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## **An Epidemiological and Health status of pit workers in a Copper mine and office Employees residing in the Vicinity**

**Tumane Rajani<sup>1</sup>, Nath Nirmalendu<sup>1</sup>, Khan Aqueel<sup>1</sup>**

1-University Department of Biochemistry, RTM Nagpur University, LIT, Mahatama Gandhi Marg, Amravaati Road, Nagpur-440033  
E mail: [rajo\\_tumane@yahoo.co.in](mailto:rajo_tumane@yahoo.co.in)

### **ABSTRACT**

*Prolonged occupational exposure to chronic high levels of copper could enhance such risk factors as lung cancer and coronary heart diseases etc. However, information regarding work related health risk assessment is still lacking in an actual copper mine. It becomes imperative to carry out a systematic investigation regarding the general health status in pit workers as well as office employees. In this context, Total (n= 164) subject were selected and categories according to their length and duration of exposure. A standard questionnaire was used to record information health status of the employees. Metal analysis and serum ceruloplasmin were determined according to their accepted standard procedure. Data analysis was carried out using SPSS (version 19) and R-3.0.1 programming language with pre-validated programs. Significant enhancement ( $p < 0.05$ ) in the blood contents of copper along serum ceruloplasmin were observed in chronically exposed pit workers as well as office employees after post adjustment with possible risk factors. The elevation in the content of copper found in chronically exposed miners and office employees were found to be positively correlated ( $r = 0.253$ ;  $r = 0.299$ ) with tenure of exposure. Sole responsible factor seems to be copper since no relationship of manganese with duration of exposure could be obtained. In conclusion, long term exposure to copper dust from the environment precipitates into copper toxicity in both miners and office employees.*

**Keywords:** Biomarkers, Copper, Ceruloplasmin, Environmental Exposure, Heavy Metals & Mine

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### **INTRODUCTION**

Mining sectors in India are plagued with several environmental and health-safety related problems [1,2]. The information regarding environmental contamination documenting definite undue exposure to the population living around the mining area is available. [3, 4, 5,6 and 7]. It may be recalled that long term exposure to copper dust from copper smelters was found to cause irritation of nose, mouth and eyes accompanied with headache, dizziness, nausea and diarrhea [8,9]. Occurrence of fever has also been observed on exposure to copper fumes [10,11]. Concerns have been expressed that exposure to chronic high levels of copper could enhance such risk factors as lung cancer and coronary heart diseases [12,13]. Both pre and post mortem studies of workers working in copper based industrial zones such as smelters were found to be susceptible to various diseases dependent on the chronicity of exposure and/ or the type of such processes as smelting, converting and plating they were engaged in [14,15]. Other studies have also implicated hepatomegaly, digestive disorders and a range of respiratory discomfort in workers from copper smelters [16]. However, information regarding work related health risk assessment is still lacking in pit workers exposed to copper dust in an actual copper mine. It becomes imperative to carry out a systematic investigation regarding the general health status of copper pit workers as well as office employees. The study primarily aims to present the general health status of copper mine workers on the basis of short and long term exposure to copper dust. A comprehensive assessment of the epidemiology and systemic contents of blood copper and such associated heavy metals as manganese and zinc along with serum Ceruloplasmin were performed in these workers at different span of exposure. Among the Indian copper complexes at Ghatsila in Jharkhand, Jhunjhun in Rajasthan and Hindustan copper limited in Madhaya Pradesh, we chose the pit workers from the latter mine as the model for this study.

## MATERIALS AND METHODS

### Selection of Subjects and Study Design:

A cross sectional study of mine workers from Malanjkhand copper Project, Hindustan Copper Limited, Dist. Balaghat, Madhya Pradesh (M. P) was made. The health status of the employees was evaluated using a standard questionnaire to record information on demographic characteristics such as age and sex match subjects, height, weight, diet and such habitual characteristics as smoking, tobacco chewing, alcohol consumption. Information about duration of exposure to Copper mining schedule of job held by miners as well as preventive measures used at job places were also sought. Data on family and personal history and present and past medical conditions, symptoms and signs related each body systems were collected. Information regarding history of chronic diseases such as neurological disorders, kidney problem, respiratory diseases, diabetes and cardiovascular diseases was also recorded. Informed consent was taken from all participants. Height and weight were measured for calculation of Body Mass Index (BMI). Inclusion and exclusion criteria were fixed before selection of the study subjects. Selected mine workers having copper dust exposure period for more than 1 year were included in this study. All selected subjects were male and female workers were excluded from these study.

Mine workers (n=87) in the age group of 20-60 years were selected. Miner who were engaged in mining activities and their duration of prolonged exposure to copper mine ranging from 1 to 40 years were considered as Experimental (EX) subjects. These subjects were further subdivided into two groups and classified according to their exposure period such as 0-10 years as Miner-I (n=16) and >11-30 years as Miner-II (n=71). For the purpose of comparison, such subject as office employees (n=47) not working in the mine but residing in the same mining environment were chosen as experimental control with age and sex match. A separate group of subjects (n=30) considered has healthy individual from non mining area (from Central part of India, Nagpur region) after age and sex match served as normal control.

### Blood sample collection

Venous blood samples were collected from all selected subjects using aseptic conditions. To minimize the possibility of blood sample contamination, workers were instructed to report for the collection before the start of the shift. 5ml metal free, sterile syringes were used for the collection of blood. 2ml Whole Blood (WB) was collected in sterile tube for the determination of metal concentration in blood. Remaining blood samples were allowed to clot and centrifuged at 1000 rpm for 5 min. Aliquots of serum samples were allowed to freeze immediately and stored at -40°C in accordance to accepted procedures. The serum samples from all subjects were used for measurement of serum Ceruloplasmin analysis.

