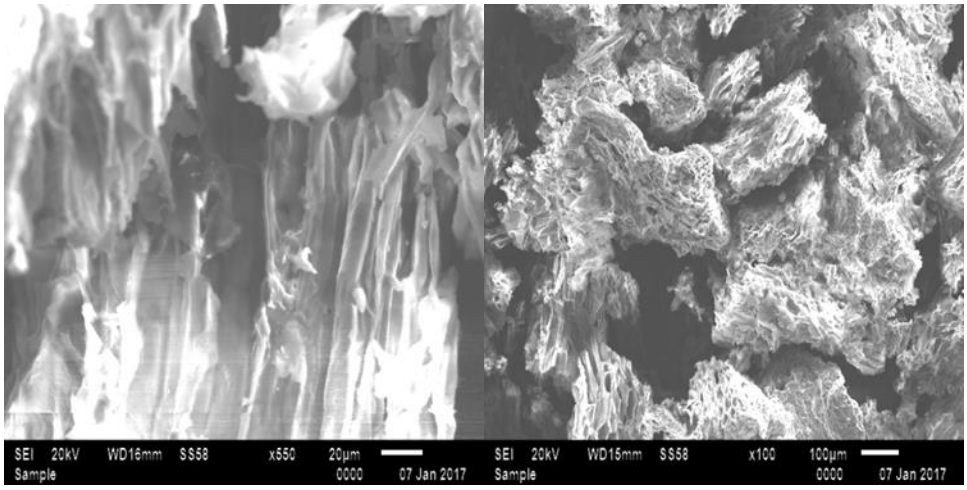


Figure.8. SEM micrographs of *Spathodea Campanulata* leaves biosorbent after biosorption.

3.7.2. Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopy (FTIR) provides structural and compositional information on the functional groups presented in the biosorbent. The functional groups present in the *Spathodea Campanulata* leaves biosorbent before and after biosorption of crystal violet was investigated by FTIR spectra within the range of 400 – 4000cm^{-1} wave number and shown in Fig. 9 and 10. The adsorption spectra of raw biosorbent (Fig.9.) shows the broad band at 664 cm^{-1} is due to C-Br stretch bands from alkyl halides. The bands at 818.82cm^{-1} denote the presence of C–Cl stretching band. The band at 904.65 cm^{-1} suggests the presence of C–H aromatics. A number of

absorption peaks, indicating the complex nature of the biosorbent and it was composed of various functional groups which are responsible for the binding of crystal violet dye. Fig.10. shows some of the absorption peaks that are shifted or disappeared and new peaks are formed. This could be due to the biosorption of crystal violet dye on surface of the biosorbent. The broad band at 560.35 cm^{-1}represents C–Br stretch bands from alkyl halides. The band at 818.82 cm^{-1} is due to the presence of C–Cl stretching band. The band at 904.65 cm^{-1} indicates the presence of C–H Aromatics mode. The band positions in the FTIR spectra of the *Spathodea Campanulata* leaves biosorbent before and after biosorption of crystal violet is presented in Table.1**.** It shows that biosorption bands shifted to higher values. These shifts may be attributed to the changes in counter ions associated with carboxylate and hydroxylate anions. This indicates that the main contributors in dye uptake may be acidic groups, carboxyl and hydroxyl group.

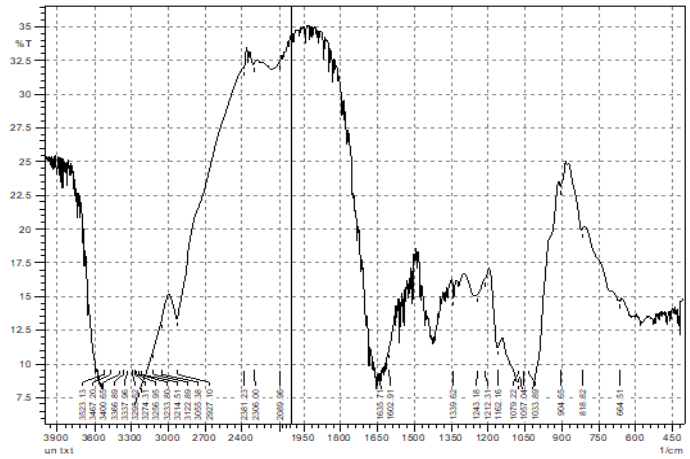


Figure.9. Untreated Crystal violet.

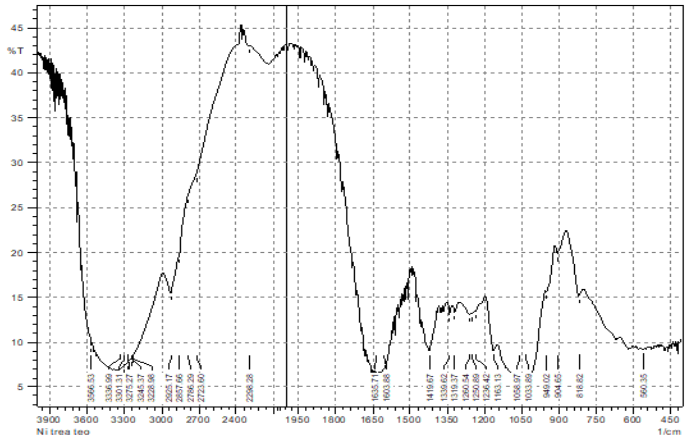


Figure.10. Treated Crystal violet.

TABLE.1. FTIR spectra of *Spathodea Campanulata* leaves biosorbent for Crystal violet.

Sl No	Peaks in untreated powder, cm^{-1}	Peaks in biosorbent Treated Crystal violet powder	Bond and Functional group Description
1	-----	560.35	C–Br stretch bands from alkyl halides
2	664	-----	C–Br stretch bands from alkyl halides
3	818.82	818.82	C–Cl stretching band
4	904.65	904.65	C–H Aromatics
5	-----	949.02	The bands observed at 949 (in IR) and at 928 cm-1(in Raman) respectively are assigned CN to be due to stretch
6	1033.89	1033.89	Si-O stretching band
7	1057.04	-----	C=S stretching modes
8	-----	1058.97	C=S stretching modes
9	1079.89	-----	C=S stretching modes
10	1162.16	-----	Aromatic C–H bending mode
11	-----	1163.12	Aromatic C–H bending mode
12	1212.31	-----	=C–H bend alkenes mode
13	-----	1236.42	
14	1243.18	-----	
15	-----	1250.89	
16	-----	1260.54	
17	-----	1319.37	
18	1339.62	1339.62	
19	-----	1419.67	C=C stretch mode
20	1602.91	-----	Alkyl C–H stretch mode
21	-----	1603.88	Alkyl C–H stretch mode
22	1635.71	1635.71	OH bending mode of vibration
23	2089.96	-----	C≡C bands of the alkynyl ligand
24	-----	2298.28	Amine N–H stretch mode
25	2306.00	-----	
26	2381.23	-----	
27	-----	2723.60	
28	-----	2786.29	
29	-----	2857.66	Asymmetric –CH2–, symmetric –CH3 and –CH2– stretching vibrations
30	-----	2925.17	Asymmetric –CH2–, symmetric –CH3 and –CH2– stretching vibrations
31	2927.10	-----	Amine N–H stretch mode
32	3055.38	-----	aromatic C–H stretching mode
33	3122.89	-----	
34	3214.51	-----	
35	-----	3228.98	

36	3233.80	-----	Amine N–H stretch mode
37	-----	3245.37	
38	3256.95	-----	
39	3274.31	-----	
40	-----	3275.17	
41	3295.52	-----	–CH stretch mode
42	-----	3301.31	
43	-----	3336.99	
44	3337.96	-----	
45	3400.65	-----	
46	3467.20	-----	OH stretch mode
47	3523.13	-----	
48	-----	3566.53	

3.7.3. X-RAY DIFFRACTION

The X-ray diffraction (XRD) is used to investigate the existence of crystallinity in biosorbent material. The X-ray diffraction diffractograms of Spathodea Campanulata leaves biosorbent before and after biosorption of Crystal violet dye is shown as plots of Fig 11, and 12. The diffracted X-ray intensities are recorded as a function of 2θ by using copper target (Cu-Kα radiation with wave length λ= 1.5492 A^0) at a scan speed of 2^0/min. XRD patterns are recorded from 3 to 90^0.

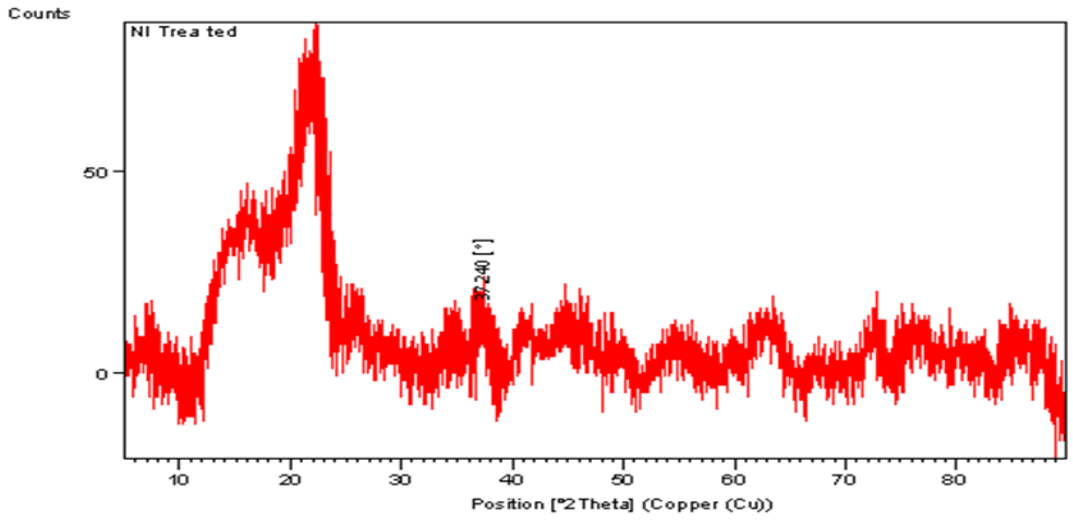


Figure.11. XRD pattern of Crystal violet treated *Spathodea Campanulata.*

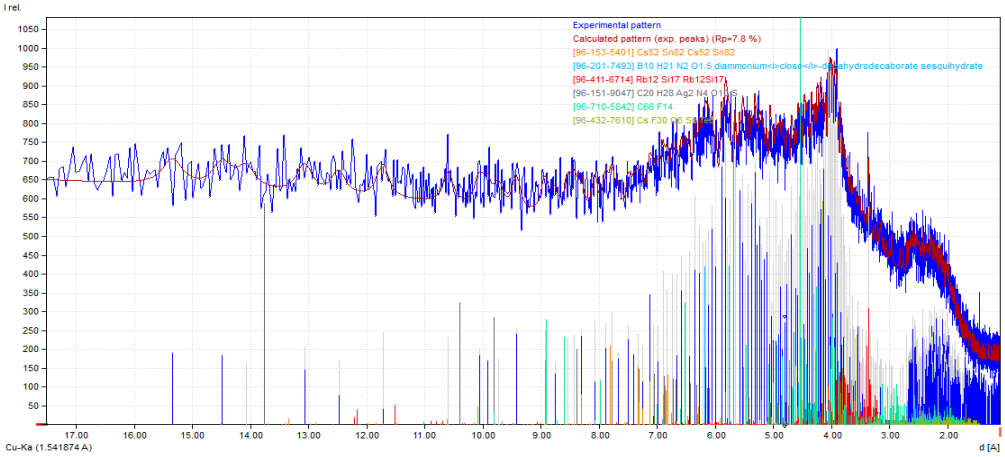


Figure.12. XRD pattern of Crystal violet treated *Spathodea Campanulata* with matching compounds.

XRD patterns for Crystal violet treated powder [Figs.11 & 12] exhibit more amorphous nature and increase in surface area and porosity. The peaks at 2θ values of 0.7400, 0.7104, 0.5667, 0.6598 and 0.6134 reflect the presence of $Rb_{12}Si_{17}$, $C_{66}F_{14}$, $Cs_{52}Sn_{82}$, $B_{10}H_{21}N_2O_{1.5}$ and $C_{20}H_{28}Ag_2N_4O_{10}S$, their corresponding d-values are 4.8072, 4.9561, 4.6587, 4.5135 and 4.2819.

4. CONCLUSION

The contact time of 30 min was sufficient for the maximum removal of crystal violet dye onto Spathodea Campanulata biosorbent. The maximum removal efficiency was predicted to be 89% for crystal violet, which was obtained at a temperature of 303.28 K, solution pH of 6.06, initial dye concentration of 20.09 mg/L and biosorbent dosage of 1.50 g. Characterization of Spathodea Campanulata biosorbent was analysed with SEM, FTIR and XRD with effectively.

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Focus Group Discussion as a data collection tool in Economics

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ABSTRACT

This paper reviews the literature on the focus group discussion as a data collection tool and provides information for a better understanding of focus group discussion by addressing the questions of what is the meaning of focus group discussion? What are logistics needed? Its advantages and limitations, how leader behaviors necessary to keep focus group discussion going? And its application in economic data collection. The secondary data were drawn from a pool of research papers and sourced from journal articles, related books, published training manual and websites that specify key elements of the focus group discussion and find that focus group discussion has different definitions, types and it is highly valued as a qualitative research data collection tool, especially for its ability to generate in-depth understandings to a topic in an effective and timely manner. It can be a cost-effective, quick approach to data collection and relatively easier to conduct. But it has some limitations and logistical difficulties involved such as groupthink and accuracy, harder to get everyone in the same place at the same time. Above these challenges, focus group discussion as a data collection tool is seen as effective and even less resource-intensive compared to other methods. Yet it requires proper planning and organization.

Keywords: Data collection, Economics , Focus Group, Qualitative research

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1. INTRODUCTION

There are two types of data; these are primary and secondary data. The primary data are those which are collected afresh and for the first time, and thus happen to be original. The secondary data, on the other hand, are those which have already been collected by someone else and which have already been passed through the statistical process. The researcher would have to decide which sort of data would be used in the study and accordingly select one or the other method of data collection. The methods of collecting primary and secondary data differ since primary data are to be originally collected, while in the case of secondary data the nature of data collection work is merely that of compilation. Data collection is a very demanding job that needs thorough planning, hard work, patience, determination, and more to be able to complete the task successfully. It starts with determining what kind of data is required followed by the selection of a sample from a certain population. After that, the researcher needs to use a certain instrument to collect the data from the selected sample.

One of the instruments that collect primary data is focus group discussion. A focus group discusses issues and questions of importance on a topic of mutual concern and its purpose of the discussion is to understand concepts and issues, to increase participation in the data collection session, and to draw on the experience and expertise of participants, discussion involves two-way communication between participants. It is how focus group discussion participants share experiences, ideas, and attitudes. Focus group discussions are a data collection method that data is collected through a semi-structured group interview process. Focus groups are generally used to collect data on a specific topic. Focus group methods emerged in the 1940s with the work of Merton and Fiske who used focus groups to conduct consumer satisfaction (Kabir, 2018).

The objective of this paper is to *reviews* the literature on focus group discussion as a data collection tool and provides information for a better understanding of focus group discussion by addressing the questions; what is focus group discussion? What are the Logistics of focus group discussion? What are the advantages and limitations of Focus Group Discussion? How leader behaviors necessary to keep FGD going? Is focus group discussion utilized in economic data collection?

Based on the theoretical review and integrative research review, the paper data and information have synthesized in the accumulated state of knowledge on similar and related data to the focus group discussion. The data for this paper were drawn from a pool of research papers. Secondary data were sourced from journal articles, related books, published training, and websites that specify key elements of the focus group discussion and explain its definition, logistics needed, advantage and its limitations, leader's behaviors, and utilization of focus group discussion as data collection tool in economics. It is expected that this paper will provide a crystal clear answer to the research questions. I focus on an understanding of the theoretical and empirical prospects of focus group discussion to propose policy recommendations.

2. DEFINITION AND TYPES OF FOCUS GROUP DISCUSSION

2.1 Definition and types of Focus Group Discussion

Focus group discussion is a technique where a researcher assembles a group of individuals to discuss a specific topic, aiming to draw from the complex personal experiences, beliefs, perceptions, and attitudes of the participants through a moderated interaction (Cornwall & Jewkes, 1995)

A focus group discussion is a loosely structured discussion among six to ten individuals that are used to gather information on a particular research or program topic (Debus, 1998).

From the above concepts, we can understand that, a focus group discussion is a qualitative research data collection technique in which a selected group of people discusses a given topic or issue in-depth, facilitated by a professional, external moderator.

Focus group analysis is the process of organizing and examining information to find meaning in it. According to Krueger (as cited in De Negri & Thomas 2003), analysis is like detective work. After conducting the focus group discussions, you must read through lots of notes, organize them, and then look for "clues" that will help you to make sense of it all. This takes a lot of time and needs that you know the information very well.

2.2 Types of Focus Group Discussion

According to Ochieng et al., (2018), there are a total of seven types of focus group discussion, of which five are main types identified in the literature, and a further two are emerging with the growth in access and variety of online platforms. First, a single focus group; is the interactive discussion of a topic by a collection of participants and a team of facilitators as one group in one place. It is the most common and classical type of focus group discussion (Morgan, 1996). It has been commonly used by both researchers and practitioners across different disciplines (Wilkinson, 1998). Second, a two-way focus group involves using two groups where one group actively discusses a topic, whereas the other observes the first group and usually, this type of focus group is conducted behind one-way glass. The observing group and the moderator can observe and note the interactions and discussion of the first group without being seen and hearing what the other groupthink or by observing their interaction often leads the second group to different conclusions than those it may have reached otherwise (Morgan, 1996).

Third, a dual moderator focus group, contains two moderators working together, each performing a different role within the same focus group and the division of roles ensures a smooth development of the session and ensures that all topics are covered. Fourth, the dueling moderator focus group involves two moderators who purposefully take opposing sides on an issue or topic under investigation (Krueger & Casey, 2000). The proponents consider that the introduction of contrary views to the discussion by the moderators is critical to achieving more in-depth disclosure of data and information (Kamberelis & Dimitriadis, 2005). Five, respondent moderator focus group, researcher hires some of the participants to take up a temporary role of moderators. Having one

of the participants lead the discussion is thought to impact the dynamics of the group by influencing participants' answers, it increasing the chances of varied and more honest responses. The other two are, mini focus group, researchers are usually faced with a situation where there is a small potential pool of participants and difficult to reach, yet the research design needs that the topic must be discussed in a group. Under these situations, researchers can only convene a small group of between two and five participants but such groups are usually made up of individuals with a high level of expertise (Kamberelis & Dimitriadis, 2005).

And the last one is online focus groups; it is not a different type of focus group discussion, it is applied within the online environment, using conference calling, chat rooms, or other online means. Online focus groups boast an impression of dynamism, modernity, and competitiveness that transcends classic problems with face to face focus group discussion (Kamberelis & Dimitriadis, 2005). However, the discussion platforms are only accessible to participants with access to the Internet and prone to technical problems such as poor or loss of connectivity and failure to capture nonverbal data (Dubrovsky, Kiesler, & Sethna, 1991).

The main means of data collection during a focus group discussion include audio and tape recording, note-taking, and participant observation. Besides, the types of questions in the focus group are to be in sequence of deductive questioning that is from general to specific, as recommended, which has: first, questions of engagement: it begins the session with questions that put the participants at ease and create a conducive environment accessible to participation. Second, questions of exploration: it is penetrating, well-constructed group members 'questions that get to the main target of the discussion. And lastly, questions of exit: it presents, if there is anything more or further suggestions regarding the topic, and check if there is missed anything (Stewart, Shamdasani, & Rook, 2007).

According to USAID (2011), there are a few common steps that are mostly taken when designing and implementing focus group discussions. These are: - first, select the team to facilitate the discussions by considered good practice to have at least two people conducting focus group discussions. One person facilitates the discussion whilst the other takes notes. Second, identify the groups and participants that will take part that is participants should be from similar backgrounds and should be able to discuss matters freely with each other. This is why focus group discussions are often carried out with separate groups. Third, decide on the timing and location of the meetings, which is generally last up to two hours. Wherever possible, they should be located in a convenient space where people can discuss matters in comfort and with some degree of privacy.

Fourth, develop a set of questions or a discussion guide; this can be as simple as a checklist containing a few broad, guiding questions. 2-3 questions are normally considered sufficient for a focus group discussion, as it is important to allow time for the questions to be discussed at length. The fifth step, conduct and record the focus group discussions, each discussion should start with a brief introduction, explaining the purpose of the session, and ensuring that participants know how the information will

be used, and also discussions should be recorded as they happen. Sixth, analyses the data; it can be done in two stages after each focus group discussions took place, the facilitation team should review the records of the discussions to identify any key themes emerging, and then once all focus group discussions have been completed, the data can be examined to look for common trends and patterns.

3. LOGISTICS OF FOCUS GROUP DISCUSSION

3.1 Time

Regardless of the number of focus group discussion meetings, it is important to consider the duration of the meetings. Participants are likely to suffer from fatigue when discussions are longer. The rule of thumb is 1–2 hr., based on the complexity of the topic under investigation, the number of questions, and the number of participants. This might differ when the group consists of younger participants such as school children, this is because children tend to have shorter attention spans and will begin to lose focus and interest in the topic quicker than adults (Gibson, 2012).

3.2 Number of Panelists

Although there is no iron-clad rule about how many focus groups are enough, the number of focus group discussions to hold depends on the type and complexity of the research topic. Focus group discussions should generate sufficient data to allow for comparisons and to generate and test theoretical ideas (Barbour 2005). A focus group typically consists of around 6 to 12 target participants engaged in a discussion with a research moderator. Whereas Prince and Davies‘(2001) explain that small-sized groups of 4 to 6 may be productive as they encourage members to actively participate in the discussion and subsequently, a considerable amount of different ideas may be generated on the topic in discussion within a certain time limit. But According to Krueger (1994) argues that focus group research should comprise a minimum of three groups.

3.3 Equipment that used for Focus Group Discussion

Equipment used in focus group discussions is in one to one focus group discussion like Tape recorder, lapel, microphone, quiet room are used whereas in other focus groups discussion like Tape recorder, oval/round table/table microphone, props such as flashcards, leaflets, access to a photocopier, Sheets of paper (A4 or letter size), Scissors, Tape or glue are required.

3.4 Moderator (Facilitator)

Focus group discussion requires a team consisting of a skilled facilitator and an assistant (Krueger, 1994).

Moderators who display an intrinsic interest in the research topic, overt friendliness, a sense of humor, an insatiable interest in people, curiosity and openness to new insights, and a willingness to listen are more likely to encourage participants to share their experiences. (Prince and Davies, 2001, p. 208)

The moderator's role is to guide the focus group discussions by questioning predetermined questions and to ensure a good quality of focus group results that address the research objectives. In other words, the moderator, who guides the discussion, encourages participants to talk freely and reveal their thoughts and feelings

about the research topic, is central to the discussion not only by managing existing relationships but also by creating a relaxed and comfortable environment for unfamiliar participants. Similarly, the assistant's role includes observing non-verbal interactions and the impact of the group dynamics and documenting the general content of the discussion, thereby supplementing the data (Kitzinger, 1995). Non-verbal data rely on the behavior and actions of the respondent's pre-focus group discussion, during, and post focus group discussion. The moderator, a well-trained expert who works from a predetermined set of focus group discussion topics, raises issues identified in a discussion guide and uses probing questions to solicit opinions, ideas, and other information, often as it relates to a project or other activity.

3.5 Note-taker roles

A wealth of valuable information can be raised during a focus group discussion, but to for it to be analyzed and used, the assistant moderator must accurately and comprehensively record it. Different data from the focus group discussion can be recorded in different ways, but generally, it will involve some kind of note-taking and also possibly audio or video recording. The note-taker may ask quick, clarifying questions to the facilitator or the participants, if needed. The note-taker may help the facilitator, if necessary, by driving out questions that are not well explored or missed and be discreet or neutral as possible that the focus group discussion participants do not surprise or worry about what is being written down while they talk.

4 ADVANTAGES AND LIMITATIONS OF FOCUS GROUP DISCUSSION

4.1 Advantage

Focus groups are a primary format for qualitative research. This is a type of research that seeks open-ended thoughts and feelings from consumers, as opposed to quantitative research that involves numerical-based data collection.

According to Asmamaw, Mohammed, & Lulseged, (2011) the most compelling reason for using focus group discussion is the need to generate discussion or debate about a research topic that requires collective views and the meanings that lie behind those views including their experiences and beliefs. Focus groups and other qualitative techniques are rich in detail and often provide surprises on what people may think of a product. Focus groups allow for broader and deeper insights into the brand and related topics versus very precise and structured quantitative research studies. It also offers an opportunity to research small children who may not read well enough to complete surveys or questionnaires. This is quite different from quantitative surveys or studies which limit respondents to single-word or short phrase multiple-choice responses. Focus groups are allowed for interactive research with a small group as opposed to individual responses on surveys. Through this interaction, moderators get the content from responses, and they get to observe the group and pick up on emotional responses, contradictions, stress, anger, frustration, enthusiasm, and other feelings that do not come through a structured questionnaire. Some companies have invested significantly in new technology and software to collect research data, but relatively low-cost focus groups remain one of the more productive consumer research techniques. Focus groups

generally require little to no technology and are often completed in just a few hours on one day.

4.2 Disadvantage

Two common disadvantages of focus groups are groupthink and accuracy. Moderators may tend to generalize or categorize individual feedback into group sentiment. Additionally, those with strong influence, such as the moderator and vocal group members, can sway the conversation and make it seem one-dimensional or quash feedback from less vocal participants.

Response accuracy is a concern because when participants know they are involved in research for a particular brand or company, they may feel less comfortable speaking out against that brand in front of the moderator and company onlookers. That is focus group discussion participants are sometimes reluctant to discuss sensitive topics in a discussion setting compared with an individual interview or a survey.

Harder to get everyone in the same place at the same time, difficulties preventing a particularly vocal or dominant participant from coercing others to agree with his/her views, data obtained are very context or specific and therefore not generalizable to other institutions or contexts

There are also several limitations to focus group discussions that are important to consider. These are: it requires advanced planning; it calls for getting together a set of people at the same place and time, which usually requires invitations to be distributed and confirmed in advance. Surveys and individual interviews also require planning to design the instruments and schedule the data collection sessions. However, the fact that FGI's require a group often implies greater coordination. Limited generalizability, is common, the prospective number of interviewees involved with a project is relatively large, and collecting the viewpoints of the limited number of people included in focus group discussions does not give evaluators a sample of sufficient size to be able to conclude the whole population. Therefore, focus group discussions are not intended to answer questions of "how much" or "to what extent" a service, result, or opinion exists in the whole population. Surveys or other quantitative data usually fill this role. It is Vulnerable to moderator bias, the moderator plays a central role throughout the focus group discussions process, especially when guiding the discussion and interpreting the results. The thoughtful design of the moderator's guide, accurate recording, systematic coding, and involvement of more than one individual in the data analysis can help minimize, but not eliminate, the risk of bias. On the other hand, in-person and telephone surveys, as well as individual interviews, also are susceptible to this kind of bias. And limited confidentiality, there are rare instances in which people may be more forthcoming in a group discussion than they would be on an individual basis; in most cases, participants will be less likely to share very personal or sensitive information when they are speaking with a group. To collect such data, evaluators can use surveys or individual interviews, where the privacy of the data collection can help to protect confidentiality (USAID, 2013).

