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Impact of Lead Exposure on Fecal Microbiome in Workers at a PVC Manufacturing Plant in Rayong Province, Thailand

Chatchawan Praditwongsin[1] and Sumeth Wongkiew[1, 2*]

[1] *Industrial Toxicology and Risk Assessment Graduate Program,*
Department of Environmental Science, Faculty of Science,
Chulalongkorn University, Bangkok, Thailand
[2] *Water Science and Technology for Sustainable Environment Research Unit,*
Chulalongkorn University, Bangkok 10330, Thailand

*Corresponding author: sumeth.w@chula.ac.th

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Abstract

Lead exposure in industrial settings poses significant health risks. The relationship between lead exposure and changes in the fecal microbial community remains largely unexplored while its adverse effects on human health are well documented, thereby creating a crucial gap in the understanding of its broader health implications. This study investigated the fecal microbiota among workers in a lead-using polyvinyl chloride factory, comparing those with blood lead levels (BLLs) above and below 3.06 µg/dL and between lead-exposed and nonexposed groups. Results revealed a strong association between lead exposure and BLLs, notably at BLLs of 3.06µg/dL, with significant shifts observed at a threshold of < 10 µg/dL. BLLs associated with alterations in the fecal microbiome. Key genera, such as *Blautia, Dorea, Streptococcus, Collinsella,* and *Bifidobacterium*, were notably affected, indicating the influence of lead on gut microbiota and potential health implications. This study identified specific microbial biomarkers of lead exposure, including an increased presence of *Blautia* and *Streptococcus* and a decrease in *Bifidobacterium* in exposed workers. These insights provide avenues for developing noninvasive diagnostics for detecting lead exposure. The results highlight the need for protective measures against lead exposure in the workplace and reveal the use of the fecal microbiome as a sensitive indicator of environmental pollutants, supporting improved safety protocols and monitoring practices in industrial environments, although further research with larger cohorts is required.

Keywords: Fecal microbiome; Industrial safety; Lead exposure; Microbial biomarkers; Occupational health

1. Introduction

Lead exposure continues to pose a significant public health risk globally. A blood lead level (BLL) of ≥ 10 µg/dL was considered a level of concern (Centers for Disease Control and Prevention, 2013). However, research indicates that BLLs of 10 µg/dL or even < 5 µg/dL still cause severe neurobehavioral disorders (Huang, 2022; Khoshnamvand *et al.,* 2021). Occupational lead exposure is notably prevalent in the United States, affecting hundreds of thousands of workers in both general and construction industries (Shaffer and Gilbert, 2018). This situation is mirrored globally, where the incidence of heavy metal poisoning, including lead, has demonstrated an increasing trend over the years (Balali-Mood *et al.,* 2021; Mills and Adderley, 2017). For instance, the Epidemiology Division of the Department of Disease Control, Ministry of Public Health, Thailand, emphasized an increase in heavy metal poisoning cases over the past decade, with lead poisoning being a significant contributor to morbidity and mortality rates (Kenyota and Jinsart, 2022; Thanapop *et al.,* 2007). Such statistics emphasize the urgent need for global and national policies aimed at controlling, reducing, and ultimately ceasing the use of lead to prevent disease and health hazards associated with its exposure.

Lead is administered to the human body through three exposure routes, namely inhalation, dermal, and ingestion (Wongsasuluk *et al.,* 2020). Industrial sources of lead exposure include electronic industries, batteries, paint production, ceramics, jewelry making, waste treatment, and lead smelting (García-Lestón *et al.,* 2011; Kenyota and Jinsart, 2022). The database from U.S. monitoring studies highlights primary industrial lead sources including lead-based paint work, metal processing, and lead-acid battery manufacturing (Koh *et al.,* 2015). Additionally, environmental exposure occurs through contaminated petrol, electronic waste, water, the food chain, and other fuel sources (Islam *et al.,* 2018; Oliveira *et al.,* 2005). For example, in the production of lead-acid batteries, hazardous processes such as lead oxide preparation and grid casting expose workers to high atmospheric lead levels, primarily through inhalation and ingestion (Kalahasthi *et al.,* 2014). Once exposed, lead predominantly accumulates in bones, teeth, and soft tissues, including the liver and kidneys, posing long-term health risks (Hemmaphan and Bordeerat, 2022; Kenyota and Jinsart, 2022). Lead toxicity, particularly its effects on the nervous system, reproductive health, endocrine disorders, and the risk of anemia and hypertension, has been well documented (Islam *et al.,* 2018; Nava-Ruiz *et al.,* 2012; Rehman *et al.,* 2018). Pregnant women and children are especially vulnerable because lead crosses the placental barrier and becomes a significant risk to fetal development and child health (Mahdi *et al.,* 2023; Rebelo and Caldas, 2016).

The association between occupational exposure and BLLs has been a focal point of research, emphasizing the chronic nature of exposure in various industries and its potential to cause severe health outcomes, including mutations and cancer (Hemmaphan and Bordeerat, 2022; Kalahasthi *et al.,* 2014; Mohammadyan et al., 2019). Studies have used logistic regression methods to investigate the association between BLLs and specific morbidities, emphasizing the need for comprehensive BLL and health symptom evaluations among workers in lead-related industries. This approach demonstrates the extensive use of lead across numerous sectors and the critical need for targeted research to understand and mitigate the health implications of lead exposure (Leelapongwattana and Bordeerat, 2020; Paoliello and De Capitani, 2007).

