

Sputum Cytology and Blood Level of Some Heavy Metals in Subjects Exposed to Quarry, Wood and Rice Dusts in Abakaliki, Ebonyi State, Nigeria

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ABSTRACT

Background: In many occupations, workers are exposed to elements contained in rice husk/dust, quarry dust and wood dust such as magnesium, lead. Magnesium, lead and silicon are heavy metals and critical pollutants of the environment with negative impact in human health. Therefore, it is utmost importance to evaluate their toxicity in the workers.

Methods: This study investigated the levels of magnesium, lead and silicon in the workers of rice mill, quarry and timber. The cellular changes in the sputum of these workers were also investigated. The experimental groups were from 18years and above and must have worked in rice mill, quarry and timber for at least two years. The sputum of the workers were collected, stained with Pap, perl's Prussian blue and Fuelgen.

Results: The blood levels of magnesium was significantly ($p < 0.05$) low in all the test samples/groups. Timber workers recorded lowest value of magnesium (1.23 ± 0.09 mmol/L), followed by rice mill workers (1.32 ± 0.26 mmol/L) and quarry workers with 1.45 ± 0.22 mmol/L when compared to the control (5.04 ± 0.70 mmol/L) group. Lead was significantly high ($p < 0.05$) in all the samples obtained from workers exposed to rice mill husk dust, quarry dust and timber wood dust. Silicon level was high in samples obtained from timber workers (19.18 ± 10.44 $\mu\text{g/dL}$) and rice mill workers (14.78 ± 6.78 $\mu\text{g/dL}$) when compared to the control (7.62 ± 1.27 $\mu\text{g/dL}$). Samples from quarry site workers had significant high silicon level (59.83 ± 18.38 $\mu\text{g/dL}$) compared to the control.

Results and Conclusion: our observations suggest that occupational exposure to rice mill dust, quarry dust and wood dust from timber workers causes a decreased blood levels of magnesium and significant high blood levels of Lead and silicon in rice mill, quarry and timber workers. However, further research is needed to confirm molecular interactions of these metals in the organisms.

Keywords: Sputum, heavy metals, quarry, wood, rice dust, Abakaliki

INTRODUCTION

Rice has been shown to contain minerals such as calcium, magnesium and phosphorus alongside with some infinitesimal amounts of iron, copper, zinc and manganese [1]. Exposure to rice mill dust is one of the most common causes of allergic rhinitis, chronic respiratory disorders, including asthma and occupational airway diseases [2]. Workers involved in the production of rice are prone to have various types of airways diseases such as pneumoconiosis, Farmer's Lung, chronic bronchitis, pulmonary fibrosis and asthma. The needle like structure of rice dust husk causes chronic irritation of bronchial which in turn result in the impairment of lung function. It also brings damages to the bronchial passage as well as the elasticity of the alveolar walls [2]. However, most occupational exposures to mill hazards can be greatly reduced or eliminated through understanding the etiology of the disease via direct or cytological examination of sputum using different stain..

The health impacts of working in stone quarrying industry have been documented [3][4]. For instance; numerous epidemiological studies have supported the association between respiratory impairment and occupational exposure to dust [3]. Individuals working in dusty environment have been found to carry the risk of inhaling particulate materials (e.g., silica) that may lead to adverse respiratory effects, such as chronic bronchitis, emphysema, acute and chronic silicosis, lung cancer which are disabling and can even be fatal [5]. Also, high prevalence of silicosis has been reported among workers engaged in quarrying shale sedimentary rock in India. The major respiratory symptoms among quarry workers include non-productive cough, chest pain, catarrh, and dyspnea [6].

Pulmonary function impairments have been reported in quarry workers [4]. The revealed that, the prevalence of respiratory morbidity among stone quarry workers was 32.5%, based on radiological study; the severity of pulmonary function impairment was significantly associated with increasing age, duration of exposure to dust, smoking status and presence of chronic obstructive airways disease on radiological study. It was shown that dusts generated from granite quarrying contain 71% silica.

Inhalation of air pollutant increases various respiratory tract diseases due to the organic dust generated from such industries [7]. This research helped to detect the abnormal cells present in the sputum, effects of dust inhalation on DNA and ferric iron.

METHODOLOGY

Study Area

This study was conducted at rice mill, Quarry and Wood industrial sites Abakaliki, the capital city of the present-day Ebonyi State in South-Eastern Nigeria, located 64 kilometres (40 mi) southeast of Enugu [8]. The state is bounded by Cross River State, Enugu State and Benue on the East, West and North respectively while both Imo and Abia States is bordering Ebonyi State on the south. The inhabitants are primarily members of the Igbo nation [9].

Study Design

Sampling was by purposive sampling method in which subjects confirmed to have worked at industries that generate dust such as quarry, timber and rice mills who met the inclusion criteria were selected. Sputum and blood samples were collected from the subjects for cytological examination and assessment of heavy metals. Their age range was of the participant from 18 years and above For the control, students and civil servants asymptomatic; asymptomatic in this context are those who were by history, physical appearance and physical examinations free of systemic disorders, and subjects taking drugs which directly or indirectly affect the cardiovascular and respiratory system were excluded from the study. For the occupational workers, they must have worked in the dust zone for at least two years and above consistently to give a substantial effect, and are not engaged in other air pollutant jobs.

