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Phenotypic and Allelic Distribution of the ABO and Rhesus Blood Groups among students at Hawassa University, Ethiopia

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ABO blood;

Allele frequency;

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Research article

ABSTRACT

A prior information on the distribution of ABO and Rh groups is important for management of blood bank and transfusion, genetic counseling, anthropological studies, to study the association of blood groups and diet; to investigate the association between blood and diseases. This study intended to estimate the frequency of ABO and Rh bloods and investigate gene diversity at both loci among students in Ethiopia. A descriptive cross-sectional survey was employed involving randomly selected two thousand thirty nine (2039) university students (1054 males and 985 females) with an age range of 18–29 years. Blood groups were determined based on agglutination reaction. The most abundant blood group was found to be O (42.47%), followed by A (27.86%), B (21.87%), and AB (7.80 %). The frequency of Rh+ and Rh- were 90.88% and 9.12 %, respectively. The combined blood types showed O+, A+, B+ and AB+ were: 38.60 %, 25.20%, 20.10% and 7.00%, respectively. Slightly different distribution pattern of ABO blood group was observed among females from Amhara region (O> B> A>AB). The distribution of ABO phenotypes from Addis Ababa and Amhara did not differ significantly from those expected under the Hardy Weinberg Equilibrium. A high level of gene diversity was observed for both loci. In general, the O blood type is most frequent and followed by A, B and AB. A similar pattern of distribution of the ABO and Rh blood groups was found in male and female study subjects. The present study will generate a baseline data that could be used in blood bank management and transfusion, genetic counseling, population genetic and anthropological studies, and for disease management.

INTRODUCTION

The knowledge on the distribution of ABO and Rh groups is important for the management of blood bank and transfusion, genetic counseling, population genetics, and anthropological studies (Liu *et al*., 2017; Canizalez-Román *et al*., 2018) and to study the association of blood groups and diet, to relate the association between blood and diseases (Puryear, 2017). Individuals with the O blood type are thought to be resistant to viral disease (Zhao *et al*., 2021) but susceptible to some bacterial infections (Harris *et al*., 2005) and Hepatitis B virus (Jing *et al*., 2020). People with the A blood group have a higher risk, whereas people with blood group O have a

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lower risk for SARS-Cov-2 infection and COVID-19 severity (Zhao *et al*., 2021).

The frequencies of ABO and Rh blood groups differ with ethnicity, geographical locations, race, population movements, natural selection and genetic drift. In the USA, and Mexico the O type is the most frequent, followed by A, B and AB (Garratty *et al*., 2004; Canizalez-Román *et al*., 2018). Nevertheless, in China (Liu *et al*., 2017), type A is the most common, followed by O, B and AB. In southeast Asia, A and B blood groups were interchangeably taking the most common blood group place, while AB was the least common (Dewan, 2015). Type O is the most frequent whereas AB is less common in most African countries (Ndoula *et al*., 2014; Anifowoshe *et al*., 2017). The frequency of Rh-blood is less or rare in African and Asian countries (Anifowoshe *et al*., 2017; Liu *et al*., 2017). The available limited data indicate that the O blood is more frequent but AB is least common in Ethiopia (Golassa *et al*., 2017; Fufa and Debelo, 2019).

To date in Ethiopia, there are limited works done on the distribution of ABO and Rh blood groups and the most are small scale in terms of sample size and regional coverage (Golassa *et al*., 2017; Fufa and Debelo, 2019). In this work, however, the subjects were originated from almost all regions in the country and relatively bigger sample size of the study subjects involved in the study. Therefore, this study intended to eatimate the distribution of ABO and Rh bloods and investigate gene diversity of both loci among students in Ethiopia. It is expected that the data may partly contribute to strategies of supply and demand of blood products in transfusion services countrywide and could have consequences in investigating vulnerability to various disease conditions known to be connected with the blood groups.

MATERIALS AND METHODS

Study area and study subjects

The study was conducted in four campuses (College of Agriculture, Main Campus, Institute of Technology and; College of Medicine and Health Sciences) of Hawassa University. It is assumed that the regional and ethnic diversity of the Ethiopian population could be represented in the university student population. In Ethiopia students from different regions are randomly allocated to federal universities in the country, we therefore, believe that, student populations in Hawassa University could represent the regional and ethnic diversity in Ethiopia. Ethiopia has eleven regional States and two city administrations.

A descriptive cross-sectional survey was employed involving randomly selected two thousand thirty nine (2039) students (1054 males and 985 females) with an age range of 18–29 years. The selected study subjects originally came from eight regional states (Afar, Amhara, Benishangul Gumuz, Gambela, Oromia, Southern Nation Nationalities and People State (SNNPRS), Somali and Tigray) as well as from Addis Ababa (AA) and Dire Dawa (the two city administrations). The inclusion criteria for the study were: Ethiopian students who are above 18 years old and willing to participate in this study.

Blood group determination

The ABO and Rh blood group testing was done in the Hawassa University students' clinics, using a commercially available kit for blood

grouping (Human Diagnostic, Germany). Briefly, blood samples were collected from the tip of volunteers' finger, a drop of anti-A, anti-B and anti-D human sera was then added into 5% suspension of the collected blood (in principle the red blood cell) in normal saline within test tubes. The mixture was gently stirred with glass rods, and blood groups of the tested individuals were determined based on agglutination reaction.

Allelic frequency and gene diversity analysis

The frequencies of the I^A allele (p_1), I^B allele (p_2) and I^O allele (p_3) were calculated based on the extension of Hardy-Weinberg Equilibrium (HWE) for multiple alleles with two co-dominant allele and one recessive allele (Hamilton, 2009). Genotypic frequencies were calculated under HWE assumptions as $[(p_1 + p_2 + p_3)^2 = p_1^2 + p_2^2 + p_3^2 + 2\,p_1p_2 + 2\,p_1p_3 + 2\,p_2p_3 = 1]$as p_1^2 (I^AI^A) + 2 p_1p_3 (I^AI^O) + p_2^2 (I^BI^B) + 2 p_2 p_3 (I^BI^O) + 2 p_1p_2 (I^AI^B) + p_3^2 (I^OI^O). Nevertheless, the Rh system alleles "D" and "d" were allocated q1 and q2, respectively, and their occurrences were also computed using HWE for two allele system $[(q_1 + q_2)^2 = q_1^2 + 2\,q_1q_2 + q_2^2 = 1\ ]$ as q_1^2(DD), 2 q_1q_2 *(Dd)*, q_2^2 *(dd)*. Gene diversity (He) was analyzed according to Nei (1973). Percentage was used to express blood group phenotypic frequencies whereas allele frequencies were estimated/projected using the assumption of Hardy–Weinberg Equilibrium (HWE). The Chi - square test was used to compare observed allelic and genotypic frequency distributions of the blood group and Rh antigens to that expected under the HWE (Hamilton, 2009). The level of statistical significance was at $p<0.05$.

Ethical Approval

The study was carried out after getting ethical approval of the Institutional Review Board (IRB) of Hawassa University (Ethiopia), College of Natural and Computational Sciences (Ref. No. IRB/203/11; Date: 05/03/2019). Accordingly, the study objectives were explained to students, and written consent for participation in the study was obtained.

RESULTS

Distribution of ABO and Rh blood groups

The frequencies of O, A, B and AB blood types among the participants were: 42.47%, 27.86, 21.87% and 7.80, respectively. The O blood group had the highest frequency while blood group AB had the least frequency (Table1). The study showed that the ABO blood group pattern was in the order of O > A > B > AB. Statistically, no significant variation was noted in the proportions of the A, B, O and AB blood groups among the considered regions ($\chi^2_{0.05,\,12} = 15.055$; $p < 0.05$). But there were slight differences in the frequencies of ABO blood types. In terms of each blood type the highest proportion of the A phenotype (30.12%), the B phenotype (25.47%) and O phenotype (44.04%) was observed in SNNPRS, Amhara and Addis Ababa, respectively (Table1). The proportion of ABO blood antigens significantly different from those anticipated under Hardy–Weinberg Equilibrium (HWE) ($\chi^2_{0.05,\,1} = 10.498$; $p < 0.05$) for the combined data set, Oromia region ($\chi^2_{0.05,\,1} = 7.304$; $p < 0.05$), SNNPRS ($\chi^2_{0.05,\,1} = 6.027$; $p < 0.05$) and 'Others' ($\chi^2_{0.05,\,1} = 4.248$; $p < 0.05$), respectively. However, the distributions in Addis Ababa ($\chi^2_{0.05,\,1} = 1.063$; $p < 0.05$) and Amhara ($\chi^2_{0.05,\,1} = 0.004$, $p < 0.05$) did not deviate from HWE.

Table- 1: Phenotypic frequency of the ABO Blood groups and Rh system based on regions/ towns

Regions	ABO Blood					Rh System	
	N**	Type A (%)	TypeB (%)	TypeAB (%)	TypeO (%)	Rh^+ (%)	R^- (%)
Addis Ababa	234	62(24.50)	52(22.22)	17(7.26)	103(44.04)	202(86.32)	32(13.68)
Amhara	691	185(26.77)	176(25.47)	44(6.37)	286(41.39)	635(91.90)	56(8.10)
Oromia	603	167(27.69)	128(21.23)	53(8.79)	255(42.29)	554(91.87)	49(8.13)
SNNPRS	437	132(30.21)	77(17.62)	36(8.22)	192(43.94)	393(89.93)	44(10.07)
Others *	74	22(28.57)	13(17.57)	9(12.16)	30(40.54)	69(93.24)	5(6.76)
Total	2039	568 (27.86)	446(21.87)	159(7.80)	866(42.47)	1853(90.88)	186(9.12)

*Afar(1,1,0,2/3,1), Benishangul Gumz (1,1,1,5/8,0), Dire Dewa (1,2,0,1/4,0), Gambela (1,1,0,1/3,0), Somali (3,2,1,4/7,3)and Tigray(15,6,7,17/44,1). Numbers in the parenthesis are the numbers of individuals with ABO/Rh system, respectively. **sample size.

The frequency of allele O was larger as compared to alleles A or B ($p_3 > p_1 > p_2$). A comparable higher level of gene diversity was found in each region for both loci (Table2).

Table- 2: Allelic frequencies and Gene diversity of ABO blood group and Rh systems

Region/City	*ABO allele*			Rh allele		*ABO*	*Rh*	*ABO**				*Rh**			
	P_1	P_2	P_3	q_1	q_2	H_e	H_e	H_S	H_T	D_{ST}	G_{ST}	H_S	H_T	D_{ST}	G_{ST}
AA	0.186	0.16	0.65	0.63	0.37	0.51	0.47								
Amhara	0.182	0.17	0.64	0.76	0.24	0.52	0.37								
Oromia	0.203	0.16	0.63	0.72	0.29	0.53	0.41								
SNNPRS	0.215	0.14	0.65	0.68	0.32	0.52	0.43								
Others	0.238	0.14	0.62	0.74	0.26	0.54	0.39	0.54	0.51	0.04	0.07	0.41	0.42	0.004	0.01

*The figures are for the entire population. H_e:gene diversity in a subpopulation; H_S: average gene diversity within subpopulation; H_T: gene diversity for the entire population; D_{ST}: gene diversity among subpopulation; G_{ST}: Gene differentiation among subpopulation; AA; Addis Ababa; SNNPRS: Southern Nation Nationalities People Regional State

The Rh+ blood group comprised 90.88% for the overall data set (Table 1). The highest frequency of Rh+ (93.00%) was observed for 'other' group and least was for Addis Ababa (86.32%). In Addis Ababa the frequencies of Rh+ and Rh- were 86.32 % and 16.68%, respectively. The largest frequency of q_1 allele and lowest level of gene diversity was observed in Amhara (Table2). The expected gene diversity was highest in Addis Ababa and the lowest from 'others' (Table 2). For the ABO locus, the highest frequency of gene diversity was observed in the 'others' groups (H_e= 0.535) but the lowest was in Addis Ababa (H_e = 0.512).

The sex specific allele frequency is similar in male and female participants (Table3). A unique phenotypic (O > B > A > AB) was observed for females in Amhara.

Table- 3: Phenotypic, allelic frequencies and gene diversity of ABO blood based on sexes [Male (M); Female (F)]

Regions	Sexes		ABO blood type				Allelic frequencies*			
		N	A (%)	B (%)	AB (%)	O (%)	p_1	P_2	p_3	H_e
Addis Ababa	M	119	31(26.05)	27(22.68)	7(5.88)	54(45.37)	0.175	0.155	0.670	0.496
	F	115	31(26.95)	25(21.73)	10(8.69)	39(33.91)	0.254	0.220	0.526	0.610
Amhara	M	325	89(27.38)	77(23.69)	22(6.76)	137(42.15)	0.189	0.166	0.645	0.521
	F	366	96(26.22)	99(27.04)	22(6.01)	149(40.71)	0.177	0.182	0.641	0525
Oromia	M	320	86(26.87)	70(21.87)	23(7.18)	141(44.06)	0.188	0.158	0.654	0.512
	F	283	81(28.62)	58(20.49)	30(10.60)	114(40.28)	0.220	0.170	0.610	0.551
SNNPRS	M	249	75(30.12)	37(14.85)	21(8.43)	116(46.58)	0.216	0.124	0.660	0502
	F	188	57(30.31)	40(21.27)	15(7.97)	76(40.42)	0.214	0.159	0.627	0.536
Others	M	39	11(28.20)	7(17.94)	4(10.25)	17(43.58)	0.215	0.153	0.632	0.531
	F	35	12(34.28)	7(20.00)	4(11.42)	12(34.28)	0.263	0.172	0.565	0.585
Total	M	1054	292(27.70)	218(20.68)	78(7.40)	466(44.21)	0.194	0.152	0.654	0.512
	F	985	276(28.02)	228(23.14)	81(8.22)	400(40.60)	0.201	0.190	0.609	0.553

*Sex specific allelic frequencies

AB negative case was not observed from Addis Ababa. The highest frequency for B+ was from Amhara while O+ was highest for Oromia. The O+ was observed more than one third of the population, while AB- was recorded in less than 1% (Table 4).

Table- 4: Phenotypic frequencies of ABO blood types based on the Rh system

Regions/town	N*	Phenotype (%)							
		A+	A-	B+	B-	AB+	AB-	O+	O-
Addis Ababa	234	52(22.22)	10(4.27)	50(21.4)	2(0.85)	17(7.30)	0(0.00)	83(35.47)	20(8.54)
Amhara	691	171(24.70)	14(2.00)	159(23.00)	17(2.5)	40(5.80)	4(0.60)	265(38.40)	21(3.00)
Oromia	603	150(24.9)	17(2.8)	117(19.4)	11(1.80)	48(8.00)	5(0.80)	239(39.40)	16(2.70)
SNNPRS	437	120(27.50)	12(2.70)	71(16.20)	6(1.40)	30(6.90)	6(1.40)	172(39.40)	20(4.60)
Others	74	21(28.37)	1(1.35)	12(16.23)	1(1.35)	8(10.81)	1(1.35)	28(37.33)	2(2.70)
Total	2039	514(25.20)	54(2.60)	409(20.10)	37(1.80)	143(7.00)	16(.80)	787(38.60)	79(3.90)

*N: Sample size (number of students examined)

The proportions of the ABO/Rh blood groups were significantly different among the five regions ($\chi^2_{0.05,\ 28} = 43.033$; $p < 0.05$). There was a clear variation in the frequency distribution of the blood types between males and female subjects among the regions. For instance, the frequency of the A+ and O+ blood type showed a difference between the male and female from Addis Ababa (Table5).

Table- 5: Phenotypic frequencies of ABO blood types based on the Rh system for male (M) and female (F) subjects

Regions/city	Sex	N*	Phenotype (%)							
			A+ (%)	A-(%)	B+ (%)	B-(%)	AB+ (%)	AB-(%)	O+ (%)	O-(%)
Addis Ababa	M	119	25(10.70)	6(2.60)	26(21.84)	1(.84)	7(5.88)	0(0.00)	46(38.65)	8(6.72)
	F	115	27(24.35)	4(3.47)	24(20.86)	1(0.86)	10(8.69)	0(0.00)	37(32.17)	12(10.43)
Amhara	M	325	82(25.23)	7(2.15)	68(20.92)	9(2.76)	21(6.46)	1(0.31)	131(40.31)	6(1.84)
	F	366	89(24.32)	7(1.91)	91(24.86)	8(2.18)	19(5.19)	3(0.81)	134(36.61)	15(4.10)
Oromia	M	320	80(25.00)	6(1.87)	65(20.31)	5(1.56)	20(6.25)	3(0.93)	131(40.93)	10(3.12)
	F	283	70(24.73)	11(3.88)	52(18.37)	6(2.12)	28(9.82)	2(0.63)	108(38.16)	6(2.12)
SNNPRS	M	249	70(28.11)	5(2.01)	35(14.05)	2(0.80)	18(6.12)	3(1.20)	104(41.76)	12(4.81)
	F	188	50(26.59)	7(3.72)	36(19.15)	4(2.13)	12(6.38)	3(1.59)	68(36.17)	8(2.25)
Others	M	41	11(26.82)	0(0.00)	7(17.07)	0(0.00)	4(9.75)	1(2.43)	17(41.46)	1(2.43)
	F	33	10(30.30)	1(3.03)	5(15.15)	1(3.03)	4(12.12)	0(0.00)	11(33.33)	1(3.03)
Total	M	1054	268(25.42)	24(2.27)	201(19.07)	17(1.61)	70(6.64)	8(0.76)	428(40.60)	37(3.51)
	F	985	246(24.97)	30(3.05)	208(21.11)	20(2.03)	73(7.41)	8(0.81)	358(36.34)	42(4.26)

*N: Sample size (number of students examined)

DISCUSSION AND CONCLUSIONS

Distribution of ABO and Rh blood groups

The frequency distribution of ABO blood group differs from race to race, population to population and differs in different geographical areas. This study could serve as an initial countrywide report, as the participants were recruited from a national university accepting students from all corner of the country and representing the Ethiopian population. Ethiopia has a rich cultural, linguistic, and ethnic diversity and is home to over 70 different ethnic groups and over 80 living languages (Pagani *et al*., 2012, 2015). Therefore, Ethiopia is an important region for studying how genetic diversity and differentiation correlate with linguistic and cultural diversity. Furthermore, the knowledge on the distribution of ABO and Rh groups is important for management of blood bank and transfusion, genetic counseling, population genetics and anthropological studies and to study the association of blood groups and diet, to relate the association between blood and diseases (Dewan, 2015; Liu *et al*., 2017; Puryear, 2017; Canizalez-Román *et al*., 2018).

The proportion of AB blood was relatively larger compared to previous studies in Ethiopia

(Tesfaye *et al*., 2015; Fufa and Debelo, 2019), this could be due to the wider sampling regions covered in this study (Table1). The distribution pattern of ABO blood group of females from Amhara seems similar to that of the Bengali population in Bangladesh (Dewan, 2015). Golassa *et al*. (2017) found two patterns of ABO Phenotypes O>A>B>AB [Nilotic people] and A>O>B>AB ['Highlanders] in Gambela, southwestern Ethiopia. The results of this study are similar to that of previous studies in Cameroon (Ndoula *et al*., 2014), Nigeria (Anifowoshe *et al*., 2017), and Mexico (Canizalez-Román *et al*., 2018). However, the distribution differs from reports made in Egypt (Abdelmonem *et al*., 2019), China (Liu *et al*., 2017), and Bangladesh (Dewan, 2015).

Most of the participants in the current study were Rh+ (90.88%), while the rest were Rh- (9.12%). In general, the frequency of Rh- blood is less or rare in African and Asian countries (Liu *et al*., 2017; Abdelmonem *et al*., 2019). Golassa *et al*. (2017), however, reported a relatively higher frequency of Rh- (19.37%) in Gambela, southwestern Ethiopia. The overall frequency of Rh+ in the current study is comparable to Egypt (Abdelmonem *et al*., 2019), whereas lower compared to Cameroon (Ndoula *et al*., 2014), and Nigeria (Anifowoshe *et al*., 2017). The proportion of Rh+ in Addis Ababa is similar to white non-Hispanic in USA (Garratty *et al*., 2004), but large relative to Gambela (Golassa *et al*., 2017). The proportion of Rh- ranged from 7-14%, which is wider relative to studies done in Ethiopia (Tesfaye *et al*., 2015; Fufa and Debelo, 2019). Such a large range in the proportion of Rh- in this study could reflect wider regional coverage – the participants are almost from all the regions of the country.

The proportions of O+, A+, B+ and AB+ were: 38.60%, 25.20%, 20.10% and 7.00%, respectively. There was variation in the distribution of ABO/Rh between regions. The proportion of B+ was higher in the Amhara but O+ was higher in the Oromia (Table 4). The O+ frequency was found to be over one third of the entire population, while AB- was recorded in less than 1% of the study population. Similarly, in Cameroon (Ndoula *et al*., 2014), the O+ blood group is highly predominant, representing about half of the entire population, while AB- is very infrequent.