In recent years, the gut microbiome has appeared as a crucial factor in human health, regulating immune regulation, metabolism, and disease susceptibility (Marchesi *et al.,* 2016). The microbial ecosystem within the gastrointestinal tract, predominantly composed of the phyla *Bacteroidetes* and *Firmicutes*, plays a vital role in maintaining health and preventing disease (Eckburg *et al.,* 2005). Gut microbiome disruptions, whether due to dietary changes, environmental pollutants, or toxic substances, such as lead, adversely affect human health (Liu *et al.,* 2021). The role of the gut microbiome in conditions ranging from inflammatory bowel disease and obesity to neurological disorders emphasized its importance as a new frontier in health research (Clarke *et al.,* 2014; Sommer and Bäckhed, 2013). This emerging field of study indicates that gut microbiome alterations could serve as indicators of environmental toxin exposure, including lead, potentially offering new avenues for diagnosis and treatment.

Exploring the intricate relationship between environmental exposure and human health has crucial implications for public health policy and occupational safety. The identification of the gut microbiome (via fecal analysis) and microbial biomarkers related to lead exposure opens new avenues for disease prevention and health promotion among vulnerable populations. Moreover, contribution to the growing body of evidence supporting the role of the gut microbiome is warranted in mediating the health effects of environmental pollutants, emphasizing the need for integrated approaches to health surveillance and environmental protection (Borre *et al.,* 2014; Larsen *et al.,* 2010). Unraveling the intricate connections between the environment, gut microbiome, and human health, these insights could inform the development of more holistic and effective public health strategies to combat the enduring challenge of lead exposure. Furthermore, understanding the relationship between lead exposure and gut microbiome alterations could contribute to the development of more effective strategies for monitoring and mitigating the health risks associated with lead in occupational settings (Hertzberg *et al.,* 2022).

Therefore, this study investigates the impact of lead exposure on the fecal microbial community among workers in a lead-using factory. This study identified specific microbial biomarkers associated with lead toxicity by comparing the microbial profiles of lead-exposed workers with those in lead-free environments. Such biomarkers could provide insights into the mechanisms by which lead affects human health and offer potential targets for therapeutic intervention.

2. Methodology

2.1 Study site and industry

This study was conducted at a prominent industrial facility located in Rayong province, Thailand. This facility specializes in producing a wide array of products, including plastic additives, catalysts, cosmetics, pharmaceuticals, medical supplies, and components for electronic devices. A critical aspect of the production process involves the incorporation of lead compounds in manufacturing polyvinyl chloride (PVC), which are essential for improving the properties of PVC pipes and fittings. These enhancements include improved lubricity, thermal stability, weather resistance, and cost-effectiveness. This study focused on a PVC production facility that uses lead in its manufacturing processes, specifically comparing the fecal microbial communities in lead-exposed workers with those in nonexposed environments.

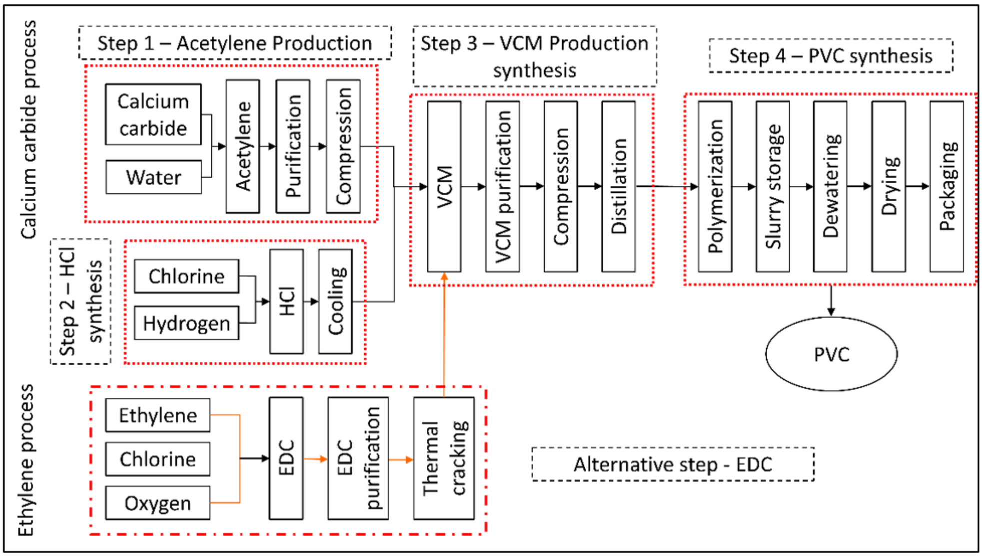

Figure 1. Industrial process of PVC production. Step 4: PVC synthesis emphasizes the stage involving lead use, where workers are exposed to lead during the packing of PVC powder. (VCM: vinyl chloride monomer, EDC: ethylene dichloride, PVC: polyvinyl chloride)

2.2 Experimental design

The experimental framework was designed to distinguish between workers exposed to lead in PVC production lines and those in control settings (nonlead-exposed), such as office environments. The experiment categorized into (1) the lead-exposed group, consisting of workers operating in areas with significant lead usage, particularly on the production floor, and (2) the control (nonlead-exposed) group, comprising workers stationed in lead-free zones, including administrative offices. The lead-exposed group comprised workers actively engaged in the PVC production line (step 4 in Figure 1), and exposure is consistent throughout their working hours. The step 4 is critical for identifying the sampling group for lead exposure categorization (Figure 1). Previously, ethylene dichloride (EDC) dehydration was used to produce vinyl chloride monomer (VCM). However, the oxychlorination process of ethylene is now predominantly used to produce VCM in Thailand (Figure 1). In contrast, the control group included employees performing administrative duties within environments free from lead exposure (not included in Figure 1), working 8 hours per day, 5 days a week, thereby eliminating direct lead contact. The duration of exposure for the lead-exposed group is directly linked to their length of service in specific roles on the production floor, working 12 hours per day, 4 – 5 days a week.

Secondary data on BLLs, obtained upon request from the company's annual health checkup records, were used to assess the risk associated with elevated BLLs. BLLs obtained from the company for this study were double-blinded; thus, the identities of the sample owners were not disclosed and unknown. The study included 15 BLLs from the lead-exposed group and 37 from the control group, totaling 52 records for secondary BLL data. Given the use of secondary data without personal identifiers, this research was deemed exempt from requiring ethical approval for research involving human subjects.

