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Bacterial contamination of coffee and personal exposure to inhalable dust and endotoxin in primary coffee processing factories in Ethiopia

Samson Wakuma Abaya[1,A-F], Magne Bråtveit[2,A,C,E-F] ✉, Abera Kumie[1,A,C,E-F], Wakgari Deressa[1,A,C,E-F], Bente Elisabeth Moen[3,A,C,E-F]

[1] *Department of Preventive Medicine, School of Public Health, College of Health Sciences, Addis Ababa University, Ethiopia*
[2] *Department of Global Public Health and Primary Care, University of Bergen, Norway*
[3] *Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Norway*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. Endotoxins from gram-negative bacteria might be released when the coffee cherries are processed and may cause respiratory health problems among workers in the coffee industry. The relationship between bacterial contamination and occupational exposure to endotoxin levels has not been thoroughly explored previously in primary coffee processing factories in Ethiopia, or elsewhere. The aim of this study was to characterize the level of personal endotoxin exposure and its relations with bacterial contamination of coffee cherries in such factories in Ethiopia.

Materials and method. A cross-sectional study was conducted from March 2020 – February 2021 in 9 primary coffee processing factories in 3 regions in Ethiopia. A total of 180 personal air samples were collected to analyze workers' exposure to inhalable dust and endotoxin. Correlation tests were performed to assess the relationship between total bacteria and endotoxin levels and between inhalable dust and endotoxin levels.

Results. The geometric mean (GM) of personal inhalable dust exposure among machine room workers and hand pickers were 9.58 mg/m^3 and 2.56 mg/m^3, respectively. The overall GM of endotoxin exposure among machine room workers and hand pickers were 10,198 EU/m^3 and 780 EU/m^3, respectively. Gram-negative bacteria were found in all 54 coffee samples. The correlation between inhalable dust and endotoxin exposure was significant ($r=0.80$; $P<0.01$).

Conclusions. About 92% of the samples from hand pickers and all samples from machine room workers exceeded the occupational exposure limit of 90 EU/m^3 recommended by the Dutch Expert Committee on Occupational Standards. Prevention and control of bacterial contamination of the coffee in primary coffee processing are suggested to reduce endotoxin exposure that might cause respiratory health problems among coffee workers.

Key words

endotoxin, exposure assessment, inhalable dust, gram-negative bacteria, coffee workers

INTRODUCTION

In Ethiopia, more than 15 million people depend directly or indirectly on coffee production for their living [1]. Coffee contributes to more than 60% of the foreign currency income for the country and is therefore considered an important sector [2].

Coffee dust is generated in coffee factories during coffee handling and processing. Studies have indicated that exposure to a high level of coffee dust is likely to cause chronic respiratory symptoms and reduced lung function [3, 4]. However, a study conducted by Abaya et al. among hand pickers in primary coffee processing factories in Ethiopia, found a higher level of chronic respiratory symptoms and reduced lung function among hand pickers compared to controls, although the dust exposure was relatively low [arithmetic mean (AM)=1.6 mg/m^3] compared to the occupational exposure limit of 5 mg/m^3 for organic dust [5]. This suggests that other factors, such as bacteria/ endotoxins, might be involved in causing these symptoms and signs in the respiratory system of these workers [6].

An association between exposure to endotoxins and respiratory symptoms has been described in several studies in the agricultural industry [7–11]. Studies have indicated that endotoxin is released into the working environment when agriculture products contaminated with gram-negative bacteria are processed in the factories [12–15]. In line with this, a study conducted in primary coffee processing factories in Tanzania found a high level of personal endotoxin exposure level among the workers [4]. This study also indicated an association between exposure to cumulative endotoxin and reduced lung function, and developing respiratory symptoms [4].