Allelic and genotypic diversity

The order of the ABO allele frequency was $I^O > I^A > I^B$, and similar to earlier studies (Ndoula *et al*., 2014; Anifowoshe *et al*., 2017), but differs from Bangladesh (Dewan, 2015), and in Egypt (Abdelmonem *et al*., 2019).The frequency of q_1 allele in this study is lower compared to that of Nigeria (Anifowoshe *et al*., 2017). However, a higher level of among subpopulation gene diversity (D_{ST}) and gene differentiation (G_{ST}) was found to be comparable to Mexican populations at both loci (Canizalez-Román *et al*., 2018). The higher level of gene diversity supports the hypothesis that Ethiopia is an important region for studying how genetic diversity and differentiation correlate with linguistic and cultural diversity (Pagani *et al*., 2012, 2015). The distribution of ABO phenotypes for Addis Ababa city and Amhara are not significantly different from those expected under the HWE. If a population is in a HWE the genotypic frequency will remain stable unless the equilibrium is perturbed. That would be a good opportunity for the management of blood banks as the frequencies of the blood groups will be stable

generation after generation (Ndoula *et al*., 2014; Canizalez-Román *et al*., 2018).

Participants from different regions showed a similar pattern of distribution for both loci which may reflect the complex processes, population admixing among the regions (Hamilton, 2009; Dewan, 2015; Canizalez-Román *et al*., 2018). Although the general pattern of distribution of the ABO and Rh blood type was similar among different populations, there are also slight differences in the frequencies of different blood types, genotypes and allele frequencies. Such differences of in the phenotypic, genotypic and allelic frequencies could be due to differences in the culture, geography, endemic diseases or population admixture (Hamilton, 2009; Dewan, 2015; Canizalez-Román *et al*., 2018). Some of these factors could put a selective pressure in favoring one allele over the other and could shape the genetic structure of the ABO and Rh loci of populations in the respective areas in a long term. Furthermore, the low level of gene differentiation observed among subpopulations, could be due to complex population admixture among regions (Dewan, 2015; Canizalez-Román *et al*., 2018).

Association between blood groups and diseases

The higher frequency of O blood group observed in this study could have an evolutionary advantage in conferring resistance to disease like malaria. In malaria prone countries of Africa group O are dominant with the distribution ranging from 40.0% to 80.0 % (Cserti and Dzik, 2007). As Ethiopia is a malaria endemic area in the sub-Saharan Africa (Anstee, 2010; Golassa *et al*. 2017), the dominance of O blood type in the present study, could be advantageous for protection against protozoan diseases (Harris *et al*., 2005; Panda *et al*., 2011). Tekeste and Petros (2010) reported a strong association between ABO blood group distribution and the prevalence of malaria in three malaria endemic areas in Ethiopia. The same authors have found among study participants with severe malaria the most frequent blood is type A, whereas among the healthy control groups the most common blood type is O. Similarly, a study done by Panda *et al*. (2011) in India found that the most common blood type is B among participant with severe malaria in while blood type O is the most frequent blood type among the healthy control groups. Both of these studies support the hypothesis that O blood confers protection to severe malaria albeit the exact mechanism of protection is not well understood and needs further investigation. A review made by Rowe *et al*. (2009) on the association between falciparum malaria and ABO blood group support the hypothesis that non-O blood groups emerging as significant risk factors for life-threatening malaria, through the mechanism of enhanced rosette formation. Although the O blood is hypothesized to give protection against malaria, it makes people susceptible *Vibrio cholera* (Harris *et al*., 2005). Therefore, this interplay between the different diseases on ABO blood type (e.g., cholera vs. malaria) could contribute to the phenomenon of a balanced polymorphism in the human population genetic structure (Hamilton, 2009).

In a nut shell, the current study established that among the various ABO and Rh blood groups, blood group O is the most common, followed by blood groups A, B, and AB with a predominance of Rh positivity. This work will

provide useful information for health institutions in the establishment of regional and national programs that speed up blood transfusions and tissue transplants needed in clinical practice. Additionally, this work is expected to generate interest in population geneticists and anthropologists to study genetic variation at ABO and Rh loci, as well as for physicians interested in the application of immunogenetics in diagnosis and clinical practice.

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Effect of Pressure on Carbon monoxide Oxidation on Titania Supported Platinum Nanoparticles Catalyst

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Activity;

CO conversion;

Particles;

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Research article

ABSTRACT

Thermographic testing methodology was developed to facilitate measurements of particle dimension and substrate influence in heterogeneous catalysts. A screening chip with several areas of less stress silicon nitride membranes which displays less heat conductivity and heat capacity was used. Heat produced during the reaction on catalysts deposited on membranes was established through IR camera which gave the value of the turn over frequency. Effect of pressure on CO conversion on titania supported Pt particles of different dimension was measured on 120 catalysts concurrently. The reaction was studied at various O_2 and CO pressures at 170 ℃ and 240 ℃. At these temperature conditions, activity increased with increase of O_2 and CO pressure, in agreement with previous reports.

INTRODUCTION

There is a fair idea on catalysis starting from ancient times. It largely contributes in a number of applications as in chemical, agricultural, food and pharmaceutical industries, manufacturing and energy transformation and environmental safety (Scheidtmann *et al*., 2001, Emmanuel, 2022). Estimates indicate that above 90% of chemical production routes depend on a single or multiple catalytic pathways (Armor, 2011, Emmanuel and Hayden, 2022). Metal catalysts largely rely on substrate, shape and dimension of supported metal nanoparticles catalyst. Reports indicate that the reactivity of properly characterised supported catalysts are important in knowing the influence of particle dimension and substrate. A good case of such a catalyst revealing a greater substrate and particle dimension influence on reactivity is supported platinum (Pt) in low temperature conversion of tiny molecules like carbon monoxide (CO) and hydrocarbons (Liu *et al*., 2010). Platinum (Pt) is among the frequently used metal catalysts in enormous applications over the decades. Dobereiner reported the activity of Pt in 1800s where it was applied in the catalysis of H_2 and

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O_2 in portable lamp (Somorjai, 1994). Besides, Pt related catalysts are applied in different reactions such as the transformation of aliphatic straight-chain organic molecules to aromatic molecules and branched molecules, in huge scale hydrogenation in chemical and petroleum-refining industries and ammonia conversion (Somorjai, 1994). More significantly, it is used for CO oxidation and unburned hydrocarbons in car emissions control (Franceschetti *et al*., 2003). Platinum catalyst, on the other hand, is the most commonly applied and active electrode in fuel cell technology (Franceschetti *et al*., 2003). Although Pt is widely used in various fields, it is an expensive precious metal and less abundant which make its application in various technologies largely challenging (Cameron *et al*., 2003). Because Pt bare high cost, the priority has been its application at the atomic scale in heterogeneous catalysis. This includes the spreading of Pt particles on high surface area metal oxide substrates like Al_2O_3, TiO_2 and Fe_2O_3 which reduce the quantity of Pt integrated in the catalyst (Somorjai, 1994). Supporting Pt lowers the catalyst expenses in addition to increasing effective surface area of the catalyst in addition, it strengthens catalyst' particles. Substrates like TiO_2 are directly involved in reaction pathways through reactants and intermediates activation thus, improving the activity. Pt catalysts on carbon is widely applied in PEMFCs technology (Emmanuel, 2022). However, application of Pt catalyst is affected because of the decline of catalytic efficiency due to particle disintegration, rusting of cathode substrate and CO inhibition (Kim and Jhi, 2011). The decline of efficiency is because of greater interaction of Pt and CO which inhibits O_2 from getting on the surface of a catalyst (Schubert *et al*., 2001; Molina *et al*., 2009; Liu et al., 2010). Carbon monoxide conversion reaction on Pt supported catalysts is widely investigated (Santra and Goodman, 2002; Liu *et al*., 2010; Slavinskaya *et al*., 2011; Allian *et al*., 2012; Dobrin, 2012). It is known that because of strong interaction with oxygen and Pt surface, O_2 adsorbs and break down to generate effective surface adsorbed atomic oxygen which combines with adsorbed CO to generate CO_2 (Bamwenda *et al.*, 1997; Haruta, 2003; Gao *et al*., 2009). Carbon monoxide conversion on Pt proceeds very efficiently with a conversion rate dependent on CO and oxygen partial pressures. Previous reports show that the reaction needs chemisorbed oxygen and CO on Pt surface, a pathway termed as Langmuir-Hinshelwood mechanism (McCash, 2001; Kolasinski:, 2002; McClure and Goodman, 2009; Liu *et al*., 2010, Santos *et al*., 2010). Studies indicate that this reaction is influenced by reactants pressure, coverage and surface temperature (Kolasinski:, 2002). However, competitive adsorption among CO and O_2 is reported. Although CO can adsorb on an O_2 occupied surface, O_2 cannot adsorb on CO occupied surface. With high CO occupied surface, the reaction is restricted by O_2 thus, raising CO pressure inhibits the reaction because no extra adsorption sites for O_2. However, at low CO occupancy, O_2 adsorption proceeds rapidly and the reaction relies on surface coverage of CO and O_2 (Kolasinski:, 2002). Given the catalytic efficiency of Pt catalyst, the reactivity is hampered by CO inhibition in gas-phase CO oxidation thus, maximum CO oxidation to CO_2 is attained at minimal CO surface occupancy (McClure and Goodman, 2009). In this regards, Pt catalysts on substrates are perceived as poor catalysts for small temperature CO conversion (Li *et al*., 2008).

Studies indicate that Fe_2O_3 supported Pt nanoparticles catalyst exhibits unusual high catalytic properties for CO conversion at small temperature (Liu *et al*., 2010). The activity is associated with the capability of Fe_2O_3 to provide active oxygen during the reaction. Titania supported Pt nanoparticles less than 5 nm show low activity for CO oxidation reaction (Rashkeev *et al*., 2007). Theoretical studies propose that Pt nanoparticles between 1 and 2 nm dimensions are more effective for CO oxidation (Dobrin, 2012). Further reports indicate that Pt particles of 2 nm dimension are the best effective for CO conversion in comparison with those of 3 nm and 5 nm dimensions (Kageyama *et al*., 2013). Electrocatalysis benefits from combinatorial synthesis and thorough characterization of oxide-supported metal nanoparticle catalysts, readily enabling high-throughput screening of various reactions using electrochemical chips. However, applying analogous methods to heterogeneous catalysis is considerably more complex. A breakthrough solution, published by Emmanuel et al. (2019), utilizes a 100-channel microreactor array and mass spectroscopy to study the H2-D2 exchange reaction on thin-film, small-area alloy catalysts. For less complex reactions in which it is not important to establish selectivity, net activity established in the heat generating reaction through infrared thermography methodology is promising. This methodology was applied for testing of catalysts in the conversion of hydrogen and octane on high area catalyst samples (Emmanuel *et al*., 2019). However, expansion of the methodology to arrays of properly characterised catalysts like metal supported electro-catalysts to achieve structure/activity influences is a challenge (Hayden, 2013). The considerably low surface area of planar catalysts needs an amplified sensitivity so as to identify the heat produced in the course of a reaction. The current study reports the impact of pressure on reactivity of titania supported Pt particles catalyst for CO conversion by applying a nano-fabricated screening chip which enables simultaneous testing of a chains of supported Pt catalysts of controlled particle dimension at different CO and O_2 pressures.

MATERIALS AND METHODS

Creation of platinum nanoparticles catalysts

A combinatorial technique centred on a high throughput physical vapour deposition (HT-PVD) developed by Brian et al. (Hayden et al., 2009) was used to create thin films of TiO_2 and TiO_2 supported Pt nanoparticles catalyst, Figure 1.

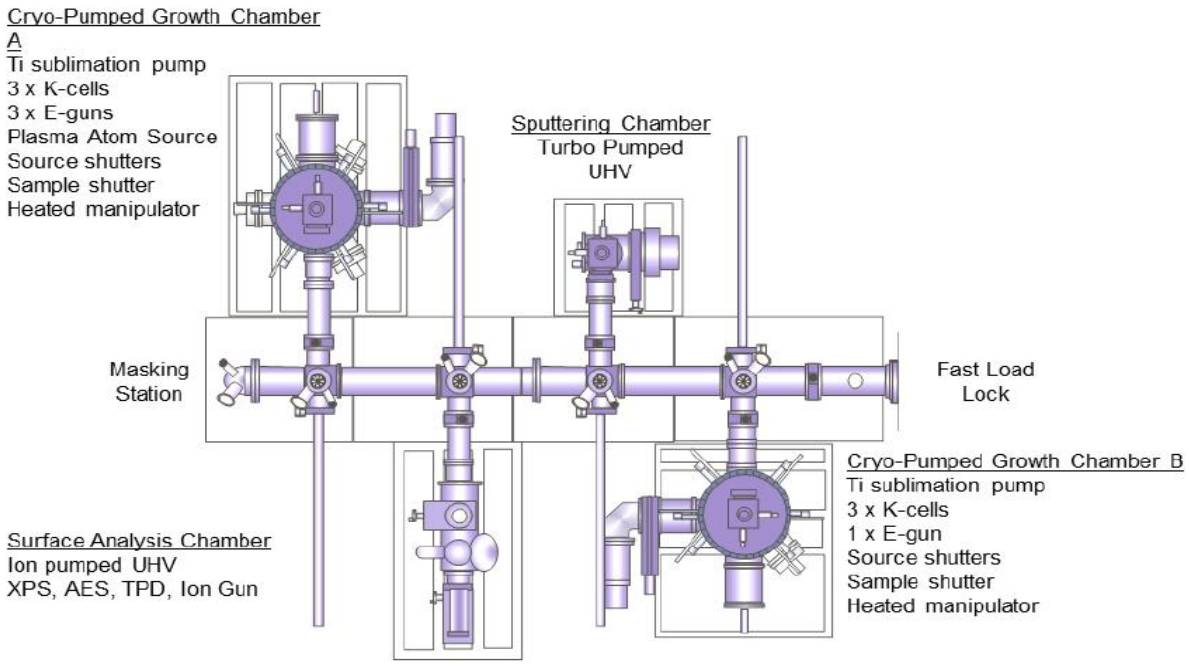

Figure 1: Schematic diagram of HT-PVD system indicating two cryo-pumped thin film synthesis chambers A and B, sputtering and surface analysis chambers and the load lock (adapted from Hannah, 2012).

This technique creates thin films through condensation of evaporated material onto a substrate. The deposition chamber consisted of three electron gun (e-gun) evaporation sources (Temescal) and three Knudsen cell (K-cell) sources (DCA). The HT-PVD system operational base pressure was 1 x 10^{-10} mbar. In the present study, electron beam sources, E-gun 1 was applied to evaporate Ti, Pt was evaporated from E-gun 3. Titania layers of about 200 nm were deposited onto a catalyst screening chip from titanium (99.995 %, Alfa Aesar metals) from E-gun 1 and oxygen (Air products, special gases, 99.999 %) at a constant pressure of 9.7 x 10^{-6} Torr at 1 sccm oxygen flow rate and plasma source, P_{rf} = 300 W at a deposition rate of 4 Å/s with substrate retained at ambient temperature throughout film creation. The widths of titania layer deposits were managed through deposition time and sample thickness was subsequently achieved via calibration of deposition rates from AFM readings. The AFM (Vecco Autoprobe M5) instrument was applied in a contact mode with a silicon cantilever, resonance frequency of 180 kHz, spring constant of 5Nm^{-1} with an estimate tip (CSC17 probe, MikroMasch) curvature of 10 nm.

Characterization of platinum nanoparticles

Characterization and distribution of particle dimension was conducted by using TEM where a small layer of TiO_2, l5-25 nm thick, was created onto small carbon coated copper TEM grids (Agar scientific). The grids bared TiO_2 produced under the same deposition conditions as a catalyst screening chip. Platinum particles

from Pt source (E-gun 3), were created onto a screening chip where TiO_2 support material was previously created through HT-PVD technique. The deposition rate of 4 Å/s were achieved by creating several thick layers from short to extended times, showing that the thickness as established on contact masked samples using AFM was relative to the deposition duration. The rate of Pt deposition (0.15 Å/s) was established through creation of continuous Pt small films, and reduced deposition durations (30 s – 360 s) applied to create Pt particles via nucleation and growth on titania substrates at 250 ℃. For surface characterization of Pt nanoparticles on titania substrate, Pt nanoparticles were grown onto Formvar® carbon coated copper grids (Agar scientific) coated with a small layer of titania 15-25 nm thick for transmission electron microscope (TEM) measurements. Characterization of particles was conducted by TEM prior deposition onto the screening chip for assurance of particles creation. Using Jeol 3010 instrument, TEM images were attained at an accelerating voltage of 300 kV containing a Gatan CCD camera for capturing images. X-ray Photoectron Spectroscope (XPS) studies were conducted in Ultra High Vacuum (UHV) system containing a twin anode X-ray source (Mg Kα and Al Kα) and a VG Clam Single Channel XPS system analyser. Depositions the substances were undertaken onto silicon nitride on silicon and on a 10 x 10 or 12 x 12 array nano-fabricated catalyst screening chip (450 µm silicon wafer thickness) on which a low pressure chemical vapour created (LP-CVD) silicon nitride membrane (300 nm and 600 nm) has been previously created. The screening chip was back etched to create individual membranes. The HT-PVD system was set up on a "wedge" deposition to generate different particle dimensions distributions throughout the support.

A silicon chip with the dimension of 35 mm x 35 mm was fabricated (450 mm thick silicon wafer) for IR thermography readings and an array of 10 x 10 silicon nitride membranes (1.5 mm x 1.5 mm) of 600 nm thickness were produced by back etching of silicon to a layer of LP-CVD silicon nitride as described earlier (Emmanuel, 2022). The membrane with 200 nm of titania substrate was optically transparent. For temperature measurement of the membrane, a small graphitic carbon layer (ca. 200 nm) was created on the back of the membrane supplying an emissivity approximate to that of a black body. Thin SiN membrane offered a support for the catalyst with small thermal mass and less thermal conductivity to the surrounding silicon chip thus, heat produced in the course of a reaction on the catalyst could subsequently increase membrane temperature.

Testing of platinum nanoparticles for catalytic activity

A screening chip with a catalyst created on the whole chip was placed on a heated sample holder with a heat shield to enable the whole chip to be heated evenly up to 250 ^{o}C. The sample holder was placed in a UHV system an IR transparent window (CaF_2) and the surface of the chip was captured (50 mm focal length camera lens) by a thermal camera (Jade III, CEDIP) operating in the spectral range of 3.6 – 5.1 µm with a thermal sensitivity of 20 mK.

Spatial resolution was 320 x 240 pixels and the whole 12 x 12 array was imaged to fill the detector. Reactions were conducted in a turbomolecular pumped UHV system with a

base pressure of 1 x 10^{-10} mbar and the temperature response of the catalyst on the membrane for a given power input (from an exothermic reaction) was established via finite element thermal modelling (Comsol Multiphisics®) (Emmanuel, 2022).

It is hypothesized that energy loss from the membrane primarily occurs via thermal conduction through the membrane itself, including the titania support and graphite layer, to the underlying silicon chip. Radiative and convective losses were estimated to be zero therefore, extra radiative and convective wastes over a few degrees temperature over the base temperature of reaction were expected to be very minimal (Emmanuel, 2022). For CO conversion reaction (ΔH = -283 $kJmol^{-1}$) (Emmanuel, 2022) and a pressure of 1 x 10^{-3} mbar, assuming each molecule is converted, the theoretical power was 2.289 x 10^{-4} Js^{-1} mm^{-2} (Emmanuel, 2022). This resulted in a calculated temperature increase of 4 °C (ΔT = 4 °C) at the center of the membrane. The chip's calculated sensitivity enabled the determination of the reaction turnover frequency (TOF) at the catalyst surface. However, the uncertainty in the thermal conductivity values of the membrane's composite layer led to an estimated error of approximately ±30% in the absolute TOF values. A detailed simulation of the temperature distribution across the 1.5 mm x 1.5 mm membrane was previously reported (Emmanuel, 2022).

RESULTS AND DISCUSSION

Images of TEM for Pt catalyst supported on titania resemble those reported earlier in terms of their shape and growth mode (Emmanuel, 2022), Figure 2. The TEM images facilitated the determination of particle dimension reliance of supported Pt in relative to the equivalent coverage of Pt created. On the Figure, particles of Pt appear in black relative to the white substrate background. At a shorter deposition period (30 seconds) in (a), particles are smaller (black). Conversely, when deposition periods escalate, particles increase in dimension because extra Pt is being added as witnessed in the dimension of particles in (b), (c) and (d), respectively, with deposition periods of 30 seconds, 2, 3.5 and 5 minutes, respectively.

In order to establish the change of particle dimension distribution after CO conversion because it was not possible to have it established directly on the chip by TEM, XPS measurements were conducted prior and after the reaction. A small change to higher binding energy was observed which was associated with final-state effect, consistent with earlier studies (Liu *et al*., 2014). The raise in the intensity was detected with increasing particle dimension, showing that Pt particles were growing in size as extra Pt was deposited. The study of Pt core level energies show a small but recognizable shift in binding energy.

Studies indicate that the binding energy shifts governed by the species to which an atom is attached and the binding energies shifts for core level electrons can emerge from initial-state or final-state effects (Attard and Barnes, 1998, Kolasinski:, 2002).

