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ACADEMIC JOURNALS expand your knowledge

Full Length Research Paper

# Impact of waste vehicle tyres incineration and heavy metals contamination of soil in some locations in Lafia, Nasarawa State

# Joseph Ikwebe<sup>1</sup> and Christopher David Bando<sup>1,2\*</sup>

<sup>1</sup>Department of Biochemistry, Federal University Wukari, Wukari, Taraba, Nigeria. <sup>2</sup>Bioresources Development Center, National Biotechnology Development Agency, Jalingo, Taraba State, Nigeria.

### Received 27 September, 2023; Accepted 10 November, 2023

This study was carried out in Lafia, to determine metallic pollution in soils due to incineration of tyre on the soils at different layers and the effect of pH on the heavy metals leaching. Soil composite samples were collected from 5 identified locations (Shabu, Wakwa Alhaji, Ombi1, Tudun Amba and Akurba) manually at various depths, 0-10, 11-20 and 21-30 cm using a stainless-steel hand auger. Atomic Absorption Spectrophotometer (AAS) was used for the analysis of the selected heavy metals. The samples showed elevated amounts of metallic depositions compared to the control sample. It was also observed that the heavy metals concentrations in the soil samples decreased with soil depth. The distribution pattern was in the following order Zn > Fe > Pb > Cd > Cu. Across all the sampling locations respectively. The pH values ranged from slightly acidic to slightly alkaline with 7.3 - 8.4 (0 - 10 cm), 7.1 - 7.4 (11 - 20 cm) and 6.9 - 6.7.4 (21 - 30 cm). The waste tyre burning serves as a potential source of heavy metals pollution to the environment.

Key words: Heavy metals, Lafia, atomic aborption spectrometer, soil, tyre.

### INTRODUCTION

Heavy metals get into the earth's crust through natural and anthropogenic activities. Natural sources could be weathering of earth's crust and anthropogenic activities including mining, soil erosion, industrial discharges, urban runoff, sewage effluents, application of pests or disease control agents to crops, air pollution etc. (Ming-Ho, 2005; Bando et al., 2023). The concentration of these heavy metals varies across different ecological environments or regions and have tendency to be cycle in the order: Industry, atmosphere, soil, water, foods and human (Morais et al., 2012). Their distribution in the environment is governed by the specific properties of the metals (Khlifi and Hemze-Chaffai, 2010). The sole purpose of designing tyres is for vehicles and not to be burned as a fuel. They constituents of tyres are hazardous to the environment and carcinogens. Tyre-

\*Corresponding author. E-mail: <u>bandomidase@gmail.com</u>.

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Figure 1. Map of Nasarawa state showing Lafia local government area. Source: Ezekiel et al. (2021).

derived fuel (TDF) also contains remnants of wire that are difficult to totally remove. In Nigeria, there is little or no records of the quantity of scrap tyres but their uses on old vehicles, burnt as fuel or either to obtain iron and steel frames and other purposes such as roasting animal skin is encountered in various parts of the country (Beetseh and Onum, 2013; Bando et al., 2023).

Indiscriminate habit of burning of waste tyres is of public health concern and a potential threat to environmental. There is high tendency of emission of heavy metals to the soil, water and air through uncontrolled, open, waste tyre incineration, its effect in the ecosystem is a major concern (Mashi et al., 2005, Bando et al., 2019). Unlike other organic toxic substances that can be destroy in the environment either through biological or chemical processes, heavy metals possess these characteristics of being persistent in the environment (Beetseh and Onum, 2013; Bando et al., 2023). Some of the metallic contaminants such as Pb, Cd, Hg, Cu and As, which are mostly found in the ecosystem are of no beneficial value in humans (Draghici et al., 2010; Vieira et al., 2011). These metals are generally considered as injurious to humans and animals as even at low concentrations they have potentials of causing adverse effects to human and animal health (Jomova and Valko, 2011; Tokar et al., 2011).

The severe degradation of air, water and soil quality in most parts of the world are attributed to the growth in the number of industries and urbanization (Bando et al., 2019). Elevated levels of heavy metals depositions in urban environments are as a result of increase of anthropogenic activities. In today's industrial society, there is no escaping exposure to toxic substances and metals. Therefore, there is need for government and other non-governmental organisations to ensure or implement monitoring programmes that will ensure the quality and safety of water, food, soil and air and the environmental health. This study aimed to evaluate the impact of vehicle tyre incineration and metallic depositions of soil in some locations in Lafia, Nasarawa State (Figure 1).

### MATERIALS AND METHODS

### Study area description

Lafia is the capital city of Nasarawa state in central Nigeria region which lies geographically 8°29'30"N and 8°31'0"E. The human population of Lafia is placed at 330,712 according to the 2006 census. It is the largest town in Nasarawa State.

### Sampling locations

The soil samples were collected from 5 identified locations of open incineration of tyres within Lafia. Table 1a shows the specific locations.

### Sample collection

Two types of soil samples were collected manually as described by

Table 1a. Sampling locations.

Incineration sites	Location	Specific address	Latitude	Longitude
Α	WakwaAlhaji	Behind Project Quarters, Shendam Road Lafia	08°30'27.1''N	008°33'47.3''E
В	Akurba	Near precious FM	08°31'29.8''N	008°33'34.8"'E
С	Ombi 1	By Nasarawa State Polytechnic	08º32'23.1''N	008°31'55.5"E
D	TudunAmba	Behind NTA, Doma road Lafia	08º28'25.7''N	008°31'32.4"E
E	Shabu	Shabu Dumpsite	08°32'20.1''N	008°31'65.3''E

Prajapati and Meravi (2014), in each location. The first from the contaminated soil at depth 0-10, 11-20 and 21-30 cm in triplicates respectively. The second were collected from a control site that appeared free from any burning activity at 250 m interval. All the soil samples were separately put in airtight transparent low-density polyethylene pouch and taken to laboratory for sample preparation and analysed.

### Sample preparation

Soil sample preparation was done in the soil science laboratory of Nasarawa State University. The soil samples were dried in an oven at 50°C for 3 days. The dried soil samples were then sieved with 5 mm mesh to remove stones, coarse materials and other debris. Twenty grams portion of the sieved soil samples were ground in a mortar and then stored in airtight polythene pouches in a desiccator (Xiandong et al., 2004).

### Sample digestion and extraction

The stock solution of each of the ground soil samples was then prepared and heavy metal analysis was carried out using a strong acid digestion method. Approximately 1.0 g of each of the prepared soil sample was weighed and placed in pre-cleaned Pyrex test tubes. Thirty-two millilitre concentrated HNO<sub>3</sub> and 8 ml concentrated HClO<sub>4</sub> were added. The mixtures were heated at 50°C for 3 h, 75°C for 1 h, 100°C for 1 h, 125°C for 1 h, 150°C for 3 h, 175°C for 2 h and 190 for 3 to 5 h until completely dried. After the test tubes are cooled 40 ml of 5% HNO<sub>3</sub> was added and heated for 1 h with occasional stirring. On cooling, the mixtures were decanted into polythene tubes and centrifuge at 3500 rpm for 10 min. Metal concentrations of the solutions were measured using Inductively Coupled Plasma-Atomic Adsorption Spectrometry (ICPAAS). The major heavy metals analysed were Cu, Pb, Cd, Fe and Zn.

### Data analysis

The data generated from the laboratory analyses of the selected heavy metals were subjected to inferential statistics using ANOVA at a significance level of  $P \le 0.05$ .

### RESULTS

Table 1b shows the mean concentration of selected heavy metals from the 5 sample sites. Zn has the highest mean concentration in mg/kg in all the sites when compared with other heavy metals, in order of C>A>B>E>D with mean values of  $9.38\pm0.16$ ,  $0.02\pm0.41$ ,  $8.84\pm0.71$ ,  $7.18\pm0.90$ ,  $7.15\pm0.08$  respectively. The second metal with high mean concentration values is Fe, in the order of E>C>B>A>D with values of  $2.06\pm0.62$ ,  $1.55\pm0.36$ ,  $1.48\pm0.34$ ,  $0.82\pm0.01$  and  $0.78\pm0.26$ , respectively. Pb mean concentration in mg/kg is high in site A ( $0.40\pm0.20$ ) and lowest in site C ( $0.00\pm0.00$ ). Fe mean concentration in mg/kg is high in site E ( $2.06\pm0.62$ ) and lowest in site C ( $0.78\pm0.26$ ). Zn mean concentration in mg/kg is high in site E ( $7.15\pm0.08$ ). Cu mean concentration in mg/kg is high in site E ( $0.0347\pm0.01794$ ) and lowest in site D ( $0.00\pm0.00$ ).

Table 2 shows the pH values of the soils at varied depth and in different sites. The pH of the soil at depth 1 to 10 cm is higher than pH at any depth with highest value of 8.410 in site C. The lowest value of the pH was recorded at site E (6.898).

### DISCUSSION

Generally, this study shows elevated amounts of all the heavy metals in the contaminated sites when compared to their controls. Zn has the highest concentrations in all sites. This implies that Zn is relatively higher in concentration in the chemical metal components of the tyres. This is in concordance with the research of Beetseh and Onum (2013). Fe is the second metal with high mean concentration in all the sites. This can be attributed to being abundant natural element of the soil in that region. The difference in the concentrations of these metals between sites could be as a result of the reoccurrence of this activity more often in one site than the others. Contaminated sites have higher concentrations compares to the control. This may be attributed to the open incineration of tyre.

The amount of the metallic deposition in site A at various depths shows high level concentration of the metals at the depths of 1 to 10 cm, and low-level concentration was observed at the depths of 21 to 30 cm. This disagrees with the submission of Raju et al. (2013) which reported an increase in the heavy metals as the depth of the sampled soil increases. However, the finding of the present study, agrees with that of Jean-Philippe (2012) which shows a remarkable decrease in heavy

0:1	Mean concentration of heavy metals (mg/kg)									
Sites (cm)	Pb	Fe	Zn	Cu	Cd					
Site A										
1-10	0.40±0.20 <sup>b</sup>	0.82±0.01 <sup>a</sup>	9.02±0.41 <sup>b</sup>	0.02±0.01ª	0.25±0.00 <sup>b</sup>					
11-20	0.37±0.16 <sup>b</sup>	0.81±0.01 <sup>a</sup>	9.01±0.28 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.23±0.00 <sup>b</sup>					
21-30	0.33±0.11 <sup>b</sup>	0.81±0.01 <sup>a</sup>	8.67±0.31 <sup>b</sup>	0.01±0.01 <sup>a</sup>	$0.20\pm0.00^{b}$					
Site B										
1-10	0.34±0.17 <sup>ab</sup>	1.48±0.34 <sup>ab</sup>	8.84±0.71 <sup>b</sup>	0.04±0.02 <sup>a</sup>	0.30±0.05 <sup>b</sup>					
11-20	0.31±0.12 <sup>ab</sup>	1.42±0.28 <sup>ab</sup>	8.73±0.64 <sup>b</sup>	0.04±0.01 <sup>a</sup>	0.29±0.04 <sup>b</sup>					
21-30	0.31±0.07 <sup>ab</sup>	1.37±0.25 <sup>ab</sup>	8.72±0.47 <sup>b</sup> 0.03±0.0		0.29±0.04 <sup>b</sup>					
Site C										
1-10	0.00±0.00 <sup>a</sup>	1.55±0.36 <sup>ab</sup>	9.38±0.16 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.13±0.06 <sup>ab</sup>					
11-20	0.00±0.00 <sup>a</sup>	1.49±0.21 <sup>ab</sup>	9.18±0.14 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.13±0.04 <sup>ab</sup>					
21-30	$0.00 \pm 0.00^{a}$	1.47±0.18 <sup>ab</sup>	8.78±0.08 <sup>b</sup> 0.01±0.00 <sup>a</sup>		0.12±0.04 <sup>ab</sup>					
Site D										
1-10	0.03±0.00 <sup>a</sup>	0.78±0.26 <sup>a</sup>	7.15±0.08 <sup>ab</sup>	0.00±0.00 <sup>a</sup>	0.12±0.06 <sup>ab</sup>					
11-20	0.03±0.00 <sup>a</sup>	0.73±0.26 <sup>a</sup>	7.12±0.06 <sup>ab</sup>	0.00±0.00 <sup>a</sup>	0.12±0.06 <sup>ab</sup>					
21-30	0.02±0.01 <sup>a</sup>	0.68±0.16 <sup>a</sup>	7.10±0.05 <sup>ab</sup>	0.00±0.00 <sup>a</sup>	0.11±0.04 <sup>ab</sup>					
Site E										
1-10	0.06±0.00 <sup>ab</sup>	2.06±0.62 <sup>b</sup>	7.18±0.90 <sup>ab</sup>	0.01±0.01 <sup>a</sup>	0.25±0.13 <sup>b</sup>					
11-20	0.07±0.01 <sup>ab</sup>	1.97±0.52 <sup>b</sup>	7.17±0.80 <sup>ab</sup>	0.01±0.01 <sup>a</sup>	0.23±0.12 <sup>b</sup>					
21-30	0.04±0.00 <sup>ab</sup>	2.01±0.54 <sup>b</sup>	7.15±0.78 <sup>ab</sup>	0.01±0.01ª	0.22±0.08 <sup>b</sup>					
Control	0.00±0.00	0.72±0.10	4.69±0.59	0.00±0.00	0.00±0.00					

Table 1b. Concentration of selected heavy metals from different sampling sites.

Mean with the same letter within a column are not significantly different at p<0.05; \*All values are means of triplicate determinations.

Table 2. pH Values of soil samples at varied depth and sites.

Site	Depth (cm)	рН
	0 - 10	7.85±0.26
Site A	11 - 20	7.38±0.36
	21 - 30	7.20±0.13
	0 - 10	7.60±0.21
Site B	11 - 20	7.24±0.13
	21 - 30	7.24±0.13
	0 - 10	8.41±0.12
Site C	11 - 20	7.42±0.61
	21 - 30	7.42±0.93
	0 - 10	7.30±0.57
Site D	11 - 20	7.24±0.13
	21 - 30	6.99±0.56
	0 - 10	7.81±0.61
Site E	11 - 20	7.12±0.10
	21 - 30	6.90±0.46

metals with regards to the soil depths.

The concentrations of heavy metals at varying depth in sites B, C, D and E followed similar pattern to site A. The pH value in general ranges from slightly acidic to slightly alkaline. This slight difference of the soil characterization may not have effect on the distribution of the heavy metals at varying depths. This agrees with the submission of Jean-Philippe (2012) which observed that the effect of pH on the bioavailability in close range is insignificant.

When compared to set standards, all the results of the present study are within maximum permissible levels by WHO/FAO. By implication, they cause no harm, but since the burning activity is continuous, soil accumulation of heavy metals may occur, and hence available to be transported by run-off water and taken up by plants.

### Conclusion

Open incineration of used tyres is not a sustainable environmental waste management practice. The heavy metals where within the acceptable set limits of WHO/FAO. However, their presence in the contaminated soil calls for concern as accumulation over time may increase the level of these metals above set limits.

### Recommendation

1. Uncontrolled burning of tyres should be discouraged.

 Better waste management practice should be adopted.
 Agricultural activities within the vicinity of contaminated area should be discouraged as the heavy metals may change the properties of the soil that might affect it uptake of minerals by plants and reduce crop productivity and its consumption might promote ill health in humans.

### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

# An assessment of health care waste generation rates in public, faith-based and private health facilities in **Douala, Littoral Region of Cameron**

## Noela E Kazeem\* and Veronica E Manga

Department of Environmental Science, Faculty of Science, University of Buea, P. O. Box-63 Buea, Cameroon.

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Healthcare wastes are potentially dangerous to both humans and the environment due to their unique characteristics. The quantity generated continues to increase in varying proportions across different healthcare facilities, partly based on ownership and management styles, which represent significant constraints on healthcare delivery. This study assessed healthcare waste generation rates and management systems in eleven healthcare facilities (representing three types of hospitals) in Douala, the Littoral Region of Cameroon. Data were collected through a quantitative survey, using questionnaires and were subjected to descriptive and inferential statistics. Comparatively, more waste was generated in Public Hospitals (2257.52 kg) than in Private Hospitals (831.2 kg) and Faith-Based Hospitals (789 kg). The median quantity of waste generated/bed/day by Private Hospitals was greater than that generated by Faith-Based and Public Hospitals, with values of 0.22 > 0.19 > 0.09 kg/bed/day, respectively. Similarly, the median quantities of waste generated/patient/day stood at 0.31 > 0.11 > 0.09 kg/patient/day for private, faith-based, and public hospitals, respectively. The linear regression model used for predicting waste generation rates by outpatients yielded R2 values in order of 0.9732, 0.9298, and 0.7275 for Private, Public, and Faith-Based Hospitals, respectively. This indicates that the number of outpatients accounts for 97, 92, and 72% of the total variance explained in solid waste generation in the hospitals. The quantity of hazardous waste ranged from 43.63 to 81.4%. In conclusion, the total hazardous waste generated is higher than the nonhazardous waste in the healthcare facilities.

Key words: Douala Cameroon, Healthcare facilities, healthcare waste, waste generation, waste composition, general waste, hazardous waste.

### INTRODUCTION

The provision of healthcare, aimed at restoring and improving health, is also responsible for generating vast quantities of waste. These wastes consist of approximately 25% non-hazardous and 75% hazardous components, respectively (WHO, 2011; Ezeudu et al., 2022). Medical waste classified as non-risk or general healthcare waste is comparable to domestic waste and mainly originates from the administrative and housekeeping functions of healthcare establishments, including waste generated during the maintenance of healthcare premises. On the

\*Corresponding author. E-mail: monikaefosi@gmail.com. Tel: +237 67477121.

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other hand, hazardous waste, consisting of infectious, pathological, chemical, sharps, and radioactive materials, has the potential to pose various health risks (WHO, 2011).

Of the two types of hospital waste, more attention is typically given to hazardous waste due to its severe impact. According to a WHO report, approximately 85% of hospital wastes are nonhazardous, while the remaining 10-25% is considered hazardous. A study conducted at the Mizan Tepi University Teaching Hospital (MTUTH) in the Bench Maji Zone, South West Ethiopia, revealed different types of wastes in the waste stream, including pathological waste (0.033 kg/bed/day), infectious waste (0.02 kg/bed/day), general waste constituting 32.3%, pharmaceutical waste (0.011 kg/bed/day, 6.7%), and sharps (0.009 kg/bed, 5.5%). This result contrasts with a World Health Organization (WHO) report, which suggests that the distribution of healthcare wastes from hospitals in developing countries is expected to be 15% pathological and infectious waste, 1% sharp waste, and 3% pharmaceutical waste.

Improper handling of hazardous components poses health risks to both humans and the environment, with the magnitude of these risks increasing as waste output rises. The amount of healthcare waste has seen an upward trajectory due to increased access to healthcare- a global development priority outlined in Sustainable Development Goal 3, which aspires to end epidemics and communicable diseases. Furthermore, improved medical diagnosis, mass immunization campaigns, and the changing pattern of diseases, such as the COVID pandemic, have contributed to increased investment in the health sector and a spike in waste generation.

The capacity of any health facility to provide high-quality healthcare is closely linked to its healthcare waste management standards (Sanida et al., 2010). According to WHO reports, the quantity of healthcare waste (HCW) produced by any medical institution depends on its size and varies from one country to another, correlating with national income and the level of development (Marinkovic et al., 2008). Previous studies have noted variations in the quantities of healthcare waste generated between developing regions and developed nations, as well as from country to country.

Marinkovic et al. (2008) observed that highly developed countries produce higher quantities of medical waste than middle and less developed countries. Diaz et al. (2008) suggested that medical waste generation in developed nations ranges from 1.2 to 200 times more than that generated in developing countries. In terms of quantities generated, WHO (2011) reported that East Asia, Eastern Europe, and the Middle East produce 1.3 kg/bed/day. Healthcare waste generation rates are given as 0.54, 0.34, 2.0, and 1.4 for Taiwan, the Philippines, Portugal, and Greece, respectively (Cheng et al., 2009; Diaz et al., 2008; Tsakona et al., 2007). Rates of hazardous components have been reported as follows: 0.25 kg/bed/day (Bangladesh), 0.4 to 1.9 kg/bed/day (Iran), and 1.4 kg/bed/day (Greece) (Patwary et al., 2009; 2011; Taghipour and Mosaferi, 2009).

The quantity of waste generated also varies depending on the department. For instance, in the Mizan Tepi University Teaching Hospital (MTUTH), Bench Maji Zone, South West Ethiopia, the gynecological ward contributed the largest portion of total waste, accounting for 5.08 kg/day (28.90%), while the office generated the lowest proportion at 0.22 kg/day (1.30%).

The issue of healthcare waste is particularly challenging in developing countries. WHO (2004) reported that, from an assessment of 22 developing countries, 18% to 64% of healthcare facilities do not use proper waste disposal methods. In Sudan, Ahmed et al., (2014) noted that healthcare waste management practices observed in Khartoum state hospitals were not fully safe and had harmful environmental effects, characterized by the absence of continuous segregation, collection, transportation, and final disposal methods for pathological and other medical wastes. Similar reports have been documented in Ethiopia (Tesfahun and Kume, 2007).

Therefore, the poor management of healthcare waste in hospitals remains a significant problem in most countries. Factors such as hospital policies and practices, staff strength, number of patients, and the type of care provided influence the quantity of healthcare waste generated. In developing countries, a range of 1-5 kg/bed/day of waste is generated, with substantial intra and inter-country specialty differences. It is reported that in rural hospitals in Africa, the total generation rate of medical waste is estimated to be between 0.3-1.5 kg/bed/day (5-20% hazardous waste) (Yadav, 2001).

Improving existing waste management practices is imperative to prevent exposure of various community groups. The availability of adequate data on waste generation and management practices in healthcare facilities plays a crucial role in planning appropriate methods. The city of Douala in Cameroon is home to various categories of healthcare facilities, including privately owned, Faith-Based, and government/public facilities. There has been a public outcry regarding the nature of services provided by these health facilities.

A survey on the level of user satisfaction with health services delivered in Douala revealed that, apart from the convenience of location for user access, which was rated moderately satisfactory, satisfaction levels for other components such as the skill and competency of medical staff, speed in completing examinations and reports, equipment for modern diagnosis and treatment, accuracy and completeness in filling out reports, friendliness and courtesy of the staff, responsiveness (waiting time) in medical institutions, and satisfaction with cost were generally rated low. These components also have a multiplier effect on the quantity of waste generated.

However, there is a paucity of data related to the

aforementioned issues at the individual hospital level. Therefore, the objective of this study was to determine the average generation of healthcare waste (HCW) per different hospital categories in Douala, analyze possible statistical differentiations among those categories, and compare calculated generation rates with other available references. This study aims to facilitate benchmarking of the facilities, allowing for comparisons of generation rates to identify possibilities for improving the efficiency of their waste management systems.

### METHODS AND MATERIALS

### Study area and period

The present study was conducted in three types of hospitals (Public, Private, and Faith-Based) in Douala, located in the Littoral Region of Cameroon. Douala is one of the most industrialized cities in the country, situated at latitude 4o 2' 53" and longitude 9° 42' 15" E, on the southeastern shore of the Wouri Coast, approximately 130 miles (210 km) west of Yaoundé. The Littoral Region is bordered by the West Region to the North, the Centre Region to the East, the South Region and the Gulf of Guinea to the South, and the South West Region to the West. The city has a projected population of 5,768,400 in 2021, compared to the 1,906,962 recorded in 2005. The area experiences a humid equatorial climate, with a mean annual temperature of 26°C, mean monthly temperatures consistently above 25°C, and daily temperatures often exceeding 31°C.

The healthcare delivery system in Cameroon is characterized by multiple providers, including the public sector, private sector, religious establishments, and private enterprises. In 1999, there were 1,689 health centers and 339 hospitals, with 67% operated by the Ministry of Public Health (MINSANTE) and the remaining managed by the private sector. Both sectors play complementary roles to improve the quality and accessibility of healthcare. Douala has ten health districts, displaying significant geographic inequalities, with some districts having a higher number of physicians per person than others, leading to poor health outcomes. About 70% of regions have a density of health personnel-to-population per 1,000 that is less than 1.5, indicating a shortage of health personnel. Health facilities in Douala, whether Public, Faith-Based, or private, are characterized by poor working and living conditions (Tandi et al., 2015).

For this study, 11 health facilities were randomly selected for both qualitative and quantitative assessments to evaluate current healthcare waste generation rates and practices. The facilities included five Public hospitals (PU1, PU2, PU3, PU4, and PU5), three Private hospitals (PR1, PR2, and PR3), and three Faith-Based hospitals (FB1, FB2, and FB3). These are not the actual names of the hospitals. All the selected hospitals provide inpatient and outpatient services at different scales, with some categorized as tertiary hospitals. The study was conducted from March to December 2021.

### Study design

A cross- sectional study was conducted in each facility to measure health care waste generation rate and describe current management.

### Source population and sampling method

Source population for this study was all 11 hospitals which are found in Douala three hospital types. The three hospital types were selected purposively based on ownership while the 11 health care facilities were selected randomly to assess health care waste generation rate and its management system. All departments which are found in these facilities were included in the study.

### Data collection tools and procedures

Data were collected through field quantifications/observations, interviews, and questionnaire survey. To quantify the amount of health care waste generated from each unit/facility, measurements were done daily for consecutive 30 days. An observational checklist was used to assess the management system in terms of segregation, collection, transportation, and treatment of health care wastes and how healthcare workers and waste handlers handled healthcare waste in all departments of the hospital. To quantify the amount of health care waste generated unit/facility, the waste was collected, sorted and weighed every day using weighing scale in the mornings (Figure 1) Waste characterization was undertaken by creating waste categories (Table 1) based on an adaptation of the health care wastes categories proposed by the World Health Organization, WHO (Pruss et al., 1999). Appropriate protective equipment (gloves, face masks) were used to manually separate the individual waste bins from each department into separate waste.

Informal interviews and structured questionnaires were used to collect data on waste practices from 335 key hospital staff and stakeholders including general supervisors, sanitation workers, Doctors and nurses who are directly responsible for the handling of various waste streams at individual facilities. The questionnaires were proportionately distributed for the based on the status of the hospitals (PU1=81, PU2=19, PU3=19, PU4=34, PU5=19, PR1= 50, PR2=19, PR3=19, FB1=55, FB2=19, and FB3 19). The questionnaires were designed to obtain information on the characteristics of each facility and the existing procedures and practices in the generation and handling of wastes produced. Observational walks were also undertaken across the entire facility to identify the number of departments, wastes collection, handling and disposal practices at the facility.

#### Data analysis

The data was subjected to descriptive and inferential analysis, Microsoft excel 2010 and SPSS version 20. Descriptive statistics included the use of, percentages, frequencies, mean, variance and standard deviation. Kruskal Wallis test, ANOVA, factor analysis and simple linear regressions were used to test if there was a significance difference between different units/facilities and ownership type with regard to total health care waste generation rate and types of waste and as models for prediction. Finally, the result was presented using tables, tables, box plots, bar charts, pie charts and graphs.

#### Data quality assurance

To assure the quality of data collected, assistant data collectors were trained. The weighing scales were calibrated every morning using a known weight before the actual measurements start. Close and routine onsite observation was made by the investigator during the collection and measurement of wastes.

### Ethical clearance

Ethical clearance was obtained from University of Buea, through the Faculty of Health Sciences. Permission for data collection was



Figure 1. Sample pictures showing waste collection and weighing.

obtained from the authorities of the different health facilities. All data collectors were reminded and provided protective devises for use while collecting and measuring healthcare wastes

### RESULTS

### Characteristics of healthcare workers

Table 2 shows the socio demographic characteristics of the respondents. There were more female health workers 230 (60.3%) compared to the males. Majority of the respondents 157 (44.6%) were between the ages of 30 and 39 years. Forty-five percent of participants in the study are in the income range of 101000 - 200000 frs CFA while only 18.4 % earn more than 401000frs a month. Most of the respondents 169(48%) were nurses while the least 29(8.2%) were laboratory scientists. The number of health care workers in the hospitals are in the order of Nurses 169 (48%) > Waste handlers 72 (20%) > doctors 36(10.3%) > Technicians/assistants 29(8.2) > health/ward lab assistants 11(3.1%)> Administrators 4 (1%). Majority of the respondents 169 (48.2%) have been employed for less than 5 years closely followed by 114(30.2) respondents employed for 5 to 10 years. Two hundred and fifteen (61.0%) of the health workers had attained tertiary education with only 12(3.2%) attaining post graduate level of education.

# Characteristics of the studied healthcare facilities of in Douala

Three are 1671 beds in the three hospitals categories for inpatient services and short stays (Table 3). A greater proportion (77.7%) of these beds is found in government health facilities, Faith Based (13.3%) and then private (9%) health facilities. Within the 30 days' study period, a total of 29777 outpatients were documented in the hospitals: 21662 (72.8%) at Public Health facilities, 5675 (19.1%) at Faith Based facilities and 2440 (8.2%) at privately owned health units (Table 4). The hospitals received a total of 4985 in patients. A majority (4356 (87.4%) of the in patients were registered in government hospitals while only 359 (7.2%) and 270 (5.4%) were respectively recorded in Faith Based and private health facilities, respectively. The studied establishments record a total number of 2390

Table 1. Description of some terms used in the study.

Waste category	Description
General waste	Domestic type of waste, packing material, wastewater from laundries
Infectious waste	Includes cultures and stocks of infectious agents from laboratories, waste from survey and autopsy on patients in isolation wards and dialysis from infected patients
Pathological waste	Consists of tissues, organs, body parts, human fetuses, blood and body fluids
Sharps	Includes items like needles, blades, broken glass etc. i.e. any item that can cause a cut or puncture
Pharmaceutical wastes	Consists of pharmaceutical products, drug and chemicals those have been returned from the wards

health personnel. In a descending order, the Public hospitals has the greatest (1753 (73.5%)) number of workers followed by the Faith Based 440 (18.4%) and then the private 197(8.2%). The total number of doctors in the government hospitals stands at 181 with an average 30.2 in each hospital.

The private hospitals have an average of 6.7 doctors per hospital while the Faith Based hospitals have an average number of 15.3 doctors per hospitals. The average number of nurses in each hospital type stands in the descending order of Public > Faith Based > private with absolute averages of 140.2, 70 and 31.2 nurses, respectively. The Public hospitals, followed by the Faith Based and then private also consistently dominate in the number of administrators, laboratory technicians, and waste handlers. Apart of the government hospitals, only the FB1 owns an incinerator.