5 LEADER BEHAVIORS NECESSARY TO KEEP FOCUS GROUP DISCUSSION GOING

The team leader is a person who supervises the research team, oversees the systematic coding and organization of information, keeps the original copies of the information, notes, and tapes in a safe place, provides guidance for interpretation, ultimately responsible for the final report, even if parts of it are written by other members of the team. Besides, the leader participates in the focus groups in one way or another i.e., the leader is a moderator, note-taker, or observer. If this is not possible, then the team leader should spend some time in the field observing the focus groups and working with the team immediately following the focus groups. In this scenario, the moderators, note-takers, and observers can help the team leader organize, read, and code the information. They can also brief the team leader on their impressions of the group, including group dynamics and nonverbal communication.

6 SUMMARY OF A STUDY THAT UTILIZED FOCUS GROUP DISCUSSION

According to Morgan (1996) suggested that focus group discussions can be used as a stand-alone method or in combination with other methods as an exploratory or as a follow-up tool. Focus groups are used for economic research situations to explore and identify issues of satisfaction for customers, staff, or suppliers and to explore views of brand and service delivery associated with the brand. It has its merits as well as its restrictions as a data collection tool, and it should primarily be used in a triangulation approach to qualitative economic researches. Triangulation, or using more than one source of data gathering technique, boosts the validity and often allows for greater generalizability. Outcomes gathered from focus group discussions alone will not completely access the natural perceptions of participants, but it can be very useful in the initial research stages or to validate the participants' perceptions on a given research topic either during a study or as an ex-post evaluation of a product or business. Emphasis should be placed on focus group discussions as a method of catching dynamic real-life interaction among participants when research topics assess individuals' attitudes, perceptions, and opinions. Focus group discussions are an efficient tool in qualitative consumer and marketing research data collection when conducted from a truly qualitative perspective through segmenting target participants into smaller subgroups according to specific, categorical attributes such as gender, job title, etc. Its success lies in the researcher's ability to utilize focus group discussions to uncover the why behind and what in participant communication while getting to the core of the producers' and or consumers' beliefs.

7 Conclusion and Recommendation

7.1 Conclusion

This paper reviews the literature on focus group discussion as a data collection tool and provides information for a better understanding of focus group discussion by addressing the questions of what is the meaning of focus group discussion? What are logistics needed? Its advantages and limitations, how leader behaviors necessary to

keep focus group discussion going? and its application in economic data collection. The accessed literature shows that focus group discussion has different definitions, types, and steps. It is highly valued as a qualitative research data collection tool, especially for its ability to generate in-depth understandings into a topic in an effective and timely manner and can be a cost-effective and quick approach to data collection. It is relatively easier to conduct as all the group participants and the moderator are readily available in one location at the same time and the types of questions in the focus group discussions are to be in sequence of deductive questioning that is from general to specific.

Regardless of some of the limitations and logistical difficulties involved such as groupthink and accuracy, harder to get everyone in the same place at the same time, this data collection tool is seen as effective and even less resource-intensive compared to other methods and requires a team consisting of a skilled moderator and an assistant. Whereas, when focusing group discussion used in a triangulation, it can help the researcher to access a deeper understanding of the phenomenon by furnishing results on social interactions to meaningful construction. Yet, it requires proper planning and organization.

7.2 Recommendation

In this paper by reviewing the available researchers' studies, I have seen focus group discussion is important in discussing issues and questions of importance on a topic of mutual concern and its purpose of the discussion is to more thoroughly understand concepts and issues. By taking the above explanation as an input for policy interventions the researcher, leader, the moderator should focus on:

Leaders and facilitators should know the research topic itself will direct the nature of focus group discussion, making it a flexible and unique tool for gaining in-depth understanding and gathering diverse group members' opinions and perspectives on a comprehensive range of subjects. Facilitators should know beforehand which topics can safely be covered within a focus group and which cannot and also should keep an eye on the note taker and allow him or her to catch up, before moving to the next question or topic. Moderators or facilitators should remind that the potential risks that groupthink can pose on the result of focus group discussion by ensuring a fair distribution of opportunities to all participants to voice out their perceptions. The note taker should be as modest and neutral as likely so that the focus group discussion participants do not surprise or worry about what is being written down when they talk. The frequent use of this tool should make a highly collaborative process for participants and an opportunity for the leader to gain multiple perspectives on an interesting topic.

Finally, I suggest that focus group discussions can also be useful when combined with quantitative studies and that this gives a strong triangulation research design.

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ASSESSING PRACTICES OF GOOD GOVERNANCE IN THE SECURITY SECTOR: THE CASE OF ADIGRAT CITY SECURITY AND ADMINISTRATION OFFICE, EASTERN ZONE OF TIGRAY, ETHIOPIA.

Mebrahtu Kiros Hagos *

ABSTRACT

The objective of this research article was to assess the practices of good governance in the security sector particularly in Adigrat City Security and Administration Office. The study used both qualitative and quantitative research methods. Primary and secondary sources of data were also used. Primary data was gathered through semi -structured interview and focus group discussions and secondary data was gathered through document analysis. Respondents were selected using probability and non- probability sampling methods. Among the probability sampling methods, simple random sampling technique was employed, and non- probability sampling method (Convenience sampling technique) was used. The qualitative data was collected from 10 respondents and the quantitative data was gathered from 122 respondents. The qualitative data was analyzed through narrative analysis and discussion and the quantitative data was analyzed through descriptive analysis techniques such as, frequency and percentage.

Keywords: Adigrat City, Good Governance, Principles of Good Governance, Security Sector and Administration.

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1. INTRODUCTION

Over the past 60 years, there have been efforts to ensure international peace and security by the United Nations. Despite tremendous efforts, conflict and instability still remains unsolved. The members of the UN therefore continued the efforts of promoting peace and security by providing two integrated perspectives. These perspectives are, first: security, human rights, and development are complementary and go hand in hand for ensuring everlasting peace. Second, ensuring rule of law and good governance are recognized as the main factors for safety, human right promoting and ensuring economic development(UN, 2010).

There is also argument within the members of the UN and other actors of the international system on the constraints of socio-economic and political progress because of insecurity and conflict. This has dramatically changed the point of discussion and expanded the scope of security from security of the state towards safety and security of people and communities. That's why states and governments have started to modify their institutions in such a way to promote security. This is because states that don't promote safety and wellbeing to their citizens are considered as having ineffective security sector thereby cannot realize visible development and protection of human rights (Fallis, 2013). As a result, good governance in the security sector as a policy concept was emerged with the aim of institutionalizing a professional security sector that is effective, responsive, efficient, legitimate, apolitical and accountable to the citizens (Ball et al., 2003).

Africa is also experiencing a daunting challenge to promote rule of law, good governance and security. The continent has experienced tremendous conflicts, human right violations and political upheavals. This problem attracted the need for strong security sector institutions among African countries through regional integration and cooperation (African Union,2013). Thought African governments tried to rearrange their security sector much has remained to be done. The security sector institutions are filled with unprofessional human resources in addition to weak management and follow up mechanisms (Cawthra, 2009).

In response to challenges of good governance in the security sector, the Ethiopian government has introduced 'the security sector reform' in 2002 under the authority of Ministry of Capacity building. The security sector reform was latter named as the Comprehensive Justice Sector Reform Program(JSRP) in 2005 (Henok ,2014). The goal of the JSRP was to ensure rule of law and good governance thereby creating fertile condition for the promotion and protection of basic human rights and freedoms of citizens. However, the security sector governance program failed to participate relevant stakeholders and gave little attention to democracy as it has been dominated by security forces like police and military forces (Wallelign, 2018).

As part of fighting the challenges of security sector governance, the Tigray regional state justice sector reform bureau also involved in solving insecurity and violence. But,

political interference, inadequate human and material resources and incompetent leadership have been the most serious problems (Tigray Bureau of Plan and Finance, 2015).

1.1 JUSTIFICATION

After the end of Cold War new perspectives emerged insisting that security and development are inseparable and indivisible. Besides, The World Bank's voices of the poor studied over 60,000 poor women and men from 60 countries and indicated that insecurity is the main challenge to reduce poverty (Caparini, 2006). The security sectors in many developing and transition countries are inefficient and ineffective. Due to lack of professionalism in the security sector and irresponsible security bodies and justice systems insecurity and violence are rampant. This overall mess happens due to weak governance in the security sector (EU, 2007).

According to Geneva Center for Democratic Control of Armed Forces (DCAF), (2015) poor security sector governance stems from neglecting the principles of good governance within a state's security sector office. Poor security sector governance affects the overall socio-political system and dynamism of a state (OECD, 2008). Ball. Bouta, Tsjeard van de Goor, and Luc, (2003) also argued that poor security sector governance leads to mess within the security sector institutions. Ineffective follow ups, lack of transparency and accountability in the security sector also create a suitable condition for corruption and mischiefs. In general, poor security threatens political, economic and social stability of a given polity (Kayhan & Hartog, 2013).

Taking a lesson from the international actors, the security sector reform or the justice sector reform program in Ethiopia was launched and the security sector institutions are reformed based on the hope that they would deliver efficient and effective services as per the country's law by inviting relevant stakeholders to take part in crime prevention ,creating professional ,accountable and transparent police officers via establishing police colleges at federal and regional governments (Henok, 2014).

However, little achievement was registered and the security threat in the state increased from time to time in both federal and regional governments. For example, in recent years (2018-2020) insecurity and violence in Tigray region have been increased. Specifically, as the home of more than 800,000 populations, the eastern zone of Tigray region has suffered from lack of good governance in the security sector administration office which resulted in instability and violence. Hence, a significant number of people are dying, losing their physical body, being confiscated their property like money, mobile phones and different materials because of high level of insecurity(Tigray Bureau of Plan and Finance, 2015) .

Pertaining to the studies little has been done on security sector governance in Ethiopia in general and in Tigray region in particular. For example, (Wallelign, 2018) studied "The security sector reform in Ethiopia particularly in Amhara National Regional State police force" and argued that the challenges of security sector reform program are

political interference, lack of adequate budget and incentives, and lack of human and material resource. His studies are limited to assess the implementation of the security sector reform program, and challenges of security sector reform program in the police force. Wallelign failed to examine the importance of stakeholders' participation in the security and administration, the role of government to control and oversight in activities of security and administration, and overall the importance of applying good governance principles in the security sector in enhancing security environment. The fact that no research regarding good governance in the security and administration office is conducted in the study area and the resulting rampant crime, loss of life and property makes the topic relevant and timely, which inspired the researcher to engage in such research activity.

1.2 Objectives of the project

The general objective of the study was to assess the practices of good governance in the security sector taking case study of Adigrat City's Security and Administration Office, Eastern zone of Tigray region, Ethiopia.

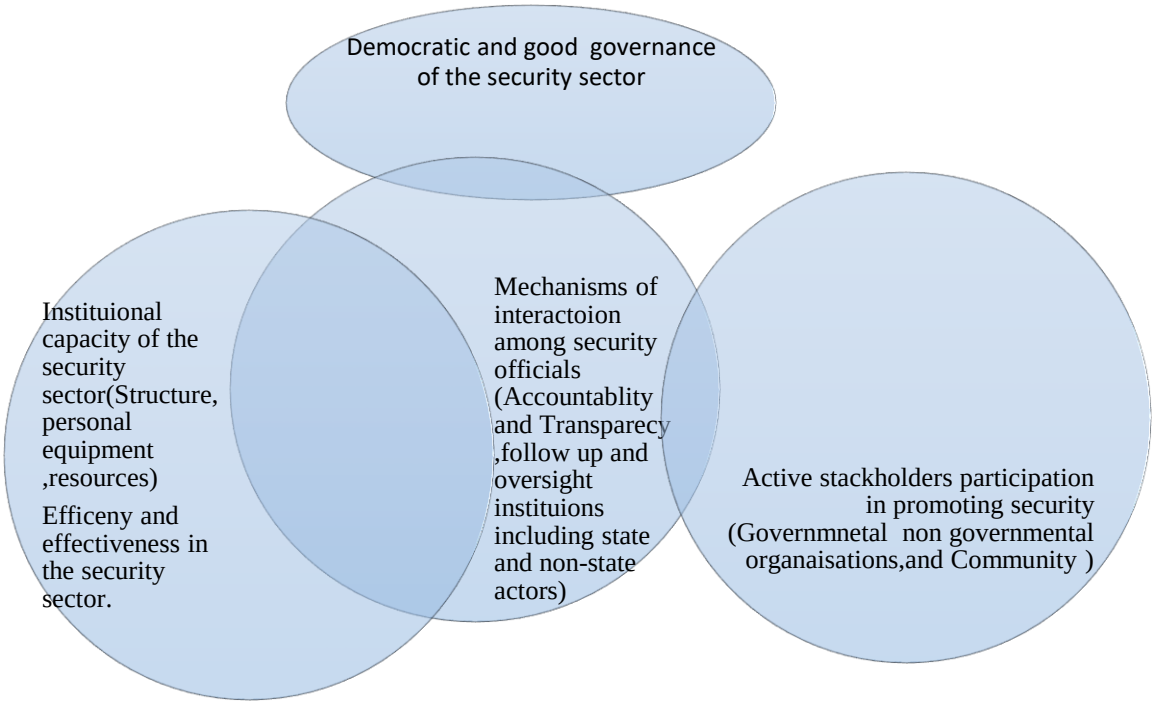
The Specific Objectives of the study are:

i. To assess the capacity of the Security and Administration Office in promoting good governance.
ii. To examine the mechanisms of interaction in the Security and Administration Office.
iii. To examine the extent of stakeholders participation in preventing security challenges in collaboration with the security and administration office.
iv. To identify the major challenges hindering good governance in the Security and Administration Office.

Research Questions

i. What looks like the capacity of Security and Administration office to function effectively and efficiently in promoting good governance?
ii. Are there sound mechanisms of interaction in the Security and Administration Office?
iii. To what extent do the stakeholders participate in preventing security problems in collaboration with the security and administration office?
iv. What are the challenges hindering good governance in the Security and Administration Office?

1.3 Conceptual framework



Source: Adopted from Ball, (2004).

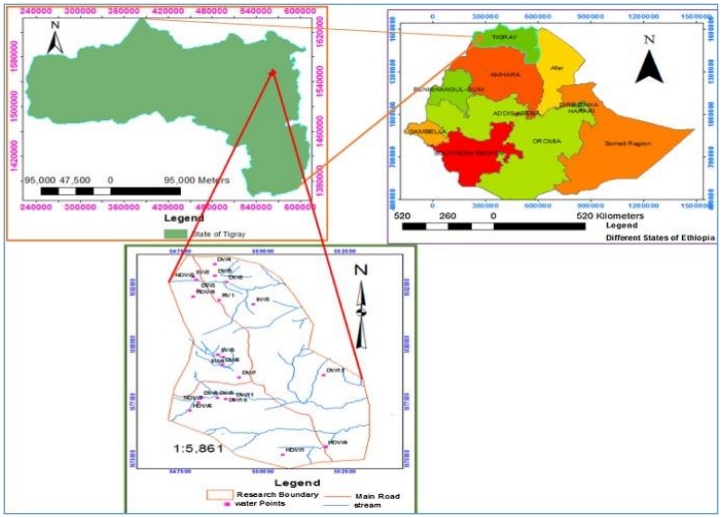
Figure.1. Conceptual framework

2. METHODS AND MATERIALS

2.1 Description of the study area

The study was conducted in Adigrat town located in the Eastern zone of Tigray region, Ethiopia. The town is the Center of Eastern zone of Tigray. It is situated between 14°15′N Latitude and 39° 27′ E Longitude with an elevation of 2,457 metres (8,061 ft) above sea level. It has 6 administrative *kebelles*. The total population of the town is 97,125 of whom 46,113 are men and 51,012 women (Adigrat town Labour and Social affairs office, 2020).

2.2 Map of the study area



Source: (Grmay , 2018).

Figure.2. Map of the study area

2.3 Research Strategy and Design

2.3.1 Research Strategy

There are two common research strategies. These are Qualitative and Quantitative. The study used both qualitative and quantitative research strategies. This is because mixed research strategies substantiates the strengths and weakness of both methods and improves the analytic power of the study (Creswell, 2007).

2.3.2 Research Design

Research design is a blue print of the methods to be employed for collecting data and the techniques to be used in analyzing data considering the research objectives and resources (Kothari, 2004). The design helps to minimize bias and maximizes reliability. It also helps to address the research questions. Thus, cross-sectional survey design was used because it is affordable in terms of money and consumes less time.

In terms of the depth of investigation, case study research design was employed because the researcher has intensely described the practice of good governance principles in the study area, that is- Adigrat security and administration office. Creswell, (2007) noted that case study design is detailed analysis of attributes rested on selected study areas.

2.4 Sampling Methods and Techniques

This study employed both non-probability and probability sampling methods. One of the types of non-probability sampling methods, known as convenience sampling was used. That means the researcher selected interviewees, FGD discussants and *kebelles* purposively because of the fact that the respondents were supposed to be better source of information about the security issues in the district. In addition, Maurer & Michelleti, (2012) argued that if respondents are selected based on better source of information and the close relationship they have with certain sector, there are chances that the

results obtained will be highly accurate with a minimum margin of error. Accordingly, from the Eastern zone of Tigray region, Adigrat town was purposively selected because of its high level of crime and insecurity (Eastern zone security and administration office, 2020). As mentioned earlier Adigrat town has 6 *kebelles*. Then out of Six *kebelles*, three *kebeles i.e Kebelle* 3,*Kebelle* 4 and *kebelle* 6 were selected still purposively based on the level of crime and insecurity they have(Ibid). Questionnaires were distributed to the respondents based on preliminary interview made by the researcher because of the fact that there is no documented list of clients per day or per week in the security and administration office of the town. The estimated numbers of clients per day are gained from the security and administration office selected as key informants. Therefore convenience sampling was employed to get the average clients per day.

TABLE 1: Sampling Technique.

Selected Town/District	Selected Institution	Average Clients per week	Sample
		50*5=250	112
Adigrat	Security and Administration Office		

Source: Survey result, 2020.

N.B -50 indicates average clients per day in the security and administration office.
-One week has 5 working days.

The sample size is determined by using Yamane's formula(1967) as cited by Israel, (1992). The formula is given by:

$$n = \frac{N}{1 + N(e)^2}$$

$$n = \frac{250}{1 + 250(0.07)^2} = 112$$

Where, n= Sample size, N=population size, e = the level of precision (93%) and sampling error which is 0.07% 1=the probability of even occurring.

There are also 10 workers in the Security and Administration Office; therefore 122 questionnaires will be distributed to the respondents, i.e 112+10=122.

2.5 Sources and Methods of Data Collection

The study used both primary and secondary data sources. Primary data sources including key informant interviews, and Focus Group Discussions (FGDs) were used. The secondary data was gathered from both published and unpublished materials.

Qualitative and quantitative methods of data collection were used to collect reliable data. To collect data by qualitative methods, the study utilized key informant interview, and Focus Group Discussions while survey technique was utilized for quantitative method. To express in detail:

2.6 Key informant interview (KII): In this study Key Informant Interview particularly-Semi-Structured interview was used because the researcher wants to know

the specific information which can be compared and contrasted with information gained in other interviews. The researcher also wants the interviewee to remain flexible so that important information can be obtained (Dawson, 2013).

Therefore, key informants who have background information on security and administration issues were purposively selected and interviewed. From the security and administration office one security and administration's unit leader, and two security and administration officers were approached. Totally, three key informants were interviewed. The researcher also made interview with one eastern zone administrative and security affairs office officer to strengthen his data. Hence, there were totally four key informants in this study. Checklist was prepared for conducting the interview.

Focus Group Discussions (FGDs): Taking age, religion, education and sex in to account the researcher carried out two Focus Group Discussion. Each FGD was composed of six participants. For this purpose, checklist was prepared and each discussion was facilitated by the researcher.

Survey: a survey is a method of obtaining large amounts of data, usually in a statistical form, from a large number of people in a relatively short period of time (Kothari, 2004).Both close ended and open ended questionnaires were used in this study. Close ended questionnaire was used because categorized data were required and the informant chooses the answer from a set of provided alternatives. Whereas open ended questionnaire were used because the researcher needs new facts to be searched out by giving to the respondent to freely express their views and ideas. Questionnaires were distributed to 122 respondents. The questionnaires were prepared in Tigrigna language which is the medium of communication for the community in the study areas. Survey was used to supplement and triangulate the qualitative data gathered through key informant interview and FGD.

Document analysis: secondary materials were reviewed to supplement the primary sources of data. The research utilized secondary information such as the Tigray region security and administration bureau annual plans and performance reports, the Adigrat town security and administration office annual plans and performance reports, magazines, newspapers, articles, research reports, published and unpublished books dealing with the nature and practice of good governance in the security sector.

2.7 Method of Data Analysis

The quantitative data collected by household survey (Close- ended questionnaire) was analyzed using descriptive statistics like frequency, percentage. Statistical Package for Social Sciences (SPSS) software version 20 was employed for analyzing descriptive statistics. The qualitative data obtained through Open-ended questionnaire, Key Informant Interview, and Focus Group Discussions was analyzed through narrative analysis and discussion.

3. RESULTS AND DISCUSSIONS

In this section, the findings of the study will be presented. Firstly, the institutional capacity of the security and administration office will be discussed. This indicates the assessment of inputs vital for the efficient and effective performance of the security and administration office of the case study. Secondly, the mechanisms of interaction employed in the security and administration of the town will be presented. Thirdly, the extent of stakeholders participation in preventing security problems will be discussed, lastly, the challenges of good governance in the security and administration office of the town will be presented.

3.1 Sex Composition of Respondents

As shown in the table below, out of the total sample respondents 74.6 %(91) were male and 25.4 %(31) were female. This indicates that majority of the respondents were male.

TABLE 2. Sex of sample respondents

Sex		Frequency	Percent
	Male	91	74.6
	Female	31	25.4
	Total	122	100.0

Source: Survey result, 2020.

3.2 Educational Status of Sample Respondents

As depicted in table 3, out of the total sample respondents, 45.1 % (55) were Diploma holders, 34.4 % (42) were 9-12 grade, 15.6% (19) were Degree and above, and 4.9 % (6) were 5-8 grade. This shows that most of the respondents who filled the questionnaires were diploma holders and 9-12 grades.

TABLE.3. Educational Status of Sample Respondents

Education status		Frequency	Percent
	5-8 grade	6	4.9
	9-12 grade	42	34.4
	Diploma	55	45.1
	Degree and above	19	15.6
	Total	122	100.0

Source: Survey result, 2020.

3.3 Adequacy of Inputs

Supportive materials are one of the indicators of institutional capacity of the security sector. This is to mean that the security sector has to be filled with adequate materials and professional security personnel to functions its duties efficiently and effectively. Security governance Scholars like N.Van .D .walle, Nicole Ball,& V.Ramachandran (2013) also noted that adequate provision of goods and services to the security providers in the security sector promotes good governance.

Accordingly, out of the total sample respondents, 43.4 %(53) disagree with the provision of necessary inputs important for promoting good governance in the security sector and 33.6% (41) strongly disagree with the availability of supportive materials in the security and administration office. And, 9.8% (12) of them were neutral on the availability of supportive materials while 7.4 %(9) strongly agree , and 5.7% (7) agree. In addition, a police officer with the rank of "Unit Leader" in the security and administration office of the town explained that:

"The security bodies in our institution don't receive adequate resources (financial, training, material) to execute their roles as professionals. They don't also receive enough professional training while they are working in their institution thus lack the necessary expertise to fulfill their functions efficiently and effectively. The police officers always ask the necessary materials needed to promote peace and security in the town. But, the security and administration office don't provide them. There is also lack of financial support, for example there is no allowance/incentive to the police officers in the security and administration office. The police officers legally have the right to get 200 birr per month for house allowance, but, peculiar to other Weredas/Districts of eastern zone of Tigray, the Adigrat security and administration office don't provide house allowance to its workers. So, mostly the workers feel discouraged of their profession which has its own negative impact on peace and security of the town (Interview, Unit Leader , Adigrat, 2020)."

TABLE 4. Sample respondents on Adequacy of Inputs

Response		Frequency	Percent
	Strongly Agree	9	7.4
	Agree	7	5.7
	Neutral	12	9.8
	Disagree	53	43.4
	Strongly Disagree	41	33.6
	Total	122	100.0

Source: Survey result, 2020.

3.4 Accountability

A sound mechanism of interaction among workers in the security sector enhances good governance in the security institutions. These mechanisms of interaction are accountability, transparency, responsiveness, oversight and checks and balances. Specifically, accountability in security sector refers to the existence of clear expectations for security provision, independent authorities be it governmental or non-governmental oversee whether these expectations are met and impose sanctions if they are not met (DCAF, 2015).

The survey result on the status of accountability in the security and administration office indicates that, out of total sample respondents 48.4 % (59) of them disagree and 31.1(38) of them strongly disagree . In addition, 10.7 %(13) of them agree, and 5.7 % (7) of them were neutral and 4.1% (5) of them strongly agree.

As per the interview conducted with a security and administration officer and with some clients, the status of accountability was not satisfactorily. One *police officer* interviewee explained that:

"Of course authorities like the peoples' representatives of the town oversee the actions and decisions of the security and administration office but it's not adequate. Some security officials coordinate with criminals and engage themselves in crime activity. Seeing this, they are not accountable to any concerned body and the people's representatives don't impose sanctions on these criminals particularly police officers. In addition, the community, the media and other non- government officials don't adequately oversight and follow up the actions and decisions of the security officials in the security and administration of the town'. (Interview, Police Officer Adigrat town, 2020)."

TABLE 5. Accountability of sample respondents

Response		Frequency	Percent
	Strongly Agree	5	4.1
	Agree	13	10.7
	Neutral	7	5.7
	Disagree	59	48.4
	Strongly Disagree	38	31.1
	Total	122	100.0

Source: Survey result, 2020.

3.5 Transparency

In the contemporary world, transparency or openness in governance is significant in the process of development. Its meaning is linked with information freely available and accessible to those who will be affected by decisions and their implementation (DCAF, 2015).