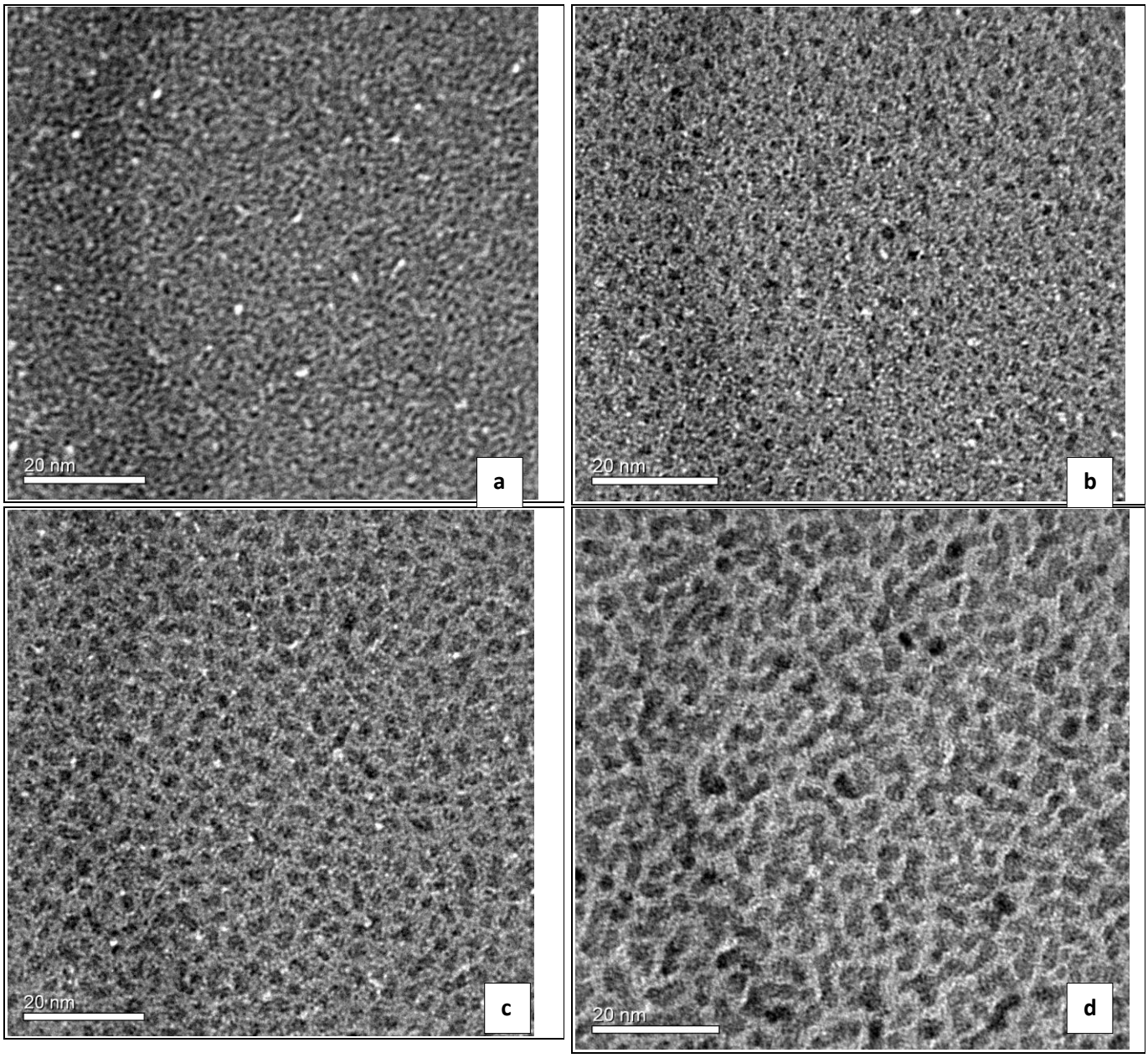

Figure 2: Images of TEM for Pt particles at different deposition periods, (a) 30 seconds, (b) 2 minutes, (c) 3.5 minutes and (d) 5 minutes with a mean particle dimension of (a) 1.6 nm, (b) 2.6 nm, (c) 4.9 nm and (d) 6.7 nm

Initial-state effects (chemical shift) are attributed to chemical bonding which largely affects electronic configuration of an atom leading into a large shift in binding energy of up to 10 eV (Emmanuel, 2022). Thus, atoms in a high oxidation state generate XPS peaks at high binding energy as compared to similar atom in a low oxidation state (Emmanuel, 2022). However, the final-state effects are because of the ejection of an electron from an atom which

corresponds to an ionic state producing a hole in place of the removed photoelectron (Emmanuel, 2022). Effect of the binding energy shift due to final-state is often a slight binding energy shift compared to that of initial-state effect. Because the core level binding energy shift for Pt particles detected in the current study is smaller, typically less than 1 eV, it can be linked with final state-effect, consistent with the earlier reports (Zhang *et al*., 1997; Guerin *et al*., 2006; Liu *et al*., 2014,). Figure 3 show the reliance of Pt $4f_{7/2}$ binding energy for Pt particle dimension recorded on the screening chip prior and after the reaction on Pt supported catalysts.

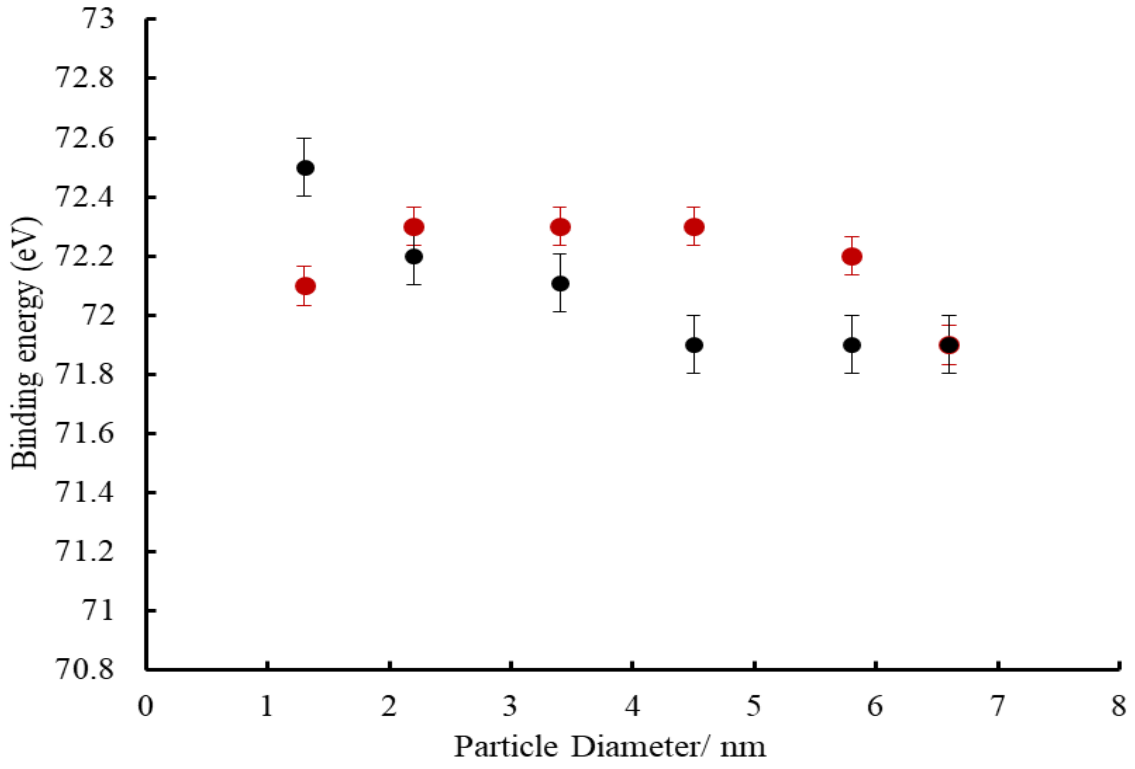

Figure 3: Binding energy of Pt particle size for the Pt 4f7/2 prior and after the catalytic reaction.

At a base temperature of 170 ºC on Pt catalyst, a pressure of a gaseous mixture was 7.2 x $l0^{-2}$, 8.4 x 10^{-2} and 1.04 x 10^{-1} mbar with O_2: CO ratio of 1:1. For a pre-exposed O_2 catalyst surface, the pressure was 1.5 x $l0^{-1}$, 1.9 x $l0^{-1}$ and 2.2 x 10^{-1} mbar with O_2: CO ratio of 2:1, 1:1 and 1:1, respectively, while on a pre-exposed CO catalyst surface the pressure was 1.5 x 10^{-1}, 1.7 x 10^{-1} and 2.2 x 10^{-1} mbar with O_2: CO ratio of 1:2, 1:1 and 1:1, respectively. Besides at 240 ℃ and O_2: CO ratio of 1:1, the pressure was 6 x 10^{-1}, 1.1 and 2.4 mbar.

The temperature was determined concurrently on the catalysts integrating over a 5 minutes interval. Catalysts were created such that particles dimension was constant throughout the rows and differed in the columns of the screening chip. The variation in temperature

across a row of similar particle dimensions was 0.2 ℃, far less than that in the columns and was attributed to a partial shielding of the gas flux at the boundaries of the sample by the holder. The increase, ΔT, in average was applied to establish the oxidation rate of CO to CO_2 at the catalyst assuming the enthalpy of reaction was ΔH = -283 kJ mol^{-1} (Emmanuel, 2022). The mass of Pt and number of Pt atoms at the surface of the particles per catalyst area of catalyst was computed from TEM images, assuming the particles were hemispherical (Hayden *et al.*, 2009, Emmanuel, 2022). This allowed the computation of TOF at Pt surface. Figure 4 presents the TOF for CO conversion reaction at 170 ℃ with the pressure of 7.2 x $l0^{-2}$, 8.4 x $l0^{-2}$ and 1.04 x $l0^{-1}$ mbar and O_2: CO ratio of 1:1.

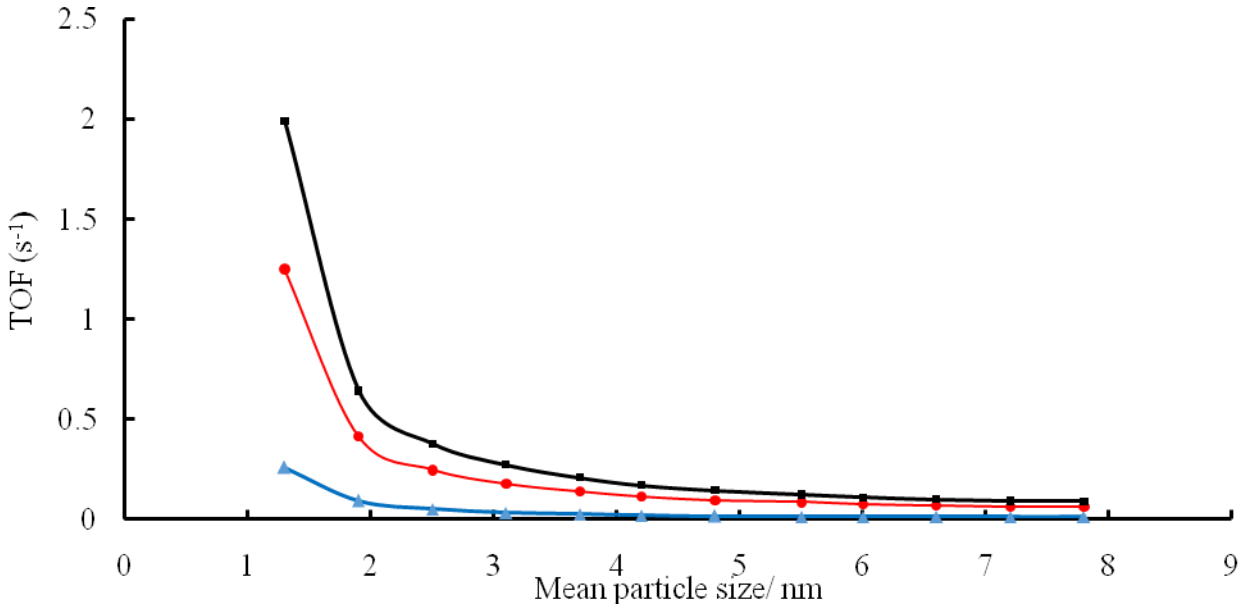

Figure 4: TOF for CO conversion on Pt particles at a pressure of 7.2 x 10^{-2} (blue triangles), 8.4 x 10^{-2} (red circles) and 1.04 x 10^{-1} mbar (black squires) with O_2: CO ratio of 1:1 at 170 ℃.

In Figure 5 is the TOF for CO conversion reaction on a pre-exposed O_2 catalyst surface with a pressure of 1.5 x 10^{-1}, 1.9 x 10^{-1} and 2.2 x 10^{-1} mbar at O_2: CO ratio of 2:1, 1:1 and 1:1, respectively, at 170 ℃. Figure 6 presents the TOF for CO conversion at 170 ℃ on a pre-exposed CO catalyst surface with a pressure of 1.5 x 10^{-1}, 1.7 x 10^{-1} and 2.2 x 10^{-1} mbar at O_2: CO ratio of 1:2, 1:1 and 1:1, respectively

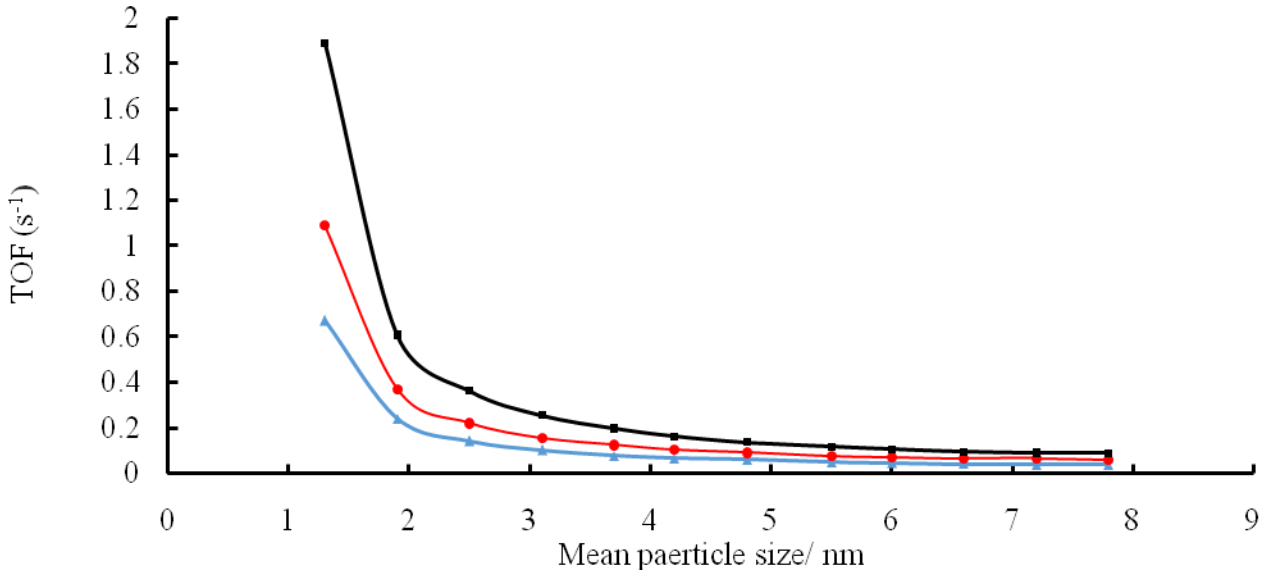

Figure 5: TOF for CO conversion reaction on Pt particles catalyst for a pre-exposed O_2 Pt surface, pressure of 1.5 x 10^{-1} (blue triangles), 1.9 x 10^{-1} (red circles) and 2.2 x 10^{-1} mbar (black squires) at O_2: CO ratio of 2:1, 1:1 and 1:1, respectively, at 170 ℃.

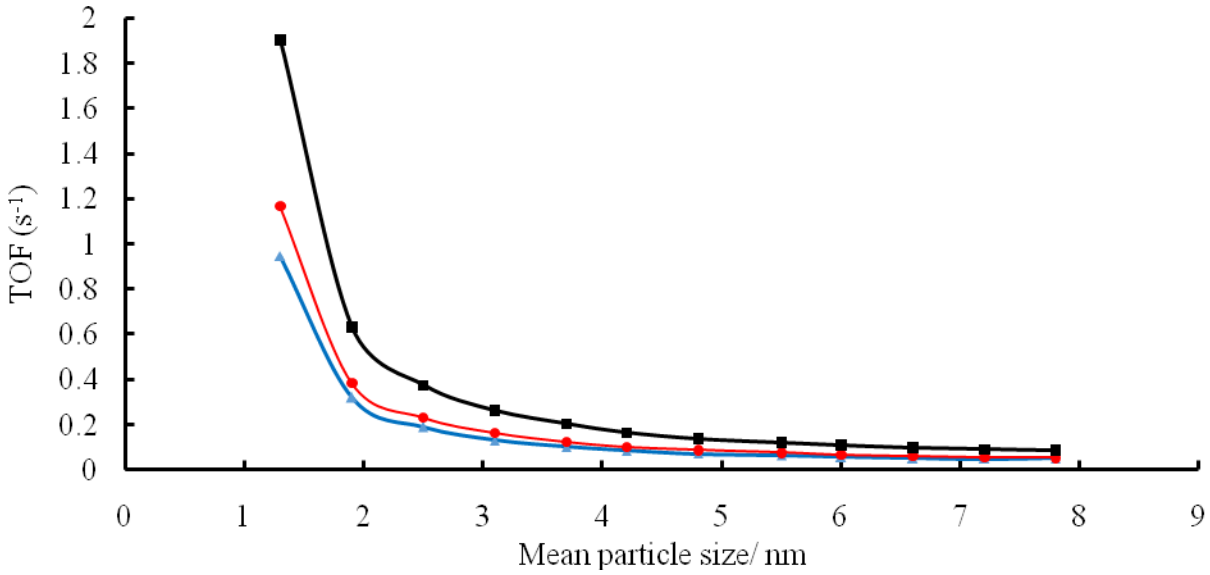

Figure 6: TOF for CO conversion on Pt particles catalyst for a pre-exposed CO Pt surface, pressure of 1.5 x 10^{-1} (blue triangles), 1.7 x 10^{-1} (red circles) and 2.2 x 10^{-1} mbar (black squires) at O_2: CO ratio of 1:2, 1:1 and 1:1, respectively, at 170 ℃.

The TOF for CO conversion reaction at 240 ℃ with a pressure of 6 x 10^{-1}, 1.1 and 2.4 mbar at O_2: CO ratio of 1:1 is also presented, Figure 7.

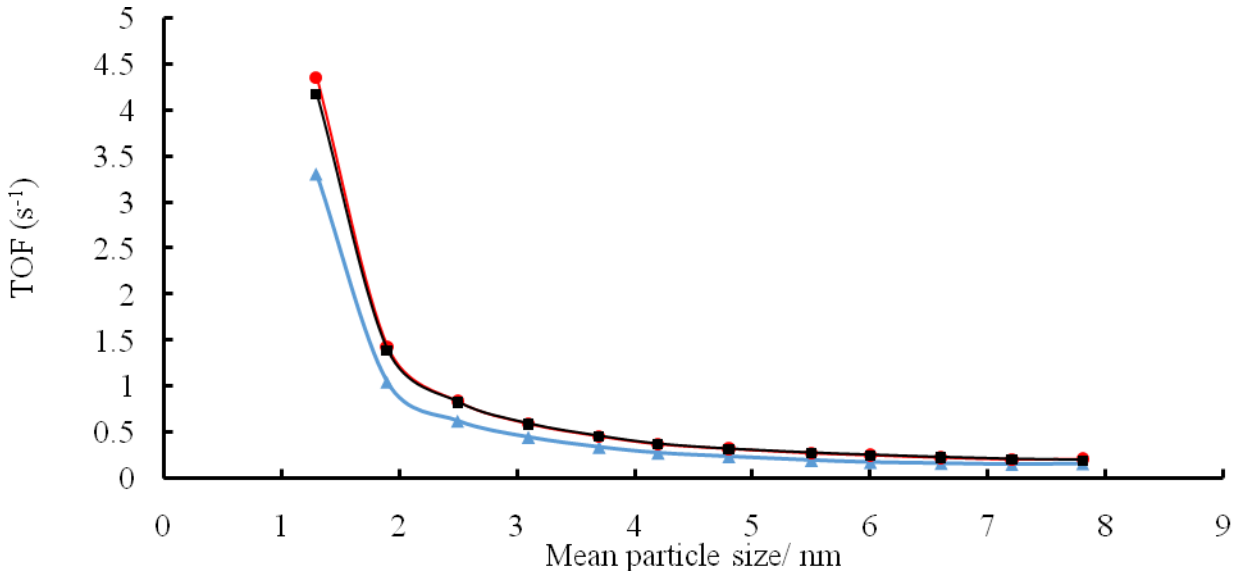

Figure 7: TOF for CO conversion on Pt particles catalyst at 240 ℃ and O_2: CO ratio of 1:1, pressure of 6.1 x 10^{-1} (blue triangles), 1.1 (red circles) and 2.4 mbar (black squires).