### Waste composition and generation rates

### Waste generation rates

Within the 30 days period, the highest quantity of waste was generated from the PU4 hospital (615.7 kg), a government owned hospital while the least was generated from a privately-owned hospital, PR2 with a total quantity of 165 kg (Table 4). Comparatively, more waste is generated in Public Hospitals (2257.52 kg) than in Private Hospitals (831.2 kg) and then Faith Based Hospitals (789 kg). For example, more waste was generated in the PR2 with fewer units. Alternatively, the average quantity of waste generated in Public Facilities > Faith Based >Private, with quantities standing at 15.06>9.2>8.8kg, respectively. The summary statistics for pair wise comparison is presented in (Table 5) in which public hospitals waste generation was statistically different (p= 0.14,  $\alpha$  =0.05) from Private and Faith Based facilities (which shows no significant differences amongst them). Government hospitals have higher number of beds, higher outpatient flow and visitors. The quantities of waste generated per bed/day varied from 0.105 to 0.26, 0.06 -0.164 and 0.11 to 0.22 or Public, Faith Based and Private Facilities respectively. The average rates were as follows: 0.299, 0.184 and 0.129 for Public Private and Faith Based facilities, respectively. For the Public Hospitals, the highest quantity of waste per bed/day (0.105 kg) was generated by the PU5 Hospital. In the private facilities the highest quantity of waste per bed (0.228 kg) was recorded at the PR3 Hospital. On average, in a descending order, more waste is generated per bed per day in public facilities > Private > Faith Based facilities respectively. Patients of government hospitals generate on average 0.087 kg every day while in Faith Based hospitals, averagely 0.74 kg of waste is generated a day.

Figure 1 shows the daily waste generation. On a daily basis, a median quantity of 25.90 kg of waste was generated by private hospitals against 24.5 kg in Faith Based (Figure 2). A very huge quantity of waste was generated by the Public Hospitals in day one (1) and 22 (as represented by the 1 and 22 outliers above the box plot). For the private hospitals, huge quantities were generated on day one and two (represented by the outliers 31 and 32) in Figure 2a. Almost same quantities are generated daily by the different Faith Based hospitals evaluated.

The median quantity of waste generated per bed per day by private hospitals is greater than that generated by Faith Based and public hospitals Figure 2b. These median values stood at 0.22 > 0.19 > 0.09 kg/bed/day respectively for the private, Faith Based and public hospitals. The median quantities of waste generated per patient per day (Figure 2c), stood at 0.31 > 0.11 > 0.09 kg/patient per day for private, Faith Based and public hospitals respectively.

# Selection of the best fit models for the prediction of hospital healthcare waste generation rate by outpatients

From the linear regression model (Figure 3), used for the prediction waste generation rates by outpatients, R<sup>2</sup> values obtained were in a descending strength of 0.9732, 0.9298, and 0.7275 for Private, Public and Faith Based hospitals respectively. This indicates that the number of outpatient accounts for 97, 92 and 72% of total variance explained in solid waste generation in the hospitals. Out patients thus fairly well predict waste generations.

Characteristics	Mid-class	Frequency	Percentage	
Condor	Male	140	39.7	
Gender	Female	213	60.3	
	Total	353	100	
	Single	90	25.5	
	Married	247	70	
Marital status	Widow/widower	16	4.5	
	Total	353	100	
	<20	3	0.8	
	20 - 29	71	20.1	
	30 - 39	157	44.6	
	40 - 49	92	26.1	
Age gloup (years)	50 50	28	7.0	
	50 - 59	20	7.9	
	60 - 69 Tatal	2	0.5	
	Total	353	100	
	<100.00	71	20.1	
		150	20.1	
	201.000 - 200.000	159 EE	45	
Income level (FRS)	201.000 - 300.000	20	15.6	
	301.000 - 400.000	3	0.9	
	2401.000	65	18.4	
	lotal	353	100	
	Doctor	36	10.3	
	Nurse	169	48	
	Health/ward asst	11	3.1	
	Administrator	4	1	
Occupation	Lah Tech/Asst	29	82	
	Waste handlers	72	20.4	
	Others	32	20.4	
	Total	352	9 100	
	Total	555	100	
	<5	169	48.2	
	5 - 10	114	32.3	
	11 - 15	28	7.9	
Years of experience	16 - 20	19	5.7	
	21 - 25	16	4.4	
	26 - 30	7	-1 2 0	
	Z0 - 50 Totol	252	2.0	
	iulai	333	100	
	Primary	55	15.5	
	Secondary	72	20.3	
Level of education	Tertiary	215	61.0	
	Postaraduato	213	2.0	
	Total	1 I 252	100	
	iulai	303	100	

 Table 2. Socio-demographic characteristics of healthcare workers.

Facility ownership	Facility name	Total number of beds	Outpatients / month	Inpatients/ month	Total number of patients	Number of staff	Number of incinerators	Doctors	Nurses	Health/w ass	Admin	Lab/tech ass	Waste handlers	Others
	PU1	630	9010	2562	11572	675	1	70	324	21	7	28	165	60
	PU2	150	3103	1006	4109	230	1(bad)	24	110	7	3	18	47	21
Public	PU3	168	2904	908	3812	208	1(bad)	21	100	6	3	17	42	19
(Government)	PU4	230	4340	1340	5680	430	1	44	206	13	5	35	88	39
	PU5	120	2305	1102	4307	210	1	22	101	7	2	17	43	18
Sub-Total		1298 (77.7)	21662 (72.8%)	4356 (87.4%)	29781 (77.3%)	1753 (73.5%)		181 Av= 0.2	841 Av= 140.2	54 Av= 9	20 Av= 3.3	115 Av=19.2	385 Av= 64.2	157 Av= 26.2
	PR1	50	106	73	179	37	0	4	17	1	1	3	8	3
Private	PR2	50	1331	101	1432	90	0	q	43	3	1	7	19	8
Tivate	PR3	50	1003	96	1099	70	0	5 7	34	2	1	6	14	6
Subtotal		150 (9%)	2440 (8.2%)	270 (5.4%)	2710 (7.0%)	197 (8.2%)		20 Av = 6.7	94 Av= 31.2	6 Av= 2	3 Av= 1	16 Av = 5.3	41 Av= 13.7	17 Av= 5.7
	EB1	73	1442	151	1503	00	1	10	13	3	1	6	18	8
Faith Based	EB2	50	2201	08	2200	100	0	10	40	3	1	Q Q	20	10
T altr Dased	FB3	100	2032	110	2142	250	0	26	119	8	3	21	50	23
Sub-total		223 (13%)	5675 (19.1%)	359 (7.2%)	6034 (15.7%)	440 (18.4%)		46 Av 15.3	210 Av= 70	14 Av= 4.7	5 Av= 1.7	35 11.7	88 Av= 29.3	41 Av= 13.7
Grand-Total		1671	20777	1985	38525	2300		2/17	11/5	74	28	166	51/	215

Table 3. Characteristics of the sampled hospitals in Douala.

# Regression models for inpatient and total quantity of waste (kg) in 30 days in the different hospitals

From the linear regression models,  $R^2$  values obtained from hospitals stood in the descending order of 0.724 > 0.3192> 0.1478 for private, Faith Based and public hospitals, respectively (Figure 4). The inpatients do not considerably predict the quantity of waste generated as do the out patients. In patients in private hospitals still produce more waste than in government hospitals.

# Quantity of waste generated in the different units in health facilities

Major units with notorious waste generations were

as follows the theater > maternity > Medicine C 4 > Radiology > Emergency > Laboratory (Table 6). Among the different types of hospitals, the quantity of waste generated following units waste generations stood in the following order, Medicine C 4 (260 kg)> Theatre (230.9 kg) > Maternity (228.9 kg); Emergency unit (175.3 kg) > Theater (158.7 kg) > Laboratory (119.6 kg) > Maternity (113.9 kg); and the Theatre (153.7 kg) > the

Hospital type	Hospitals	Total Waste (Kg) in 30 days	Total daily weight of waste generated (Kg/day)	Waste generated (Kg/bed/day	Kg/Patient/ day
	PU1	484.72	16.2	0.026	0.042
	PU2	387.7	12.9	0.086	0.094
Public	PU3	389.1	13	0.077	0.102
	PU4	615.7	20.5	0.089	0.108
	PU5	380.3	12.7	0.105	0.088
Total (average)	5	2257.52 (451.50)	75.3(15.06)	0.383 (0.299)	0.434 (0.087)
	FB1 Hospital	344.7	11.5	0.158	0.217
Faith based	FB2	246.8	8.2	0.164	0.106
	FB3 Hospital	197.5	6.6	0.066	0.092
Sub-Total (average)	3	789 (263)	26.3 (8.8)	0.388 (0.129)	0.415 (0.138)
	PR1	324.2	10.8	0.216	1.8
Private	PR2	165	5.5	0.11	0.114
	PR3	342	11.4	0.228	0.308
Sub-total (average)	3	831.2 (277.1)	27.7 (9.2)	0.554 (0.184)	2.222 (0.741)

**Table 4.** Waste generations' rates in the different hospital.

**Table 5.** Krustal-wallis pair wise comparison for total waste generation in surveyed health facilities in Douala.

Groups comparison	Test statistics	Std. error	P. value
Public facilities	9.4	2.052	0.017 <sup>a</sup>
Faith Based facilities	3.6	2.44	0.083 <sup>b</sup>
Private facilities	4.3	2.61	0.094 <sup>b</sup>

Laboratory (115.7 kg) > the Radiology (112.4 kg for Public, Private, and Faith Based, respectively. The least quantity of waste (82.2 kg) was generating from the Hemodialysis center. None of the private hospitals owns a Covid-19 Unit or a Mortuary and thus no waste generated. Among the Public hospitals, less is generated at the PU1 Hospital when compared to the PU4. Within the private health establishments, waste production per unit is highest at PR3. Among the Faith Based hospitals, unit generation is highest at FB2 Hospital.

### Types of waste generated in the Hospitals

A quantitative assessment (Table 7) indicated that a majority of the waste in the different categories was general waste. General waste ranges from 0.17 to 0.32, 0.16 to 0.25, and 0.17 to 0.28 kg/day for the Public, Private

and Faith Based facilities, respectively. The quantity of hazardous waste ranges from 40.7 to 81.4%. The percentages show that the average value of the hazardous component of the total healthcare waste was > 50% in the different hospital types. The hazardous waste included Infectious (materials contaminated with blood, cultures and stocks of infectious agents, waste from patients in isolation wards, swabs, bandages, urine faeces and body secretion) Pharmaceutical and Pathological waste such as body parts, chemicals used in pathological activities, needles, syringes expired, used and contaminated drugs and vaccines. Materials used in the handling of pharmaceutical products such as vials, connecting tubing were also generated in these unites, and Sharps. The infectious waste range as follows 0.7 to 0.18, 0.11 to 0.13, and 0.06 to 0.15 kg/day for the Public, Private and Faith Based facilities, respectively. The quantities of sharps ranged from 0.03 to 0.7 for the public, 0.02 to 0.06 for the



**Figure 2.** A: Total daily waste generated by the different hospital types; B: Total daily waste generated per bed by the different hospital types; C: Total daily waste generated per patient by the different hospital types.

Private and 0.02 to 0.03 for the Faith Based facilities. More sharps were generated in the public than the other facilities. Pharmaceutical waste ranges from 0.01 to 0.14, 0.03 to 0.06 and 0.05 to 0.07 kg/day respectively for the Public, Private and Faith Based facilities. From one-way analysis of variance, there are a significant difference (0.001<0.05) in the categories of waste generated among different hospitals while there was no (0.323 > 0.05) there was no significant difference in categories of waste generated at different units in hospitals (Table 8).

### Perceptions on types of waste generated

The study revealed that in all the hospitals, there were multiple responses regarding perceptions of the types of wastes generated. Many participants indicated that all forms of waste were generated in the hospitals in different

perceived proportions of the categories. At least 58.3% perceived the waste generated to be hazardous (infectious, highly infectious, pathological, sharps, pharmaceutical, etc.), while a maximum of 68.7% perceived it to be nonhazardous/nontoxic (Table 9). Observations in some cases revealed general waste mixed with hazardous waste, making them hazardous. Figure 5 shows that in the different types of hospitals, it was perceived that all categories of waste were generated. A chi-square test revealed no evidence of a significant association (P= 0.185, r = 0.05) between the type of hospital and the nature of the waste generated (Table 10). From Principal Component Analysis, two principal factors were extracted explaining the reasons for the nature of waste generated in the hospitals. The two factors explained 43.9% of the total variance. Principal Component One contributed 24.8% of the total variance, while Principal Component Two contributed 19.1% (Table 11).



Figure 3. Regression models for outpatient and total quantity of waste (kg) in 30days in the different hospitals.



Figure 4. Regression models for inpatient and total quantity of waste (kg) in 30days in the different hospitals.

Factor one was moderately loaded by characteristic such as Follow up on clinical waste to landfill (0.780), Recording of clinical waste data (0.709), Problems in clinical waste management (0.686). *Factor two* comprised of Frequency on clinical waste management training (0.724), units (0.529), which were positively loaded while waste accessible to all persons and scavengers (was negatively loaded). Before Principal component analysis was performed, the Kaiser-Meyer-Olkin Measure of Sampling Adequacy and the Bartlett's Test of Sphericity were carried out (Table 12). A value of 0.530 was obtained for the Kaiser-Meyer-Olkin Measure of Sampling Adequacy which was low but permits PCA analysis and the Bartlett's Test of Sphericity gave a significant result.

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Hospital type	Sampled hospitals	Anapath	COVID-19 unit	Emergency	Hemodialysis	Laboratory	Maternity	Medicine C4	Mortuary	Pharmacy	Purification chamber	Radiology	Theatre
	PU1	14.1	13.14	12.2	9.7	9.7	13.1	22.8	10.2	9.2	0	12.7	26.7
	PU2			37		32	61	38.2	21	24		42.7	63.8
Public	PU3			31.4		34.4	59.4	59.4	29.4	32.1		57.2	41.8
	PU4	28.5	80.64	48.9	27.9	20	46.8	121.8	42.2	42.4	19.8	42.1	38.4
	PU5	12.1		13.4	8.1	4	26.9	17.8	4.2	5.9		8.4	58.2
Sub-total		54.7	93.78	142.9	45.7	100.1	207.2	260	107	113.6	19.8	163.1	228.9
	PR1	8.8		82.3	23.1	43.2	32.4	30.6	0	21.1	23.1	2.6	57
Private	PR2			18		25.4	15.5	5.4		28.8		10.7	44.7
	PR3			75		51	66			39		54	57
Sub-total		8.8		175.3	23.1	119.6	113.9	36		88.9	23.1	67.3	158.7
	FB1	26.6		13	13.4	38.1	15.6	28.1	25.4	16	19.4	28	35.6
Eaith based	FB2			23.8		55	48	17.4		19		46	55
Failin based	FB3			17.6		22.6	30.1	20		25.7		38.4	63.1
Sub-total		26.6		54.4	36.5	115.7	93.7	65.5	25.4	60.7	19.4	112.4	153.7
Grand total		90.1	93.78	372.7	82.2	335.4	414.8	361.5	132.4	263.2	62.3	342.8	541.3

Table 6. Total quantity of waste (/kg) generated in the different units in health facilities in Douala/30 days.

