Assessing the Biochemical and Reproductive Biomarkers to Evaluate Health Consequences of Heavy Metals Exposure among Male Brick kiln Workers in District Layyah, Punjab Pakistan

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“In the Name of Allah, the most Beneficent, the most Merciful”

Dedicated to my parents,

whose boundless love and

sacrifices

have paved the path to my

success.

Your dedication to my

education

will forever be a source of

inspiration.

Thank you for believing in me

and my aspirations.

DECLARATION

I here by declare that the work presented in the following thesis is my own effort and the material contained in this thesis is original. I have not previously presented any part of this work elsewhere to any other degree.

Kibria Hassan

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List Of Abbreviation

WHO	World health organization
PM	Particulate matter
CCAC	Climate and clean air coalition
BTK	Bull's trench kiln
VSBK	Vertical shaft brick kiln
FCBTK	Fixed chimney bull's trench kiln
MFCBTK	Modified fixed chimney bull's trench kiln
CBK	Contemporary brick kilns
TBK	Traditional brick kilns
NOx	Nitrogen oxides
CO	Carbon monoxide
CO_2	Carbon dioxide
SO_2	Sulfur dioxide
BC	Black carbon
CH_4	Methane
NO	Nitric oxide
N_2O	Nitrous oxide
SO_X	Sulfur oxides
SPM	Suspended particulate matter
GHGs	Emission of greenhouse gases
H_2S	Hydrogen sulfide
VOCs	Volatile organic compounds
NAAQS	National ambient air quality standards
Cu^2	Copper ion
PAHs	Polycyclic aromatic hydrocarbons
DNA	Deoxy ribonucleic acid
Cr	Chromium
Cd	Cadmium

Pb	Lead
Ni	Nickle
DHS	Demographic & health surveys
CBC	Complete blood count
AAS	Absorption spectroscopy
BMI	Body mass index
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
WBC	White blood cell count
HGB	Hemoglobin
RBC	Red blood cell
DBP	diastolic blood pressure
SBP	Systolic blood pressure

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Abstract

The current study was designed to examine the impact of brick kiln emissions containing heavy metals on the reproductive health and biochemical status of brick kiln workers in Layyah, Pakistan. This study involved (n=300) workers and (n=200) non workers. Demographic data, health history and body mass index (BMI) were assessed. Blood samples were collected to determine heavy metals concentration, hematological profile and liver function test. Blood was centrifuged and plasma was collected and kept at -20^{o}C to study biochemical variables between two groups. The results showed a significant decrease in BMI and DBP while increase in SBP and blood sugar among workers. Analysis of heavy metals in blood showed an elevated level of lead and cadmium in workers as compared to non-workers. Increased white blood cells (p=0.004), platelet count (p=0.001), alkaline phosphatase (p=0.001), alanine transaminase (0.000), aspartate aminotransferase (0.001), bilirubin total (p=0.000) and oxidants level while decreased hemoglobin (p=0.001), red blood cells (p=0.001), albumin (p=0.003), protein (p=0.001) and antioxidant enzyme were evident among workers as compared with the non-workers. Significant increase in total cholesterol (p=0.003), low-density lipoprotein (p=0.000) and triglyceride (p=0.001) while significant decrease in high-density lipoprotein (p=0.000) and testosterone levels (p=0.001) were seen among workers as compared with non-workers. Present study demonstrate increase in heavy metal burden in blood of brick kiln workers and caused reproductive health issues due to higher oxidative stress conditions. Alternate technology is needed to be developed and brickkilns should be replaced.

INTRODUCTION

Human activities such as industrial processes, transportation and agriculture release pollutants that contaminate our water, air and soil. This pollution puts our environment in danger and can have harmful effects on human health and ecosystems. The industrial revolution brought great advancements in technology and society but it also led to the release of harmful pollutants into the air, impacting human health (Manisalidis *et al*., 2020). Global environmental pollution is a significant international public health concern. Efforts are now being made worldwide to reduce environmental degradation through eco-friendly technologies and regulations (Luka *et al*., 2018). However, the growing demand for industrialization continues to contribute to daily environmental degradation and pollution. We must find sustainable solutions to protect our environment and health (Ismail *et al*., 2012).

Air Pollution

According to the World Health Organization (WHO), air pollution is defined as any physical, chemical or biological substance is in the air, indoors or outdoors that changes the normal properties of atmosphere (Pariyar *et al*., 2013). When different pollutants are released into the atmosphere it leads to air pollution, which is harmful to people, other living things and the environment (Kim *et al*., 2015). Air pollution is widely recognized as one of the major risks to human health (Tan *et al*., 2021). Air pollution is one of the man-made difficulties that is now impacting countries all across the world (Ismail *et al*., 2012). Air pollution in brick kilns is caused by both stack emissions and fugitive emissions

(Skinder *et al*., 2014). The release of these hazardous chemicals by the brick kiln sector considerably increases the pollution. Brick kiln emissions are a significant cause of air pollution and health problems. (Khan *et al*., 2019). Air pollution is a major issue that may have a negative impact on both human health and the environment. When certain air pollutants are inhaled, they can trigger inflammation and the release of harmful oxygen radicals, causing damage to local tissues and leading to respiratory distress (Shaikh *et al*., 2012). The consequences of air pollution are far-reaching including a shortage of oxygen for animals to breathe, reduced visibility, eye irritation, an increase in upper respiratory infections and unpleasant odors. The impact of poor air quality extends beyond respiratory issues. It has been observed that air pollution is associated with an increased incidence of respiratory illnesses like asthma pharyngitis, emphysema, bronchitis, and allergic reactions (Parvez *et al*., 2023). Moreover, the harmful effects of air pollution can extend to other vital systems in the body such as the central nervous and cardiovascular systems (Pariyar *et al*., 2013). Numerous epidemiological studies conducted worldwide have shown a correlation between deteriorating air quality and a higher prevalence of various health conditions. These include pulmonary fibrosis, bronchitis, asthma, decreased lung function, allergic rhinitis, eye irritation, cough, pharyngitis, emphysema and low birth weight (Rehman *et al*., 2013). According to the Global Burden of Disease (GBD) report in 2017, air pollution was responsible for approximately 4.9 million deaths worldwide (Tan *et al*., 2021). More than two million of these fatalities were directly connected to lungs and respiratory system impairment induced by air pollution. Among the deaths caused by air pollution, around 2.1 million were attributed to fine particulate matter (PM), while approximately 0.47 million were due to ozone exposure (Kim *et al*., 2015). However, the WHO reports that air pollution kills around three million people per year. Approximately 800,000 of these deaths are premature deaths due to outdoor air pollution, which causes cardiovascular disease, lung cancer and respiratory problems. The impact of air pollution is particularly severe in South Asia where an estimated 150,000 deaths occur annually (Pariyar *et al*., 2013). These statistics highlight the significant health consequences associated with air pollution, especially in developing countries, and emphasize the urgent need for effective measures to address this problem.

Brick Kilns

The brick kiln industry, which is considered a small-scale or cottage industry, provides seasonal employment despite significant investment (Sanjel *et al*., 2016). This industry has experienced rapid growth due to the increasing demand for bricks driven by economic growth, urbanization, and population growth in countries like Pakistan, China, India, and Bangladesh. Bricks have been widely used as a construction material for a long time (Asif *et al*., 2021). Brick manufacturing is a large and established industry in several Asian countries (Skinder *et al*., 2014). However, brick manufacture has significant environmental, human health, economic and social consequences. The manufacturing process releases pollutants such as carbon oxides, sulfur oxide, nitrogen oxide, organic matter, dust, polycyclic aromatic hydrocarbons, hydrogen sulfide and which can harm both humans and the environment (Parvez *et al*., 2023). The improper construction of brick kilns can release fumes containing gases like carbon monoxide (CO), carbon dioxide (CO_2) and nitrogen oxides (NOx), contributing to ozone formation. Brick kilns are also significant sources of greenhouse gases (Shaikh *et al*., 2012). The smoke and dust emitted from brick kilns contribute to air pollution negatively impacting air quality, vegetation and human health (Asif *et al*., 2021). Every year, around 1.5 trillion bricks are produced globally, with the majority about 90%, made in Asia (Parvez *et al*., 2023). There are roughly 300,000

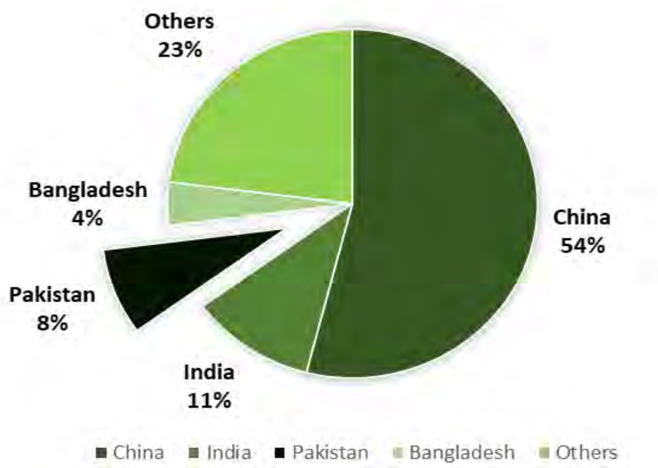


Figure 1. Worldwide bricks production status

brick kilns worldwide, with 75% of them located in Pakistan, Bangladesh , China and India (Raza & Ali, 2021).

There are roughly 152,700 operational kilns in South Asia alone, which contribute extensively to the worldwide brick kiln sector. This sector employs approximately 16 million people and 500,000 animals and makes up more than 21% of global brick making (Parvez *et al*., 2023). These numbers highlight the financial significance of the brick industry. Pakistan is South Asia's third-largest brick manufacturer, producing about 45 billion bricks annually (Tan *et al*., 2021). According to an April 2018 assessment by the Climate and Clean Air Coalition (CCAC), the country has around 20,000 brick kilns located in both urban and rural locations (Khan *et al*., 2019). With about 7,000 brick-making units in operation, Pakistan employs around 100,000 permanent workers in this industry. The majority of the brick kilns, around 5,000 are located in cities and towns throughout the province of Punjab (Raza & Ali, 2021). These kilns vary in size with smaller ones producing 400,000 to 600,000 bricks monthly, while larger kilns around Lahore can produce 800,000 or more bricks per month. The number of baked bricks extracted daily, known as "nikasi" typically ranged from 20,000 to 30,000 (Ercelawn & Nauman, 2004).

Categories of brick kilns

Brick kilns can be divided into two main types: continuous kilns and intermittent kilns. In intermittent kilns, bricks are burnt in batches. Scotch, Clamp, Down Draft and Scove kilns are examples of intermittent kilns. These kilns operate by firing a certain number of bricks at a time. On the other hand, continuous kilns have a fire that is always burning. In these kilns, bricks are heated, fired and cooled instantaneously in different parts of the kiln. Examples of continuous kilns include Hoffmann kiln, Tunnel kiln, Bull's trench kiln (BTK), Zigzag kiln and vertical shaft brick kiln (VSBK) (Rajarathnam *et al*., 2014). In Pakistan, brick kilns can be broadly categorized into two types: Traditional Brick Kilns (TBK) and Contemporary Brick Kilns (CBK). Traditional Brick Kilns mainly consist of Fixed Chimney Bull's Trench Kiln (FCBTK), which is an older technology still widely used in many parts of Pakistan. Technologically refined brick kilns include the Modified Fixed Chimney Bull's Trench Kiln (MFCBTK), Hoffman Kiln, Tunnel Kiln and VSBK (Khan *et al*., 2019). Many brick kilns in Pakistan are open-air Bull Trench Kilns that are located outdoors. These kilns vary in size, ranging from small ones employing around 8-10 workers to large-scale operations with over 100 workers. It is essential to note that the levels of airborne particles at these kilns are much greater, about 7-8 times higher than at other types of kilns such as (VSBK).

Bricks Making Process

Brick manufacturing is considered a study of civilization since straw and mud bricks have been used for thousands of years. (Rameshbhai & Gopalkrishanan, 2019). The raw substances used for making bricks are sediments clay or soil from rivers, which contain fine particles (Skinder *et al*., 2014). Bricks are primarily made from soil, and additives are added to enhance their strength. After the bricks are made, they are burnt for a set amount of time in brick kilns. These bricks, after burned, are used in construction. (Khan *et al*., 2019).

Fuel Used by Brick Kilns

Many brick kilns use fuels like slack coal, assam coal, lignite and other sources that have high sulfur content and ash content ranging from 25% to 30%. In terms of fuel composition, approximately 70% of coal, 24% sawdust and the residual 6% consists of wood and other materials are used by brick kilns (Skinder *et al*., 2014). These kilns commonly use various fuel sources such as recycled motor oils, wood, fuel oils, diesel, coals, tires, plastics and waste materials (Pariyar *et al*., 2013). During the brick production process, a typical brick kiln that produces 800,000 bricks consumes a large amount of rubber for ignition and burns around eight tons of low-quality coal or 20 drums of used vehicle oil. These burning releases toxic pollutants like CO, NOx and dioxins. Brick manufacturers frequently use waste materials as fuel, such as wood shavings, scrap wood, and sawdust, which include resins and chemical compounds. They use cheaper waste materials such as used motor oil, plastics, old tires and garbage as alternate source. Furthermore, traditional kilns have inefficient energy usage due to poor heat distribution and inadequate insulation, resulting in higher fuel consumption, increased emissions and longer firing times (Sanjel *et al*., 2016).

Coal

In addition to coal, brick kilns use various biomass fuels like bagasse, rice husk, dry dung, firewood and other agricultural residues for firing bricks (Rajarathnam *et al*., 2014). In some places, low-grade carbonaceous resources including bagasse, wood, sawdust and rice husk are used in place of coal either partially or completely. When coal is burned in these kilns, it produces high levels of sulfur dioxide and black carbon which are harmful pollutants (Skinder *et al*., 2014). The combustion of both biomass fuels and coal in brick kilns leads to the emission of black carbon (BC), PM, SO_2, CO and NOx. The release of these pollutants, including CO_2 from coal combustion can have harmful effects on human health and influence to air pollution (Parvez *et al*., 2023).

Biomass Burnings

When biomass, such as forest wood is burned in various industrial activities, it results in the emission of greenhouse gases (GHGs). This not only increases the concentrations of GHGs like CH_4, CO, CO_2, nitrous oxide N_2O, nitric oxide (NO) and NOx in the air but also contributes to deforestation (Bhat *et al*., 2014). Both non-trace and trace gases, including CH_4, CO_2, NO_X, NO and N_2O are produced during biomass burning. Furthermore, hazardous fumes including suspended particulate matter (SPM) rich in carbon particles as well as high concentrations of sulfur oxides (SO_X) and CO are emitted during biomass combustion. In the context of brick manufacturing, NO_X emissions are mainly the result by the oxidation of air nitrogen during the combustion of biomass (Sanjel *et al*., 2016).

Rubber tires

When rubber tires are used as fuel in brick kilns, the emissions from the kilns include not only CO but also other pollutants. These contaminants include tiny dust particles, hydrocarbons, SO_2, NOx, fluoride compound and a limited amount of hazardous dioxins (Pariyar *et al*., 2013).

Wood

Brick kilns use a combination of fuel wood obtained from forests and coal in different proportions for baking bricks (Bhat *et al*., 2014). Many brick kilns in Pakistan use wood and coal, exposing kiln workers to high amounts of air pollution and associated harmful health effects (Shaikh *et al*., 2012). On average, each kiln burns around 350 tons of wood per year leading to significant deforestation if the number of kilns increases (Pariyar *et al*., 2013). Previous investigations have connected wood smoke exposure to a 70% higher chance of developing chronic obstructive pulmonary disease (Shaikh *et al*., 2012).

Brick Kilns Emissions

Brick kiln emissions typically contain fine particles from dust and coal as well as organic stuff. SO_2, NOx, hydrogen sulfide (H_2S) and CO are also present in trace levels (Skinder *et al*., 2014). The processes and gases of brick kilns contain fly ash, volatile organic compounds (VOCs), NOx, SO_2, CO_2, CO and PM. These emissions frequently exceed WHO and National Ambient Air Quality Standards' (NAAQS) standards (Raza & Ali, 2021). According to studies, the manufacture of 1000 bricks emits 0.64-1.4 kg of PM 0.52-5.9 kg of SO2 and 6.35-12.3 kg of CO (Parvez *et al*., 2023).

Clay

Clay dust produced in brick kilns is a mixture of inorganic components such as free silica, iron oxide, alkalis, lime, sodium chloride, magnesium carbonate, calcium sulfate, calcium carbonate and varying quantities of organic elements (Shaikh *et al*., 2012).

SPM

The presence of suspended particulate matter (SPM) in brick kiln chimney gases is mostly generated by incomplete fuel combustion but it can also be caused by fine coal ash, dust and burnt clay particles (Sanjel *et al*., 2016).

Particulate Matter

Particulate matter (PM) in the air is a combination of solid and liquid particles that may vary in size and chemical content throughout time and distance. Sulfates, nitrates, elemental and organic carbon, organic molecules such as polycyclic aromatic hydrocarbons (PAHs), biological chemicals such as endotoxin and cell fragments and metals such as iron, nickel, zinc, copper, and vanadium are among the chemical components of PM (Kim *et al*., 2015). Some types of particulate matter that are of concern include toxic metals like lead, mercury, PAHs and persistent organic pollutants like dioxins (Curtis *et al*., 2006).

Particulate matter can have various effects on the body, particularly on the respiratory and cardiovascular systems. It can cause systemic oxidative stress, inflammation and increased blood pressure. Exposure to PM has been linked to changes in serum metabolites and the possibility of inducing apoptosis through inflammation and oxidative stress potentially involving TNFα or mitochondria (Kim *et al*., 2015; Tan *et al*., 2021). High levels of PM exposure have been linked to negative consequences such as infant low birth weight, pre-term births and even infant and fetal mortality. Exposure to PM can affect lung development in children resulting in reversible impairments in lung function, a decreased rate of lung growth over time and long-term abnormalities in lung function (Kim *et al*., 2015). According to studies, the levels of PM in the air surrounding brick kiln regions might be three times greater during the off-season of brick kilns (Pariyar *et al*., 2013). Particulate matter, especially $PM_{2.5}$ is a main contributor to respiratory issues such as asthma, coughing, chronic bronchitis and reduced lung function. $PM_{2.5}$ is particularly dangerous since it may penetrate deep into the respiratory system, causing premature death and respiratory illnesses. It has also been related to attacks of asthma and cardiac issues (Parvez *et al*., 2023). Inhaling $PM_{2.5}$ can cause minor symptoms such as chest discomfort and pain, shortness of breath, wheezing, and coughing (Kim *et al*., 2015). PM_{10} another type of particulate matter emitted from brick kilns can directly impact human health by passing through the nose and entering the respiratory system, potentially leading to conditions like asthma and bronchitis (Pariyar *et al*., 2013).