The relationship between bacterial contamination and endotoxin exposure is not yet clear. No correlation between gram-negative bacterial count and endotoxin level was found in a study conducted in chicken and cattle husbandry confinement buildings [16, 17]. However, another study has indicated a weak correlation between Gram-negative bacterial levels and endotoxin levels in a live poultry market [18]. To

✉ Address for correspondence: Magne Bråtveit, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
E-mail: Magne.Bratveit@uib.no

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the best of our knowledge, no other studies have assessed the relationship between bacterial contamination and endotoxin exposure in primary coffee processing factories. Furthermore, the endotoxin exposure level in primary coffee processing factories in Ethiopia is not known. Thus, the aim of this study was to characterize personal inhalable dust and endotoxin levels, as well as to assess the relationship between the bacterial contamination of coffee and the endotoxin level. This knowledge will be beneficial for developing methods to prevent the potential adverse respiratory health effects among coffee workers.

MATERIALS AND METHOD

Characteristics of factories covered by the study. An institutional-based cross-sectional study was conducted from March 2020 – February 2021 in 9 selected primary coffee processing factories in the following regions of Ethiopia: Oromia, Addis Ababa, and Southern Nations, Nationalities and Peoples' Region (SNNPR) – 3 factories from each region. These regions were selected because they are the locations of almost all primary coffee processing factories in Ethiopia..

The study included all types of primary coffee factories; old and new (i.e., year established, 1952–2012) as well as small and large sized (i.e., production capacity 4.5 to 126 tonnes of coffee/day). If the coffee processing factories also processed other agriculture products and/or were involved in secondary coffee processing, such as roasting and grinding, they were excluded because they did not represent the majority of primary coffee factories in Ethiopia.

Characteristics of the study population. The number of personal dust and endotoxin samples was calculated based on Rappaport and Kupper who suggested repeated samples from 5–10 randomly selected individuals per exposure group [19]. In this study, each exposure group was composed of workers with similar tasks and sharing the same working environment [19]. In primary coffee processing factories in Ethiopia, 3 main departments – hand pickers, transporters, and machine room workers – have distinct characteristics in terms of tasks performed, and are assumed to constitute 3 similar exposure groups. However, transporters are not appropriate for this study as they have several tasks in a day and mostly work outside the machine room. This makes it difficult to link their endotoxin exposure to specific coffee contamination. Therefore, the 2 remaining departments (i.e., machine room workers and hand pickers) were considered for the study.

The machine room department refers to people working within a 5-meter radius from the huller machine, and includes machine operators, cleaners, and workers who feed the hopper. Hand pickers are mainly women involved in manually sorting and removing defective and discoloured coffee beans.

In each factory 5 coffee workers were randomly selected for dust sampling from the workers' registration list from each of the 2 departments. Thus, 10 persons were involved from each factory, and a total of 90 workers from the 9 primary coffee processing factories were involved in this study. These workers were re-selected for repeated dust measurement; which made the total sample size 180.

Endotoxin – sample collection and analysis. Personal endotoxin and dust sampling were conducted with an IOM sampler using Whatman Glass Microfiber Filters GF/A 25 mm with Side Kick Casella (SKC) pumps with a flow rate of 2 l/min. Pumps were calibrated before the start of sampling and at the end of sampling. During sampling, the pumps were checked every second hour. Field blanks were used to correct any weight changes during sampling. After sampling, the cassettes were capped and transported as hand luggage by airplane to the laboratory in Denmark in a sealed box suitable for preventing damage or disturbance.

Full-shift exposure measurements (range: 194–505 min) were conducted on randomly chosen days of the week, and repeated sampling on the same workers conducted the following day. The inhalable dust samples were analyzed gravimetrically using a standard microbalance scale AT261 Mettler Toledo with a detection limit of 0.01 mg/m^3 at the Aarhus University Laboratory in Denmark. Endotoxin samples were extracted by immersing the filters in 5 ml of pyrogen-free water (PFW) with 0.05% (v/v) Tween-20 [20]. All extracts of samples were stored at –20 °C until analysis in freezers.

Samples were then shaken for 60 minutes on a shaker. The extracts were analysed for endotoxin using the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test [21]. The LAL kit was obtained from the Lonza Group.

Bacterial contamination – sample collection and analysis. Samples of coffee cherries were collected twice a day (i.e., at the start of coffee processing and after the lunch break) from the machine room (the hopper) and once a day from the hand pickers area. Thus, three coffee cherry samples were collected each day. A minimum of two sampling days were required in each coffee factory, thus 6 coffee cherry samples were collected from each coffee factory. Thus, a total of 54 coffee samples were collected from the 9 coffee factories for bacterial analysis.