Ethical consideration

Ethical approval was obtained from the Nnamdi Azikiwe University, Faculty of Health Science and Technology ethical committee (FHST/REC/024/625).

Sputum collection and processing

Sputum was collected from each subject by coughing into a universal/sterile container. A drop of fresh sputum samples were added on a grease free slide using pasture pipette. Then a smear was made on the slide and fixed immediately in 95% ethanol for 30 minutes and arranged in a staining rack and stained with the following stains Fielgen stain, Perl's Prussian stain and Papanicolaou stain as describe by Bancroft and Marilyn [10] and Ovwioro, [11].

Blood sample collection and Determination of lead, silicon and magnesium

Blood sample was collected, and shared into 2 containers of EDTA and plain contain

The concentration of lead, silicon and magnesium were determined using varian AA240 Atomic Absorption Spectrophotometer (AAS) technique was used to determine the concentration of each analyte according to the method described by Association of Analytical Chemistry [12].

Data analysis

Data were entered and analysed using SPSS version 22. Descriptive statistics was used to summarize the data from questionnaire. Descriptive statistics was employed to determine the level of heavy metal in the blood and morphology of cells in sputum samples. Numerical data was presented as mean (SD) or median (IQR) depending on their normality distribution. Categorical data were presented as frequency (percentage)

RESULTS

Magnesium, lead and silicon level in individuals exposed to timber, quarry and rice dust.

As shown in figure 4.1, the mean levels of magnesium reduced significantly ($p < 0.05$) across all test samples/groups with the lowest value recorded in timber (1.23 ± 0.09 mmol/L), rice (1.32 ± 0.26 mmol/L) and then quarry (1.45 ± 0.22 mmol/L) when compared with the control (5.04 ± 0.70 mmol/L) group which were individuals not expose to neither timber, quarry nor rice dust.

The result obtained from lead estimation (figure 4.2) indicated that there was significantly high ($p < 0.05$) mean concentration of lead in all the samples obtained from individuals exposed to dust from various sites with those from quarry site (20.28 ± 5.90 $\mu\text{g/dL}$), followed by those from rice mill (9.82 ± 3.73 $\mu\text{g/dL}$) and then timber when compared to that of control (0.06 ± 0.03 $\mu\text{g/dL}$).

The silicon level obtained from the study and presented in figure 4.3 indicated a non-significant increase ($p > 0.05$) in silicon level of samples obtained from timber (19.18 ± 10.44 $\mu\text{g/dL}$) and rice mill (14.78 ± 6.78 $\mu\text{g/dL}$) compared to control (7.62 ± 1.27 $\mu\text{g/dL}$) group. Meanwhile, the samples obtained from quarry site had a mean silicon level of 59.83 ± 18.38 $\mu\text{g/dL}$ which was significantly ($p < 0.05$) higher compared to the mean control.

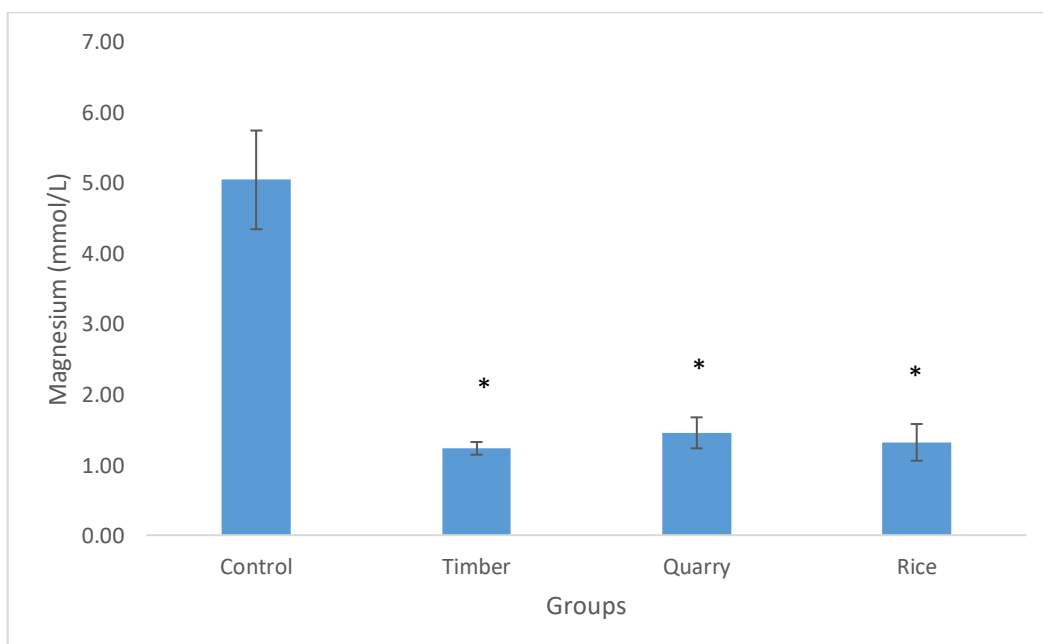


Figure 4.1 Mean level of Magnesium in individuals exposed to timber, quarry and rice dust. Values are Mean \pm SD those with asterisks are significantly lower compared to the control.