Nitrogen Oxide (NOx)

NOx is emitted from various natural and anthropogenic sources. However, in rural areas, traditional brick kiln industries contribute to higher concentrations of NOx in the lower part of the atmosphere. Releases of NOx also play a significant part in the formation of ozone. Rural areas near brick kilns often have elevated levels of ozone due to these emissions. When a small quantity of NOx is present in the atmosphere, it reacts with solar rays leading to the production of ozone (Sanjel *et al*., 2016). NO_2 and NOx are components that mostly disturb the respiratory tract and alveoli in the lungs. It can also enter the bloodstream and cause tissue hypoxia by joining with hemoglobin (Tan *et al*., 2021).

Sulphur oxides (SOx)

SO_X is a word used to describe sulfur oxide emissions, especially SO_2 and SO_3. SO_X emissions from brick manufacturing have been a source of concern. When it comes to man-made sources, coal combustion accounts for 74% of SO_X emissions, industry 22% and transportation 2%. SO_2 is a substantial pollutant that is discharged directly into the atmosphere by a variety of processes, particularly those involving the burning of petroleum and coal. The primary sources of SO_2 emissions are the combustion of fossil fuels and the smelting of sulfide ores (Bhat *et al*., 2014). SO_2 is currently a major air pollutant in developing nations, contributing to urban pollution as well as regional acid rain (Sanjel *et al*., 2016). SO_2 is the major cause of concern in brick manufacture. It is a major source of air pollution, especially in developing nations, where it contributes to both urban pollution and deposition of acids. When SO2 is ingested, it dissolves in the respiratory system's aqueous surfaces, forming sulfite and bisulfite, which pass through the respiratory tract cells and circulate throughout the body. SO_2 stimulates the respiratory tract and can potentially affect the cardiovascular system (Tan *et al*., 2021). Acute SO_2 exposure causes bronchial constriction, airway constriction, increased pulmonary resistance, raised airway responsiveness and metabolic alterations. Chronic SO_2 exposure causes mucosal tissue inflammation and raised secretions.

Ozone (O_3)

Ozone (O_3) may immediately reach the deep respiratory tract and injure the mucosa, adverse asthma symptoms and decreasing lung function. (Tan *et al*., 2021).

Carbon mono oxide (CO)

In brick kilns, high levels of carbon monoxide (CO) are produced due to poor kiln design, leading to incomplete coal combustion. This can have negative effects on the central nervous system, causing symptoms like headache, dizziness, nausea, and shortness of breath. Carbon monoxide is quickly absorbed by the alveoli, capillaries, and even the placental barrier, binding with hemoglobin and reducing the supply of oxygen to body tissues resulting in tissue hypoxia (Tan *et al*., 2021).

Harmful Effects of Brick Kilns Emission on Environment

In Pakistan, there is a concern about brick kilns being located close to agricultural lands, which adds pollutants to the food chain (Ismail *et al*., 2020). These kilns use coal, wood, tires and furnace oil for their operations resulting in the production of pollutants like NOx, COx, SOx and PM (Ismail *et al*., 2012). These emissions from brick kilns contribute to ozone layer depletion and the occurrence of acid rain. Poisonous gases in the atmosphere react with raindrops and then generate weak acids that can damage plant leaves, stems and animal skin. Harmful gases from brick kilns can flow into rivers and seas causing harm to aquatic life. These chemicals can also be absorbed by the soil, changing its pH and leading to soil degradation (Khan *et al*., 2019). Acid rain, which is caused by these pollutants, is a severe hazard to human health since it infects not only the air we breathe but moreover the water and food we eat. The increase in pollutant concentration in the air has made acid rain a major concern (Ismail *et al*., 2012). Excessive use of soil in brick production causes soil degradation and creating various environmental problems (Khan *et al*., 2019). Brick kilns mainly use coal as fuel leading to excessive wood consumption and deforestation, which contributes to air pollution, soil erosion, health issues and climate change (Khan *et al*., 2019). The environmental implications of brick kilns include both short-term and long-term. In the short term, vegetation suffers, crop yield is harmed and plant health suffers. In the long term, ozone depletion, global warming, photochemical smog, decreased land fertility and lower groundwater levels become significant concerns (Pariyar *et al*., 2013).

Harmful Effects of Brick Kilns Emissions on Humans

Humans are exposed to various environmental contaminants from the air, water, and food we consume (Woodruff *et al*., 2008). Toxic substances released by brick kilns adversely affect the soil, plants, animals and people living nearby, brick workers, children, and women being most affected (Skinder *et al*., 2014) . Gases like CO, NOx, and SOx can lead to dizziness, headaches, fatigue, lung irritation, bronchitis, other respiratory issues, and impact the brain and heart (Bhat *et al*., 2014). Many respiratory diseases both acute and chronic are linked to inhaling harmful substances at the workplace, such as pneumoconiosis, hypersensitivity pneumonitis, lung cancer. Among full-time workers, around 2.5 per 10,000 develop nonfatal respiratory diseases due to their occupation (Rehman *et al*., 2013). Poisonous gases like NOx, SO_2, CO_2, CO and PM can cause central nervous system, cardiovascular and various respiratory disorders when inhaled by humans. Emissions from brick kilns can contribute to respiratory diseases such as emphysema, lung cancer, coughing, wheezing, chronic bronchitis, asthma and difficulty in breathing. In addition to respiratory diseases, harmful gases from brick kilns can cause irritation of the nose, eyes and throat (Khan *et al*., 2019). These harmful substances also have an harmful impact on the human reproductive system resulting in infertility, birth abnormalities, spontaneous abortions and early miscarriages. The male reproductive system is particularly affected during spermatogenesis. Furthermore, these pollutants can cause immune system disruption by creating excessive quantities of reactive oxygen species which can cause cell damage through decomposition of protein, nucleic acid degradation or lipid peroxidation. (Jahan *et al*., 2016). Overall, emissions from brick kilns can have wide-ranging effects on human health, affecting various body systems and causing multiple health issues. Brick kilns that use diesel for combustion can produce harmful substances known to be genotoxic, cytotoxic, carcinogenic causing damage to DNA and cells. Workers in such kilns may also be exposed to heat and radiation, which might result in genetic changes. DNA strand breakage, DNA-protein cross-links and a variety of lung disorders such as radiation pneumonitis, fibrosis, and widespread alveolitis are all possible causes (Kaushik *et al*., 2012). Working in this hazardous environment causes a variety of health problems and musculoskeletal problems in brick kiln workers. Repetitive work in prolonged awkward positions can lead to discomfort, fatigue and health problems especially for those involved in manual tasks (Sain & Meena, 2018). The combination of exposure to harmful substances and physical strain can have significant impacts on the health of workers in brick kilns. Brick kiln workers often suffer from musculoskeletal disorders and discomfort due to the heavy physical work and awkward postures involved in manual handling. Unlike other occupational disorders caused by exposure to toxic substances, the musculoskeletal issues in brick kiln workers which effect of prolonged periods in uncomfortable positions leading to significant health problems (Inbaraj *et al*., 2013). Molding and firing of bricks are critical processes in the brick kiln industry. The firing work requires round-the-clock teamwork in extremely hot environments, with temperatures ranging from 90˚C to 120˚C (Sanjel *et al*., 2016). Excessive heat exposure can lead to various heat-related disorders including heat rash, heat stroke, exhaustion and dehydration posing risks to workers health and safety. Due to heat, brick kiln laborers may experience tiredness earlier during their work shifts which can lead to carelessness and affect their ability to focus on safety protocols. This can further increase the likelihood of workplace injuries and accidents. Brick kiln workers face several health challenges that affect their well-being in different ways. Due to the extreme heat in the kilns, workers may find it difficult to concentrate on their tasks and may not prioritize safety protocols. A core body temperature above 38°C indicates heat stress which can be harmful. The lack of sufficient and clean water supply as well as inadequate toilet facilities, raises concerns about gastrointestinal health among brick kiln workers. Additionally, the low wages and high levels of pollution in their workplace contribute to mental health issues.

Certain job roles, such as bakers, brick unloaders and ash handlers expose workers to higher levels of pollutants. Inhaling these pollutants can lead to skin and eye irritation as well as respiratory diseases like pneumoconiosis and silicosis due to exposure to siliceous dust. Overall, brick kiln laborers face a variety of health difficulties including respiratory problems, injuries, gastrointestinal issues, musculoskeletal illnesses, reproductive health issues and mental health challenges. These health problems highlight the importance of better conditions for workers and safety requirements in the brick kiln industry (Sanjel *et al*., 2016).

Heavy Metals

Pollution from industry outlets, chimneys and contaminated liquids gradually introduces heavy metals into the soil. The brick kiln manufacturing process also contributes to the presence of heavy metals, particularly in the microenvironment surrounding the kilns, affecting vegetation, soil and humans (Ismail *et al*., 2020). Heavy metals like lead found in brick kiln soil can have negative effects on the male reproductive system. They can affect the hypothalamic-pituitary-gonadal system or have a direct impact on spermatogenesis resulting in low sperm quality (Jahan *et al*., 2016). Furthermore, excessive amounts of heavy metals in the soil can cause a variety of health problems including neurological, hematological, gastrointestinal and immunological difficulties as well as neoplasms. Heavy metals mainly affect the body by interacting with heme making enzymes, thiol-containing antioxidants and enzymes. Silica and heavy metals can both harm free radicals and cause oxidative stress (Kaushik *et al*., 2012).

Arsenic

Arsenic is a silent threat as it has no smell or taste. Inorganic arsenic is a cancer-causing substance known as a carcinogen and can lead to skin, lung, liver and bladder cancer (Bhat *et al*., 2014). Even at lower levels of exposure it can cause nausea, vomiting, and harm to our blood cells and heart rhythm. It also harms blood vessels and causes "pins and needles" sensations in the hands and feet (Mahurpawar, 2015). It is essential to avoid exposure to arsenic to protect our health.

Cadmium

Cadmium and cadmium compounds are harmful to our health and known to cause cancer. Smokers are at higher risk of exposure to cadmium compared to non-smokers. Exposure to high amounts of cadmium can cause serious lung damage. Ingesting extremely high amounts might cause gastrointestinal irritation which may cause vomiting and diarrhea. Long-term cadmium exposure can cause renal illness, lung damage and weaker bones because it accumulates in the kidneys (Mahurpawar, 2015). Cadmium salts that are soluble in water can be hazardous to many organs including the lungs, liver, heart, kidneys, brain, testes, and central nervous system. Cadmium does not produce free radicals directly although it does induce lipid peroxidation in tissues shortly after exposure (Pariyar *et al*., 2013). To protect our health, it is important to minimize exposure to cadmium and its compounds.

Chromium

Chromium (Cr) exists in two biologically active oxidation states: Cr (III) and Cr (IV). Cr (VI) is more lethal and causes greater oxidative stress making it harmful to our health (Pariyar *et al*., 2013). Chromium (VI) compounds are poisons and recognized human carcinogens, but Chromium (III) is a vitamin that our body needs. High concentrations of Chromium (VI) in the air can cause ulcers, nasal irritation, runny nose and breathing issues such as asthma or wheezing. Skin exposure to Chromium (VI) can result in skin ulcers, intense redness and swelling indicating an allergic response. Long-term Chromium (VI) exposure can cause liver, circulatory, renal and nerve tissue damage as well as skin irritation (Mahurpawar, 2015). Chromium has been associated with allergic dermatitis and is dangerous and carcinogenic in humans as well as animals. To preserve our health, we must limit our exposure to hazardous types of chromium particularly Chromium (VI) (Pariyar *et al*., 2013).

Lead

Lead is a toxic metal. Inhaled lead can accumulate in different parts of the body including the lungs, blood, liver, bones, soft tissue, nervous, cardiovascular and reproductive systems. In adults exposure to lead has been associated with symptoms such as memory issues, loss of concentration as well as joint and muscle pain (Manisalidis *et al*., 2020). High lead exposure can have severe consequences on health, damaging the brain and kidney even leading to death. Men exposed to high lead levels may damage the organs responsible for sperm production (Mahurpawar, 2015). Lead is a significant environmental contaminant that can result in hematological, gastrointestinal and neurological diseases. Prolonged lead exposure can also cause reproductive disorders, renal problems and hypertension. It affects nerve conduction, calcium balance, enzyme function and stimulates the production of binding proteins (Pariyar *et al*., 2013). Lead has harmful impacts like it involves the formation of ROS and the oxidative stress production (Pariyar *et al*., 2013). In men, lead exposure can lead to reduced libido, sperm abnormalities (reduced motility and numbers, abnormal morphology), chromosomal damage, infertility, prostate dysfunction and changes in testosterone levels. Women may experience infertility, miscarriage, premature labor, pregnancy-related hypertension and preterm birth due to lead exposure. Lead can disrupt hormonal balance and fertility in both genders affecting libido, spermatogenesis in men and the ovarian cycle in women leading to adverse pregnancy outcomes. It is vital to reduce lead exposure to protect overall health and reproductive well-being (Rameshbhai & Gopalkrishanan, 2019).

Mercury.

High concentrations of mercury exposure can result in lifelong brain, renal and fetal harm. It can damage brain function resulting in symptoms such as tremors, shyness, irritation, abnormalities in vision or hearing and memory issues. Short-term exposure to high quantities of metallic mercury vapors can induce lung damage, vomiting, blood pressure, nausea, diarrhea, eye irritation and skin rashes (Mahurpawar, 2015). Reducing exposure to mercury is essential to protect our health and well-being.

Selenium

Short-term oral selenium intake can cause vomiting, nausea and diarrhea. If exposed to excessive levels of selenium over a long period, it can lead to a condition called selenosis, which shows signs like hair loss, brittle nail and neurological issues. Inhaling high levels of selenium in the air can cause bronchitis, difficulty in breathing, respiratory tract irritation, and stomach pains in the short term. Long-term exposure can cause bronchial spasms, lung irritation, and prolonged coughing symptoms. To avoid potential issues with health, it is essential to limit your exposure to a large amount of selenium (Mahurpawar, 2015).

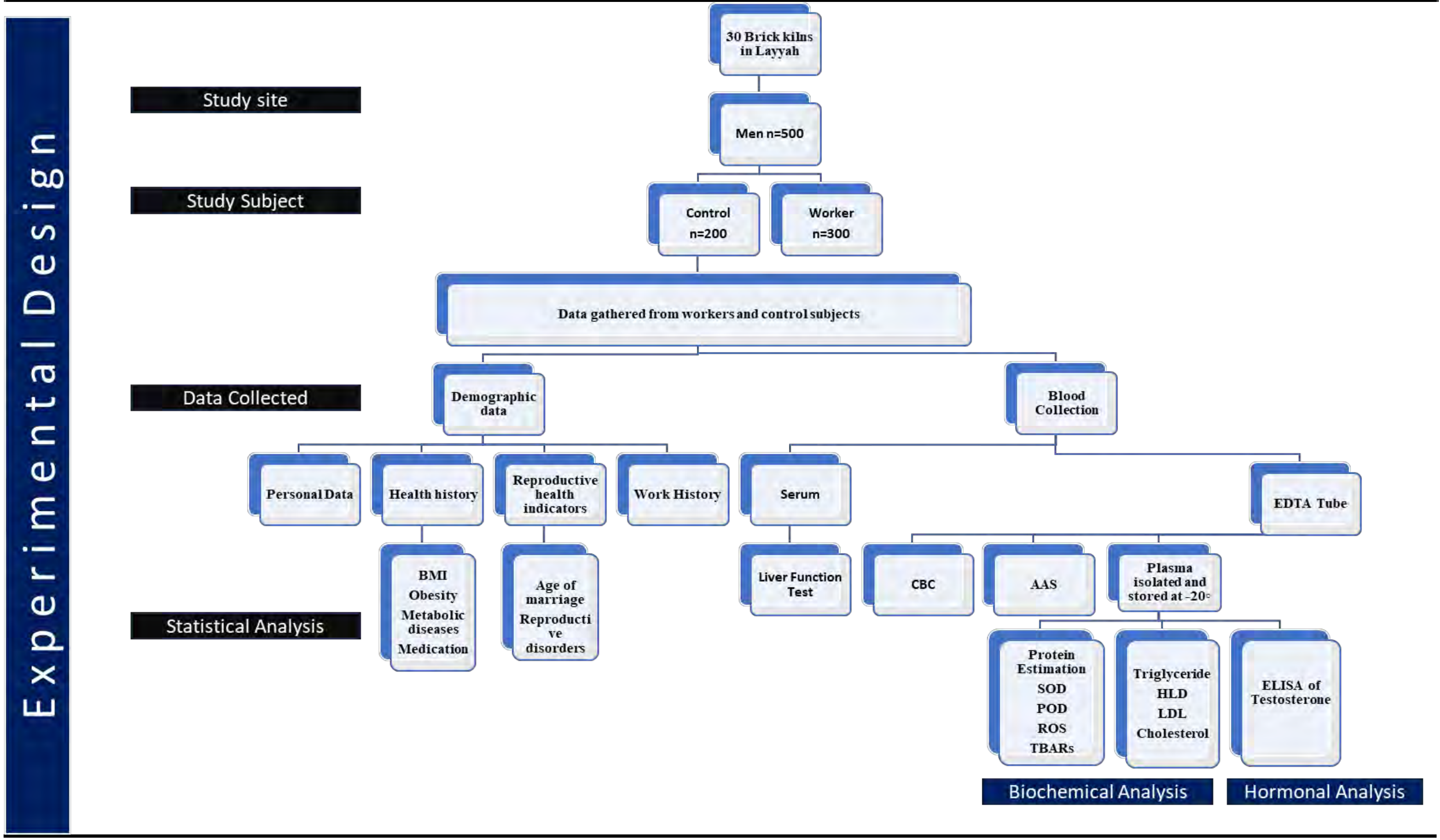
Nickel

Nickel is known to be a carcinogen, particularly in workers exposed to it in nickel refineries where it can lead to nasal and lung cancers. It is also a common cause of allergic contact dermatitis affecting around 4-9% of people with this type of skin condition. The carcinogenic effects of nickel may be linked to its ability to enhance the manufacture of ROS which can harm tissues including DNA. Additionally, nickel may lead to the accumulation of iron in the body further causing damage to lipids in the body (lipid peroxidation) and leading to the formation of harmful ROS (Pariyar *et al*., 2013).