The summary result presented in table 6 indicates that 40.2 % (49) of the respondents replied disagree with the existence of transparency, 31.1% (38) replied strongly

disagree, 18 % (22) of them replied agree and 10.7 % (13) of them remain neutral. Additionally, as per to the researchers Focus Group Discussion on transparency in the security and administration office the discussants noted that that:
"In the security and administration office of Adigrat town, there is no transparency about almost all decision makings as well as about implementations of laws and programs. Most information is not accessible to the media and ordinary citizens. That's why the community and Medias don't know about the activities of the security and administration office the town. Because of lack of transparency, the security and administration of the town cannot be easily monitored and understood by the entire society. The communication between the community and the security and administration office is too weak. They don't work cooperatively. This is the result of weak community policing in the town (FGD, Adigrat town 2020)."

TABLE 6. Transparency and response of sample respondents

Response		Frequency	Percent
	Agree	22	18.0
	Neutral	13	10.7
	Disagree	49	40.2
	Strongly Disagree	38	31.1
	Total	122	100.0

Source: Survey result, 2020.

3.6 Responsiveness

As shown in the table below, most of sample respondents, i.e. 49.2 % (60) of them replied disagree with responsiveness in the security and administration office, 9.8% (12) of them strongly disagree, and 30.3 % (37) of them agree. Furthermore, 5.7% (7) of them were neutral and 4.9 % (6) of them strongly agree with fast service delivery to clients in the security and administration office. To supplement the study with qualitative data the researcher also conducted an interview with security and administration worker. Accordingly, the interviewee said that:
"The security and administration office of Adigrat town is not sensitive to the different security needs of all parts of the population and they don't perform their missions in the spirit of a culture of service. The service delivery to clients in the institution is very poor. This might happen because of lack of adequate provision of material resource, motivations, impartiality, and respect for human rights (Interview, Security and administration worker Adigrat, 2020)."

TABLE 7. Responsiveness of sample respondents

Response		Frequency	Percent
	Strongly Agree	6	4.9

	Agree	37	30.3
	Neutral	7	5.7
	Disagree	60	49.2
	Strongly	12	9.8
	Disagree		
	Total	122	100.0

Source: Survey result, 2020.

3.7 Rule of Law

Ensuring rule of law in the security sector entails impartial treatment of all citizens, equality of all citizens irrespective of their differences based on different backgrounds ,respect for human and democratic rights enshrined in the international bill of human right (Fallis, 2013). Hence, out of total sample respondents, 44.3 % (54) responded disagree with the existence of rule of law in the security and administration office of the town, 34.4% (42) of them replied strongly disagree, and 5.7 %(7) of them replied agree. Furthermore, 10.7 % (13) of them remained neutral and 4.9 % (6) of them replied strongly agree. As per the FGD conducted with clients, the essence of rule of law is more of theoretical than practical in the case study's security and administration office. The FGD discussants explained that:

"Rule of law is the most abused issue in the security and administration office of Adigrat town. All citizens are not seen equal in the security and administration office especially in police stations. The workers especially police officers are not subject to laws and regulations. The police officers are not impartial. They practice preferential treatment to clients who need service from the office. The police officers are not consistent with the international and national human right norms and standards. There is violation of human rights like the right to equality in the security and administration office (FGD, Adigrat town, 2020)."

TABLE 8. Rule of Law and response of Sample Respondents

Response		Frequency	Percent
	Strongly Agree	6	4.9
	Agree	7	5.7
	Neutral	13	10.7
	Disagree	54	44.3
	Strongly Disagree	42	34.4
	Total	122	100.0

Source: Survey result, 2020.

3.8 Oversight and follow-up mechanisms

According to Wanandi, (1998) security management and oversight bodies, such as parliament, justice sector and civil society, the community and the media are responsible to make oversight and follow up actions in different public sector institutions. Laying on this perspective, the oversight and follow-up mechanisms in the security and administration office of Adigrat town was assessed. Thus, the summary result presented in table 9 indicates that, 43.4 %(53) of respondents disagree with oversight and follow up mechanisms in the security and administration office of the town, 22.1% (27) of them strongly disagree, and 18.9 %(23) of them agree. But, the rest 15.6 %(19) remained neutral. According tom the interview conducted with a client in the security and administration office about the oversight and follow up mechanism. The interviewee explained that:

"Actors' oversight and follow up practice in the institution is very poor. The people's representatives of the town rarely follow up the security and administration office of the town. The relation between the people's representatives and the security bodies is weak. This opens a door for security bodies to coordinate themselves with criminals and engage in criminal activities. But some measurements were made by the people's representatives in coordination with the community because of the alarming level of insecurity in the town. For example, last year in 2019, a significant number of police officers were caught coordinating with criminals and become responsible and sanctions were imposed on them (Interview, Client, Adigrat town, 2020)."

TABLE 9. Oversight and follow up mechanism and response of sample respondents

Response		Frequency	Percent
	Agree	23	18.9
	Neutral	19	15.6
	Disagree	53	43.4
	Strongly	27	22.1
	Disagree		
	Total	122	100.0

Source: Survey result, 2020.

3.9 Stakeholders Participation

Active stakeholders' participation is the key to ensure good governance not only in security sector but also in other public sectors. The engagement of civil society and the media in security affairs enables the perspectives of ordinary people and important sub-sections of the population (such as women and minority groups) to be incorporated into policymaking and oversight processes (Berhe et al., 2015). Having this in mind, the respondents were asked about the level of stakeholders' participation. Thus , the

survey result in table 10 shows that 52.5 % (64) of respondents replied Low stakeholders participation, 31.1% (38) of them replied Very Low stakeholders participation, and 16.4 %(20) of them replied Medium stakeholders participation. The interviewee serving as a security and administration unit leader also elaborates that:
"Stakeholders like the community, governmental and non-governmental organizations have less opportunity to participate in decision-making. They community residing in the town has also less interest to participate in security provisions(Interview ,Unit Leader , Adigrat Town,2020) .This is similar with Ball's et al (2003) argument that low stakeholders' participation in decision making process in the security and administration office leads to low oversight and follow up mechanisms."

TABLE 10. Response on Stakeholders participation

Response		Frequency	Percent
	Medium	20	16.4
	Low	64	52.5
	Very Low	38	31.1
	Total	122	100.0

Source: Survey result, 2020.

3.10 Challenges Hindering Good Governance in Adigrat Security and Administration Office

The survey result depicted in figure 2 indicates, out of total sample respondents, 54.9 %(67) of them replied agree that lack of accountability is the main challenge hindering good governance. 19.7% (24) of them replied strongly agree, and 14.8 %(18) of them strongly disagree. Besides, 5.7 % (7) of them responded disagree and 4.9 %(6) of them were neutral about the constraint of accountability in the security and administration office of the town.



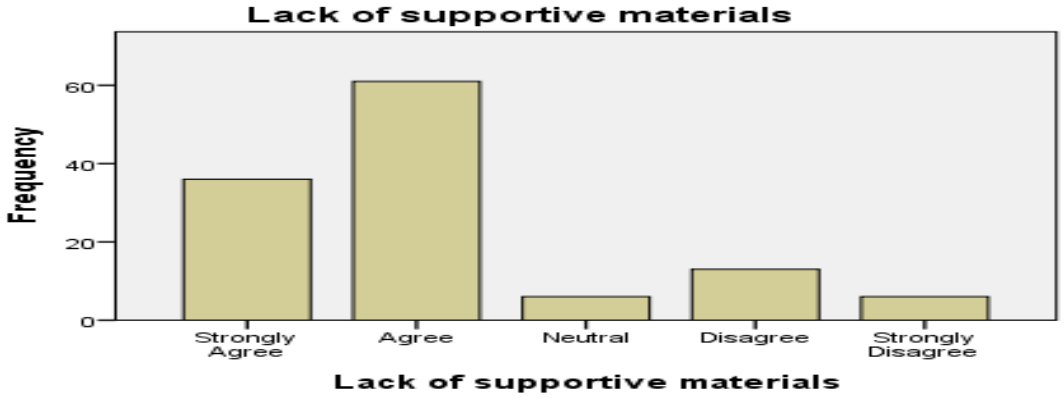
Figure.2. Lack of accountability

Source: Survey result, 2020.

3.11 Lack of Inputs

Good governance can't be achieved without adequate material support (inputs) in every public sector. The summary result in figure 3 shows that out of the total sample respondents, 50 %(61) of them replied agree, 29.5% (36) of them responded strongly agree, 10.7 %(13) of them responded disagree, 4.9 % (6) of them replied strongly disagree, and 4.9 %(6) of them were neutral.

The survey result shows most of respondents agree lack of material support is the main factor hindering good governance in the security and administration office of the town. Other also strongly agrees that there is shortage of material support important for ensuring good governance in the security administration office of the town.

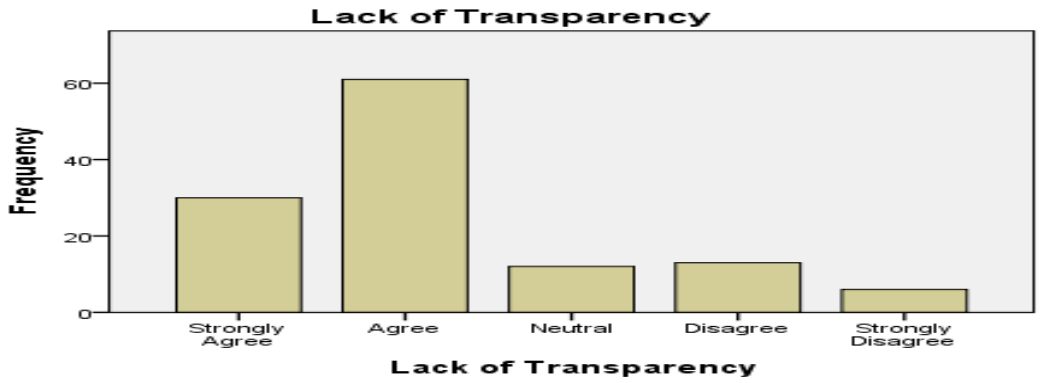


Source: Survey result, 2020.

Figure.3. Lack of supportive material

3.12 Lack of Transparency

As it is depicted in the figure below, out of the total sample respondents, 50 %(61) of them replied agree, 24.6% (30) of them strongly agree, and 10.7 %(13) of them replied disagree. Besides, 4.9 % (6) of them responded strongly disagree, and 9.8 %(12) of them remained neutral. The survey result is depicted figuratively as follows:

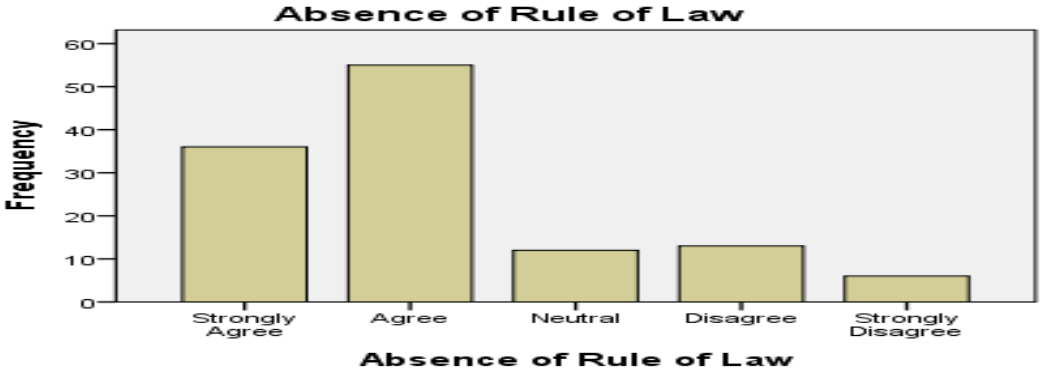


Source: Survey result, 2020.

Figure.4. Lack of transparency

3.13 Absence of Rule of Law

The respondents were asked to mention the challenges hindering good governance in the security and administration office .Accordingly, out of the total sample respondents, 45.1 %(55) of them responded agree, and 29.5% (36) of them replied strongly agree. In addition, 10.7 %(13) of them replied disagree, 4.9% (6) of them responded strongly disagree, and 9.8 %(12) of them replied Neutral.

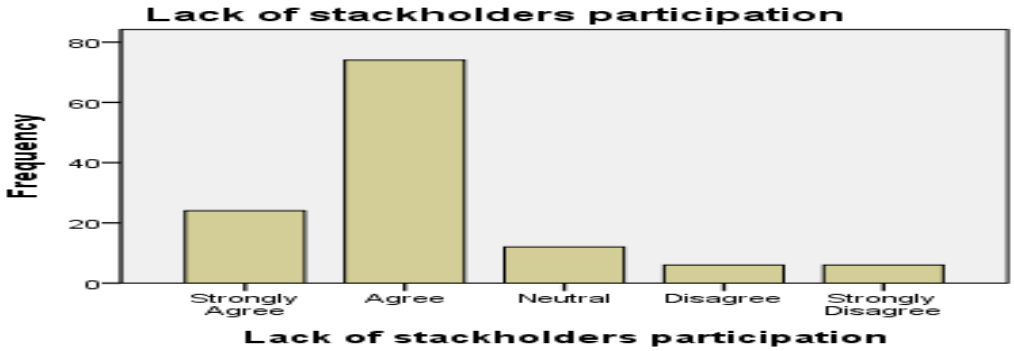


Source: Survey result, 2020.

Figure 5. Absence of rule of law

3.14 Low Stakeholders Participation

The other factor assessed in hindering good governance in the security and administration office is lack of stakeholders' participation. Accordingly, out of the total sample respondents, 60.7 %(74) of them responded agree, 19.7% (24) of them responded strongly agree, 4.9 %(6) of them replied disagree, 4.9% (6) of them replied strongly disagree, and 9.8% (12) of them replied Neutral.



Source: Survey result, 2020.

Figure.6. Lack of stakeholders' participation

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The findings of the study reveals that the practices of good governance in the security and administration office do not brought promising results. The institutional capacity of the security and administration office is not strong. That means the institution doesn't have adequate inputs, the workers particularity police officers don't have motivation like allowances/incentives. There is high shortage of necessary materials important for security provision. The security workers feel discouraged because of the overall lack of attention given to them.

Mechanism of interaction in the security ad administration office is also weak. That means the workers in the security and administration office are not accountable, transparent and responsive. Absence of rule of law is also highly prevailed in the institution. There is also low level of legislative, media and community follow up and oversight mechanisms which negatively affects check and balances.

In addition, the stakeholders' participation to promote good governance in the institution is low. In general, Institutional capacity, strong mechanisms of interaction and community participation in the security sector are not satisfactorily. These components are intertwined and thus highly reinforce to each other. If these components of security sector are not implemented efficiently and effectively, good governance in the security and administration office will not be achieved.

The Likert scale result of the study revealed that, lack of adequate inputs, low level of accountability and transparency, absence of rule of law, and low level of stakeholders' participation are the major challenges hindering good governance in the security and administration office.

4.2 Recommendation

In order to ensure good governance in the security and administration office of Adigrat town, the following recommendations are forwarded.

The institutional capacity of the security and administration office of the town should be strengthened. That means, adequate inputs like material support, financial support (budget), and frequent training for workers to enhance their knowledge, skills, and attitude should be provided.

The mechanisms of interaction within the security and administration office of the town should be enhanced. For instance, Rule of Law should be ensured, accountability and transparency should be prevailed, oversight and follow up mechanisms from all stakeholders like the legislative of the town, the community and the media should be strengthened.

For enhancing the oversight mechanisms, the security and administration office should engage stakeholders in decision making process, and awareness rising campaign or training should be made so that the stakeholders especially the community could actively participate in decision making process.

The major challenges of good governance in the security and administration office, such as inadequate inputs, lack of accountability, low level transparency, absence of rule of law, and low level of stakeholders' participation should be solved as soon as possible before they brought serous insecurity and violation of human wellbeing.

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KINETIC STUDY OF ADSORPTION OF CONGO RED DYE ON WOOD CHARCOAL OF ADANSONIA DIGITATA (BAOBAB)

Mastan Rao Kotipalli*, Samwel Anderson Mazengo, Emiliani Mwaliga Kikambako and Alfreda Narzis Kinunda

ABSTRACT

Activated charcoal prepared from Adansonia Digitata (baobab) wood has been used for the removal of congo red dye (CR) from simulated aqueous solutions employing adsorption method. The effect of adsorption was studied by using different parameters such as pH(2-8), contact time(30-120min), initial dye concentration(50-150mg/L) and adsorbent dosage(0.5-2.0g). The extent of adsorption was determined by employing UV-Vis spectrometer. The experimental data was verified by Freundlich model and found to be in agreement. Under the experimental conditions the parameters at the maximum adsorption ability have been determined for the dye and reported.

Keywords: adsorption, congo red dye, baobab wood charcoal.

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1. INTRODUCTION

Most of the dyes are complex organic molecules. Since dyes are synthetic aromatic compounds they are normally stable and difficult to be degraded (yenikaya, et al., 2010). Several industries release dyes into atmosphere causing harmful effects to the living beings. According to the chemical structure, dyes can be divided into azo dyes, aryl dyes, acridine dyes etc. Azo dyes are widely used in industrial activities (Benkhaya et al., 2020). Dyes are used in production of the essential consumer products of modern life such as textile, leather, cosmetic, foods and beverages, papers and pulp which cause the environmental pollution and threat to the ecological balance (Ulas, et al., 2017). Dyes are used in academic as well as research institutions and normally released into atmosphere after the study is over (Yusuph, 2017).

Synthetic dyes are extensively used for coloring and printing in variety of industry (mohanraj, 2009). The dye based effluent is a considerable source of non-aesthetic pollution. The presence of small amount of dye (below 1 ppm) is also clearly visible. Many researchers found that colorant may cause problems in aquatic ecosystem in several ways (yenikaya, et al., 2010). Worldwide, infectious diseases such as water-borne diseases are harmful to the children under five years old and more people die from unsafe water annually than from all forms of violence (Edogbanya, et al., 2013a). The problem of water pollution has become an issue drawing global attention. Every day, several million tons of sewage, industrial and agricultural waste is discharged into the world's waste water bodies (WWAD, 2006). There are several ways for removal of dyes. Adsorption has been considered as one of the safest and simple methods for the removal of textile dye effluents due to its low cost, easy availability, simplicity of design, high efficiency, ease of operations and ability to treat wide variety of dyes (Ramalakshmi et al., 2012). Activated carbon is a carbonaceous adsorbent with a highly crystalline form and internal pore structure (Kavitha 2019). A wide variety of activated carbon products are available depending upon a starting raw material and activation techniques (Gupta, 2003). Mihayo et al., studied the adsorption effect of methyl red dye on baobab pericarp charcoal and found that adsorption is considerable.

In the present study, adansonia digitata (baobab) wood charcoal is considered for the adsorption of simulated congo red dye solutions. Congo red dye was discovered on 1884 which was used to dye cotton but has been replaced with dyes which are more resistant to light and washing. (Rodriguez, 2018). The baobab tree are mostly found in the equatorial area such as Gabon, Congo, Zaire and the area with marked dry season (Gebauer, et al., 2002) such as Dodoma region in Tanzania. It is a tree of multiple uses and it is observed that all parts of the tree are useful. Generally baobab fruits and seed oil are used for human consumption and the rest of the tree parts are used as traditional medicine (simon, 2015). Baobab belongs to genus: Adansonia and Species: Adansonia digitata. In the present study, congo red is used as a simulated adsorbate and baobab wood charcoal as adsorbent.

2. MATERIALS AND METHODS

2.1 Preparation of dye solution

Stock solution of dye concentration 1000mg/L was prepared by dissolving 1g of 100% pure congo red dye in 1000mL of distilled water by using standard flasks. Solutions of different concentrations were prepared by dilution method between 20 and 200mg/L using the above stock solution of the dye. All chemicals of 99.9% purity have been used in the present study.

2.2. Preparation of adansonia digitata wood biosorbent

Hard wood of adansonia digitata has been collected from a single tree in Dodoma region at Kongwa district. After removing the bark, small chips of the baobab wood are pulverized into powder using grinding machine and then sieved through a pore size of about less than one millimeter. The dried sample was treated with sulfuric acid with constant stirring. Then the sample was kept in hot air oven at 200±50K temperature for twelve hours. The sample was washed with distilled water and soaked in sodium bicarbonate solution and then allowed to stand overnight to remove the residual acid from the pores of carbon. The sample was washed again with distilled water, until the pH of the adsorbent becomes neutral (7±0.2). Then the sample was dried in a hot air over at 100±5℃ for twelve hours. The dried sample was ground to get a powder of particle size about 125-250µm range and stored in an airtight container for further experiments.

2.3 Batch mode biosorption studies

A standard calibration plot between the adsorbate concentration and absorbance was drawn for computing the concentrations from batch mode experiments. Batch biosorption equilibrium experiments were conducted in 250mL conical flasks at a constant agitation speed (180rpm). All the experiments were carried out at room temperature (30^0C). The concentrations of the dyes both before and after adsorption were determined using Agilent 60 Cary UV-Vis spectrophotometer, USA through monitoring the absorbance for the dye used. All the spectra were recorded for the absorbance of congo red dye at a wave-length of 497nm. In the present study, the effect of initial concentration of the dye, adsorbent dosage, pH of solution and contact time on adsorption has been studied. The effect of initial dye concentration was studied in the concentration range 50-150mg/L. The effect of pH on adsorption process was studied in the pH intervals of 2, 4, 6 and 8. The effect of mass of adsorbent was studied in the range of 0.5gram to 2.0gram at constant dye concentration 100mg/L and optimized conditions. The effect of contact time was studied in the constant dye concentration of 100mg/L and time range from 30 minutes to 120 minutes.

3. RESULTS AND DISCUSSION

3.1 Effect of initial concentration of dye

The effect of initial dye concentration was studied in the concentration range 50-150mg/L. The extent of adsorption of dye per gram of activated carbon was calculated and reported in Table No:1. A plot is drawn between adsorbate concentration and adsorption ability and shown in Fig.1.
x/w = extent of adsorption (mg/g), C_i = initial concentration of dye (mg/L), C_f = final concentrations of dye (mg/L) , w = dry weight of the activated carbon (g), C_i-C_f = x

TABLE 1: Data showing initial concentration of dye solution and adsorption ability

Sl. No.	C_i (ppm)	w (g)	Absorbance	C_f (ppm)	C_i-C_f (ppm)	x/w (mg/g)
1	50	1.0	1.0397	39.9148	10.0851	10.0851
2	100	1.0	1.9922	73.6914	26.3085	26.3085
3	120	1.0	2.4159	88.71631	31.2836	31.2836
4	150	1.0	3.0336	110.6206	39.3794	39.3794

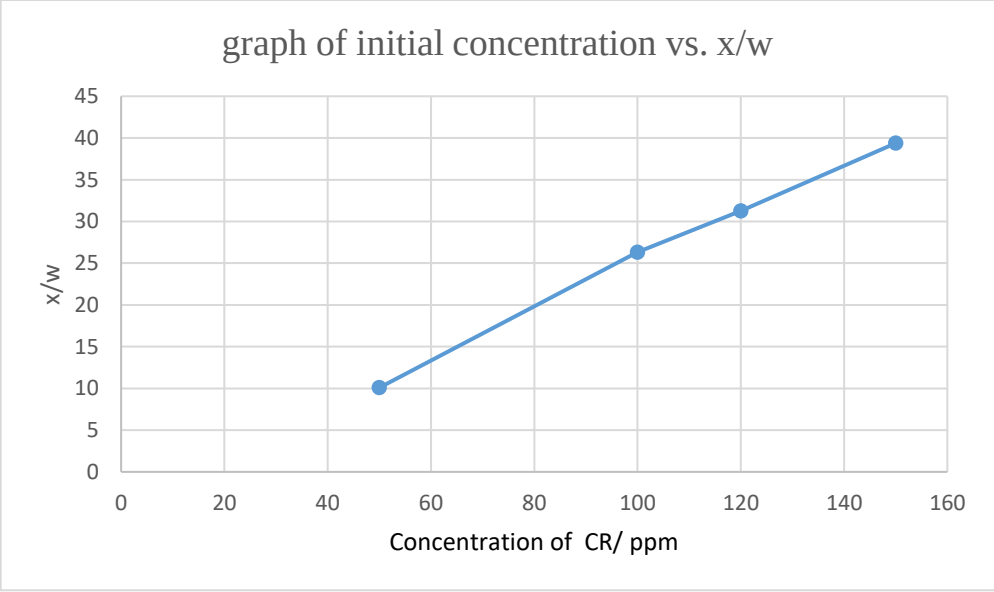


Figure 1. Adsorbate concentration vs. Adsorption ability

It is observed from the graph that as the initial concentration of adsorbate increases, adsorption ability increases to a certain extent and thereby the increase is decreased.

This may be due to the saturation of the active sites of the adsorbent upon adsorption of the dye molecules.

3.2 Adsorption isotherm

Adsorption isotherm has been studied on the extent of adsorption of CR dye on the adsorbent by using Freundlich adsorption model. According to the Freundlich isotherm model, the amount of adsorption at low concentration varies linearly, at moderate concentration becomes dependent on the Freundlich constant and at higher concentration becomes independent of the adsorbate concentration. From the Fig: 1 above, it is observed that the experimental results obey the Freundlich model satisfactorily. Further it is verified by plotting a logarithmic graph (Fig: 2) and found to be a straight line which is expected of the above results.
x/w – extent of adsorption, C_i – equilibrium concentration of CR dye in aqueous medium.

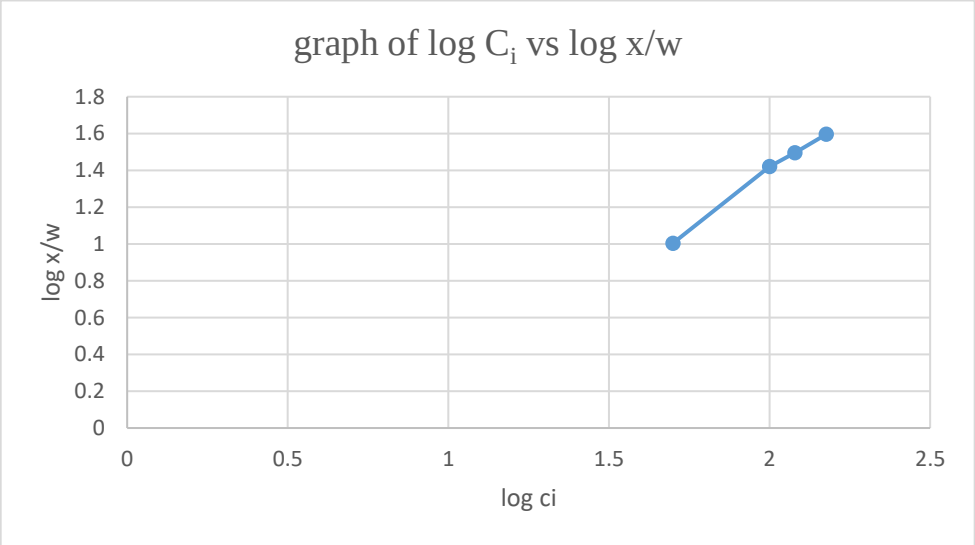


Figure 2. Freundlich adsorption isotherm

3.3 Effect of adsorbent dosage

The effect of mass of absorbent was studied in the range of 0.5g to 2.0g at constant adsorbate concentration of 100mg/L. The extent of adsorption of dye per gram of activated carbon was calculated and reported in Table No: 2. A plot drawn between adsorbent dosage and adsorption percentage is shown in the Fig.3. It was observed that the extent of adsorption increases with an increase in the mass of adsorbent (activated carbon) from 0.5 -2.0 gram which may be due to the availability of unsaturated surface area (active sites). Further the values of C_i-C_f /w are decreasing which can be attributed to the less concentration of dye molecules available per gram of adsorbent. Under the

experimental conditions, a maximum of 44.85% of dye is removed by the adsorbent from the solution under consideration.

