Journal of Bioresource Management

Volume 7 | Issue 3 Article 8

Microbial Diversity in the Sawmill Environment: Implications on the Health of Sawmill Workers and Merchants, Nigeria

Ebere J. Okafor-Elenwo EJ
Igbinedion university okada, ebjulein2010@yahoo.com

Odaro S. Imade Os
Igbinedion university okada

Osazee E. Izevbuwa EO
Igbinedion university okada, osazee.izevbuwa@iuokada.edu.ng

Recommended Citation

Okafor-Elenwo, E. J., Imade, O. S., & Izevbuwa, O. E. (2020). Microbial Diversity in the Sawmill Environment: Implications on the Health of Sawmill Workers and Merchants, Nigeria, *Journal of Bioresource Management, 7* (3).
DOI: https://doi.org/10.35691/JBM.0202.0143
ISSN: 2309-3854 online
(Received: Aug 12, 2020; Accepted: Oct 5, 2020; Published: Sep 30, 2020)

Microbial Diversity in the Sawmill Environment: Implications on the Health of Sawmill Workers and Merchants, Nigeria

Cover Page Footnote

We are appreciative of the laboratory staff of the microbiology laboratory of igbinedion university okada

MICROBIAL DIVERSITY IN THE SAWMILL ENVIRONMENT: IMPLICATIONS ON THE HEALTH OF SAWMILL WORKERS AND MERCHANTS, NIGERIA

EBERE JULEIN OKAFOR-ELENWO, ODARO STANLEY IMADE AND OSAZEE EKUNDAYO IZEVBUWA

Department of Biological Sciences, Igbinedion University Okada, Edo State, Nigeria

*Corresponding author: osazee.izevbuwa@iuokada.edu.ng

ABSTRACT

There is an increasingly high suspicion of occupational health diseases amongst workers in sawmill factories. Hence this study aimed to determine the microbial diversity in some sawmill factories in Okada, Edo State, Nigeria to evaluate potential implications of the factory's processing area on the health of the workers and wood merchants. Bacteria and fungi count in the bioaerosols within the processing area of each factory was performed with the passive air sampling technique. While in the sawdust samples, microbial counts were carried out with the pour plate technique. Identification of the microbes was performed with macroscopic and microscopic examinations as well as standard phenotypic tests. Mean total viable count (TVC) and total fungi count (TFC) of bioaerosols in the air within the processing area of the sawmill factories were found to be greater than 4162.99 CFU/m^3 while mean total coliform count (TCC) was reported as 756.28 CFU/m^3. Fisher (F) one-way ANOVA test of the TVC and TCC dataset of bioaerosols from the four sawmill factories indicated no significant difference ($p = 0.77$ and 0.83 for TVC and TFC respectively) in the mean TVC and TFC of the bioaerosols. Bacterial (*Micrococcus, Staphylococcus, Bacillus, Klebsiella* and *Serratia*) and fungal (*Saccharomyces*, *Aspergillus*, *Cladosporium* and *Penicillium*) species were isolated from the bioaerosols and sawdust samples. Results of this study indicated that workers in the sawmill factories and other wood merchants who visit sawmill processing area are most likely exposed to airborne contaminants that may cause occupational diseases such as ophthalmic irritations and dermatitis. Hence it is recommended that sawmill workers and other visitors wear personal protective gadgets in the processing area of the sawmill factories.

Keywords: Occupational health, sawmill, bioaerosol, sawdust, microbes.

INTRODUCTION

Sawmill factories generate large quantities of organic dust (Demers et al., 2000; Straumfors et al., 2018) that easily disperses into air and has been found to pose a high risk of occupational diseases such as asthma, frequent headaches, chronic bronchitis, phlegm, nasal symptoms and ophthalmic irritations (Demers et al., 1997; Alwis et al., 1999; Idu et al., 2019). The airborne dust is often associated with microorganisms, referred to as bioaerosols (Sawane and Sawane, 2017), which may cause a myriad of adverse health effects on the workers and wood merchants. Several studies have shown that bacteria and fungi are the main airborne microorganisms that are associated with sawdust. Some microbial contaminants of sawdust that have been reported include *Micrococcus, Staphylococcus, Klebsiella*, *Aerococcus, Moraxella*, *Sphingmonas*, *Bacillus*, *Enterobacter*, *Aspergillus*, *Cladosporium* and *Penicillium* (Oppliger et al., 2005; Park et al., 2010; Baranu and Edmund, 2019; Idu et al., 2019). This is because before processing the wood, it is stored outside the factory premises thereby creating a favorable environment for the