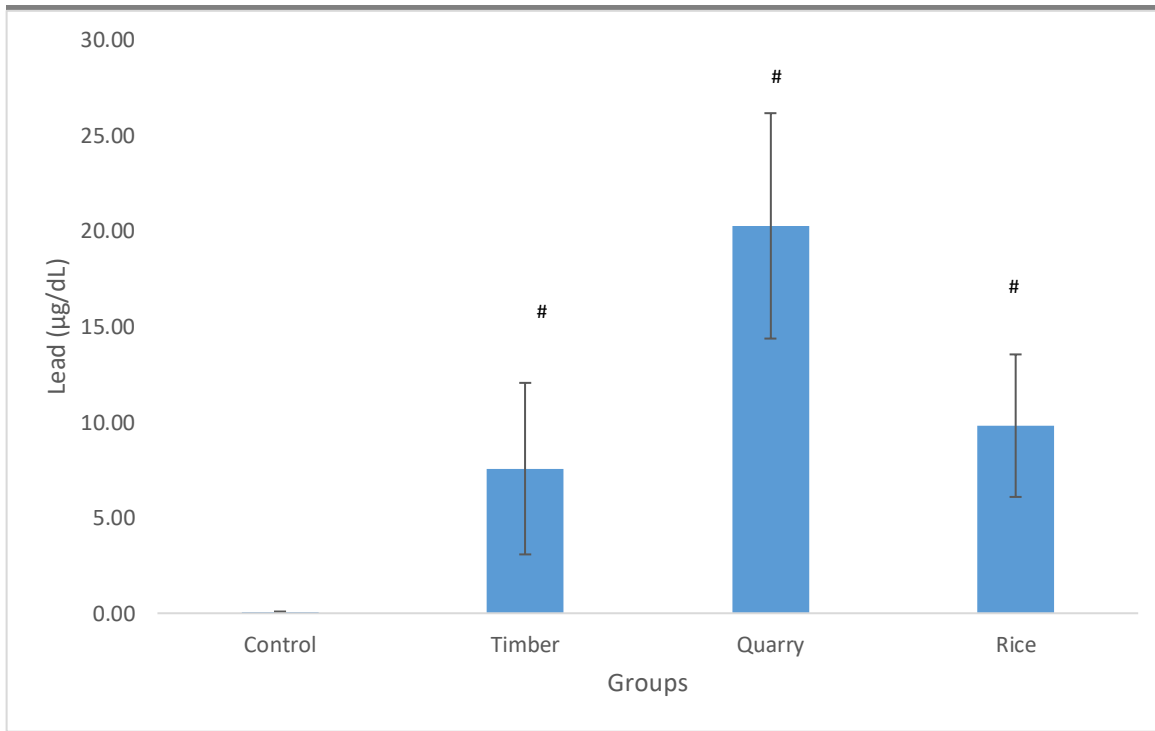


Figure 4.2 Mean level of Lead in individuals exposed to timber, quarry and rice dust. Values are Mean±SD those with asterisks are significantly higher compared to the control.