Aim and Objectives

The study was performed to evaluate the health of brick kiln workers. The objectives are as follows:

- Assess any changes in toxic metal levels in the blood due to exposure to brick kiln emissions.
- Identify the level of oxidative stress caused by exposure to heavy metals from brick kiln emissions.
- Analyze the impact of brick kilns emitting heavy metals on reproductive hormones.
- Investigate if heavy metal exposure from brick kilns is associated with changes in BMI and lipid profile levels.
- Conduct liver function tests on individuals exposed to brick kiln emissions.
- Examine the correlation between brick kiln emissions and socio-demographic factors within individuals subjected to emissions from brick kilns.


Experimental Design
Study site
Study Subject
Data Collected
Statistical Analysis
30 Brick kilns in Layyah
Men n=500
Control n=200
Worker n=300
Data gathered from workers and control subjects
Demographic data
Blood Collection
Personal Data
Health history
Reproductive health indicators
Work History
Serum
EDTA Tube
BMI
Obesity
Metabolic diseases
Medication
Age of marriage
Reproductive disorders
Liver Function Test
CBC
AAS
Plasma isolated and stored at -20
Protein Estimation
SOD
POD
ROS
TBARs
Triglyceride
HLD
LDL
Cholesterol
ELISA of Testosterone
Biochemical Analysis
Hormonal Analysis

MATERIALS AND METHODS

Study design and ethical clearance

The study was carried out at the Reproductive Physiology Laboratory, Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan. The aim was to understand how the pollutants from brick kilns affect the health of male workers. Data collection and blood sampling took place from January 2023 to April 2023 at different brick kiln locations, including Pahar Pur, Kot Sultan, Chowk Azam, Noshera, and Karor Lal Esan in district Layyah after the approval from the Bio-Ethical Committee of the Department of Zoology, Quaid-i-Azam University, Islamabad. Additionally, the participants in the study willingly agreed to take part and signed consent forms, indicating their understanding and willingness to be involved in the research.

Selection of brick kiln sites

For this study, we focused on different brick kilns in District Layyah, Punjab. There were around 187 brick kilns in the area. Fixed Chimney Bull's Trench Kilns and zigzag technology were mostly used to produce bricks. We conducted a survey and chose specific brick kilns based on their production capacity and operations. We visited 65 brick kilns but 30 owners were willing to participate in our study. They agreed to be part of the research and provide valuable information for our investigation.

Figure 2. Brick kiln site (Layyah) showing type of kilns and bricks making process.

Description of study area

District Layyah is in southern Punjab and is surrounded by district Jhang to the East, Dera Ghazi Khan to the West, Bhakkar to the North, and district Muzaffargarh to the South. The district consists of three tehsils: Layyah, Chaubara, and Karor Lal Esan. According to the 2017 National Census of Pakistan, the population of Layyah was approximately 1.82 million, compared to 1.12 million in 1998, with a growth rate of 2.59. The geographical coordinates of Layyah are between 30° 45' to 31° 24' North latitudes and 70° 30' to 71° 47' East longitudes. The elevation of the district is about 500 feet above sea level. The eastern part of the district is characterized by sandy desert, which leads to sandstorms due to extreme temperatures during the summer season. The climate in this area is dry, and rainfall is infrequent. Summers are long and humid, making up about half of the year, while winters are short. Throughout the year, the temperatures range from 7 to 41°C.The study area is known for its rich and fertile fields, citrus gardens, and historical sites. It has extensive agricultural lands and access to a wide canal system, allowing for the cultivation of various crops. The region is particularly well-known for producing grams (chickpeas). To facilitate the research, the researchers utilized Geographic Information System (GIS) software, specifically ArcGIS, to develop sampling sites on a map. Figure 3 illustrates the locations of the brick kiln sites where the sampling was conducted.

Regional Map of Layyah, Punjab Pakistan

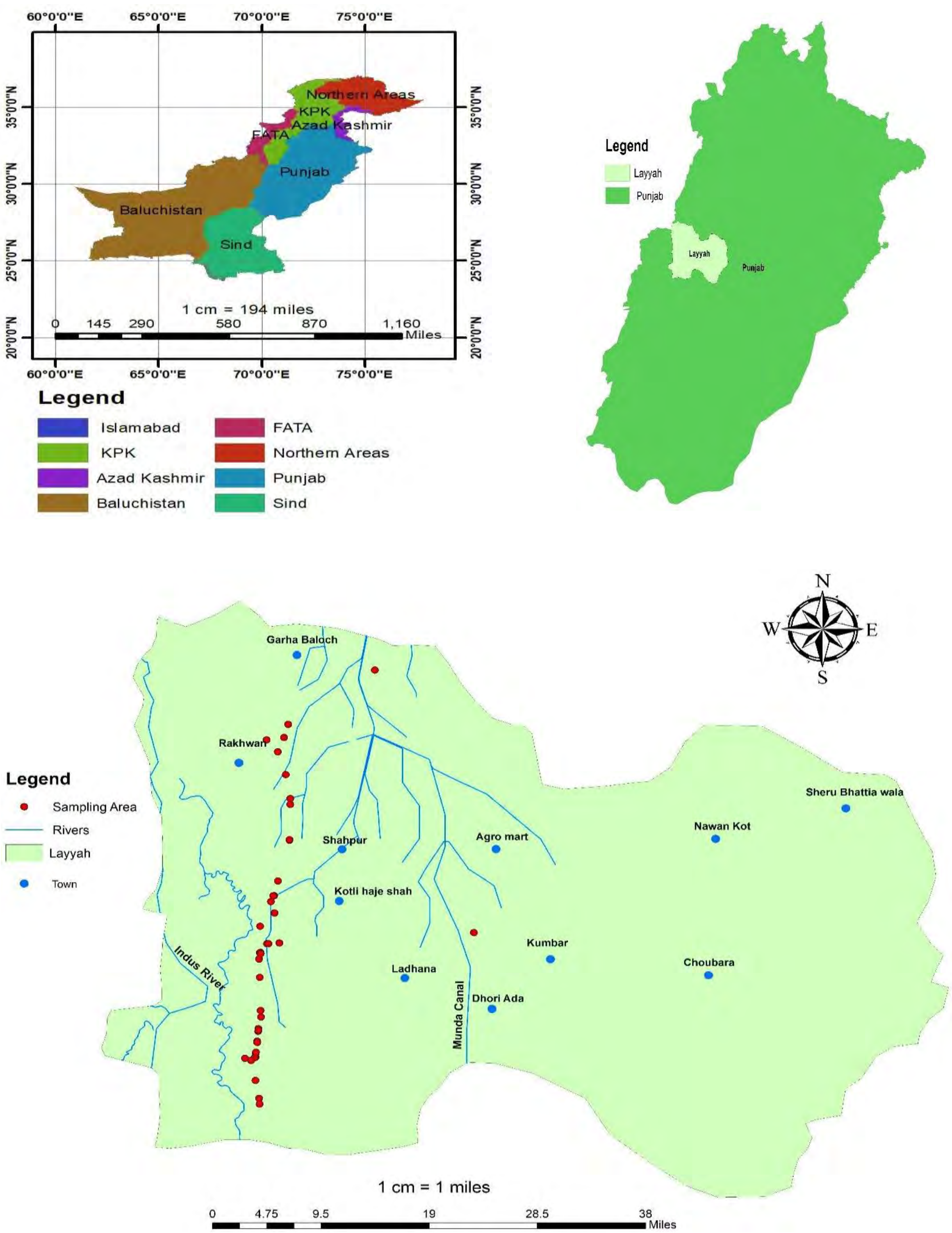


Figure 3. Area map of sampling sites of District Layyah Punjab Pakistan.

Questionnaire design

The researcher carefully studied current literature on the issue before performing the study and designing a questionnaire. The questionnaire was designed in a "mixed" format, which means it included both open-ended and closed-ended questions to capture both qualitative and quantitative data. The initial portion of the questionnaire collected socio-demographic information from participants such as their height, weight, age, education status, marital status, employment position, wealth quintile, and previous medical history. The following section of questionnaire covered a variety of aspects, such as work history, any previous diseases or health issues, smoking status or habits, reproductive history, the nature of the job, the duration of exposure to brick kiln pollutants and the participants' knowledge of other diseases associated with such work. By using this well-structured questionnaire, the researchers were able to collect valuable data, both in qualitative and quantitative forms, to thoroughly explore the impact of brick kiln contaminants on the health and reproductive well-being of male workers in selected areas in Punjab's District Layyah.

Participants selection

This study focused on adult males since they are the ones who are most subjected to brick kiln emission. Men do the majority of the activities associated in the brick kiln the industry, such as loading, unloading, baking and fire. As a result, the researcher included adult male brick kiln workers (n=300) who had worked in brick kilns for at minimum 50 years. To compare the effects, a control group of males (n= 200) was also included in the study. The control group served as a comparison group to assess the potential impacts of working in brick kilns on the health and reproductive well-being of the workers.

Interviews

Before completing the scientific investigation, the researcher made certain that all subjects provided informed permission. Following that, the individuals were questioned using a systematic and verified questionnaire. The questionnaire was designed to gather various demographic details from each participant, making it easier to understand their backgrounds and potential influences on their health. The demographic information collected through the questionnaire included height, weight, age, education level, family size, marital status, the number of children, any record of diseases in the family, current medications, smoking habits, duration of exposure to brick kiln emissions, the amount of time spent on job whether they used protective equipment while working and the nature of their job tasks. By using this comprehensive questionnaire, the researchers aimed to gather essential information about the participants, which would help in analyzing the potential health effects of working in brick kilns and their reproductive health.

Inclusion criteria

In this study, healthy participants from various ethnic backgrounds were included in the study group.

Exclusion criteria

Workers beyond the age of 80 were excluded from the present study.

Grouping of participants

After gathering the necessary information from the participants, the males were separated into two distinct groups based on their characteristics. First group was labeled as Control males (CM). These were individuals who did not work in brick kilns and served as the control group for comparison. The second group was comprised of male workers who were actively working in brick kilns. This group was referred to as brick kiln male workers (AM).

Collection of demographic data

Once the participants were selected, they were interviewed using a self-reported questionnaire (SRQ). This questionnaire included various demographic details such as age, gender, ethnicity, height, weight, education level, diet and the duration of their work in brick kilns. Additionally, the questionnaire collected information on their daily working hours and their disease history.

Blood Samples Collection

Blood samples were taken from both brick kiln workers and control members after a written consent was signed. A 5 mL syringe was used to draw the blood, which was then divided into four portions. Three of these parts were placed in tubes coated with EDTA (a chemical that helps preserve the blood). The blood in the EDTA tubes was used for three different purposes. First, it was used for complete blood count (CBC) to assess various blood parameters. Second, the blood in these tubes was used to assume the levels of heavy metals using a technique called Atomic Absorption Spectroscopy (AAS). Blood in the third tube was separated into plasma and kept at (-20 °C) till further analyzed. The fourth part of the blood was collected in yellow-top tubes. These tubes were used to conduct liver function tests which help to evaluate the health of the liver.

Body mass index

The body mass index (BMI) of all participants was determined by dividing their weight in kilograms by the square of their height in meters.

BMI= weight (kg)/ height(m^2)

1. Underweight body group (BMI less than 18.5)
2. Normal body weight group (BMI less than 24)
3. Overweight body group (BMI greater than 24)
4. Obese body weight group (BMI greater than 30)

Measurement of Blood Pressure

Blood Pressure was measured while workers were sitting after a 5-minute rest interval, and the stated result is the average of three measures obtained via a mercury-based Sphygmomanometer on the right arm.

Measurement of blood sugar

Blood sugar is measured with the Life Check TD-4141 glucometer.

Hematology Analysis

Blood samples were stored in lavender-colored vacutainers containing a substance called EDTA K3, which helps preserve the blood for analysis. The hematology analysis was conducted using an automated hematology analyzer at Al Ahmad Laboratory in Karor Lal Esan district, Layyah.

Liver function test (LFTs)

The blood samples collected were stored in red-top tubes, specifically for obtaining serum (the liquid part of blood). Liver function tests were conducted using a Chemistry analyzer at Al Ahmad Laboratory in Karor Lal Esan district, Layyah. The data collected include Alkaline phosphatase (ALP), Alanine transaminase (ALT) Aspartate aminotransferase (AST), Bilirubin Total and Albumin.

Analysis of Heavy metals (Atomic Absorption)

The present study's methodology was based on (Ishaq *et al.,* 2010; Tripathi *et al.,* 1997) with a few adjustments.

Acid Digestion

The acid digestion process for the samples involved four steps:

Washing Step

All the equipment to be used was soaked in a 10% nitric acid solution overnight. Then, the equipment was washed with a 69% nitric acid solution and dried using absorbent paper. The equipment was kept overnight after washing.

Pre-digestion:

5 ml of 69% nitric acid was added to 0.5 ml of whole blood until the solution turned transparent. This is known as pre-digestion.

Digestion

Manual digestion was performed by boiling the mixture of whole blood and nitric acid at 400°C until the solution volume reduced by half.

Filtration

The samples were then filtered using Whatman filter paper to separate any solid particles. Finally, distilled water was added to the filtrate to reach a final volume of 15 ml which was used for further analysis using Atomic Absorption Spectroscopy (AAS).

Formulation of metal standards (Cd and Pb)

The following method was used to make the standard solution for each metal salt at a concentration of 1000 ppm:

Molecular weight of salt = X (Molecular weight of sample) / 0.1

To make the 1000 ppm solution, X grams of the metal salt were mixed in 100 ml of distilled water. Next the instrument (AA 40 FS Fast Sequential Atomic Absorption Spectrometer) was calibrated using multiple concentrations ranging from 1 to 50 ppm. The calibration was done using the formula:

$C_1V_1 = C_2V_2$

Where:

C_1 = Concentration of the standard's stock solution

V_1 = Volume of the standard's stock solution

C_2 = Required concentration for the calibration curve

V_2 = Required volume for the calibration curve

Using this calibration curve, the number of heavy metals in the samples were determined in ppm (parts per million). To present the results in a more familiar unit, µg/dL (micrograms per deciliter) was used. This conversion allows for easy interpretation and comparison of the measured concentrations with standard reference values.

Biochemical Analysis

The activity of oxidants (reactive oxygen species and thiobarbituric acid reactive species) and antioxidant enzymes (catalase and sodium peroxidase) in blood plasma from control and brick kiln male workers was measured using a UV Spectrophotometer (Agilent 8453).

Catalase (CAT) activity

Used Reagents

1. Plasma 2ml
2. Phosphate buffer solution 2.5ml (50 mM, pH=7)
3. H_2O_2 1ml (30 mM)

Procedure

The activity of catalase (CAT) was calculated in this study using a method of (Aebi, 1974). The CAT activity was measured by determining the decrease in absorbance induced by hydrogen peroxide (H_2O_2) consumption. To assess CAT activity, 2 ml of diluted plasma (25µl of plasma mixed with 2.5 ml of 50 mM phosphate buffer solution at pH 7) and 1 ml of 30 mM H_2O_2 were used. An Agilent 8453 spectrophotometer was used to obtain absorbance values at 240 nm at 15-second intervals for 1 minute. In addition, instead of plasma, blanks were run with pure water. The unit of CAT activity was defined as a 0.01 per minute change in absorbance.

Superoxide dismutase activity

Used Reagents

1. Homogenate 0.3ml
2. Sodium pyrophosphate 1.2ml (0.052 mM; pH 7.0)
3. Phenazine methosulphate 0.1ml (186 μM)
4. NADH 0.2ml (780 μM)
5. Glacial acetic acid 1ml

Procedure

In the current study, the activity of superoxide dismutase (SOD) was evaluated using the methods developed by (Kakkar *et al*., 1995). To evaluate SOD activity, a combination of 0.3 ml of the sample, 1.2 ml of sodium pyrophosphate buffer (0.052 mM; pH 7.0), and 0.1 ml of phenazine methosulphate (186 M) was prepared. The reaction was started by adding 0.2 ml of NADH (780 M) to the mixture. After 1 minute, 1 ml of glacial acetic acid was added to stop the reaction, and absorbance readings were recorded at 560 nm. The results are given in units per milligram of protein.

Peroxidase activity (POD)

Used Reagents

Plasma	0.1 ml
Guaiacol	0.1 ml of 20 mM
H_2O_2	0.3 ml of 40 mM

Phosphate buffer 2.5 ml of 50 mM

Procedure

The reaction solution was made up of 0.1 mL of plasma, 0.1 mL of 20 mM guaiacol, 0.3 mL of 40 mM H2O2, and 2.5 mL of 50 mM phosphate buffer at pH 5.0. The absorbance of the reaction mixture was measured at 470 nm after one minute. Based on the study of (Chance & Maehly, 1955), POD activity was defined as an absorbance change of 0.01 per unit per minute

Glutathione reductase activity (GR)

Used Reagents:

1. Buffer 250 mmol/l potassium phosphate pH 7.3: 0.5 mmol/l EDTA
2. GSSG 2.2 mmol
3. NADPH 0.17 mmol in 0.1 % sodium carbonate
4. Plasma 0.04 ml

Procedure

The reaction solution contained 250 mmol/l potassium phosphate at pH 7.3 and 0.5 mmol/l EDTA, as well as 2.2 mmol of GSSG and NADPH (0.17 mmol in 0.1% sodium carbonate). To evaluate glutathione activity, 0.04 ml of plasma was combined with 1 ml of substrate solution (0.5 ml buffer and 0.5 ml GSSG), followed by 0.2 ml of NADPH (0.1 ml NADPH and 0.1 ml ddH_2O). The absorbance of the mixture was measured at 340 nm after 1 minute and for a total of 5 minutes.