2.3 Fecal sample collection and risk assessment

This study collected fecal samples as part of the company's annual health checkup, with 10 samples from the lead-exposed group and 12 samples from the control (nonlead-exposed) group. The identities of the sample providers were unknown. Fecal samples, residual from routine health checkup procedures and not identified by any linking codes to ensure double-blinded anonymity. The samples were labeled using Lead and Control for both the lead-exposed and control groups, respectively. Given the use of residual fecal samples without personal identifiers in this context, this research was deemed exempt from requiring ethical approval for research involving human subjects.

A comprehensive analysis was conducted in these samples to compare the fecal microbial community compositions between the exposed and nonexposed groups. Risk assessment was conducted using odds ratios derived from BLL data, with levels ranging from 3.06 (the average level in the industrial area of Rayong province, 2014) (Sripaung, 2020) to 4, 5, 10, and 15 µg/dL, aiming to identify the potential health risks associated with lead exposure in the workplace. The odds ratio (OR) was used to compare the odds of a health outcome (BLL) occurring in the exposed group (a/b) to the odds in the control group (c/d) as follows:

$$\text{Odds Ratio (OR)} = (a/b)/(c/d)$$

Where:
a is the number of adverse outcomes (BLL > cut-off) in the exposed group,
b is the number of non-outcomes (BLL < cut-off) in the exposed group,
c is the number of adverse outcomes (BLL > cut-off) in the control group,
d is the number of non-outcomes (BLL < cut-off) in the control group.

2.4 Microbial community analysis

Metagenomic DNA extracted from the fecal samples was analyzed to characterize the microbial community structure. The 16S rRNA gene was amplified, targeting the V3 – V4 variable regions, and sequenced on an Illumina MiSeq platform at the Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand), as described by Wongkiew *et al.* (2023). This sequencing effort provided a detailed overview of the microbial diversity and composition present in the fecal samples of both groups.

2.5 Statistical analysis and data visualization

The Mothur software suite (Schloss *et al.,* 2009) was used for bioinformatic analysis of the microbiome data, with STAMP for examining taxonomic and functional profiles for further statistical evaluations (Parks *et al.,* 2014). Predictive functional analysis based on 16S rRNA gene data was performed using Tax4Fun2 (Feng *et al.,* 2017), providing insights into the potential metabolic pathways influenced by lead exposure. This comprehensive methodological approach ensures a robust analysis of the effect of lead exposure on the fecal microbiome of workers, integrating advanced chemical analysis techniques with in-depth microbial community profiling to investigate the health implications of occupational lead exposure.

3. Results and Discussion

3.1 Cut-off BLLs associated with occupational lead exposure

This study identified a pronounced association between occupational lead exposure and low BLLs, particularly highlighting those < 10 µg/dL as a critical cut-off level (see odds ratio in Table 1). Notably, a BLL of 3.06 µg/dL, which represents the average level among lead-exposed workers in Rayong province in 2015 (Sripaung, 2020), demonstrated a strong association. While cut-off levels exceeding 10 µg/dL also indicated an association (with ratios of > 1), these results were not statistically conclusive, indicating that BLLs above this threshold may be rare in this lead-using manufacturing process where lead exposure only slightly elevates BLLs. Consequently, using a cut-off of > 10 µg/dL may not be an effective baseline for evaluating workers in lead-related industries, although it could be appropriate for identifying toxic threshold levels. In contrast, establishing a baseline at 3.06 µg/dL provided a more effective surveillance indicator for the initial monitoring of BLLs within the lead industry.

Consistent with other research, results from this study support the rationale for setting BLLs associated with lead exposure at more conservative levels rather than near harmful levels of > 10 µg/dL. This approach aligns with the consensus that no safe threshold for lead exposure exists. The evidence from this study aligns with previous studies indicating that lower levels (e.g., 2 – 5 µg/dL) are more precisely measurable and provide a benchmark for successful prevention strategies (Gilbert and Weiss, 2006), which is crucial for preventing several diseases. For instance, a study in the United States emphasized the cardiovascular effects of lead exposure at levels as low as 0 – 10 µg/dL, revealing a significant association between BLLs and key cardiovascular markers, such as diastolic blood pressure and high-density lipoprotein cholesterol, across various occupational groups (Obeng-Gyasi *et al.,* 2018). Similarly, Balachandar *et al.* (2020) revealed that males with occupational lead exposure demonstrated significantly higher BLLs (BLLs: 4.8 – 66.4 µg/dL), lower sperm counts, reduced sperm motility, and elevated serum prolactin levels compared with their unexposed counterparts (BLLs: 1.3 – 28.2 µg/dL). Moreover, these results, along with environmental exposure levels, indicate that nephrotoxic effects from lead persist even at relatively low exposure levels, typically associated with environmental rather than occupational levels. Specifically, workers with higher BLLs (high concentrations of 4.6 µg/dL) demonstrated more significant estimated glomerular filtration rate reductions and a 49% higher risk of chronic kidney disease (CKD) compared with those with lower BLLs (1.5 – 2.9 µg/dL). Importantly, the risk of CKD was apparent at a median lead level of 2.9 – 4.6 µg/dL, emphasizing the potential health effects at levels currently considered low (Harari *et al.,* 2018).

In conclusion, the research contributes to the growing body of evidence that lead exposure has no safe level, emphasizing the need for measures to reduce lead exposure across all occupational settings. Future studies should further investigate the cardiovascular and other health effects of lead at levels of < 3.06 µg/dL to inform more protective public health policies and occupational standards. Given the observed adverse effects at BLLs of < 10 µg/dL, particularly near the 3.06 µg/dL mark, the results advocate for a reevaluation of occupational and public health standards for lead exposure. The subsequent section investigates the effect of BLLs, specifically at 3.06 µg/dL, on the fecal microbial community among the lead-exposed group, aiming to elucidate the association between BLLs and fecal microbiota composition, which could be biomarkers and predict microbial pathways associated with lead exposure.