propagation of these microorganisms (Oppliger et al., 2005). Occupational diseases have increasingly become a source of concern in the world. For instance, in South Korea, the third most common source of occupational diseases is derived from bioaerosols generated from sawmill factories and other workplaces (Park et al., 2010). Occupational health problems associated with bioaerosols generated from sawdust in sawmill factories situated in Switzerland have also been reported (Oppliger et al., 2005). Idu et al. (2019) also indicated occupational health problems in a Nigerian sawmill factory. In order to prevent occupational health problems associated with the sawmill workplace, there is a need to regularly establish occupational exposure levels of the bioaerosols and have a knowledge of airborne contaminants in sawmills. Hence, this study was carried out to determine the microbial diversity in some sawmill factories in Okada, Edo State, Nigeria with a view to evaluate potential implications of the factory's processing area on the health of the workers and wood merchants.

MATERIALS AND METHOD

Experimental Design

Four sawmill factories situated in Okada, Ovia North-East local government area, Edo State, South-Southern Nigeria were visited between the months of May to July 2020 for sample collection. Each sawmill factory was visited twice weekly for sample collection. In each of the sawmill factories, air and sawdust samples within the vicinity of the sawmill factories were collected. Overall, 24 sawdust samples and 24 bioaerosol samples were collected from all the four sawmill factories examined. One-way analysis of variance of the sample sizes was carried out with the 'completely randomized design' technique. Counts of total viable bacteria (TVC), total coliform bacteria (TCC) and total fungi (TFC) was performed on the samples.

Microbial Analysis of Bioaerosol Samples

Bacteria and fungi count in the bioaerosols within the processing area of each factory was performed with the passive air sampling technique or the settle plate method (Pasquarella et al, 2000). Sterile duplicate nutrient agar (NA), MacConkey agar (MA) and Sabourad's dextrose agar (SDA) Petri dishes held at a height of one meter above the floor of the processing area and one meter away from any obstacle in the processing area were exposed to air for one hour to enable organic dust that is suspended in the air to settle on the Petri dishes. Each exposed Petri dish had a diameter of 8.5 cm with an equivalent area of 60.053 cm^2. After exposure, the NA and MA Petri dishes were then incubated at 37^{o}C for 24 hours, while the SDA Petri dishes were incubated at room temperature for 72 hours. Colony counts (TVC, TCC and TFC) on the Petri plates were respectively expressed as colony-forming units per cubic meter (CFU/m^3) according to the equation stated below (Fekadu and Getachewu, 2015).

$$N = 5a \cdot 10^4 (bt)^{-1} \quad (1)$$

N: microbial CFU/m^3 of indoor air, a: number of colonies per Petri plate, b: dish surface (cm^2), t: exposure time (minutes).

Microbial Analysis of Sawdust Samples

Isolation of bacteria and fungi in the sawdust samples collected from the processing area was carried out with the pour plate technique (Public Health England, 2014). Serial dilution of samples was made up to 10^{-5}, with the first dilution made by mixing 25 g of sawdust sample with 225 ml of sterile 1.5% peptone water in a sterile tube. One milliliter of each serially-diluted sample was separately poured into three different duplicate sterile

petri dishes and then respectively mixed with 19 ml of sterile NA, MA and SDA media. The NA and MA Petri dishes were then incubated at 37^{o}C for 24 hours while the SDA Petri dishes were incubated at room temperature for 72 hours. After incubation, microbial colonies were counted with the colony counter and counts on NA, MA and SDA Petri plates, which were expressed as colony-forming units per gram (CFU/g) and were respectively reported as TVC, TCC and TFC.

Identification of Microbial Colonies

Identification of the bacterial colonies was performed with standard phenotypic tests (Barrow and Feltham, 2003). The phenotypic tests that were performed on the bacterial colonies included Gram staining, coagulase, catalase, citrate, oxidase, indole, methyl red and Voges Proskauer tests. Identification of fungi was mainly done by macroscopic and microscopic examination using the 'manual of medically important fungi' as a guide (Larone, 1995).

Parasitological Examination

Sawdust samples were observed for the presence of protozoan parasites with the zinc sulphate centrifugal floatation method as previously prescribed by Parameshwarappa et al. (2012).