Table 2. Adsorbent dosage vs. adsorption ability data

Sl.No.	C_i (ppm)	w (g)	Abs	C_f (ppm)	C_i-C_f (ppm)	x/w	% Removal
1	100	0.5	1.9926	73.7056	26.2943	52.5886	26.29%
2	100	1.0	1.666	62.1241	37.8758	37.8758	37.88%
3	100	1.2	1.5244	57.1028	42.8971	35.7476	42.89%
4	100	1.5	1.4694	55.1524	44.8475	29.8983	44.85%

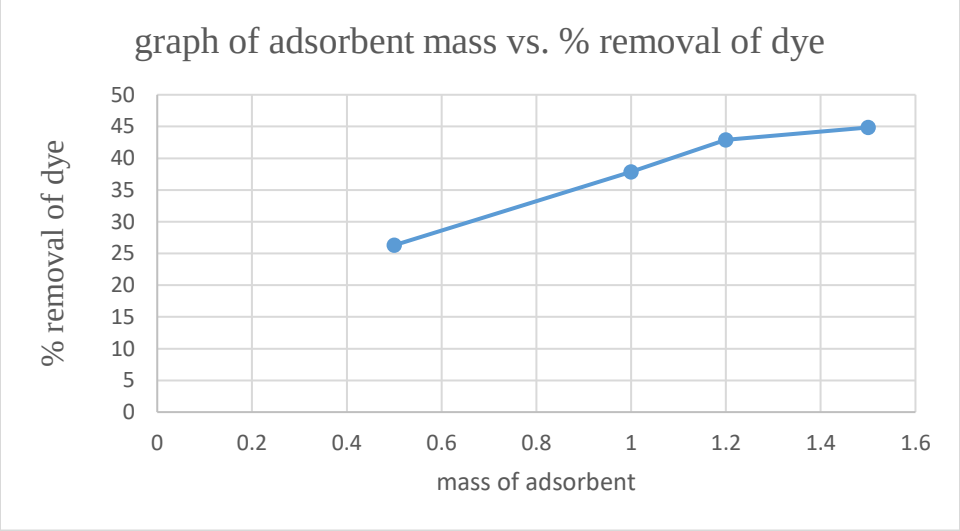


Figure.3. Graph between adsorbent dosage and % removal of dye

3.4 Effect of pH

The effect of pH was studied at a constant concentration of 100mg/L of adsorbent and pH range 2-8. The pH of different solutions under examination was adjusted using sulphuric acid by employing pH/OPR & EC/TDS/NAC pH Meter. The solutions were allowed to stand under optimized conditions and the spectra were recorded. The extent of adsorption of dye per gram of activated carbon was calculated and reported in table No.3.

x/w = extent of adsorption (mg/g), C_i = initial concentrations (mg/L), C_f = final concentrations of dye (mg/L) , w = dry weight of the activated carbon (g)

The percentage of removal of the dye was calculated as, $q\% = \frac{(Ci-Cf)}{Ci}$.

TABLE 3. Data showing the variation of absorbance with change in pH at a constant mass of adsorbent.

Sl.No.	C_i/ppm	w (g)	pH	abs	C_f	C_i-C_f	x/w	% Removal
1	100	1.51	2.18	0.8258	32.3297	67.6702	44.8147	67.67%
2	100	1.51	4.41	1.4909	55.9148	44.0851	29.1954	44.09%
3	100	1.50	6.22	1.9989	73.9290	26.0709	17.3806	17.38%
4	100	1.51	8.45	2.3612	86.7766	13.2234	8.75722	8.76%

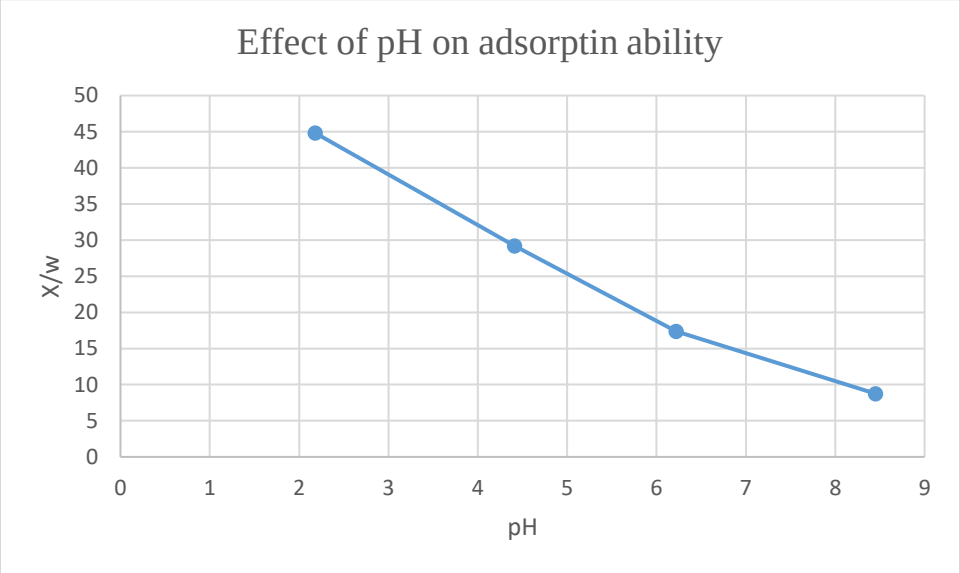


Figure.4. Effect of pH on the adsorption ability

The adsorbate shows a maximum adsorption at pH 2.18 with maximum percentage of adsorption of 67.67%, because it works efficiently on the acidic medium than in a basic medium. Congo red is an anionic azo dye; hence at lower pH it shows higher adsorption due to the positively charged surface of the adsorbent.

3.5 Effect of contact time

The effect of contact time was studied at a constant concentration of 100mg/L and time range from 30 minutes to 120 minutes. The extent of adsorption of dye per gram of activated carbon was calculated and reported in Table no: 4 below.
The percentage of color removal in the dye solution after adsorption was calculated as $q\% = \frac{(Ci-Cf)}{Ci}$.

TABLE 4. Contact time vs. adsorption ability data

Sl. No.	C_i/ppm	w/g		Time(min)	abs	C_f	C_i-C_f	x/w	% Removal
1	100	1.1		30	1.584	59.2305	40.7695	37.0631	40.77%
2	100	1.0		60	1.115	42.6170	57.3829	57.3829	57.38%
3	100	1.1		90	0.401	17.2659	82.7340	75.2127	82.73%
4	100	1.0		120	0.218	10.7978	89.2021	89.2021	89.20%

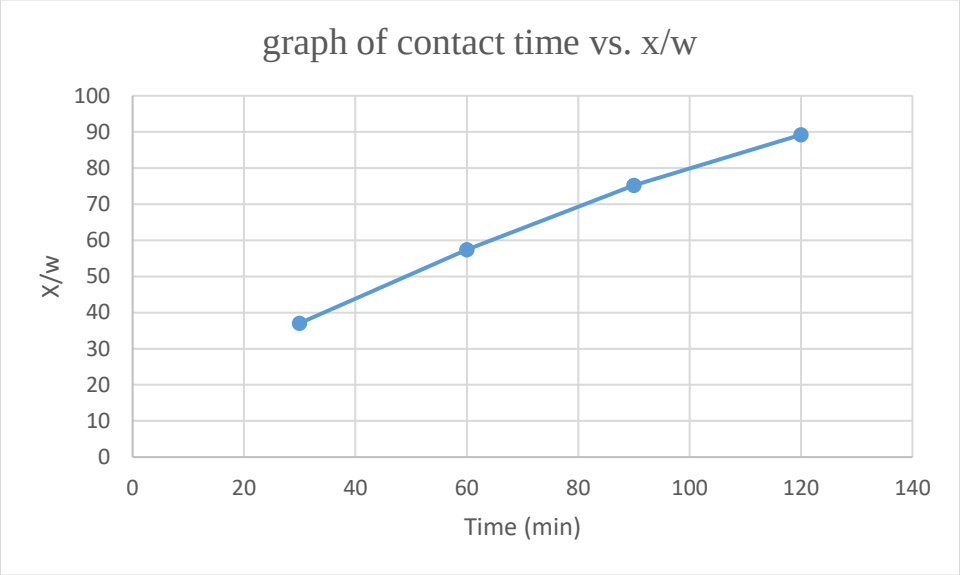


Figure.5. Effect of contact time on the adsorption ability

The removal of congo red dye by activated carbon as a function of time was observed. Initially, the adsorption of dye is considerable and increasing during the time interval of 50 to 150 minutes. It is predicted that the adsorption of congo red dye will be adsorbed more on baobab charcoal under extended experimental conditions. The adsorption efficiency was found to be 89.2% which is found to be maximum under the experimental conditions.

4. CONCLUSION

The data obtained from the adsorption studies showed that the maximum removal efficiency was found to be 67.67% at a temperature of 303K, at solution pH of 2.18, initial dye concentration of 100mg/L and biosorbent dosage of 1.5g. A contact time of 120min was sufficient for the maximum removal of congo red dye from aqueous solution using baobab wood charcoal under the above optimized conditions.. The experimental data of biosorption of the dye on the baobab wood charcoal biosorbent

fits well with the Freundlich adsorption model. This indicates that the biosorption of the dye on the baobab charcoal is favorable. It is observed that the adsorption of congo red dye can be further increased upon allowing it for longer period under different experimental conditions. Further investigation of the adsorption characteristics like surface area of the adsorbent and surface characteristics etc., are suggested to understand the adsorption mechanism.

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Bovine Tuberculosis in Ethiopia – A Review On Risk Factors And Its Public Health Importance

Faizullah Peer* and Gizachew Fentahun**

ABSTRACT

Bovine tuberculosis, a chronic infectious zoonotic disease of domestic animals and humans is characterized by formation of granulomas in tissues especially lungs, lymph nodes, liver, intestines and kidneys. Mycobacterium bovis an acid-fast bacterium is the main etiological agent and transmitted both by erogenous and enterogenous routes. In Ethiopia, bovine tuberculosis is endemic and mostly transmitted to humans by ingestion of unpasteurized contaminated milk and close contact with infected animals. Disease causes development of miliary tubercular lesions, chronic cough, and obstruction of air passages, alimentary tract and blood vessels and enlargement of lymph nodes. A spectrum of Cell-Mediated Immune Responses (CMI) predominate infection, projecting the role of macrophages and T-cell populations. In advanced stage, there is increased humoral response. Tuberculin tests; single intra-dermal and comparative intra-dermal tests and other highly sensitive delayed type hyper-sensitivity tests are used for diagnosing tuberculosis (TB) in live animals, TB eradication and also for international trade. Vaccination of calves with attenuated bovine-strain of tuberculosis bacterium, known as Bacillus of Calmette and Guerin (BCG) and testing and culling of infected animals are important measures in bovine tuberculosis (BTB) control and prevention in endemic areas like Ethiopia. Additionally, pasteurization of milk is important before human consumption to reduce public health risks.

Keywords: Tuberculosis, Bovines, Risk factots, Ethiopia.

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1. INTRODUCTION

Bovine tuberculosis (BTB) a chronic infectious zoonotic disease of worldwide occurrence is caused by *Mycobacterium bovis*. BTB can have an impact on the ecosystem by transmission of the disease to wild life. It is primarily of economic importance having considerable direct effect on milk and meat production besides animal reproduction (Admassu et al., 2015).

Ethiopia, one among the nations of African continent possesses largest number of bovine population (58 million), has a record of low livestock productivity among other factors is attributed to the low genetic potential and performance, poor nutrition, different diseases, traditional husbandry practices and inadequate skilled manpower, (Akalu., 2017). BTB in Ethiopia is detected on the basis of tuberculin skin testing, abattoir meat inspection and very rarely on bacteriological techniques. A prevalence of BTB ranging from 3.4% (in small holder production system) to 50% (in intensive dairy productions) and 3.5 to 5.2% in slaughterhouses across the country has been recorded (Akalu, 2017). Pasteurization of milk, tuberculin testing followed by culling of infected animals in developed countries has reduced the incidence of disease in humans contrary to the underdeveloped nations of Africa where both human (85%) and animal populations (82%) are inhabitants of prevalent disease or only partially controlled areas (Palmer et al., 2012). This is because of close human-animal interaction and shift from extensive rural production system into the combined urban and peri-urban intensified livestock husbandry practices leading to the transmission of zoonotic diseases (Mbugi et al., 2012)..

Bovines are infected by infected cattle excreting organisms in the exhaled air, sputum, feces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes. Disease can also be disseminated by animals having gross lesions communicating with airways, skin or intestinal lumen and cattle in early stage of the disease before visible lesions. Experimentally infected animals excrete organisms up to 90 days post infection.

Th1-induced cell mediated immunity is triggered by Mycobacterial infection with release of cytokines like tumor necrosis factor-α, Interleukin-12 (IL-12) and interferon gamma (IFN-γ,(insert bracket) essential for macrophage activation. Depending on the quantum of cytokines macrophages may kill and eliminate bacteria or bacteria may lie dormant or disease may develop if bacteria are not controlled by the macrophages (Welsh et el., 2005).

With this background, the different aspects of Bovine Tuberculosis (TB) need to be reviewed.

2. MATERIALS AND METHODS

2.1 Etiology

Mycobacterium is a non-spore forming, nonmotile, obligate aerobic, non-capsular acid-fast bacteria. They are facultative intracellular microbes, thin, rod shaped, slightly curved, measuring 1-10µm in length and 0.2 - 0.6 µm in width having slow generation time of about 15-20 hours. Mycolic acid, a waxy coat lipid enriching cell wall of the mycobacterial cell wall contributes to its acid fastness, hydrophobicity, resistance against many disinfectants, common laboratory stains, antibiotics, physical injuries and slow growth rate by restricting uptake of nutrients. (Birhanu *et al;* 2015)

Mycobacterium bovis is the main etiological agent of bovine tuberculosis, can best survive in frozen tissue. An adverse effect of tissue preservative *i.e*. sodium tetraborate on its viability has been recorded. (Birhanu et al., 2015) though bacteria can survive for various months especially in cold, dark and moist environmental conditions. Survival at 12˚C - 24˚C (54˚F - 75˚F) varies from 18 - 332 days depending on sunlight exposure. From soil or grazing pasture there is infrequent isolation of this organism. Organisms stored artificially can be cultured for approximately two years, remains viable between 4 - 8 weeks in 80% shade. The incubation period of *M. bovis* is 3 weeks (Verma *et al*., 2014).

2.2 Epidemiology

Tuberculosis (TB) a chronic disease of wide range of mammals including humans, cattle, deer, llamas and other wild animals of various age groups are susceptible. Primarily disease affects the cattle, other domestic and wild animals as well as human beings. Disease found throughout the world is more prevalent in Africa, parts of Asia and America, with high rate of prevalence in the tropical and sub-tropical countries (Verma *et al*., 2014). Based on comparative tuberculin skin test, a prevalence of 6.9% of bovine tuberculosis in small holder dairy farms, 12.9% in traditionally managed extensive production systems and 24.6% in intensive dairy farms has been recorded in different areas in Ethiopia (Shitaye *et al*., 2007).

Bovine TB, a globally distributed disease has been eradicated from Antarctica and those countries such as Caribbean islands, parts of South America and Australia by following strict test and slaughter policies, but it is a major health problem in India. Predisposing factors like changing environmental conditions, topography, anthropogenic variables, seasonality, immunosuppression, long antibiotic therapy and working conditions increase chances and severity of infection. Infected cattle are the main source of infection for other cattle, when organisms are excreted in exhaled air,

sputum, feces, milk, urine, vaginal and uterine discharges and discharges from open peripheral lymph nodes (Ameni*et et al*., 2011).
Genetic, behavioral, biological or environmental factors influence epidemiology of bovine TB which have effect on transmission, establishment of infection and expression of disease (Vial *et al*., 2011). Male badgers are more affected as compared to female. Males are at higher risk of mortality promoting gender influence while risking transmission of TB to cattle (Graham *et al*., 2013). It is more prevalent in dairy workers exposed to poor control areas of bovine tuberculosis. The pulmonary form of disease is more severe in occupational groups, working with animals infected with *M. bovis* on farm or slaughter houses, than the alimentary form of disease. Bovine tuberculosis herd prevalence has been positively related with Mycobacterium Tuberculosis Complex (MTC) is also correlated positively with size of land, number of imported cattle and presence of wild host but not with isolation of cattle as well as density of cattle. Hereditary and maternal influences have a great role in the incidence of TB in cattle progeny. (Verma *et al*., 2014) besides factors associated with tuberculosis influencing the occurrence of disease such as sex, breed and social management of livelihood conditions (Acevedo *et al*., 2013).

2.3 Transmission

Bovine tuberculosis caused by *M. bovis* is a zoonoticdiseases in which both natural and anthropogenic movement of animals has influenced the epidemiology (Kruse *et al*., 2004). In many parts of the world, badgers, brush-tail opossums, wild boars, deer and other wildlife species constitute a wildlife reservoir of the pathogen. Thus, the natural movement of these reservoir animals increases the spread of the disease to domestic animals and thereby, its public health impact.
Transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans. *M. bovis* infection is transmissible from cattle to humans directly by aerogenous route and through direct contact with materials contaminated with nose and mouth secretions from an infected herd of cattle. Research findings revealed that individuals at risk are persons directly in contact with potentially infected animals such as veterinarians, abattoir workers, meat inspectors, autopsy personnel, farmers, milkers, animal keepers, animal dealers, laboratory personnel and owners of potential tuberculous pets (e.g. monkeys) (Yumi *et al*., 2007) (missing in references). Indirectly, man acquires the disease from animal sources by ingestion of meat and meat products from slaughtered infected cattle and consumption of unpasteurized infected milk.

2.4 Clinical signs

TB a chronic debilitating disease occurs in cattle. No symptoms occur in the early stage of disease. However, in late stage, there is progressive emaciation, a mild fluctuating fever, weakness and inappetence. When infection is present in the lung, then dyspnoea, moist cough or tachypnoea may occur. In the terminal stage, animals become extremely emaciated and develop acute respiratory distress. Involvement of respiratory tract and its role in pathogenesis

of disease is evidenced by the predominant distribution of lesions present in upper respiratory tract, lung and tonsils in affected human as well as in animals (Van Rhijn *et al*., 2008).
Some cows with extensive miliary tubercular lesions are clinically normal but in most cases progressive emaciation unassociated with other clinical signs occur, in spite of good appetite. A capricious appetite and fluctuating temperature are commonly associated with disease. The hair coat may be rough. Affected animals tend to become more docile and sluggish but eyes remain bright and alert. These general signs often become more pronounced after calving. Pulmonary involvement is characterized by chronic cough due to bronchopneumonia. Cough occurs only once or twice at a time and is low, suppressed and moist which is easily stimulated by squeezing the pharynx or by exercise and is most common in morning and in cold weather (Van Rhijn *et al*., 2008).
In advanced cases, air passages, alimentary tract, or blood vessels may be obstructed by enlargement of lymph nodes. Lymph nodes of the head and neck may become visibly affected and sometimes rupture and drain. Involvement of the digestive tract is manifested by intermittent diarrhoea and constipation in some instances (Van Rhijn *et al*., 2008).
There may be chances of bloat occuring due to pressure of enlarged mediastinal glands on the oesophagus. The enlargement of retropharyngeal glands results in dysphagia. Extreme emaciation and acute respiratory distress may occur during the terminal stages of tuberculosis. Lesions on the female genitalia may occur, while male genitalia are seldom involved. Tuberculosis mastitis is of major importance because of danger to public health and of spread of disease to claves and difficulty of differentiating it from other forms of mastitis (Radostits *et al*., 2000).

2.5 Associated risk factors

2.5.1. Risk factor for cattle

Animal level risk factors are cattle breeds, genetic resistance, physiological state of the animal, age, sex, stress, concurrent infection, immune status and body condition score (BCS) (Elias *et al*., 2008). Several past and recent studies have shown that susceptibility to bovine TB can vary between cattle breeds with suggestions that indigenous zebu cattle are more resistant to BTB than exotic breeds (Zeru *et al*., 2014). This fact is substantiated by the lower prevalence recorded in several studies and it is evident where European breeds of cattle have been used to establish a dairy industry. Genetically improved cattle may suffer more severely from deficient housing and malnutrition and thus be more prone to infection (Gebrez gabiher *et al*., 2014).
Studies conducted in different areas of our country also confirm variation in susceptibility to bovine TB among cattle breeds (Elias *et al*., 2008). Risk factors at herd level are herd size, types of farming practice and housing of cattle, geographical origin, history of bovine TB in the herd and human antecedent of tuberculosis in the household, contact between animals and with wildlife reservoirs, introduction of cattle in a herd, herd movements and trading, lack of performance of diagnostic tests, the use of hired/ shared bulls, manure and environmental persistence of *M. bovis* (Gumi *et al*., 2012). In Ethiopia, as wildlife habitats are not fenced, there is intensive interaction between a fast-growing human population and livestock and wildlife competing for scarce grazing land. Wildlife and, in particular, herbivores sharing pastures with cattle

might therefore be at risk for bovine TB transmission. Domestic animals were sharing grazing land in close proximity with wildlife in the area where wild animals live (Mamo *et al*., 2013).

2.5.2. Risk factors for human

Consumption of raw milk and meat in Ethiopian societies, a habit traditionally accepted is the main risk factor for *M. bovis* infection in humans (Namomsa *et al*., 2014) as *M. bovis* has been detected from raw milk confirming the existing problem and the potential risk of the infection in humans. Moreover, poverty, malnutrition, HIV infection, illiteracy, the consumption of raw milk (unpasteurized milk), uncooked or poorly cooked meat, work condition and close contact to livestock and using cow dung for plastering wall or floor are the main risk factors which contribute to the acquisition *M. bovis* infections in both urban and rural human populations in Ethiopia (Otte *et al*.,2002). Because of these factors cases of human TB of animal origin will continue to pose a serious public health problem, especially in areas where raw milk or its products are commonly consumed (Namomsa *et al*., 2014).

Intensification of livestock production has promoted close physical contact between the owner and his cattle, especially at night and thus facilitates the transmission of bovine TB and in turn intensifies TB as zoonosis. Moreover, there is habit of chewing and spitting tobacco to their cattle among Ethiopian farmers. These factors have increased risk of transmission for *M. tuberculosis* as well as for *M. bovis* at the human–livestock interface through inhalation of cough spray. (WHO, 2012).

2.6 Diagnosis

2.6.1. Acid-Fast Staining

Acid fast staining is commonly used to detect Mycobacteria in clinical samples by zeil-Neelsen or fluorescent staining for the diagnosis of Bovine TB and to monitor treatment progress. The lipid content ranging from 20-40% of the dry weight, in the cell wall of the acid-fast bacteria including *M. Bovis*, enables these organisms to resist decolorization with acidified organic solvents. They take up this stain only and appear as short red or pink rods on microscopic examination (*Sharma et al*; 2005).

Primarily, from the TB suspected tissues proper stained smears are made for preliminary examination. Smear made on a grease free clean slide from scrapings of cut surface tissue is air dried and fixed on flaming for one or two seconds, is treated with concentrated carbol fuchsin by heating and finally decolorized with a sulfuric acid and alcohol solution. Malachite green or methylene blue is commonly used as a counter stain (Birhanu *et al*., 2015). The stained smears are examined under an ordinary light microscope for the presence of acid-fast bacilli., The bacteria appear as red, colloidal or bacillary cells, 1-3 microns in length, as single rods or in clumps (WHO, 2013).

2.6.2. Culturing

Mycobacteria can be isolated by culture on an egg based media, Lowenstein-Jensen (LJ) medium. *M. bovis* has sparse, thin growth on glycerol containing media that is

called dysgenic, however, it grows well on pyruvate containing media without glycerol (Patterson and Grooms, 2000).

2.6.3. Tuberculin Test

Tuberculin testing is the standard method used internationally for the detection of bovine tuberculosis where intra-dermal injection of bovine tuberculin as purified protein derivative (PPD) is given in the mid neck region or in the skin of the caudal fold in single intra- dermal testing, SID, after the preparation of the site properly. The results are recorded by observing a swelling (a delayed hypersensitivity reaction at the site) 72 hours later. Comparative intra-dermal testing can also be performed by the same procedure where PPD and avian tuberculin is given intra-dermally at two different sites in the mid neck region or skin of the caudal fold. followed by observing a swelling at the site of injection. However, higher doses of tuberculin may be used in the caudal fold because skin of the neck is more sensitive than caudal fold (OIE, 2009).

Swelling at the site of injection or delayed hypersensitivity reaction can be noticed after 3-6 weeks in an animal or herd who had been very recently in contact with infected animals to avoid the probability of false negatives. SID cannot be used in the eradication of bovine TB as the sensitivity of the test is less than 100%. Tuberculin test becomes non responsive in chronically infected animals with severe pathology should also be considered. The tuberculin test has not been well validated in most non-bovid and non-cervid species (OIE, 2009)

2.6.4. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA, helpful in anergic cattle and deer, is a suitable antibody-detection test and can be a complement, rather than an alternative, to tests based on cellular immunity. It has an advantage of its simplicity, but it is less sensitive mostly because of the late and irregular development of the humoral immune response in cattle during the course of the disease. It is poor in specificity, is also poor in cattle when complex antigens such as tuberculin or *M. bovis* culture filtrates are used. However, a comparison of antibody levels to PPD-B and PPD-A has been shown to be useful in increasing specificity in the ELISA (Chambers, 2013).

However, using a combination of different antigens including proteins, improvement may be possible. The test is also helpful for *M. bovis* infections in wild life. This test is now used for elephants and non-human primates in the USA and is approved for use in badgers by United Kingdom (Lyashchenko *et al*., 2004).

2.7 Treatment

Isoniazid, combination of streptomycin and para-aminosalicylic and other acids are commonly practiced in human tuberculosis whereas in animals it is not a favorable option for the treatment or eradication of the disease especially in developed countries and is not economical. The disease requires long term therapy and the therapeutic regimen is not followed judiciously in animals, chances of development of multidrug resistant (MDR), extremely drug resistant (XDR) and even totally drug resistant (TDR) bacterial strains becomes obvious. Vaccination of calves with BCG and testing and culling is the viable option for prevention and eradication of tuberculosis (Verma *et al*., 2014).