3.2 Top microbial community abundances in workers with BLLs above and below 3.06 µg/dL

The analysis of all samples (Figure 2A) revealed that the most abundant genera at the operational taxonomic unit (OTU) included *Blautia* (phylum *Firmicutes*; class Clostridia; order Lachnospirales; family *Lachnospiraceae*), *Dorea* (Firmicutes; Clostridia; Lachnospirales; *Lachnospiraceae*), Streptococcus (Firmicutes; Bacilli, Lactobacillales; *Streptococcaceae*), *Collinsella* (Actinobacteriota; Coriobacteriia; Coriobacteriales, *Coriobacteriaceae*), and *Bifidobacterium* (Actinobacteriota; Actinobacteria; Bifidobacteriales; *Bifidobacteriaceae*). The overall microbial community structure did not significantly differ between samples with BLLs above and below 3.06 µg/dL (Figure 2B), but certain genera demonstrated distinct variations.

The high average relative abundances of *Blautia*, *Dorea*, and *Streptococcus* were 41.5%, 6.4%, and 6.48%, respectively, in fecal samples with BLLs of > 3.06 µg/dL. This contrasts with the lower relative abundances of *Blautia* (18.5%), *Dorea* (2.4%), and *Streptococcus* (1.8%) in samples with BLLs of < 3.06 µg/dL. Conversely, samples with BLLs of < 3.06 µg/dL exhibited higher abundances of *Collinsella* (24.3% for BLL of < 3.06 µg/dL vs. 13.1% for BLL of >3.06 µg/dL) and *Bifidobacterium* (24.3% for BLL of < 3.06 µg/dL vs. 1.9% for BLL of > 3.06 µg/dL). These major differences in microbial abundance indicate distinct bacterial groups that may be associated with the response of the gut microbiome to varying BLLs, particularly at the critical cut-off of 3.06 µg/dL, emphasizing the underlying biological processes.

This study investigated the effect of BLLs of > 3.06 µg/dL on the gut microbiome and revealed distinct microbial compositions between individuals with higher and lower BLLs. A notable dominance of the

Table 1. Associations between occupational blood lead levels (BLLs) in lead-exposed and control (Control) groups.

Groups	BLL cut-off (µg/dL)	BLL > Cut-off (µg/dL)	BLL < Cut-off (µg/dL)	Odds ratio	95% CI
Lead-exposed	3.06	15	0	Strong association (undefined)	-
Control		14	23		
Lead-exposed	4	13	2	12.0	(2.3 – 61.5)*
Control		13	24		
Lead-exposed	5	12	3	10.8	(2.5 – 46.4)*
Control		10	27		
Lead-exposed	10	7	8	7.2	(1.7 – 30.8)*
Control		4	33		
Lead-exposed	15	2	13	2.7	(0.3 – 21.1)
Control		2	35		

* Indicate a significant value of the odds ratio of >1, showing that lead exposure is associated with BLLs above the cut-off level

phylum Firmicutes, particularly genera, such as *Blautia*, *Dorea*, and *Streptococcus*, with *Blautia* being significantly prominent, was observed in those with BLLs of > 3.06 µg/dL. Conversely, individuals with BLLs below 3.06 µg/dL demonstrated a higher abundance of the phylum Actinobacteriota, especially the genera *Collinsella* and *Bifidobacterium*. Research, primarily in mouse models, revealed that *Bifidobacterium* and *Lactobacillus* possess lead-binding and resistance capabilities, potentially explaining their reduced presence in individuals with higher BLLs (Yu *et al.,* 2021). These genera are recognized for their probiotic qualities, including the production of short-chain fatty acids and immune system benefits (Yu *et al.,* 2021). Experimental exposure of mice to heavy metals, such as cadmium and lead, caused an increased abundance of families, such as *Lactobacillaceae* and *Erysipelotrichaceae*, aligning with results that higher BLLs correlate with an increase in certain microbial families and genera, including *Blautia* and *Streptococcus* (Breton *et al.,* 2013). This indicates that elevated BLLs affected the gut microbiome composition, with potential implications for host metabolic and immune health.