At each temperature, there is an increase in TOF as reactants pressures increase revealing that Pt catalyst is more active for CO conversion at higher pressure. For a pre-exposed O_2 catalyst surface at 170 ℃, a similar trend in TOF of Pt catalyst was observed by increasing pressure, Figure 5. The same behaviour in TOF was noticed for a pre-exposed CO Pt catalyst surface at 170 ℃, Figure 6. Again, reactants pressure led to a significant increase in activity of Pt catalyst for CO conversion with increasing pressure at 240 ℃ as shown by the values of TOF in Figure 7. Thus, the highest TOFs of Pt particles catalyst were achieved with increasing reactants pressure at higher temperature. Therefore, at each temperature, there is a linear relationship between the TOFs and pressure for CO conversion on Pt catalyst. The activity trend achieved in the current study is consistent with earlier findings which addressed the dependence on O_2 and CO pressure of the reaction rate for CO conversion on Pt catalysts (Li *et al*., 2013, Berlowitz *et al*., 1998). For a pre-adsorbed O_2 surface, TOF increased by raising CO pressure at O_2: CO ratio of 1:1, pressure of 2.2 x 10^{-1} mbar and on a Pt surface pre-adsorbed with CO, a similar trend was achieved, consistent with earlier reports (Berlowitz et al., 1998; Johanek *et al*., 2004; Li *et al*., 2013). TOF of Pt catalyst at 240 ℃ and higher reactant pressures resulted in the highest TOFs although a slight decline in activity was observed at 2.4 mbar, Figure 7. For example, TOF declined from 4.355 s^{-1} at 1.1 mbar to 4.174 s^{-1} at 2.4 mbar on Pt particle dimension of 1.3 nm. The fall of activity is attributed to CO inhibition of Pt catalyst surface

prohibiting O_2 adsorption and breakage on the catalyst surface, an important reaction pathway thus, decreasing Pt activity, in agreement with previous findings (McClure and Goodman, 2009). Results show that CO conversion on Pt catalyst is influenced by reactants pressure and surface temperature (Kolasinski:, 2002). For instance, at 170 ℃ and O_2: CO ratio of 1:1, Figure 4, TOF raised from 0.259 s^{-1} at 7.2 x 10^{-2} mbar to 1.991 s^{-1} at 1.04 x 10^{-1} mbar on Pt particle dimension of 1.3 nm. A similar raise in activity with increasing reactants pressure was attained on a pre-adsorbed O_2 Pt catalyst surface where TOF increased from 0.671 s^{-1} at 1.5 x 10^{-1} mbar to 1.889 s^{-1} at 2.2 x 10^{-1} mbar on Pt particle dimension of 1.3 nm, Figure 5. Besides, TOF increased from 0.946 s^{-1} at 1.5 x 10^{-1} mbar to 1.903 s^{-1} at 2.2 x 10^{-1} mbar on Pt particle dimension of 1.3 nm for a pre-adsorbed CO Pt surface, Figure 6. However, a competitive adsorption exists among CO and O_2 towards Pt surface, though CO can adsorb on an O_2 occupied surface, O_2 cannot adsorb on a CO occupied surface. Therefore, at high CO occupied surface, the reaction is restricted by O_2 thus, raising CO pressure prevents the reaction because there are no extra adsorption sites for O_2. Contrary, at low CO occupied surface, oxygen adsorption occurs rapidly and the reaction relies on the surface coverage of CO and O_2 (Kolasinski:, 2002). Usually, higher CO conversion to CO_2 is attained at situations that allow least CO surface occupancy (McClure and Goodman, 2009). In addition to temperature and pressure, CO conversion on Pt is dependent on particles dimension where the smallest particles exhibit higher activity. Although there is no common agreement from literature about the influence of particle dimension for supported Pt catalyst, results show that Pt particles within 1.1 nm and 10 nm, for CO conversion at different temperatures the activity increases with decreasing particle dimensions (Li *et al*., 2013). This is because for particle dimension of lower than 10 nm, the comparative number of kink sites, steps and corners raises monotonically with declining dimension and the low-coordinated surface atoms bare huge difference in the capability to react with molecules from the gas phase thus, accelerating the reaction (Overbury *et al*., 2006; Li *et al*., 2013). However, it is necessary to relate the activity trend with pressure at 170 ℃ and that attained at 240 ℃. Results show higher activity of Pt catalyst at 240 ℃ as reflected in TOF with increasing pressure, for instance, TOF increased from 3.312 s^{-1} at 6.1 x 10^{-1} mbar to 4.355 s^{-1} at 1.1 mbar on Pt particle dimension of 1.3 nm. Studies show that Pt surface is exceptionally active at high reactant gas pressure and temperature because at low temperatures, the CO repressed regime takes over and the reaction is prohibited by adsorbed CO which preventing adsorption and breakage of O_2 thus, leading to low catalytic activity of Pt catalyst (Gao *et al*., 2009, McClure and Goodman, 2009, Liu *et al*., 2010). However, at all pressure and temperature conditions investigated in the current study, CO conversion rate increased with increasing pressure. Such activity trend illustrates the influence of pressure and temperature for CO conversion on Pt nanoparticles catalyst in addition to Pt particles dimension. This behaviour is consistent with earlier findings on the reliance of pressure of O_2 and CO of the reaction rate for CO conversion on a similar system (Berlowitz *et al*., 1998; Li *et al*., 2013). These result can be considered in the light of the influence of pressure to explain high activity of Pt catalyst at high pressure and temperature

among other factors (Li *et al*., 2013). The findings indicate that at a particular temperature, an increase of pressure yields the highest activity compared to that obtained at low pressure and temperature. Thus, these results provide the evidence of the influence of pressure on CO conversion on Pt particles catalyst at a given reaction temperature.

CONCLUSION

The CO conversion on titania supported Pt catalyst of different particle dimension between 1.3 nm and 7.8 nm at different reactants pressures was measured simultaneously on 120 catalysts at 170 and 240 ℃ and at different reactants pressures. The XPS studies indicated a small change of particle dimension after the reaction. At each temperature studied, a linear relationship between TOF for CO conversion on Pt catalyst and pressure was attained. However, the highest TOF for CO conversion on Pt was observed at higher pressure and temperature of 240 ℃, consistent with earlier findings on similar catalyst system (Li *et al*., 2013, Berlowitz *et al*., 1998). However, a slight decline in activity was observed with a pressure of 2.4 mbar at 240 ℃ which is attributed to CO poisoning of Pt catalyst, consistence with earlier findings (McClure and Goodman, 2009). Besides, the smallest Pt particles attained the higher TOFs, in agreement with earlier reports.

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Plant Species Composition and Community Perception Towards Landscaping Work Executed at Hawassa University-Main Campus, Ethiopia

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Research article

ABSTRACT

Landscaping is making visual improvements to academic institutions, urban and other private organizations with plants and attractive materials. People all over the world are attracted to green and beautiful landscapes which initiate them to be happy and creative. Especially students in higher educational institutions who spend much of their time focused on their studies need of clean, green and attractively well-designed landscape in their campus. So far data on plant species composition and perception of university towards landscape work is lacking. With this understanding, the study was conducted to investigate plant species composition and community perception towards landscaping work executed at Hawassa University's main campus using questionnaires, interviews, observation and group discussion methods. In addition, vegetation data were collected using systematic sampling method. In the study, 221 respondents were drawn from academic staff, administrative workers and students using random sampling method. Key informants were selected using purposive sampling method. In order to identify the plant species, systematic sampling along the transect line was employed. Twelve (12), 40m X 40 m (i.e. 1600 m^2) area quadrat plots were laid and all the plants species in the quadrats were collected and identified. The distance between each transects and quadrat was 200 m and 100m, respectively. 52 plant species with 44 genera and 29 families were recorded. The study showed that, the most dominant family was *Fabaceae* with 9 species followed by *Cupressaceae* as well as *Moraceae* with 4 species each respectively. The results revealed that, campus landscaping work was the most significantly important and made the campus more attractive by plantation (61.1%), landscaping (27.1%) and walkways (5.7%). Plantation could also modify the micro-climate of the campus environment (94.3%) due to the plants photosynthesis process which produces O_2 and takes in Co_2 from the atmosphere. In general educational institutions landscaping need to be encouraged to enhance the teaching-learning process, and to make the working environment attractive. A great educational environment is guided by the most powerful teacher of all, nature itself.

INTRODUCTION

The global physical landscape is changing due to the process of urbanization. According to the United Nations Department of Economic and Social Affairs (2014), about 54% of the world's populace resided in city/urban regions in 2014. Lands are modified to make useful for residential, commercial, public gatherings, industrial and institutional zones; encouraging population migration to gain access to these facilities. Gradually the shrinking of urban green spaces/areas results in the loss of

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biodiversity and disturbance to the natural ecosystem (Izyan *et al*., 2017). The ecologicalization of plant life landscape want to ensure the rich species and diverse shapes of campus flora, and the plant configuration is affordable, the usage of vegetation to decorate the campus environment to ensure the stability and balance of campus environment and reach the coordination of artificial environment and natural environment (Habib *et al*., 2016). The campus planning and panorama layout should fairly adopt circulate concept, ecological recuperation idea and occasional carbon idea to make college campus come to be harmonious ecological campus from macro views (Horhota *et al*., 2014). A development of green infrastructure (GI) on the University compound has been acknowledged as one of the foundations of Education for Sustainable Development (ESD), an initiative that could provide various benefits to the campus community. The students and the campus community are the primary beneficiaries in several aspects which contribute to the enhancement of knowledge, skills, as well as physical and mental replenishment from sustainable landscaping, greening, and beautification work (Izyan *et al*., 2017). Landscaping is the process of making visual improvements to academic institutions, urban and other private organizations with plants and attractive materials (Amy, 2009).

Landscaping, greening and beautification in better instructional establishments and faculties can be traced to the very starting of formal education, thinking about it very vital components of the total plan (Jellico, 1966; Boughman, 1992). Landscaping, beautification and greening are completed to create the high-quality aesthetic feature, appropriate environment and to supply a naturalized placing the usage of nicely matched herbal or man-made materials. The desired quality of beautification can be made via the affiliation of plant life, shrubs, timber, lawns, fences, houses, pavement, and water (Boughman, 1992; McConnell *et al*., 2003). Large bucks have been spent on landscaping to fulfill mankind’s preferred fine and beautiful environment. Plantation of wood, vegetation, shrubs and water offer residing quarters with non-violent and comfy surroundings that are pondered in houses, organizations, authorities places of work, church homes, and academic facilities (Boughman, 1992). An adorable bodily environment satisfies the student's emotional and cloth wishes and stimulates non secular increase. A scholar's capability to look and admire physical splendor is nurtured via the manner of his/her surroundings. Actual colleges have the potential for attracting beauty to high school community and then transmitting it to the related buddies (Brown, 1983).

A college campus made attractive by landscaping, greening and beautification is a concept to all of the university network and the parents. The purpose of landscaping at University and faculties are to beautify the surrounding physical surroundings, to offer shade, assist the coaching mastering strategies and to inspire students to love and admire the environment (Malone and Tranter, 2003; Dyment and Bell, 2007; Habib *et al*., 2016), because the campuses are social group, and that they play a giant position in converting peoples’ life-style. According to Wells (2000), panorama may also alter the micro-climate of the environment surrounding the campus due to the plants photosynthesis system which produces oxygen and absorbed carbon dioxide from the

atmosphere. Landscape and plants gives an appealing element through the way of aesthetic value (Rasidi *et al*., 2013). An attractive landscaping, greening and beautification plays a vast position in minimizing emotional stress, negative emotions, physical pressure, growth fine thoughts among college students and initiate the scholars' interest toward getting to know, due to the fact, flowers affect human psychology (Han, 2009). Maintainable landscaping and beautification practices can help to lessen pressure and improve the overall health of humans living in town, college, campus and other organization environments, and may be significantly less expensive to hold. Therefore, developing an advantageous dating between a man and the environment may want to have extensive benefit closer to mental balance, enhancing the behavior and improving health situations. Furthermore, interaction with none restrict with lovely outside environment may want to reduce mental strain in humans daily lifestyles (Harting *et al.*, 1991; Kaplan, 1995; Kaplan *et al*., 1998; Wells; 2000; Taylor *et al*., 2001).

A University campus is just like a small city with infrastructure (such as buildings, transportation, electricity, roads, beautiful and pleasant landscape and green compound) because of the complete function, the construction of campus panorama area have to create locations to carry all kinds of sports and make sure that campus space has attractive elements (ICADCE, 2015). The school grounds, with adequate care (clean, attractive, comfortable, and orderly surroundings) can make major contributions to student progress. Preserving a sustainable landscaped campus environment play a considerable position meets the requirements of diverse verbal exchange between teachers and college students under the assorted social historical past of more than one value. the distance spirit can well arouse the feel of identification of instructors and students to campus panorama cause them to experience pride to analyze, live, contact, leap forward in a campus landscape environment, be unconsciously encouraged through the edification of information and boom the experience of belonging of campus are (ICADCE, 2015). Greening is the process of planting trees, herbs and shrubs to transform the living environment into more health, beautiful and suitable place (Douglas, 2003; Ryrie, 2004). A beautiful and green campus in the end offers an institution the opportunity to take the lead in redefining its environmental way of life and growing new paradigms through developing sustainable answers for the environmental, social and economic needs of mankind. Therefore, the main purposes of the study were to assess the community perception towards landscaping work executed at Hawassa University main campus.

MATERIALS AND METHODS

Study Area

The study was conducted in the main campus of Hawassa University. Hawassa University (HU) was established at Hawassa on the 25th of April 2000. Hawassa University has seven functional campuses, namely; College of Agriculture (AC), College of Medicine and Health's Sciences Campus (CMHS), Institute of Technology (IOT) and the Main Campus (the seat for the top administration) are located in Hawassa City. The remaining three, namely Wondo Genet Campus (WCFNR), Awada Campus and Bensa Daye Campus are situated out of Hawassa city.

Hawassa University (HU) is a comprehensive university that is engaged in the provision of all-round education, research, training and community service through its diversified areas of the academic units. Hawassa University has recently established three Centers: "Center of Excellence for Teacher Education and Educational Leaders, Academic Center of Excellence in Human Nutrition, and Science, Technology, Engineering, and Mathematics (STEM) Center". It is located 275 km South of Addis Ababa, the capital city of Ethiopia in the Sidama region, Hawassa city. The main campus was established in 2000. Geographically
it lies at 07^0 05' north, $38^0$29' east and at 1697m above sea level. The rainfall pattern is characterized by bimodal distribution with spring/"belg" and summer (main) rainy seasons. However, the rainfall is continuously disbursed with one dry season. The mean annual temperature is 19.5^0c with the minimum average ranging from 9.3^0C at some point of December to 140 ^{0}C at some stage in July and maximum temperature varies from 24 °C throughout July to 29.2 °C at some stage in March. The land shape is obvious with reddish volcano soil which is right for construction. The present study was conducted at the main campus of Hawassa University.

Methods of Data Collection

The study was conducted in Hawassa University's main campus from 2017 - 2019 to study the perception of its community on the beautification, greening, and landscaping work that had been done. The data were collected by using both questionnaires and semi-structured interviews (Martin, 1995) and site observation. Systematic random sampling techniques were employed to select lecturers, third-year main campus students, administrative staffs, key-informants and campus cleaners as representative samples for data collection. A total of 221 informants were randomly selected and 10 key informants were included. Questionnaires were prepared and distributed for two hundred twenty-one (221) respondents to collect data on the perception of the community on landscaping and its contribution on education quality, job creation and beautification work in the campus. The amassed plant specimens were pressed, dried and recognized by experienced botanists using the vegetation of Ethiopian and Eretria extent one to eight (Azene *et al*., 1993; Edwards *et al*., 1995; Hedberg *et al*., 2003; Hedberg *et al*., 2004), then mounted on herbarium sheets and deposited in the lab. In order to collect the plant species, purposive sampling was used and a total of 12 quadrants, 40m X 40m (i.e. 1600 m^2 area) along transect line were used. The distance between, each transects and quadrants were 200m and 100m, respectively. From the total of 12 quadrants, all the plants species in the quadrants were collected and identified.

Data Analysis

The collected quantitative data were organized, summarized and subjected to descriptive statics.

RESULTS AND DISCUSSION

The study was conducted at Hawassa University's main campus to assess the perception of Hawassa University's community towards the landscaping work executed at its main campus carried out from 2017 to the present. Since its establishment in 2000, the main campus was bare land with hot and

intolerable climatic conditions, not sufficiently attractive for the teaching-learning process and for the administrative worker, academic staffs, and students to accomplish their regular work effectively (Figure 1). Thanks to landscaping and greening works done, currently, the seat, shade area and recreational area made a suitable environment for the service intended (Figure 1 and 3). The respondents were asked whether they have been ever observed landscape and beatification work in governmental institution in Ethiopia. The result indicates that 78.6% of the respondents' agreed as they are familiar with landscaping, greening and beautification works in governmental institution in Ethiopia while 21.4% of the respondents responded as not observed landscaping and beautification works elsewhere (Table 1). The respondents observed beautification work in a governmental institution such as university (52.2%), Federal office (18%), Schools (14.6%), region office (8.2%) and less in Zone office 7.0%). The majority of the University community (77.1%) agreed and was satisfied with the beautification, greening and landscaping work of the University whereas 22.9% of the respondents claim that as there are some issues that need more attention than beautification and landscaping work (Table 1).

Figure 1. Comparison between past (Top) and present (bottom) appearance of the main campus

The respondent agrees that campus beautification and landscaping work was the most important and made the campus more attractive by plantation (61.1%), landscaping (27.1%), walk ways (5.7%), seat and shades (4%), and small water pools compared to the previous situation (Figure 1, 3 and Table 1). Each tree helps to fight global warming via reducing the quantity of greenhouse gases within the environment. Vegetation in the campus environment provides color to break out the warmth of the sun and additionally they provide green areas for rest, relaxation and pastime or recreation (Behe *et al*., 2005). The respondents also rated the change in the main campus environment as there is drastic change (75.8%) while the rest claimed as beautification, greening and landscaping work doesn't include all the campus areas (Table 1).

Table 1: The Perception of respondents towards beautification and landscaping work done in the main campus

Information	Category	Percentage (%)
Have you ever observed land scape & beatification work in governmental institution in Ethiopia	Yes	78.6%
	No	21.4%
Types of institutions landscape and beatification carried out	Schools	14.6%
	Federal office	18.0%
	Zone office	7.0%
	Regional office	8.2%
	University	52.2%
Are you satisfied with the landscaping and beautification work in the campus	Yes	77.1%
	No	22.9%
Which landscaping and beautification work is most attractive?	Landscaping	27.1%
	Walk ways	5.7%
	Seat & shades	4.0%
	Small water pools	0.0%
	Plantation	61.1%
How do you rate the change on the campus environment?	Have change a lot	75.8%
	Not that much	17.1%
	No change at all	7.1%
Plants planted have significant role in micro-climate modification	Yes	94.3%
	No	5.7%
Landscaping work increases the beauty of the campus	Agree	97.7%
	Disagree	2.3%
Landscaping work has created suitable environment for teaching learning process	Agree	91.4%
	Disagree	8.6%
Landscaping work has positive contribution for education quality	Agree	74.5%
	Disagree	25.5%
Landscaping increases plantation and infrastructure of the campus	Agree	87.1%
	Disagree	12.9%
The seat, shade area and recreational area made a suitable environment for service intended	Agree	91.4%
	Disagree	8.6%
Landscaping work create job opportunity for citizens	Agree	96.2%
	Disagree	3.8%

Attractively designed and well-maintained physical environment of the campus can help to create a positive paramount impression, set up a peaceful mood, and increase assets value. The present study finding also agreed with this concept that, majority of respondents (94.3%) confirmed as plantation have a significance role for environmental micro-climate modification. Because, plants regulate the temperature, it is good for refreshment, provide clean air and also good for all image of the campus. Hence, almost all respondents (97.7%) agree with landscaping, greening and beautification work increases the beauty of the main campus (Table 1). Besides creating excellent working environment for the University community, the landscaping, greening and beautification work also contribute a lot for the teaching-learning process (91.4%) and education quality (74.5) through modifying micro-climate, providing shade, recreation areas and serving as reading area (Table 1).

With the improvement of socialization, integration and popularization of higher education, the substances of education so come to be more massive and they are not confined to books and school rooms. The campus surroundings that students stay in their everyday life are the materials of training as nicely (Hartig *et al.*, 1991; Tennessen and Cimprich, 1995; Habib *et al*., 2016). The modified physical environment surrounding the campus promotes efficiency in the teaching-learning process and contributes to education quality as well as contributes to their aesthetic development. Almost all respondents confirmed that, before the start of beautification and landscaping work, it was difficult to stay at the office, even students were traveling to Lake Hawassa side areas seeking shade and comfortable reading and seating area due to hot temperature. Nowadays, landscaping and beautification work increase plantation and infrastructure of the main campus (87.1%) and almost all respondents agreed (91.4%) with the opinion that the seat, shade, and recreation area made a suitable environment for the service intended (Figure 1, 3 and Table 1). On the other hand, beautification, greening and landscaping work create job opportunities for citizens (96.2%) (Figure 3, 4 and Table 1).