### DISCUSSION

# Waste generation rates and composition by health facilities

More waste is generated in public than private and Faith Based health facilities. The quantity of waste generated in hospitals was related to the number of units in those hospitals. For example, more waste was generated in the PU1 hospital and PU4 with more units (15 units each) than in PR1 and PR2 with few units. This result agrees with the findings of Marinkovic et al. (2008) who reported that the amount of HCW production depends on the size and the type of medical institution and differs from country to country based on their national income or their level of development. The higher quantity of waste generated in the PU1 and PU4 could be related to the fact that in these hospitals have more units and would have therefore invested more money in the health system leading to larger amounts of medical waste generation. In this study,

within a period of 30 days PU4 hospital generated 615.7 kg of HCW PR2 generated 165 kg. In a study to assess current practices of waste management in teaching hospitals and the presence of incinerators in densely populated area in Pakistan, Khalid et al. (2021) overall higher significant (P< 0.017) mean ranks for public hospitals than private.

The average total hospital healthcare waste generation rate estimated in kg/bed/day was 0.164 kg kg/ bed/day in this study is smaller compared with the generation rate in Iran (2004) 2.71

Hospital type	Hospital	General	Infectious	Sharps	Pharmaceutical	Pathological	Average hazardous (%)
	PU1	0.32±0.01	0.08±0.02	0.7±0.01	0.04±0.01		71.9
	PU2	0.25±0.08	0.18±0.06	0.02±0.01	0.14±0.06	_	57.6
Public	PU4	0.27±0.02	0.16±0.03	0.03±0.01	0.05±0.01	0.04±0.01	5 <u>0</u> .9
	PU3	0.18±0.13	0.16±0.06	0.05±0.01	0.06±0.01	0.002±0.001	60.2
	PU5	0.17±0.03	0.07±0.03	0.03±0.01	0.01±0.006	0.007±0.001	40. 7
	PR1	0.16±0.08	0.11±0.01	0.02±0.01	0.06±0.01	0.009±0.001	55.70
Private	PR2	0.25±0.02	0.16±0.03	0.06±0.01	0.05±0.01	0.04±0.01	52.42
	PR3	0.20±0.02	0.13±0.08	0.06±0.06	0.03±0.005	0.02±0.004	51.41
	FB1	0.17±0.05	0.06±0.02	0.03±0.004	0.05±0.01	0.04±0.01	43.63
Faith Based	FB2 Hospital	0.22±0.04	0.13±0.09	0.03±0.02	0.05±0.03	0.06±0.03	81.4
	FB3 Hospital	0.28±0.11	0.15±0.10	0.02±0.01	0.07±0.03	0.03±0.02	53.69

Table 7. Quantitative categories of waste generated in the hospitals.

Table 8. A comparison of the different categories of wastes generated within and across the different hospital.

ANOVA						
		Sum of squares	df	Mean square	F	Sig.
	Between groups	316.649	12	26.387	2.995	0.001
HOSPITAL	Within groups	1965.062	223	8.812		
	Total	2281.712	235			
	Between groups	245.273	12	20.439	1.147	0.323
UNIT	Within groups	3973.117	223	17.817		
	Total	4218.390	235			

Table 9. Nature of waste generated in the hospitals.

Nature of waste	Frequent	Percent
General (Non-hazardous)	206	58.3
Infectious	243	68.7
Highly infectious	162	45.8
Pathological waste	143	54.3
Sharps	62	22.1
Pharmaceutical wastes	113	42.7
Total	353	100

kg/bed/day, UK (3.3 kg/bed/day), Norway (3.9 kg/bed/day) and Kuwait (7.0–10.0 kg/bed/day) as it can be seen in Bdour et al. (2007) (37, 54). The reason for this is the higher the per capita gross domestic product (GDP), the higher quantity of hospital healthcare waste which is related to the high supply and provision of healthcare services. The study conducted in Ethiopia (2011) show a higher waste generation rate range (0.75–10.47 kg/bed/ day), but the results of this study are comparable with those reported in Turkey (2010) 2.35 kg/bed/day (31, 58). With the exception of this small discrepancy, the findings are in agreement with the fact that in developing countries the overall healthcare waste generation rate is smaller than in developed nations. As the healthcare delivery system of the country is similar across the regional states, the findings of this research may serve for all hospitals



Figure 5. Nature of waste generated per hospital type.

 Table 10. Chi-squate test of association between nature of waste generated and type of hospital.

Chi-Square Tests						
	Value	df	Asymp. Sig. (2-sided)			
Pearson Chi-Square	11.313 <sup>a</sup>	8	0.185			
Likelihood Ratio	11.462	8	0.177			
Linear-by-Linear Association	0.479	1	0.489			
N of Valid Cases	310					

Table 11. Component matrix for two principal components extracted explaining quantities of waste produced.

Component metrix	Compo	onent
	1	2
Follow up on clinical waste to landfill	0.780	0.327
Recording of clinical waste data	0.709	0.033
Problems in clinical waste management	0.686	-0.303
Hospital	0.454	0.007
Quantity of clinical waste generated kg per day	0.420	-0.182
Frequency on clinical waste management training	0.110	0.724
Waste accessible to all persons and scavengers	0.097	-0.709
Unit	-0.015	0.521
Total variance explained		
Total	1.985	1.531
% of variance	24.808	19.134
Cumulative %	24.808	43.942

Kaiser-Meyer-Olkin measure	0.530	
Bartlett's Test of Sphericity	Approx. chi-square	233.425
	Df	28
	Sig.	0.000

 Table 12. Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's Test of

 Sphericity prior Principal component analysis.

in similar settings. The generation rates of total number of patients (in and outpatient) estimated in kg/patient/day was not significantly different when compared between private and government hospitals. On the contrary, the generation rates of inpatients estimated in kg/bed/day were significantly higher in private hospitals than government hospitals. This is owing to the fact that patients who have access to private hospitals have high incomes and can make a significant contribution to the generation rate of healthcare waste.

US hospitals generate an estimated 6,670 tons of healthcare waste per day (Rutala and Mayhall, 1992), 3,8 kg/bed/day in Portugal (Alvim-Ferraz et al., 2000) and 1 kg/bed/day is generated in Thailand (Kerdsuwan, 2000). This indicates that the quantity of waste generated per bed per day in hospitals in Douala is far less that that generated in the Developed countries. This could be related to the unavailability of medical equipment's and/or the inability of patients to purchase requested materials. Marinkovic ' et al. (2008) had earlier reported that highly developed countries have a larger production of medical waste than middle developed and developing countries. More waste is generated by inpatients in private hospitals, than other hospitals which could be related to the fact than a majority of those who make use of private hospitals are the economically viable individuals who can afford to pay for all services demanded. A study conducted in Jordan (2007) confirmed that there was high statistically significant (linear) correlation between the number of inpatients and the amount of daily healthcare waste generated. The hospital waste generation prediction models can help to optimize healthcare waste management systems, set guidelines and evaluate the prevailing strategies for healthcare waste handling as well as disposal.

### Nature of waste generated in the Hospitals

The highest quantity of infectious waste generated varied with hospital type. This variation is highly influenced by the management of noninfectious waste as it mixing with hazardous waste makes it to become hazardous. According to OTA (1992) it is also challenging to determining which portion or components of healthcare waste is infectious due to its inherent heterogeneous nature and definitional problems. Furthermore, no tests currently exist to objectively determine whether waste is infectious or not (Rutala and Mayhall, 1992). This might have affected the quantities of infectious wastes reported by the hospitals. Chi-square test revealed no evidence of a significant association (P = 0.185, r = 0.05) between the type of hospital and the nature of the waste generated. This means that all hospitals generated similar types of wastes. Within the hospitals, the highest quantities of sakear et al. (2006) similarly pointed out that in Bangladesh, Laboratories and diagnostic centers produce the highest quantities of HCW.

From Principal Component Analysis, the two principal factors that were extracted explained only 43.9 % of the total variance. This indicates that HCW in Douala is much more complex than to be totally explained by the variables considered in this study. Those who followed up the clinical waste to landfill, Frequency on clinical waste management training, just as recording of clinical waste data daily generated more wastes. This ensures proper management. According to WHO (2011)poor management of health care waste potentially exposes health care workers, waste handlers, patients and the community at large to infection, toxic effects and injuries, and risks polluting the environment. It is essential that all medical waste materials are segregated at the point of generation, appropriately treated and disposed of safely.

### Categories of waste generated in the hospitals

The proper management of waste generated in medical facilities depends to a large extent on strong knowledge on the type of waste generated, the administration and organization of the health facilities concerned. From the results a quantity of hazardous waste ranges from 43.63 to 81.4%. According to a WHO report, around 85% of the hospital wastes are actually non-hazardous or general wastes, and the remaining 10-25% is hazardous in nature (Mukesh, 2001). However, the result from these hospitals identified that of total stream of health care wastes was lower than hazardous. This result was comparable with a result obtained in Nigeria where 41% of the total health care waste generated was hazardous (Ogbonna, 2013). But it was much bigger than a result identified in Sudan where only 20% of the total health care waste stream

generate are hazardous (Ahmed et al., 2014). This could be attributed to inappropriate segregation practice of health care wastes generated in the hospital.

### Conclusion

More waste is generated in Public Hospitals (2257.52 kg) than in Private Hospitals (831.2 kg), with Faith-Based Hospitals generating even less (789 kg). Specifically, Public Hospital PU1 dominated in generating the highest quantity of infectious waste, while private hospitals produced the least infectious waste. Figure 1b illustrates that the median quantity of waste generated per bed per day in private hospitals. These median values are 0.22 > 0.19 > 0.09 kg/bed/day, respectively, for private, Faith-Based, and public hospitals. Similarly, the median quantities of waste generated per patient per day are 0.31 > 0.11 > 0.09 kg/patient per day for private, Faith-Based, and public hospitals.

Interestingly, inpatients do not significantly influence the quantity of waste generated compared to outpatients. Linear regression models show R2 values in the order of 0.724 > 0.3192 > 0.1478 for private, Faith-Based, and public hospitals, respectively.

The major units with notorious waste generation, in descending order, are the theater, maternity, Medicine C 4, Radiology, Emergency, and Laboratory. The total hazardous waste generated in this study surpasses the nonhazardous waste, aligning with expectations for healthcare facilities in developing countries. However, the magnitude of hazardous waste generated exceeds the estimate set by the World Health Organization (WHO).

### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

# Revitalizing maize production through managing biological N fixation, soil acidification and nitrous oxide emission from legumes in tropics

# Daniel Markos<sup>1\*</sup> and Tarekegn Yoseph<sup>2</sup>

<sup>1</sup>Hawassa Agricultural Research Center, P. O. Box- 06, Hawassa, Ethiopia. <sup>2</sup>Hawassa University, College of Agriculture, P. O. Box - 05, Hawassa, Ethiopia.

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Legume associations address challenges related to soil fertility and land degradation commonly encountered in maize monocultures. However, it's important to note that legumes contribute to the production of both nitrous oxide (N<sub>2</sub>O) and hydrogen ions (H+). In our examination of 693 manuscripts published between 1941 and 2023 to document the benefits of legumes in maize-based cropping systems, we identified and included 195 credible journal publications in our analysis. The findings revealed that, apart from fixing 50 to 320 kg nitrogen (N) ha<sup>-1</sup>, legumes offer various non-N advantages. When compared to maize grown after maize, a 2.4 to 173% greater yield of maize was recorded, corresponding to a 9 to 96.7% increase in biomass when maize is grown after legumes. The most substantial increases were observed in farms employing reduced tillage, residue retention, and suitable legumes. Intercropping maize with legumes, as opposed to solitary maize production, resulted in 4.3 to 80% higher biomass, 5 to 14.8% higher grain yield, and 5 to 29.5% higher profit. However, it's important to acknowledge that acid and N2O production ranged from 0.2 to 2.7 mol H+kg<sup>-1</sup> biomass produced and 5.6 kg N<sub>2</sub>O ha<sup>-1</sup>, respectively. Implementing compatible cropping systems, increasing soil N mineralization, and recycling crop residues can enhance biological N2 fixation, reduce acid buildup in soils, mitigate N<sub>2</sub>O emissions, and simultaneously improve maize yield. In conclusion, the review underscores the necessity for location-specific cropping system standards and regulations to ensure the sustainability of maizelegume cropping systems.

Key words: Cropping system, legumes, nitrous oxide, residue management, soil acidification.

### INTRODUCTION

According to van Dijk et al. (2021), there is projected to be a 35 to 56% increase in global food demand from the agricultural sector between 2010 and 2050 to accommodate an additional 3.5 billion people (Borlaug, 2007). Muhie (2022) suggests that by 2050, the production of maize, wheat, and rice alone should rise by 70% to meet

\*Corresponding author. E-mail: <u>danielmarkos2012@gmail.com</u>.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> the demands of the world's rapidly expanding population. This necessitates an increase in maize yields while simultaneously reducing greenhouse gas emissions globally. Low soil fertility has consistently posed a significant challenge to maize production, historically limited by the lack of varieties adaptable to environmental shocks such as heat, drought, salinity, and acidity.

Additionally, nutrient exhaustion and declining soil fertility have been linked to the practice of growing maize after maize (Thierfelder et al., 2013). Therefore, improving soil fertility and establishing sustainable cropping systems are crucial for increasing maize yields for smallholder farmers in the tropics (Stoorvogel et al., 1993).

In such circumstances, increasing the adoption of legume technology is a well-considered initial step toward addressing unsustainable cropping systems. Intensifying cropping systems in developing nations could yield significant increases in productivity. Nitrogen, a vital element for soil and plant systems, is cycled from the environment. Approximately 78% of the nitrogen available for cycling is present in the atmosphere in a form that most organisms cannot utilize. In terrestrial and aquatic ecosystems, nitrogen continues to be the limiting factor for growth (Dalton and Krammer, 2006). This limitation is primarily due to the fact that, aside from N-fixing bacteria, most living organisms find the gaseous form of N2 unavailable for use. The atmospheric nitrogen must be converted from its N<sub>2</sub> form to ammonia (NH<sub>3</sub>) and nitrate by energy-releasing biotic processes, such as the Nitrogenase enzyme complex, which has iron or nickel catalysts, to become biologically accessible (Unkovich et al., 2008; Lasaletta, 2014).

It is worth noting that the addition of N<sub>2</sub>-fixation from nonbiological sources, such as meteor streaks, cosmic radiations, forest fires, volcanic eruptions, and thunderstorms, contributes as much as 10 to 20 million metric tons of nitrogen. However, this nitrogen is not directly available to plants (Bezdicek and Kennedy, 1998; Table 1).

Terrestrial plants require between 150 and 200 million tons of mineral nitrogen (N) annually (Unkovich et al., 2008). To meet this demand, the industrial Haber-Bosch process produces over 100 million tons of fertilizer nitrogen, constituting only 20% of the world's total nitrogen requirement (Table 1). An example is the necessity for nitrogen (N) in cereal cropping systems, which is addressed by applying N fertilizers produced through industrial processes involving high temperatures (400 to 450°C) and high pressures (200 atm) in the presence of iron or nickel catalysts. These processes also annually consume 3 to 5% of the world's natural gas (Bezdicek and Kennedy, 1998; Myrold and Bottomley, 2007). However, the industrial production of inorganic nitrogen releases 2.25 to 10 kg of CO2 for every kilogram produced (Ecoinvent). Despite the energy costs, rising manufacturing expenses, and environmental concerns associated with industrial nitrogen production, biological  $N_2$ -fixation has been advocated as a solution since the 1970s (Phillips, 1980).