Bacillus Calmette Guérin (BCG) vaccine is the most popular and effective vaccine against TB in humans and has an advantage for its use in cattle since the vaccine is safe, inexpensive and is commercially produced for human application on a large scale. The vaccine has shown variable levels of efficacy, protecting humans against pulmonary TB in children and adults, ranging from 0% - 80% (Parlane and Buddle, 2015). However, in animals vaccination with BCG sensitizes animals to the tuberculin skin test, and vaccinated animals will therefore, exhibit false positive results in classical skin test for a significant period after vaccination. Therefore, test and slaughter based control strategies using tuberculin skin testing is a favored option over BCG vaccination in many countries including Ethiopia (Ameni *et al*., 2010).

2.8. Control and Prevention

Tuberculosis a zoonosis, causes severe loss of productivity in infected animals needs to be prevented and controlled. However, control measures are not applied or are applied inadequately in most developing countries because of financial constraints, dearth of trained professionals and lack of political will. Moreover, in both the animal and public health sectors of national governments and donor agencies underestimate the importance of tuberculosis as zoonotic disease. Standard public health measures should be applied to manage patients with contagious *M. tuberculosis* to stop person-person transmission (Theon *et al*., 2009). Due to the limitations in the treatment of tuberculosis in animals, cattle should not be treated at all and farm animals suffering from tuberculosis must be slaughtered (culled). Moreover, *M. bovi*s is resistant to pyrazinamide, a drug widely used in the treatment of human tuberculosis caused by M. tuberculosis Complex (Krauss *et al*., 2003).

3. CONCLUSION AND RECOMMENDATIONS

Conclusions.

1 Tuberculosis is a zoonotic disease transmissible to human beings and extra pulmonary TB including BTB remains a significant problem in under developed countries.

2 Disease transmission, a most important factor in the spread of tuberculosis among humans, is mainly because of traditional drinking of raw milk without pasteurization and meat and is the chief source of dissemination besides aerosol transmission. Educate people about pasteurization infrastructure and public health importance and the hazards of raw milk consumption.

3 Antimicrobial therapy in animals is not a favored option because of limitations of longterm treatment and resistance to drugs. Culling of infected animals by test and slaughter and strict meat inspection at slaughter houses is the only means of disease eradication in animals.

4 Conducting intensive surveillance programs including on-farm visits, individual testing of cattle systematically including infected as well as in contact animals, quarantine, controlled movement of infected animals and their removal.

5 Advancement in the diagnostics comprising of conventional, immunological and molecular techniques and vaccine development has revolutionized the detection and control of TB in humans and domestic and wild animals as well, especially bovines. This can be a better option in the control and eradication of the disease.

Based on the above conclusions, the following recommendations are forwarded

- A Calves born from known TB dams should be separated to avoid transmission of the tuberculosis through infected milk.
- T B Test positive animals should be culled and is important that calves being reared as herd replacements be fed on tuberculosis-free milk, either from known TB free animals or pasteurized milk.
- C Infrastructure for pasteurization should be developed and pasteurized milk should be supplied for public consumption.
- D People around country should be educated about public health importance and the hazards of raw milk and meat consumption.
- E Vaccination against tuberculosis should be made mandatory for humans and animals because of zoonotic nature of the disease.
- F Bio-security measures and protective clothing should be worn to minimize interaction between domestic animals and wildlife animals during handling of the diseased animal and infected carcasses.
- G Carcass of infected animals should be disposed as per law of the state preferably, buried deep under the ground.

Abbreviations

BTB……. Bovine tuberculosis

CMI…….. Cell-Mediated Immune responses

BCG……. Bacillus of Calmette and Guerin

TB……. Tuberculosis

M.bovis……. Mycobacterium bovis

OIE……. Office of de epizootic

WHO…… World Health Organization

AFB…… Acid fast bacilli

IL…… Interleukin

IFN-γ……Interferon gamma

ZN……Ziel-Neelsen

PPD…… Purified protein derivative

IU…… International Units

ELISA…… Enzyme-Linked Immunosorbent Assay

ML…… Milliliter

USA…… United States of America

USDA…… United States drug and administration

HIV…….. Human immune virus

MDR…… Multidrug resistant

XDR…… Extreme drug resistant

TDR…… Totally drug resistant

MTC…… Mycobacterium Tuberculosis Complex

BCS…… Body condition score

AIDS…… Acquired immune deficiency diseases

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Role of grafting on tomato production: Review

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ABSTRACT

Tomato an important vegetable crop is a rich source of red pigment carotenoid, lycopene, vitamins and minerals. Its world production is reached to 182,256,458 tons in 2018 with productivity of 38.269 t ha^{-1}. The contribution of Ethiopia for World market in case of tomato is insignificant due to its low production of about 43,816 tons in 2018 with area coverage and productivity of 7089 ha and 6.18 t ha^{-1} respectively. The low production and productivity of tomato may be associated with abiotic and biotic factors. Grafting positively affects the life of plants in different ways. As it is indicated by various findings, grafted tomato plant was well performed both in growth and eventually in yield related parameters without significant quality reduction in most cases. To boost the production of tomato crop in the areas where there are different biotic and abiotic factors, using grafting technology with disease resistant and abiotic factors tolerant rootstock is the noble solution. Especially in Ethiopia, grafting which is almost new technology for the country can improve tomato production and productivity. To adopt such important technology in Ethiopia, it is necessary to conduct researches regarding tomato grafting. Furthermore, the rootstocks with desired characteristics should be identified within the species through researches.

Keywords: Grafting, Growth, Yield

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1. INTRODUCTION

Back ground and Justification

Tomato (*Lycopercicon esculentum* Mill.) is belongs to the *Solanaceae* family and it is the most important vegetable crops produced throughout the world for its nutritional and economic importance. Central and South America are believed to be center of origin and diversity of the crop (Nelson, 2008). It distributed to Europe and Asia in the early and mid-1960s (Asfew Zeleke and Eshetu Derso, 2015).

Tomato varies in shape, size, and color depending on the varieties. It varies from small cherry like, fresh market and processing types. It is served raw, baked, stewed, fried, and processed to pickles, sauce, juice and concentrated pastes (Zeleke and Derso, 2015).

A very valuable constituent of tomato is the red pigment carotenoid and lycopene, which are important anti-oxidants (Nasir *et al*., 2015). Tomato seeds contain high-quality plant proteins that can be supplemented into various food products. Studies have revealed that it is economical to utilize protein isolated from tomato seeds, as a substitute for wheat flour used in bakery products since it contains most essential amino acids at higher content (Organization for Economic Co-operation and Development (OECD), 2010). It is an excellent source of vitamin C, biotin, molybdenum, vitamin K, vitamin A (in the form of beta-carotene), vitamin B, vitamin E, foliate, and niacin. It is also a good source of protein, chromium, pantothenic acid, molybdenum, choline, zinc, and iron (Zeleke and Derso, 2015). Besides the use of tomatoes and tomato products for direct human consumption, tomatoes and its by-products serve as raw materials for several secondary products (Sogi *et al*., 2005).

In addition to the mentioned importance, tomatoes have also economic importance for smallholder farmers in Ethiopia as they are high value crops. The high yielding potential coupled with its high price, tomato is an important income source for small scale farmers in the country (Melese, 2017).

Moreover, commercial farms of tomato are also recently established in the central rift valley areas, which create job opportunity for young people in the area (Tefera *et al*., 2013). Therefore, if the commercial scopes of tomato production are appropriately used it can reduce unemployment and increase the daily income of the produces and employees.

The world tomato production is reached to 182,256,458 tons in 2018 with productivity of 38.269 t ha^{-1}. Ethiopia recorded low production of 43,816 tons in the same year, with the area coverage and productivity of 7089 ha and 6.18 t ha^{-1} respectively (FAOSTAT, 2019). As a result, the contribution of Ethiopia for world market is very low. The low production and productivity of tomato is associated with abiotic and biotic factors that hamper the production of tomato ((Estan *et al*., 2004; Castillo *et al*., 2007). From the biotic factors, soil borne diseases like root knot, fusarium, wilt and bacterial wilts and

insect pest like mite, aphids, leaf borers, armyworms, weevils, wireworms and etc. are very important (Capinera, 2001).

The most known abiotic factors that reduce the production of vegetables including tomatoes are problems associated with salinity and high temperatures (Castillo *et al*., 2007). These problems are especially serious, as tomato is mostly produced by irrigation in lowland areas with high temperatures and evaporations that results deposition of salts and thus salinity (Baye, 2017).

In this regard, various research results demonstrated that grafting of tomato on resistant variety of eggplant reduce the growth and yield reduction associated with salinity and other biotic and abiotic problems (Colla *et al*., 2014; Semİz *et al*., 2015; Kariada and Aribaw, 2017). Therefore the objective of this paper is to review on role of grafting on tomato production.

2. Role of Grafting Technology on Tomato Production

2.1. Description of the Tomato Plant

Tomato is a flowering vegetable plant of the nightshade family (Solanaceae) and cultivated widely for its edible fruits (Petruzzello, 2019). Tomato plants are branched, spreading and 60– 180 cm in height. The plant is somewhat trailing when fruiting, while a few forms are compact and upright. Leaves has hairy characteristics with strong odorous and also it is pinnately compound, and up to 45 cm (18 inches) long. The flowers are five-petaled yellow with 2 cm (0.8 inches) across and clustered nature. The fruit berries are varying in diameter from 1.5 to 7.5 cm (0.6 to 3 inches) or more. They are typically different in color such as red, light red or yellow, green and purple varieties are the common. They vary in shape from almost spherical to oval and elongate to pear-shaped. Each fruit contains at least two cells of small seeds surrounded by jellylike pulp (Petruzzello, 2019).

Tomatoes have two types of growth patterns, determinate and indeterminate (Dessalegn, 2002). The main differences between two categories are that determinate tomatoes establish the flowers and fruit at the tip of the branches. Thus plants of this group of tomatoes are usually smaller in size and the growth is terminated with the flower production. The fruits of such group ripen earlier and usually uniformly.

On the other hand, indeterminate tomatoes give flowers and fruit at the location where two branches meet each other. Unlike the determinants, the branches keep growing and as a result, they are usually larger in height and form vines. Some tomato varieties share the habits between the two forms (Rashid and Singh, 2000).

2.2. Ecological Requirement of Tomato Plant

Tomato is usually grown as an annual for both fruits as well as seed purpose. It is warm-season crop and requires relatively warm weather and much sunlight. In cooler climate the crop is therefore grown in hot houses. The temperature requirements of tomato vary with its developmental stage like germination, vegetative growth, flower and fruit set stages. An optimum temperature for seed germination is close to 29 ºC. Tomato growth best at the temperature ranges of 21-24 ºC. Temperatures below 10 ºC and above 38 ºC are not suitable for growth and development of tomatoes as they slow down physiological activities (Krishna dubey, 2012).

Tomato plant doesn't withstand frost and high humidity and thus they require low to medium rainfall (Bighaat, 2020). In addition, bright sunlight, especially at the time of fruit set, is required for the development of attractive dark red colored fruits (Newenhouse, 2010). Tomato requires well-drained loamy soils which are rich in organic matter content. The pH of the growing media/soil should be at the range of 6.5-7.5 (Ramasamy, 2004).

The plants are susceptible to several pests and diseases, including bacterial wilt, early blight, mosaic virus, Fusarium wilt, nematodes, and tomato hornworms (Capinera, 2001). Many of these problems can be controlled with crop rotation, the use of fungicides and pesticides, and the planting of resistant varieties. The tiny currant tomato (*S. pimpinellifolium*) is a closely related species and has been used by breeders to hybridize several pests- and disease-resistant tomato varieties (Petruzzello, 2019).

2.4. Vegetable Grafting

Grafting is a process by which a piece of scion is attached to a rootstock in such a way that the cambiums of both scion and rootstock come in firm contact, so that the new secondary tissue resulting from cambial cell division in the scion and rootstock is closely knitted (Kumar, 2011; Malik, 2000). When the two genotypes are compatible, the vascular bundles reconnect at the graft union and the wounded surfaces of the scion and rootstock meet without presenting a barrier for water and nutrient translocation.

Vegetable grafting is an important component of sustainable horticulture as it increases the vigor of the crops and resistance against diseases, tolerance to temperature, salinity, flooding and drought, and increase nutrient uptake (Colla *et al*., 2014). Commercial grafting of vegetable plants began in Korea and Japan in the late 1920s when watermelon plants were grafted onto squash rootstocks for Fusarium wilt resistance (Grieneisen *et al.,* 2018).

Since then, research on vegetable grafting has substantially increased. Vegetable grafting is mainly applied to *Solanaceae* and *cucurbitaceous* crops, primarily tomato, eggplant, pepper, watermelon, cucumber, and melon (Goreta *et al.,* 2008). Currently, in many countries in Latin America, Europe, and Asia, grafted plants represent a large percentage of the tomato industry (Leonardi and Romano, 2004; Grieneisen *et al.,* 2018). However, information is still needed to answer the questions like how to graft vegetables, which one is compatible to which, how to develop and exploit the vegetable grafting techniques and understand the potential contribution of vegetable grafting for sustainable vegetable production (Giuseppe and Cardaralli, 2014).

2.5. Effects of Grafting on Growth, Yield and Fruit Quality of Tomatoes

2.5.1. Grafting methods used for tomato

Grafting of tomato plants involves the joining of two seedlings of different plants, with one serving as the rootstock and the other as the scion (Grieneisen *et al.,* 2018). It can be either with other crops of similar species like *Solanaceae* families or self-grafting (Poudel and Lee, 2009; Kumar, 2011).

Different types of grafting techniques including whip grafting, tongue, and cleft grafting can be used for tomato grafting. According to Koirala (2018) whip/Clip grafting technique is the simplest method to join a stock and scion and suitable for

herbaceous plants. For successful grafting, the stock and scion should be less than 1 inch in diameter and of equal thickness (Kumar, 2011).

In the case of whip grafting long, diagonal cuts of equal lengths are made on root stock and scion so as to exactly fit the cut surfaces. The two pieces of stock and scion should be hold together with plastic tape or other tying materials. When the diameter of stock and scion are not equal, the scion should be placed to one side of the stock to ensure cambial contact on at least one side (Grieneisen *et al*., 2018). According to Kumar (2011), greenhouse tomatoes, eggplants, cucurbits such as zucchini, pumpkin, and squash are some examples of vegetable crops that can be grafted by whip or splice grafting.

2.5.2. Effects of grafting on growth of tomatoes

Grafting influences most of the growth performance of vegetable crops including tomatoes (Kumar *et al.,* 2011). Based on the rootstock species and variety, grafting can improves the growth and development of tomato plants which associated with higher concentration of chlorophyll fluorescence and photosynthetic pigments in leaves due to better nutrient translocation and availability (Rivero *et al.,* 2004; Leonardi and Giuffrida, 2006; Kumar *et al.,* 2011; Draie, 2017). Therefore, grafted tomato plants generally produces more vigorous, higher branch number, longer stem and highest aboveground dry weight of the vegetative organs compared to non-grafted plants (Khah *et al.,* 2006 and Yaris, 2011; Draie 2017; Ulas *et al.,* 2019). In addition to growth it also influences days to physiological maturity and days to first harvest of tomatoes (Pogony *et al*., 2005; Nkansah *et al.*, 2013).

2.5.3. Effects of grafting on yield and quality of tomatoes

Yield and quality of tomatoes is influenced through various agronomic practices including grafting. Pogony *et al.* (2005) investigated the effects of grafting on yield and quality of greenhouse tomato. According to the results of the authors, fruit yield was significantly higher on grafted tomato plants than on Non-grafted one. Nkansah *et al.* (2013) also reported that grafted tomatoes on *solanum* rootstocks performed better in terms of growth, yield, disease incidence and flood tolerance. Colla *et al.* (2008) concluded from researches they conducted in soilless media that higher marketable fruit yield was harvested from grafted tomato.

Pék *et al*. (2007) reported the production of significantly more number of fruits on grafted tomato plants in five consecutive harvests than Non-grafted plants. Therefore, grafting improves the yield and yield components of tomatoes including flowers number per cluster, marketable and unmarketable fruit yield (Khah, *et al.,* 2006; Nkansah *et al.,* 2013) that can be partly through the increased fruit number and partly by increase in fruit weight (Pogony *et al.,* 2005).

Furthermore, although higher total costs in grafted tomatoes, particularly labor costs; the difference in revenue was large enough to make grafted tomato significantly more profitable (Wang and Chen, 2012; Milenković *et al.*, 2018).

Not only the quantity but also the quality of tomato can be influenced by grafting of on different root stocks whereby fruit quality is described by size, firmness, pH content, total soluble solid content and other physical and chemical properties. In this regard,

Pogony *et al.* (2005) reported that soluble solids (Brix°), carbohydrate and organic acids concentrations and the ratio of carbohydrate and acid content of fruits harvested from grafted tomatoes were lower than those from Non-grafted ones. According to Nkansah *et al.* (2013) fruit TSS didn't have significant differences on grafted and non-grafted fruits. According to Poudel & Lee (2009) the fruit size of tomato grafted on eggplant rootstock was significantly smaller than non-grafted and self-grafted.

Both horizontal and vertical diameter of the fruits of non-grafted and self-grafted possessed significantly higher values than tomato grafted on eggplant rootstock. Therefore, although fruit quality values were lower in grafted plants, these values were still satisfactory and lied within the adequate ranges (Khah *et al.,* 2006 and Milenković *et al.*, 2018).

2.5.4. Effect of grafting on salt tolerance of tomato plants

Salinity is among abiotic factors that affect the overall life of plant through imposing both osmotic and specific ion stresses (Castillo *et al.,* 2007). Tomato growth is sensitive to salt concentration above 2.5dS/m, which causes yield reduction (Dessalegn 2002; Ezin *et al.*, 2012; Zhai *et al.,* 2015; Umar *et al.,* 2018). Furthermore, the increment of salinity cases blossom end rot that can result fruit quality loss (Zhai *et al.,* 2015 and Adams *et al.*, 2008).

Grafted plants deploy numerous and diverse physiological and biochemical mechanisms to cope with salt stress (Korrekturen, 2012). It is also reported that grafting tomato on salt tolerance root stock is considered as the best solution (Fernandez-Garcia *et al.,* 2004). In this regard, Mahmoud (2014) observed significantly better vegetative growth, yield and water use efficiency of tomato plants which were grafted on salt-tolerant varieties compared to those none grafted plants.

Generally, the grafted plant on salt tolerant root stock still produced higher yield while the yield of non-grafted plants was reduced with the increment of salt level (Mahmoud, 2014; Korrekturen, 2012; Semİz *et al*., 2015). Similarly, tomato plants grafted on salt-tolerant rootstock withstand the salt stress and gave better yield than Non-Grafted one where the ability of the plant to withstand the stress depends on the type of rootstock (Bahadur *et al.,* 2015).

2.5.5. Role of grafting tomato in organic farm

The accumulation of harmful chemicals that results from pesticide application throughout the world is increasing from time to time. Despite, converting crop production from conventional to organic farming could reduce the persistent and harmful pesticides from entering the environment (Chait, 2019).

The technology of vegetable grafting is the best way of reducing pesticide contamination of crops and environment since it can be considered as non-chemical pest control methods (Aktar *et al.,* 2009; Martin, 2009). Therefore, it is effective integrated pest management (IPM) tool for the management of soil-borne diseases (Louws *et al.,* 2010; Guan *et al.,* 2012; Kyriacou, 2017). Various researches also recommended the technology as one of the best methods to be included in organic farming practices (Aktar *et al.,* 2009; Martin, 2009; Pogony *et al.,* 2005; Kyriacou, 2017).

Despite the relatively high cost of grafted transplants, due to increased labor and input for their production, grafting has evolved into a unique cultural practice that helps to reduce pesticide use, enhances yield and production efficiency, and improves economic viability in sustainable vegetable production under both open field and protected culture systems (Kubota *et al*., 2008).

2.6. Vegetable Grafting in Ethiopia

In Ethiopia there are abundant production lands which have different agroecology. The country has quality groundwater potential of the country is about 2.6 billion cubic meters (Agency, 2012)

The elevation ranges from -47 up to 3000 m.a.s.l. allows the productions various tropical subtropical and temperate crop such as onion, tomato, garlic, shallot, capsicum, , cole crops, Ethiopian kale, head cabbages, muskmelon, watermelon, pumpkin, beetroot, carrot, snap bean, sweetpotato, Irish potato, anchote, colocosia, yam, taro, moringa etc. throughout the country. Although grafting is known technology in Ethiopia its applicability to vegetables is not yet adopted (Yassin & Hussen, 2015).

3. CONCLUSION

Grafting positively affects the life of plants in different ways. As various researches indicate grafted tomato plant was well performed both in growth and eventually in yield related parameters without quality reduction in most cases. To boost the production of tomato crop in the areas where there are different biotic and abiotic factors it is necessary to use grafting technology with rootstock which is resistant to disease and tolerant to abiotic factors that challenging the crop production in the world. Especially in Ethiopia the yield reduction due to different factors can get solution by using grafting which is almost new technology for the country.

4. Future Prospect

In Ethiopia the culture of grafting vegetable is not yet adopted. Even though, some researchers and MSc. Students are trying to conduct the research on grafting. To adopt such important technology, it is necessary to conduct researches regarding tomato grafting. Furthermore, the rootstocks with desired characteristics should be identified within the species through resarches.

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In-vitro embryo production and transfer technology in cattle: An updated review article

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ABSTRACT

The use of in vitro technique with domestic animal gametes and the methods of embryo manipulation for assisted reproduction is currently the most advanced method in cattle breeding with regard to scientific research and practice. In vitro embryo production (IVP) is the process of production of live embryos in the laboratory rather than in vivo by collecting oocytes from slaughterhouse ovaries as well as from live donor cows. IVP entails the completion of three biological steps that are now relatively well established in cattle: oocyte in vitro maturation, in vitro fertilization and embryo culture. Oocyte maturation is a complex process involving nuclear and cytoplasmic maturation in appropriate media. To enhance successful fertilization of matured Oocytes, sperm cells must be motile, have the ability to undergo capacitation and express the acrosome reaction. In vitro embryo culture systems depend on multiple parameters such as the composition of culture media and gases. The most common media used in culture systems are synthetic oviduct fluid (SOF), potassium simplex optimization medium (KSOM), and complement receptor 1 (CR1aa). Embryos of appropriate quality can be transferred directly to recipient cows or frozen for future use. Embryo transfer (ET) is a multi-step process that involves the production and collection of pre implantation embryos from genetically superior females and the subsequent transfer of those embryos into reproductively healthy females for the purpose of establishing pregnancies and producing live offspring. ET technology is an important tool to improve livestock at faster rate as well as gives an opportunity to utilize the genetic contribution of both male and female at the same time. Selection of recipient and proper recipient herd management are vitally important to the success of transferred cow embryos. There are two methods of transferring embryos; surgical and non-surgical. There are problems associated with in vitro embryo production. Low success rates limit the contribution that the IVP technique can make, especially to genetic improvement.

Keywords: *Bovine, Embryo transfer, In vitro, embryo production, Review*

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1. INTRODUCTION

The development and application of assisted reproduction technologies like artificial insemination(AI), estrus synchronization, embryo transfer, in vitro fertilization, transgenesis, cloning and semen sexing in animal husbandry can be expected to bring about a faster multiplication and propagation of superior genetic animal species (Manik *et al*., 2003; Mondal *et al*., 2014). The use of in vitro technique with domestic animal gametes and the methods of embryo manipulation for assisted reproduction is currently the most advanced method in cattle breeding with regard to scientific research and practice (Samardzija *et al.*, 2015).

In vitro embryo production (IVP) is the process of production of live embryos in the laboratory rather than in vivo by collecting oocytes from slaughterhouse ovaries as well as from live donor cows (Bilodeau-Goeseels and Panich, 2002; Chaubal *et al.*, 2006). IVP is not a single step process but it comprises in vitro maturation (IVM) followed by sperm capacitation, in vitro fertilization (IVF) of matured oocytes and then in vitro culture (IVC) of fertilized oocytes for 7 days till the stages of morula or blastocyst (Lojkic *et al.,* 2014). In vitro embryo development is strongly influenced by events occurring during oocyte maturation, fertilization and the subsequent development of the fertilized oocytes. So, improving the efficiency and identifying the sources of variations between IVF systems or between different laboratories are more important when routinely producing blastocysts from individuals of high genetic merits (Abd El-Aziz *et al.,* 2016).

Embryo transfer is a multi-step process that involves the production and collection of preimplantation embryos from genetically superior females (called donors) and the subsequent transfer of the harvested embryos or in vitro produced embryos into reproductively healthy females (called recipients) for the purpose of establishing pregnancies and producing live offspring (Youngs, 2007). Embryo transfer (ET) is universally recognized as the final and most critical stage in an in vitro fertilization (IVF) program (Neithardt *et al*., 2005). Recipients play a critical role in the success of ET program (Bó *et al*., 2002). Recipients must be synchronous with the stage of development of embryos that had been previously frozen (Mapletoft, 2006). Prior to transfer, harvested embryos must be evaluated using a microscope in order to assess their developmental stage and quality. The International Embryo Transfer Society has adopted a uniform standard for evaluating and describing cattle embryos (Fufa *et al.,* 2016). Therefore the objective of review is to identify the role of in vitro embryo production and embryo transfer in genetic and production improvement in cattle as well as its application.

2. OVARIAN DYNAMICS IN CATTLE

The cow is a polyestrous animal; therefore, once estrous cycles are established, they continue indefinitely unless interrupted by pregnancy. In the non-pregnant cow, ovulation occurs at approximately 21-day intervals. Cellular division of oocytes (oogenesis) begins early in fetal life.

Division is arrested at the diplotene stage of the first meiotic division, only to resume at the onset of the estrous cycle in follicles destined for ovulation. At birth, the ovaries of the female calf contain all the oocytes she will ever produce from 200,000 up to about half a million (Ball and Peters, 2004). These immature oocytes remain in a resting phase known as germinal vesicle (GV) stage (Landim-Alvarenga and Maziero, 2014). Oocytes are produced and matured, involving nuclear and cytoplasmic maturation, in the ovary (Sirard and Coenen, 2006). The process of nuclear maturation begins when meiosis resumes from the diplotene stage, signaled by chromosome condensation and germinal vesicle breakdown (GVBD). It corresponds to the reversal of the first blockage of meiosis from the germinal vesicle stage until the second blockage at metaphase II. After GVBD, the oocyte goes through the first meiotic division and then rapidly passes through metaphase II of second meiotic division where a second meiotic arrest occurs (Kubelka *et al*., 2000; Hyttel *et al.*, 2000). By the end of the second meiotic division, homologues of oocyte chromosomes (2n) have separated into two nuclei with n chromosomes each. The cytoplasm divides asymmetrically generating two cells, one keeping almost all the cytoplasm, the secondary oocyte and the other very small, first polar body. Soon after, the second meiotic division begins and remains in MII until the fertilization or parthenogenesis activation (Quetglas *et al*., 2010).