About 25 grams of coffee cherries were collected for one sample. The coffee cherries were collected by the principal investigator, together with 1 senior microbiologist. The coffee cherries were collected in a sterile plastic bag, labelled and transferred to ice boxes. The samples were transported from the different sample sites to the Regional Public Health Microbiology Laboratory in Addis Ababa by using a triple package. Samples were analysed within 2 hours after arrival in the laboratory. The coffee cherries were transferred to a flask containing 225ml of sterile buffered peptone water (1% peptone, 5% weight per volume NaCl) and swirled gently for 20 min using an orbit shaker (lab-line instruments.inc) [22]. Peptone water was used to make a serial dilution (1:10) for each sample. The serial dilution was made to 10^{-3} for each sample to obtain the appropriate number of colonies, ranging from 30–300. From each dilution, 1ml of the sample was plated onto plate count agar (PCA) (Park Scientific, USA) as described by the FDA [23]. The plates were then incubated at 37°C for 72hrs. All distinguishable colonies were counted. The better of 2 consecutive dilutions were used, as n_1 and n_2 to calculate the results. The total bacteria colony count was presented per gram of coffee beans (CFU/g). Bacteria were identified as gram-negative and gram-positive bacteria based on the standard Gram-stain technique [24].

A senior microbiologist from Laboratory of the Health Bureau of the Addis Ababa City Administration was involved

in the identification of bacteria. All the stained slides were cross-checked by another microbiologist from the Ethiopian Public Health Institute.

Data analysis. The results were described using arithmetic mean, standard deviation, geometric mean, and geometric standard deviation. Correlation tests were performed to check the relationship between the number of colonies and endotoxin units, as well as between the inhalable dust level and endotoxin units on natural logarithm-transformed data. For each day of measurement in the respective factories, the AM of endotoxin was calculated for the machine room and hand pickers, respectively. Similarly, for each day of measurement in the respective factories, the AM of colony count for the machine room and hand pickers was calculated, respectively. In total, 18 AMs for each group were included in the correlation analysis. (i.e., 9 machine rooms and 9 hand pickers departments times 2 days of measurements). A correlation was ran between these endotoxin estimates and the AM of colony count for the corresponding day and department. The analyses were performed using SPSS version 26.

Exposure limits. Endotoxin results obtained in this study were compared with the Dutch Expert Committee on Occupational Standards which recommends 90 Endotoxin Units (EU)/m³ as the occupational exposure limit for endotoxin [25]. Dust exposure was compared to the Norwegian occupational exposure limit of 5 mg/m³ for organic dust [26].

Ethical considerations. Ethical approval was obtained from the Department of Preventive Medicine Ethics Committee and Institutional Review Board (IRB) of the College of Health Sciences of Addis Ababa University. Informed consent was obtained from participants. Participation in this study was completely voluntary. The study did not involve any inhumane treatment of the participants who did not experience any physical harm, social discrimination, psychological trauma, or economic loss due to the present study.

RESULTS

A total of 180 inhalable dust samples were collected from 9 primary coffee processing factories. Seven samples were excluded from the analysis, 3 due to pump failure and 4 inhalable dust. Exposure among machine room workers and hand pickers were 9.58 mg/m³ (range 2.24–57.35 mg/m³) and 2.56 mg/m³ (0.73–7.02 mg/m³), respectively (Tab. 1). The overall GM endotoxin exposure among machine room workers and hand pickers were 10,198 EU/m³ and 780 EU/m³, respectively.

Correlation between dust and endotoxin. The overall correlation between inhalable dust and endotoxin exposure was significant, with a correlation coefficient (r) of 0.80 ($P < 0.01$) (Fig. 1). The correlation was higher among workers in the machine room department compared to hand pickers (r=0.63 and r=0.52, respectively).