Trees for Landscaping and Beautification

The present study revealed that greening and plantation (94.3%) significantly modify the micro-climate of the environment in the study area (Figure 3 and Table 1). Plants are the “lung of urban areas’’ (McPherson, 2005) due to their ability to remove pollutants from the atmosphere that is breathed by acting as natural filters. Powe and Wills (2004) reported that plants generate health benefits by reducing the mortality rate and reducing visits to the hospital. Ornamental plants made a more pleasant environment for people (Akbari, 2002) and people walking around green areas showed reduced stress levels (Lohr *et al*., 2007; Ulrich, 1989). Higher education institutions’ students’ under exam stress had increased positive feelings and reduced fear and anger when they had view of plants (Frank, 2003).

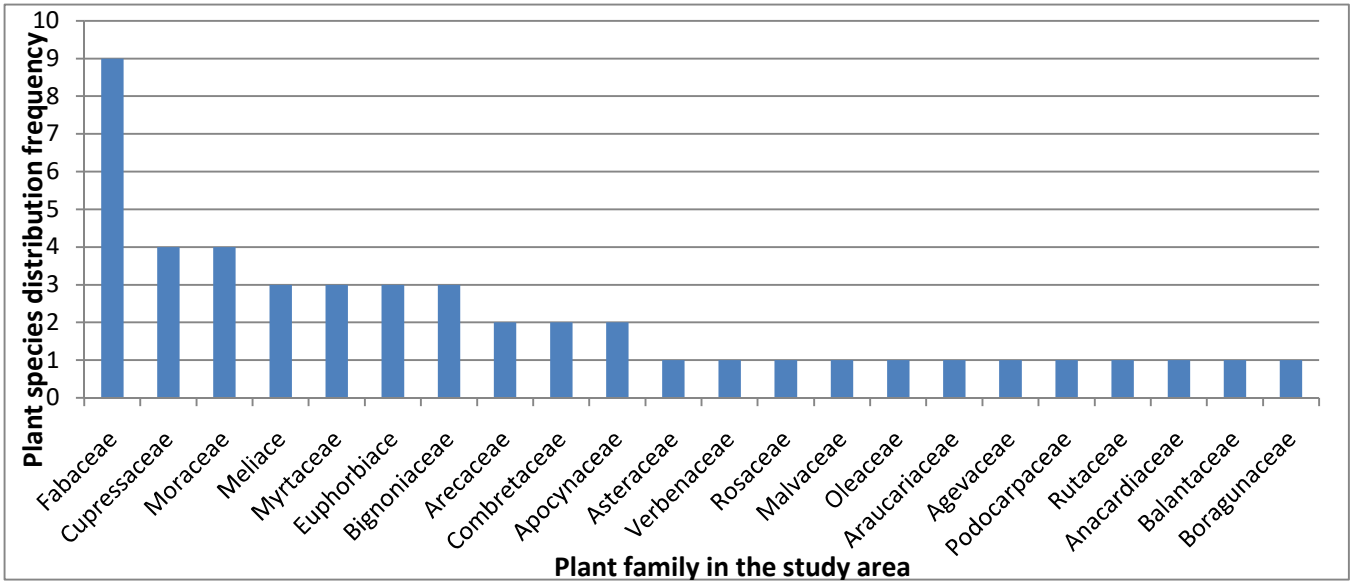

Figure 2. Plant Family and species distribution frequency from the study area (both exotic and indigenous plants, see Appendix)

In the present study area, both exotic and native plants were used (Appendix). Plantation trees used in the study area includes; *Junipers procera*, *Jacaranda mimosofolia*, *Ficus elastic*, *Phoenix reclenata*, *Borassus aethiopum*, *Acacia species*, *Nerium oleander*, *Hibiscus syriacus*, *Cupressus sempervirens pyramidalis*, *Graviellia robusta*, Araucaria species to mention a few (Appendix).

The majority of plants species used for plantation in the main campus belongs to twenty (29) families; dominated by the Fabaceae family with nine (9) species and Cupressaceae and Moraceae with four (4) species each (Figure 2 and Appendix). The main reason for choosing plants for landscaping such as providing the screen, blocking the unwanted view, modifying temperature, stabilizing soil, and aesthetic value(overall habit or shape of the plants and their foliage, flower, fruit, and bark).Thus, diversity of plant species and combination of plant forms, foliage, flowers, fruit and bark can result in creative, artistic display and beautiful landscape composition. Plant species indigenous to the country should be incorporated into the landscaping and beautification work. Compared to exotic plants, indigenous plant species carries a variety of environmental benefits ranging from reducing erosion, shade, micro-climate modification, and these plants would require less overall maintenance, less watering and less intensive management than exotic species currently used on campus landscaping. The plantation of indigenous trees is very important to conserve the plants species and their survival rate is higher compared to exotic plants. The present study finding indicates that the study area is dominated by exotic plants (Appendix). Therefore, the researchers recommend that planting indigenous plant is more important than exotic trees for sustainable and long-term usage of the landscaping. Because, having a larger proportion of native plants would be less expensive to upkeep in the long-run.

Effect of Landscaping and Beautification on Teaching-learning Processes

There is direct dating among the construction of verbal exchange space and the realization of the instructional feature of campus landscape, high-quality campus communication area offers a great location for various communique activities in the campus, makes it convenient for carrying out sports which include instructional alternate, extracurricular practice, extracurricular coaching, out of doors exercise, undertaking and sightseeing. The current university college students present range and randomness and numerous forms of conversation space with wealthy gradation meet the requirements of various varieties of customers. From this perspective, campus panorama surroundings, higher realizes the function for education carrier (ICADCE, 2015). Because college days are a vital duration to form the view of existence values, excessive great campus area environment has a lively effect on shaping the thoughts of university college students (Habib *et al*., 2016).The present study finding reveals that campus landscaping, beautification, and greening had a significant effect on supporting the teaching-learning process, job opportunity and recreation, 91.4%, 96.2%, and 91.4% respectively (Figure 3 and Table 1). Plantation and greening assist the teaching- learning process through providing shade, recreational place, increasing staff and students stay at the campus, reading space and modifying the temperature of the environment (Figure 3 and Table 1). Interplay with natural factors such as vegetation, water source or different appealing regions could lessen psychological pressure in humans each day lifestyles. Decreased psychological strain could permit advantageous attitudes to broaden and stimulate students learning interest towards studying (Kaplan *et al*., 1998; Han, 2009). Beautification components, especially flora have active psychological relationship with people, could assist in mental stability, improving the behavior and health conditions (Wells, 2000; Taylor *et al*., 2001)..

Figure 3. Landscape designing and walk ways in the main campus

Humans have better standard health and immunity to diseases while dwelling in an area with considerable inexperienced space, and patients recover faster when a view of a natural environment is available (Maas *et al*., 2006; Maller *et al*., 2002). Beautiful environment enhance the aesthetic value of the campus environment and reduce the surrounding temperature and help to get clean air (Malone and Tranter, 2003). Hence, almost all respondents (97.7%) of the present study appreciate the landscaping and beautification work done in the study area (Table 1).

Job opportunities

Before landscaping and plantation work started Hawassa University's main campus was bare lands, hot and inconvenient for teaching-learning process, academic staff, students and administrative workers to accomplish their daily activity properly. The majorities of main campus students especially prefer to seat and read at Lake Hawassa sides. Nowdays, the campus appears as one of the most beautiful and attractive campuses in the country (Figure 1 and 3).Even the campus is becoming preferable a film shot and another activity area. Beautification, greening and landscaping work in the campus started in 2012 G.C and continue to present with the support of the Germany Government that support around 74 million birr, and beatified by Flora Agricultural state PLC AA (personal communication with campus beatification officer). Since beautification started, different activities were carried out in the main campus such as planting trees, grasses and protecting them watering, mulching, weeds avoiding, shortening the grass with a sickle, scissors and machines. The study result confirmed that the campus beautification work has created a great job opportunity (96.2%) for more than 80 workers now (Figure4 and Table 1). However, different challenges interrupt the beautification and greening activities (Table 2). The price paid and works are not related and workers are disadvantageous. The study suggests that sustaining the landscaping, beautification, greening and encouraging the workers are crucial (Figure 3 and 4). Awareness raising activities should be given to the students and campus societies to protect and proud off the campus beautifying and greening.

Figure 4. Partial view of landscaping, beautification and greening workers in the main campus.

Landscaping and beautification requires significant hand work and the use of a system, relying on the nature of site grounds. However the use of educated labor and higher equipment may also lessen the fee. From 1% - 4% of the price range for the campus, the school invested on campus beautification. Therefore, setting up an endowment could create a higher financial supply to be available for campus beautification and up maintenance (Larry, 1992).

Seating Areas

The respondents' were asked to which facilities they would like to be incorporated to the site and indicated that incorporating seating regions need to be a design priority. Respondents were verified the maximum popular option benches (85%), as they would love to look benches brought to the area, indicating a preference to make the space greater interactive and welcoming. at some stage in the focal point group, participants emphasized seating areas as an amazing manner to get students to note and admire the ecological layout features of the site (Figure 5).

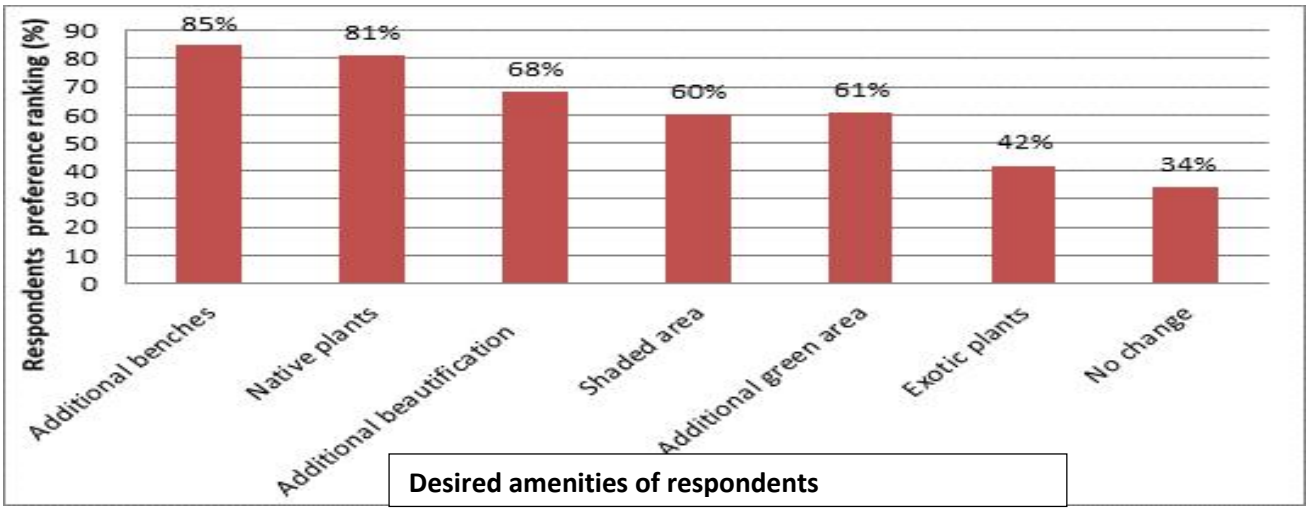

Figure 5. Desired amenities of respondents in the study area

The respondents point out that, comfortable areas where students and the community could seat and examine or do work are the seating regions ought to be as a minimum partly offset from the on foot paths and avenue facets so that they may be not be disturbed by high pedestrian site visitors. Figure 5 above also indicates that, green space and landscaping involving native plants species (81%) are more appreciated by respondents. The findings indicate thatthere is support for additional beautification, landscaping and greening with less exotic plant species (Figure 5 and Appendix).

Constraints of Landscaping, Beautification and Greening in Hawassa University Main Campus

Different challenges interrupt the beautification and greening activities (Table 2). Semi-structured interview and questionnaire reports indicate that, the main challenges of landscaping, greening and beautification work of the study area were lack of budget (77.66%), lack of beautification equipment's (76.10%), absence of nursery on the main campus (56.23%), lack of planting materials, lack of

awareness and lack of trained human power (54.73%), (53%)and (51.%) respectively (Table 2).

Table 2: Constraints of landscaping, beautification and greening work in the study area

Challenges	Frequency	Percent (%)	Rank
Knowledge gap in beautification	51	52.33	6th
Lack of training for workers	52	51.10	7th
Lack of water availability	37	41.20	9th
Destruction by animals	43	49.12	8th
Lack of budget	82	77.66	1st
Lack of awareness for University Community	45	53.00	5th
Lack of beautification equipment's	71	76.10	2nd
Knowledge gap in plant breeding	31	19.10	10th
Absence of nursery in main campus	55	56.23	3rd
Lack of planting materials	57	54.73	4th

CONCLUSIONS

A development of green infrastructure on University compound has been acknowledged as one of the foundations of Education for Sustainable Development, an initiative that could provide various benefits to the campus community. The investigation was carried out to assess plant species composition and the perception of Hawassa University community towards the landscaping work executed at its main campus since 2017 to present. Besides creating excellent working environment for the University community, the landscaping, greening and beautification work contribute a lot for teaching-learning process and education quality through modifying micro-climate, providing shade, recreation place, and serving as reading space. Nowadays, landscaping and beautification work increase plantation and infrastructure of the main campus and almost all respondents agreed with the opinion that the seat, shade, and recreation area made a suitable environment for the service intended. The finding of the study also indicates that, the majorities of respondents agree and appreciate that landscaping and beautification work creates enormous job opportunities for citizens. We recommend that sustainable landscaping works to enhance the teaching-learning process make the campus more attractive and refresh the mind of workers and students as it potentially contributes to the quality of education. Therefore, all University communities should protect/be proud of the greening and beautification work done. Protection and taking care of the plants should be given higher priority. Furthermore, the University should use this beautiful environment as an income source (place to shoot films and photos). Planting different plant species should continue in consultation with experts in the area (botanists, environmentalists, and horticulture).

Conflict of Interests

The authors declared that there is no any conflict of interests.

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Appendix. List of plants, their local name, family, frequency of occurrence

No	Scientific name	Family	Local name	Habit	Frequency	Origin
1	*Acacia abyssinica Hochst*	Fabaceae	Baziragirar	Tree	3	Indigenous
2	*Acacia seyal DC.*	Fabaceae		Tree	6	Indigenous
3	*Acacia tortilis Forssk.*	Fabaceae		Tree	1	Indigenous
4	*Afrocarpus falcatus (Thunb.) C.N.Page*	Podocarpaceae	Zigiba	Tree	45	Indigenous
5	*Agave Americana L.*	Agevaceae		Tree	10	Exotic
6	*Araucaria biramulata Buchh.*	Araucariaceae			11	Exotic
7	*Azadirachta indica A.Juss.*	Meliaceae	Neem	Tree	1	Exotic
8	*Balanitesa egyptiaca (L.) Delile*	Balanitaceae		Tree	2	Indigenous
9	*Bauhinia sp L*	Fabaceae		Tree	6	Exotic
10	*Borassus aethiopum Mart.*	Arecaceae	Zembaba	Tree	44	Indigenous
11	*Caesalpinia pulcherrima (L.) Sw.*	Fabaceae		Tree	6	Exotic
12	*Calforniaspps*	Geraniaceae		Tree	4	Exotic
13	*Callistemon citrinus (Curtis) Skeels*	Myrtaceae		Tree	30	Exotic
14	*Casimiroa edulis La Llave*	Rutaceae		Tree	1	Exotic

15	*Combretum molle R.Br. ex G.Don,*	Combretaceae		Tree	3	Indigenous
16	*Cordia africana Lam.*	Boraginaceae	Wanza	Tree	21	Indigenous
17	*Croton macrostachyus Hochst.*	Euphorbiaceae	Bisana	Tree	2	Indigenous
18	*Croton megalocarpus Hutch.*	Euphorbiaceae	Kenya bisana	Tree	1	Exotic
19	*Cupressus lusitanica Mill.*	Cupressaceae	Yefenjitid	Tree	15	Exotic
20	*Cupressus sempervirens pyramidalis*	Cupressaceae	Italian cypress	Tree	53	Exotic
21	*Delonix regia (Boj.ex Hook.)Ref.*	Fabaceae	Yedirezaf	Tree	12	Exotic
22	*Duranta erecta L*	Verbenaceae	-	Tree	>100	Exotic
23	*Ekebergia capensis Sparrm.*	Meliaceae	Lol	Tree	5	Indigenous
24	*Eriobotrya japonica Loquat*	Rosaceae		Shrub	4	Exotic
25	*Ficus elastic Roxb. ex Hornem*	Moraceae	Yegomazaf	Tree	1	Exotic
26	*Ficus sycomorus L*	Moraceae	Shola	Tree	5	Indigenous
27	*Ficus vasta Forssk*	Moraceae	Worka	Tree	4	Indigenous
28	*Grevillea robusta R.Br.*	Proteaceae	Abeba	Tree	45	Exotic
29	*Hibiscus rosa-sinensis L.*	Malvaceae		Shrub	16	Exotic
30	*Jacaranda mimosifolia D.Don.*	Bignoniaceae	Yetemenjazaf	Tree	70	Exotic
31	*Jatropha curcas L.*	Euphorbiaceae		Tree	5	Exotic
32	*Juniperus procera Hochst ex Engl*	Cupressaceae	Tid	Tree	16	Indigenous
33	*Leucaena leucocephala (Lam.) de Wit*	Fabaceae		Shrub	3	Exotic
34	*Mangifera indica L.*	Anacardiaceae	Mango	Tree	5	Exotic
35	*Melia azandrach L.*	Meliaceae		Tree	14	Exotic
36	*Moringa stenopetala (Baker f.) Cufod.*	Moringaceae	Shiferaw	Tree	1	Exotic
37	*Musa paradisiaca L.*	Musaceae	Banana/Muz	Tree	20	Exotic
38	*Nerium oleander L.*	Apocynaceae	-	shrub	80	Exotic
39	*Olea europaea ssp. cuspidata (Wall.exG.Don) Cif.*	Oleaceae	Woyra	Tree	15	Indigenous
40	*Phoenix reclinata Jacq.*	Arecaceae	Saticho	Tree	64	Indigenous
41	*Pinus patula L.*	Pinaceae		Tree	8	Exotic
42	*Plumeria alba L.*	Apocyanaceae	Yebereha rose	Shrub	3	Exotic
43	*Psidium guajava L.*	Myrtaceae	Zeyituna	Tree	2	Exotic
44	*Senna siamea (Lam.) Irwin et Barneby*	Fabaceae		Tree	24	Exotic
45	*Sesbania sesban (L.) merr.*	Fabaceae		Shrub	2	Indigenous
46	*Spathodea campanulata P.Beauv.*	Bignoniaceae		Tree	19+ 24	Exotic
47	*Syzygium jambos L*	Myrtaceae	Yeferenjigoya	Tree	9	Exotic
48	*Terminalia brownie Fresen*	Combretaceae		Tree	42	Indigenous
49	*Thevetia peruviana (Pers.) K.Schum.*	Apocynaceae			14	Exotic
50	*Thuja orientalis L.*	Cupressaceae	Yefenjitid	Tree	11	Exotic
51	*Trilepsum madagascare*	Moraceae		Tree	18	Indigenous
52	*Vernonia amygdalinaDelile*	Asteraceae	Grawa	Tree	5	Indigenous

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Ovine Eimeria infection, OPG and determinants in and around Gondar town, Ethiopia

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KEYWORDS:

Eimeria;

Gondar;

OPG;

Prevalence;

Sheep;

Ethiopia

Research article

ABSTRACT

The study aimed to assess the prevalence of ovine *Eimeria* infection, assess the putative risk factors, and the intensity of infection. A cross-sectional study design was employed and the study was carried out from November 2017 to May 2018. A total of 422 sheep were selected by systematic random sampling technique, and from these animals, faecal samples were collected and examined for *Eimeria* oocysts. Of the selected and examined sheep 132 (31.3%, 95% CI=26.8-35.7) were found infected by *Eimeria* species. Eimeria infection prevalence was significant (P <0.05) higher in lambs/young than the adult, in females than males, and in poor body condition than in medium body condition sheep. Moreover, the prevalence of Eimeria infection was significantly (P <0.05) higher in sheep with soft faeces than in normal faeces sheep and semi-intensive than extensive sheep production. The intensity of *Eimeria* species infection was influenced by the age, sex, body condition, fecal consistency, and production system of sheep (P< 0.05). The overall mean Oocysts per gram of faeces was 2390.6 (95% CI=2007.5-2773.8). The mean OPG was significantly higher in lambs, with poor body conditions and female sheep. In addition, it was higher in sheep with soft faeces and an extensive production system. In conclusion *Eimeria* species infection was an important problem of sheep production in the study area. Generally, this study's results provided useful information to design and implement appropriate control strategies. Finally, it is recommended that further study identify the species of *Eimeria* circulating in the areas.

INTRODUCTION

Ethiopia is home to a large and diverse livestock resource (Gizaw *et al*., 2010); and the country has around 17 million sheep (CSA, 2021). This means sheep represent an important segment of the livestock system in the country. Sheep are important sources of income for the agricultural communities, represent one of the country's major sources of foreign currency through the export of skins and meat, and are a source of animal protein. They also play a major role in the food supply and social well-being of rural communities living in conditions of extreme poverty, which is the particular case in parts of Ethiopia (Dagnew *et al*., 2017; Gizachew *et al*., 2014). The major constraints for sheep production in Ethiopia are feed shortage, grazing land limitation, problems related to Veterinary Services, and diseases (Welday *et al*., 2019; Kenfo *et al*., 2018; Nigussie *et al*., 2013).