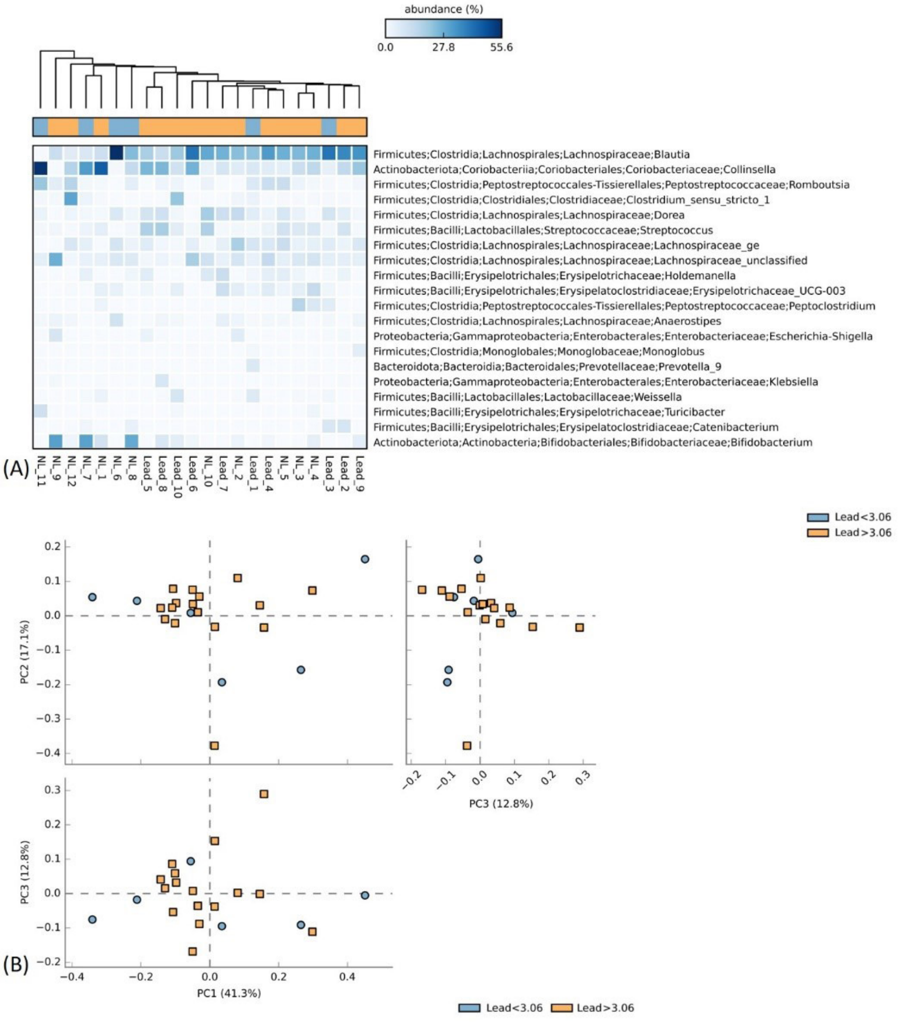

Figure 2. (A) Heatmap of the most dominant microbial genera (with a relative abundance of > 5% in at least one sample), and (B) Principal Component Analysis (PCA) illustrating the similarity in microbial community composition between groups with BLLs above (Lead > 3.06 µg/dL) and below 3.06 µg/dL (Lead < 3.06 µg/dL) based on White's nonparametric t-test (two-sided)

3.3 Top microbial community abundances between the lead-exposed and control groups

Regarding the lead-exposed and control groups, the most abundant genera remained consistent with the BLLs, including *Blautia*, *Dorea*, *Streptococcus*, *Collinsella*, and *Bifidobacterium*, with only a slight shift in microbial abundance observed (Figure 3A). The overall structure of the microbial community did not demonstrate significant differences between the lead-exposed and control groups (Figure 3B); however, certain genera, such as *Blautia* and *Bifidobacterium*, exhibited distinct variations.

In the fecal samples of the lead-exposed group, the average relative abundances of *Blautia*, *Dorea*, and *Streptococcus* were 28.5%, 5.9%, and 6.0%, respectively. Compared with the BLL cut-off, the relative abundances of *Blautia* (20.8%), *Dorea* (5.2%), and *Streptococcus* (4.6%) in the control group samples did not significantly differ from those in the lead-exposed group. Furthermore, samples from the lead-exposed group demonstrated *Collinsella* abundances (15.0% for lead-exposed vs. 17.0% for control), with no significant difference, and *Bifidobacterium* abundances (2.22% for lead-exposed vs. 9.2% for control), with a notable difference. Such significant differences in microbial abundances indicate that distinct bacterial groups may be associated with the response of the gut microbiome to industrial lead exposure. This exploration could provide insights into the interactions within the gut microbiota affected by lead exposure, emphasizing the underlying biological mechanisms.

Analyzing the dominant fecal genera in workers exposed to lead reveals intriguing relationships between gut microbiota and human health, particularly concerning metabolic and inflammatory diseases. Specifically, the genera *Blautia*, *Streptococcus*, and *Dorea* were highly abundant, indicating dominant clues. This study revealed the complexity of the association of *Blautia*, despite its probiotic properties, with various physiological conditions, including obesity, diabetes, cancer, and specific inflammatory disorders, such as irritable bowel syndrome and ulcerative colitis (Liu *et al.,* 2021). *Streptococcus* and *Dorea* were in higher abundance in breast cancer survivors without obesity in the present study, thereby supporting their contributions to host health (Smith *et al.,* 2023). Moreover, the significant presence of *Dorea* in patients with diarrhea-predominant irritable bowel syndrome, which is characterized by symptoms, such as abdominal pain and flatulence due to gas production, is associated with increased intestinal permeability (Maharshak *et al.,* 2018). The notable enrichment of *Streptococcus* in patients with CKD indicates a relationship between kidney injury and *Streptococcus*-mediated immune dysregulation (Zhao *et al.,* 2021). In contrast, a higher abundance of *Bifidobacterium* in the control group induced significant beneficial changes in the gut microbiota, thereby reducing pathogenic or opportunistic bacteria and potentially alleviating obesity-related health complications (Naumova *et al.,* 2020). However, an increase in *Collinsella*, a genus of bacteria that has been related to atherosclerosis, was found, indicating a condition of the buildup of fats, cholesterol, and other substances in and on the artery walls, which caused heart disease and may not be associated with both lead or control group (Frost *et al.,* 2019). However, this complexity emphasizes the need for detailed analysis that extends beyond genus level generalizations to the effects of specific species or strains on health.

Changes in gut microbiota including *Blautia*, *Dorea*, *Streptococcus*, *Collinsella*, and *Bifidobacterium* affect human health and may interact with environmental factors such as lead exposure (Shao and Zhu, 2020). Elevated BLLs, a major workplace risk, could exacerbate or regulate these microbial alterations. For instance, increased *Blautia* may combine with lead exposure to affect obesity risk while improving metabolic disorders such as diabetes (Liu *et al.,* 2021). In workers with high BLLs, changes in *Dorea* and *Streptococcus*, which regulate immunological regulation and gut permeability, can exacerbate illnesses such as irritable bowel syndrome and chronic kidney disease (Smith *et al.,* 2023). Lead, which affects cardiovascular health, may also alter *Collinsella* and cardiovascular risks (Frost *et al.,* 2019). Conversely, a rise in *Bifidobacterium*, which maintains a healthy gut environment, may protect against lead-related metabolic diseases (Giambò *et al.,* 2021). This interaction highlights the need to include environmental contaminants including lead when studying microbiome alterations and health.