Bacterial contamination of coffee and endotoxin. Gram-negative bacteria were found in all 54 coffee samples. The overall colony counts were significantly higher ($P<0.001$) in coffee samples from the machine room (265×10^6 CFU/m³) than from the hand-picking area (144×10^6 CFU/m³) (Tab. 2). There was a significant correlation between bacterial colony count in number and endotoxin levels (EU/m³; r=0.74; $P < 0.01$) (Fig. 2).

Table 1. Personal inhalable dust and endotoxin in nine primary coffee processing factories in Ethiopia

Region	Factory	Department	Inhalable dust (mg/m³)					Endotoxin (EU/m³)				
			NW	NS	AM	Range	GM(GSD)	NW	NS	AM	Range	GM(GSD)
SNNPR	A	Hand picker	5	9	1.90	0.82-2.79	1.77(1.52)	5	9	440	295-695	419(1.36)
		Machine room	5	10	6.85	2.24-12.88	5.93(1.79)	5	10	6840	1459-20228	4628(2.48)
	B	Hand picker	5	10	1.73	0.76-3.1	1.62(1.49)	5	10	488	31-1068	365(2.72)
		Machine room	5	10	6.16	2.37-14.29	5.42(1.70)	5	10	22382	2058-80571	11614(3.63)
	C	Hand picker	5	10	1.80	0.73-2.76	1.67(1.52)	5	10	1662	505-4561	1312(2.03)
		Machine room	5	9	8.98	4.56-16.27	8.17(1.57)	5	9	19175	8672-26265	18033(1.48)
Oromia	D	Hand picker	5	10	2.40	1.30-3.48	2.29(1.40)	5	10	1056	386-2445	888(1.82)
		Machine room	5	10	10.78	2.64-23.14	7.77(2.39)	5	10	18478	3520-51707	12332(2.64)
	E	Hand picker	5	10	3.93	2.16-5.70	3.78(1.31)	5	10	2664	355-6298	2121(2.23)
		Machine room	5	10	16.54	8.58-35.33	15.18(1.51)	5	10	36537	8989-82292	30638(1.95)
	F	Hand picker	5	10	2.81	1.59-5.00	2.59(1.49)	5	10	1201	354-2826	925(2.18)
		Machine room	5	8	11.33	4.40-20.16	10.07(1.68)	5	8	24585	8389-42401	21590(1.75)
Addis Ababa	G	Hand picker	5	10	2.37	1.05-3.40	2.20(1.48)	5	10	107	15-230	83(2.27)
		Machine room	5	10	10.85	3.07-39.23	8.00(2.14)	5	10	2671	199-11795	1495(3.16)
	H	Hand picker	5	10	5.04	3.69-7.02	4.90(1.23)	5	10	3813	903-6269	3290(1.88)
		Machine room	5	7	35.42	9.65-57.42	30.88(1.86)	5	7	33142	8597-47608	29436(1.80)
	I	Hand picker	5	10	3.85	1.79-5.09	3.71(1.38)	5	10	1254	312-2047	1064(1.93)
		Machine room	5	10	13.68	6.01-25.76	12.30(1.62)	5	10	6864	1585-14317	5884(1.84)
Hand pickers in all factories			45	89	2.88	0.73-7.02	2.56(1.67)	45	89	1421	15-6289	780(3.46
Machine room workers in all factories			45	84	12.71	2.24-57.35	9.58(2.12)	45	84	18321	199-82292	10,198(3.49)

NW - number of workers; NS - number of samples; AM - arithmetic mean; GM - geometric mean; GSD - geometric standard deviation; SNNPR - Southern Nations - Nationalities& Peoples' Region