The progression of meiosis that characterizes nuclear oocyte maturation, does not by itself guarantee further embryonic development; additional cytoplasm modifications is also necessary (Ferreira *et al*., 2009). Cytoplasmic maturation includes structural and molecular changes occurring in the oocyte from the GV stage to the end of MII. The ultra-structural changes in the cytoplasm include migration of several of the organelles. The mitochondria and Golgi complex, which are located in the periphery of the oocyte in immature oocytes, move to aper nuclear distribution. The cortical granules, originating from the Golgi complex, and originally situated in the center of the oocyte migrate to the periphery and become anchored to the oocyte membrane (Landim-Alvarenga and Maziero, 2014).

At ovulation the ovum or egg is collected by the infundibula end of the oviduct or fallopian tube. It is transported down the oviduct towards the uterus possibly by a combination of cilia (hair-like) action and muscular contractions. Transport through the oviduct appears to be under the control of ovarian steroid hormones since estrogens reduce and progesterone increases the speed of passage of ova through the oviducts. Fertilization normally occurs in the ampulla section of the oviduct close to the junction with the isthmus. In the cow, the ovum enters the uterus 4 -5 days after ovulation (Hill, 2017).

Mammalian spermatozoa acquire motility and part of their capacity to fertilize the ovum, during their passage through the epididymis (Mújica *et al*., 2003). However, before spermatozoa are able to fertilize the ovum, they have to also undergo a further series of maturational changes in the female tract. These processes are known as capacitation and the acrosome reaction and are thought to require about six hours in the cow. The acrosome reaction is necessary to allow penetration of the oocyte by the sperm. Normally, only one sperm is able to pass through the zona, but when more enter, a process known as polyspermy, the resultant embryo is non-viable. Penetration by the fertilizing sperm (pronucleus) stimulates the resumption of the second meiotic division of the oocyte and the extrusion of the second polar body. Fertilization is completed with the fusion of the haploid male and female pronuclei, a process known as syngamy (Gordon, 2003).

The ovum begins to divide mitotically, a process known as cleavage, immediately after fertilization is complete. Division continues so that a solid cluster of cells or blastomeres known as a morula (mulberry shape) is formed by five or six days. From about day 6 after fertilization, the ovum begins to hollow out to become a blastocyst. By day 16, the embryo is sufficiently developed to signal its presence to the maternal system and prevent the luteolysis by mechanism of hormonal changes (Hunter, 2003).

3. IN VITRO EMBRYO PRODUCTION

The first calf from IVF, which is called "Virgil", was reported in 1982 after aspiration of an in-vivo matured oocyte, i.e. an oocyte collected immediately before ovulation, which was submitted to IVF and immediately transferred to a recipient. At that time it was restricted to research investigations and was applied to rescue follicular oocytes of slaughtered donors but it has since become an increasingly important tool employed commercially for assisted production in many farm species and leads to the beginning of IVF as a tool for production in the cattle industry (Velazquez, 2008; Parrish, 2014). In cattle, besides used as research tool in slaughtered donors, IVP has become important for the production of embryos from live donors as an alternative to or integrated with multiple ovulation embryo transfer (MOET) because of the advantages and flexibility that it offers (Bizelew and Getahun, 2016). IVP entails the completion of three biological steps that are now relatively well established in cattle: oocyte in vitro maturation, in vitro fertilization and embryo culture (Samardzija *et al.*, 2015). However, primary oocytes collection should be added upstream of these major steps and embryo management should be added downstream to give a complete overview of the whole process. Once the immature oocytes have been removed from the ovary, they are matured, fertilized, and cultured in vitro for up to seven days until they develop to a stage that is suitable for transfer or freezing (Bizelew and Getahun, 2016).

Superovulation for invitro embryo production

Superovulation is when an animal is induced (usually through use of injectable hormones) to ovulate multiple ova (the hormonal treatment for harvesting increased

number of oocytes from the ovary than normal). This is usually done on animals with superior genetics, but any cycling female can be super ovulated (Stenbak *et al*., 2001). It is important to recognize that super stimulatory protocols used for the production of embryos in vivo are different than those used to produce embryos in vitro with oocytes obtained via ovum pickup (OPU). The objective of superovulation in MOET programs is to maximize the number of ovulations without compromising embryo quality, whereas super stimulatory treatments prior to OPU are aimed at increasing the number of follicles suitable for puncture, preferentially with a diameter between 5 to 10 mm (Karadjole *et al*., 2010).

There are two glycoprotein hormones that may be administered to induce superovulation. The hormone follicle stimulating hormone (FSH) is the hormone naturally responsible for recruitment and growth of ovarian follicles during the estrous cycle. Super ovulatory treatments of donor females with FSH are typically initiated on days 8-10 of the estrous cycle in order to stimulate follicles at the start of the second follicular wave when a dominant follicle is not present (Tesfaye *et al.,* 2016). Because the half-life of FSH is short (~ 4 to 6 hours) in cattle, FSH must be given as a series of multiple injections administered over a period of several days. A typical superovulation regimen for beef cattle involves a series of eight injections of FSH administered at 12-hour intervals over a 4- day period. The FSH may be given as a constant dose (all eight injections are the same amount of FSH) or as a descending dose (FSH doses get smaller over time). An injection of PGF2 should be given in conjunction with the 6th (or 7th or 8th) dose of FSH in order to induce luteolysis and enable the donor to exhibit estrus (Youngs, 2007). The other hormone is pregnant mare serum gonadotropin (PMSG); more recently termed equine chorionic gonadotropin (eCG) (Sendag *et al*., 2008). PMSG is a glycoprotein hormone found in the blood of the mare between days 40 and 130 of gestation and is unique among gonadotrophins in possessing both FSH and luteinizing hormone (LH) biological activities within the one molecule (Gordon, 2003).

Oocyte collection

The oocytes for in vitro production of bovine embryos could be harvested from slaughterhouse ovaries as well as from live donor cows (Chaubal, 2006).

For the setup of the techniques as well as for the large scale production of average genetic merit embryos, large quantities of material can be obtained at low cost by collecting ovaries at slaughterhouses (Mermillod *et al*., 2006). Slaughterhouse derived ovaries contain a highly heterogeneous population of oocytes that are recovered regardless of follicular dynamics and may come from cattle with diverse reproductive backgrounds. Production of embryos from slaughterhouse ovaries begins with oocytes collected from all follicles visible on the surface of the bovine ovary. Oocytes obtained from slaughtered animals are variable in their developmental competence after IVM (Bilodeau-Goeseels and Panich, 2002). Bovine ovaries obtained from abattoirs should transport to the laboratory in physiological saline with antibiotics (penicillin and streptomycin) at 37 °C within 3 h of slaughter. Cumulus oocyte complexes (COCs) can be aspirated from 2 to 8 mm diameter follicles using an 18G needle attached to a

vacuum pump. Then aspirated follicular fluid should transfer from the collection tube to a perti dish (Karadjole *et al*., 2010). One of the main disadvantages of collection of ovaries from slaughter house is the lack of repeatability. Once the cow has been slaughtered there can be no further collection of oocytes from the same donor. In conjunction with in IVP, OPU technique provides an alternative means to increase the number of offspring from genetically valuable cows (Galli *et al*., 2001).

Oocyte recovery from live donors

Oocytes are more frequently recovered from live animals by transvaginal ultrasound-guided oocyte collection or OPU. The technique of ultrasound-guided transvaginal follicular aspiration is a noninvasive procedure for recovering oocytes from antral follicles in live animals. Using transvaginal OPU ova can be harvested repeatedly from the same cow, potentially providing many more ova (Qi *et al.*, 2013). According to Ball and Peters (2004) an eight-year-old cow has produced 176 embryos during 158 weekly collections and aspiration of ovum twice per week may be better than once per week as it is more likely to pre-empt the development of a dominant follicle that could have a negative effect on results. In vitro fertilization in conjunction with ovum pick-up (OPU) has become important for the production of embryos from super stimulated donors (Galli *et al*., 2001).

The equipment required for oocyte recovery in live donors consists of an ultrasound apparatus whose transducer has a built-in needle guide, echogenic needle (18G disposable needle, attached to the stainless steel guidance), sterile collection tube and suction pump. After epidural anesthesia of the donor, the transducer is introduced into the vagina and the ovary to be aspirated is moved per rectum to a position just anterior to the vaginal fornix. Thus, follicles can be imaged and the needle guide used to pass an echogenic needle through the vaginal wall into the ovary. Target follicles are positioned and aspirated by means of a foot-controlled vacuum into a sterile collection tube with aspiration medium Tissue Culture Medium 199 (TCM 199) hepes supplemented with antibiotics, heparine and bovine serum albumin attached to a suction pump to ensure constant aspiration pressure. The follicular fluid obtained by aspiration should transferred into an embryo transfer filter (Ball and Peters, 2004; Getz, 2004; Galli and Lazzari, 2005).

Table **1**: the recovered oocytes are identified under a stereomicroscope, counted and morphologically classified into four categories.

Grade	Morphology
1	Oocytes completely surrounded with more than three layers of cumulus cells and evenly granulated ooplasm that completely filled the zona pellucida
2	Oocytes with one to three cell layers and with evenly granulated ooplasm
3	Partially denuded oocytes with unevenly granulated ooplasm
4	Completely denuded oocytes, degenerated oocytes, expanded oocytes

Source: Karadjole *et al*., (2010)

Oocyte maturation

Mammalian oocytes are arrested at the diplotene stage of the first meiotic division. In response to the preovulatory surge of gonadotrophins some of the oocytes undergo resumption of meiosis characterized by germinal vesicle breakdown, chromosome condensation, formation of the first meiotic spindle, expulsion of the first polar body and arrest in metaphase of the second meiotic division. These events are defined as oocyte maturation and lead to an ovulated fertilizable oocyte (Ball and Peters, 2004). Therefore the goal of IVM is to cause an immature oocyte from a developing follicle to resume meiosis and render itself capable of fertilization, arrested at the second metaphase step of meiosis just like in vivo matured counterpart (Gibson, 2014).

The oocytes are subsequently matured for 24-26 hour in vitro. The maturation of oocytes invitro mainly depends on medium components (cell culture medium, serum, and antibiotics) and incubation conditions. The medium component includes TCM-199 supplemented with sodium pyruvate, porcine follicular stimulating hormone (p-FSH), estradiol, epidermal growth factor (EGF), fetal calf serum (FCS) and gentamycin (Shabankareh, 2014). But oocytes are very sensitive to temperature shocks so it is important to monitor carefully the collection procedure temperatures as fluctuations can easily occur (Youngs, 2007). Thus oocytes should be cultured at 38.5°C under 5% CO2 and 95% air atmosphere with saturated humidity for 24 hours (Gibson, 2014).

Evaluation and selection of oocytes

The success of IVM of bovine oocytes is measured by morphological assessment of proliferation of cumulus oophorus and corona radiata cells, thickness and compactness of the cumulus investment and the homogeneity of the ooplasm. The degree of nucleus maturation is measured as a percentage of oocytes in the stage of metaphases II (MII) with the first polar body expelled (Lojkic *et al*., 2014). Consequently, morphological evaluation alone is insufficient to distinguish competent oocytes that have the ability to bring about full-term pregnancy. On the other hand, only 30-40% of the zygotes obtained after in vitro maturation and fertilization (IVF) will reach the blastocyst stage in culture. This is probably due to the quality of the oocytes at the beginning of maturation, and indicates the need for the development of other approaches. Different selection criteria have been used to predict the quality of these oocytes and to improve the embryo development such as oocyte diameter, brilliant cresyl blue (BCB) test and the follicle diameter (Shabankareh, 2014).

Sperm preparation and in vitro fertilization (IVF)

In vitro capacitation procedures are aimed at stimulating the sequence of events that normally occur in the cow's reproductive tract. This involves the removal of seminal proteins and other substances that coat the sperm membrane of the ejaculated semen. To do this, sperm cells are subjected to thorough washing and exposure in media with elevated ionic strength (Duran, 2000). Frozen semen is generally used and a selection step is required to enrich the sperm suspension in living and motile spermatozoa. In the swim-up method, spermatozoa are deposit at the bottom of a culture medium containing tube and allowed to swim in the medium. The top fraction, containing motile sperms is then collected after a given incubation time. Alternatively, motile sperms

may be purified by centrifugation in a discontinuous density gradient. The motile fraction is collected at the bottom of the tube after centrifugation (Mermillod *et al*., 2006).

The sperm concentration used for IVF ranges between 0.5 and 2x106 spermatozoa per ml in fertilization medium containing caffeine, heparin and bovine serum albumin (BSA). This concentration should be optimized for each male and even for each ejaculate from a given male. The fertilization rate increases together with sperm concentration. However, after a given value, an increasing proportion of the oocytes become polyspermic. A compromise has to be established for high fertilization rate with low polyspermy rate (Gibson, 2014; Pereira *et al*., 2005).

The IVF is a mixed cultivation of mature oocytes with spermatozoa in strictly controlled laboratory conditions. The co-incubation of spermatozoa with oocytes lasts for 24 hours, at 39°C with 5% CO2 in the air and 80% humidity after which the first mitosis occurs. The success of IVF of bovine oocytes is estimated 48 hours following fertilization by a number of cleaved embryos and by identification of male and female pronucleus which may be visible after 18-22 hours of coincubation (Samardzija *et al.*, 2015).

Embryo culture

The last stage of in vitro production of bovine embryos is the culturing of embryos for 7 days, from the zygote until the blastocyst stage when such embryos may be transferred into a recipient or frozen and stored in liquid nitrogen (Rynkowska *et al*., 2011). During IVC, the embryo is passing through four important developmental stages: the first mitosis, activation of embryonic genome, compaction in morula stage, the formation of the blastocyst with differentiation of their cells (Samardzija *et al.*, 2015).

In vitro embryo culture systems depend on multiple parameters such as the composition of culture media and gases (Kocyigit, 2016). An efficient culture system for in vitro embryo development should be formulated to protect from intracellular stress and include all demands of the embryo thereby embryo can maintain viability. Various approaches have been employed to improve the culture systems of bovine embryos in vitro. The plurality of research toward improving embryo development in vitro has focused on culture composition, soluble chemicals and surrounding medium components (Lonergan and Fair, 2014).

There are several articles on the composition of embryo culture media and the role of specific medium components in supporting mammalian embryo development in vitro. It is essential to appreciate that the culture media are only one part of the overall culture system. Optimal culture media for embryo production appear to require a combination of various factors such as hormones, cytokines, growth factor, antioxidants, vitamins, enzymes and macromolecules (Rezaei and Chian, 2005; Block and Hansen, 2007). The formulations of culture media are a key aspect of embryo culture (Gordon, 2003).

The most common media used in culture systems are synthetic oviduct fluid (SOF), potassium simplex optimization medium (KSOM), and complement receptor 1

(CR1aa); nevertheless, other media, such as G1.1/G2.2, complement receptor 2 (CR2aa), and TCM199, (Farin *et al*., 2001).
There are different culture systems available for in vitro fertilized oocytes. They can be classified according to their formulation as follows: In vivo, where fertilized oocytes are placed in the oviduct of a synchronized female and grown for 6-8 days and can be performed across species, e.g., bovine or equine embryos can be successfully cultured in the sheep oviduct; Co-culture, where fertilized oocytes are placed in culture drops containing cells from the oviduct to try to mimic the maternal environment (Galli and Lazzari, 2005); Semi-defined medium, where fertilized oocytes are placed in culture drops containing serum but the composition of the serum is variable and usually unknown; Defined medium, where fertilized oocytes are placed in culture drops where concentrations of all the components are known including growth factors; or Sequential culture, where embryos are placed in culture medium containing the nutrients necessary for one specific stage of development, mimicking the maternal environment (Mermillod *et al*., 2006, Feugang *et al*., 2009).

Table **2**: Supplementation with factors towards improving defined media

Factor	Action	Reference
Colony-stimulating factor 2	Blocked apoptosis in bovine embryos through regulatory actions on apoptosis genes.	Loureiro *et al.*, 2011
Epidermal growth factor	Besides its role on proliferation, migration and differentiation, EGF is involved in reduction of apoptosis during preimplantation and placentation.	Gordon, 2003
Fibroblast growth factor 2	FGF2 receptor activity were necessary for optimal development	Fields *et al.*, 2011
Preimplantation factor	Involved in modulation of local immunity, promoting decidual proadhesion molecules and enhancement of trophoblast invasion.	Stamatkin *et al.*, 2011
Insulin, transferrin, sodium selenite	Insulin exerted its actions on glucose transport and AA uptake along with antioxidant protection from transferring and selenium.	Gordon, 2003
GH and IGF-I	In combination stimulated embryonic development.	Moreira *et al.*, 2002
IGF-II, FGF2, TGFβ1, CSF2	Results from the combination were similar to culture under serum conditions	Neira *et al.*, 2010
leukemia inhibitory factor (LIF)	LIF receptors have been detected in embryos of different species that is considered essential for blastocyst development such as hatching rate, cell count and cryotolerance	Kocyigit and Cevik, 2015
Activin A	Use of activin A shortened the lag phase and was also beneficial at later stages; however, more rapid rates of development were associated with higher rates of apoptosis.	Gordon, 2003
Thyroid hormone	On the bovine embryo TH is involved in lowering apoptotic rates as well as improved hatching rates post thawing for cryopreserved embryos.	Ashkar *et al.*, 2010

In culturing of embryos the factors should be considered includes gas phase, pH, osmolality, temperature, water purity and sterility. Gas phase adjusted in embryo culture can be done using determined concentration of gases. The two most commonly used gases are 5% CO2 in air, 5% O2 and 88% to 90% N2. This gas mixture allows for better embryo development because it's thought to be more similar to the condition found in the oviduct and uterus (Sirard and Coenen, 2006; Karadjole *et al*., 2010). The pH of the culture medium should be between 7.2 and 7.4 and
Osmolality should be between 260 – 280 mOsm/kg. The culture temperature should be the same temperature found by the embryo in the oviduct and uterus. In the cow, the normal temperature ranges from 38.5 to 39 oC and so this temperature should use during culture. Resistivity of water must be less than 18 mOhm and procedures should be carried out taking care of keeping the culture sterile, using sterile techniques and antibiotics if necessary (Cevik *et al*., 2009).

EMBRYO EVALUATION

Embryos are classified and evaluated by morphological examination at 50 to 100 X magnification in small culture dish according to the manual of the International Embryo Transfer Society. The overall diameter of the bovine embryo is 150 to 190 um, including a zona pellucida thickness of 12 to 15 mm. The overall diameter of the embryo remains virtually unchanged from the one-cell stage until blastocyst stage. An ideal embryo is compact and spherical. The blastomeres should be of similar size with even color and texture. The cytoplasm should not be granular or vesiculated. The perivitelline space should be clear and contain no cellular debris. The zona pellucida should be uniform; neither cracked nor collapsed and should not contain debris on its surface. Embryos of good and excellent quality and at the developmental stages of late morula to blastocyst yield the highest pregnancy rates. It is advisable to select the stage of embryo development for the synchrony of the recipient (Mapletoft, 2006).
Embryos are graded on a scale from one (excellent) to four (poor) with no or few extraneous degenerated cells (table 3).

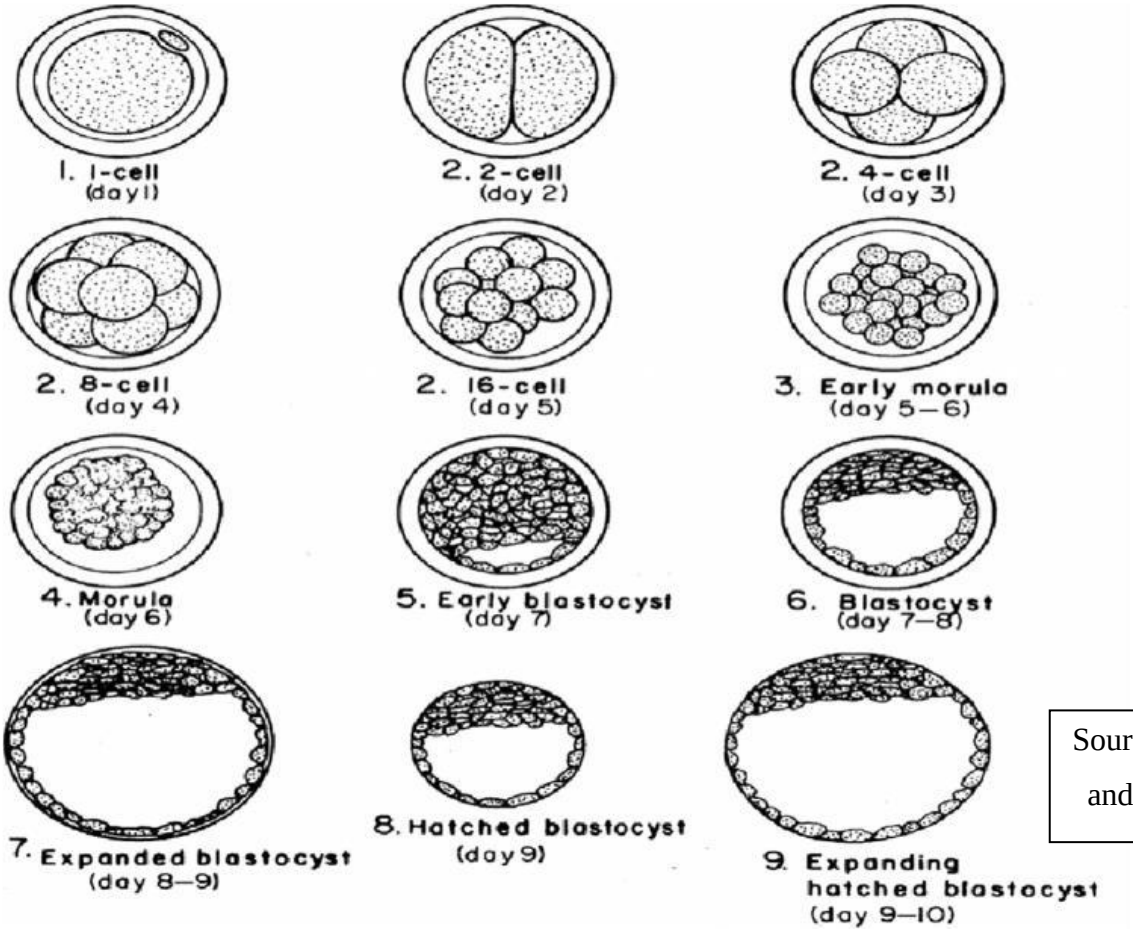
Table 3: Embryo quality grades

Grade	Description
1	Excellent or Good: Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Structural Irregularities should be relatively minor, and at least 85% of the cellular material should be intact, viable embryonic mass. This judgment should be based on the percentage of embryo cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a Petri dish or a straw.
2	Fair: Moderate irregularities in overall shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass.

3	Poor: Major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass.
4	Dead or degenerating: Degenerating embryos, oocytes or 1-cell embryos. These embryos are non-viable.

Source: Genzebu, (2015); Mapletoft, (2006)

Embryos of appropriate quality (1 or 2 preferably) can be transferred directly to recipient cows or frozen for future use (Genzebu, 2015). The visual assessment of an embryo's morphologic characteristics is a subjective evaluation. The best predictor of an embryo's viability is its stage of development relative to the given day after fertilization (Phillips and Jahnke, 2016).



Source: Stringfellow and Seidel, (2010)

Figure 1: Illustration of normal bovine embryonic development

EMBRYO TRANSFER

Embryo transfer technology is an important tool to improve livestock at faster rate as well as gives an opportunity to utilize the genetic contribution of both male and female at the same time. With the help of ET or MOET techniques faster improve of livestock, rapid expansion of elite animals, genetic gain, accelerated herd development and conservation of rare genetic stocks could be achieved (Bizelew and Getahun, 2016).
ET does not have been only done immediately after collection of embryo. However, it can be also a frozen, either conventionally (slow freezing using ethylene-glycol) or by vitrification, which ensures safe storage and better management of the genetic resources (Saragusty and Arav, 2011). ET of in vivo (or in vitro) produced embryos to the uterus of a recipient cow is easy and reliably done by transcervical intrauterine deposition, with more than 60% of pregnancy rates. Use of OPU for collection of oocytes and the following in vitro embryo production technique make the MOET more effective since it waives superovulation and AI treatment (Presicce *et al*., 2011).

The first successful transfer of mammalian embryos was performed by Walter Heape in 1890. Heape transferred two four-cell Angora rabbit embryos into synchronized Belgian doe, which subsequently gave birth to four Belgian and two Angora young (Betteridge, 2003; Nagassa, 2016). However, the first successful cattle embryo transfer was reported 70 years later (Youngs, 2007). Today, more than ¾ million bovine preimplantation embryos are transferred each year throughout the world (Thibier, 2006).