Further research has shown that symbiotic and nonsymbiotic relationships mediated bv Rhizobium. BradyRhizobium, and free-living bacteria in soils produce between 100 and 175 million metric tons of nitrogen annually, saving approximately US\$10 billion on fertilizer nitrogen annually (Freiberg et al., 1997; Chafi and Bensoltane, 2003). The effects of legumes for nitrogen fixation go beyond improving soil fertility. Legumes reduce reliance on nitrogen derived from industrial methods that use significant amounts of fossil fuels by fixing nitrogen from N2 (Herridge and Rose, 2000). Thus far, biofertilizers have improved the yield of legumes and reduced the need for synthetic nitrogen fertilizer required in most parts of the world, benefiting many cropping systems through this biological process.

Legumes directly contribute to daily nutritional uses, providing food or feed values, health benefits, or protein supplements. They serve as sources of proteins, amino acids, chlorophyll, or urea, all essential for life on earth (Buren and Rubio, 2017). Additionally, most legumes offer several non-nitrogen benefits, such as being sources of carbon, phosphorus, and other nutrients.

In the simplest terms, biological nitrogen fixation (BNF) by legumes has been described as the second most important biological process on Earth, following photosynthesis (Cheng, 2008). However, it's important to note that energy is required for both photosynthesis and the nitrogen fixation process. The triple-bonded nitrogen atoms constituting gaseous N2 must be separated to reduce 1 mole of N2 to ammonia, a process requiring 147 kCal, 16 moles of ATP, or 20 kg CO2 kg-1 N fixed (Herridge and Brock, 2016). The symbiotic nitrogen fixation process in legumes is considered "greenhousegas neutral" since they acquire all the required carbon directly from the atmosphere through photosynthesis (Ecoinvent Centre, 2010). Therefore, developing optimal legume management scenarios under various genetic and environmental conditions is essential to minimize N2O emissions and soil acidification.

While fixation by legume-cereal cropping systems may reduce nitrogen (N) and carbon (C) losses, effective management practices play a crucial role in achieving this outcome (Gregorich et al., 2005; Li et al., 2015). Several scientists (Starling et al., 1998; Ohyama et al., 2009) have observed inconclusive or negative BNF results in various field investigations when starter-N applications were applied for legumes. Consequently, the use of biofertilizers is becoming increasingly necessary for legumes or their symbiotic relationships with cereals.

The adoption of the sustainable crop production technique involving biological nitrogen fixation by legumes within well-designed cropping systems should be standardized across various agro-ecologies (Lasaletta,

Type of fixation	N <sub>2</sub> fixed (10 <sup>12</sup> g per year, or 10 <sup>6</sup> metric tons per year)
Industrial N fixation	About 50 to 100
Non-biological N fixation	
Combustion	About 20
Lightning	About 10 to 12
Sub-total	- 30-32
<b>Biological N fixation</b>	
Agricultural land	About 90
Forest and non-agricultural land	About 50
Sea	About 35
Sub-total	About 175

**Table 1.** Estimated proportion of industrial, biological and non-biological N fixation (Bezdicek and Kennedy, 1998; Lasaletta, 2014).

2014). This is because the cultivation of legumes stores nitrogen, enhances biodiversity, and sequesters carbon, all of which can benefit partner crops planted alongside legumes in intercropping or rotation systems (Ecoinvent Centre, 2010). The inclusion of legumes in cropping systems contributes to a reduction in the carbon footprints of agricultural products, promoting sustainability (Peoples et al., 2009a; Gan et al., 2011). Historical data from the 1950s indicated that leguminous foods, fodder, and green manures provided approximately half of the necessary nitrogen in several European nations (Gan et al., 2011). Therefore, leveraging leguminous biological nitrogen fixation presents both an opportunity and a necessity to reduce reliance on synthetic nitrogen fertilizer, mitigate environmental impact, and address the negative consequences of climate change on agricultural productivity.

However, there has been a lack of examination and documentation for grassroots utilization in tropical regions regarding how much nitrogen can be fixed by specific legumes in cropping systems under different conditions and how much of this fixed nitrogen can be utilized by subsequent or companion maize crops. In order to establish productive maize-based legume cropping systems, the purpose of this paper is to evaluate and explore quantifications in nitrogen fixation, acidification, and nitrous oxide (N<sub>2</sub>O) production resulting from diverse legume species. The aim is also to identify key characteristics for further investigation.

### MATERIALS AND METHODS

The selection of documents for review was conducted through a literature search using keywords such as "legumes," "N fixation," "soil acidification," "formation of nitrous oxide," and "cropping systems," with a specific focus on quantifying the benefits. Additionally, references from papers published in internationally renowned journals, the international legume database, progress reports,

journals, and websites spanning the period from 1941 to 2023 were consulted. Following the removal of articles of a more general nature and eliminating obvious duplicates that were less relevant to tropical agriculture, the number of remaining publications (693) was reduced (Figure 1). The identified papers, such as those by Ellert and Janzen (2008), Peoples et al. (2009b), Njira (2016), Kermaha et al. (2018), were prioritized, and approximately 86 special journal issues addressing the topic were considered. The review revealed complexities in nitrogen fixation and its quantification, soil acidification, and the formation of nitrous oxide from legumes. It also brought to light some emerging realities and trends in the field.

### FINDINGS AND DISCUSSION

### Production of nitrogen by legumes

There is intense competition among crops growing in the same environment for limited nutrients, moisture, sunlight, and space. This competition highlights the effects of cropping systems on related legume crops and their biological nitrogen fixation (BNF) production. While legumes are capable of fixing atmospheric nitrogen for their own benefit, they can also transfer nitrogen to companion crops in the same growing season or to subsequent crops in the following season. This capability allows for planning and utilizing legume-derived nitrogen for non-legume crops, presenting a sustainable agricultural production method with significant potential for reducing the use of chemical pesticides and fertilizers. Incorporating legumes into cropping systems focused on maize, whether as fallows, green manures, rotations, intercrops, or alley crops, has been demonstrated as a viable approach (Peterson and Russelle, 1991; Smil, 1999; Giller, 2001).

### Legume sole crops

The atmosphere contains between 79 to 80% nitrogen,



Figure 1. Screening of studies via databases and registers between 1941 to 2023.

meaning that most legumes obtain more than 70% of their nitrogen needs from the atmosphere (Ahmed et al., 2005). After seed harvests, legumes such as cowpea, pigeon pea, green gram, and groundnuts generated positive net nitrogen balances of up to 136 kg N ha<sup>-1</sup> (Peoples and Craswell, 1992). Recently, it was discovered that biological nitrogen fixation (BNF) accounted for between 50 and 60% of the nitrogen requirement in legumes (Salvagiotti et al., 2008). The amount of nitrogen fixed varied widely depending on crop management conditions, with the percentage of transfer to related crops ranging from 0 to 70%, as noted by several writers (Anglade et al., 2015). The net nitrogen balance would decrease even more from 28 to 104 kg N ha<sup>-1</sup> if residues were removed from the field (People and Craswell, 1992). These differences are explained by the legume's variety, maturity period, and biomass production (Rao and Dart, 1987; Seymour et al., 2015; Table 2). Rhizobium inoculation could enhance this process by increasing N2 fixation, plant yield, and seed quality (Bambara and Ndakidemi, 2010).

Productivity and quality benefits following Rhizobium inoculation were attributed to increased soil pH, nitrogen, calcium, and salt levels (Bambara and Ndakidemi, 2010). For staple cereal crops, BNF serves as a necessary and affordable substitute for industrially produced nitrogen fertilizers (Brockwell and Bottomley, 1995; Bezdicek and Kennedy, 1998; Carlsson and Huss-Danell, 2003; Galloway et al., 2008; IAASTD, 2008; Weil and Brady, 2017). According to Buchi and his associates, biological fixation allowed Lathyrus sativus, Pisum sativum, Vicia sativa, Vicia villosa, or Vicia faba to fix more than 100 kg ha<sup>-1</sup> of nitrogen (Buchi et al., 2015). They found that the percentage of nitrogen obtained from atmospheric N<sub>2</sub> varied from 0 to 100% both between and among species

in a brief growing season, providing valuable data for application in later cropping systems. The amount of nitrogen fixed in single crops was also higher than in legume-cereal combinations, according to the results, due to higher biomass accumulation in sole crops compared to mixed cropping systems (Anglade et al., 2015). *Trifolium pratense L.*, white clover (*Trifolium repens L.*), and alfalfa (*Medicago sativa L.*) produced total BNF values of 465, 252, and 102 kg N ha<sup>-1</sup>, respectively. Faba bean (*Vicia faba L.*), field pea (*Pisum sativum L.*), and lentil (*Lens culinaris Medik.*) produced total BNF values of 165, 111, and 52 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively (Table 2).

Due to their limited access to land, manpower, and capital, small-holder farmers are attracted to dual-purpose legumes that can be utilized for both food and feed (Giller, 2001). Forage legumes, mucuna, and long-duration pigeon pea are a few examples of dual-purpose legumes with low harvest indices (Giller, 2001). Therefore, to increase the biomass available for fodder, weed suppression, and soil fertility enrichment, it would be preferable to cultivate dual-purpose genotypes with early maturity duration rather than developing new varieties of cowpea and pigeon pea with a high harvest index trait or with extra-early or extra-short maturity duration (Giller, 2001).

### Crop rotation

Many nations have standardized the rotation of grains with legumes through rules and regulations, such as the states (PCO, 2021), Uzbekistan (InforMEA, 2007), and Brazil (Sparovek et al., 2012). This is likely due to the fact that, as highlighted by Zablotowicz et al. (2011), legumes

Сгор	N fixed	Reference
Alfalfa	90 - 220	Bell and Nutman (1971)
Beans	20 - 80	Havlin et al. (2014)
Lupins	60 - 100	do
Black gram	100	Mugwe et al. (2011)
Cowpea	90	do
Fenugreek	45	do
Lentil	40 – 68	do
Chickpea	40 – 50	Seymour et al. (2015)
Faba bean	130	do
Mung bean	112	do
Groundnut	150	do
Clover	100 -150	Aranjuelo et al. (2009)
Cluster bean	60 – 150	Meena et al. (2017)
Soybean	100–150	do
Cowpea	47 – 105	Giller (2001)
Pigeon pea	13 – 167	do
Cowpea	90	Wetselaar et al. (1973)
Field pea	65 -100	Peoples et al. (2009)
Groundnuts	33 – 124	Nyemba and Dakora (2010)
Mung bean	60	Shaha et al. (2003)
Field pea	30 – 140	Nutman (1965)
Pigeon pea	20 – 124	Njira et al. (2012)
Pigeon pea-groundnuts	42 - 82.8	Mhango (2011)
Pigeon peas	133	Sen (1958)

Table 2. Biological nitrogen fixation (kg N ha $^{\text{-1}}$ ) by legume species grown as sole crops.

constitute a significant source of protein for both human and animal nutrition. In addition to providing plants with nitrogen from the atmosphere, rotating cereals with legumes helps prevent the accumulation of weeds, pests, and crop diseases. Legumes contribute to improved soil structure and are therefore preferred for rotational cropping due to their large tap roots, which can extend deep into the soil profile (Ofori and Stern, 1987). The benefit of grain legumes to the yield of the companion crop is greater than the benefit brought through the carryover of nitrogen in the soil (Zablotowicz et al., 2011). Grain legumes can boost yields in subsequent cereal crops by up to 1.6 t ha<sup>-1</sup> (Ofori and Stern, 1987; Preissel et al., 2015).

According to writers such as Berg (1997), wheat (Triticum aestivum) yielded 3,070 kg ha<sup>-1</sup> year-1 after five years of alfalfa, 2,580 kg ha<sup>-1</sup> year<sup>-1</sup> after milk vetch, and 950 kg ha<sup>-1</sup> year<sup>-1</sup> after grass. Reports from other authors indicate that the productivity of maize increased by 46% when soybeans were grown following maize (Yusuf et al., 2009). Another study found that the production of maize increased by 28 and 21%, respectively, in the year

following the planting of soybean and cowpea compared to growing maize again (Kureh et al., 2006). Following two rotational years after soybean and cowpea, maize yield increased by 85 and 62%, respectively, compared to sole maize cropping (Kureh et al., 2006). Conversely, in another maize-soybean rotation experiment, maize crops adversely affected soybean nodulation in the subsequent seasons (Charles, 1990).

In plots treated with uniform residue, rotating maize with velvet bean, cowpea, and soybean produced advantages in biomass production of 41, 18 and 9%, respectively, in sandy soils of Moniya area at Oyo state in Nigeria (Uzoh et al., 2019). Even in plots untreated with residue mulch in the same area, rotating maize with velvet bean, cowpea, and soybean resulted in biomass production advantages of 96.7, 55.5 and 23.9%, respectively. These authors attributed the advantages to significantly increased total soil nitrogen, exchangeable potassium, magnesium, and cation exchange capacity arising from legume-cereal rotations. In plots treated with residue, a yield advantage of 122.4, 3.7 and 2.4% was measured due to rotating maize with velvet bean, cowpea, and soybean; in plots

untreated with residue, there was a yield advantage of 173, 61.8 and 71% in respective order in sandy soils of Moniya area at Oyo state in Nigeria (Uzoh et al., 2019).

The study by Lengwati et al. (2020) showed that the symbiotic nitrogen contribution from groundnut, black gram, cowpea, mung bean, or bambara groundnut was about 20 kg N ha-1 for the succeeding maize crop. Eaglesham et al. (1981) reported that 24.9% of nitrogen fixed by cowpea was transferred to the succeeding maize crop. Up to 35% of nitrogen in maize grown after pigeon pea was obtained from nitrogen fixation, and part of the fixed nitrogen was due to root excretion, nitrogen leached from leaves, and leaf fall (Eaglesham et al., 1981). In some environments, faba bean has shown a greater effect than other legumes on the yield of subsequent cereal grain crops (Hauggaard-Nielsen et al., 2012). In another maizesoybean rotation experiment, Gomez (1968) observed maize yields similar to those of sequential maize fertilized with nitrogen. Caldwell (1982) also obtained a 14% yield increase over nitrogen treatments for maize following soybean. These differences in nitrogen transferred to the succeeding maize crop could be attributed to inherent differences in the potential of legume species and varieties to fix atmospheric nitrogen.

One of the essential prerequisites for biological nitrogen fixation is the prevalence of Rhizobia in great numbers in the surrounding area of legume roots. In this regard, Brockwell et al. (1988) showed that the actual densities of nodule bacteria are in the order of 10^6 to 10^9 organisms per milliliter of soil solution in the rhizosphere. Such large amounts are because Rhizobia originating from nodule disintegration of the previous legume crop are likely to form an important component of the Rhizobial populations and act as a source of molybdenum, phosphorus, cobalt, iron, zinc, sulfur, and nitrogen for subsequent legume crops (Brockwell et al., 1988).