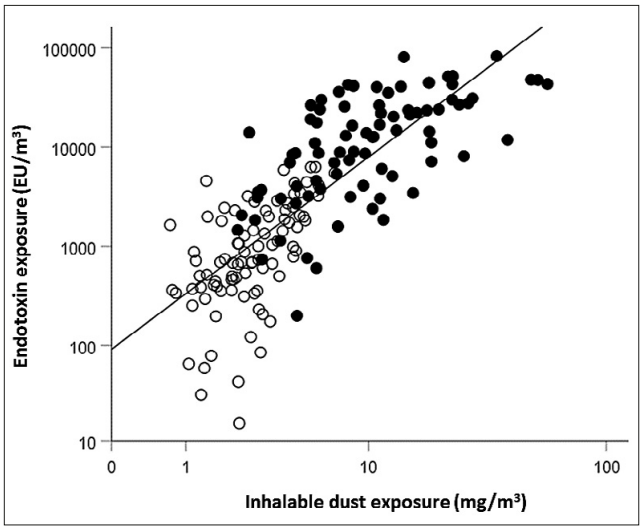

Figure 1. Correlation between 173 personal samples of inhalable dust (mg/m^3) and endotoxin (EU/m^3) among hand pickers (open circles) and machine room workers (closed circles) in 9 coffee factories

Table 2. Microbial load in coffee bean samples from nine primary coffee processing factories in Ethiopia

Factory	Department	n	Colony count
A	Hand picker	2	109×10 ^{6}
	Machine room	4	261×10 ^{6}
B	Hand picker	2	130×10 ^{6}
	Machine room	4	276×10 ^{6}
C	Hand picker	2	165×10^6
	Machine room	4	274×10^6
D	Hand picker	2	149×10^6
	Machine room	4	252×10^6
E	Hand picker	2	167 x10^6
	Machine room	4	275×10^6
F	Hand picker	2	109×10^6
	Machine room	4	270×10^6
G	Hand picker	2	162×10^6
	Machine room	4	252×10^6
H	Hand picker	2	211×10^6
	Machine room	4	265×10^6
I	Hand picker	2	100×10^6
	Machine room	4	275×10^6
All hand picker samples		18	144×10^6
All machine room samples		36	265×10^6

n=number of coffee samples

DISCUSSION

The GM of personal endotoxin exposure among machine room workers and hand pickers were considerably higher than the occupational exposure limit of 90 EU/m^3 recommended by the Dutch Expert Committee on Occupational Standards [25]. Similarly, GM of personal inhalable dust exposure among machine room workers was higher than the recommended Occupational Exposure Limit (OEL) of 5 mg/m^3 [26]. Gram-negative bacteria were found in all coffee samples and there was a strong correlation between colony count in number and endotoxin levels in the coffee factories.

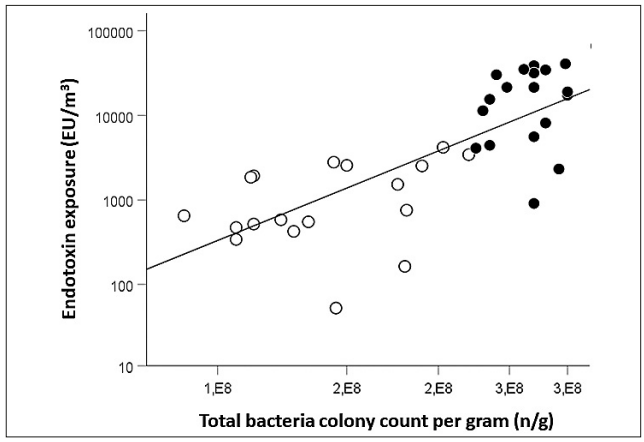

Figure 2. Correlation between total bacteria colony count per gram in coffee bean samples and personal endotoxin exposure (EU/m^3) among hand pickers (open circles) and machine room workers (closed circles) in nine coffee factories

In this study, the GM personal endotoxin exposure among machine room workers was much higher than in the study conducted among coffee workers in Tanzania reporting a GM of 3,556 EU/m^3 [4]. This difference might be due to the differences in coffee type, coffee processing method at the farm, and coffee handling in the coffee factories. In addition, the number of machines in the room could contribute to this difference. In Ethiopia, all machines were located in 1 big hall whereas in 2 out of 4 of the studied coffee factories in Tanzania, the machines were located in different rooms.

Personal endotoxin exposures among hand pickers in the current study were higher than the study by Moen et al. among hand pickers in Tanzania (183 EU/m^3) [27]. The difference could be partly related to the study design, in the present study 9 different factories were included, while in the previous study, only 1 coffee factory was included. In addition, the coffee type and processing method could contribute to the difference. Furthermore, the difference could be related to sitting position – all hand pickers in Tanzania sit on a chair and sort the coffee beans on a long table or a conveyor belt, whereas the majority of hand pickers in Ethiopia perform their work while sitting on the ground.