Reduced glutathione (GSH)

Used Reagents

1.	Plasma	100 µl
2.	deionized water	800 µl
3.	Trichloroacetic acid	100 µl (50 %)
4.	Tris-EDTA buffer	800 µl (0.2 M, pH 8.9)
5.	DTNB reagent	20 µl
6.	GSH	0.0307g
7.	EDTA	0.4 M

Procedure

The reduced glutathione (GSH) level was measured using a method that was determined by (Dominiczak, 1999) includes various changes, including the use of Ellman's reagent (DTNB). 100 µl of plasma was combined with 800µl of deionized water and 100 µl of 50% trichloroacetic acid to determine GSH levels in plasma samples. After mixing for 10-15 minutes using a mixer, the mixture was centrifuged for 15 minutes at 3000 rpm. The 400 µl supernatant was then mixed with 800 l of tris-EDTA buffer (0.2 M, pH 8.9) and 20 µl of the DTNB reagent. Using a vortex, the resultant solution was thoroughly mixed. For comparison, distilled water was used to make blanks. There were also standard tubes made with 0.0307 g of GSH in 100 ml of EDTA (0.4 M) solution. Within 5 minutes of adding DTNB to the standard tubes and plasma samples, the absorbance at 412 nm was determined. This method allows for the quantification of reduced glutathione in the samples.

Estimation of ROS

In this study, the estimated number of reactive oxygen species (ROS) was determined using a procedure previously devised by (Hayashi *et al*., 2007). Firstly, a solution called R_1 was prepared, containing 100 g/ml of DEPPD dissolved in sodium acetate buffer (0.1M) with a pH of 4.8. Secondly, R_2 solution with a concentration of 4.3M was prepared by dissolving ferrous sulfate in sodium acetate buffer. To establish a calibration curve, a standard solution of H_2O_2 was used. The measurements were taken using a UV Spectrophotometer (Agilent 8453) to quantify the number of ROS present in the samples based on the readings obtained from the calibration curve.

Lipid peroxidation assay

Used Reagents

1. Phosphate buffer 0.29ml (0.1 M; pH 7.4),
2. Plasma 0.1ml
3. Ascorbic acid 0.1ml (100 mM)
4. Trichloroacetic acid 0.5ml (10 %)
5. Thio barbituric acid 1 ml 0.67 %

Procedure

In this study, malondialdehyde (MDA) in blood plasma was measured using the TBA method. 0.29ml of 0.1 M phosphate buffer (pH 7.4) and 0.1ml of 100 mM ascorbic acid were added to a 0.1ml plasma sample. In a shaking water bath, the mixture was incubated for one hour at 37°C (Wright *et al*., 1981). To stop the reaction after 60 minutes, 0.5ml of 10% trichloroacetic acid was added. Then the reaction mixture was then incubated for 20 minutes at 95°C in a boiling water bath with 1ml of 0.67% trichloro barbituric acid. After cooling in a crushed ice bath, the samples were centrifuged at 2500X g for 10 minutes to separate the supernatant. A spectrophotometer (Agilent 8453) was used to measure the optical density of the samples and the reagent blank at 535nm. Using a particular formula, the TBARS concentration was determined and represented as TBARS per minute per milliliter of plasma at 37°C.

Concentration of the test = $Abs_{test} - Abs_{blank} / 1.56*1000000$

Total protein measurement

The total protein content in the serum was measured using a kit provided by AMP kits from AMEDA Labordiagnostik GmbH (Austria).

Principle of Assay:

When Cu^{+2} ions react with peptide bonds in an alkaline solution, they produce a chelate known as the biuret reaction. The amount of color produced indicates the concentration of protein in the sample. This reaction results in the formation of a complex between copper and protein, and the stronger the color, the greater the protein concentration in the sample. The reaction takes place at a pH greater than 12.

Used Reagents

Potassium sodium tartrate	21 mM/L
Potassium iodide	6 mM/L

Copper sulphate	6 mM/L
Sodium hydroxide	0.75 M/L

Standard:

Bovine serum albumin	7 g/dL

Procedure

Standards for calibration were created by adding 20 µl of the standard solution provided in the kit to 1.0 ml of the reagent. For the samples, 20 µl of serum was mixed with 1.0 ml of the reagent, and for the reagent blank, 20 µl of distilled water was used instead of serum. All samples were then incubated at 37°C for 10 minutes. The change in absorbance of both the standards and samples was determined at 546 nm with a chemistry analyzer. The concentration of the unknown samples is determined using a specific formula based on the absorbance values obtained from the measurements.

$$A_{sample} / A_{standard} \times C_{standard} = \text{g/dl Total protein}$$

Lipid profile

A lipid profile is a set of tests conducted to measure the levels of cholesterol and other fats in the blood. This profile includes the measurement of the following components:

Triglycerides

Total cholesterol

Low-Density Lipoprotein (LDL)

High-Density Lipoprotein (HDL)

Blood plasma samples were analyzed using a chemistry analyzer from (AMP diagnostic) and AMP diagnostic kits (AMEDA Labordiagnostik GmbH, Austria) Low-density lipoprotein (Cat # BR3302) TC (Cat # REF104989993203), High-density lipoprotein (Cat # 104989993193) and TG (Cat # REFBR4501) were measured in blood plasma.

High density lipoprotein

HDL is often referred to as "good cholesterol" because it plays a beneficial role in the body by helping to remove LDL (low-density lipoprotein) cholesterol, which is considered harmful. This action of HDL helps reduce the risk of heart diseases. Healthy levels of HDL in the blood typically range from 40 to 60 mg/dL.

Total cholesterol

The measurement of total cholesterol in the body provides an indication of the overall cholesterol level. For adults, a desirable total cholesterol level is less than 200 mg/dL.

Triglycerides

Excess calories are converted by the body into triglycerides, which are subsequently stored as fat in cells. High levels of triglycerides are commonly found in obese individuals or animals. A triglyceride level of 60 mg/dL or above is in the protective range. This means that having triglyceride levels at or above this level is associated with a lower risk of certain health issues.

Principle

Lipoprotein lipase (LPL) is an enzyme that breaks down plasma triglycerides into free fatty acids (FFA) and glycerol. Glycerol is phosphorylated in the presence of glycerol kinase (GK) and adenosine triphosphate (ATP) to produce adenosine diphosphate (ADP) and glycerol-3-phosphate (G-3-P). As a result of the oxidation of G-3-P, hydrogen peroxide and dihydroxyacetone phosphate (DHAP) are produced. To measure the triglyceride content in a sample, a combination of phenol and 4-aminoantipyrine (4-AA) is mixed with hydrogen peroxide (H_2O_2) under the action of peroxidase (POD). This reaction produces a red chromogen, the concentration of which is inversely proportional to the number of triglycerides present in the sample. Therefore, the more triglycerides in the sample, the less intense the red color formed during the reaction. This method allows for the quantification of triglycerides in biological samples.

Triglyceride + $3H_2O \rightarrow$ 3FFA + glycerol

GK

ATPGK + Glycerol $\rightarrow$ ADP + glycerol − 3 − p

GPO

Glycerol − 3 − p + O_2GPO $\rightarrow H_2O_2 + DHA$

H_2O_2

4 − phenol + 4 − AA $\rightarrow$ H, O + Quinoneimine

Bring the reagent and sample to room temperature.

Read against reagent blank.

Pipette as follows.

Amount of chemicals for Triglycerides.

	Blank	Sample	Standard
Reagent	1.0mL	1.0mL	1.0mL
Sample	----	10µL	----
Standard	----	----	10µL

Gently mixed

Incubate at 37 C for five minutes.

Absorbance of sample and standard was observed.

Cholesterol

Principle

Enzymatic based colorimetric technique with cholesterol esterase and cholesterol oxideoxidase was used.

CHE

Cholesteryl esters + H_2O → fatty acids + cholesterol

CHO

O_2 + Cholesterol → H_2O + cholest − 4 − en − 3 − one

Phenol +2H_2O_2 +4-aminoantipyrine → quinonimine dye-4-en-3-one + H_2O

(Red colored)

A linear relationship is present between cholesterol concentration and color intensity.

Manual Procedure

Wavelength 500nm (Hg-546nm)

Temperature 20-25°C /37°C

Cuvette 1cm

Pipette into cuvette.

Concentration of chemicals for cholesterol.

	Reagent blank (RB)	Test	Standard
Cholesterol	1000µL	1000µL	1000µL
Bring up to the temperature of determination. Then Add			
Standard	----	----	10µL
Sample	----	10µL	----

Incubate for 5 min at 37°C. Against reagent blank read the absorbance of the test andstandard

High Density Lipid (HDL)

Principle:

There are two steps in distmel reaction assay:

(1) By using cholesterol esterase, oxidized cholesterol and then catalase chylomicron VLDL and LDL are eliminated.

Cholesterol

Cholesteryl ester → cholesterol + fatty acids

Cholesterol oxidase

O_2 + Cholesterol → cholesterol + H_2O_2

Catalase

$2H_2O_2 \rightarrow H_2O + O_2$

2) After detergents in 2-Reagent have released-Cholesterol, HDL-Cholesterol measurement was done. Catalase is inhibited by sodium azide in the 2-Reagent.

Cholesterol esterase

Cholesterol ester → fatty acids + cholesterol

Cholesterol oxidase

O_2 + Cholesterol → H_2O_2 + cholesterol

Permidase

$2H_2O_2$ → qumonc pigment + $4H_2O$

Procedure

Wavelength 620nm, 630nm, 670nm,

Temperature 37°C

Optical path 1cm

Measurement against reagent blank and substrate.

Concentration of the chemicals for HDL.

	RB	**Test**	**Cal**
Reagent 1	300 µL	300 µL	300 µL
Sample	-	5 µL	-
Calibrator	-	-	5 µL
Mix well, incubate 5min at 37°C, and then add			
Reagent 2	100 µL	100 µL	100 µL

Mix well**;** incubate calibrator after 5 minutes absorbance of A (T) and calibrator A against the blank reagent.

Low Density Lipid (LDL)

Principle

The LDL cholesterol in plasma determination occurs in two steps. In the first step, all other lipoproteins present in the sample, such as HDL and VLDL, are completely masked, leaving only LDL unmasked. Then, by adding the R2 reagent, Cholesterol esterase (CE) and Cholesterol oxidase (CO) specifically react with the unmasked LDL, enabling a direct and accurate measurement of the LDL cholesterol component. This method allows for a simplified and efficient assessment of LDL cholesterol levels in plasma.

CE

Cholesterol Ester + H_2O → Cholesterol + Fatty Acid

CO

Free cholesterol + O_2 → delta 4 - cholestone + H_2O_2

POD

$2H_2O_2$ + 4-AA + TODB → Quinone Dye + $4H_2O$

The LDL in the samples is directly proportional to the intensity of the color developed in the sample.

Procedure

Wavelength: 600nm (sub 700nm)

Temperature: 37°C

Cuvette 1 cm light path

Read against reagent blank.

Pipette as following:

Concentration of the chemicals for LDL.

	Blank	**Calibrator**	**Sample**
Reagent 1	220uL	220uL	220uL
Distilled water	3uL	-----	-----
Calibrator	-----	3uL	-----
Sample	-----	-----	3uL

Mix gently.

Incubate for 5minutes at 37°C.

Read and record absorbance of calibrator and sample.

Add:

Reagent 2	**220uL**	**220uL**	**220uL**

Mix gently.

Incubate for 5 minutes at 37°C.

Read and record absorbance of calibrator and sample.

Hormonal Analysis

Quantitative Determination of Testosterone Concentration

Testosterone levels were determined by quantitatively using EIA kits from Bio check Inc, USA. The assay works based on a specific ability of antibody to bind with testosterone molecules in the sample. By measuring the signal produced during the reaction, the concentration of testosterone in the sample can be accurately determined.

Principle of the Test:

The testosterone ELISA worked on a competitive principle. Micro well plates were coated with goat anti-rabbit antibodies, forming a solid phase. HRP-labeled testosterone, testosterone calibrator and a specific antibody were added, leading to the formation of a secondary antibody. The amount of HRP-labeled testosterone was inversely proportional to the testosterone concentration in the plasma. After removing the unbound testosterone-HRP, substrates (Chromogen A and Chromogen B) were added and the absorbance value was calculated. By plotting a concentration-absorbance curve, the testosterone content of samples can be calculated.

Procedure:

To measure T levels in human plasma, the required number of coated wells were secured in the holder. Standards, specimen and controls (19µl) were dispensed, followed by addition of Testosterone-HRP conjugate Reagent (100µl) and Rabbit anti-Testosterone Reagent (50µl) and a thorough mixing for 30sec. The microtiter well plate was incubated at 37°C for 90min, followed by cleansing 5 times with distilled or deionized water and administration of TMB Reagent (100µl), after gentle mixing for 10sec, the micro well plate was incubated at 18-25°C for 20min. Lastly, stop solution (100µl) was augmented to stop the reaction and was mixed gently for 30sec. Later, within 15min, the absorbance was read at 450nm using microtiter well reader. The results were expressed in ng/ml.

Statistical analysis

The data is represented as the mean and standard error of the mean (Mean±SEM). Descriptive statistics of socio-demographic variables were computed as mean, standard deviation, and percentage. An unpaired student t-test in SPSS 22 were used to compare test and control participants in terms of heavy metal burden, blood parameters, liver function test, antioxidant enzymes profile, and hormonal concentration.

RESULTS

Area distribution of workers

Brick kiln workers residing at various sites in Layyah District, Punjab, come from different towns and tehsils. The number of workers at each brick kiln site varied and this distribution is illustrated in the following figure.

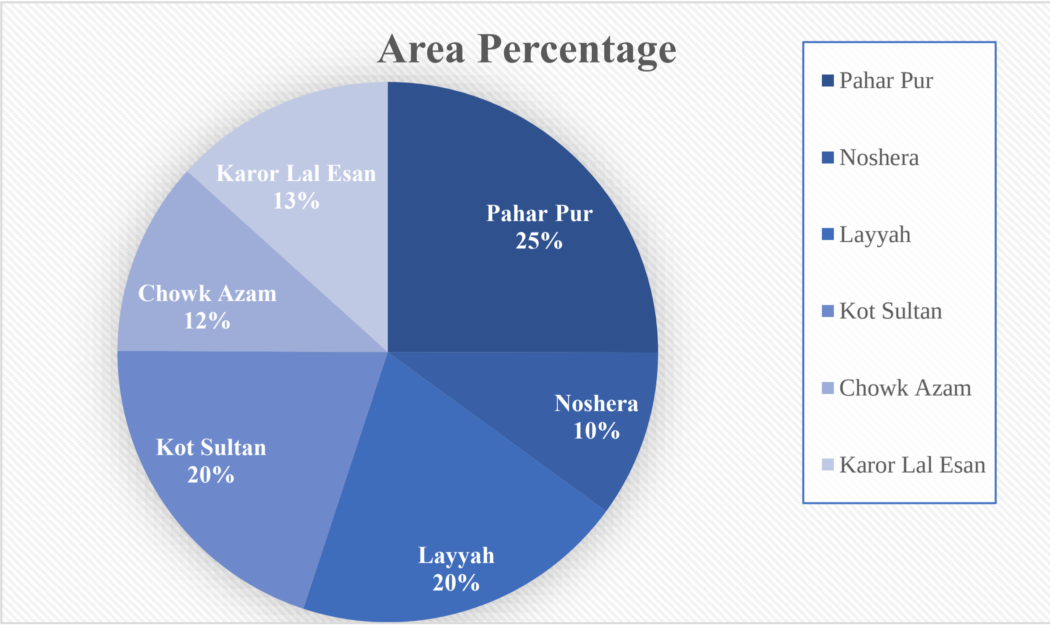


Figure 4. Area distribution of male workers working at brick kiln sites in district Layyah

Language of workers

The subjects working at brick kiln sites spoke different languages.

- Saraiki
- Punjabi
- Pashto

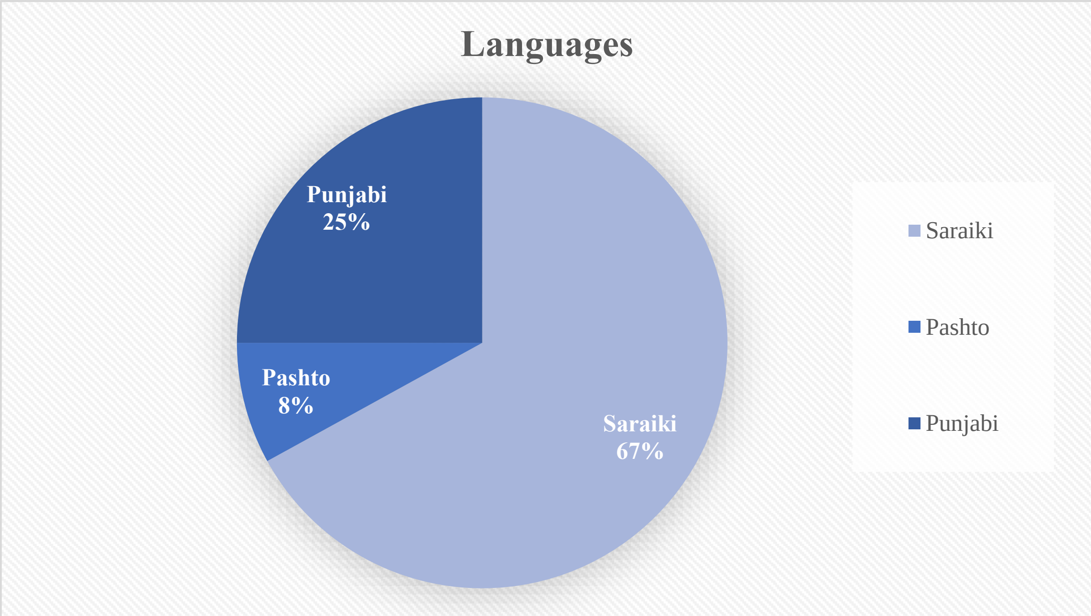


Figure 5. Pie chart showing languages spoken by male workers at brick kiln sites.