Lifestyle factors, including dietary choices, physical activity, and exposure to environmental toxins, have significant effects on the regulation of these microorganisms. Diets that have a high amount of fiber might enhance the growth of good bacteria such as *Bifidobacterium* (Fu *et al.,* 2022). Conversely, diets that are heavy in fat may promote the growth of *Collinsella*, which can worsen cardiovascular risks (Prins *et al.,* 2023). Gaining a deeper understanding of these relationships through future studies may result in specific dietary recommendations or lifestyle treatments to control or reduce the impact of these microbial effects on health.

Therefore, this investigation indicates the need for further research to emphasize the functional activities of the intestinal microbiome. Moving beyond simple compositional analysis will elucidate the complex interactions between the gut microbiota and host health, paving the way for developing targeted therapeutic interventions

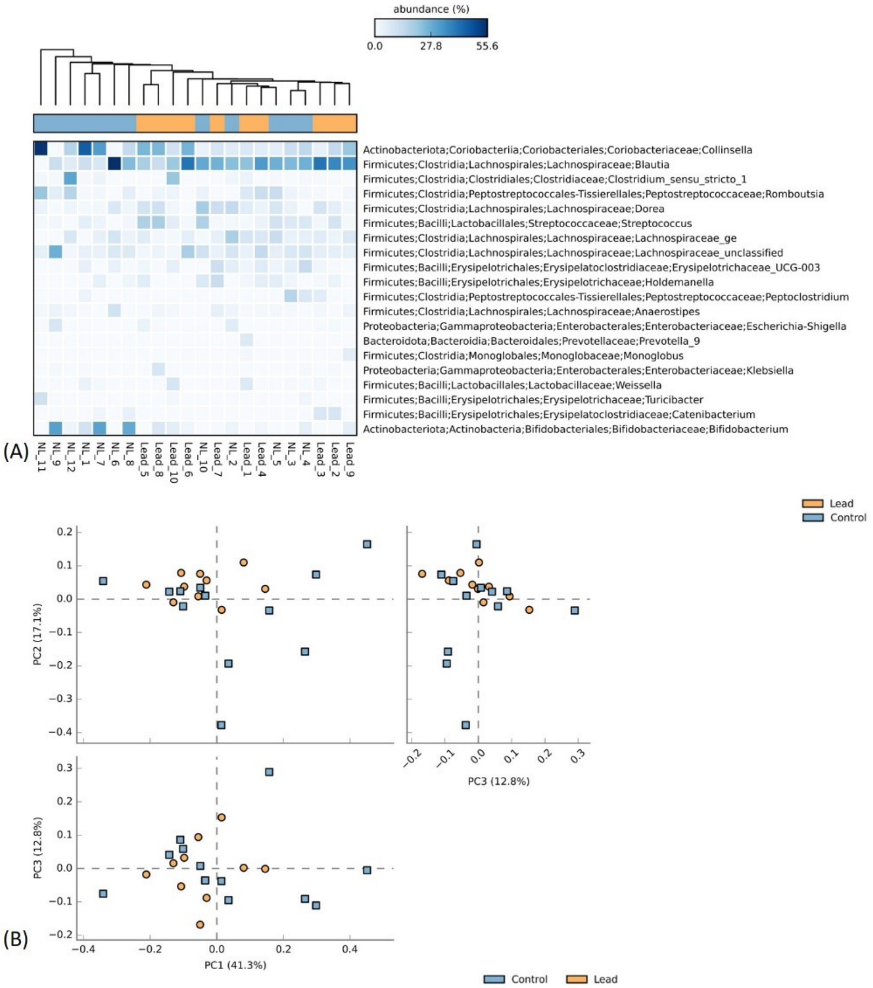

Figure 3. (A) Heatmap of the most dominant microbial genera (with a relative abundance of > 5% in at least one sample), and (B) Principal Component Analysis (PCA) illustrating the similarity in microbial community composition between lead-exposed (Lead) and control (Control) groups based on White's nonparametric two-sided t-test

that consider the significant effects of lead exposure and BLLs. Subsequent investigations should prioritize many crucial domains in order to have a deeper comprehension of the influence of gut microbiota on overall well-being. Studying the impact of particular diets on important bacteria such as *Blautia* and *Bifidobacterium* could provide valuable insights for designing dietary interventions. Conducting cohort studies might be beneficial in establishing causal links between changes in microbiota and chronic illnesses. Utilizing advanced metagenomic approaches may reveal the precise pathways via which microbiota impact health, paving the way for the development of medicines based on microorganisms.

3.4 Biomarkers associated with the above cut-off BLL of 3.06 ug/dL and lead-exposed groups

Biomarker analysis revealed that *Blautia* was the only genus significantly dominant in individuals with BLLs of > 3.06 µg/dL. *Streptococcus* and *Dorea* also revealed distinct average abundances in the group with BLLs of >3.06 µg/dL, whereas *Collinsella* and *Bifidobacterium* were notably abundant in the group with BLLs of < 3.06 µg/dL, although these differences did not reach statistical significance at a p-value of < 0.05. Most high-abundance genera (as shown in Figures 2 and 3) were not identified as significant biomarkers (p > 0.05) when comparisons were made using either a BLL cutoff of 3.06 µg/dL or between the lead-exposed and control groups. However, within these groups, *Blautia* and *Monoglobus* emerged as the most significant biomarkers (p < 0.05) in the BLLs of > 3.06 µg/dL and in the lead-exposed groups, respectively (Figure 4). Among the less abundant genera, Monoglobus is a significant biomarker in the lead-exposed group (0.01% in control vs. 0.7% in lead-exposed). A previous fecal microbiome study indicated a significant negative correlation between Monoglobus and immune-response-related genes in cigarette smokers (Meng *et al.,* 2022), with a decrease observed after consuming metal-containing food such as Zn in piglets (Xiao *et al.,* 2023).