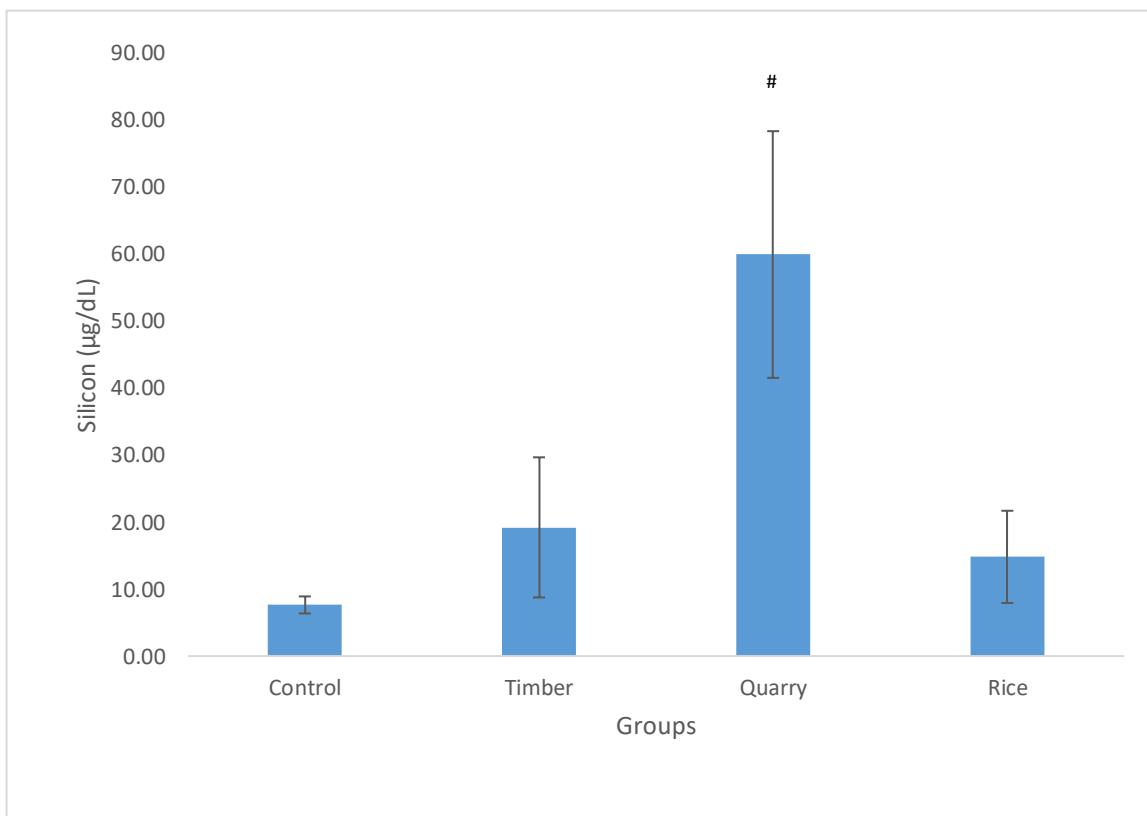


Figure 4.3 Mean level of Silicon in individuals exposed to timber, quarry and rice dust. Values are Mean±SD those with asterisks are significantly lower compared to the control.

The effects of rice husk dust on rice mill Occupational worker.

The cellular pattern of Sputum sample from rice mill workers revealed the presence of granular debris and irregular cytoplasmic outline (Figure 4.4 A and B). Figure 4.4 C and D revealed the presence of cells with scanty cytoplasm and cohesive clusters of elongated cells respectively. Our results also showed ill- defined,

feathery cytoplasm (Figure 4.4 E). Our observation shows the cellular pattern sputum sample from rice mill workers showing reactive bronchial cell with nuclear enlargement (figure 4.4F) and multinucleation (figure 4.4G). The pap stained sputum sample also shows nuclear moulding and inflammatory cells (figure 4.4H and I).

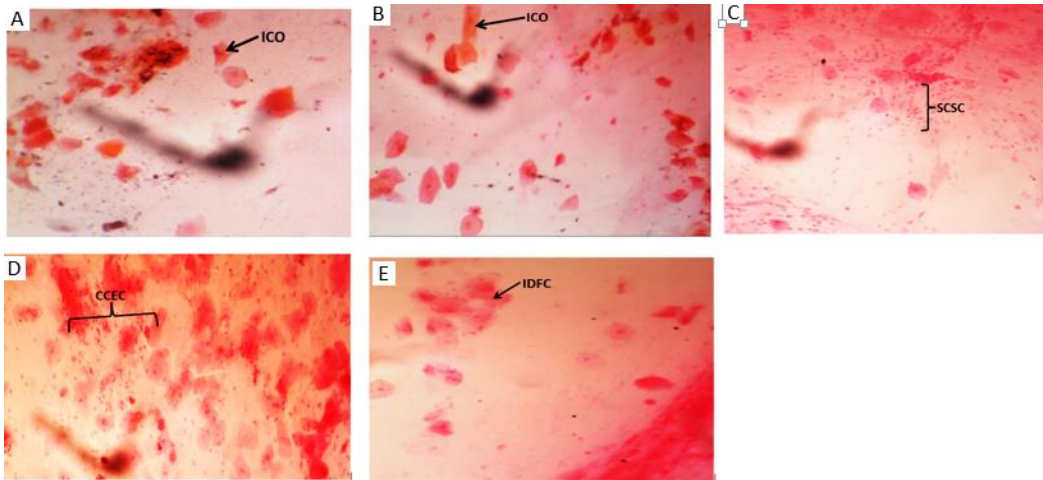


Figure 4.4 Photomicrograph of RICE Sputum samples from rice mill workers (A) showing granular debris and irregular cytoplasmic outline. (B) Showing irregular cytoplasmic outline. (C) Showing single cells with scanty cytoplasm Stain. (D) Showing cohesive clusters of elongated cells. (E) Showing ill- defined, feathery cytoplasm. (Pap stain X10).

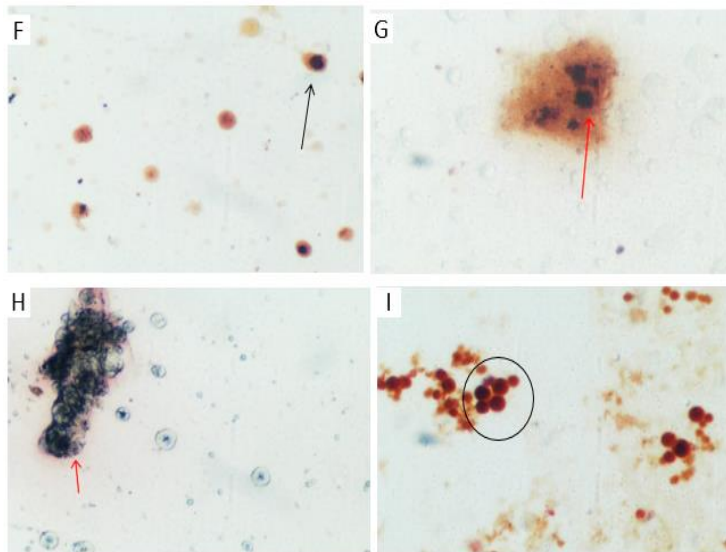


Figure 4.4 (F) showing nuclear enlargement (arrow). (G) Sputum sample showing multinucleation (arrow). (H) Sputum sample showing a possible nuclear moulding (arrow). (I) sputum sample showing inflammatory cells (Pap x10).