Statistical Analysis

The NCSS (version 12) data analysis software was used to carry out descriptive statistics of TVC, TCC and TFC. Shapiro–Wilk normality test, Levene test of homogeneity, Fisher (F) one-way ANOVA test for normally distributed datasets with equal variances and Kruskal–Wallis nonparametric one-way ANOVA tests were also performed with NCSS. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

RESULTS

Microbial Counts in Bioaerosols and Sawdust Samples

Table 1 represents the counts of bacteria and fungi found in the processing area of sawmill factories situated in Okada, Ovia North-East LGA, South-Southern Nigeria. Mean TVC and TFC of bioaerosols in the air within processing area of the factories were found to be greater than 4162.99 CFU/m^3; while in the sawdust, mean TVC was recorded as 2150000 CFU/g and mean TFC as 92500 CFU/g. Mean TCC of the bioaerosols was reported as 756.28 CFU/m^3 while mean TCC of sawdust samples was 215000 CFU/g. The mean TVC and TFC datasets of bioaerosols obtained from all the sawmill factories were normally distributed ($p = 0.94$ and 0.95 for TVC and TFC respectively) with equal variance ($p = 0.62$ and 0.68 for TVC and TFC respectively). Fisher (F) one-way ANOVA test of the TVC and TCC dataset of bioaerosols indicated no significant difference ($p = 0.77$ and 0.83 for TVC and TFC respectively) in the mean TVC and TFC of the bioaerosols. The mean TCC dataset of bioaerosols was non-normally distributed ($p = 0.02$) with equal variance ($p = 0.07$). Kruskal-Wallis ANOVA test of the TCC dataset indicated a significant difference ($p = 0.04$) in the median TCC dataset of aerosols. Fisher (F) one-way ANOVA test also showed that the mean TVC, TCC and TFC datasets of sawdust obtained from all the sawmill factories showed no significant difference ($p = 0.63$, 0.51 and 0.73 for TVC, TCC and TFC respectively).

Table 1: Microbial counts obtained from sawmill environment

Microbial counts in samples					
Bioaerosols in the processing area of all sawmill factories			Sawdust in the processing area of all sawmill factories		
Mean TVC N = 24 (CFU/m^3)	Mean TCC N = 24 (CFU/m^3)	Mean TFC N = 24 (CFU/m^3)	Mean TVC N = 24 ($\times 10^6$ CFU/g)	Mean TCC N = 24 ($\times 10^5$ CFU/g)	Mean TFC N = 24 ($\times 10^4$ CFU/g)
> 4162.99 ± 0.00	756.28 ± 266.47	> 4162.99 ± 0.00	3.37 ± 0.22	2.15 ± 0.80	9.25 ± 2.56

TVC: total viable count; TCC: total coliform count; TFC: total fungi count. Mean count represented as mean ± standard error of mean. N is the total number of samples examined.

Identity of Microbes in Bioaerosols and Sawdust Samples

The microbial community in the bioaerosols and sawdust samples present in the sawmill factories include bacteria, fungi and parasites. The bacteria that were identified in the bioaerosols included *Micrococcus, Staphylococcus, Bacillus, Klebsiella* and *Serratia* species, while *Saccharomyces, Aspergillus* and *Penicillium* species were the fungi that were identified. The main bacteria identified in the sawdust included *Micrococcus, Staphylococcus, Klebsiella* and *Bacillus* species, while the fungi identified were *Saccharomyces, Aspergillus, Cladosporium* and *Penicillium* species. *Entamoeba* species was the main parasite found in sawdust. The main bacteria and fungi that were present in the air were similar to those that were isolated from the sawdust indicating that the airborne microbes may most likely originate from the wood that was processed in the sawmill factories.

Table 2: Phenotypic characterization of microbial isolates in sawmill environment