Despite its relatively low abundance, *Monoglobus* may serve as a promising biomarker, indicating its potential role in lead-exposed hosts. However, given their relative abundances, *Blautia*, *Dorea*, *Streptococcus*, *Collinsella*, and *Bifidobacterium* could be more effective primary biomarkers. Confirmatory research on these biomarkers should be conducted with a larger sample size to validate these results.

3.5 Predictive microbial functional pathways associated with lead exposure in workers

Specifically comparing those with BLLs above and below 3.06 µg/dL, the industrial workers demonstrated different microbial functional pathways associated with lead exposure. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG), this study categorized the pathways based on their prevalence within each group at a cut-off level of 3.06 µg/dL, providing insights into how lead exposure may affect the functional capabilities of the gut microbiome.

The heatmap (Figure 5) revealed a significant distribution of pathway prevalence, with degradation of aromatic compounds being the most prevalent, for the group with BLLs of > 3.06 µg/dL compared with those < 3.06 µg/dL. This was followed by important pathways, such as lysine degradation, phenylpropanoid biosynthesis, d-alanine metabolism, drug metabolism by cytochrome P450, xenobiotic metabolisms by cytochrome P450, retinol metabolism, lysosome pathway, and *Staphylococcus aureus* infection. This study identified significant pathways, emphasizing how lead exposure potentially affects these metabolic processes.

The degradation of the aromatic compounds pathway (ko01220) was more prevalent in individuals with BLLs of > 3.06 µg/dL, indicating an adaptive microbial response to environmental pollutants, possibly due to occupational exposure to organic solvents in addition to lead. This pathway plays a crucial role in bioremediation by breaking down various aromatic pollutants and has implications for biofuel and chemical production from biomass

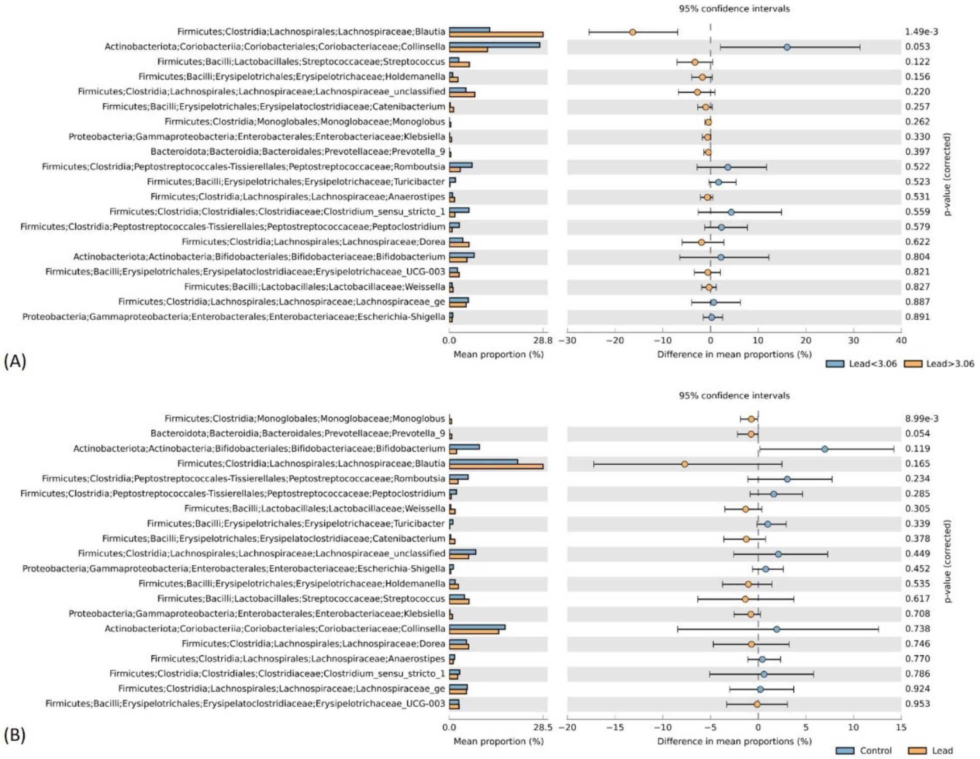

Figure 4. Differential abundances of microbial communities at the genus level (with a relative abundance of over 5% in at least one sample) between groups with blood lead levels above (Lead > 3.06 µg/dL) and below 3.06 µg/dL (Lead < 3.06 µg/dL) (A) and between lead-exposed (Lead) and control (Control) groups (B) based on White's nonparametric t-test (two-sided)

(Saeki and Emura, 2002). The lysine degradation (ko00310) and phenylpropanoid biosynthesis (ko00940) pathways demonstrated variations between the groups, indicating alterations in amino acid metabolism and secondary metabolite synthesis, which are crucial for cellular functions and plant stress responses, respectively. The presence of these pathways indicates that lead exposure indirectly affected essential metabolic processes and the body's ability to handle other environmental toxins (Wang *et al.,* 2022; Yan *et al.,* 2020). The study investigated the metabolism of xenobiotics and drugs by cytochrome P450 (ko00980 and ko00982), which is a critical process for detoxifying foreign compounds, including heavy metals such as lead. The workers with higher BLLs demonstrated significant activation of this pathway, reflecting the body's attempt to mitigate lead toxicity (Tian *et al.,* 2015). Retinol metabolism (ko00830) is another pathway affected by lead exposure, which is essential for vision, cellular growth, and immune regulation. The observed increase in this pathway among workers with higher BLLs may indicate a compensatory mechanism to maintain vitamin A levels despite potential lead-induced disruptions (Defo *et al.,* 2014).