An intercrop of maize and soybean rotated after wheat resulted in nitrogen uptake of 100 kg N ha<sup>A-1</sup>, which is twice that following maize alone (Searle et al., 1981). Such high nitrogen uptake was due to the suppression of Rhizobial multiplication during the non-legume cropping season, resulting in poor survival of introduced Rhizobia. This manifested that residual nitrogen input and the nitrogen fixation capacity of the preceding legume crops have a direct effect on the total nitrogen uptake of the following maize crop. Other authors also showed that nitrogen recovery by the succeeding crops may reach as high as 12% of the residual nitrogen at the maturity of the cropping season (Mayer et al., 2003). Soils may be devoid of Rhizobium to form an effective symbiosis with a legume when the legume succeeds a non-leguminous crop like maize, which requires planning inoculations for subsequent legume crops. Hence, supporting high vields of the succeeding legume becomes impracticable without re-inoculation, largely because the Rhizobia have to live saprophytically.

### Relay cropping

Relay cropping is a common practice in industrial agriculture where intercropping falls short due to difficulties associated with machinery use for weeding, fertilizer application, and harvesting of intercrops (Peoples et al., 2009b). The advantages from legumes relay cropping are due to the addition of organic carbon and mineralization of nitrogen from residual legume biomass that could, in turn, support the growth of subsequent non-legume crops (Zablotowicz et al., 2011).

In Australia, non-legume crops required nearly 60% less nitrogen (N) fertilizer when planted with relay-cropped legumes, which yielded an average of 225 kg N ha<sup>-1</sup> (Zablotowicz et al., 2011). Legume crops frequently contribute to yields of cereal crops that are comparable to those obtained from applying 30 to 80 kg of N fertilizer ha<sup>-1</sup> (Peoples et al., 2009a).

Additionally, the authors estimated that root biomass contains 16 to 77% of total plant N, a percentage that is not typically included in calculations of N fixation (Peoples et al., 2009a). Grain legumes could provide a minor positive N balance even with significant grain N exports if estimates of root biomass are included in the N estimation process (Figure 2). Nevertheless, the majority of this nitrogen is exported from the farm as protein-rich seeds, which causes poor or negative net nitrogen balances in the soil (Peoples et al., 2009b). Due to their greater root: shoot ratios than annual species, perennial legumes typically have higher below-ground nitrogen (N) as a percentage of total plant N (average of 43%) than do annual grain legumes (average of 32%) (Antos and Halpern, 1997). But as plant allocation to roots rises during drought, environmental factors may also have an impact on root biomass and root architecture (Zablotowicz et al., 2011).

### Intercropping systems

Smallholder farmers in the tropics intercrop leguminous and non-leguminous crops to mitigate the risks of crop failures associated with monocultures and ensure stable income and nutrition (Francis, 1986). Examples of intercropping systems include maize-pigeon pea, maizecommon beans, maize-cowpea, sorghum-cowpea, maizegroundnuts, maize-lablab, millet-groundnuts, and ricepulses (Matusso et al., 2012). Researchers in the tropics have reported yield advantages of 7.5 and 5% from intercropping maize with common beans and cowpea, respectively, compared to sole maize cropping, corresponding to 29.5 and 5.9% profit in that order (Hidoto and Markos, 2019). In other studies, maize production improved by 25 and 88% after intercropping mucunamaize and cowpea-maize, respectively (Whitbread and Pengelly, 2004). Franzluebbers et al. (2016) also observed 30% more efficient productivity of millet due to millet-



Figure 2. Average amount of N fixed by legumes under relay cropping (Zablotowicz et al., 2011).

Table 3. ∟	eaf litter and	I nutrient relea	ased from	selected	legumes (	Addo-Qua	ve et al.,	2011).
							, ,	

Crop	Leaf litter (t ha-1)	Nitrogen (kg ha <sup>-1</sup> )	Phosphorus (kg ha <sup>-1</sup> )	Potassium (kg ha <sup>-1</sup> )
Chickpea	1.1 - 1.7	7 - 14	3 - 5.5	8 – 20.0
Lentil	1.2 - 1.6	8 - 10	3.5 - 4.5	12.5 - 19
Pigeon pea	1.3 - 2.8	8 - 16	2.5 - 5	13.5 - 24

cowpea intercropping compared to sole millet planting. Thus, the nitrogen (N) nutrition of cereals is improved due to the transfer of biologically fixed N from associated legumes when cereals and legumes are grown in an intercropping system (Willey et al., 1983; Meena et al., 2015). This brings the possibility of minimizing the presentyear N demand by about 25 to 50%, possibly due to the transfer of N from legumes to non-legumes through excretion by plant roots, release from decaying roots and falling leaves, and leaching of N from leaves of component legumes, which is a better way to reduce environmental damage from nitrate (NO<sub>3</sub>) leaching and nitrous oxide (N<sub>2</sub>O) emission (Table 3). The process involves rhizodeposition of low and high molecular weight N and C compounds, which are later used by non-legume crops like maize (Wichern et al., 2007). Indeed, intercropping contributes fresh organic matter to the rhizospheres, enhancing nutrient mineralization due to changes in organic matter decomposition rates (Mobasser et al., 2014). However, limited information is available on the effect of Rhizobia inoculation on the chemical composition of the rhizosphere of intercropped plants (Mobasser et al., 2014). The yield advantage of intercropping maize and common beans under conservation agriculture was 14.8% higher compared to intercropping under conventional agriculture, corresponding to a 39.4% profit (Hidoto and Markos, 2019). Intercropping maize with pigeon pea resulted in 52.6, 4.3 and 80% higher biomass, grain yield, and land equivalent ratio, respectively, in the southern central rift valley of Ethiopia (Hidoto and Daniel, 2019). This could be attributed to the moisture-conserving and microclimate-modifying attributes of conservation agriculture, along with the biological nitrogen fixed and transferred from pigeon peas. The quantity of nitrogen fixed by legumes in cereal-legume intercrops depends on plant species, plant morphology, density, and growth habit of the component crops (Stern and Ofori, 1987). Intercropping sorghum with groundnut, green gram, and cowpea reduced the mineral nitrogen requirement by 61, 83, and 38 kg ha<sup>-1</sup>, respectively, for the subsequent crop (Nair et al., 1979).

The partial soil nitrogen balance study conducted by Kermaha and his colleagues indicated that intercrops produced 14 to 21 kg N ha<sup>-1</sup>, and sole legumes yielded 8 to 23 kg N ha<sup>-1</sup>, but these values were smaller than those of sole maize receiving nitrogen fertilizer, which ranged from +7 to +34 kg N ha<sup>-1</sup> (Kermaha et al., 2018). Another study by Li and his colleagues reported a production contribution of 15% nitrogen for the intercropped cereal (Li et al., 2009). Cowpea, mung bean, or groundnuts were reported to accumulate nitrogen in the range of 80 to 350 kg N ha-1 year-1 (Table 3).

Some authors suggested that 40% of nitrogen could be fixed by legumes biologically without nitrogen fertilizer in

Crean	N <sub>2</sub> fixe	d (%)	N2 fixed (kg/ha)		
Сгор	Mono crop Intercrop		Mono crop	Intercrop	
Soybean/non-nodulating	42	23	71	17	
Pea/barley	62	84	115	81	
Cowpea/maize	28	34	22	10	
Pea/mustard	48	50	71	62	
Pigeon pea/sorghum	74	55	169	124	
Pea/oat	27	52	22	30	
Lentil/flax	77	85	14	8	
Pea/rape	38	33	41	27	
Pea/mustard	28	34	20	18	
Pea/oat	80	86	50	16	
Pea/rape	78	88	20	27	
Rice/bean/maize	32	75	30	39	
Cowpea/rice	32	30	35	32	
Faba bean/barley	74	92	79	71	
Pea/barley	68	84	213	74	

**Table 4.**  $N_2$  fixation by grain legumes grown in monoculture or intercropped with nonlegumes (van Kessel and Hartley, 2000).

intercropping systems of soybean with cereals and 30% in monocrops (Osunde et al., 2004). Mucuna harvested in 12 weeks supplied about 160 kg N ha<sup>-1</sup> when intercropped with maize (Sanginga et al., 1996). Eaglesham et al. (1981) recorded that cowpea fixed about 41 kg N ha<sup>-1</sup> when intercropped with maize, and approximately 24.9% of the nitrogen fixed by cowpea was transferred to intercropped maize (Eaglesham et al., 1981).

The contribution of nitrogen from groundnut to the growth of maize in intercropping systems is equivalent to the application of 96 kg N ha<sup>-1</sup> at a ratio of plant population densities of one maize plant to four groundnut plants (Mandimba, 1995). Osunde et al. (2004) found that without the addition of fertilizer, the proportion of nitrogen derived from N2-fixation was about 40% in the intercropped soybean and 30% in the sole crop. These contributions result from root excretion, nitrogen leached from leaves, and leaf fall. In another study, the nitrogen input from groundnut to the growth and yield of maize in an intercropping system was equivalent to the fertilization of 96 kg of nitrogen/ha at a proportion of plant population densities of individual maize plants to four groundnuts plants (Mandimba, 1995). However, there is an opportunity cost of space or time when legumes are integrated with other crops in the cropping system, which has been considered a major constraint to the adoption of legumes in cropping systems. Successful adoptions are more likely when legumes serve multiple purposes of producing a net positive nitrogen balance while still producing feed or food (Ghosh et al., 2007). The total amount of nitrogen fixed per unit area in maize-legume cropping systems is often lower than in sole legume cropping due to decreased legume

population densities and increased competition for light, moisture, and nutrients among leguminous and nonleguminous crop components (Table 4). An intercropping system could also produce higher nitrogen fixation at times when limited resources are used effectively among companion crops (Table 4). In another study, intercropping pigeon pea with other legumes suppressed biological nitrogen fixation (BNF) (Njira et al., 2012). However, when pigeon peas were intercropped with maize, the slow growth of pigeon pea offered little competition, allowing the BNF released from the legume component to be efficiently used by intercropped maize, resulting in better system productivity (Giller, 2001; Hidoto and Markos, 2019).

While intercropping generally results in a reduction in the amount of nitrogen fixed relative to a legume monocrop, it represents an agronomically important input of nitrogen compared to a sole cereal crop and enhances the use of available nutrients and water (Hauggaard-Nielsen et al., 2003). Hence, a properly designed maize-legume intercropping system can be considered productive and sustainable, making an invaluable contribution to food and nutrition security. Besides improved soil fertility, the intercropping system offers benefits such as resource facilitation, enhanced crop productivity, increased soil and water conservation, and protection against crop pests and diseases (Dahmardeh et al., 2010; Lemlem, 2013). Furthermore, intercropping reduces soil erosion and nutrient leaching, suppresses weeds and pathogens, and provides food and shelter for beneficial insects (Dahmardeh et al., 2010; Lemlem, 2013). However, intercropping with perennials like Leucaena leucocephala resulted in a respective decrease of 38%, 34%, and 29%

in maize, black gram, and cluster bean yields compared to pure crops (Ghosh et al., 2007), highlighting the need for the selection of an appropriate legume-maize cropping pattern.

### Green manures

Green manures are cover crops incorporated into the soil at the maximal stage of biomass production of legumes (Ahmed et al., 2005). These manures are primarily meant to improve soil fertility. Tropical green manures, such as Canavalia, Crotalaria, and Mucuna, commonly fix over 100 kg N ha<sup>-1</sup> per year, all of which are known to produce a more positive N balance than grain legumes (Ahmed et al., 2005). Green manures are more commonly used in temperate systems because of lower land pressures and because they could be grown during the colder winter months when crop production is not possible. In tropical systems, relay green manures are less common due to high land pressures, labor shortage, the inability to produce crops year-round in some regions, or the lack of moisture to support green manure growth during the dry seasons (Giller, 2001; Choudhury and Kennedy, 2004). The intercropping of green manure crops to supply nitrogen to simultaneously growing cash crops has also been adopted in some systems (Yonevama et al., 1987). The aquatic fern, Azolla, and its symbiotic association with the cyanobacteria Anabaena provide an example of a green manure that is used exclusively as a source of nitrogen when intercropped in lowland rice systems. With 80-95% of Azolla, rice-Azolla green manures could fix approximately 30 kg N ha<sup>-1</sup> (Yoneyama et al., 1987; Choudhury and Kennedy, 2004). Some constraints to more widespread adoption of Azolla are pest pressures, phosphorus limitation, and limited irrigation availability in some regions (Giller, 2001).

## Alley cropping

Alley cropping involves the use of perennial woody or shrub legumes between "alleys" of non-legume crops (Ghosh et al., 2007), where pruning from the legumes has been used as livestock forage or incorporated into the soil as a source of nitrogen for non-legume crops. Inclusion of perennials in cropping systems provides additional ecological benefits due to their extensive rooting systems that persist across multiple cropping seasons (Ghosh et al., 2007). Perennials could lessen soil erosion, access nutrients and water from deeper soil pools, provide critical microbial habitat between annual cropping seasons, and increase soil organic matter (Giller, 2001). *Leucaena* and *Gliricidia* are two common leguminous alley crop species practiced in sub-humid environments. Ghosh et al. (2007) also reported that the highest earnings can be achieved

when Leucaena is grown in alley cropping with cluster bean and black gram than growing of sole maize or Leucaena intercropped with Leucaena. sorahum increased sorghum yields by 73%, as compared to sorghum grown without N fertilizer, and yields were 43% greater than with a low rate of N fertilizer application (Ghosh et al., 2007). Phiri and Snapp (1999) reported that maize production was enhanced by 24.4% after Sesbania sesban-maize cropping system. Alley-cropped legumes could fix between 200 and 300 kg N ha<sup>-1</sup> per year (Giller, 2001). A 1-year Sesbania sesban alley crop increased the yields of succeeding maize crops by 50-80%, and a 2-year alley crop showed yield increases of 150 to 270% (Nair et al., 1999). The residual benefits of different legumes were observed for 4 years after alley cropping, and yields were three times greater than monocropped maize. Some of the challenges in the adoption of alley cropping systems include the competition of the legume with the cash crop for moisture in dry years, the labor required for pruning, and the use of land by a non-cash crop (Ghosh et al., 2007). Selection of species that have complementary rooting systems with cash crops (that is, a deep-rooted perennial legume cropped with a shallow-rooted annual), and species that grow at a manageable pace to supply nitrogen while not requiring excessive pruning inputs, are important considerations in the selection of legume species for allev cropping (Ghosh et al., 2007), Lastly, while reliable data on the contributions of non-symbiotic diazotrophs is limited, there are circumstances where it may be possible to increase nitrogen fixed by these microbes (Giller, 2001).