According to Gizaaw *et al*. (2013), one of the research gaps is sheep diseases, of which

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parasites are among the most important diseases. Among parasitic diseases, Coccidiosis is an important protozoan parasitic infection that is responsible for low productivity, impaired growth, and mortality in sheep populations (Ayana *et al*., 2009). It is caused by a protozoan parasite of the genus *Eimeria* that affects young animals in particular (Hendson and Agnes, 2002). They are highly species-specific, meaning the species of *Eimeria* that infect sheep will not infect goats or cattle and vice versa (Constable *et al*., 2017; Aitken, 2007). Coccidiosis in sheep was reported in various parts of the world (Khan *et al*., 2011; Bukar, 2007; Toulah, 2007). Also reported from Ethiopia (Lakew and Seyoum, 2016; Ayana *et al*., 2009), but in the country, there are some areas with limited information about the prevalence of *Eimeria* species infection in the sheep. There is no study of *Eimeria* species infection prevalence of sheep in and around Gondar. Therefore, the aims of this particular study were to estimate the prevalence of *Eimeria* species infection, to identify the putative risk factors and to assess the intensity of infection in sheep in and around Gondar.

MATERIALS AND METHODS

Study Area

This study was carried out in and around Gondar town (Azezo, Tseda, Maksegnit, Angereb, Ayra and Fenter Kebeles) from November 2017 to May 2018. Gondar town is located in Amhara regional State, North-West Ethiopia. The study area is located at an latitude and longitude of 12.30 - 13.80 °N, 35.30 - 35.7°E; and its altitude is on average about 2220 meters above sea level. The mean annual rainfall and temperature of the area were 1172mm and 19.7ºC, respectively (National Meteorological Agency, 2017).

Study animals

The study animals of the study were sheep flock reared under an extensive and semi-intensive management system. All grazing age groups, local breed,, and both sexes of sheep were considered for the study (Breed information was obtained with personal communication with the owners).

Study design

Across sectional study design was employed to estimate the prevalence of *Eimeria* infection of sheep in and around Gondar town. During this study faecal consistency, age, sex and body condition of the sheep, the hygienic status of the animal house and management system were considered as potential risk factors (independent variables) for *Eimeria* infection (dependent variable) of sheep. The study animals were divided into three age groups, namely: lamb (less than 6 months old), young (6-12 months old), and adult (over 12 months old) as described by ESGPIP (2009) and Gatenby *et al*. (1991). Faecal consistency was examined and classified as normal (Formed pellets), soft (Pellets not formed) as described by (Platzer *et al.,* 2005). Body conditions of the study animals were scored as poor, medium, and good as described by Russel (1991).

Sample size determination & sampling techniques

The required sample size for this study was computed by considering 50% prevalence of ovine *Eimeria* spp. infection, to obtain the

maximum sample size. The formula described by Thrusfield (2018) was used to compute the sample size required for the study. The study considered a 95% confidence interval and 5% absolute precision. Therefore, 384 sheep were required for the study; but 422 animals were selected and studied. A systematic random sampling method was used to select the study animals from the target sheep flock.

Study methodology

Faecal sample collection

Faecal samples, about 10gm, were collected directly from the rectum of the sheep by using arm-length plastic gloves, and placed in screw capped universal sample bottle. Each samples was labeled with the necessary information about the animal and the sample (i.e. age, sex, faecal consistency and body condition score) were recorded on the format prepared for this study. Moreover, the hygienic status of the animal and the management system was carefully observed. Finally, the samples were packed in icebox with ice packs and transported to the parasitological laboratory of the Faculty of Veterinary Medicine, Gondar University. The samples were kept in refrigerator at 4ºC until examined. Collected samples were examined within 24 hours for the presence or absence of Eimeria oocysts as described previously (Taylor *et al*. 2016; Zajac and Conboy, 2011).

Qualitative and Quantitative faecal examination

From the collected faecal samples, 3gm was weighed and properly mixed with 42ml flotation fluid (Saturated salt solution). Then sieved by tea strainer and filled to test tube, and then centrifuged at 1,500 rpm for 3 minutes. The sample taken out of the centrifuge and the supernatant was examined for Eimeria oocysts under 10X and 40X microscopic magnifications as described by Urquhart et al. (1996). Those faecal samples positive in the qualitative examination were subjected to quantitative examination. For this examination, McMaster egg counting technique (Urquhart *et al*., 1996; MAFF, 1986) was used to determine the number of oocysts per gram of faeces and assess the intensity of infection.

Data management and analysis

Collected data were entered into a Microsoft Excel spreadsheet, cleaned, coded, and then summarized by descriptive statistics like percentages or proportion and mean. The prevalence of *Eimeria* infection was computed by dividing the number of positive animals by the total number of animals examined and multiplied by 100. The association of the various risk factors considered for this study was analyzed by univariable logistic regression analysis. Those non-collinear factors with a p-value of ≤ 0.25 in the univariable logistic regression analysis were further analyzed by multivariable logistic regression analysis. The dependability of the fitted model was further evaluated using the receiver operating characteristic curve (ROC). Finally, the model fitness was assessed by the Hosmer-Lemeshow goodness fit test as described by Dohoo *et al*. (2009). The mean number of oocysts per gram of faeces was computed by considering those sheep positive in the qualitative examination for oocysts. Oocysts per gram of faeces was determined and then after, to normalize the data were log-transformed ($\log_{10+1}$). The transformed data were analyzed by using a t-test for the existence of associations between oocysts per

gram of faeces (OPG) and the risk factors were analyzed by paired t-test. For the data analysis, STATA 14.2 software (Stata Corp 4905 Lakeway Drive, College Station, Texas 77845 USA) was used.

RESULTS

Qualitative faecal examination results

From a total of 422 examined sheep 132 (31.3%, 95% CI=26.8-35.7) were infected by *Eimeria* species. The association of risk factors for infection of sheep by *Eimeria* species was shown in table 1.

Table- 1: Results of Logistic regression analysis of risk factors for Eimeria infection in sheep in and around Gondar

Variable	Category	No. examined	Prevalence		Univariable		Multivariable		
			No. (%) positive	95% CI	OR	P-value	OR	95% CI	P-value
Age	Lamb	96	56 (58.3)	48.4-68.3	6.3	≤0.001	5.4	3.2-11.9	≤0.001
	Young	151	44 (29.1)	21.8-36.4	1.8	0. 022	2.8	1.3-4.2	0.005
	Adult	175	32 (18.3)	12.5-24.0	Rf.	-	Rf	-	-
Sex	Male	237	96 (19.5)	13.7-25.2	Rf.	-	Rf	-	-
	Female	185	36 (40.5)	34.2-46.8	2.8	≤0.001	3.8	1.7-4.9	≤0.001
Faecal consistency	Normal	242	56 (23.1)	17.8-28.4	Rf.	-	Rf.	-	-
	Soft	180	76 (42.2)	35.0-49.5	2.4	≤0.001	5.7	2.6-7.1	≤0.001
BCS	Poor	147	73 (49.7)	41.5-57.8	3.6	≤0.001	2.2	1.1-3.0	0.030
	Medium	275	59 (21.5)	16.6-23.3	Rf.	-	Rf	-	-
Management system	Extensive	244	43 (17.6)	12.8-22.4	Rf.	-	Rf	-	-
	Semi-intensive	178	89 (50.0)	42.6-57.4	4.7	≤0.001	5.7	2.6-7.1	≤0.001
Total		422	132 (31.3)	26.8-35.7		-	-	-	-

BCS= Body condition; NB. OR= Odds ratio; CI= Confidence interval; Rf=Reference category

All the independent variables were non-collinear [gamma value (γ) value fall between -0.6 and +0.6], and the univariable analysis p-value was <0.25. Hence, all the risk factors were subjected to multivariable analysis. The final model had Hosmer-Lemeshow χ^2 (8) = 13.66, P= 0.091, and ROC= 0.7621 that there is no significant difference between the observed and predicted values.

Quantitative faecal examination result

The minimum and maximum numbers of Oocysts per gram of faeces (OPG) were 300 and 12,500, respectively. The overall mean OPG of faeces was 2390.6 Oocysts/gram of faeces (Table 2).

Table- 2: Mean OPG and t-test analysis of risk factors for intensity of *Eimeria* species infection

Risk factors and its levels		No examined	No positive	Mean OPG	95% CI	t-value	P-value
Sex	*Female	237	96	2618.9	2146.4-3091.3	4.95	≤0.001
	*Male	185	36	1781.9	1172.0-2391.9	Rf.	
Age	-Lamb	96	56	3294.8	2638.3-3951.3	8.01	≤0.001
	-Young	151	44	1920.5	1324.7-2516.2	2.44	0.015
	-Adult	175	32	1454.7	914.8-1994.6	Rf.	
Body condition	*Poor	147	73	3266.6	2714.7-3818.4	7.12	≤0.001
	*Medium	275	59	1306.8	931.1-1682.5	Rf.	
Faecal consistency	-Soft	180	76	3008.7	2462.5-3554.9	4.84	≤0.001
	-Normal	242	56	1551.8	1107.0-1996,6	Rf.	
Management system	*Extensive	244	43	2061.6	1421.5-2701.8	Rf	
	*Semi-intensive	178	89	2549.6	2067.9-3031.2	7.62	≤0.001
Overall		**422**	**132**	**2390.6**	**2007.5-2773.8**		

DISCUSSION AND CONCLUSIONS

The prevalence of *Eimeria* species infection of sheep in and around Gondar was 31.3%. All risk factors considered during this study for *Eimeria* species infection of sheep were significantly ($p< 0.05$) influencing infection of sheep by *Eimeria* species. Lamb and young sheep were 5.4 and 2.8 times more likely to be exposed to *Eimeria* species infection than the adult sheep, respectively. Similar observations were reported from various areas of the world (Elkhatam *et al*., 2020; Etsay *et al*., 2020; Paul *et al*., 2020; Kiltuet al., 2016; Nourollahi-Fard *et al*., 2016; Lakew and Seyoum, 2016; Wang *et al*., 2010; Maingi and Munyua, 1994; Kanyari, 1993). Infection of sheep followed by the development of species-specific immunity, and so, sheep infected and immune to one species of *Eimeria* may not be re-infected again by the same species of *Eimeria*. This difference might be related to earlier infection and the development of immunity in adult sheep (Matos *et al*., 2018; Constable *et al*., 2017; Rehman *et al*., 2011; Yakhchali and Golami, 2008). The other justification could be there is no active immunity to *Eimeria* species in younger naïve sheep (Paul *et al*., 2020).

This study showed that *Eimeria* infection was significantly ($P< 0.05$) higher in female sheep than in male sheep as also reported by various authors (Elkhatam *et al*., 2020; Etsay *et al*., 2020; Paul *et al*., 2020; Sharma *et al*., 2017; Kiltu *et al*., 2016; Khan *et al*., 2011; Rehman *et al*., 2011; Sharma *et al*., 2009; Yakhchali and Golami, 2008), which reported sex influences the prevalence of ovine Coccidiosis. This might be attributed to sex-related factors including the physiological stress encountered by the ewe (i.e. pregnancy; lambing, and suckling the newborn lamb), which was the reason for the ewe being more susceptible to *Eimeria* infections (Heidari *et al*., 2014; Lopes *et al*., 2013).

A significantly higher ($P< 0.05$) prevalence of *Eimeria* infection was found in semi-intensive than extensive production systems of sheep, which is also reported in various areas of the

world (de Macedo *et al*., 2020; Lakew and Seyoum, 2016; Kanyari, 1993). In an extensive management system, animals are freely moving in larger areas, and hence, the chance of infection is decreased as compared to the semi-intensive management system. It is known that a semi-intensive management system (i.e. where high animal population density occurs) contributed to the propagation of *Eimeria* species (de Macedo *et al*., 2020). As the flock size of sheep increases, there is greater contamination of feeding and watering troughs (Yakhchali and Rezaei, 2014). Hence, confinement was found to contribute to high prevalence (Kanyari, 1993).

In this study, a significant association was documented between body condition score and *Eimeria* infection. Similarly, Lakew and Seyoum (2016) and Khan et al. (2011) reported a higher infection rate in sheep with poor body condition scores. The poor body condition in sheep might be due to the immunosuppressive effect of concurrent disease problems and/or nutritional scarcity. All these can negatively influence the animal feed intake and weaken the immunity, and the sheep become highly susceptible and get infected (Constable et al., 2017).

During this study, a significant ($P< 0.05$) association of *Eimeria* species infection was observed in sheep with soft faecal consistency than the normal faeces. This finding is in a general agreement with reports from various parts of the world (Khodakaram-Tafti and Hashemnia, 2017; Lakew and Seyoum, 2016; Yakhchali and Rezaei, 2014; Yakhchali and Golami, 2008). Infection by most of the pathogenic *Eimeria* species leads to the destruction of intestinal epithelial cells and induces enteritis followed by diarrhea (Constable *et al*., 2017; Chartier, and Paraud, 2012; Wang *et al*., 2010; Urquhart *et al*., 1996).

The mean oocysts count from infected animals was 2390 OPG (range 300 to 12,500). The mean was meaningfully higher ($p< 0.05$) in lamb and young, female, and poor body condition sheep. In addition, it was statistically significant ($p< 0.05$) in sheep with soft faecal consistency and semi-intensively managed (Table 2). Higher oocyst counts were reported in lamb and/or young sheep (Chartier and Paraud, 2012; Arslan *et al*., 1999; Maingi and Munyua, 1994; Kanyari, 1993) and the semi-intensive production systems (Chartier and Paraud, 2012) from various areas. The negative association between OPG and the age of sheep was a result of acquired immunity (Kanyari, 1993). Factors inducing stress, for example, increased number of animals in an area like in the semi-intensive production system, and loss of body condition might increase the oocysts output.

In a nut shell, the present study revealed that the prevalence of *Eimeria* species infection in sheep is higher. And it was significantly associated with various host-related (i.e. Age, sex, body condition, and faecal consistency) and environmental factors (i.e. Production system). So, this result provided useful information to design and implement appropriate control strategies. Finally, it is recommended that further to study identify the species of *Eimeria* circulating in the areas.

Limitation of the study

The major limitation of the study was lack of facilities for Eimeria spp. identification.

List of Abbreviations

CSA: Central Statistical Authority; OPG: Oocysts per gram of faeces; ROC: Receiver operating characteristic curve.

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Determination of the Levels of Some Selected Metals in *Ocimum lamiifolium* in Wolaita Zone, Southern Ethiopia

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KEYWORDS:

Adverse effects;

FAAS;

Medicinal plants;

Herbal remedies;

Permissible limits

Research article

ABSTRACT

Medicinal plants have global applications in the treatment of diverse types of human animal diseases. Among the medicinal plants of Ethiopia, *Ocimum lamiifolium* Hochst. Ex Benth (Damakese, in Amharic) is one of the well celebrated and most widely used home remedy for the treatment of a disease locally known as "Mitch" which is characterized by headache, fever, inflammation, joint pain, sweat, loss of appetite, etc. The aim of the present study on this medicinal plant was to determine the levels of heavy and trace metals in the leaves using the flame atomic absorption spectrometer (FAAS), which is nov AA model. The sampling technique used to carry out the analysis was purposive for the community in the selected area use the plant widely to treat different diseases. In addition, for each of three kebeles, selected from Duguna Fango District, three sites were selected to homogenize the samples. The concentrations or levels of heavy and trace metals, Cd, Co, Pb, Cr, Cu and Zn, in the leaves of the selected medicinal plant were found to be (in mg/L) 0.0489, 0.0579, 0.0936, 0.153, 0.214 and 0.847, respectively. The results revealed that the selected medicinal plant accumulated these metals at different concentration levels in different sites. The results also confirmed that the concentration levels of the metals in the leaves of the selected medicinal plant were not higher than the globally accepted permissible limits. Thus, the results indicated that the medicinal plant under the study is safe for medicinal uses. Furthermore, monitoring such medicinal plants for heavy and trace metals concentrations is of great importance in protecting the community from the adverse effects of the heavy metals..

INTRODUCTION

Background of the study

Medicinal plants play an important role since prehistoric time as they are used in traditional medicine and also as home remedies. Environment, pollution, atmosphere, soil are some of the issues, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Traditional medicines include herbal medicines composed of herbs, herbal materials, and finished herbal products, that contain as active ingredient parts of plants, or other plant materials, or combinations of all mentioned (WHO, 2005).

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Herbal medicines usually refer to plant-derived substances that occur in nature and are utilized with little or no industrial processing for treatment of illnesses (Tilburt and Kaptchuk, 2008). Herbal medicines are formulated using various parts of plants, including leaves, roots, barks, fruits, and seeds. Due to their natural origin, many people who use herbal remedies believe they are safer than conventional pharmaceutical products. The WHO reckons that over 80% of the population in Africa and other developing countries depend on herbal remedies for their healthcare needs (WHO, 2005). For many people in Africa, the high costs of Western pharmaceuticals make modern health care services inaccessible. As a result, they heavily rely on herbal medicine and medicinal plants to fulfill their primary health care needs. In addition, western pharmaceuticals are most of the time inaccessible to most people in Africa and so herbal medicines have become one of the major options for treating various diseases (Debas *et al*., 2006).

When consumed in excess, lead (Pb) can increase blood pressure and lead to serious damage to vital organs such as the kidney and the brain. Cadmium (Cd) poisoning is linked with a number of respiratory disorders, renal failures and cardiovascular issues. Although zinc (Zn) is an essential mineral, overdosing on it can result in symptoms such as fever, nausea, and general weakness. Though iron deficiency causes anemia, too much iron is predominantly dangerous in young children and could cause gastrointestinal and skin problems (Baker *et al*., 2010).Therefore, it is necessary to measure and establish the levels of heavy and trace metals in the herbal plants as these elements when consumed at higher levels become toxic. Thus, the objective of the present study was to determine the levels of selected heavy and trace metals in the leaves of *Ocimum lamiifolium* plant using flame atomic absorption spectrophotometer

Statement of the problem

The use of herbal medicines is rapidly expanding around the world. Many individuals now turn to herbal medicines or associated products for their healthcare within various national healthcare systems. However, mass media coverage of adverse events is often exaggerated, leading to the negative perceptions of herbal medicines in general, rather than focusing on the specific causes behind these events.

Currently, most adverse associated with the use of herbal medicines are attributable either to poor product quality or the improper usage. In order to expand knowledge about genuine adverse reactions to herbal medicines, and to avoid wasting scarce resources for identifying and analyzing adverse events, events resulting from such situations will need to be reduced or eliminated.

Ocimum lamiifolium, among vital medicinal plants, is used to treat various ailments such as cough, headache, eye infections, abdominal colic, bloat, inflammation, joint pain, etc. Thus, it is used by most people in wolaita zone for the treatment of mentioned diseases. The level of heavy and trace metals in herbal medicines beyond the permissible limit is a matter of great concern to public safety all over the world (Khan *et al.*, 2008). The problem is more pronounced in the case of Ethiopia because the herbal medicines used by the society without realizing the concentration of toxic heavy metals as well as the trace metals. World Health

Organization (WHO) basically recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium, and lead which amounts to 1.0 ppm, 0.3 ppm and 10 ppm, respectively (WHO, 2006). The common conception among the population is that "natural" means "safe" and that drugs of natural origin are harmless and have no risk associated with their use, does not match reality. Some medicinal plants have inherent toxicity and herbal medicines, like any medicine, have side effects that can cause many diseases (Lanini *et al*., 2009). Thus, the current study focuses on the determination of the levels of heavy and trace metals in the leaves of *Ocimum Lamiifolium* that is grown in Duguna Fango district in order to protect the individuals from their adverse effects when used beyond the permissible limits

Objectives of the study

The study was carried out with the objectives of determining the levels of selected heavy and trace metals (lead, cadmium, chromium, cobalt, copper, and zinc) in *Ocimum lamiifolium* using FAAS technique and comparing the levels of the mentioned metals present in the leaves of *Ocimum lamiifolium* with the permissible limits of WHO standard and other international standards.

Significance of the study

Society has increasing curiosity in the therapeutic use and benefits of herbal remedies. However, there is a wide spread misconception that natural herbs and plants are inherently safe. There is also insufficient information available on the safety of traditional herbs and their products. Therefore, this study helps provide important evidence on the levels of selected heavy and trace metals in *Ocimum lamiifolium* grown in the study area so that the society could be free of the potential health risks caused from the excessive uptake of the heavy and trace metals in the herbal medicines. On the other hand, the results of this study could be used as reference for other researchers who want conduct the similar studies on the same plant growing in different parts of the country.

Scope of the study

This study was restricted to the investigation of concentrations of the selected heavy and trace metals found in *Ocimum lamiifolium* grown in Duguna Fango District, Wolaita zone, Southern Ethiopia using the widely used analytical technique called spectroscopy specifically using the analytical instrument flame atomic absorption spectrophotometer. The metals Pb, Cd and Cr were selected for they are more toxic, and the metals Co, Cu and Zn were selected merely to represent trace elements. Furthermore, leaf part of the selected medicinal plant was taken to carry out the analysis for the society use this part of the plant to treat different diseases.