The *S. aureus* infection pathway (ko05150) exhibits an association between heavy metal exposure, such as lead and cadmium, and the risk of both superficial and severe infections, including those caused by methicillin-resistant strains. This connection indicates that higher BLLs elevate the risk of infection (Eggers *et al.,* 2018). The research results coincide with Bishayi and Sengupta (2003), revealing that groups exposed to arsenic and lead had higher *S. aureus* bacteria levels in their blood and slower spleen response in clearing these bacteria, compared with the nonexposed group ($p < 0.05$ for arsenic and $p < 0.025$ for lead), emphasizing the need for lead exposure control in work and environmental areas.

The FoxO signaling pathway (ko04068) is crucial for regulating apoptosis, cell cycle, glucose metabolism, and oxidative stress resistance. Its activation in those with higher BLLs indicates that lead exposure may alter cellular stress responses and longevity, indicating that oxidative stress contributes to cellular dysfunction and diseases (Paithankar *et al.,* 2021). Chemical carcinogenesis via DNA adduct formation (ko05204) is a key process by which environmental carcinogens, such as lead, drive cancer development. These adducts disrupt DNA replication and repair, causing mutations and, potentially, cancer, indicating that individuals with higher BLLs face a heightened risk of lead-induced carcinogenic effects (Langie *et al.,* 2015).

KEGG pathway analysis reveals significant insights into the biological effect of lead exposure on human health. These pathways collectively highlight the multifaceted nature of lead toxicity, encompassing increased infection risks, oxidative stress-related cellular dysfunction, and enhanced cancer risk, emphasizing the critical need for effective monitoring and mitigation strategies against lead exposure. Given the small sample size of this study, the results linking BLLs to shifts in microbial community composition and KEGG pathway analysis should be interpreted with caution, as they may not fully represent broader population dynamics or capture the full spectrum of metabolic pathways affected by lead exposure.

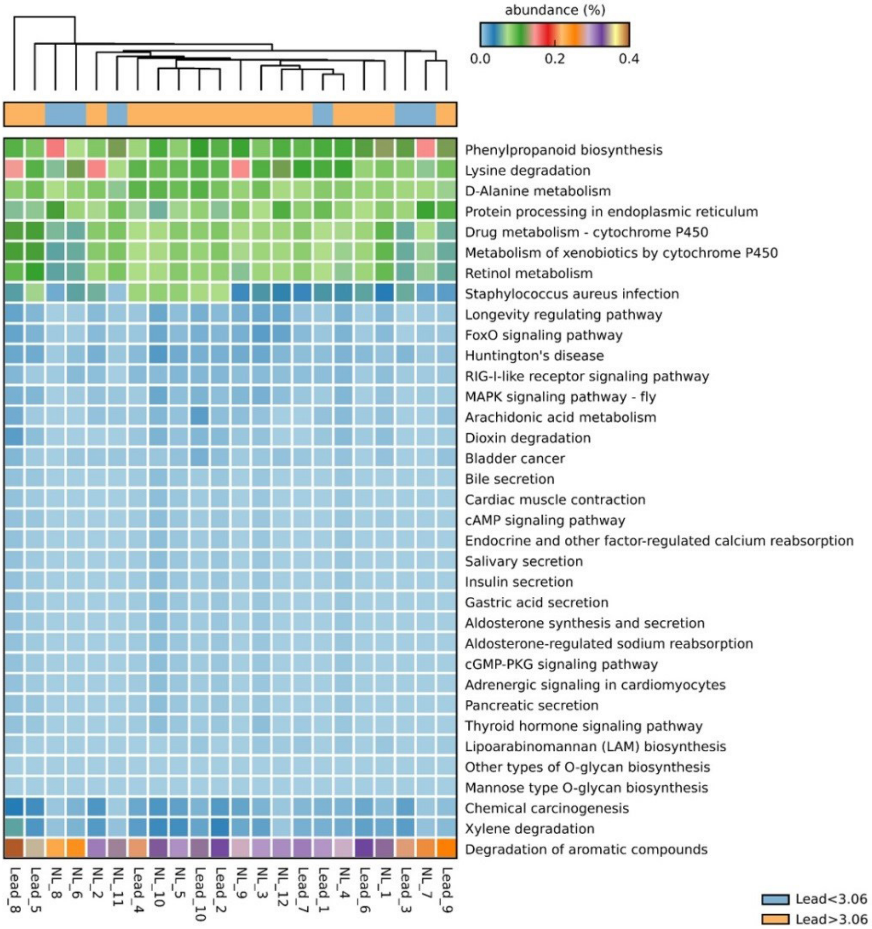

Figure 5. Heatmap depicting the microbial pathways in the fecal microbiome of lead industry workers, categorized by blood lead levels above and below 3.06 µg/dL. Pathways were predicted using Tax4Fun2, with significant differences between groups identified through ANOVA followed by Tukey–Kramer post hoc analysis ($p < 0.05$) in STAMP

4. Conclusion

This study investigated the fecal microbial community among workers in a lead-using factory, differentiating between BLLs above and below 3.06 µg/dL and those exposed and those not exposed to lead. This study revealed that lead exposure was significantly associated with BLL of < 10 µg/dL, with a strong association at 3.06 µg/dL. Lead exposure at BLL of 3.06 µg/dL cut-off alters the fecal microbial composition, suggesting the effect of lead on fecal and gut microbial dynamics and host health outcomes. Moreover, the study determined specific microbial biomarkers associated with lead exposure, emphasizing the increased presence of *Blautia* and *Streptococcus* and the decreased abundance of *Bifidobacterium* in exposed workers. These microbial signatures provide avenues for developing noninvasive diagnostic tools for lead exposure. The results indicate the need for protective measures against lead exposure in industrial settings and validate using fecal microbial as bioindicator of environmental pollutants. This study enhances the understanding of microbial responses to heavy metal exposure but emphasizing the health risks of occupational lead exposure, advocating for improved safety protocols and monitoring.

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