### Forage legume – grass mixtures

In grass-legume mixture systems, choosing appropriate cultivars and species may enhance nitrogen fixation (N2 fixation) and nitrogen transmission (N<sub>2</sub> transmission) (Jørgensen et al., 1999). For instance, in white clover monoculture, the percentage of nitrogen derived from biological nitrogen fixation (BNF) ranged from 75 to 94%, while in combinations of white clover and ryegrass; it was between 85-97%. Compared to legume monocultures, grass-legume combinations exhibit comparatively higher nitrogen fixation, which may be explained by increased competition from non-nitrogen-fixing plants for soil nitrogen. Nitrogen losses into the environment could occur even while the ecosystem had high nitrogen inputs (from relationships) (Scherer-Lorenzen et al., 2003). An abundance of grasses in the feed combination could enable a highly competitive uptake of mineral nitrogen from the soil, preventing such nitrogen losses (Scherer-Lorenzen et al., 2003). Forage legume BNF can differ based on species, cultivar, soil nutrient content, climate, and prevailing environmental factors. Legumes fix different amounts of nitrogen, which can be explained by the

Crop	pH (a) (before legume)	pH (b) (after legume)	% Change	Source
Vicia faba	6.00	5.64	-6.0	Yan et al. (1996)
White clover	4.97	4.27	-14.1	
Lotus	4.97	4.34	-12.7	Managhan at al. (1000)
Lucerne	4.97	4.44	-10.7	Monagnan et al. (1996)
Caucasian clover	4.97	4.41	-11.3	

Table 5. Soil pH prior and after legume growth without lime application.

multitude of factors controlling BNF (Jørgensen et al., 1999).

### Production of acids by legumes

Legumes have been shown to acidify their rooting medium through field tests, solution cultures, and greenhouse investigations (Table 5). Although several nutrient cycles can produce acids, the carbon and nitrogen cycles are thought to generate the most acids in pasture and agricultural ecosystems (Helvar and Porter, 1989). McLay et al. (1997) tested ten legume species (pilosus, yellow, white, and narrow-leafed lupins, faba beans, field peas, grass peas, chickpeas, common vetch, and lentils) for their ability to produce acid. They found that the species' ability to do so varied greatly, with proton production ranging from 77 to 136 cmol kg<sup>-1</sup> dry matter (McLay, 1997). Additionally, their research revealed that field peas had the least potential for acidification, while chickpea and narrowleafed lupin had the largest acidification potential. It was also recognized that acid production by nitrogen-fixing legumes ranges from 0.2 to 2.7 mol H+ kg<sup>-1</sup> biomass produced. The condition is worsened in continuous legume cultivation, sole cropping of legumes with high nitrogen fixation potential, and in conditions where no residue is incorporated back (Yan et al., 1996; Monaghan et al., 1998) (Table 5).

Due to the excess uptake of nutrient cations over anions from the soil solution, the net efflux of hydronium (H<sub>3</sub>O+) ions from plant roots into the rhizosphere, and the leaching of nitrate (NO<sub>3</sub>- N), perennial legumes acidify the rooting zone more than annuals (Jarvis and Robson, 1983; Helyar and Porter, 1989; Loss, 1992). When the pH of the soil falls below 4.5, most plant nutrients become less available, making it harder to grow food crops. In humid tropical locations, aluminum and certain micronutrients become more soluble and harmful to plants that have acute issues (Harter, 2007).

It takes 15 to 36 kg of calcium carbonate (CaCO3) to neutralize the acidity produced by 1,000 kg of grain legumes, but it takes 53 to 100 kg of CaCO3 to neutralize the acidity produced by 1,000 kg of bean shoots (McLay et al., 1997).

According to Helyar (1991), preventing nitrate leaching

is a crucial step in reducing soil acidity. Therefore, some corrective measures to prevent the accumulation of soil acidity in legume fields include growing acid-tolerant crops, applying lime, burning crop residue and leftovers, limiting the amount of ammonium-based nitrogen fertilizer to what plants actually need, following recommended tillage intervals, using cover crops during the off-season, rotating with cereals, and incorporating residue, which are important.

### Production of nitrous oxides by legumes

Two-thirds of all anthropogenic N<sub>2</sub>O emissions are caused by agricultural operations. N<sub>2</sub>O has 300 times the potential to cause global warming than carbon dioxide. Legume cultivation produces more N<sub>2</sub>O emissions than it can reduce CO<sub>2</sub> emissions from (Lugato et al., 2018), hence the role of legumes shouldn't be undervalued. The addition of fertilizer N (the largest magnitude), the development of legumes that fix atmospheric N, and the inoculation of legume residue into the soil through the microbiological processes of ammonification, nitrification, and denitrification have all contributed to an increase in nitrous oxide emissions from agricultural soils (Chafi and Bensoltane, 2003). Rather than the actual process of N fixation, the impact of legumes on N2O emissions is due to the release of excess N through the rhizodeposition of soluble N compounds and the breakdown of nodules (Rochette and Janzen, 2005). Up to 5.6 kg N<sub>2</sub>O-N ha<sup>-1</sup> of nitrous oxide was released by vetch, alfalfa, and lupin (Pattey et al., 2008), 4.9 N2O-N ha<sup>-1</sup> by Ellert et al. (2008), and 0.5-24 N<sub>2</sub>O-N ha-1 by Barton et al. (2011). Variations in these amounts were attributed to factors such as temperature, pH, soil water-holding capacity, irrigation techniques, fertilizer rate, tillage techniques, soil type, and oxygen concentration, availability of carbon, vegetation, land-use practices, and chemical use. But the amounts of nitrous oxide that legumes produce are less than those released in industrial processes, fuel combustion, synthetic fertilizers, N-leaching, and runoff in magnitude. Indeed, if appropriate management was implemented, biological N fixation might be regarded as one of the biological ways to minimize the usage of fertilizers and perhaps reduce N<sub>2</sub>O emissions (Shah, 2014). Lower N

Cropping sy	/stem					N₂O-N (kg/ha)	% change
Alfalfa	Alfalfa	Alfalfa	Wheat	Barley	0 N	4.90	315.3
Alfalfa	Alfalfa	Alfalfa	Wheat	Barley	Manure	8.66	633.9
Alfalfa	Alfalfa	Alfalfa	Wheat	Barley	Ν	7.11	502.5
Alfalfa	Alfalfa	Alfalfa	Wheat	Barley	N and manure	12.13	928.0
Corn	Wheat	Corn	Wheat	Barley	0 N	1.39	17.8
Corn	Wheat	Corn	Wheat	Barley	Manure	2.08	76.3
Corn	Wheat	Corn	Wheat	Barley	Ν	6.54	454.2
Corn	Wheat	Corn	Wheat	Barley	N and manure	8.67	634.7
Faba bean	Wheat	Faba bean	Wheat	Barley	0 N	1.18	0.0

**Table 6.** Nitrous oxide (N<sub>2</sub>O) annual emissions measured based on year-round estimate N (N)  $ha^{-1}$  over 3 yr (Ellert and Janzen, 2006).

fertilizer rates combined with legume intercropping with sugarcane decreased N losses (N<sub>2</sub>O emissions), but did not increase sugar yields in the first year (Smith and Conen, 2004).

Regardless of the presence of legumes, N<sub>2</sub>O emissions decreased by 50 to 70% in the second year with a 67% N treatment (Elizabeth et al., 2016). Comparing N<sub>2</sub>O emissions between mono-cropped faba bean and unfertilized wheat, authors found that faba bean fields had three times greater N<sub>2</sub>O emissions than unfertilized wheat plots (441 vs. 152 g N2O ha<sup>-1</sup>, respectively); in contrast, when faba bean was mixed with wheat, cumulative N2O emissions fluxes were 31% lower than that of N-fertilized wheat (Senbayram et al., 2016).

If mineral N inputs to intercropping or rotation could be decreased, or if N mineralization from legume residues is synced with the N need of the cereal crop, then N<sub>2</sub>O emissions from intercropping might be minimized. The release of nitrous oxides from fertilizers combined with manure was greater than that from N fertilizer applied alone. On the other hand, alfalfa released twice as much nitrous oxide than cereal single crops. In a similar vein, Table 6 shows that nitrous oxide output was highest from N fertilizer compared to manure, but lowest from the control. Thus, intercropped legumes can influence N<sub>2</sub>O emissions in two ways: either by directly supplying organic N or by adjusting the degree to which plants and microorganisms compete for soil N, for instance by serving as extra N sinks before nodulation. However, adding synthetic N to readily degradable crop wastes may increase N<sub>2</sub>O emissions (Baggs et al., 2000). Therefore, adding grain and legume residue aids in reducing the amount of N<sub>2</sub>O released. It is commonly recognized that the quantity and quality of crop residues (C: N ratio, lignin and cellulose content), soil texture, mulch location (surface mulching or integration), soil moisture, and temperature regimes all affect how much N2O is released from the soil (Li et al., 2016). Therefore, the management of the agroecosystems in which legumes are grown determines the impact of legumes in lowering GHG. Anyway, when economically relevant rates of N fertilizer are applied, the benefits of introducing legumes into crop rotations become noteworthy. Low-land rice cultivation techniques that flood their fields create anaerobic conditions that may lead to gaseous N losses (Havelin et al., 2014).

### Bottlenecks in biological nitrogen fixation

Numerous agricultural techniques, including tillage, crop rotation, residue retention, and continuous cropping, alter the microbial communities in the soil; however, various microbial groups may react in different ways (Lindstrom and Mousavi, 2019). Ineffective strains, low populations of microorganisms, high levels of contaminants, exposure to high temperatures, storage in unfavorable conditions, use of suboptimal doses, poor adhesive quality, negative effects of plant protection chemicals, exposure to low soil moisture, acidity or alkalinity, low availability of phosphorous and molybdenum, presence of high native populations, or the presence of bacteriophages could all be reasons for a lack of response from the application of biofertilizers.

### Nitrogen in the soil

For the majority of legumes, 5–15 kg N from different fertilizer sources are utilized during planting as starter N. This initial N promotes quick nodule production, strong growth, and subsequent N fixation. On the other hand, N fixation is decreased by high residual or additional N levels. This is because there is rivalry for photosynthate between NO<sub>3</sub>- reduction and N<sub>2</sub> fixation reactions when there is excess NO<sub>3</sub>- availability, which lowers nitrogenase activity and inhibits N<sub>2</sub> fixation (Havlin et al., 2014). For leguminous crops, N fertilization is typically not advised due to this negative effect.

However, there can be circumstances in which N must be applied, such as to cereals in rotations or mixed cropping, and fertilizer might then have an impact on the legume crop's ability to fix N. The corrective measures, which comprise the development of grain legumes that are less sensitive to mineral N or apply small amounts of soil or foliar N fertilizer, may increase yield without reducing the amount of N fixed are promoted (Hardarson et al., 1991; Boote et al., 1978).

### Other soil nutrients

Compared to legumes in fertile fields (-0.8 to +2.2%; 23 to 85%), those in poorly fertile fields exhibited considerably reduced shoot  $\delta$ 15N enrichment (-2.8 to +0.7‰) and a higher %Ndfa (55 to 94%). However, due to higher shoot dry matter and N yields, the N2 fixed was higher in fertile fields (16 to 145 kg N ha<sup>-1</sup>) than in poorly fertile fields (15 to 123 kg N ha<sup>-1</sup>) (Kermaha et al., 2018). Similar environmental conditions that are required for the host plant's healthy growth, vigor, and production of dry matter promote N<sub>2</sub> fixation by Rhizobium bacteria in leguminous plants. Dependent on biological N<sub>2</sub> fixation, plants require extra P for signal transduction and membrane production, as well as ATP for nodule formation and function. The concentration of phosphorus in the nodule is frequently much higher than in the tissue of the shoot or root. Additionally, Al-Niemi et al. (1997) proposed that even in cases where plants have received ordinarily sufficient P levels, bacteroids may be P limited. Considering this necessity for symbiosis, strategies that increase legumes' uptake and utilization of P are crucial. According to Israel (1987), there was a considerable reduction in host plant growth and biological N2 fixation when there was a severe phosphorus deficiency. This suggests that N<sub>2</sub> fixation requires more phosphorus for efficient functioning than host plant growth and nitrate assimilation. Reduced N2 fixation would arise from shortages in molybdenum, iron, phosphorus, magnesium, and sulfur since these elements are components of the Nitrogenase complex, which facilitates N<sub>2</sub> fixation. Molybdenum is a major component of nitrogenase since N fixation requires more of it than the host plant can provide (Verma et al., 2015).

### Soil acidity

Acidic soils hinder N fixation and agricultural productivity. Low levels of soil-available P and some micronutrients in acidic soils inhibit the growth of the related N-fixing Rhizobium, which lowers the BNF in the soil (Vincent, 1990; Giller, 2001). Rhizobia can thrive best at a pH of 6 to 7 (Giller, 2001). Nonetheless, species that grow slowly and produce alkali, like *BradyRhizobium japonicum*, are more tolerant of lower pH values, whereas species that grow quickly and produce acid, like *R. leguminosarum*, are more tolerant of higher pH values (Krieg and Holt, 1984). Certain Rhizobia may, in fact, withstand large quantities of aluminum (AI) at low pH in both solution media and soil, according to certain research. On the other hand, vulnerable species, low phosphorus levels, high levels of acidity, and high amounts of aluminum caused a 40% decline in population, retarded growth, and frequently prolonged the lag period (Munns and Keyser, 1979). Low pH prevented the development of nodules, and nodulation failure in these soils is typically ascribed to Rhizobia's low survivability or inability to proliferate in the rhizospheres (Munns, 1978). This is due to the fact that low soil pH is typically linked to mineral toxicity for Rhizobia and nutritional deficiencies. For instance, toxicity results from low molybdenum levels or excessive iron solubility in acidic environments (Munns, 1978). Therefore, some environmental factors are detrimental to Rhizohium life (for example, if the pH of the soil is higher than 7 for R. lupini or less than 5.5 for R. meliloti). Soil amendments were necessary in these cases to guarantee Rhizobia establishment. Using Rhizobium species and acid-tolerant legume cultivars are among the necessary management alternatives; also, soil liming should be restricted to reaching a pH where accessible manganese or aluminum levels are no longer harmful (Vincent, 1990; Giller, 2001).

### Unsuitable microbial factors

Poor quality inoculants, inability to compete with local Rhizobia, suppression by native microbial flora, or inoculants' inability to thrive in low pH and dry soils are some possible reasons for BNF failure (Graham, 1981). Certain Rhizobia are promiscuous (able to nodulate with native Rhizobium species), while others are nonpromiscuous (could only nodulate with the variety-specific bacteria). Therefore, in non-promiscuous legume cultivars, the biological N fixation would be reduced in the absence of a particular Rhizobial strain. Microbes such as parasites like bacteriophages and bellovibrios and predators like protozoa and amoebae may control the amount of Rhizobia in tropical soils (Keya and Alexander, 1975). Consequently, inoculation is required whenever new leguminous crops are brought to a region in order for hostspecific Rhizobia to regularly develop for the development of new cultivars (Montanez, 2000).

Furthermore, many soils have a high concentration of inefficient Rhizobia that might cause nodulation without benefiting the host. In these circumstances, it becomes necessary to replace the ineffective native *Rhizobia* with very large inoculums of a very effective and competitive strain. In order to decrease inorganic N inputs and increase legume production, research institutes (such as IITA) appropriately began dispersing promiscuous legume germplasms (nodulated by broad host-range Rhizobia strains) (Schulze, 2004; Ndakidemi et al., 2006; Li et al., 2015; Arreseigor et al., 1997). Accordingly, the amount of Rhizobia already present in the soil, the availability of soil N, and the crop's need for N all influenced how the legumes responded to inoculation (Montanez, 2000).

### Tillage

Nodulation and BNF are positively impacted by reduced tillage. This is because tillage promotes the soil's organic matter to become more mineralized, which makes a lot more nitrate available and may inhibit nodulation and N fixation.

According to Alves et al. (2002), it indicates that, in terms of BNF, no-tillage circumstances are favored over frequent tillage operations.