Samples	Representative isolates	Morphology on Petri plates	Microscopy		Biochemical characteristics of bacterial isolates							Identified microbes
			Bacteria	Fungi	CO	CA	OX	CI	I	MR	VP	
Bioaerosols in the processing area of sawmill factories	1	Mucoid colony on NA plate	Gram positive cocci		-	+	+	-	-	+	-	*Micrococcus* spp.
	2	Mucoid colony on NA plate	Gram positive cocci in clusters		-	+	-	-	-	-	+	*Staphylococcus* spp.
	3	Dry colony on NA plate	Gram positive rods		NP	+	-	-	NP	-	+	*Bacillus* spp.
	4	Red colony on NA plate	Gram negative rods		NP	+	-	+	-	-	-	*Serratia* spp.
	5	Mucoid colony on NA plate	Gram negative rods		NP	+	-	+	-	-	+	*Klebsiella* spp.
	6	Whitish colony on SDA plate	Gram positive buds	Unicellular oval cells that appeared purple upon Gram staining with some cells exhibiting budding								*Saccharomyces* spp.
	7	Greenish colonies on the surface of SDA plate		Multiple phialides arising from a single conidiophore carrying chains of conidia	NP	NP	NP	NP	NP	NP	NP	*Penicillium* spp.
Sawdust in the processing area of sawmill factories	1	Mucoid colony on NA plate	Gram positive cocci		-	+	+	-	-	+	-	*Micrococcus* spp.
	2	Mucoid colony on NA plate	Gram positive cocci in clusters		-	+	-	-	-	-	+	*Staphylococcus* spp.
	3	Dry colony on NA plate	Gram positive rods		NP	+	-	-	-	-	-	*Bacillus* spp.
	4	Whitish colony on SDA plate	Gram positive buds	Unicellular oval cells that appeared purple upon Gram staining with some cells exhibiting budding	NP	NP	NP	NP	NP	NP	NP	*Saccharomyces* spp.
	5	Black colonies on the surface of SDA plate		Conidiophores arising from septate hyphae with central vesicles that were completely filled with conidia.	NP	NP	NP	NP	NP	NP	NP	*Aspergillus* spp.
	6	Greenish colonies on the surface of SDA plate		Multiple phialides arising from a single conidiophore carrying chains of conidia	NP	NP	NP	NP	NP	NP	NP	*Penicillium* spp.
	7	Greenish colonies on the surface of SDA plate		Septate hyphae with conidiophores that have medium to long chain conidia	NP	NP	NP	NP	NP	NP	NP	*Cladosporium* spp.

Co, Ca, Ox, Ci, I, Mr and Vp indicate coagulase, catalase, oxidase, citrate, indole, methyl red and Voges Proskauer tests respectively. NP means that the test was not performed. +: positive test. -: negative test.

DISCUSSION

The TVC and TFC in the aerosols within the processing area of all the sawmill factories were greater than (>) 300 CFU/Petri plate with an equivalent mean TVC and TCC that was > 4162.99 CFU/m^3 (Table 1). The count of Gram-negative bacteria as expressed by the mean TCC was estimated at 756.28 CFU/m^3. The results agreed with the findings of Alwis et al. (1999) who obtained bioaerosol levels of microbes in the range of $10^2 – 10^4$ CFU/m^3. It was also similar to those of Park et al. (2010) who reported mean bioaerosol levels of 10^3 CFU/m^3 for bacteria and fungi respectively, as well as to the work of Oppliger et al. (2005) who obtained bioaerosol levels in the range of $10^3 – 10^4$ CFU/m^3 for TVC and TFC including a mean value of 10^2 CFU/m^3 for Gram-negative bacteria in Swiss sawmill factories. The microorganisms associated with bioaerosols in the sawmill factories may be largely due to microbes that are propagated in the bark of the trees during growth in the forest and subsequent storage in the sawmill factories before sawing (Oppliger et al., 2005). Significant exposure of workers and merchants to bioaerosols in the sawmill factories often occurs from the release of high concentration of airborne microbes into the air during sawing of the wood. In line with the findings from this study, Idu et al. (2019) also reported a high concentration of bacteria in the range of $10^6 – 10^7$ CFU/g of sawdust.

The bioaerosol levels obtained in the sawmill factories examined were compared to recommended exposure limits for bioaerosols in indoor air and occupational environments. Results of the present study indicated that the total microbial load (TVC and TFC) exceeded WHO (Nevalainen and Morawaska, 2009) and Korean (Park et al., 2010) recommended limits for indoor air (1000 CFU/m^3 and 800 CFU/m^3 for WHO and Korea respectively). Findings from this study also indicated the bioaerosol concentrations (Table 1) were above the occupational exposure guidelines of Switzerland (Oppliger et al., 2005) stipulated for airborne fungi (≤ 1000 CFU/m^3) and may be above the recommended values for total bacteria count (TVC) (≤ 10000 CFU/m^3 for TVC) but were within the recommended limit for Gram-negative bacteria (TCC) (≤ 1000 CFU/m^3 for TCC). The high concentration of total viable bacteria and fungi in the air within the processing area of sawmill factories in Okada, Edo State, Nigeria may most likely induce occupational asthma and other respiratory diseases in workers and merchants if adequate precautionary measures are not regularly observed in the work area during sawing of wood.