The effects of quarry dust on quarry Occupational worker.

Analysis of sputum sample of quarry workers showed cohesive clusters of elongated cells and granular debris (Figure 4.5A). Our results also revealed spindle shape cell with irregular outline and granular debris (Figure 4.5B). Also observed were cells with irregular outline and granular debris (Figure 4.5C). The sputum sample of quarry workers also showed cohesive clusters of elongated cells and granular debris (Figure 4.5D). Our results also showed cohesive clusters of irregular shaped cells and granular debris (Figure 4.5E). Sputum sample of quarry Occupational worker showed a marked nuclei halo with mild deposition of asbestos bodies and degenerated cytoplasmic background epithelium (Figure 4.5F). Our observation also showed a marked koilocytosis with asbestos deposition (Figure 4.5G). Marked perinuclei halos of the epithelium with

ferrogenous bodies were observed (Figure 4.5H). Stained sputum smear showed anucleated squamous cell, alveolar and squamous cell (figure I to K) respectively. Also observed is reactive squamous cell with nuclear enlargement and inflammatory cells (figure L and M) respectively. In Figure 4.5N, inflammatory cells were observed.

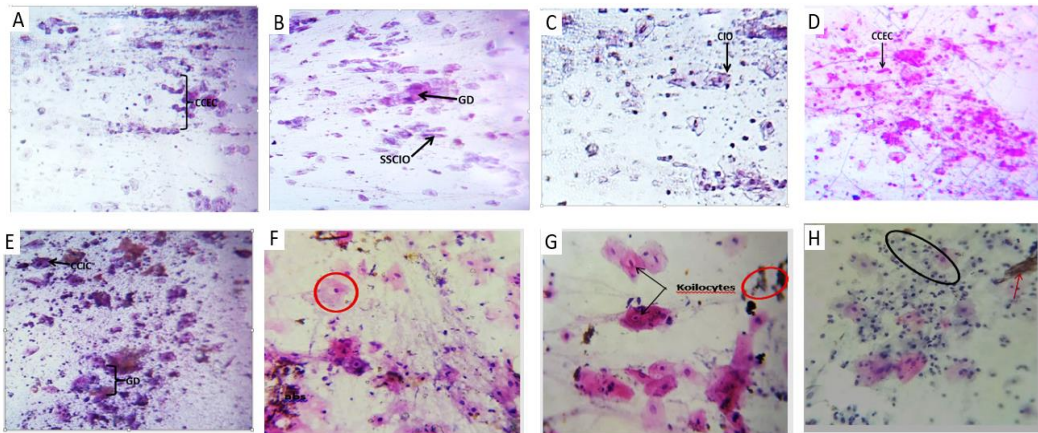


Figure. 4.5 Photomicrograph of Sputum sample of quarry workers (A) showing cohesive clusters of elongated cells and granular debris. (B) Showing spindle shape cell with irregular outline (SSCIO) and granular debris (GD). (C) Showing cells with irregular outline (CIO) and granular debris (GD). (D) Showing cohesive clusters of elongated cells and granular debris. (E) Showing cohesive clusters of irregular shaped cells and granular debris. (F) Showing a marked nuclei halo (red oval) with mild deposition of asbestos bodies (abs) with degenerated cytoplasmic background epithelium (G) Showing a marked koilocytosis with asbestos deposition (oval). (H) Showing a marked perinuclei halos of the epithelium (black oval) with ferrogenous bodies (red arrow) (Pap stain X100).