MATERIALS AND METHODS

Study Area

Description of the Study Area

The study was conducted in three selected kebeles (the smallest administrative unit) from Duguna Fango Woreda of Wolaita zone, which is found in Southern Nations, Nationalities, and Peoples Regional (SNNPR) state. The area is located at 431Km south of Addis Ababa and 82

Km from Hawassa, between 6°40' - 7°58'N latitude and 37°4' - 37°56'E longitude with a total land area of 46,660 hectares. Wolaita Zone has 16 woredas (districts) and 3 town administrations. The Wolaita people are one of the indigenous people of Ethiopia who have their own culture, tradition, political legacy and kingdom. The study area lies at an altitudinal range between 1000 – 2500 meter above sea level and have agro ecologies of dega (high land), woynadega (mid altitude) and kola (low land) with a average annual temperature of l9.5°c and annual rainfall that varies from 750-1350mm according to the projected CSA final report of 2019.

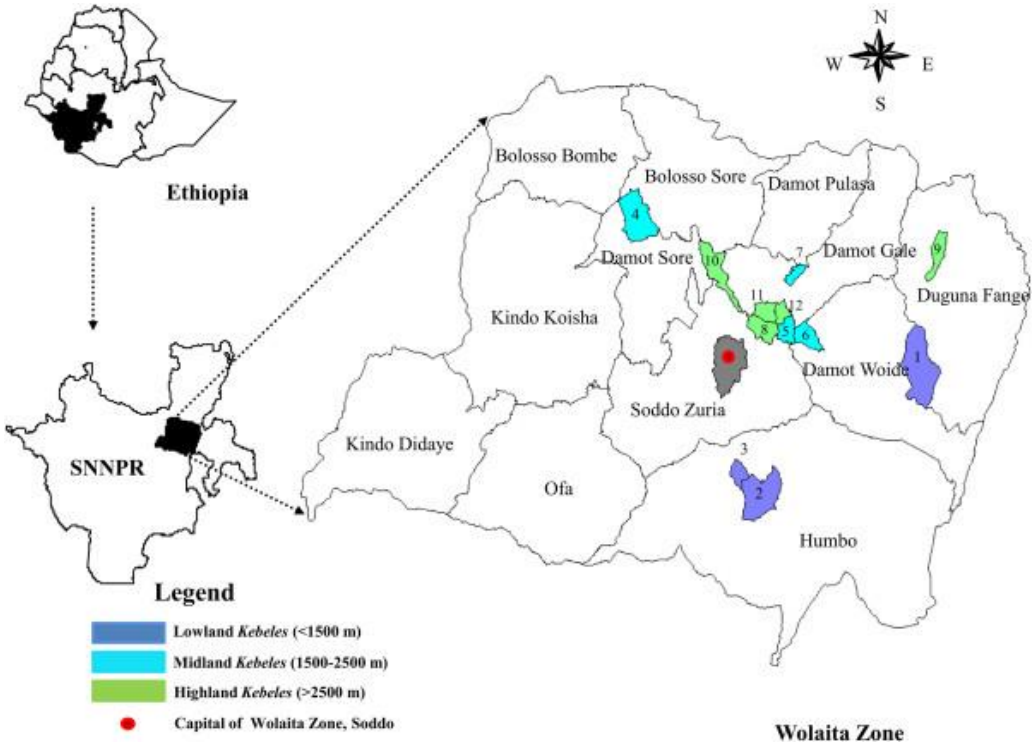

Figure 1: The map of Ethiopia, SNNPR and Wolaita zone (Adapted from Wikipedia)

Instrument and apparatus

Heavy and trace metals determination in the leaves of *Ocimum Lamiifolium* was done using atomic absorption spectroscopy (AAS). Flame atomic absorption spectrometer (FAAS) (Germany, novAA) is a suitable technique for determining metals at parts per million (ppm) concentration levels with good precision for many elements. FAAS offers air-acetylene and/or nitrous oxide flame atomizer. FAAS technique delivers fast analysis of 10-15s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost. As indicated in Figure 3, a typical AAS consists of radiation (energy) source, atomization compartment, monochromator, detector and data readout system.

Plant material was sectioned using a stainless steel axe and Teflon-coated (SSAT) knife, then dried in an air-circulating oven on porcelain supports. Samples were subsequently ground and homogenized using a blending device and ceramic pestle and mortar. A digital analytical

balance was used for accurate sample weighing. Microwave digestion was performed in 100 mL round-bottom flasks with ground-glass fittings and reflux condensers (Gallenhamp, England). Borosilicate volumetric flasks (50, 100, and 250 mL) were employed for sample dilution and the preparation of metal standard solutions.

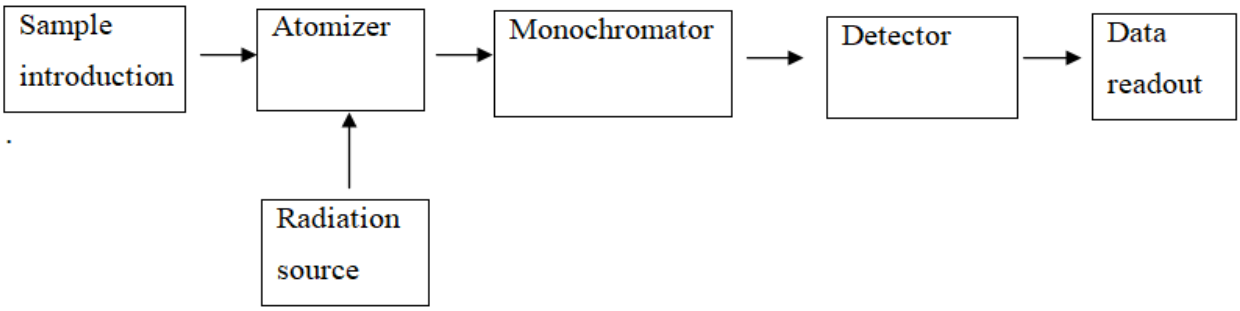

Figure 2: Basic components of atomic absorption spectrophotometer

Chemicals and reagents

Analytical grade chemicals were purchased from Sigma-Aldrich Company found in Germany. 69% nitric acid (HNO_3), 70% perchloric acid ($HClO_4$) and 30% hydrogen peroxide (H_2O_2) were used for digestion in microwave digester, while multi-element standard solution was used as a reference material. Stock standard solution for each metal cadmium (Cd), lead (Pb), zinc (Zn), cobalt (Co), chromium (Cr) and copper (Cu) with a concentration of 1000mg/L was used to prepare intermediate or working standard solutions of 10mg/L for the calibration standards of each metal. Throughout the study, deionized water was used. All glass wares were soaked in 5% (v/v) HNO_3 overnight then rinsed with deionized water and dried using laboratory dryer prior to use.

Experimental work procedures

Sample collection and preparation

The samples of fresh leaves of *Ocimum Lamiifolium* were collected from three different kebeles, which are Aruse weyde, Edo mazegaja and Dendo Koysha, in Duguna Fango district from uncultivated fields. From each kebele, three sites were selected to collect the plant leaves in order to homogenize the sample. Samples were placed in plastic bags and labeled, and brought to the laboratory. Samples were washed with distilled water, and first air-dried at room temperature and oven was used for further drying and placed in dust free environment; then ground in to fine powder manually using a porcelain mortar and pestle and allowed to pass through a sieve of 0.5mm mesh size. The powdered samples were put in plastic containers and kept in a dry, cool closet until they were analyzed. The plant species was collected from different localities based on its availability and knowledge of the societies regarding its medicinal values of the plant.

Optimization of the digestion procedure of samples

Achieving optimal sample digestion is essential for accurate analysis. Key criteria for optimum digestion include minimal reagent volume and digestion time, a clear solution with minimal residue, low digestion temperature, and procedural simplicity. The digestion procedure was optimized by varying parameters such as reagent volume, digestion temperature, and digestion time. Based on visual assessment of the resulting solutions, the optimal and appropriate digestion conditions (Table 1), were selected for subsequent FAAS analysis.

Sample digestion

One gram (1 g) of each powdered sample was accurately weighed using a calibrated digital analytical balance and transferred into a 250 mL beaker. To each sample, an optimized mixture of 69% concentrated nitric acid (HNO3), 70% perchloric acid (HClO4), and 30% hydrogen peroxide (H2O2) was added, following the optimized digestion procedure. After cooling for 30 minutes, distilled deionized water was added to dissolve any precipitate, followed by gentle swirling. The resulting solution was filtered into a 50 mL volumetric flask using Whatman filter paper number 41 to remove any suspended matter. The filter paper was subsequently rinsed with distilled deionized water until the volume reached the mark. Each bulk sample was processed in triplicate. The digested and diluted sample solutions were then stored in plastic sample bottles for FAAS analysis.

Chemical analysis

Instrument operating conditions

Intermediate standard solutions (10 mg/L) were prepared from 1000 mg/L atomic absorption spectroscopy (AAS) stock solutions. These intermediate standards were further diluted using distilled deionized water to create five working standards for each target metal. Flame atomic absorption spectrophotometry (FAAS), equipped with a deuterium arc background corrector and an air-acetylene flame system, was used to analyze six metals. An external calibration curve was used for quantification, and all instrument parameters (burner and lamp alignment, slit width, and wavelength) were optimized for maximum signal intensity. AAS is a quantitative method that measures the concentration of the element by passing light in specific wave length emitted by a radiation source of a particular element through cloud of atoms from a sample. Atoms absorbed light from an energy source known as hollow cathode lamp (HCL). In FAAS, the reduction in light intensity reaching the detector is directly proportional to the concentration of the target element in the original sample. A typical FAAS instrument consists of a light source, a sample atomizer, a monochromator, a detector, and a data processing system. Three replicate measurements were performed for each sample. The hollow cathode lamp for each metal was operated at the manufacturer's recommended conditions, using the respective primary source line for analysis. Acetylene and air flow rates were carefully controlled to ensure optimal flame conditions. The absorption mode of the instrument was used to analyze all six target metals (Pb, Zn, Cu, Co, Cr, and Cd).

Instrument Calibration

Calibration curves were cautiously prepared to determine the concentration of the metals in the sample solution. Before the commencement of the experiment, the instrument (i.e. flame atomic absorption spectrometer [FAAS]), was

standardized using five series of working standards. The working standard solution of each metal was prepared from the 10 mg/L intermediate standard solutions of their respective metals. Wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curve for each of the metals were identified and presented (Table 4).

Method detection limits (MDL)

The method detection limit (MDL), also known as the limit of detection (LOD), represents the minimum concentration of an analyte that can be reliably detected by an analytical method with a specified level of confidence. The limit is statistically determined as the lowest possible concentration distinguishable from a blank, typically with 95% confidence. The MDL/LOD is often defined as the point where the signal-to-noise ratio exceeds 3, but is not necessarily a precisely quantified value. It can be calculated by multiplying the standard deviation of the reagent blank (Sblank) by three: MDL = 3 × Sblank (Chen, 2007).

Method validation

Method validation is essential to confirm that an analytical method is suitable for its intended purpose. Given the lack of certified reference materials for the leaf and seed samples, the efficiency of the optimized digestion procedure was assessed by spiking 1 g Ocimum lamiifolium leaf samples with known concentrations of each target metal. Percentage recovery, a crucial parameter for method validation, was calculated by comparing the measured concentrations in spiked and non-spiked samples, which were digested and analyzed under identical conditions. Then the percentage recovery of the analyte was calculated by:

$$\text{Percentage recovery} = \frac{\text{Cm in the spiked samples} - \text{Cm in the non-spiked samples}}{\text{Amount added}} \times 100\%$$

Where, Cm = Concentration of metal of interest (Adapted from: IJRPC (2014), 4(1), 202-216)

Statistical Analysis

All measurements were done in triplicates and expressed as mean ± standard deviations. Data was analyzed using analysis of variance (ANOVA) at level of 5% ($p \leq 0.05$) followed by least significant difference Post Hoctest in Microsoft Excel for the determination of statistical significance of a given metal across the samples, not within a given sample. Data was further manipulated with Origin Pro 2020b SrOH(1) for windows version software program.

RESULTS AND DISCUSSION

Optimization of working procedures

As indicated in Table 1 below, eight optimization procedures were used to get the optimum digestion conditions. The procedures used in steps one through four were not chosen as optimum conditions for they used maximum reagent volumes and the highest digestion temperature as well as long time even if some of the procedures gave clear and colourless solutions. In steps six up to eight, the volumes of reagents were relatively low, but they took place at relatively long times. Therefore, procedure five was chosen as optimum condition for the digestion, because it took place at relatively minimum reagent volumes, low digestion time to give clear and colorless

solution.

Table- 1: Methods tested during optimization of the digestion procedure for the samples of the leaves of *Ocimum Lamiifolium*.

No.	Wt. (g)	Volume of reagents (mL)			Max. Temp. (°C)	Time (min)	Results
		HNO_3	$HClO_4$	Total			
1	1.0	4	3	7	200	60	Clear but turbid
2	1.0	5	2	7	200	60	Clear but yellowish
3	1.0	5	1	6	200	60	Clear but pale yellow
4	1.0	3	2	5	200	10	Clear and colourless
5	**1.0***	**3***	**2***	**5***	**150***	**20***	**Clear and colourless**
6	1.0	3	2	5	140	60	Clear and colourless
7	1.0	4	1	5	130	40	Clear and light yellow
8	1.0	3	2	5	140	30	Clear and light yellow

*Optimum digestion conditions

The results of the analytical recovery test

The validation of the method was tested by spiking the samples with a standard of known concentration of the analyte metals. As depicted in Table 2 below, the results indicated that the concentrations of elements determined are in agreement within the acceptable range for all metals, that is 80-120%. Hence, the digestion method was efficient because the values of the percentage recoveries lied within the acceptable range.

Table- 2: Analytical recovery results obtained for the validation of the optimized procedure of plant samples.

Metal	Concentration in non-spiked sample (mg/L)	Amount added (mg/L)	Concentration in spiked sample (mg/L)	Percentage recovery (%)
Cd	0.05	0.03	0.08±0.01	100±0.025
Co	0.06	0.04	0.095±0.01	87.5±0.028
Cr	0.15	0.14	0.28±0.007	92.9±0.078
Cu	0.21	0.19	0.39±0.01	94.7±0.11
Pb	0.09	0.08	0.17±0.007	100±0.049
Zn	0.85	0.82	1.68±0.02	101±0.49

Instrument operating conditions

The operating conditions for the instrument were prepared for each metal at an appropriate wave length, slit width, current and IDL (Table 3). Intermediate standard solutions of 10mg/L were prepared using 100 mL flask from stock standard solution that contained 1000 mg/L of soluble salts of $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Co(NO_3)_2$, $Zn(NO_3)_2$, $Cu(NO_3)_2$ and oxide of chromium for each metal of interest. MDL was calculated by

multiplying the standard deviation of blank solution by three ($MDL = 3S_{blank}$).

Table- 3: Instrument operating conditions for the analysis of metals in the samples of selected plant.

Element	Wavelength (nm)	Slit width (nm)	Current (mA)	IDL* (mg/Kg)
Cd	228.80	0.70	2.00	0.0001
Zn	213.90	0.70	2.00	0.0001
Cu	324.80	0.70	2.50	0.0001
Co	240.70	0.20	5.00	0.00075
Cr	357.90	0.70	4.04	0.00005
Pb	283.3	0.7	2	0.003

*Instrument Detection Limit

Concentrations of working standard solutions and correlation coefficients of calibration Curves

Working standard solutions were prepared from intermediate standard solutions containing 10mg/L, which was prepared from stock standard solutions, by diluting with deionized water to obtain five working standards for each metal of interest as indicated in Table 4. The table also showed that the correlation coefficients of calibration curves of all six metals were closer to one. Thus, these results confirmed that there are strong linear relationships between two variables, which are absorbance and the concentrations of working standard solutions.

Table- 4: Concentrations of working standard solutions and correlation coefficients of the calibration curves for the analysis of plant samples.

Metal	Concentrations of working standard solution	Correlation coefficient
Cd	0.5, 1.0, 1.5, 2.0, 2.5	0.999
Co	0.1, 0.5, 1.0, 1.5, 2.0	0.9989
Cr	0.5, 1.0, 1.5, 2.0, 2.5	0.9988
Cu	0.1, 0.5, 1.0, 1.5, 2.0	0.9977
Pb	0.5, 1.0, 1.5, 2.0, 2.5	0.9959
Zn	0.5, 1.0, 1.5, 2, 2.5	0.9974

The determination of heavy and trace metals in *Ocimum Lamiifolium*

As indicated in Table 5, all six metals (Cd, Pb, Co, Cr, Cu, Zn) were detected in all samples of the selected medicinal plant with variable concentrations in the sites. The results revealed that the concentration of zinc was the highest and that of cadmium was the least of all the metals. Furthermore, the results revealed that there were variable concentrations of each analyte metal in the selected sites.

Concentration trends of metals in Dendo koysha

As depicted in Table 5, zinc had the highest concentration among the metals and the metal with the next highest concentration was copper. The fact that zinc had the highest concentration among these metals was also true in other herbal medicinal plants based on different literatures (Baye and Haymete, 2010). But, cadmium and cobalt had the same concentration, which is 0.04 mg/L; similarly chromium and lead had the same concentration, that is, 0.10 mg/L.

Concentration trends of metals in Aruse weyde

The concentration of the metals in this sample site varied from 0.05 to 0.78 mg/L. The metal with the highest concentration and the one with the least concentration were zinc and cadmium, respectively. The concentrations of Co, Cr, Cu and Pb were 0.06, 0.18, 0.25, and 0.08, respectively (Table 5). The concentrations of the selected metals varied in different sites may be due to the difference in soil types, ecological locations, etc. (Ambaye and Mussa S., 2015).

Concentration trends of metals in Edo mazegaja

Based on the results given on Table 5, the concentrations of the metals varied from 0.05 to 0.54 mg/L. Though the concentrations differed from the other sample sites in this study, the metals with the highest and the least concentrations were zinc and cadmium, respectively. The concentration of Co, Cr, Cu and Pb were 0.07, 0.17, 0.19 and 0.10, respectively. Here, also the concentration differences of the selected metals from the other sites were observed due to the variation of different factors like ecological locations.

Comparison of the concentration of each metal among the sample sites and with different permissible limits

Cadmium

As indicated in Table 5, the concentration of cadmium ranges from 0.04 mg/L to 0.05 mg/L. This result showed that there was no such much difference in the concentration of Cd in all the sites selected. Other literatures showed that the concentration of cadmium in other herbal medicines varied between 0.0045 mg/L and 0.0091 mg/L; for instance, in champion leaf, its concentration was 0.0068 ppm (Baye and Haymete, 2010). However, the concentration of cadmium in the study area was well below the permissible limit set by WHO and other organizations. The permissible limit of cadmium in medicinal plants set by WHO, China and Thailand, was 0.3 mg/L, which is equivalent to 0.3 mg/L (FAO/WHO, 2006). The literatures suggested that it is safe for consumption if its level is less or equals to this permissible limit.

Cobalt

The concentration of cobalt in the studied medicinal plant ranges from 0.04 mg/L to 0.07 mg/L. This result indicated that there was variation in the concentration of cobalt in the sample sites. However, the difference in the concentration is not such much great. For herbal plants, the WHO/FAO has not set any regulation limit for cobalt. But, according to Jabeen *et al*. (2010) the concentration of cobalt in different plant samples ranges from 0.18 to 0.4 mg/L 9. Thus, the obtained result of cobalt is not greater than these results.

Chromium

As depicted in Table 5, the concentration of chromium ranges from 0.10 mg/L to 0.18 mg/L. This range indicated that there was a concentration variation of chromium in different sites of the selected area. The research done on the heavy metal analysis of seven herbal medicines reported that the concentration of chromium ranges from 0.04 ppm to 0.20 ppm (Baye and Haymete, 2010). So, these values are comparable with the concentrations of chromium in the current study. The permissible limit for chromium in herbal medicinal plants has not been set by the WHO yet. However, 2.0 ppm was set by Canada as the permissible limit of chromium in raw medicinal plant. High intake of chromium is reported to have a toxic effect, causing skin rash, kidney and liver damage, cancer of the lungs and nose irritations (Khan *et al*., 2008).

Copper

As indicated in Table 5, the concentration of copper varied between 0.19 mg/L and 0.25mg/L. This result indicated that there is a concentration difference of the metal in different sites which may be related with different factors like soil type difference. Regulatory limits for copper in herbal medicines have not yet been established by the WHO/FAO However, China and Singapore in 2008 set the permissible limits of 20 mg/Land 150 mg/L, respectively (Jabeen *et al.*, 2010). Thus, the concentration of copper in the studied medicinal plant was well below than these limits.

Lead

The results in Table 5 showed that the concentration of lead in the study area varied between 0.08 mg/L and 0.1 mg/L. From this, it is obvious that there was almost uniform pattern in the distribution of lead. The concentrations of lead varied between 0.02 mg/L and 0.09 mg/L in other medicinal herbs as indicated in the report of the Journal of Scientific and Engineering Research, 2016, 3(2). So, the result of lead in the present study was approximately equals to the above values. The WHO (2006), Malaysia, China and Thailand (2008) set the permissible limit for lead in medicinal herbs as 10 mg/L (Khan *et al*., 2008). So, the results of the study area showed that the concentration of lead was well below the permissible limit.