Micrococcus, *Staphylococcus*, *Bacillus*, *Klebsiella* and *Serratia* species were the bacteria that were isolated from the air and sawdust samples collected from the sawmill factories (Table 2) while the fungi that were found include *Saccharomyces*, *Aspergillus*, *Cladosporium* and *Penicillium*. The parasite that was detected in sawdust samples was mainly *Entamoeba* species. Park et al. (2010), Oppliger et al. (2005), Idu et al. (2019) as well as Baranu and Edmund (2019) have all reported similar microorganisms in other sawmill factories. The bacteria in the organic dust may infiltrate into the lungs and release endotoxins that may most likely cause adverse health effects in humans that are exposed to sawdust. The airborne fungi may also cause chronic infections such as pulmonary aspergillosis and aspergilloma, particularly in immune-compromised humans that are exposed to sawdust.

CONCLUSION

High levels of bacteria and fungi were present in bioaerosols of the organic dust generated by processing operations in all the sawmill factories examined as indicated by WHO and other international

occupational exposure limit guidelines. Thus, workers in these sawmill factories and other wood merchants who visit the sawmill processing area are most likely exposed to airborne contaminants that may cause occupational asthma, ophthalmic irritations and other respiratory diseases as well as dermatitis. Hence it is recommended that sawmill workers and other visitors within the processing area wear personal protective gadgets such as nose masks, respirators, helmets and safety boots.

REFERENCES

Alwis KU, Mandryk J and Hocking AD (1999). Exposure to biohazards in wood dust: bacteria, fungi, endotoxins, and (1→3)-beta-Dglucans. J Occup Environ Hyg., 14 (9): 598 – 608.

Baranu BS and Edmund E (2019). Microorganisms Isolated from Sawmill and Poultry Farm and their Long-Term Health Effects in Human Health. Int J Curr Microbiol Appl Sci., 8 (3): 809 – 821.

Barrow GI and Feltham RKA (2003). Cowan and Steel's Manual of Medical Bacteria. 3rd ed. Cambridge University Press: London.

Demers PA, Teschke K, Davies HW, Kennedy SM and Leung V (2000). Exposure to dust, resin acids and monoterpenes in softwood lumber mills. Am Ind Hyg Assoc J., 61: 521 – 528.

Fekadu S and Getachewu B (2015). Microbiological assessment of indoor air of Teaching hospital wards: a case of Jimma University specialized hospital. Ethiop J Health Sci., 25 (2):117 – 122.

Idu EG, Nwaubani DA and Inyang MP (2019). Isolation, characterization and identification of bacteria emanating from sawdust generated in Ahiake saw mill, Umuahia, Abia State, Nigeria. Int J Sci Eng Res., 10: 1547 – 1555.

Larone DH (1995). In: Medically important fungi - a guide to identification. ASM press: Washington, DC.

Nevalainen A and Morawaska L (2009). Biological agents in indoor environments. Assessment of health risks, work conducted by a WHO expert group between 2000 – 2003, QUT, 2009.

Oppliger A, Rusca S, Charrière N, Vu Duc T and Droz PO (2005). Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills. Ann Occup Hyg., Annals of Occupational Hygiene 49 (5): 385 – 391.

Park H, Park H and Lee I (2010). Microbial exposure assessment in sawmill, livestock feed industry, and metal working fluids handling industry. Saf Health Work. 1 (2): 183 – 191.

Parameshwarappa KD, Chandrakanth C and Sunil B (2012). The prevalence of intestinal parasitic infestations and the evaluation of different concentration techniques of the stool examination. J Clin Diagn Res., 6 (7): 1188 – 1191.

Pasquarella C, Pitzurra O and Savino A (2000). The index of microbial air contamination (review). J Hosp Infect., 46 (4): 241 – 256.

Public Health England (2014). Preparation of samples and dilutions, plating and sub-culture. In: Microbiology Services Food Water and Environmental Microbiology Standard Method. Public Health England: London.

Sawane A and Sawane M (2017). Impact of bioaerosol exposure on respiratory health of saw-mill workers. Natl J Physiol Pharm Pharmacol., 7 (10): 1036 – 1040.

Straumfors A, Olsen R, Daae HL, Afanou A, McLean D, Corbin M, Mannetje AT, Ulvestad B, Bakke B, Johnsen

HL and Douwes J (2018). Exposure to wood dust, microbial components, and terpenes in the Norwegian sawmill industry. Ann Work Expo Health., 62 (6): 674 – 688.