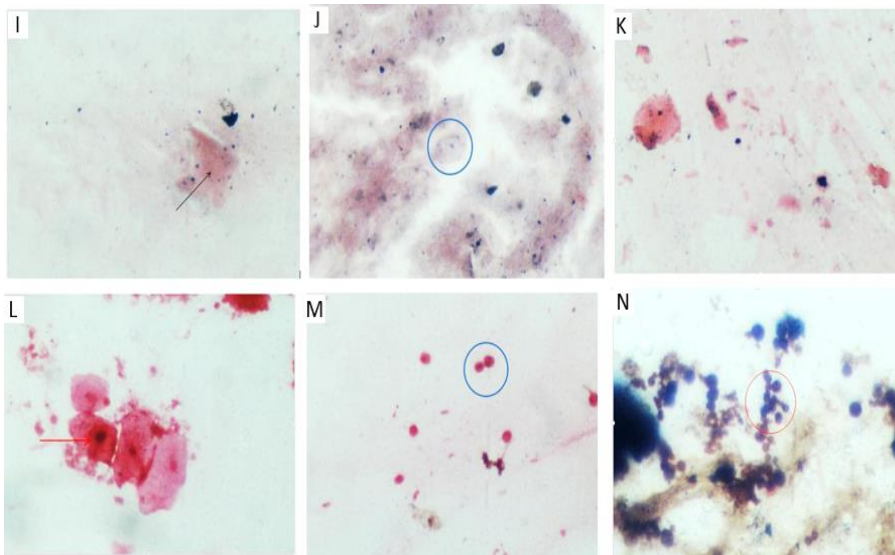


Figure 4.5 (I-N) Sputum smear of quarry workers (I) showing an anucleated squamous cell (arrow) (J) showing an alveolar macrophage (K) showing a squamous cell (L) showing a reactive squamous cell with nuclear enlargement (arrow). (M) Showing inflammatory cells (circle). (N) Showing inflammatory cells (circle). (Pap stain x10)

The effects of wood dust on the occupational workers.

Figure 4.6A - F are results obtained from staining the sputum sample of occupational wood workers. Figure 4.6A revealed the presence of Langhan's giant cell with peripherally placed nuclei. Also observe were the presence of squamous cell metaplasia with atypia (Figure 4.6B). Figure 4.6C showed cells with adenocarcinoma in the wood sputum sample of occupational wood workers. Also observed was squamous cell

carcinoma in the sputum sample (Figure 4.6D). There were presence of degenerated cells in the sputum samples (Figure 4.6E and F).

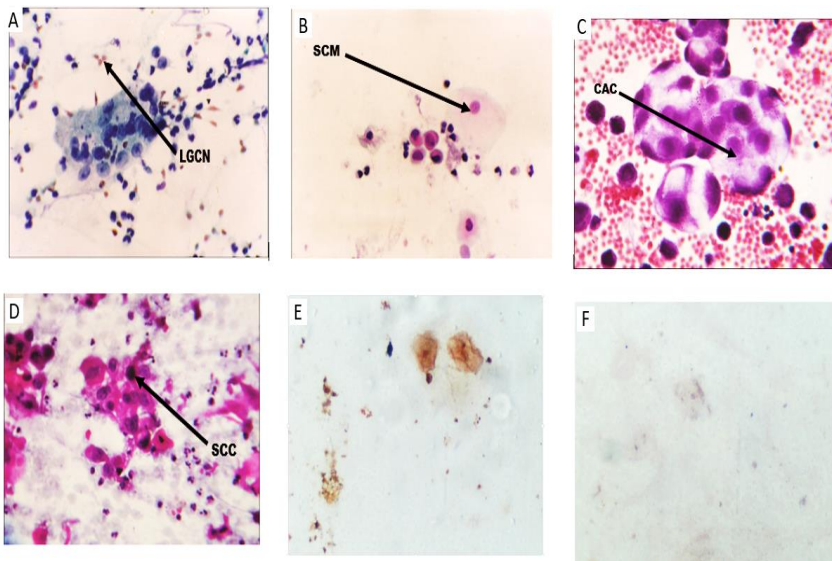


Fig.4.6 Photomicrograph of sputum sample of wood/timber worker (A) showing Langhan's giant cell with peripherally placed nuclei (Pap stain, x400). (B) Showing squamous cell metaplasia with atypical (Pap stain, x400). (C) Showing cells of adenocarcinoma (Pap stain, x400). (D) Showing squamous cell carcinoma (Pap stain, x400). (E) Showing degenerated cells (Pap stain, x10) (F) showing degenerated cells (Pap stain, x10).

Assessment of ferric iron deposition on the sputum sample of rice husk dust on rice mill Occupational worker, quarry industry and timber (wood dust)

Our results revealed that sputum smear from occupational rice mill workers (Fig.4.7A –D)) stained positive to Perl's Prussian blue. This suggests ferric iron deposition. It was also observed that sputum samples of quarry workers stained to Perl's Prussian blue stain (Fig.4.8 A-D).

Results revealed that Perl's Prussian blue stained sputum smear of wood worker with unclear positivity due to acellularity (Fig.4.9A). Perl's Prussian blue stained sputum smear of wood worker with interfering positivity from the background (Arrow shows deposits of iron) (Fig.4.9B). Perl's Prussian blue stained sputum smear of wood worker with interfering positivity from the background. Arrow shows deposits of iron (Fig.4.9C)