Sociodemographic data

The brick kiln workers had an average age of 38.02±0.76 years, while the control individuals had an average age of 40.04±0.98 years.Our findings revealed that the majority of men working at brick kiln sites had a healthy weight, but a small percentage (1.6%) were classified as obese, 8.6% were overweight, and 32% fell into the overweight category. Among these brick kiln workers, a significant proportion (85.3%) were married, while the remaining 14.7% were unmarried.Regarding sleep duration, most brick kiln workers reported sleeping routine between 5 to 10 hours (93.3%). Furthermore, about 52.4% of the workers had not received formal education. When it came to food preferences, an overwhelming majority (96%) of brick kiln workers preferred vegetables as their primary dietary choice.

Table 1. Demographic data of men working at brick kiln sites.

Socio-demographic parameters				
Parameters	**Control**		**Workers**	
Age(years)	**n**	**Percentage**	**n**	**Percentage**
18-27	60	30	75	25
28-37	46	23	85	28.3
38-47	25	12.5	76	25.3
48-57	52	26	35	11.6
58-67	14	7	19	6.3
67+	3	1.5	10	3.3
Age (average)	40.04±0.98		38.02±0.76	
BMI				
Underweight	17	8.5	96	32
Normal	108	54	173	57.6
Overweight	54	27	26	8.6
Obesity	21	15	5	16
Marital Status				
Married	129	64.5	256	85.3
Unmarried	71	35.5	44	14.7
Sleep Duration(hour)				
<5	4	2	17	5.6
5-10	174	87	280	93.3
10+	22	11	3	1
Education Status				
Illiterate	11	5.5	157	52.4

Primary	0	0	73	24.3
Middle	0	0	29	9.7
Matriculation	4	2	34	11.3
Intermediate	69	34.5	7	2.3
Bachelors	35	17.5	0	0
Masters	65	32.5	0	0
Higher studies	16	8	0	0
Food				
Vegetables, Meat	128	64	12	4
Meat	72	36	0	0
Vegetables	0	0	288	96

Health history of brick kiln workers

People who were exposed to smoke emitted from brick kilns experienced multiple health issues.. These are shown in following table;

Table 2. Health history of brick kiln workers in district Layyah.

Parameter	Control			Workers
Health History	**n**	**Percentage**	**n**	**Percentage**
Bone pain	0	0	105	35
Fever	0	0	71	23.6
Chest tightness	0	0	50	16.6
Shortness of breath	0	0	49	16.3
Stomach problem	11	5.5	40	13.3
Cough	3	1.5	39	13
Asthma	0	0	33	11
Kidney problem	0	0	25	8.3
Allergy	4	2	16	5.3
Urination problem	0	0	16	5.3
Stomach ulcer	0	0	15	5
Hepatitis	0	0	15	5
Heart problem	0	0	8	2.6
BP issues	0	0	7	2.3
Leg pain	0	0	6	2
Weakness	0	0	5	1.6
Anemia	0	0	5	1.6
Headache	0	0	4	1.3
Anxiety	0	0	4	1.3

Eyesight problem	0	0	4	1.3
Lesions	0	0	4	1.3
Deafness	0	0	3	1
Joints pain	0	0	3	1
Cold	0	0	2	0.6
Diabetes	9	5	2	0.6
Flu	0	0	2	0.6
Tiredness	0	0	2	0.6
Throat problem	0	0	2	0.6
Migraine	2	1	3	0.6
Piles	0	0	1	0.3
Depression	0	0	1	0.3
Liver Cancer	0	0	1	0.3
Jaundice	0	0	1	0.3
Aggressiveness	0	0	1	0.3
Thyroid	0	0	1	0.3
Lesions	0	0	1	0.3
Numbness	0	0	1	0.3
Arm pain	0	0	1	0.3
Bleeding from nose	0	0	1	0.3
No	170	85	44	14.6
Medication				
Yes	22	15	27	10.4
No	8	4	225	75
No need	170	85	44	14.6

Work history of brick kilns wokers

The people in this group were employed at very low wages, earning between PKR 10,000 to 20,000 per month. Majority (77%) of them were getting salaries in the range of 10,000 to 15,000 PKR. Out of the 300 workers, 57.7% worked as molders, 23.7% were carriers, 16% were bakers and 2.6% held the position of munshis (accountants).On average, these individuals had been living near brick production sites for around 37 years and their job tenure averaged at 15 years. These workers faced exposure to various harmful substances such as smoke, smog, dust and fuel.The use of personal protective equipment (PPE) was not a common practice among the kilns visited. None of the male workers were observed using any form of PPE at their workplace.

Table 3. Work profile and occupational history of brick kilns workers

Work history				
Parameters	**control**		**Workers**	
Income	**n**	**Percentage**	**n**	**Percentage**
<10,000	0	0	19	6.3
10,000-15,000	19	9.5	231	77
16,000-20,000	60	30	47	15.7
21,000-25,000	51	25.5	30	1
26,000-30,000	21	10.5	0	0
31,000-35,000	49	24.5	0	0
Work type				
Molder	0	0	173	57.7
Carrier	0	0	71	23.7
Bakers	0	0	48	16
Munshi	0	0	8	2.6
Job	200	100	0	0
Year of living in vicinity				
1-10	5	2.5	0	0
11-20	8	4	15	5
21-30	54	27	96	32
31-40	47	23.5	84	28
41-50	21	10.5	56	18.7
51-60	51	25.5	29	9.7

60+	14	7	20	6.6
Work duration(years)				
1-10	NA	NA	111	37
11-20	NA	NA	119	39.7
21-30	NA	NA	47	15.7
31-40	NA	NA	19	6.3
41-50	NA	NA	4	1.3
Exposure time(hours/day)				
1-4	NA	NA	3	1
5-8	NA	NA	152	50.7
9-12	NA	NA	141	47
13-18	NA	NA	4	1.3
Exposure material				
Smoke, Smog, Dust	NA	NA	173	57.6
Smoke, Smog, Dust, Fuel	NA	NA	127	42.4
Personal protective equipment usage				
Gloves	NA	NA	0	0
Mask	NA	NA	0	0
Head Covers	NA	NA	0	0
Respirators	NA	NA	0	0

Smoking status

A significant portion of the workers (53.7%) were found to be addicted to tobacco, consuming it in different forms such as cigarettes, naswar (a mixture of calcium oxide, tobacco leaves, and wood ash), hukkah (a tobacco mixture) and opium (a dried latex from opium poppy seed capsules). The average age at which these addictions began was 20 years. Breaking down the numbers, 12.3% of workers smoked 1 to 10 cigarettes, 11.3% used one packet of naswar, 6.3% indulged in hukkah 6 to 10 times and a small 0.6% were addicted to opium.

Table 4. Consumption of different addictive components by brick kiln workers.

Smoking status of brick kiln workers				
Parameters	**Control**		**Workers**	
Addiction	**n**	**Percentage**	**n**	**Percentage**
Yes	50	25	161	53.7
No	150	75	139	46.3
Addiction types				
Cigarette	38	19	49	16.4
Naswar	7	3.5	83	27.7
Huqqa	5	2.5	27	9.3
Opium (Pellets)	0	0	2	0.6
Age at starting addiction(years)				
<10	0	0	3	1
10-20	21	10.5	103	34.3
21-30	24	12	50	16.7
31-40	5	2.5	5	1.7
Age at starting addiction(average)	23.08±0.8		19.87±0.4	
Daily intake				
Cigarette(number)				
1-10	24	12	37	12.4
10-20	11	5.5	10	3.4
20+	3	1.5	2	0.6
Naswar (packets)				

1/4th	1	0.5	16	5.4
½	2	1	33	11
1	4	2	34	11.3
Huqqa				
1-5times	1	0.5	8	2.7
6-10times	4	2	19	6.3
Opium (Pellets)				
1-2	0	0	2	0.6

Heavy metals

The concentrations of Pb and Cd in whole blood were considerably higher in brick kiln workers than in the control group. Significant rise in level of lead was evident in male worker group (8.56±0.03 µg/dL) in contrast to control group (1.37±0.03 µg/dL). male workers showed significantly greater amounts of Cd in their blood (p=0.002) than control individuals.

Table 5. Mean±SEM of heavy metals (lead and cadmium) concentration in whole blood of control and brick kiln workers.

Parameters	Control	Workers	P value statistics
Lead(µg/dL)	1.37±0.03	8.56±0.03**	0.004
Cadmium(µg/dL)	1.41±0.03	4.13±0.20**	0.002

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

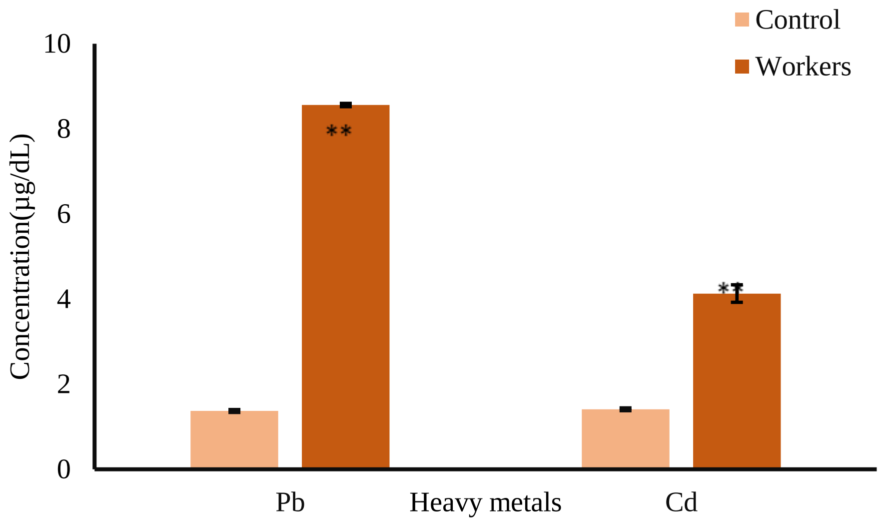


Figure 6. Comparison of heavy metals burden in blood plasma among control and workers.

Body mass index

There was an evident reduction (P=0.001) in the BMI (Kg/m^2) of workers who were exposed to brick kiln conditions as compared to men who were not exposed. In the control group, the average value of BMI was (24.71±0.23Kg/m^2) while among brick kiln workers, it was (20.75±0.22Kg/m^2) and this difference was determined to be statistically significant with a p-value of p=0.001.

Table 6. Comparison of BMI (Kg/m^2) between control and brick kiln male workers

	Control	**Workers**	**P values**
BMI(Kg/m^2)	25.16±0.20	20.43±0.22***	p= 0.001

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

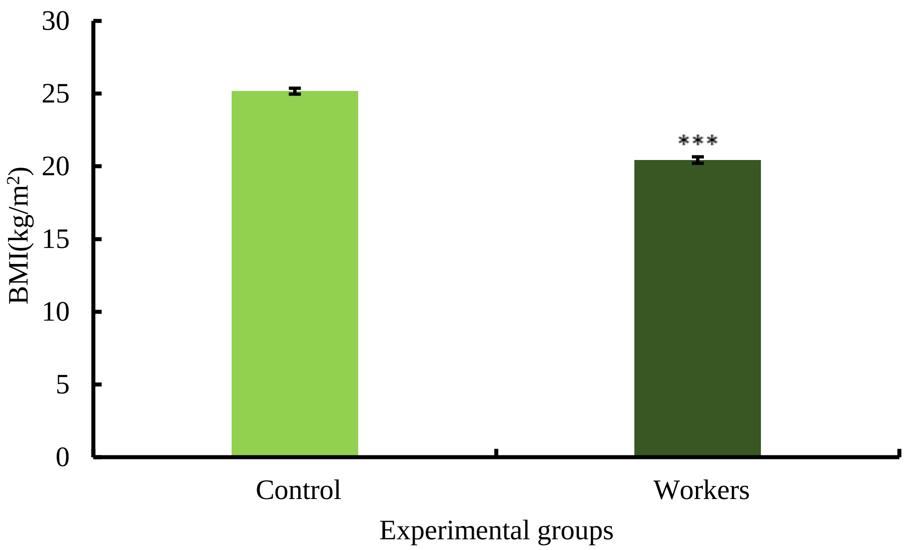


Figure 7. Body mass index (Kg/m^2) of control and male workers.

Blood Pressure

There was a significant increase in systolic and diastolic blood pressure in the working group as compared to the control group. In control , the average SBP was (105.50±0.080mmHg) while among brick kiln workers it was (123.43±0.073mmHg). In the control group DBP was (69.48±0.079mmHg) while among workers it was (89.000±0.081mmHg).

Table 7. Systolic and diastolic blood pressure (mmHg) of workers and non-workers.

Parameters	Control	Workers	p value statistics
Diastolic blood pressure	69.48±0.079	89.00±0.081***	0.001
Systolic blood pressure	105.50±0.079	123.43±0.073**	0.006

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

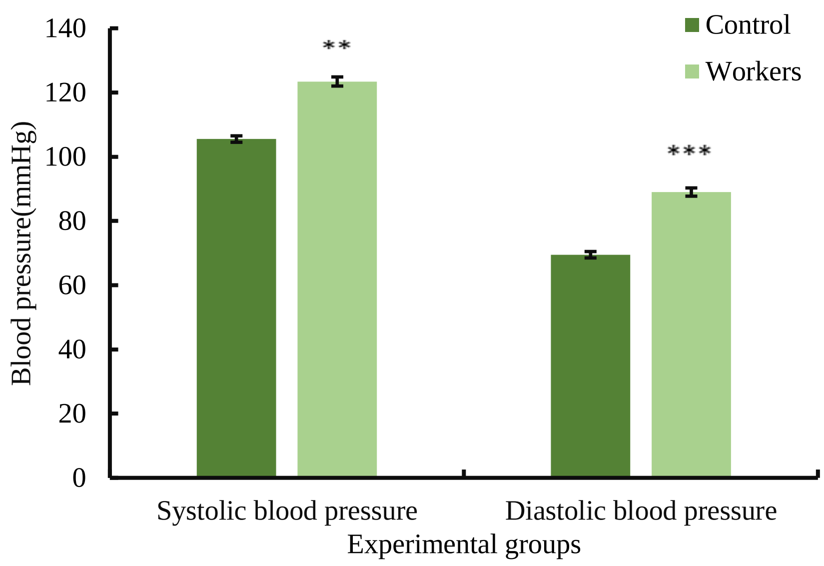


Figure 8. Systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) of workers and control.

Blood Sugar

Among the control subjects, the blood sugar level was measured at (90.09±0.22 mg/dL) while for brick kiln workers, it was notably lower at (83.84±0.20mg/dL) (as shown in figure).

Table 8. Blood sugar level (mg/dL) of workers and control.

Parameters	Control	Workers	p value statistics
Blood sugar(mg/dL)	90.09±0.22	83.84±0.20***	0.001

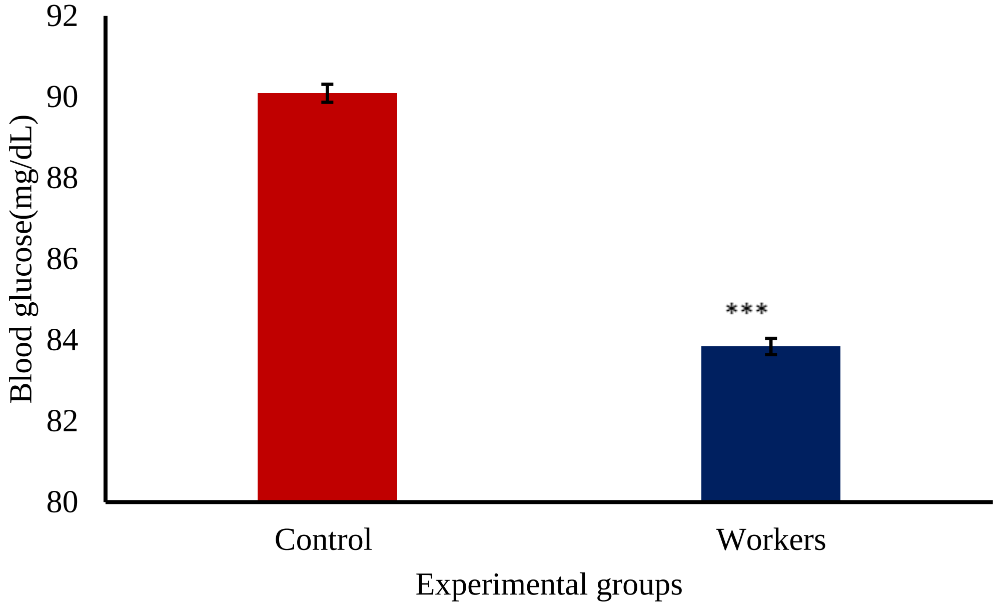


Figure 9. Blood sugar level (mg/dL) of male workers and non-workers.

Blood Parameters (White blood cells)

The results of the complete blood analysis revealed significant differences in the white blood cell counts between the control and worker subjects, as shown in Table 9. Notable findings include: An evident increase (p=0.021) in LYM# levels in workers compared to the control group. A significant rise in MID# among brick kiln workers, with a p-value of p=0.008. A high increase in LYM% was observed in brick kiln workers, with p=0.001. A significant boost in MID% with a p-value of p=0.003 was seen in workers compared to the control group. A highly significant increase in GRA# was observed among the exposed group, with a p-value of p=0.002. GRA% concentration increased from (48.37±0.51%) to (58.34±0.53%) in the exposed group.

Table 9. Blood profile (white blood cells) of workers and control.