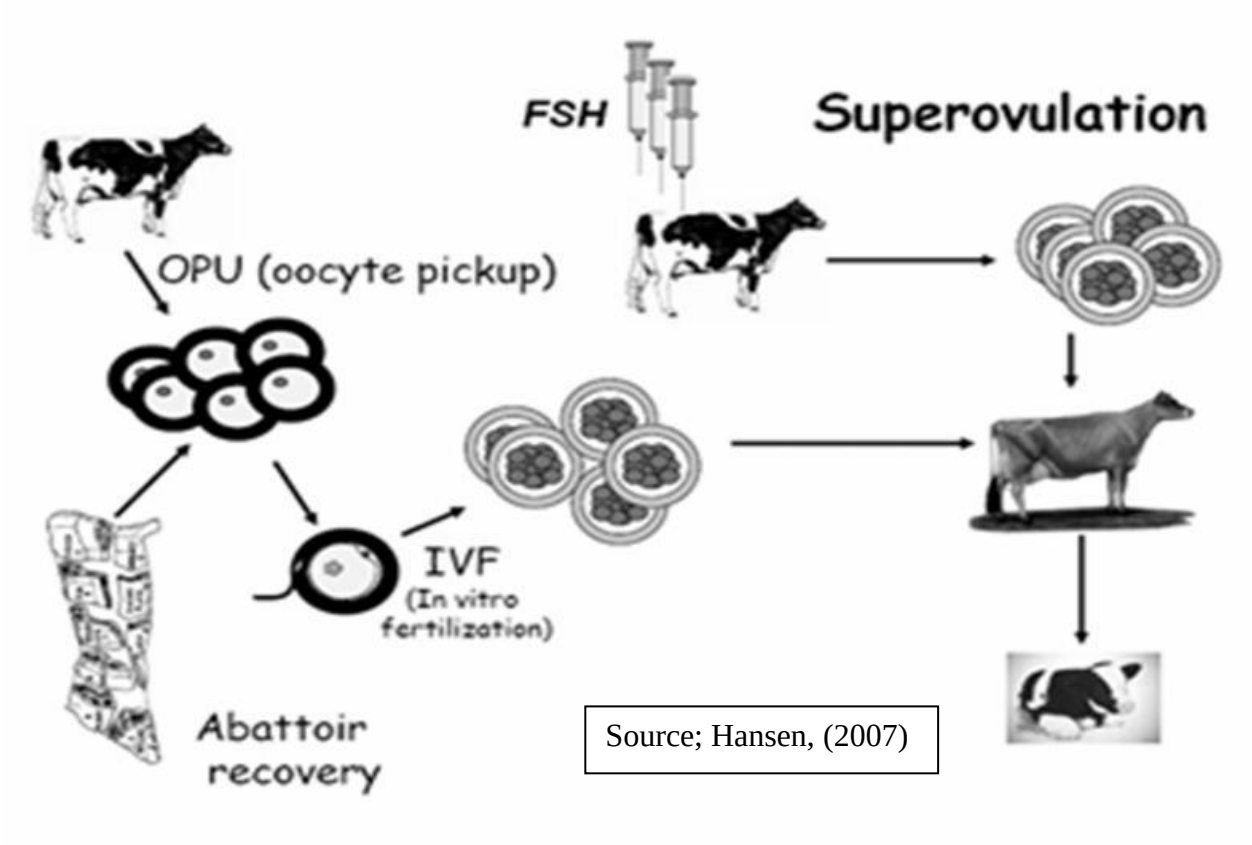


Figure 2: the production of embryo invitro (oocyte collection and IVF) and embryo transfer

Selection of recipients

Recipient (surrogate) cows are vitally important to the success of transferred cow embryos (Stroud, 2010). The surrogate contributes no genetic material to the offspring; she does exert important maternal environmental influences which affect the physical makeup (phenotype) of the calf. For example, her milk production will affect calf weaning weight. There is also a maternal environmental effect on calf birth weight. Thus she must be on a proper plane of nutrition (body condition score 6 for beef cows and dairy body condition score 3 to 4) (Marahall and Minyard, b2002). Generally females ideal as recipients are as follows; (1). Cows 3 to 8 years old make good recipients once they have a good calving record (have no history of calving problems). Cows are suited for embryos of relatively high birth weights and cows have more milk and less difficulty with calving than heifers. (2). Heifers are good recipients providing they have reached their breeding weight (around 65-70% of mature weight) and are cycling. Heifers are easier to manage than cows because they are not lactating and have higher fertility than cows. But it is more difficult to transfer embryos non-surgically in heifers than in cows. (3). First calvers are still growing and are under more stress than the rest of the herd due to the fact that they have to raise a calf. For these reasons they

should be under an intensive management and receiving enough good quality feed to use as recipients (Bova-Tech Ltd, 2012). (4). Females should be reproductively healthy (e.g., they should have two functional ovaries plus normal oviducts, uterus and cervix), free of reproductive tract adhesions, and free of ovarian follicular cysts (Youngs, 2007; Larson, *et al*., 2010). (5). One must be cautious if candidates for recipients are not lactating (and not pregnant); because this frequently means that they were culled for reasons that will make them subfertile (George *et al*., 2005).

Management of recipients

Proper recipient herd management is critical to embryo transfer success. Once elected, the animals are separate from the rest of the herd to guarantee they are away from the bulls. If animals are estrous synchronized 20 days later and an ultrasound examination should performed to reassure that the animals are not pregnant (Genzebu, 2015). A cow performs her best when she is in moderate body condition thus must be maintained on a good nutrition program (Stroud, 2010). Recipients must be synchronous with the stage of development of embryos that had been previously frozen. Recipients can be made available by maintaining a large herd to obtain natural heats or by estrus synchronization, which is much more economical. Today most recipients are synchronized regardless of whether or not embryos are transferred on farm (Mapletoft, 2006).

After transfer of embryo, the recipient should be left undisturbed as much as possible. Preferably, they should be put in a separate pasture so they are not stressed during gathering and sorting of the non-exposed recipients for further ET procedures. Make sure these cows do not lose weight during the first 90 days after transfer or have any major stressful event. The first trimester of pregnancy is the most sensitive to abortions and early embryonic death. Simply changing diet can cause early embryonic death, presumably from rumen pH changes (Stroud, 2010).

Transfer of embryo

Various methods of transferring embryos have been developed. The efficiency of transfer depends on many factors, but the most important are the experience and skill of the person(s) doing the transfer. There are two methods of transferring embryos; surgical and non-surgical. The surgical methods have in the past given higher and more consistent pregnancy rates. However, skillful technicians can now produce similar high pregnancy rates with non-surgical methods. Therefore, this method has become widely adopted today (Duran, 2000).

Surgical embryo transfer

Embryos can be transferred via mid-line abdominal incision to cows under general anaesthesia, but through flank incision is far more practical. Recipients are placed in squeeze chutes that give access to either flank. The corpus luteum is located by rectal palpation and the flank ipsilateral to the corpus luteum is clipped, washed with soap and water, and sterilized with iodine and alcohol. About 60 ml of 2 percent lidocain is given along the line of the planned incision (Tesfaye *et al*., 2016). Having scrubbed,

the surgeon makes a skin incision about 15 cm long, high on the flank, just anterior to the hip. Muscle layers are separated, and the peritoneum is cut. The surgeon inserts a hand and forearm into the incision, locates the ovary, usually about 25 cm posterior to the incision, and visualizes or palpates the corpus luteum. Then uterine horn is exteriorized by grasping and stretching with the thumb and forefinger. A puncture wound is made with a blunted needle through the wall of the cranial one third of the exposed uterine horn, because it is very fragile. Using about 0.1 ml of medium in a small glass pipette, an assistant draws up the embryo from the storage container. The pipette is then inserted into the lumen of the uterus, and the embryo is expelled. It takes some experience to be confident that the embryo has been deposited in the lumen. The incision is then closed, using two layers of sutures. With practice, the surgery takes about 15 minutes (Marahall *et al*., 2002; Nagassa, 2016).

Non-Surgical embryo transfer

Nonsurgical transfer is preferable, because it is less expensive; it is quicker and does not involve surgical procedures. This may also avoid the need for veterinary supervision, which is required for surgery in many countries (Carlos, 2014). For non-surgical transfer, the deposition of the embryos in the uterus horn in the side of corpus luteum is performed under rectal control with a transfer catheter. Care must be taken especially with young animals to avoid any injuries in the still narrow uterus (Small *et al*., 2004; Melissa, 2009). The equipment required for non-surgical embryo transfer includes petridish, stereomicroscope, transfer straw, transfer gun; transfer sheath and transfer pipette (Trasorras *et al*., 2010).

First, it is necessary to palpate ovaries accurately in order to select the side of ovulation. Pregnancy rates are markedly lowered if embryos are transferred to the uterine horn contralateral to the corpus luteum (Melissa, 2009). Also, recipients should be rejected if no corpus luteum is present or pathology of the reproductive tract is noted. The second step is to pass the embryo transfer device through the cervix. The petridish containing the embryo is placed under the stereomicroscope to load in a 0.25 ml transfer straw. The straw is then loaded into a transfer gun and covered with a transfer sheath. After the straw is loaded the transfer gun will be introduced into the vagina of recipient. The tip of the transfer gun is guided through the cervix by transrectal manipulation and positioned into the uterine horn. Heifers present a special challenge because of the small cervix. The best training prior to undertaking non-surgical embryo transfer is experience in artificial insemination. The third step with non-surgical transfer is to be able to insert the tip of the instrument into the desired uterine horn quickly, smoothly and atraumatically (Trasorras *et al*., 2010; George *et al*., 2005). The uterine lumen should be lined-up prior to transfer so as to prevent trauma to the endometrium during passage. The embryo is deposit slowly and firmly while slightly withdrawing the tip of the transfer pipette. Great care must be taken to not cause damage to the lining of the uterus (Melissa, 2009). After transfer appropriate management of the recipient is crucial for good result including definitive pregnancy

diagnosis in four to six weeks after embryo transfer and good nutrition program especially in the last three months of pregnancy (Stroud, 2010).

MERITS AND DEMERITS OF IN VITRO FERTILIZATION IN EMBRYO TRANSFER

The production of embryos using IVF presents two main advantages over the conventional approach. First, it prolongs reproductive life of cows that do not respond to superovulation treatment or produce only unfertilized ova. It is, therefore, an integral tool to keep selected cows within a breeding scheme as part of a selection program (Bousquet *et al*., 2003). Second, IVF can be applied during different stages of reproduction, including the prepubertal period and during the first 3 months of pregnancy. With the utilization of prepubertal heifers, it is possible to decrease the age of the donor female use, thereby contributing to a decreased generation interval. Using ultrasound guided trans vaginal collections, oocytes for IVF can be collected in heifers at ≤ 6 months of age (Galli *et al*., 2001). New hormonal treatments and preparation of oocyte of donor animals are promising approaches to increase oocyte competency. In a recent study, up to 80% of oocytes collected from 14 to 16 month old heifers produced blastocysts after IVF (Blondin *et al*., 2002). In addition to those merits the implementation of IVF technology allows the use of several bulls with one donor, since the control of fertilization is made in vitro. This can be realized in one session of fertilization as opposed to conventional ET, which is limited to one bull per session (Bousquet *et al*., 2003). And also IVP waives superovulation and AI treatment (Presicce *et al*., 2011).

There are problems associated with in vitro embryo production. Low success rates limit the contribution that the procedures can make, especially to genetic improvement. Specific problems include a high incidence of polyspermy during in vitro fertilization (Mizushima and Fukui, 2001) and low oocyte yield per ovary when aspiration is continuously used to increase the number of
oocytes per session or cow (Machado *et al.*, 2006). Thompson and Peterson (2000) also stated that there are significant post-day 35 fetal losses and found that much of this loss is due to failure of normal allantoic development within the conceptus. Potential welfare problems related to specifically to OPU have been referred to as a problem but do not appear to be of great concern. But much of the greater concern is the effect on offspring that are actually brought to term, and here there is indeed a welfare issue. Most serious is the birth of unusually large calves resulting from in vitro embryo production. Associated with this have been reports of breathing difficulties, reluctance to suckle and sudden perinatal death of calves (Ball and Peters, 2004).

CONCLUSION

Animal biotechnologies related to reproduction have contributed too many improvements in different important traits in livestock because reproduction lies at the heart of any livestock breeding enterprise and is vital to maintain or increase the number of animals required for production. Among the reproductive technologies,

animal breeders have made widespread use of assisted reproductive technologies to accelerate genetic improvement programs aimed at obtaining more, better and cheaper food products. Assisted reproductive technologies like in vitro embryo production and embryo transfer have contributed to animal breeding programs faster transmission of desirable trait or genetic improvement by increasing offspring of selected males and females. Successful establishment of procedures for in vitro production of bovine embryo even if IVM, IVF and in vitro embryo culture systems vary greatly among different laboratories have direct effect towards the success of embryo transfer result. However, embryo transfer and in vitro production of bovine embryos are still not widely used despite its potential benefits. In developing countries such as Ethiopia, this is mainly due to absence of the necessary facilities and infrastructure.

Therefore, on the basis of this conclusive remark, the following points are forwarded:

- Taking maximum attention in all segmented process of IVP to reach for success. Collections of oocytes, IVM, IVF and IVC all are have their own contribution in success.
- Taking maximum attention in all segmented process of embryo transfer to reach for success. Management and selection of the recipients and transfer of the embryos are all important.
- There should be well trained technician for invitro embryo production and embryo transfer technology.
- There should be of clear policy and commitment from the government about assisted reproductive technology.
- Awareness should be created in the community and companies about the profitability of invitro embryo production regulated embryo transfer and Investors should be encouraged to invest on these technologies.
- Coordination should exist between industry, universities and institutions for technology of IVP and ET.

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STUDY OF ADSORPTION EFFICACY OF ADANSONIA DIGITATA (BAOBAB) WOOD BLENDED WITH MUSA ACUMINATA STEM POWDER

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ABSTRACT

In continuation of our research work in the area of developing adsorbents from agricultural wastes, a mixture of adsorbents is chosen in the present study. A novel adsorbent obtained by blending adansonia digitata (baobab) wood and musa acuminata stem (1:1 w/w) has been used for the removal of congo red dye using simulated aqueous solutions employing adsorption method. The degree of adsorption was studied by using different parameters such as pH (2-8), contact time (50-150 min), initial dye concentration (50-150 mg/l) and adsorbent dosage (0.5-2 g). The extent of adsorption was determined by employing UV-Vis spectrometer. The experimental data was verified by Freundlich model and found to be in agreement. Under the experimental conditions the parameters at the maximum adsorption ability have been determined for the dye and reported.

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Keywords: biosorption, congo red dye, baobab wood adsorbent and musa acuminata stem adsorbent

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1. INTRODUCTION

The removal of color from dye-bearing effluents is posing a threat nowadays due to the limitations of conventional treatment methods. It is evident that low-cost adsorbents have demonstrated considerable removable capabilities for dyes. A review of feasibility of agricultural waste adsorbents for dye removal shows that most of them act as substitutes for expensive activated carbon adsorbents but complete dye removal is not possible (Kayado et al., 2015). Bagasse and corncob wastes have shown that removal of dyes is favorable (R.S.Juang et al., 2002). The presence of tiny amounts of dye in water (less than 1 ppm) is also highly visible and undesirable (L.Lian et al., 2009). Recent advances in remediation of synthetic dyes from waste waters using sustainable and low cost adsorbents show the impact and prospects (Sharf et al., 2019). Dyes are synthetic aromatic compounds that are stable and hardly undergo natural degradation (yenikaya, et al., 2010). Most of the dyes are complex organic molecules. Azo dyes are the mostly used in industrial activities. Dyes are used in production of the essential consumer products of modern life such as textile, leather, cosmetic, foods and beverages, papers and pulp which cause the environmental pollution problems and threat to the ecological balance (Ulas, et al., 2017). Dyes are produced, utilized and partially released into atmosphere after the application is over. Synthetic dyes are extensively used for coloring and printing in variety of industry (mohanraj, 2009). Biosorption of textile effluents was studied extensively and reported (Omprakash et al., 2018). Many researchers found that colorant may cause problems in aquatic ecosystem in several ways (yenikaya, et al., 2010). Worldwide, infectious diseases such as water-borne diseases are a serious threat to children under five years old and more people die from unsafe water (Edogbanya, et al., 2013a). Every day, more than two million tons of sewage, industrial and agricultural waste is discharged into the world's waste water bodies (WWAD, 2006). There are several ways for removal of dyes; adsorption has been considered as one of the safest and simple methods for the removal of textile dye effluents due to its low cost, easy availability, simplicity of design, high efficiency, ease of operations and ability to treat wide variety of dyes (Ramalakshmi et al., 2012). Activated carbon is a carbonaceous adsorbent with a highly crystalline form and internal pore structure (Kavitha 2019). A wide variety of activated carbon products are available depending upon a starting raw material and activation techniques (Gupta, 2003).

In continuation of our investigation using adansonia digitata stem adsorbent for the removal of methylene blue (Mihayo et al., 2020), favorable adsorption was observed for congo red dye also (Mastan Rao K et al., 2021). The present study attempts to make a blended adsorbent obtained from adansonia digitata (baobab)

wood powder and musa acuminata stem powder for the adsorption of simulated congo red dye solutions. The Baobab tree mostly found in the equatorial area such as Gabon, Democratic Congo, Zaire and the area with marked dry season (Gebauer, et al., 2002) such as Dodoma region in Tanzania. Generally baobab fruits and seed oil are used for human consumption and the rest of the tree parts are used as traditional medicine (simon, 2015). Baobab belongs to genus: adansonia and species-adansonia digitata. Several varieties of musa acuminata are grown in Tanzania. An attempt is made to study the adsorption of this biosorbent towards removal of organic dye.

2. MATERIALS AND METHODS

2.1 Preparation of dye solution

Stock solution of dye concentration 1000 mg/l was prepared by dissolving 1g of 100% pure congo red dye in 1000ml of distilled water by using standard flasks. Solutions of different concentrations were prepared by dilution method between 20 and 200 mg/l using the above stock solution of the dye. All chemicals of 99.9% purity have been used in the present study.

2.2 Preparation of adsorbent from adansonia digitata wood and musa acuminata stem powder

Hard wood of adansonia digitata has been collected from a single tree in Dodoma region at Kongwa district. After removing the bark, small chips of the baobab wood are pulverized into powder using grinding machine and then sieved through a pore size of about less than one millimeter. Musa acuminata stem was collected from Msalato (Tanzania) area and washed with distilled water to remove dirt and was cut into small particles size in the range of 5 to 7 cm. The sample was dried in an oven until constant weight and it was cooled for one and half hour and crushed into fine powder. Both the samples obtained from baobab and musa acuminata are mixed in 1:1 weight ratio, dried and ground to get a powder of particle size about 125-250 µm range and stored in an airtight container for further experiments.

2.3 Batch mode biosorption studies

A standard calibration plot between the adsorbate concentration and absorbance was drawn. Batch biosorption equilibrium experiments were conducted in 250 ml conical flasks at a constant agitation speed (180 rpm). All the experiments were carried out at room temperature (30^0C). The concentrations of the dye both before and after adsorption were determined using Agilent 60 Cary UV- Vis spectrophotometer, USA through monitoring the absorbance for the dye used. In the present study, the effect of initial concentration of the dye, adsorbent dosage, pH of solution and contact time on adsorption have been studied. The effect of initial dye concentration was studied in the concentration range 50-150 mg/l. The effect of pH on adsorption process was studied in the pH intervals of 2, 4, 6 and 8. The effect of mass of adsorbent was studied

in the range of 0.5gram to 2gram at constant dye concentration 100 mg/l and optimized conditions. The effect of contact time was studied in the constant dye concentration of 100 mg/l and time range from 50 minutes to 200 minutes.

3. RESULTS AND DISCUSSION

3.1 Effect of initial concentration of dye

The effect of initial dye concentration was studied in the concentration range 50-150 mg/l. The extent of adsorption of dye per gram of adsorbent was calculated and reported in Table 1. A plot is drawn between adsorbate concentration and adsorption ability and shown in figure.1.

Table 1. Initial dye concentration versus. absorbance data

S. No.	C_i (ppm)	m (g)	absorption	C_f (ppm)	C_i-C_f = x	x/m	log C_i	log x/m
1	50	1	0.6212	44.3	5.70	5.70	1.699	0.756
2	100	1	1.9877	77.5	22.55	22.55	2.000	1.353
3	120	1	3.2156	90.5	29.55	29.55	2.079	1.470
4	150	1	3.8836	117.3	32.67	32.67	2.176	1.514

x/m=extent of adsorption (mg/g), C_i=initial concentration of dye (mg/l), C_f = final concentrations of dye (mg/l) , w = dry weight of the adsorbent (g)

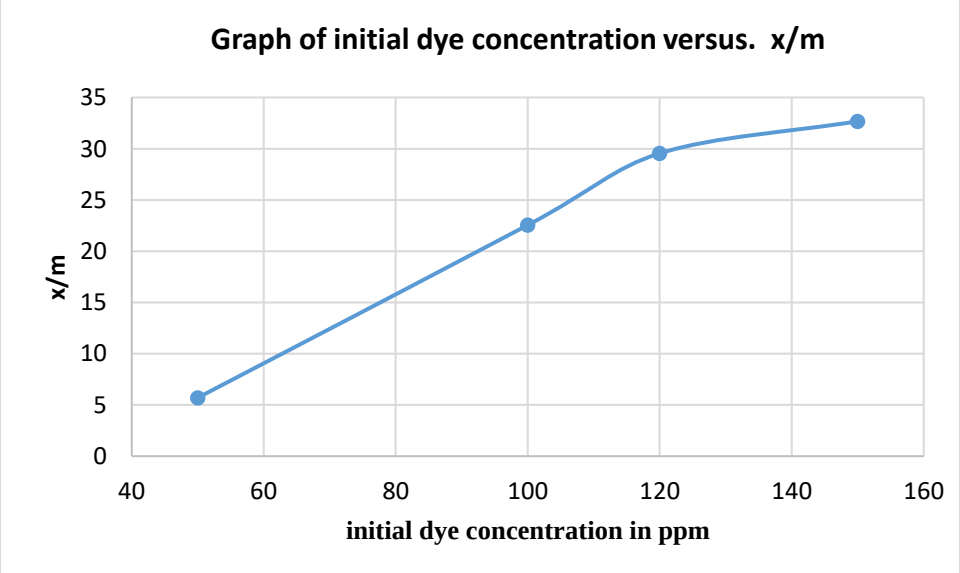


Figure 1. Graph of initial dye concentration versus. adsorption ability

3.2 Adsorption isotherm

Adsorption isotherm has been studied on the extent of adsorption of CR dye on the adsorbent by using Freundlich adsorption model. According to the Freundlich isotherm model, the amount of adsorption at low concentration varies linearly, at moderate concentration becomes dependent on the Freundlich constant and at higher concentration becomes independent of the adsorbate concentration. From the figure 1 above, it is observed that the experimental results obey the Freundlich model satisfactorily. Further it is verified by plotting a logarithmic graph (figure 2) and found to be a straight line which is expected of the above results.

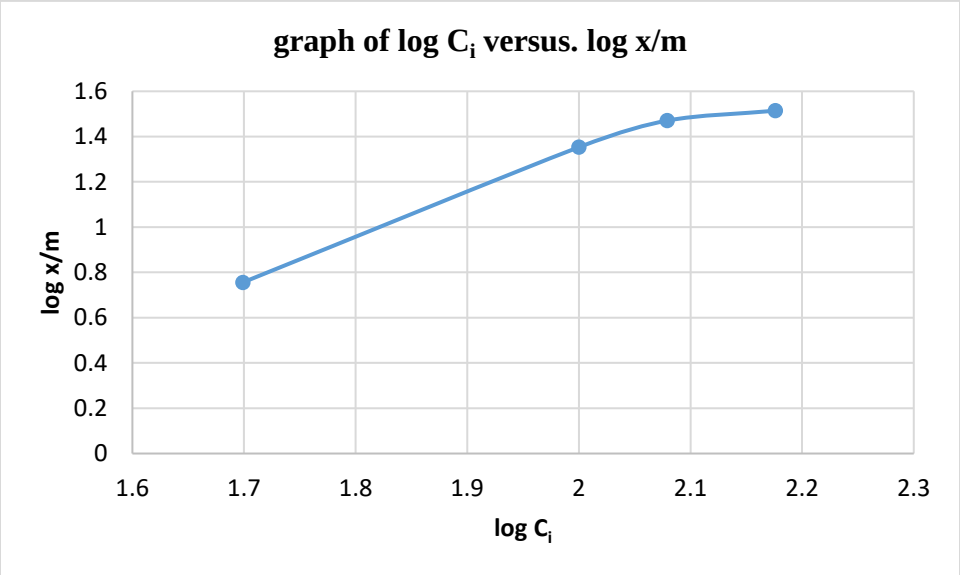


Figure 2. Freundlich adsorption isotherm for the adsorption of congo red dye on adsorbent.

It is observed from the graph that as the initial concentration of adsorbate increases, adsorption ability increases to a certain extent and thereby remains constant. This may be due to the saturation of the active sites of the adsorbent upon adsorption of the dye molecules.

3.3 Effect of adsorbent dosage

The effect of mass of absorbent was studied in the range of 0.5 g to 1.5 g at constant adsorbate concentration of 100 mg/l. The extent of adsorption of dye per gram of adsorbent was calculated and reported in Table 2. A plot drawn between adsorbent dosage and adsorption percentage is shown in the figure 3.

Table 2. Data showing adsorbent dosage % removal of adsorbate

S. No.	C_i (ppm)	m (g)	absorbance	C_f	C_i-C_f = x	x/m	% removal
1	100	0.5	2.193	80.81	19.19	38.39	19.19
2	100	1.0	1.867	69.23	30.77	30.74	30.74
3	100	1.2	1.733	64.52	35.48	29.56	35.47
4	100	1.5	1.527	57.20	42.80	28.54	42.80

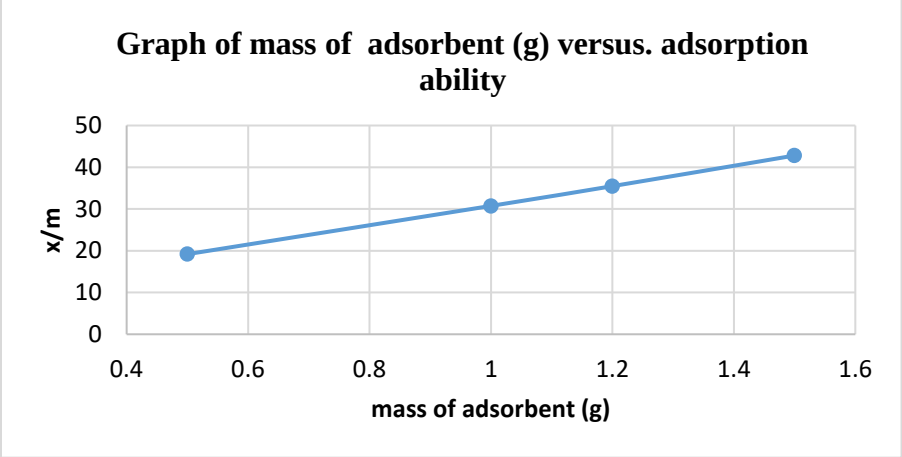


Figure 3. Graph of mass of adsorbent versus adsorption ability

It was observed that the extent of adsorption increases with an increase in the mass of adsorbent from 0.5 -2.0 gram which may be due to the availability of unsaturated surface area (active sites). Further the values of (C_i-C_f)/m are decreasing which can be attributed to the less concentration of dye molecules available per gram of adsorbent.

3.4 Effect of pH

The effect of pH was studied at a constant concentration of 100 mg/l of adsorbent and pH range 2-8. The extent of adsorption of dye per gram of activated carbon was calculated and reported in Table 3.

Table 3. Effect of pH on the adsorption ability of the adsorbent

S. No.	C_i (ppm)	m (g)	pH	absorbance	C_f (ppm)	C_i-C_f = x	x/m	% removal
1	100	1.51	2.38	0.8998	34.9539	65.0461	43.3641	65.04
2	100	1.50	4.45	1.5847	59.2411	40.7589	27.1726	40.75
3	100	1.50	6.24	2.1563	79.5106	20.4894	13.6596	20.48
4	100	1.50	8.14	2.4313	89.2624	10.7376	7.1584	10.73

x/w = extent of adsorption (mg/g), C_i = initial concentrations (mg/l), C_f = final concentrations of dye (mg/l) , m = dry weight of the activated carbon (g), the percentage of adsorption of the dye $q\% = \frac{(Ci-Cf)}{Ci}$.