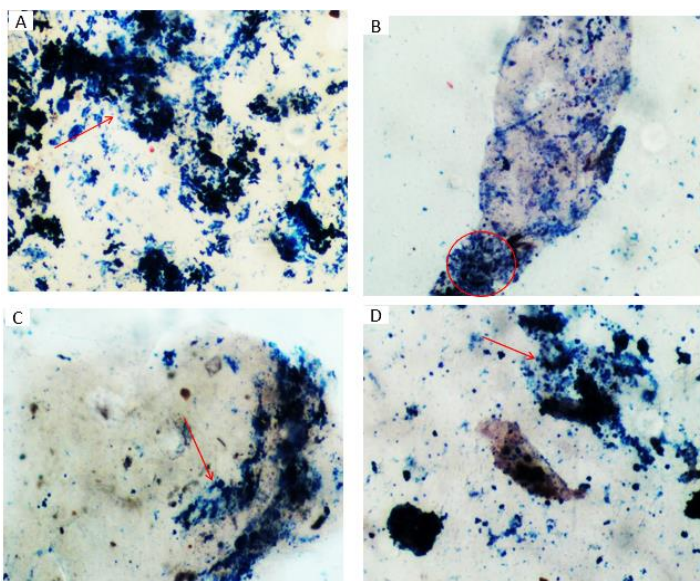


Fig.4.7 (A-D) showing sputum smear of rice mill workers stained blue (circle and arrows) (Perl's Prussian blue x10).

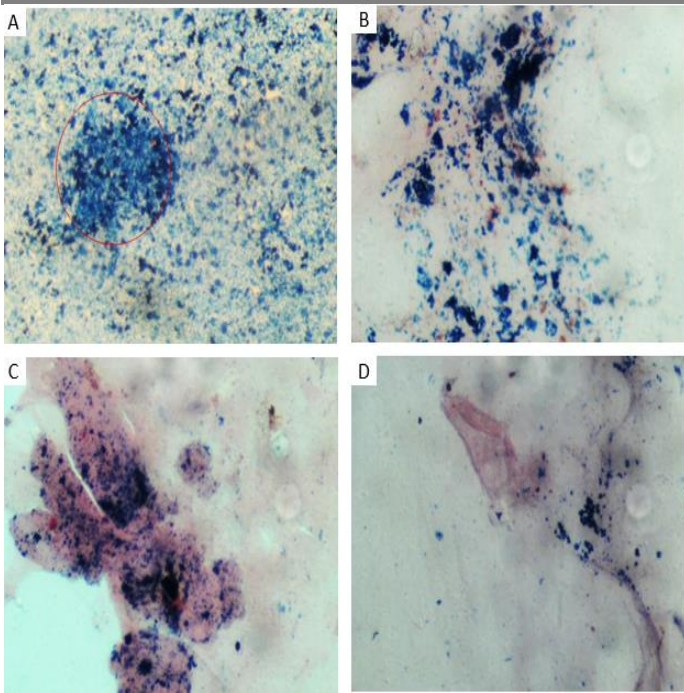


Fig.4.8 (A-D) showing sputum smear of quarry workers. (A-B) stained blue (circle and arrows) while (C and D) stained slightly blue (Perl's Prussian blue x10).

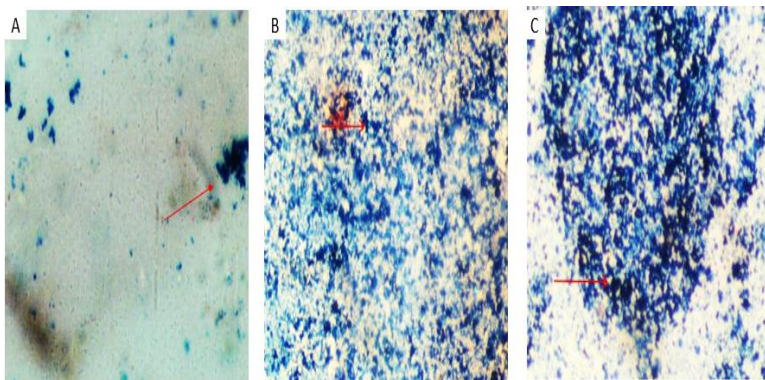


Fig.4.9 (A-C) showing sputum smear of timber workers. (A) Showed unclear blue staining cells (arrow). (B and C) stained blue depicting Perl's positive (Perl's Prussian blue x10).

Test for DNA in the sputum sample of rice husk dust on the occupational workers of rice mill husk, timber (wood dust) and quarry industry

Feulgen stain was used to visualize DNA in cells of sputum smear of rice mill workers. The Feulgen stain highlight DNA in the sputum (Fig.4.10 A). Our observation showed A fuelgen stained cells in the sputum smear with perinuclear halo (Fig.4.10B). C A fuelgen stained sputum smear showed anucleated squamous cells (Fig.4.10C). Our result also show positive fuelgen stained squamous epithelial cell with a slightly increased nucleus (Fig.4.10D).

Fig.4.11 is the results of fuelgen stained sputum smear of quarry workers which revealed anucleated squamous cells (Fig 4.11A - C).

Fig.4.12 show our observation on a fuelgen stained sputum smear of wood workers. In Fig.4.12A and B, anucleated squamous cells were observed suggesting that there is no DNA/nuclei in the sputum. Fig.4.12 C revealed fuelgen stained sputum smear of a normal cell. We also observed fuelgen stained sputum smear revealing anucleated squamous cells (Fig.4.12 D and E). Fig.4.12 F showed a fuelgen stained sputum smear showing normal squamous cells.