Blood parameters	Control	Exposed	P values statistics
WBC (1×10^3)	8.51±0.09	8.81±0.05**	p=0.004
LYM# (1×10^3)	2.10±0.03	2.51±0.04*	p=0.021
MID# (1×10^3)	0.62±0.02	0.77±0.02**	p=0.008
GRA# (1×10^3)	4.05±0.05	4.76±0.18**	p=0.002
LYM (%)	25.60±0.30	29.16±0.31***	p=0.001
MID (%)	6.07±0.11	7.91±0.11**	p=0.003
GRA (%)	48.37±0.51	58.34±0.53**	p=0.002

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

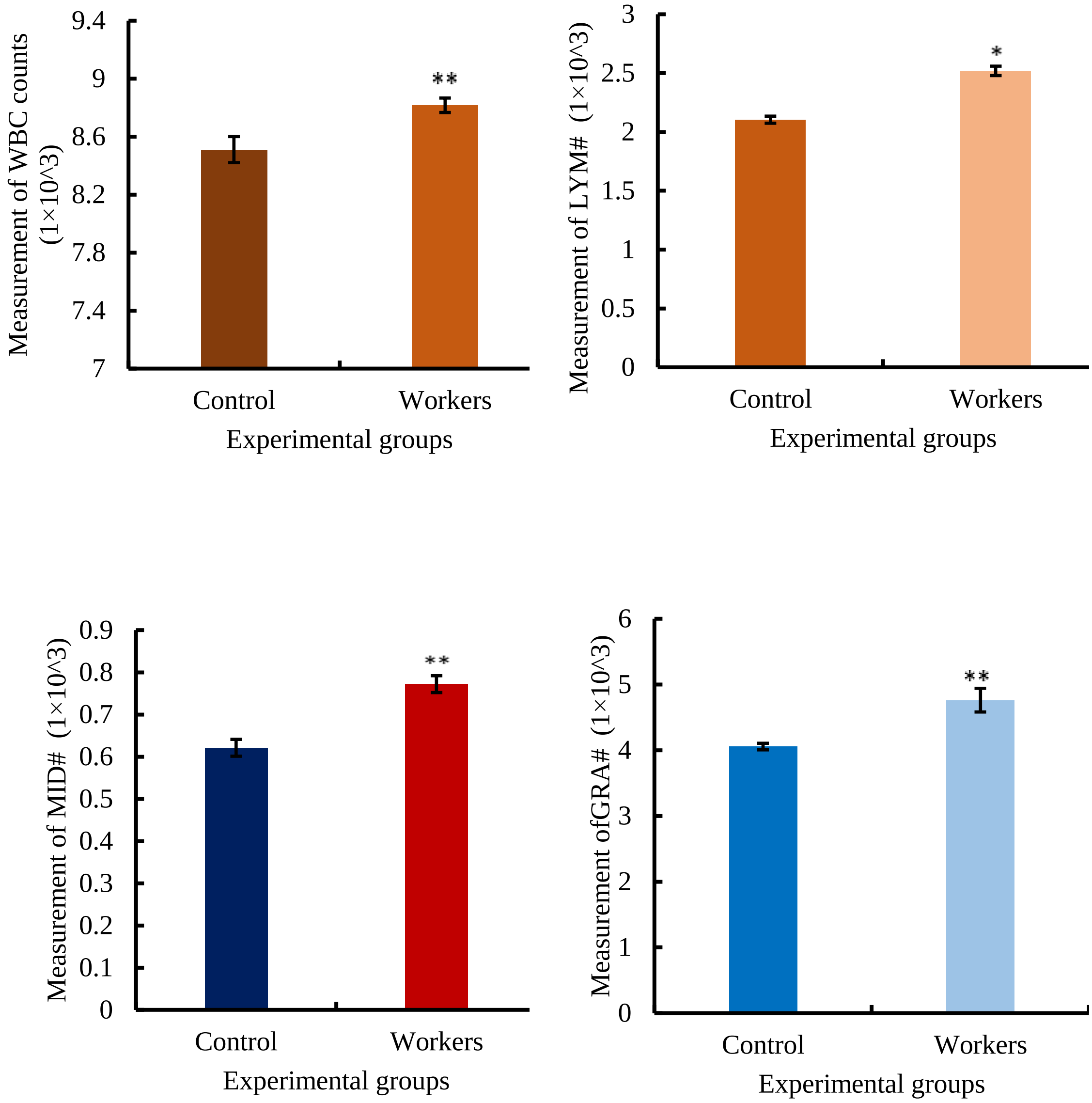


Figure 10. Comparison of white blood cell count ($1×10^3$), lymphocytes# ($1×10^3$), mid-range absolute count# ($1×10^3$) and granulocytes ($1×10^3$) number in blood plasma among male workers and control

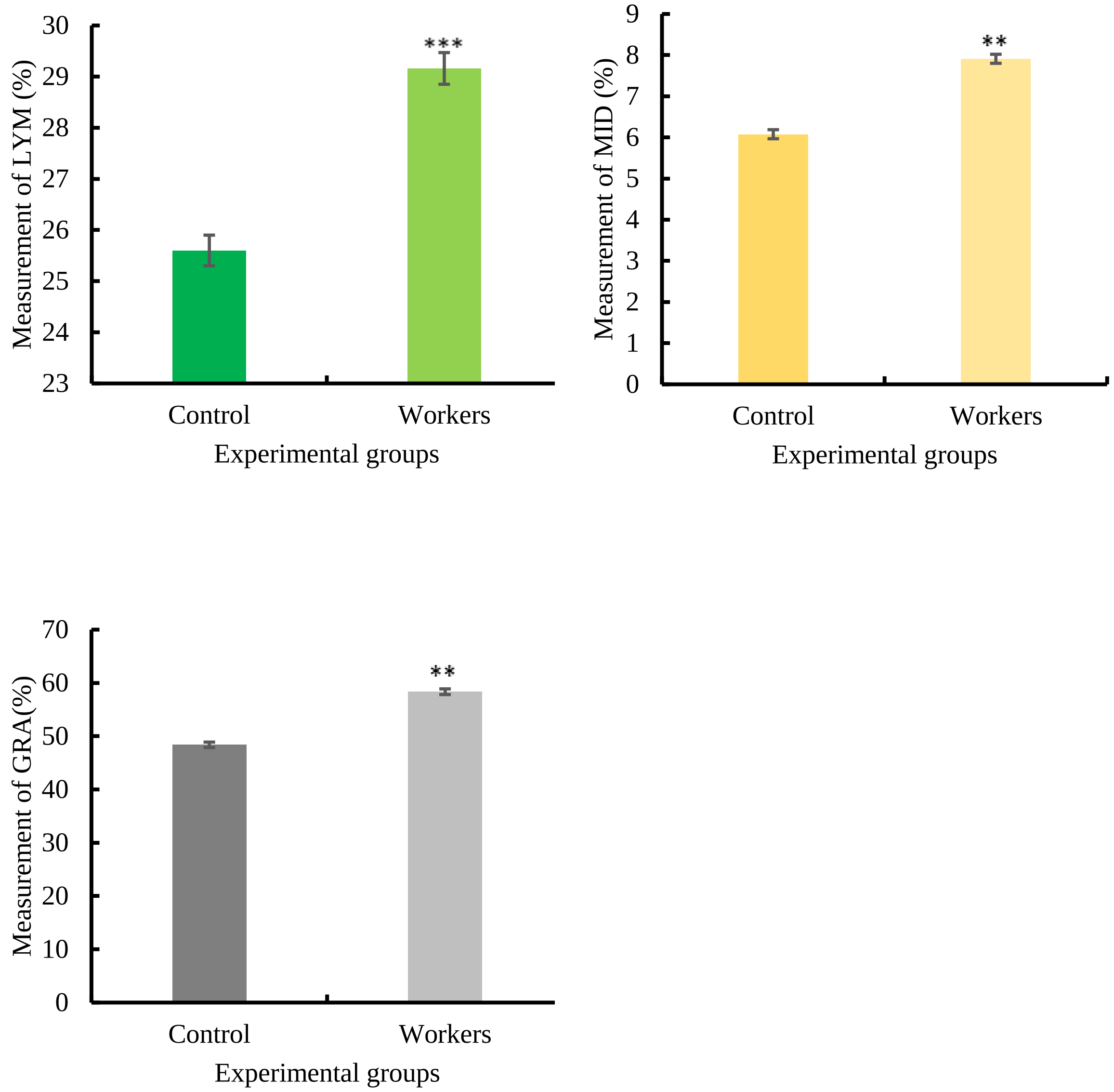


Figure 11. Comparison of lymphocytes (%), mid-range absolute count (%) and granulocytes (%) number in blood plasma among male workers and control

Blood Parameters (Red blood cells)

A significant decrease (p=0.001) in HGB levels was evident in workers compared to the control group. A significant decline in the number of RBCs was noted among brick mkiln workers, with a p-value of p=0. 001. A highly significant decrease in total hematocrit (HCT) concentration was observed in brick kiln workers, with a p-value of p<0.001. Significant reductions in MCV and MCH with p-values of p=0.002 and p=0.001, respectively, were noted in male workers compared to the control group. Significant increases in RDW-SD and RDW-CV with p-values of p<0.001 and p=0.001 respectively, were observed in male workers when compared to the control group. A significant decrease in MCHC with a p-value of p=0.002 was found in the exposed group.

Table 10. Blood profile (red blood cells) of workers and control.

Blood parameters	Control	Exposed	P values statistics
RBC (1×10^6)	4.94±0.32	4.30±0.37***	p=0.001
HGB (g/dL)	14.02±0.10	11.31±0.10***	p=0.001
HCT (%)	43.18±0.26	34.77±0.27***	p=0.000
MCV (fL)	93.01±0.24	84.04±0.26**	P=0.002
MCH (pg)	29.77±0.18	23.81±0.19***	p=0001
MCHC (g/dL)	33.57±0.08	30.96±0.09**	p=0.002
RDW-SD (fL)	48.83±0.26	51.42±0.37***	p=0.000
RDW-CV (%)	9.63±0.04	12.75±0.06***	p=0.001

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

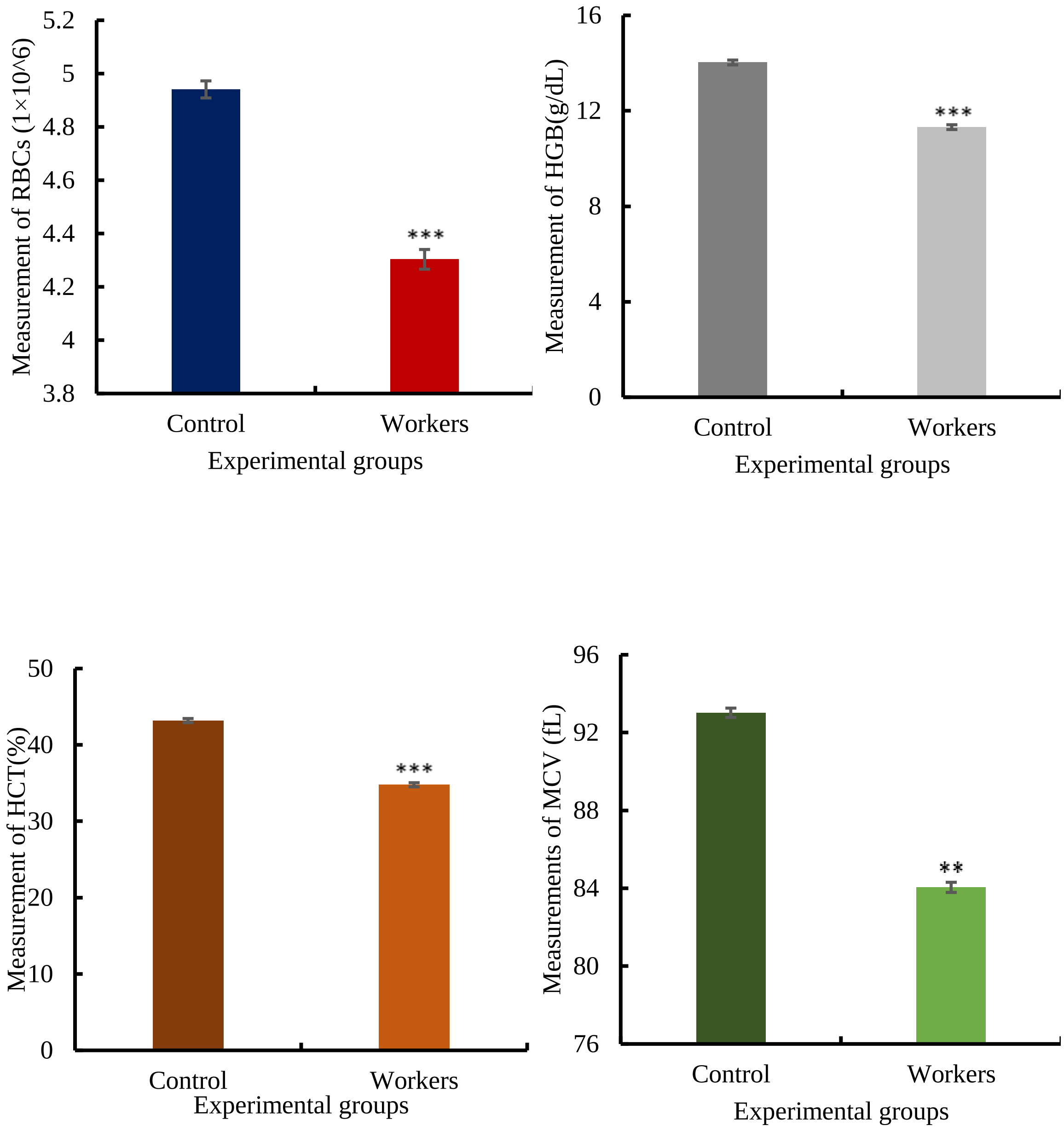


Figure 12. Comparison of red blood cell (1×10^6), hemoglobin (g/dL), hematocrit (%) and mean corpuscular volume (fL) number in blood plasma among male workers and control.

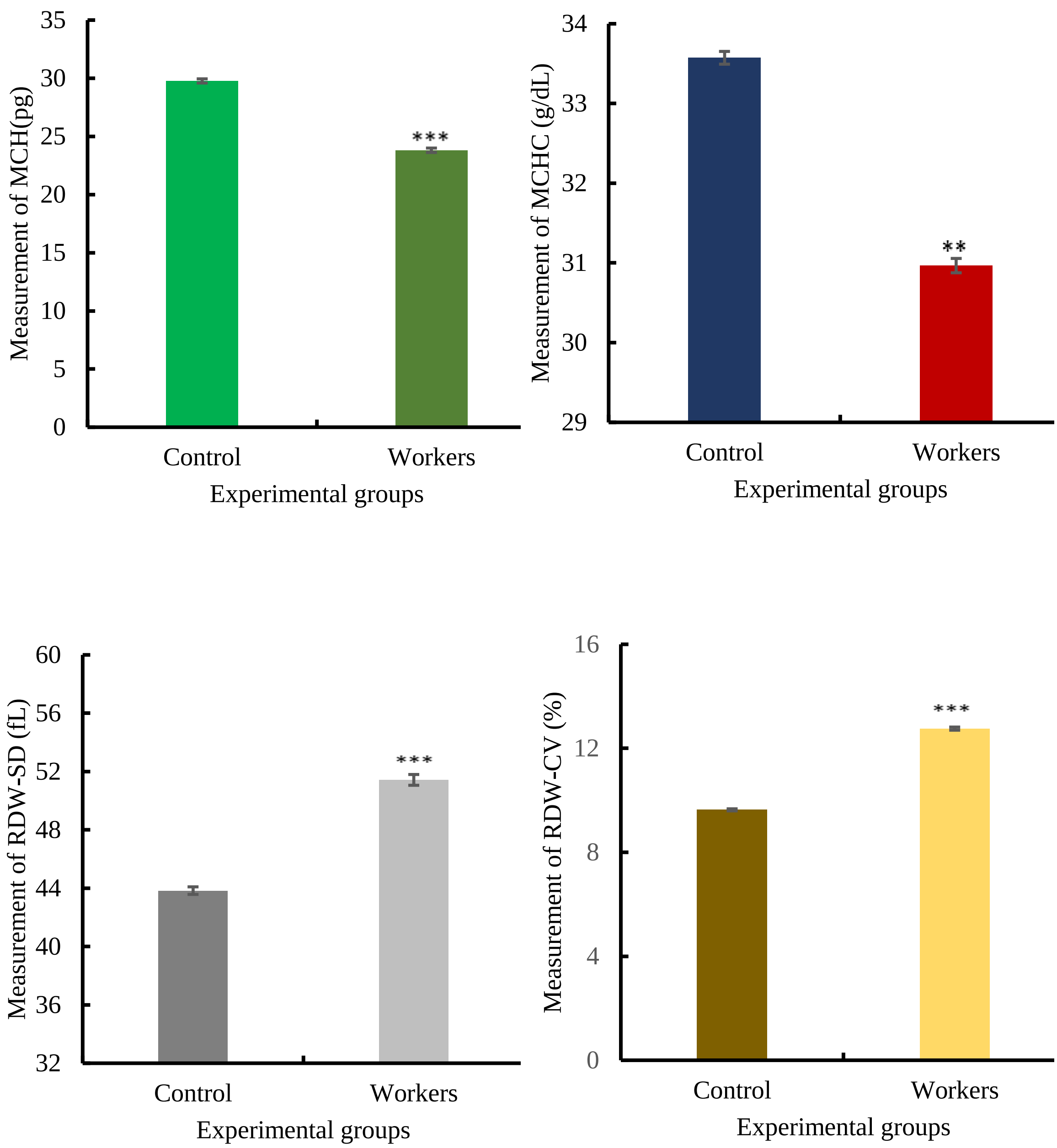


Figure 13. Comparison of mean corpuscular volume (pg), mean corpuscular hemoglobin concentration (g/dL), red blood cells distribution width-standard deviation (fL), red blood cells distribution-coefficient of variation (%) number in blood plasma among male workers and control.

Blood Parameters (Platelets)

A significant increase in PLT with a p-value of p=0.001 was seen in workers compared to the control group. Highly significant increases in MPV and PDW with p-values of p<0.001 and p=0.001 respectively, were observed.PCT concentration increased from (0.17±0.0002%) to (0.22±0.003%), and P-LCR concentration decreased from (25.30±0.41%) to (13.65±0.26%) in the exposed group.

Table 11. Blood profile (platelets) of workers and control.