In comparison to findings in conventional tillage without residue retention, Kihara et al. (2011) showed increased nodule numbers, nodule dry weights, and percent of N derived from the air in legumes under decreased tillage with residue retention. The study was conducted in Kenya. Reduced tillage will result in reduced rates of nitrification and mineralization, as well as greater N immobilization and a greater potential for denitrification, which will reduce the amount of accessible N. Remedial actions such as reducing tillage may thereby increase N requirement and N2 fixation.

Therefore, until a new equilibrium between residue input and the rate of decomposition is attained, conservation and zero tillage management strategies will stimulate  $N_2$ fixation (Table 7).

### Residues from cereal crops

It took until recently to identify soil organic carbon (SOC) limits, above or below which crop output could be negatively impacted or at which no or strong response to nitrogen treatment could be realized (Dobberman, 2005). However, it seems that when N application rates rise, so do the N fertilizer replacement values for organic inputs. According to Pikula et al. (2016), all organic residues, whether it comes from legumes or not, has a N fertilizer replacement value (NFRV). According to Mrabet et al. (2003), the amount of N, organic carbon, and particulate organic matter varies with the residue level. According to some recent estimates, increasing soil organic carbon (SOC) concentrations may help narrow worldwide output gaps and lessen the need for N fertilizer (Oldfield et al., 2019). Meki et al. (2012) found that in the Upper Mississippi River Basin, removing maize residue decreased soybean yield, N fixation, uptake, losses, and soil storage by 8, 6, 7 and 5%, respectively. This ultimately decreased the amount of nutrients available for loss, leading to a 9% decrease in N losses compared to leaving the residue in place. When *diazotrophs* used the carbon in the straw from wheat crop with a yield of 2 t/ha, they produced 50–150 kg N/ha, which drove N fixation (Kennedy and Islam, 2001).

This is because these resources are used by the tiniest and most prevalent N-fixing microorganisms in the soil, which include bacteria, *actinomycetes, fungi, algae, and protozoa*. Because they actively participate in the nutrition cycle, the decomposition of organic materials, N fixing, and *P solubilization*, they are crucial to the fertility of the soil. According to some research, adding organic wastes (biosolids, slurry), synthetic fertilizers, and bettering soil biological processes involving BNF and *mycorrhizae* can all improve soil fertility. This is one method of doing this. The retention of soil carbon and nitrogen is enhanced by the use of organic residues with a high C to N ratio, such as grain residues and during integrated nutrient management (INM).

### Residues from legume crops

Legume residues can be used as a source of carbon and nitrogen for companion non-legume crops because they have a low carbon-to-nitrogen ratio and a large amount of nitrogen. This allows them to release nitrogen more quickly than cereal residues with lower nitrogen content (Hestermann et al., 1986: Bruulsema and Christie, 1987: Yano et al., 1994; Mubarak et al., 2002). According to other writers, 44% of the nitrogen fixed by legumes was still present in the soil (Sawatsky and Soper, 1991). Additionally, the authors demonstrated that 8.6 to 12.1% of N is recovered by the next cereal crop from legume shoot residue, and 8.2 to 10.6% is recovered from microbial biomass (Sawatsky and Soper, 1991). Hence, increased release from low carbon/N shoot and root led to increased N availability for crops that came after legumes. It was documented that the residue from peanuts contributed approximately 11.2% nitrogen for subsequent wheat (Yano et al., 1994); the residue from red clover and alfalfa contributed approximately 22.7 kg N ha<sup>-1</sup> for subsequent maize (Bruulsema and Christie, 1987); the residue from crimson clover contributed approximately 19.4 kg N ha<sup>-1</sup> of nitrogen (Hestermann et al., 1986); and the residue from peanuts contributed approximately 7.9 kg N ha<sup>-1</sup> (Mubarak et al., 2002). Compared to cereal crops grown in the same environment, the amount of mineral N in the root zone after legumes is frequently 30-60 kg N ha<sup>-1</sup> higher (Dalal et al., 1998). Using a 15N label to include the residue from legumes revealed that 10 to 34% of the legume N could be recovered in the next crop of rye or wheat, 42% in rice, and 24% recovered from velvet beans by the corn crop (Ambrosano et al., 2005). Legumes' deep root systems, which accessed nutrients from lower soil layers, are to blame for this. When fed to the soil in the short term, soybean residues at harvest are lignified (10% lignin) with C/N ratios of approximately 45:1,

Crop -	N₂ fixed (%)			N₂ fixed (kg/ha)		
	СТ	ZT	% change	СТ	ZT	% change
Chickpea	34	28	-17.6	32	27	-18.5
Soybean	73	88	20.5	180	232	22.4
Soybean	73	88	20.5	91	156	41.7
Chickpea (1994)	31	40	29.0	9	11	18.2
Chickpea (1995)	12	17	41.7	4	5	20.0
Pea	48	79	64.6	ND*	ND	ND
Lentil	62	72	16.1	ND	ND	ND
Soybean <i>cv</i> S12	87	91	4.6	33	47	29.8
Soybean <i>cv</i> S15	86	88	2.3	39	44	11.4

**Table 7.** Influence of conventional tillage (CT) and zero tillage/minimum tillage (ZT/MT) practices on  $N_2$  fixation by grain legumes (van Kessel and Hartley, 2000).

\*Not determined.

which tends to immobilize N and release it for plant uptake in the long run (Toomsan et al., 1995). As active N<sub>2</sub> fixation peaks between growth stages V2-V3 and R5-R6, residue inclusion for improved N should be carried out during the legume flowering period. Grain legume residues collected during flowering phases have a narrow C/N ratio, which speeds up decomposition and increases SOM. This affects soil aggregations and reduces soil bulk density. This is because of N fixation, deep rooting, leaf shedding ability, and mobilization of insoluble soil nutrients (Ofori and Stern 1987). Straw application dramatically boosts N2-fixing activity of photosynthetic bacteria and *Rhizobial* populations, according to several field and greenhouse investigations.

Nonetheless, N increases of 2 - 4 mg N g<sup>-1</sup> straw added were observed in marijuana trials (Santiago et al., 1986). Furthermore, even when the residues are returned to the soil, there is typically a net removal of N from the field (Giller et al., 1994). Legumes like soybeans are very effective at translocating their N into the grain, ranging from 50-150 kg N ha<sup>-1</sup> (Matusso et al., 2014).

### Temperatures

Temperatures outside of the ideal range may impact Rhizobia's ability to nodulate, fix nitrogen, and survive in soil, potentially hindering N fixation. Many bacteria that develop in root nodules prefer temperatures between 25 and 30 degrees Celsius. The majority of Bradyrhizobium strains, however, are said to withstand high soil temperatures, with a maximum growth range of 30 to 40 degrees Celsius. Thus, variations in soil temperature can affect Rhizobia's ability to survive and persist in soil. Nodulation might not occur below 15 degrees Celsius (Elkan, 1987). The fact that these conditions arise during the dry off-season when crop hosts may not be growing in the field exacerbates the effects of high soil temperatures. The recommended management choice is to place the inoculum in deeper soil layers when topsoil temperatures are high and use surface mulches to conserve moisture and reduce soil temperature (Roughley, 1980).

### Soil moisture

For N fixation, both high and low moisture stress are detrimental. Low moisture stress inhibits nodulation by affecting Rhizobia colonization and infection of root hair, as well as nodule activity and function, and Rhizobia survival in soil (Davey and Simpson, 1990; Graham, 1992). Two common bean (Phaseolus vulgaris L.) cultivars, Carioca and EMGOPA-201, were used to investigate the effects of water stress on N2 fixation and nodule structure. The results showed lower nodule dry weight, lower shoot dry weight, host cell vacuolation, loss of the peribacteroidal membrane, degradation of cytoplasm host cells, and senescence of bacterioids with their release into intercellular spaces. According to Lucrecia et al. (2003), water stress changed the structure of cultivars' nodules, inhibited Nitrogenase function, and decreased the amount of intercellular glycoprotein.

*Rhizobia's* growth and activity are impacted by flooding the soil because it decreases the gas exchange between the soil and bacteria or plant nodules. *Rhizobial* strains are aerobic heterotrophs, which explain why. Furthermore, in water, *Rhizobium spp.* quickly lose vitality. According to Osa-Afiana and Alexander (1979), when soils were flooded, the population of soybean *Rhizobia* decreased by a factor of 150 (from 6.0 x 108 to 4.0 x 106 cells per gram of soil), while the population of *R. trifolii* was reduced by a factor of 300 (from 1.3 x 108 to 4.2 x 104 cells per gram of soil). Nitrate absorption is impacted by the *rhizosphere's* decreased O<sub>2</sub> content after floods. First, nitrate might be utilized in hypoxic roots as an electron acceptor instead of O<sub>2</sub>. Second, compared to nitrate absorption and assimilation, the respiratory energy requirements for N<sub>2</sub> fixation and assimilation are greater (Bacanamwo and Purcell, 1999). As a result, plants that rely on N2 fixation and have hypoxic roots are severely impacted. According to Reyna et al. (2003), waterlogging in soybean root nodules decreased nitrogenase activity and permanently changed the ultrastructure of the cells. According to Oosterhuis et al. (1990), soybeans typically do not fully recover from flooding injury. This can result in a reduction in soybean yield of 17 to 43% during the vegetative growth stage and 50 to 56% during the reproductive stage. Reduced root and shoot growth, nodulation, N fixation, photosynthesis, biomass buildup, stomatal conductance, and plant death from diseases and physiological stress are the main causes of yield losses (vanToai et al., 2003).

### Salinity

According to Munns (2002), salinity inhibits plants' capacity to absorb water, which lowers the growth rate and results in a number of metabolic changes similar to those brought on by water stress. It requires a combination of stress-tolerant Rhizobia and cultivars to maximize biological nitrogen fixation (BNF) under salinity circumstances.

# Enhancing biological N fixation while reducing acidification and $n_2$ o production

Legume crop productivity and biological N<sub>2</sub> fixation would be improved by using appropriate agronomic and plant protection practices, enhancing soil N mineralization, and recycling crop residues. Legume-supported crop systems have the potential to reduce N and C losses, but overall management plays a significant role in achieving this.

1) Finding the germplasm that has the highest potential for this trait, this necessitates addressing BNF's low heritability and comparable characteristics' evidence that BNF traits are quantitatively inherited and environmentinfluenced (Schulze, 2004).

2) Finding the best possible balance between crop management, biological inoculant, and variety (Ndakidemi et al., 2006).

3) N in the cropping system should be optimized (Ndakidemi et al., 2006), since excessive N fertilizer application above crop needs has deteriorated soil, water, and air quality.

4) The use of cover crops would lower N<sub>2</sub>O emissions and soil acidification, and cropping systems might be

intensified by optimizing tillage, using residue or fertilizer, and designing appropriate cropping systems (Gregorich et al., 2005; Li et al., 2015).

5) *Rhizobia*'s survival would have been decreased by the negative effects of temperature, which can be mitigated by surface mulching, inserting inoculum in deeper soil layers, and choosing heat-tolerant strains (Michiels et al., 1994).

6) *Rhizobia* populations and nitrogen fixation were protected from the negative effects of moisture stress by choosing strains of bacteria that can withstand moisture stress, mulching the soil, and irrigation (Hunt et al., 1981). 7) By reducing calcium deficiency and aluminum toxicity, liming the soil, adding compost, or using acid-tolerant legume cultivars all contribute to improving *Rhizobia* survival in the soil and enhancing nodulation and N<sub>2</sub>-fixation in acidic soils (Giller, 2001).

8) To prevent P deficit and promote nodulation, N<sub>2</sub> fixation, and *Rhizobia*l growth, P fertilizers should be added, effective mycorrhiza should be inoculated, and P-efficient cultivars should be used (Cassman et al., 1981).

9) Choosing salt-tolerant strains improves nodule activity, respiration by *Rhizobia*l bacteria, and nodulation while lowering salt stress (Delgado et al., 1994).

10) Increasing root infection, nodulation, and nodule activity by the breeding of cultivars less sensitive to the mineral N (Arreseigor et al., 1997).

11) *Rhizobia* and agrochemicals placed separately reduce the negative effects of fungicides, insecticides, and herbicides while promoting nodulation,  $N_2$  fixation, and *Rhizobia*l growth, as well as plant growth (Mallik and Tesfai, 1993).

12) Research focused on particular strains of *Rhizobia*l lowers natural *Rhizobia*'s competitiveness and its ability to prevent inoculation (Dowlig and Broughton, 1986).

### Silent features

A synthesis of the existing knowledge and identification of knowledge gaps has led to the determination of research requirements for the following areas of future study:

1) Is it possible to quantitatively divide a specific legume's contribution to subcomponents in various agro-ecologies for sustainable farming?

2) Due to the sensitivity of microorganisms, research on nodulation and  $N_2$  fixation in conditions of salt or drought has not advanced significantly. What may significantly increase its impact?

3) If under-sowing, intercropping, catch cropping, cover cropping, crop rotation, and double cropping on both tilled and untilled soils are taken into consideration, what would be the outcome of quantifying BNF throughout multicropping and various locations?

4) Rhizosphere acidification, acid phosphatase secretion, altered architecture at low P, increased P transport and

use-efficiency, and functional variations in mycorrhizal symbioses are all factors contributing to legumes' improved uptake and utilization of P. Which procedure has greater significance than the others?

5) Different native *Rhizobia* can be found in a range of global habitats. Developing effective inoculation tactics requires an understanding of local populations and *Rhizobia* biodiversity. The diversity of *Rhizobia* is not, however, completely understood.

6) There is little quantitative data on the mineralization patterns of crop residues from intercrops of legumes and cereals (Njira, 2016). How may we optimize use and mineralization?

7) The discharge of greenhouse gases from agricultural land adds to global warming. Legume fields emit less N<sub>2</sub>O when managed differently (Mania *et al.*, 2019). The amount of the emission reduction, though, has not yet been measured across maize based legume cultures.

8) Is it possible to create post-emergence herbicides that effectively control weeds in grain legumes without suppressing BNF?

9) How can we improve the N economy by postponing nodule degeneration?

10) Should we improve brown manuring or add green manure pulse crops for tropical Africa?

### CONCLUSION AND RECOMMENDATIONS

This review aimed to incorporate the advantages of legume-based cropping systems for maize production while highlighting their drawbacks. The analysis then suggested cropping system techniques needed to address the drawbacks of monoculture. Allowing farmers to plant whatever they want whenever they want and letting nature choose what works best will simply worsen the already degraded soils and broken agricultural systems. Therefore, in mid- to highland locations, it is possible to reduce nitrous oxide emissions and soil acidification while improving N fixation by rotating maize with grain legumes, forage legumes, green manures, or cover crops every year. Maize can be relayed, double cropped, or simultaneously interplanted with legumes that complement it in moist mid-lands. Compatible legumes could be interplanted with maize in arid midlands. But legumes should be cycled in lowland areas where one crop may be grown in a season. In this final section, we propose a cropping system act (tillage act, rotation act, intercropping act, double cropping act, and cover cropping standards) relevant to each locality in maize-based production systems. This will enforce the practices needed to lower production costs and achieve sustainable maize production, which suppresses resource abuse and environmental degradation. This is because each recommended cropping system had its own recommendation domain. The legislations and practices can be utilized for grassroots land planning as well as for regulatory purposes at the local government level.

### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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