Zinc

Table 5 indicated that the concentration of zinc in the study area ranges from 0.54 mg/L to 1.21 mg/L. When compared to the variation in the concentration of other metals, the difference is great in the case of zinc as well as its concentration was high. The permissible limit for zinc in herbal medicines set by WHO/FAO is 50 mg/L. Though there is little information

about its toxicity, consumption of zinc beyond the permissible limit may result in toxic effect on the immune system (Waheed and Fatima, 2013). The concentration of zinc in the studied medicinal plant was very much below than this value.

Table- 5: Mean concentrations of metals (mg/L) in the studied medicinal plant in the samples collected from different sites.

Metals	Concentrations of metals in three sites (mg/L) Mean±SD		
	Dendo Koysha	Aruse weyde	Edo Mazegaja
Cd	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Co	0.04 ± 0.01	0.06 ± 0.00	0.07 ± 0.01
Cr	0.10 ± 0.01	0.18 ± 0.03	0.17 ± 0.02
Cu	0.20 ± 0.02	0.25 ± 0.01	0.19 ± 0.00
Pb	0.10 ± 0.01	0.08 ± 0.02	0.10 ± 0.06
Zn	1.21 ± 0.13	0.78 ± 0.06	0.54 ± 0.06

CONCLUSION

The contamination of herbal medicines is due to the accumulation of the metals in different parts of the medicinal plants. Hence, the concentrations of the metals should be measured in order to be free of their toxic effects.

In the present study, the analysis of heavy and trace metals in the selected medicinal plant was made by using FAAS by following the optimized digestion method. The digestion method was optimized by changing the parameters until clear and colourless solution was obtained. The concentrations of all the selected metals in the medicinal plant, *Ocimum Lamiifolium*, were determined. The concentrations of Zn, Cu, Cr, Pb, Co and Cd are 0.847, 0.214, 0.153, 0.0936, 0.0579 and 0.0489, respectively.

In the studied area, the concentration of zinc was the highest of all the metals. However, its concentration was not above internationally accepted permissible limits. In the medicinal plant under study, the concentrations of some metals were below and that of others were nearly equal to internationally accepted permissible limits so that this showed the plant is safe for medicinal uses.

Furthermore, the efficiency of the digestion method was confirmed by percentage recoveries which were within accepted ranges, that is, 80 – 120%.

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Synthesis, Growth and Characterization of Magnesium Chloride Doped L-Alanine Cadmium Chloride Single Crystal: For Nonlinear Optical Application

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KEYWORDS:

Solution method;
Doping;
Crystal Growth;
Harmonic generation;
Nonlinear optics

Research article

ABSTRACT

The aim of this research was to synthesize and characterize pure and magnesium chloride ($MgCl_2$) doped L–alanine cadmium chloride (LACC) single crystals. Pure and $MgCl_2$ doped LACC single crystals were synthesized by solution method with slow evaporation solution growth technique at room temperature. The single crystal X-ray diffraction studies of pure, 1 and 2 mol% $MgCl_2$ doped LACC single crystals revealed monoclinic crystal structure with C_2 space group. The optical properties of pure and $MgCl_2$ doped LACC single crystals investigated by UV–VIS/NIR spectrometer confirmed that the crystals were transparent in the wavelength range of 230-1100 nm. The optical band gap energy of pure and doped LACC single crystals were found to have the same value of 5.4 eV. The energy dispersive X-ray analysis indicated the incorporation of magnesium and chlorine atoms in LACC single crystal. The second harmonic generation efficiency of 1 and 2 mol% $MgCl_2$ doped LACC crystals were analyzed by Kurtz-Perry powder technique and found to be 1.75 and 2 times greater than that of the standard potassium dihydrogen phosphate crystal, respectively.

INTRODUCTION

The core attention of nonlinear optical (NLO) studies is to modify the phase, frequency or amplitude of intense electromagnetic input field using NLO materials for photonic applications (Boyd, 2019). Nonlinear optical materials have been the subject of much research in recent years due to their potential uses on optical computing, laser technology, harmonic generation, optical communication, optical data storage technology, signal processing and manipulation. Therefore, currently there is a need to produce high efficiency single crystals of NLO materials (Marudhu *et al*., 2013; Raguram *et al*., 2016). Much attention has been paid to organic NLO materials due to their promising properties, such as fast optical response time and high nonlinearity, compared to the inorganic materials. The aggregate of materials which have large nonlinear optical properties with resistance to physical and

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chemical attack has led to the investigation of semi-organic materials (Kaliammal *et al*., 2020). Amino acids are favorable organic materials displaying specific features of interest, such as molecular chirality which secures non-centrosymmetric crystallographic structure, absence of strongly conjugated bonds which leads to wide transparency ranges in the visible and ultra-violet spectral regions and zwitter ionic nature of the molecule which favors crystal hardness for applications in devices (Natarajan *et al.*, 2006; Raguram *et al*., 2016). L-alanine is an amino acid group that forms a chains of complexes upon reaction with different acids.

Reports are available on L-alanine based NLO crystals such as: L-alanine cadmium chloride (Dhanuskodi *et al*., 2007), L-alanine acetate (Kumar *et al*., 2005), L-alanine Hydrogen chloride (Rose *et al*., 2010), L-alanine sodium nitrate(Fleck and Petrosyan, 2009), L-alanine potassium chloride (Prabha and Palaniswamy, 2010) and L-alanine maleate (Karunanithi *et al*., 2012). L-alanine cadmium chloride is an amino acid derivative NLO material with high second harmonic generation (SHG) efficiency and it crystallizes in the monoclinic crystal system with non-centrosymmetric space group (Jothimani and Selvarajan, 2017; Radhika *et al*., 2013).

Few reports are available on the synthesis of L-alanine cadmium chloride single crystals by solution growth method. As far as the authors are aware, there is no report on magnesium chloride doped L-Alanine cadmium chloride single crystals. Since doping can influence many of the useful properties like, optical transparency, second harmonic generation (SHG), crystalline perfection which may in turn influence the physical properties depending on the degree of doping and the accommodating capability of the parent crystal (Shkir *et al*, 2014). Magnesium chloride-doped L-alanine cadmium chloride single crystals were grown using slow evaporation, and their structural, optical, second-harmonic generation (SHG), and compositional properties were characterized.

MATERIALS AND METHODS

In this work, analytical grade L-Alanine (Sisco Research laboratories, 99%), cadmium chloride (Uni-CHEM, 99%) and magnesium chloride (Uni-CHEM, 98%) were used directly without further process. The parent compound was synthesized by taking an equimolar ratio of L-alanine and cadmium chloride that is obtained by dissolving 8.0901 g L-Alanine and 22.8353 g cadmium chloride. The calculated amount of L-alanine has been dissolved with distilled water in a beaker and placed on a magnetic hot plate regulated at 30°C then cadmium chloride was added. It has been stirred continuously for four hours to obtain homogenous supersaturated solution. Afterwards the solution has filtered by using Whatman filter paper into a 500 m1 beaker. The filtered solution has been kept free from dust and other contamination by covering it with porous cover so the rate of evaporation could be minimized. 1 mol% and 2 mol% $MgCl_2$ doped L-Alanine cadmium chloride single crystals were synthesized by adding 2.0333 g and 4.0666 g $MgCl_2$ into the parent compound solution respectively. A similar stirring process has been followed for 1 and 2 mol% of $MgCl_2$ doped L-alanine cadmium chloride. After a period of 30 days, optically clear transparent crystals were harvested from supersaturated solution. Accordingly, the grown pure, 1 and 2

mol% $MgCl_2$ doped LACC crystals were shown in Fig. 1.

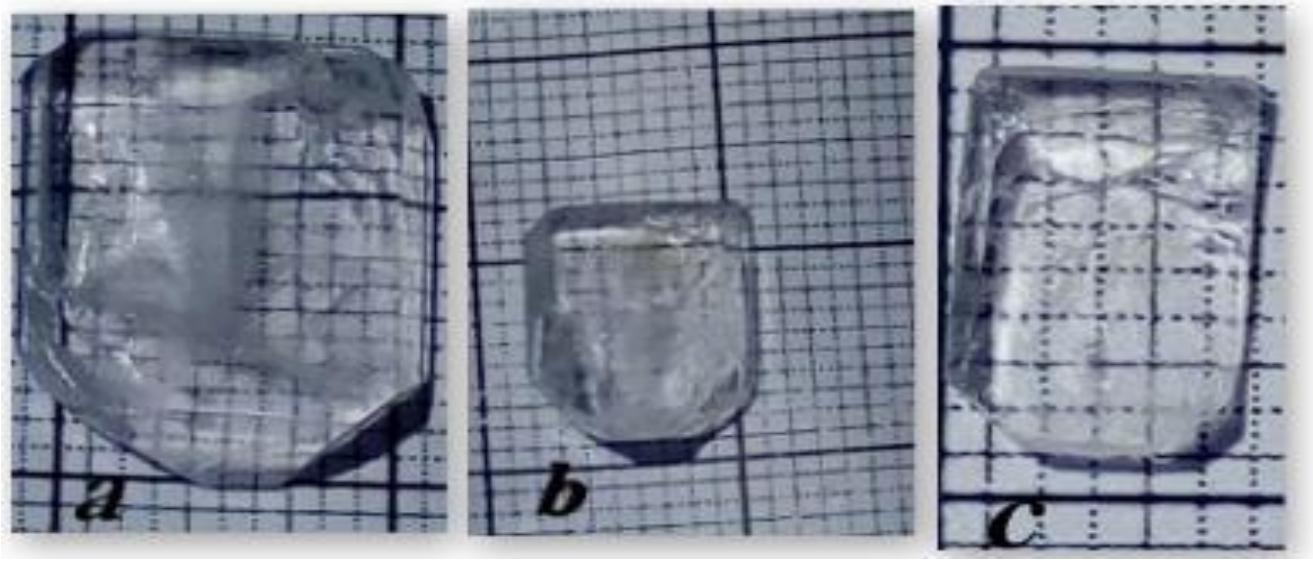

Fig. 1. Photograhp of (a) undoped, (b) 1 mol% $MgCl_2$ doped and (c) 2 mol% $MgCl_2$ doped LACC single crystals respectively.

RESULTS AND DISCUSSION

Single crystal X-ray diffraction

Single-crystal X-ray diffraction (XRD) analysis using a Bruker AXS Kappa APEXII diffractometer (Mo Kα radiation, λ = 0.07107 nm) revealed that 1 and 2 mol% $MgCl_2$-doped LACC crystals adopted a monoclinic structure (with interaxial angles in a crystal lattice of α≈ β ≈ 90° and γ ≈ 116.41°). The calculated lattice parameters (Å) were a = 16.282, b = 7.261, c = 8.008 for 1 mol% and a = 16.286, b = 7.268, c = 8.011 for 2 mol% $MgCl_2$ doping. The unit cell parameters and crystal structure for pure LACC crystal in (Å) were a = 16.298, b = 7.259, c = 7.981 and monoclinic respectively as reported by other authors (Dhanuskodi *et al*., 2007). It is observed that both undoped and $MgCl_2$ doped LACC crystals crystallize in same structure however, slight change in the lattice parameters were detected from the doped crystal compared to the pure LACC crystal. The changes in the lattice parameter may be due to the incorporation of $MgCl_2$ in LACC crystal lattice. The grown crystals belongs to space group C_2 which is recognized as non-centrosymmetric**,** thus satisfying an essential material criteria for the SHG activity of the crystal (Fentaw *et al*., 2019).

UV–Vis NIR analysis

To assess its optical properties, the transmission, cutoff wavelength, and band gap energy of the NLO single crystal were determined. In this study these optical parameters have been studied by using Perkin Elmer Lambda 35 UV-VIS-NIR spectrophotometer in the wave length range between l90-ll0 nm to cover near ultraviolet, visible and near IR regions. The recorded spectrums were shown in Fig.2.

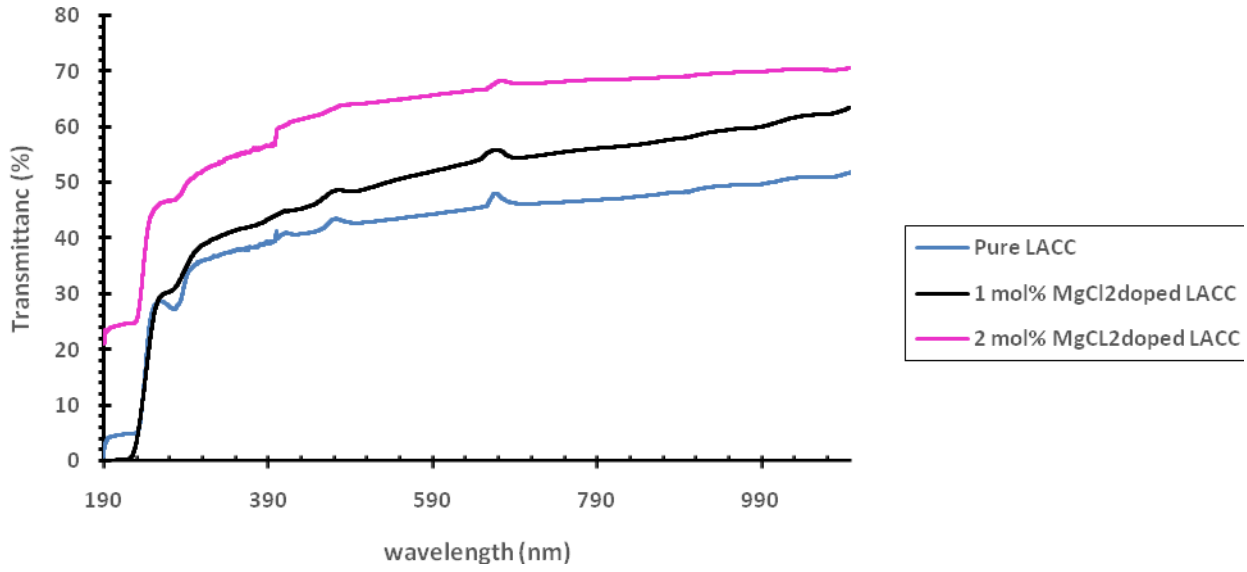

Fig.2. Optical transmittance of pure, l and 2 mol % of $MgCl_2$ doped LACC crystals.

From the spectrum it is detected that the crystals were transparent in the wavelength range 230 - 1100 nm. NLO materials can be of practical use only if they have a wide transparency range, thus the optical transmittance range of the grown crystals make them convenient materials for nonlinear optical applications (Ahlam *et al., 2013;* Charoen-In *et al.*, 2010). The result confirmed that the lower cutoff wavelength of the crystals were almost the same irrespective of the dopant concentration, however, the percentage of transmittances have increased for 1mol% and 2 mol% $MgCl_2$ doped LACC crystal. The increase in optical transmittance could be due the enhancement in the crystalline quality and absence of major defects as crystalline defect affect the optical properties (Jothimani and Selvarajan, 2017). The band gap energy was calculated using the relation $E_g = 1240/\lambda_{min}$, where λ_{min} is the cut-off wavelength of the light which is 230 nm and it was found to be 5.4 eV for pure and doped crystals (Raguram *et al.*, 2016).

Energy dispersive X-ray spectroscopy (EDX) analysis

Energy dispersive X-ray spectroscopy (EDX) is a very useful instrument that used to identify the elemental composition of the samples. The incorporation of 1 and 2 mol% of $MgCl_2$ into the crystal lattice of LACC was confirmed by JEOL-6390LV scanning electron microscope attached to EDX and it is shown in Fig.3. The EDX spectrum confirmed existance of expected elements such as oxygen, chlorine, magnesium and cadmium in both 1 and 2 mol% of $MgCl_2$ doped LACC crystal. The molecular weight and atomic percentage of identified elements in the doped NLO samples were presented in Table1. The measurable (quantitative) and qualitative EDX results clearly showed increase in the atomic percentage of Mg and Cl signifying

incorporation of the $MgCl_2$ in the LACC single crystal structure.

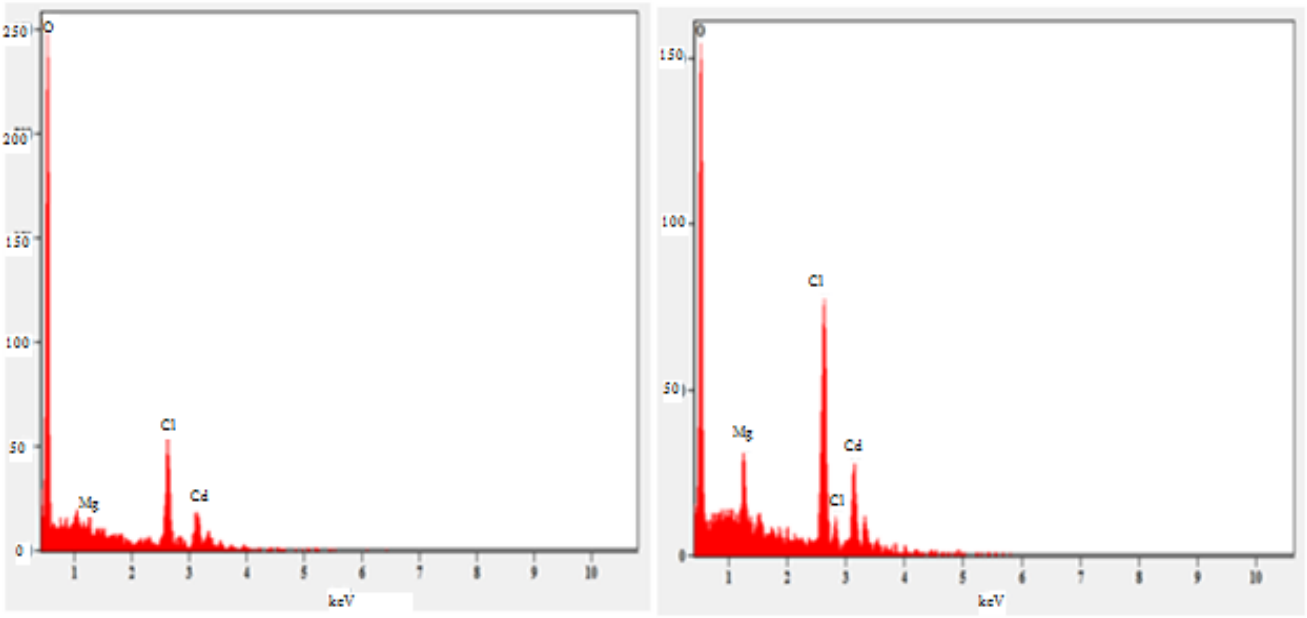

Fig. 3. EDX spectrum of 1 and 2 mol% of $MgCl_2$ doped LACC crystals respectively.

Table 1: Energy dispersive X-ray (EDX) analysis

Elements	1mol%$MgCl_2$+LACC		2mol%$MgCl_2$ +LACC	
	Weight%	**Atom%**	**Weight%**	**Atom%**
OK	74.28	91.19	62.49	82.57
MgK	0.72	0.57	5.41	4.70
ClK	10.20	5.65	16.38	9.77
CdL	14.80	2.59	15.72	2.96
Total	100.00	100.00	100.00	100.00

Second Harmonic Generation (SHG) test

The second harmonic generation efficiency analysis of the samples were carried out by Kurtz-Perry powder technique by packing very fine powder of $MgCl_2$ doped LACC sample in a microcapillary tube and a fundamental beam (1064 nm) from Nd-YAG laser was incident on the $MgCl_2$ doped LACC crystals (Kushwaha *et al*., 2011). A second harmonic generated green light beam (532 nm) was emerged from the samples. To evaluate second-harmonic generation (SHG) properties, potassium dihydrogen phosphate (KH_2PO_4, KDP) powder was used as a standard reference material. The transmitted beam voltage through KDP was then measured to be 12 mV. In comparison, the

transmitted beam voltages through the 1 and 2 mol% $MgCl_2$-doped LACC samples were 21 mV and 24 mV, respectively. The SHG efficiency of the 1 and 2 mol% $MgCl_2$-doped LACC crystals was thus calculated to be 1.75 and 2 times greater than that of the KDP reference material. The SHG efficiencies of pure LACC single crystal was 0.87 times greater than that of the standard KDP crystal reported by (Kalaiselvi *et al*., 2013). The enhancement of SHG efficiency may be either due to the improved in crystallinity or the change in electronic structure of LACC crystals due to the doping of $MgCl_2$ (Bhagavannarayana and Kushwaha, 2010). The current results indicate that 1mol% and 2mol% $MgCl_2$ doped LACC crystals are the better candidates for NLO applications than the undoped LACC single crystal.

CONCLUSION

Pure and $MgCl_2$-doped (1 and 2 mol%) LACC single crystals were grown by slow evaporation at room temperature. Characterization included single-crystal XRD (confirming a monoclinic structure, space group C2), UV-Vis-NIR spectroscopy (showing high transmission across the UV-Vis-NIR range, enhanced by $MgCl_2$ doping), energy-dispersive X-ray spectroscopy (EDAX, confirming $MgCl_2$ incorporation), and second-harmonic generation (SHG) efficiency measurements (1.75× and 2× greater than KDP for 1 and 2 mol% doping, respectively). These results suggest that both pure and $MgCl_2$-doped LACC crystals are promising candidates for nonlinear optical applications.

Acknowledgement

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