Blood parameters	Control	Exposed	P values statistics
PLT (1×10^3)	242.98±4.14	297.89±4.59***	p=0.001
MPV (fL)	6.58±0.041	7.55±0.043***	p=0.000
PDW (%)	12.91±0.132	19.00±0.137***	p=0.001
PCT (%)	0.17±0.0002	0.22±0.003*	p=0.018
P-LCR (%)	25.30±0.41	13.65±0.26**	p=0.002

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

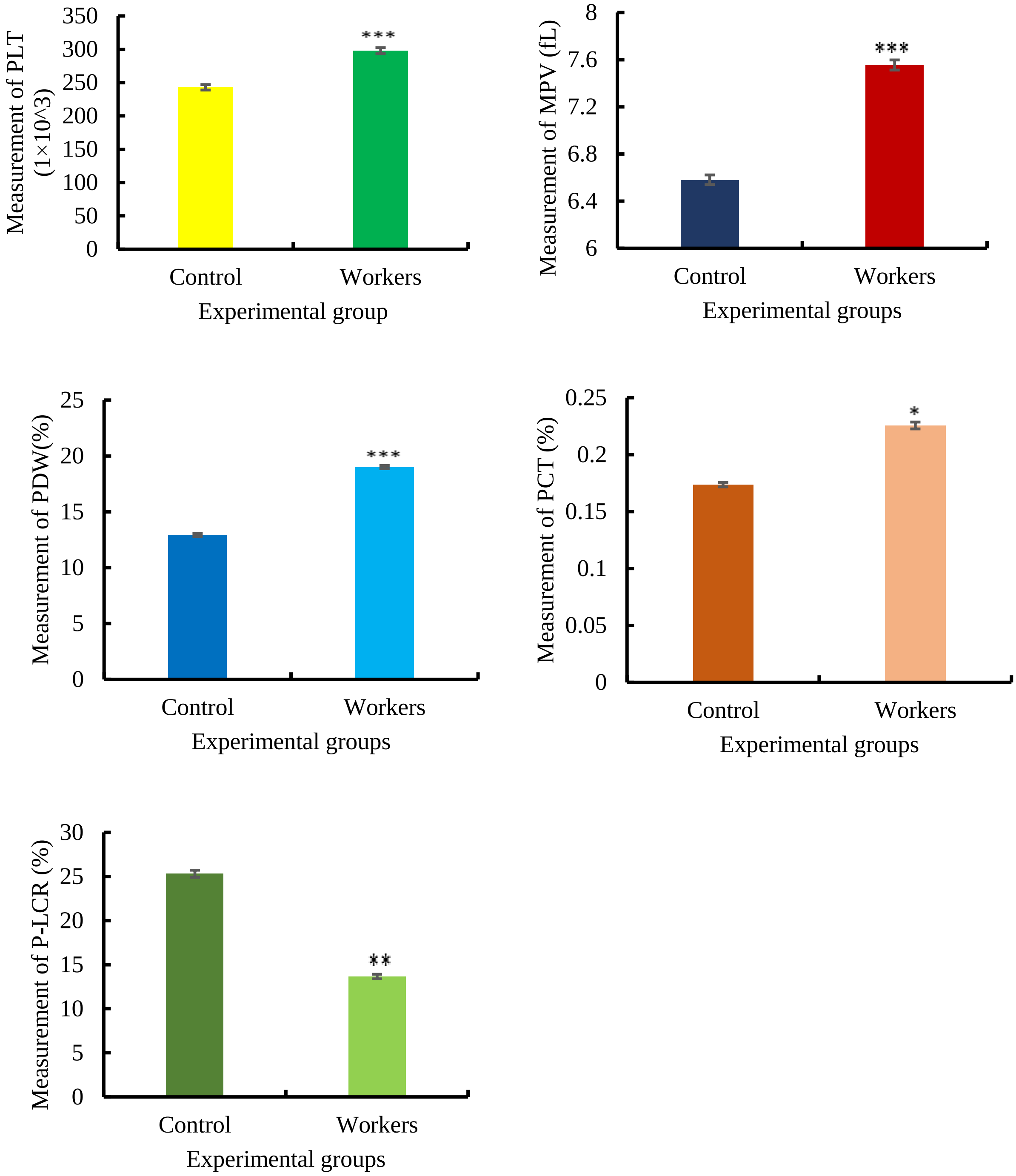


Figure 14. Comparison of platelets (1×10^3) (%), mean platelet volume (fL), platelet distribution width (%), plateletcrit (%), platelets- large cells ratio (%) number in blood plasma among male workers and control

Liver Function Tests

Workers exhibited a significant increase in ALP levels, with a p-value of p=0.001, when compared to the control group. A highly significant rise in ALT and AST levels was observed in the exposed group with p-values of p<0.001 and p=0.001, respectively. Bilirubin concentration also showed a substantial increase, from (0.47±0.24 mg/dL) to (1.94±0.90 mg/dL) among workers. Moreover, brick kiln workers experienced a significant reduction in Albumin levels, with a p-value of p=0.003. There was a highly significant decrease in Protein levels among brick kiln workers (p=0. 001)

Table 12. Effect of heavy metal burden on Liver Function of brick kiln workers and control

Liver Function Tests	Control	Workers	P values statistics
ALP (U/L)	105.13±0.31	126.36±0.33***	0.001
ALT (U/L)	48.38±0.10	55.80±0.13***	0.000
AST (U/L)	29.66±0.12	37.82±0.13***	0.001
Bilirubin Total (mg/dL)	0.47±0.01	1.94±0.05***	0.000
Albumin (g/dL)	4.39±0.21	3.88±0.18**	0.003
Protein (g/dL)	8.10±0.09	7.38±0.06***	0.001

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

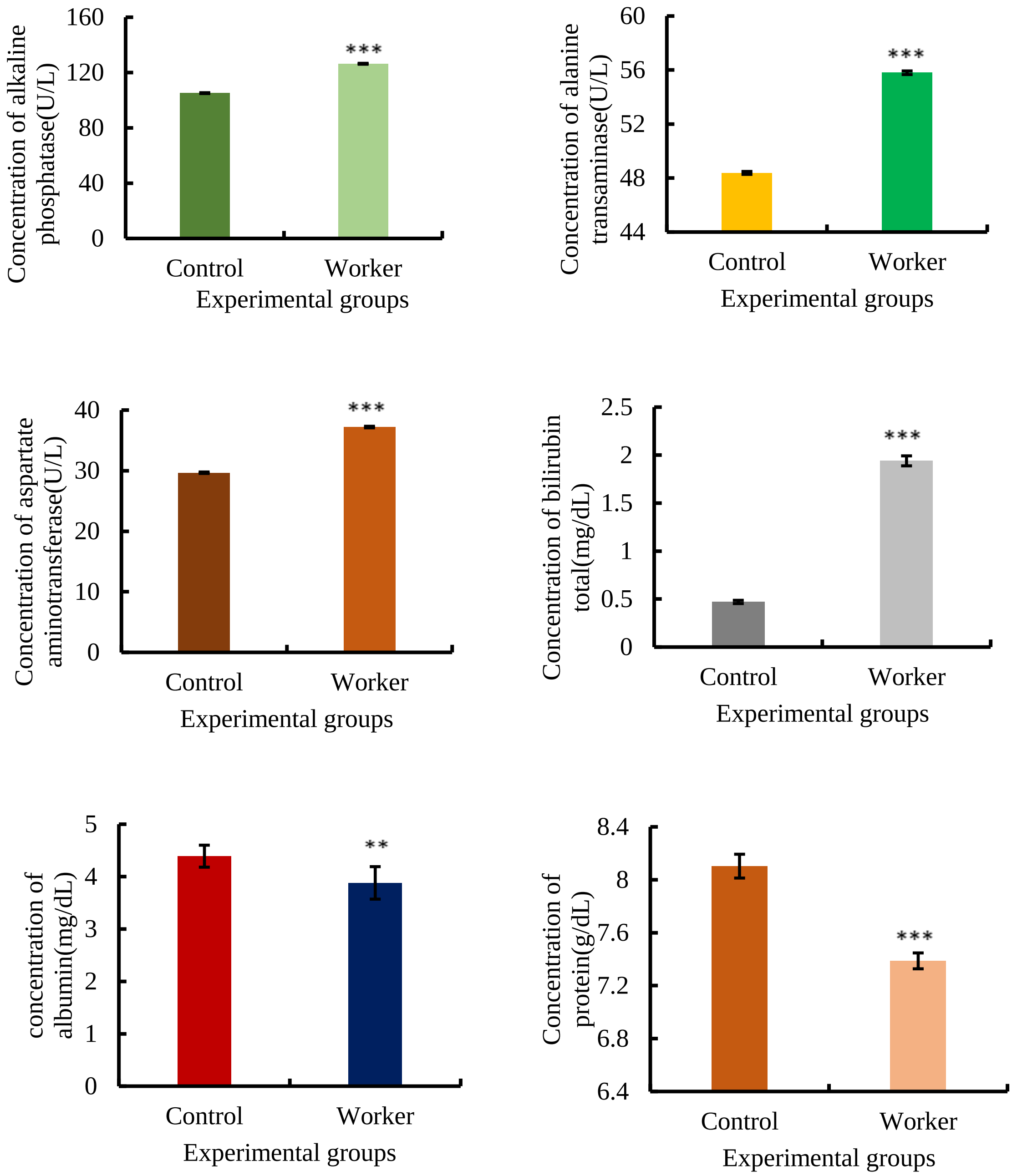


Figure 15. Comparison of alanine transaminase (U/L), aspartate aminotransferase (U/L), alkaline phosphatase (U/L), bilirubin total (mg/dL), albumin (mg/dL) and protein (g/dL) in blood plasma among male workers and control.

Lipid Profile

Among the control subjects, the Total Cholesterol level was measured at 243.68±4.59 mmole/L, while for brick kiln workers, it was notably higher at (304.54±4.61 mmole/L). The levels of triglycerides were (127.27±2.00 mmole/L) for brick kiln workers and (315.47±2.58 mmole/L) for control males. The LDL level was found to be increased ($p<0.001$) in workers when compared to the control group, and there was a highly significant increase in HDL level among workers with a p-value of $p<0.001$.

Table 13. Effect of heavy metal burden on lipid profile of brick kiln workers and control.

Lipid Profile	Control	Workers	P values statistics
Total Cholesterol (mmole/L)	243.68±4.59	304.54±4.61**	0.003
Triglyceride (mmole/L)	127.27±2.00	315.47±2.58***	0.001
LDL (mmole/L)	76.33±1.11	161.49±4.73***	0.000
HDL (mg/dL)	79.96±0.69	58.78±0.52***	0.000

*($p<0.05$), **($p<0.01$) and ***($p<0.001$) values for comparison among workers and control.

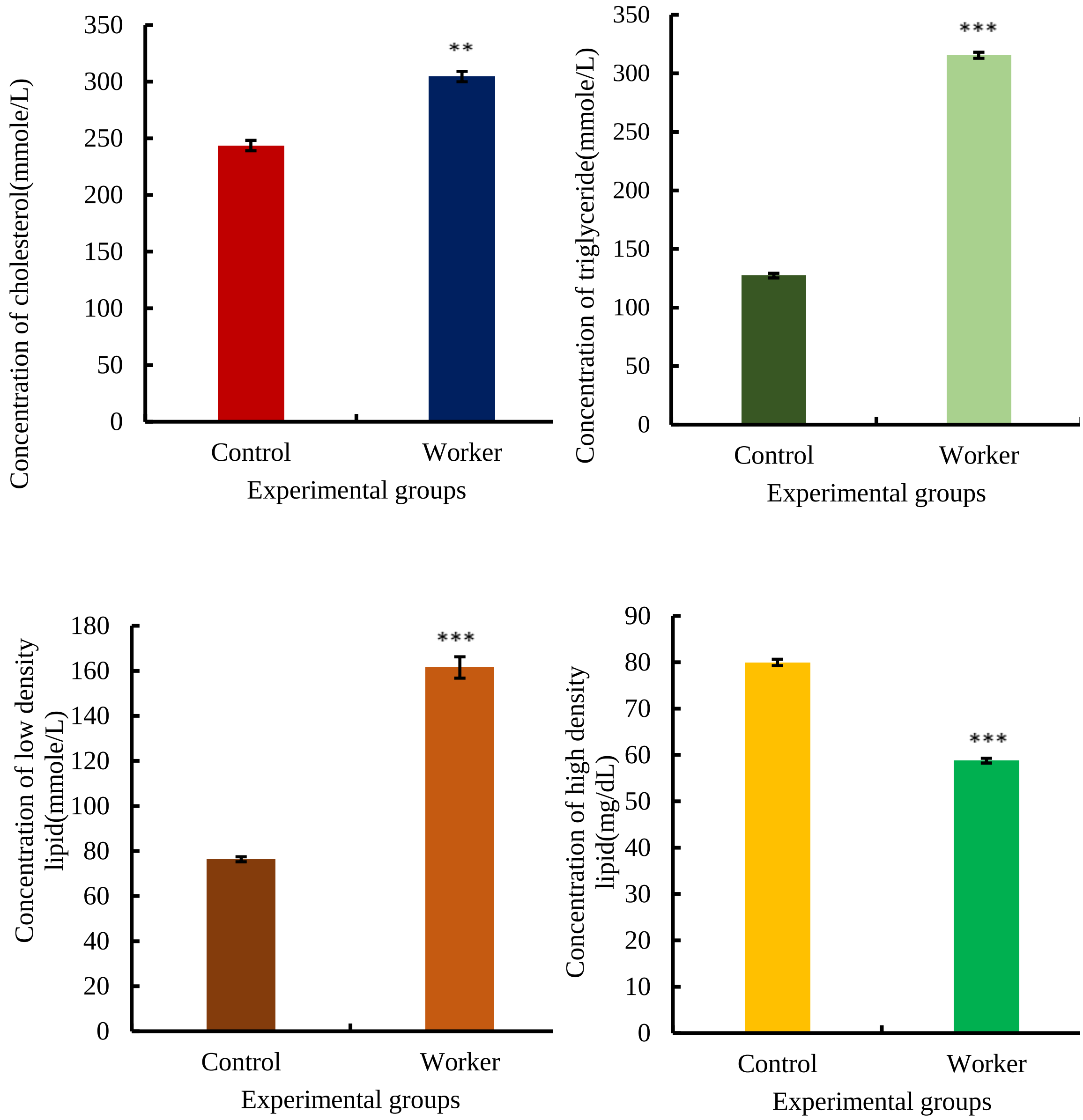


Figure 16. Comparison of cholesterol (mmole/L), triglyceride (mmole/L), low-density lipid (mmole/L) and high-density lipid (mg/dL) in blood plasma among male workers and control

Biochemical Analysis

The biochemical studies showed a significant decrease (p=0.001) in level of SOD from results of (16.04±0.04U/min) in control group to (13.81±0.05 U/min) in worker group. A significant rise in (p=0.004) in ROS number was evident in the worker group as compared to control. The mean serum POD level for worker group was (19.52±0.042 nmole) with significant level of p=0.016 as compared to control group with mean (22.72±0.049 nmole). Significant increases in TBARS with p-values of p=0.004 was observed in male workers when compared to the control group. A significant decrease in GSH with a p-value of p=0.001 was found in the exposed group. A significant increase in CAT with (p=0.004) was seen in workers compared to the control group.

Table 14. Effects of heavy metals burdens on biochemical profile of male workers and control

Parameters	Control	Workers	P value statistics
CAT(U/min)	1.402±0.018	1.003±0.014**	0.009
SOD (U/min)	16.04±0.048	13.81±0.055***	0.001
POD (nmole)	22.72±0.49	19.52±0.42*	0.01
GSH (mM/l)	18.79±0.06	11.05±0.04***	0.001
ROS (µmole/min)	2.31±0.01	3.48±0.02**	0.004
TBARS (mM/mg/protein	2.71±0.05	5.91±0.04**	0.004

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

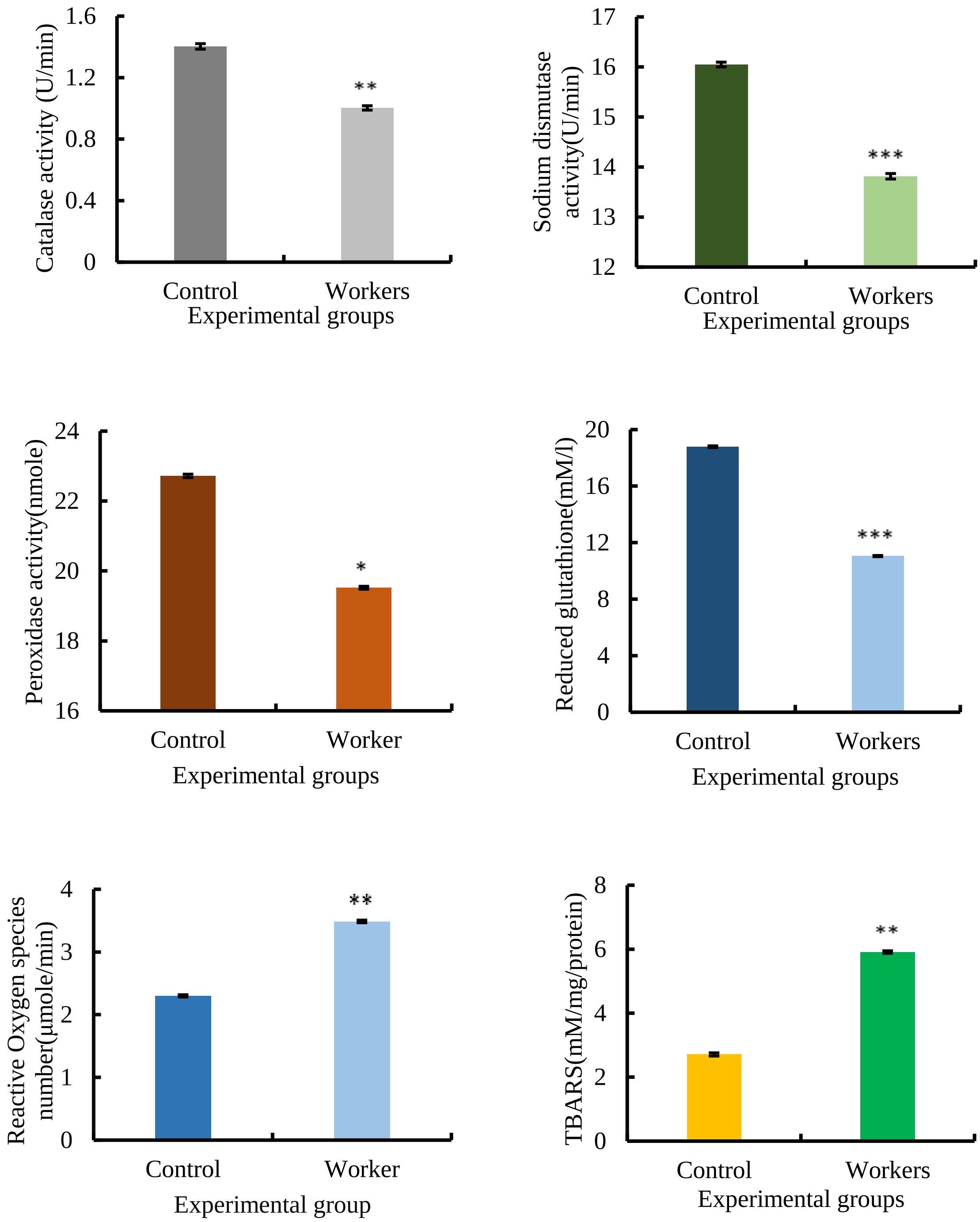


Figure 17. Effect of heavy metal burden on Catalase (U/min), Sodium dismutase (U/min), Peroxidase activity (nmole), reduced glutathione (mM/l), Reactive Oxygen species (µmole/min) and Thio barbituric acid reactive species(mM/mg/protein) in blood plasma among male workers and control.