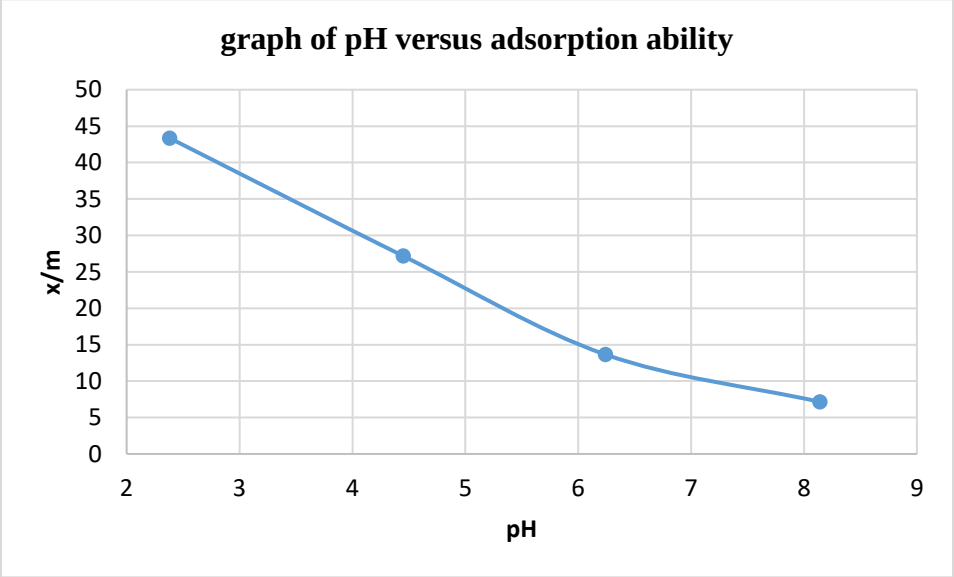


Figure 4. The Graph of pH versus adsorption ability.

The adsorbate shows a maximum adsorption at pH 2.46 with maximum percentage of adsorption of 98.9%, because it works efficiently on the acidic medium than in a basic medium. Congo red is an anionic azo dye; hence at lower pH it shows higher adsorption due to the positively charged surface of the adsorbent.

3.5 Effect of contact time

The effect of contact time was studied at a constant concentration of 100 mg/l and time range from 50 minutes to 120 minutes. The extent of adsorption of dye per gram of activated carbon was calculated and reported in Table 4 below.

Table 4. Effect of contact time on adsorption

S. No.	C_i (ppm)	m (g)	time (min)	absorbance	C_f (ppm)	$C_i - C_f = x$	x/m	% removal
1	100	1.0	30	1.328	50.13	39.31	39.31	39.30
2	100	1.1	60	1.023	39.31	60.69	55.18	60.69
3	100	1.1	90	0.664	26.60	73.40	66.72	73.39
4	100	1.1	120	0.302	13.78	86.22	78.40	86.22

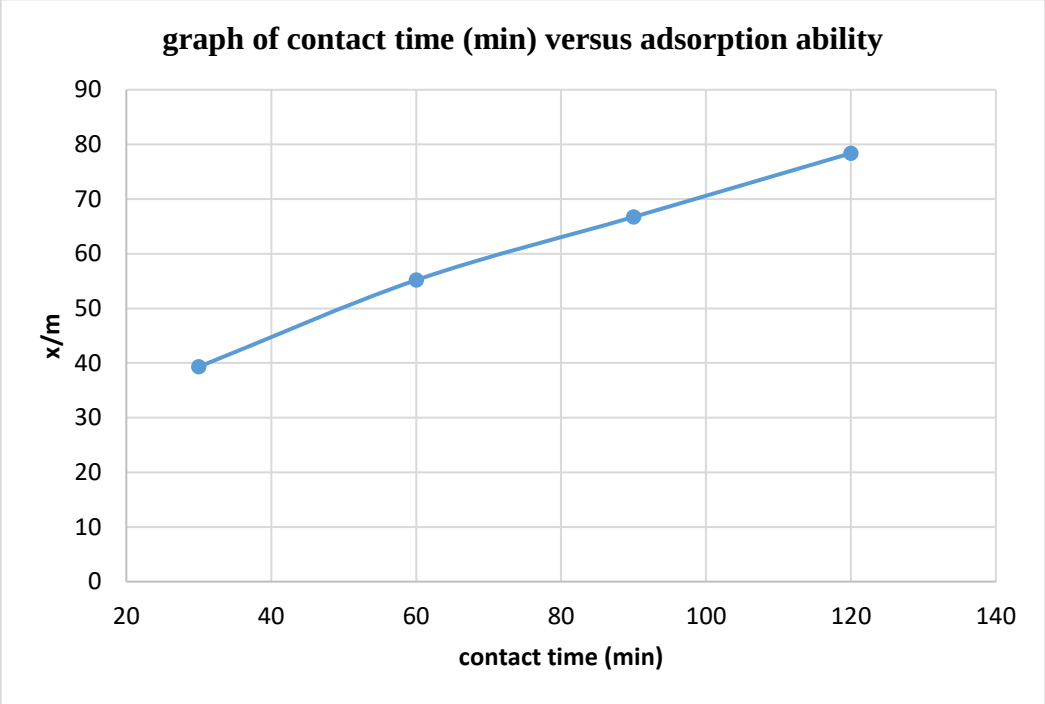


Figure 5. Graph of contact time (min) versus adsorption ability.

As seen in figure 5, it is evident that time has significant influence on the adsorption of dye at the initial concentration of 100 mg/l. The removal of congo red dye by the adsorbent blend as a function of time was observed. Initially, the adsorption of dye is considerable during the time interval of 50 to 150 min and later, the adsorption process becomes less significant and remains nearly constant. The adsorption efficiency was

found to be 98.25%. Based on these results 120 minutes was taken as the maximum time required to reach equilibrium state in batch adsorption experiment.

4. CONCLUSIONS

The data obtained from the above studies showed that a contact time of 150 min was sufficient for the maximum removal of congo red dye from aqueous solutions using baobab wood charcoal mixed with musa acuminata stem powder. The experimental data of biosorption of the dye on the biosorbent fits well with the Freundlich adsorption model. This indicates that the biosorption of the dye on the biosorbent is favorable. The maximum removal efficiency was found at a temperature of 303K, at solution pH of 2.38, initial dye concentration of 150 mg/l and biosorbent dosage of 4.0g under the experimental conditions. Further investigation of the adsorption characteristics like surface area of the adsorbent and adsorption thickness is suggested to understand the adsorption mechanism.

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EVALUATION OF FACTORS DETERMINING THE HOUSEHOLD'S WILLINGNESS TO PAY FOR IMPROVED SOLID WASTE MANAGEMENT SERVICES: THE CASE OF LOGIA TOWN.

Seid Ahmed

ABSTRACT

This paper evaluated the factors determining the households WTP for improved solid waste management services in Logia town. In this study 201 respondents are included to analyses the household's willingness to pay for improved solid waste management services in the logia town using the Contingent Valuation Method (CVM) techniques. The Sampling technique respondents were selected by lottery method (simple random methods) because of the population homogeneity. Both primary and secondary sources of information were collected and the study was applied a binary logit model. Out of 201 respondents, about 65.17% are willing to pay for improved SWM services with an average maximum willingness to pay is 13 Birr per month this shows households have an interest to contribute to environmental protection. The result shows that respondents age, distance between resident area and waste disposal facility, unpaid options (all with negative), sanitary supervisor visit, dwelling type and attitude (awareness) (all with positive) have significant effects on the willingness to pay for improved solid waste management services. In conclusion, the majority of the households are willing to pay for improved solid waste management services, which implies that any policy effective for improved solid waste management services in the study area is needed.

Keywords: Contingent valuation Method, Solid Waste, Willingness to Pay, Binary Logit Model, afar region

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1. INTRODUCTION

Nowadays, the world's environment getting polluted and its comforting ability for life becomes decline. The most dynamic engines for environmental pollution are rapid urbanization, fast expansion of urban, agricultural and industrial activities, which motivated by population growth. Economic growth in less developed countries has led to the huge movements of people from less urban areas to urban areas for searching better life. Because of this, the numbers of persons, additional to the urban population in undeveloped countries create challenges to governments, urban planners, urban administrations and some others. Due to this, urbanization and high rate of population growth are especially accountable for high aggregate rate of solid waste management (SWM) service supply. Even though advanced countries generate greater quantities of solid waste than developing countries (Solomon C, 2011).

Effective solid waste collection and disposal is a vital component of public services provisions and should take priority particularly in emerging cities. Because falling to have such services can result in many unfavorable outcomes in the long run; this may have serious adverse effects on public health and the environment. But the expansion of such services provisions is often a challenging task for governments of developing nations due to heavily burdened, and stretched financial and economic resources that lead to relatively high opportunity cost of funds (Murad et al, 2007).

Solid waste management is one of the developmental challenges facing city authorities worldwide, especially in most developing countries (UNEP, 2013). Poor solid waste management, coupled with inadequate financial resources, has led to indiscriminate dumping of solid waste into open spaces and drainages, blocking pipes and causing flooding, environment pollution and public health issues (UNEP, 2013).

Rapid urbanization has made solid waste management a serious problem in poor and less industrialized countries. Waste management is becoming a very serious problem in Ethiopia also. For this, we must evaluate the factors determining the households' willingness to pay for this service. The information can be used to raise people's welfare by introducing cost recovery by assigning into households' willingness to pay. The problem in less industrialized countries is further difficult because of increasing greatly the size and arrangement of waste being generated and the demand for waste recovery service in municipal areas by rapid growth in population and urbanization. This implies that the problem of SWM in the unindustrialized world is more important than developed countries, because as long as humans have been living in settled communities, waste generation has been a clear and serious issue in all nations (Zerbock, 2003).

According to Schubeler, Wehrle, and Christen (1996) municipalities in developing countries spend a large proportion of their budgets on the management of solid waste materials. This problem is growing due to inappropriate planning by waste management authorities, inadequate governance, lack of resource availability and unsuccessful management in fast growing cities of the developing countries.

There are a number of problems associated with the inappropriate waste management mechanism in the densely populated areas. The open waste amounts create health problems and leachate pollutes the underground water, ultimately causing waterborne diseases. This study seeks to evaluate factors that determine households WTP for improved solid waste management services in Logia town Afar Region.

2. Methodology

2.1 Description of the study area

Afar is located in eastern part of Ethiopia. The region has common boundaries with the state of Eritrea in the north-east, with Tigray in the north-west, with Amhara in the south-west, with Oromia in the south, with the state of Somalia in the south-east and with the republic of Djibouti in the east. According to Ethiopian statistical authority

(CSA) has estimated the July 2008 E.C populations of the afar administrative region at 1449,000 of which 137,000 are urban residents. The breakdowns by gender 803,000 are males and 646,000 are females. The study was conducted in Dubti Woreda at one city administrations (Samara-logia). Logia is one of the rapidly growing towns in the Afar Region, Ethiopia, some 241 mi (or 388 km) North-East of Addis Ababa, at Latitude: 11°47'55.44" Longitude: 41°0'43.49" and the total population of logia town in 2007 was 14038. Out of the population, 7,411 are males and 6,627 are females. The average number of households in logia town is 3.1. (CSA, 2 007).

2.2 Sample size and Sampling technique

This study was used two-stage sampling techniques. In the first stage, from 34 woredas, one woreda (Dubti) and one city administrations (Samara-logia) were selected purposively. In the second stage households were selected randomly from each kebeles from logia town. I used simple random sampling to select the sample of the study with the help of Cochran sample size determination techniques.

Cochran formula allows you to calculate an ideal sample size given a desired level of precision, desired confidence level, and the estimated proportion of the attribute present in the population. Cochran's formula is considered especially appropriate in situations with large and unknown populations. A sample of any given size provides more information about a smaller population than a larger one, so there's a 'correction' through which the number given by Cochran's formula can be reduced if the whole population is relatively small.

The Cochran formula is: $n_0 = \frac{Z^2 pq}{e^2}$

$$n_0 = \frac{(1.418)^2(0.5)(0.5)}{0.05^2} = 201.072 \approx 201$$

Where

n_0 The sample size

Z^2 The normal curve that cuts off an area α at the tails (1 –α equals the desired confidence level).

p The (estimated) proportion of the population which has the attribute in question.

q 1 – p.

e^2 The desired level of precision (i.e. the margin of error).

2.3 Research Design and Methods

In this study both qualitative and quantitative approaches were used. This study employed cross sectional study design because the data collection was limited to a single time period for each household head.

3.5 Data type and collection

In this study both primary and secondary sources of data were used. Both quantitative and qualitative were collected for the evaluation of factors determining the household's willingness to pay for improved solid waste management services.

3.6 Method of Data Analysis

Both descriptive and inferential statistics was used. In addition to this binary logistic regression was used. The logistic regression model is used to describe data and to explain the relationship between one dependent binary variable and one or more nominal, ordinal, interval independent variables. The willingness to pay question was the dichotomous choice format, the Binary logit model was better for analysis in this study.

Table1. Definition, descriptive statistics and expected sign of variables

Variable	Definition	Measurement	Expected sign
PSWM	Price of SWM	In Birr/ETB	-
IncomHh	Income of households	In Birr/ETB	+
EdunHh	Educational level	In year	+
Famsize	House hold size	Number	-
SanitAdvis	Sanitary supervisor advise	Number	+
DwelType	Dwelling type	1 for Own house,0 otherwise	+
HouseOwner	House ownership	1 for Yes, 0 Otherwise	+
AttSWM	Attitude about SWM	1 for positive,0 otherwise	+
Age	Age household head	In Year	-
Gender	Sex of household head	1 for female, 0 otherwise	-/+
UnpaidSWM	Unpaid Options to SWM	1 for yes, 0 otherwise	-
DistancSWM	Distance from disposal services	In kilo meter	-

3.7 Model specification

3.7.1The binary logit model

Willingness to pay question was a dichotomous choice, i.e. 'yes'/'no' thus a binary logit model (Greene 2003) can be applied in the analysis of factors associated with respondent's willingness to pay for improved solid waste management services.

The probability **P** that the respondent will give a 'yes' response, i.e. willing to pay is given as follows:

$$\mathbf{P[yes]} = \frac{\mathbf{1}}{\mathbf{1+exp}(-\boldsymbol{bx})} \quad \ldots\ldots\ldots\

The probability that the respondent will give a 'no' response, i.e. not willing to pay is given as follows:

$$\mathbf{P\ (no) = 1\text{-}P\ (yes)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$\mathbf{P[no]} = \frac{\mathbf{1}}{\mathbf{1+exp}(\boldsymbol{bx})} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Handling of (2) and (3), gives

$$\mathbf{1\text{-}P\ (yes)} = \frac{\mathbf{1}}{\mathbf{1+exp}(\boldsymbol{bx})}$$

$$\frac{\boldsymbol{p(yes)}}{\mathbf{1} - \boldsymbol{p(yes)}} = \mathbf{exp}(\boldsymbol{bx})$$

Where, exp (bx) is the ratio of the probability of a 'yes' to the probability of a 'no' response.

The logarithm of the odds ratio is given as follows:

$$\mathbf{ln}\left[\frac{\boldsymbol{p(yes)}}{\mathbf{1}-\boldsymbol{p(yes)}}\right] = \boldsymbol{bx}$$

$$\boldsymbol{WTPwm} = \boldsymbol{B0} + \boldsymbol{B1X1} + \boldsymbol{B3X3} \ldots\ldots \boldsymbol{Bnxn} + \mathrm{Ɛ}$$

Where $\mathbf{B_0}$ is the intercept, $\mathbf{B_1}$ is the coefficient associated with the price **X1**, and **Bn** a vector of regression coefficients associated with other characteristic of the respondent **Xn** and **Ɛ** is the error term which is logistically distributed.

4. Results and discussion of findings

4.1 Descriptive analysis of the survey data

This study found that Out of the total 201 respondents, about 61.19% were male. Around 47.76% of the respondents are house owner and the remaining 52.24% are not house owner. The higher number of the house owners in the study are pay for

environmental quality improvement. The respondents overall attitude of the quality of their environment and SWM, almost are positives.

The existence of unpaid waste disposal option in their area about 66.17% of them has the opportunity to unpaid options, this indicate almost of solid waste is uncollected (i.e. waste may be dumped in river, bury, burning and removed in other forms). And the remaining respondents have no unpaid options, this indicates that there is a willingness for the improved of SWM options in logia town.

Sex of households head	**frequency**	**percent**
Female	78	38.81%
Male	123	61.19%
House ownership	**frequency**	**percent**
Yes	96	47.76%
No	105	52.24%
Environmental attitude	**frequency**	**percent**
Positive	121	60.20%
Negative	80	39.80%
Unpaid waste disposal options	**frequency**	**percent**
Yes	133	66.17%
No	68	33.83%

Table1. Summary of categorical variable

The mean age of respondents for sample was around 46 years with a minimum 29 of and a maximum 0f 68 years, this therefore, shows that respondents are at the critical age to know the advantage of environmental quality and hence make smart decisions about solid waste management. The average years of schooling were 7 years and about 6.97% of the respondents did not attend any formal education, about 65.69% at primary level, 19.01% at secondary level and 7.47% at university/college level respondents are attended in formal education. The average monthly total household income for the

sample was birr 2552.1 with minimum of birr 500 and maximum of birr 6450.The average family size of the survey households was 3 which is similar to the National census result of 3.1 for logia town in 2007, with minimum of 1 and maximum 5.The respondents dwelling type, around 53.23% of respondents are living in their own occupied, 40.3% of them rented from private and 6.47% are live in the relative house without payments. The Average household solid waste produced per month was about 1.4 sacks with minimum 0.50 of maximum of 2 sacks it is too small compared to Yonas B. (2010); Muhdin H. (2016) and others studies, because respondents were not estimate perfectly how much they produce solid wastes per month. The Sanitary supervisor visit received per month, about 36.32% answered no supervisor visit per month, 46.27% once per month, 9.45% twice per months and 7.96% three times per month. The Distance between Households resident area and Municipal waste Disposal Facility, the average was 2.2 km with a minimum 1km and a maximum 4km. Almost 50.75% of respondents are currently payer for SWM and when respondents are asked about current price for SWM the average was 18.3 birr with a minimum 0 birr and a maximum 50 birr. Respondents suggested that there is unfair payment system for SWM even if they are pay without any services per months.

Variable	**Obs.**	**Mean**	**Std. Dev.**	**Min**	**Max**
Age	201	45.85075	9.582672	29	68
Education	201	7.079602	3.548751	0	17
Household size	201	2.696517	1.054722	1	5
Income	201	2552.075	1428.542	500	6450
Amount pay	201	18.30846	7.373898	0	50
Distance from waste municipal disposal facility	201	2.223881	.8857917	1	4

Sanitary supervisor visit	201	.8905473	.8763334	0	3
Dwelling type of respondents	201	2.064677	1.233206	1	5

Table 2. Summary of continuous variable

The willingness to pay question

The willingness to pay question was the dichotomous choice format. The respondents were asked whether they would have WTP for improved solid waste management or not. The respondents who was say 'yes' would ask how much ETH birr they pay in each month. The respondents who would not have WTP for improved solid waste management services were asked to give the reason(s). The respondents were asked a series of socioeconomic questions (education, gender, income and household size, asset ownership, age and marital status). They would asked about their apartment and the travel time from their residence to the nearest waste collection point. The respondents were asked whether the activities of sanitary supervisors were strongly handled in their areas and whether they are happy with private sector participation in household waste management.

As the survey shows that out of 201 respondents, about 65.17% are willing to pay for improved SWM services (table 3); this is more than the current solid waste management disposal status for respondents. And the remaining are said 'no' willingness to pay for solid waste management services.

Table3. Households' willingness to pay for improved solid waste management services.

Willingness to pay	Frequency	Percent
Yes	131	65.17%
No	70	34.83%

Total	**201**	**100%**

Although the respondents were free to give reasons for their willingness or unwillingness to pay for the improved solid waste management options, most of the respondents gave similar reasons. Those respondents who have WTP for SWM are for the sake of keeping their environment clean and healthier. And about 34.83% of respondents who were not have WTP for SWM options reason out that not satisfied with the current SWM services, lack of information on SWM services, they believe that general taxes should cover the cost of services and they can't have enough money to pay for the full cost.

Table 4, Monthly maximum willingness to pay

	Mean	**Std. Dev.**	**Min**	**Max**
Maximum willingness to pay	12.71	12.35	0	60

The average maximum willingness to pay is 13 birr with a minimum 0 birr and a maximum 60 birr WTP for improved solid waste management options, this shows households have an interest to contribute for environmental protection. The respondents said that this is our desires to pay for the services option with pleasure rather than the current services. (Table, 4).

Table 5. The logistic regression results.

Variable	Coefficient	Standard error	Significant
Age	-.0482701	.0198709	0.015*
Gender	.1003459	.3936558	0.799
Education	.1228228	.0694446	0.077*
Famsz	-.3034194	.1790778	0.090*
Income	.0000246	.0001669	0.883
Amountpay	.0294013	.0250539	0.241
Kmfar	-.6040216	.207324	0.004*
Ownership	.061723	.4625283	0.894
Unpaid	-.8821662	.388459	0.023*
Supervisit	.454222	.225685	0.044*
Dwelling	.4127323	.1958351	0.035*
Attitude	1.569827	.4116413	0.000*
Cons	2.0482	1.34057	
Statistic Summary			Significance Level
No. of Obs. 201			* Significance at 10%
Log likelihood = -101.24547 Pseudo R2 = 0.2207			
LR chi2(12) =57.35 Prob > chi2 =0.0000			

4.2 Logistic regression analysis

The logistic regression results are presented in table 5. I found that multicollinearity is not a serious problem in my data set because all independent variables have less than 10 and greater than 1 VIF value. Testing heteroskedasticity shows no problem because the probability value of chi-square is statistically insignificant it is 0.061, greater than 0.05. And there is no omitted variable. In order to test for the goodness of fit, the pseudo R2 was used. The pseudo R2 is 0.22 which implies that 22% of the variation in the probability of WTP or not is explained by the variables included in the model.

The estimated result showed that respondents age, distance between resident area and waste disposal facility, unpaid options and family size (all with negative signs),

sanitary supervisor visit, dwelling type, attitude and education (all with positive signs) have significant effects on the willingness to pay for improved solid waste management services. This result is similar with earlier findings (Tolina, 2006; Aklilu, 2002; Hagos, 2003 Yohanes, 2010). And respondents level of education, household size, income size, This result in similar with earlier findings (Yohanes Berihun,2010); amount to pay (price), house ownership, gender similar with earlier findings(Hagos ,2003) are do not significantly influence willingness to pay for improved solid waste management option.

However, age of respondents as estimated has a negative and significant effect on WTP. This shows that as peoples grow older their WTP for waste management options become diminish. This possibly can be the result of older respondents adapted to free government services in the past government. But, the younger peoples are to be expected to be more familiar to cost sharing like for education and health care services etc. The distance between resident area and waste disposal facility as estimated has a negative and significant effect on WTP. This shows that as peoples are far away from any services their Willingness to consume become low, then as the distance between respondents area of resident and municipal waste disposal facility become far-off the WTP for this solid waste management services become low.

The existence of unpaid waste disposal opportunity in a given area negatively affects their WTP for improved solid waste management services. As the estimated result show that the WTP for improved solid waste management services and the existence of unpaid waste disposal opportunity in a respondent area negatively related. The 66.17% of respondents has unpaid options (dumping in river, bury and burning) to avoid their waste. This indicates respondents may have low WTP for other solid waste management options.

The attitude(awareness) of respondents which show that individuals who are aware and has positive attitude with environmental protection have a greater position towards the

WTP for improved solid waste management than those who are not aware. This shows that the environmental protection awareness in study area is positive factor for WTP for solid waste management services. The existence of sanitary supervisor visit in the society increases the awareness and the importance of environmental care for the weal fare improvement. Then, as the estimated result show the role of sanitary supervisor visit positively affects the WTP for improved solid waste management services. Finally, as the estimated result shows the dwelling type of respondents positively significant for the WTP for improved solid waste management services in the study area. This suggested that peoples who live in their own house have more WTP for improved solid waste management services than who live rented from private/ live in relative hose without payment.

Table 6. The logistic regression for categorical variables to report odds ratio

Logistic Regression			**No of obs = 201** **LR chi2(5) = 28.19** **Prob > chi2 = 0.0000** **Pseudo R2 = 0.1085**		
Log likelihood = -115.82596					
WTP	**Odds ratio**	**std.err.**	**z**	**p>|z|**	**[93% conf. Interval]**
Gender	1.399453	.5108727	0.92	0.357	.7222675 2.711555
Ownership	.8777328	.3622638	-0.32	0.752	.4155147 1.854122
Unpaid	.4366683	.1549335	-2.34	0.020	.2295895 .8305224

Dwelling	1.377036	.2401194	1.83	0.067	1.003991
	1.88869				
Attitude	3.131768	1.145549	3.12	0.002	1.614186
	6.076109				
-cons	.8067211	.480167	-0.36	0.718	.27438
	2.371889				

Interpretation

An examination of the odds ratio coefficient for unpaid showed that peoples those have unpaid options for solid waste disposal were less likely **[Exp (β) = 0.43]** willing to pay for improved solid waste management services in the logia town than those have not an unpaid options for solid waste disposal. And the examination of respondent's attitude those have a positive attitude for solid waste management were three times more likely **[Exp (β) = 3.13]** willing to pay for improved solid waste management services in the logia town than those have a negative attitude for solid waste management services.

4.3 Conclusion

The survey result shows that the majority, which 60% of total respondents states that they have positive concerns for environmental protection and well-being. About 40% of respondents are not satisfied with the current SWM services and they are not worried about environmental protection. During the survey the respondents were also asked about the performance of a sanitary supervisor visit to their area, 36% of respondents answered there is no any sanitary supervisor conduct about SWM.

The study concludes that it is an essential to prepare a new solid waste management of the society in the study area. Based on this around 65.2% of the respondents are willing

to pay for improved solid waste management option like a door-to-door waste collection. The average willingness to pay is ETB 13 Birr. This shows households have an interest to contribute of environmental service, even more than the existing fee.

Binary logistic regression was employed to identify factors affecting households WTP for improved solid waste management system. The outcome shows that eight variables are found to be statistically significant. These are age, distance between respondent resident and waste disposal facility, existence of unpaid options, sanitary supervisor visit, respondents dwelling type and household's attitude about solid waste management ,family size and education.

4.4 Recommendation

Based on the findings the following recommendations were made to improve SWM in Logia town.

- Awareness creation and training should be given to the community about the importance of solid waste disposal through the community service.
- Increasing the community participation in local solid waste management decision making activities is better.
- Entrepreneurs and innovators should be encouraged to develop improved methods for waste collection and management.
- Policy makers should consider important variables like age, distance between respondent resident and waste disposal facility, existence unpaid option, sanitary supervisor visit, respondents dwelling type and households attitude.

- The municipal authority should control those individuals who collect unwilling payment without service from the households.
- The municipal authority should prepare the opportunity to households would receive planned and properly scheduled waste management services.
- Regular support and monitoring should be given to the business enterprise households engaged in solid waste management system.

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