The GM of personal inhalable dust among the machine room worker (9.58 mg/m$^{3)}$ was also higher than that reported for total dust exposure among comparable job groups in Tanzanian primary coffee factories (GM 2.5 mg/m^3) [4]. The difference might be related to the difference in coffee type, coffee processing, and machine type.

The GM personal inhalable dust among the hand pickers (GM 2.56 mg/m^3) was below the recommended Norwegian occupational exposure limit [26]. This was consistent with previous studies conducted in Tanzania and Ethiopia among hand pickers that reported 0.9 mg/m^3 and 1.08 mg/m^3, respectively [27, 28]. However, the endotoxin exposure level among hand pickers was significantly higher than the recommended limit of 90 EU/m^3 recommended by the Dutch Expert Committee on Occupational Standards [25].

Another factor that may contribute to the generally higher exposures in the present compared to previous studies is the difference in sampling efficiency between the dust samplers used. In the present study, dust and endotoxins are sampled by IOM inhalable samplers, while closed-face cassettes for 'total' dust samplers have been used in previous exposure studies in coffee factories [28]. It is well recognized that

the 'total' dust samplers are known to underestimate the inhalable dust levels, especially for larger particle sizes within the inhalable dust fraction [28, 30].

In the current study, the overall correlation between inhalable dust and endotoxin exposure was strong (r = 0.8). This finding was consistent with previous studies in wood and cotton processing plants that assessed the correlation between inhalable dust and endotoxin [30, 31].

This study showed the presence of gram-negative bacteria in all coffee samples. In a previous study by the authors, the results indicated the presence of gram-negative bacteria at the pre-processing stage before the coffee enters the primary coffee factories [32]. This indicates that coffee can be contaminated with gram-negative bacteria at any stage of coffee processing. The availability of gram-negative bacteria in the final stage of coffee processing might not cause risk to public health due to the high temperatures used during roosting. However, this could increase the risk of respiratory problems for coffee workers as these gram-negative bacteria release endotoxins upon disruption of the intact bacteria (death, cell lysis) [6, 33].

The current study also indicates that there was a strong correlation between the 'total' bacteria colony count and endotoxin levels. This finding was consistent with previous studies reporting positive correlations between the number of gram-negative bacteria and endotoxin concentration [34, 35]. These findings indicate the importance of proper prevention and control measures to reduce coffee contamination by gram-negative bacteria, thereby reducing endotoxin exposure at workplaces. The results also indicated that the bacterial contamination of coffee samples in the machine room is significantly higher than coffee samples from the hand-picking area. This may be related to the coffee cleaning: coffee samples in the machine rooms were cleaned before the parchment is removed from the coffee beans, while the coffee samples in the hand picking go through a series of cleaning, screening, sorting, and grading processes.

One of the limitations of this study was that it attempted to establish a relationship between bacterial load and endotoxin. However, bacterial load included both gram-positive bacteria and gram-negative bacteria, but only gram-negative bacteria are responsible for endotoxin exposure. Therefore, future studies should isolate gram-negative bacteria and determine their association with endotoxin levels. Another limitation of the study is the assignment of the CFU in the departments to all workers in the department on the same day. One of the strengths of this study, however, is that dust samplers were used that meet the CEN/ISO/ACIGH inhalable dust criteria [36] instead of the 'total' dust samplers that do not comply with this definition [37].

CONCLUSION

About 92% of samples from hand pickers and 100% of samples from the machine room exceeded the occupational exposure limit of 90 EU/m^3 recommended by the Dutch Expert Committee on Occupational Standards. The GM personal inhalable dust among the hand pickers was below the recommended Norwegian occupational exposure limit. There was a strong correlation between inhalable dust and endotoxin exposure. Similarly, there was also a strong correlation between colony count and endotoxin levels. Gram-negative bacteria were found in all coffee samples, which indicates the need for the prevention of bacterial contamination of coffee at each stage of coffee processing to reduce endotoxin levels.

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