Hormonal Analysis

The concentration of testosterone in plasma of workers group presented by mean value of (1.08±0.04ng/ml) was significantly decrease (p=0.001) from that of control group (1.40±0.05ng/ml).

Table 15. Effects of heavy metal burden on testosterone level (ng/ml) of male workers and control

Hormone	Control	Worker	P value
Testosterone (ng/ml)	1.40±0.05	1.08±0.04***	0.001

*(p<0.05), **(p<0.01) and ***(p<0.001) values for comparison among workers and control.

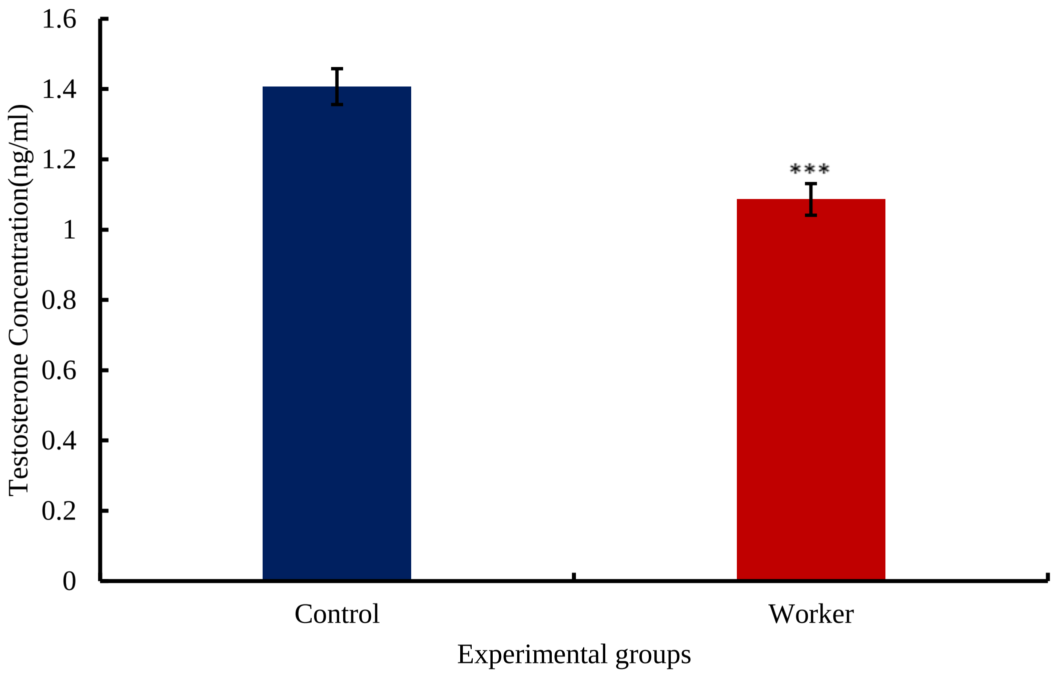


Figure 18. Comparison of testosterone (ng/ml) concentration in blood plasma among male workers and non-workers.

DISCUSSION

Brick kilns are a major source of pollution, polluting the air (Khan *et al*., 2019). This pollution is not only harmful to humans and animals, but it is also harmful to plants. The pollution has gases like CO_2, CO, SO_X, NO_X and tiny particles called $PM_{2.5}$. These substances are harmful to health and can even lead to death. (Rauf *et al*., 2022). Our study showed that male brick kiln workers experienced various health issues such as body pain, asthma, allergies, cough, chest tightness, shortness of breath, fever, backbone pain, tuberculosis, kidney problems, and hepatitis. Those exposed to prominent levels of $PM_{2.5}$ over extended periods were particularly prone to skin problems (Rauf *et al*., 2022). Long term exposure to $PM_{2.5}$ negatively affected the outer skin layer by disrupting cholesterol levels in the epidermis (Liao *et al*., 2020). Respiratory problems were also prevalent among workers experiencing frequent cough (50%), chronic cough (11.6%), persistent phlegm (11.6%), regular phlegm (21.6%), chronic wheezing (15%), shortness of breath of grades I and II (38.3%), frequent wheezing (20%) and self-reported asthma (3.3%) (Raza & Ali, 2021). The occurrence of lower back pain and tenderness was notably elevated among workers engaged in tasks like spading and mould evacuating (Sain & Meena, 2018). A study in Nepal found that brick kiln employees had pain at a rate of 58-73%, making them eight times more likely to suffer discomfort than non-brick workers (Joshi *et al*., 2013). Unfortunately, workers were not using protective equipment like masks or gloves and they molded bricks using their bare hands. This increased their health risks by exposing them to numerous toxins by inhaling, digesting or direct skin contact. The sanitary conditions were poor, with inadequate hygiene, nutrition, education and awareness about health. Education levels varied between the control group and those exposed to pollution, with many exposed workers being illiterate. Among the 300 individuals who participated in our study, a significant number of adult male workers were addicted to various substances like cigarettes, naswaar (a mixture of calcium oxide, tobacco leaves, and wood ash) and hukkah (tobacco mixture). Previous research also found a high prevalence of smoking among brick kiln workers (Ali *et al*., 2023). This situation indicates that workers in brick industries are susceptible to lung disorders such as silicosis, pneumoconiosis and musculoskeletal disorders (Khan & Vyas, 2008). Based on our findings, 54% of kiln employees were illiterate, which is associated with previous research by where 84% of brick kiln workers were found to be illiterate.

In current study, we used AAS (Atomic Absorption Spectroscopy) to analyze heavy metals in blood samples. We found a significant increase in the levels of Cd and Pb in all the samples from brick kiln workers compared to the control group**.** In current study, we analyzed heavy metal levels in whole blood and identified a significant increase in heavy metal levels among workers exposed to pollutants from brick kilns. Notably, there was an excessive amount of cadmium. Previous research has linked occupational or environmental exposure to cadmium with an increased risk of cancers in various organs such as the pancreas, lung, breast, prostate, nasopharynx and urinary bladder (Genchi *et al*., 2020). Furthermore, the results from atomic absorption spectroscopy indicated higher levels of lead in the human subjects studied. Lead poisoning in humans leads to detrimental effects on the nervous, renal and hematologic systems. Symptoms can also include hypertension, anorexia, abdominal pain, vomiting and infertility. Humans typically absorb lead through dietary intake, respiration, and the gastrointestinal system. However, in occupational exposures, breathing is a major route of entry (Kumar & Singh, 2023).

We noticed a significant decrease in the BMI among the participants working at the brick kilns. The low BMI values among these workers suggest that they have poor health conditions and weakened immune systems which could make them more susceptible to various issues such as allergies, musculoskeletal problems, respiratory disorders, stomach issues and liver and kidney problems. Our findings support with a previous study of Li *et al*. (2022) which found an inverse association between heavy metals (Pb, Cd, and Hg) and the risk of peripheral or abdominal obesity. This finding is consistent with earlier research including a study by Padilla *et al*.,(2010). Some animal studies have even indicated that lead might lead to weight reduction by disrupting the hypothalamic dopaminergic system (Leasure *et al*., 2008). higher concentrations of lead and cadmium in the environment are associated to higher systolic and diastolic blood pressures. The current findings showed an increase in SBP and DBP in workers as compared to control. Our findings are supported by Ataro *et al*. (2018) where increase in SBP and DBP was noted among garage workers as compared to control. Another study findings suggested that increase in SBP and DBP occurred in occupational workers subjected to lead as compared to control (Rapisarda *et al*., 2016). Schuhmacher *et al*. (1994) findings indicate that lead and cadmium exposure caused an increase in SBP and DBP. Our findings revealed a decrease in blood sugar levels among the workers compared to the control group. This statement is consistent with the results of Kshirsagar *et al*. (2015) where battery manufacturing workers also exhibited decreased blood sugar levels in comparison to controls.

The analysis of blood parameters in our study showed a decrease in the levels of RBCs, Hb, MCV, MCH and MCHC along with rise in levels of WBC, LYM, PLT and MPV. These modifications might be caused by heavy metals released from brick kilns. This result is consistent with a previous study that found decreased RBC levels in brick kiln workers when compared to control groups. A study conducted by Kargar-Shouroki *et al*. (2023) on battery workers similarly reported significant decreases in RBCs, Hb, MCV, MCH and MCHC along with an raise in total WBC count compared to controls. Additionally, Kshirsagar *et al*. (2016) observed significantly reduced levels of Hb, MCHC, MCV, MCH and RBC count, accompanied by an elevated WBC count, in battery manufacturing workers in India when compared to a control group. Velickova, (2017) confirmed that miners who have experienced prolonged occupational exposure to heavy metals show raised levels of heavy metals (such as cadmium, zinc, and lead) in their blood. This increased heavy metal content has led to changes in certain hematological parameters, including erythrocytes and leukocytes, which can contribute to the development of diseases with complex causes. Lead exposure can also have negative effects on lymphocyte function, cytokine production, and immunoglobulin secretion, impacting both cellular and humoral immune responses (Chwalba *et al*., 2018). Dobrakowski *et al*. (2016) reported an increased count of lymphocytes, leading to a rise in total white blood cell (WBC) count despite a decrease in granulocyte count due to lead exposure. Our results are consistent with findings of Dongre *et al*. (2011) where decreased levels of Hb, Hct, MCV, MCH, MCHC and RBC count along with lower total WBC count in 30 automobile lead-exposed workers compared to control group.

Oxidative stress is identified as a key indicator causing the toxicity of heavy metals. ROS are free radicals that are continuously generated during normal oxidative metabolism. Additionally, they can be produced by various xenobiotic substances, including heavy metals (Sun *et al*., 2022) Notably, ROS generation is not solely a result of regular physiological processes; it can also be prompted by external factors like heavy metal exposure and other contaminants that accumulate within the body. This includes occupational exposures and the presence of metal pollution in the work environment, as shown by (Khan *et al*., 2023). In current study, a significant reduction was observed in the levels of antioxidant enzymes such as SOD, POD, CAT and reduced GSH in the group of workers. This finding indicates that oxidative stress had an impact on these workers, potentially due to the exposure to heavy metals and other contaminants in their work environment. Our findings are consistent with previous research conducted by Onah *et al*. (2023) which demonstrated significant decrease in the serum activities of antioxidant enzymes like SOD, CAT, and GST, along with a substantial increase in the serum level of MDA in a group exposed to lead. Similarly, in our study, we observed an increase in levels of TBARS and ROS among the workers compared to the control group. Our findings are consistent with the findings of Aydin *et al*. (2004), which identified higher activities of P-MDA, a product of lipid peroxidation, in workers exposed to cement compared to a control group. Our observations are also supported by the work of Igharo *et al*. (2020) where the impact of heavy metal toxicity on bronze workers was evident through the increase in ROS, reflected by a slight but insignificant rise in MDA and a decrease in total antioxidant capacity (TAC). Numerous studies suggest that oxidative stress is a pertinent mechanism of Cd toxicity in humans, as indicated by (Moitra *et al*., 2014). Goyal *et al*. (2021). found that even low-level occupational exposure to Cd could lead to oxidative stress in workers, primarily due to a decrease in antioxidant enzymes and an increase in lipid peroxidation. Additionally, study has shown that exposure to coal dust and combustion products during thermal plant activities can induce oxidative stress due to increased lipid peroxidation and decreased antioxidant activity, as demonstrated by (Kaur *et al*., 2013).

Our findings demonstrated a significant rise in the levels of ALP, AST, ALT, and total bilirubin. In previous study conducted by Onah *et al*. (2023), elevated serum ALT and AST activities were observed in workers exposed to lead, indicating hepatocellular damage, while increased serum ALP and GGT (gamma-glutamyl transferase) activities were also seen. Our findings are consistent with the results of Dongre *et al*. (2010) which reported significantly higher levels of AST, ALT, ALP, and total bilirubin in automobile industry employes exposed to lead compared to controls. Similarly, Dioka *et al*. (2004) found that lead exposure could lead to liver dysfunction among Nigerian artisans. (Mazumdar & Goswami, 2014) reported similar outcomes in Indian plastic industry workers. Furthermore, in our study there was a decrease in albumin and protein levels within the worker group. Our finding are supported by Al Salhen, (2014) where cement industry workers' plasma concentrations of total protein, albumin, and globulin were found to be significantly lower.

Our current findings revealed a rise in plasma levels of total cholesterol, LDL and triglycerides, along with a reduce in HDL. Study shows that exposure to Cd can lead to alterations in lipid metabolism and contribute to the development of cardiovascular diseases (CVD) such as hypertension, atherosclerosis, cardiac arrest and stroke (Igharo *et al*., 2020). Our findings are comparable with those of David *et al*. (2020) who discovered higher levels of total cholesterol, LDL, and TG in brick kiln workers when compared to controls.Similarly, Firoozichahak *et al*. (2022) reported lower HDL levels and higher LDL levels in exposed subjects compared to non-exposed subjects. Sharma *et al*. (2012) found a similar pattern in battery workers, with increased total cholesterol, TG, LDL levels and decreased HDL levels compared to control. Moreover, previous studies have suggested that continuous exposure to cadmium can lead to an increase in both total cholesterol and LDL cholesterol concentrations (Igharo *et al*., 2020).

In our findings, decreased level of testosterone was observed in the blood plasma of brick kiln workers compared to the control group. This reduction in testosterone levels could be due to the high concentration of heavy metals present in their blood, which in turn may lead to increased production of ROS resulting in reproductive dysfunctions. The impact of Cd as an endocrine disruptor is well-recognized due to its adverse effects on reproduction. It has been shown to disrupt processes like steroidogenesis and spermatogenesis in both laboratory animals and humans. Cd ability to bind with androgen and estrogen receptors further contributes to its endocrine-disrupting effects (Ciarrocca *et al*., 2013). Pb exposure has also been linked to subclinical testicular damage and the development of ROS. Such factors are thought to be harmful to spermatogenesis and sperm function, potentially leading to male infertility (Balachandar *et al*., 2020). Our findings are supported by the prior research of David *et al*. (2022) which observed a decrease in testosterone levels among brick kiln workers. Jahan *et al*. (2016) reported decreased testosterone levels in brick kiln bakers and carriers when compared to a control group**.** Tutkun *et al*. (2018) findings indicate that lead exposure caused damage to testicular Sertoli and Leydig cells and caused a decrease in testosterone levels Similarly, Yu *et al*. (2010) evaluated the effects of Pb exposure on serum sex hormone levels in male workers and observed that testosterone concentrations were significantly lower in the exposed group.

Conclusion

The study concluded that brick kiln individuals experienced lowered body weight and had multiple health issues including skin allergies, respiratory problems, asthma, stomach liver and kidney problems. The average values of lead and cadmium were found to be higher in workers. Increased heavy metal level in blood of workers caused alteration in blood parameters, increase liver enzyme production, decrease in antioxidant enzyme levels, increase in oxidant production, decrease HDL, increase LDL, Cholesterol, TG, decreased bilirubin, protein and decrease in testosterone production. Therefore, it is determined that long -term compromised antioxidant enzymes level in blood, increased production of ROS, higher oxidative stress and disturbed production of gonadotropins and sex steroids serve as contributing factors in disturbing the metabolic health of brick kiln men, putting them at the risk for the development of other health problems that ultimately affect their reproductive outcomes.

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QUESTIONNAIRE

Date of sample collection		Background area		Sample no Location/City	
Ethnicity/ Language	Participant's name	Age		Marital status	Gender ☐ M ☐ F
Height	Weight			Sleep duration/day	
Physical appearance	Deep voice Facial hair appearance Acne Hair growth	Education Status	Illiterate Primary Matriculate Intermediate	Monthly Income	Less than 10,000 10,000-15,000 15,000-20,000 20,000-30,000
Earlier health history	T.B/fever/cough/cold/stomach problems/any allergy/shortness of breath/chest/asthma tightness/any type of bone pain/hepatitis/kidney problem (urination problem) Operation (if any)				
Food preference				Medication (if any)	
Type of work at brick Kiln	Carriage and Placement Molders Bakers			Exposure material	1.Fuel 2.Smoke 3.Radiations 4.Smog
Work history	- in Years			Exposure hours/day	Years of living in vicinity
Any addiction	Cigarette/Naswaar/huqqa/Charas/any other?			Exposure type	1.Fuel 2.Smoke 3.Radiations
Smoking Status:	Age at Starting Smoking/addiction			Intake/day	
Use of PPE	Gloves Mask Head Cover Respirators				