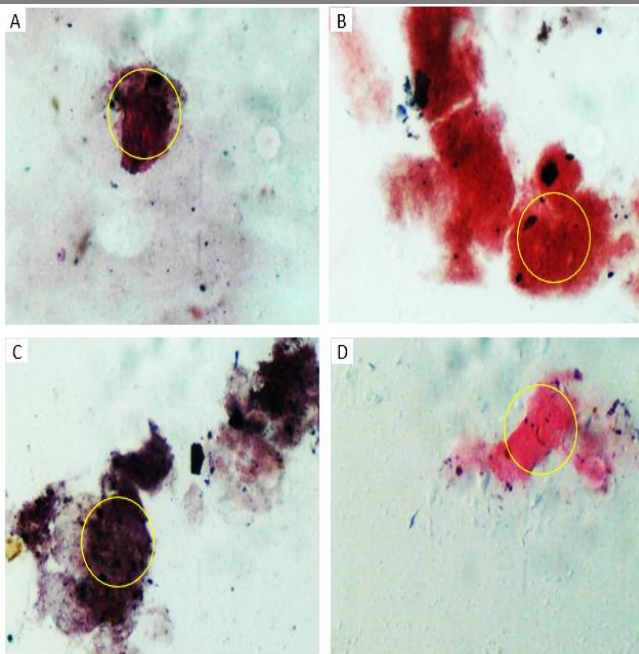


Fig.4.10 sputum smear of rice mill workers (A) showing Fuelgen staining reaction (circle). (B) Showing Fuelgen reaction on perinuclear halo (circled). (C) Showing anucleated squamous cell. (D) Showing a squamous epithelial cell with a slightly increased nucleus (circle) (fuelgen stain x40).

DISCUSSION

Magnesium is an electrolyte that plays part in many reactions in human body, which affect cellular function [13]. Our results revealed significant ($p < 0.05$) reduction in serum magnesium in the workers of timber (1.23 ± 0.09 mmol/L), rice mill (1.32 ± 0.26 mmol/L) and quarry (1.45 ± 0.22 mmol/L) which depicts Hypomagnesemia. Hypomagnesemia is a disturbance in electrolyte triggered by low level of serum magnesium in the blood [14]. Depletion of magnesium has been associated with several diseases and hypomagnesaemia has also been previously observed during treatment of diseases such as cancers [13]. The present research suggests that rice dust/husk, wood dust and quarry contain low amount of magnesium.

Lead (Pb) is a toxic metal naturally deposited in the earth's crust with widespread use. Pb causes environmental contamination, and human exposure results in significant health problems [15]. Our results exhibited that exposure to rice husk dust, wood dust and quarry significantly increased lead (Pb) levels in the blood which induced toxic effects on the cells (figure 4.2). Our results suggest that rice husk dust, wood dust and quarry contain Pb. Absorbed Pb are usually distributed through red blood cells within the organisms [16] [17]. Pb in the cells, mostly bound to haemoglobin not red blood cell membrane [18]. Hematopoietic structure represents critical Pb toxicity target which leads to anaemia [18][19][20][21][22]. Previous studies have linked Pb toxicity to cardiovascular, and nervous system disorders [19][20][23]. A study on sub-chronic oral Pb toxicity showed that kidneys, blood, and liver were main targets [24].

Weathering of rocks and other biogenic substances naturally releases silica into the environment and the main exposure route is inhalation which is firmly linked to occupational exposure [25]. Our research revealed non-significant ($p > 0.05$) increase in blood silicon levels in timber workers (19.18 ± 10.44 $\mu\text{g/dL}$) and rice mill workers (figure 4.3), depicting that rice husk dust and wood dust can increase blood silicon levels. This also suggests that rice husk and wood dust contain silicon. Samples obtained from quarry workers had significant increase in blood silicon level (figure 4.3), depicting that stone/quarry dust can increase blood silicon levels. This also suggests that quarry dust contains significant amount of silicon which caused the significant increase in silicon in the workers. Xue et al. [26], previously reported that Silica is abundant in the environs, and majority of the earth's crust consist of silica containing minerals. Occupational exposure to silica is associated with fibrotic lung disease (Silicosis), lung cancer, and obstructive pulmonary disease and increased risk of tuberculosis [25].

CONCLUSION

In conclusion, the study underscores the significant decreased in the level of magnesium associated with occupational exposure to rice mill, quarry and timber workers. The observed significant decrease in magnesium levels of rice mill, quarry and timber workers, with a corresponding increase in lead and silicon levels is indicative of the high risk faced by workers under prolonged exposure, leading to heightened susceptibility to respiratory diseases such as silicosis, chronic bronchitis, emphysema and incidence of lung cancer. The histology which is associated with granular debris, irregular cytoplasmic outline, nuclear enlargement, multi-nucleation, nuclear moulding and inflammatory cells were all indicative of cellular abnormalities of the lungs. Therefore, the need for urgent intervention and increase awareness of safety measures are imperative in reducing these occupational hazards in order to safeguard the health and wellbeing of individuals working in these industries. However, further research is recommended to confirm the molecular interaction of these elements in the intracellular and extracellular compartment of organisms.

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