### Determination of Serum Ceruloplasmin Oxidase Activity:

Ceruloplasmin catalyzes the oxidation of p-Phenylenediamine dihydrochloride,  $C_6H_4(NH_2)_2 \cdot 2HCl$  (PPD). The rate of formation of the coloured oxidation product is proportional to the concentration of serum ceruloplasmin. Its absorbance was measured at 530nm by UV-spectrophotometer. Serum Ceruloplasmin Oxidase activity was calculated in g/lit according to the accepted procedure [17].

### Estimation of Copper, Manganese and Zinc in blood by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES):

The metal content of the blood samples was analyzed through the Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). It is a multi element analysis technique that uses inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. A detector measures the intensity of the emitted light and calculates the concentration of the particular element in the sample. Metal concentration was determined according to Bazzi *et al.*, 2008[18] and Henk, 2003 [19]. Experiment set was prepared for determination of copper, Manganese and Zinc by using 2 ml blood sample with concentrated Nitric acid (30ml) in a glass beaker. This mixture was digested on hot plate at 150°C for 30 minutes. Digestion solution (upto 0.5ml) was filtered through whatman filter paper no. 40 and filtrate was diluted with nitric acid (0.5%) and volume was make up to 25-30 ml in marked graduated glass tube. Instead of blood sample, ultrapure water was used in another set of experiment followed by the same procedure. This set of experiment was used as blank. The final solution was aspirated in ICP-AES after Zeeman background correction. Each sample was checked after running of separately prepared mix standard in ICP-AES. Metal concentration of copper, Manganese and Zinc was calculated in PPM or mg/L.

### Statistical Analysis:

Data analysis was carried out using SPSS (version 19) and R-3.0.1 programming language with pre-validated programs. Descriptive statistics for demographic characteristics according to study groups were done by using one-way analysis of variance followed by Tukey's post-hoc comparison and Chi-square test and percentage wise distribution of health and diseases status of subjects of the study groups. Pearson's correlation coefficients of adjusted metal concentration with duration of exposure were prepared using R 3.0.1 and MedCalc software.

Age, BMI and habitual characteristics i.e. smoking, tobacco chewing and alcohol consumption was considered as the possible risk factors and the confounders, analysis of covariance (ANCOVA) was used for each parameter independently to adjust these confounders and to determine the true effect of exposure in all subjects of study groups. One way analysis of variance (ANOVA) was used to calculate statistically significant difference in the overall mean adjusted values of parameters across study groups was evaluated across the study groups followed by Tukey's post-hoc comparison analysis.  $p$  value of  $<0.05$  was considered statistically significant for all the analysis.

## RESULTS

The demographic characteristics among various chosen groups are depicted in Table 1. It was found that there were no statistically significant differences in age and BMI of office employees and chronically exposed mine workers (Miner-II) as compared to normal control. Miners with short term exposure (Miner-I) however, showed significantly lesser age and BMI as compared to rest of the groups.

The percentage wise distribution of educational status, dietary and other habitual characteristics of the study groups have been represented in Table 2. The questionnaire based study showed that all subjects of such groups as normal control and office employees were mostly literate while Miner-I and Miner-II were found to be semiliterate (Educated upto primary or higher secondary levels). It was also observed that except for Miner-I all other groups were predominantly non vegetarian in dietary habits. Further, there were no apparent differences in smoking and alcohol consumption between the groups. Significant percentage of pit workers i. e both (Miner-I and Miners-II) groups were found to consume tobacco orally as compared to office employees and normal control.

The health and disease status of subjects within the study groups have been depicted in Table 3. It was found that chronically exposed miners (Miner-II) expressed significantly elevated blood pressure (33.8%), accompanied with bone and joint pain (40.8%), chest pain (22.5%) along with some complaints of backache (19.7%), abdominal pain (11.2%) and browning of hair (7.04%). This group was also found to suffer from diabetes (21.1%) and Ischemic Heart disease (IHD) (15.5%). On the other hand, mildly exposed miners (1-10 years) complained of chest pain (43.7%), abdominal pain (43.7%) headache (43.7%), Backache (37.5%) as well as some browning of hair (25%) with no indication of hypertension, diabetes and IHD. It may also be perceived that office employees in mines recorded elevated blood pressure (25.5%) and some proneness to diabetes (12.8%).

Examination of Blood copper and other associated metals as well as serum ceruloplasmin denoted that in chronically exposed mine workers as well as office employees residing in the mining area possessed significantly higher contents of copper, manganese and ceruloplasmin ( $p < 0.05$ ) as compared to normal control. It may be seen that the level of blood zinc across the study groups was not found to be significantly altered as analysed through one way ANOVA (Table 4). Analysis of covariance (ANCOVA) used to adjust the above parameters against possible risk factors established the earlier observation of higher contents of copper and ceruloplasmin in Miner-II and office employees when compared to normal control. However, the content of manganese was not found to be significantly altered in either control groups (Normal and experimental). Further, Tukey's post-hoc comparisons showed that the aforementioned parameters did not differ between office employees and chronically exposed miners (Miners -II) (Table 5).

The scatter plot of adjusted blood copper levels with duration of exposure on subjects from either Miner-II or office employees (Fig. 1 & 2) demonstrated that there was a positive correlation ( $r = 0.253$ ;  $r = 0.2999$ ) between the content of blood copper with years of exposure, in a statistically significant manner ( $p < 0.05$ ).

On the other hand, when the same analysis was extended to the study of adjusted level of blood manganese with duration of exposure in Miner-II as well as office workers, it was found that an insignificant but negative correlation ( $r = - 0.0828$ ) and an insignificant but positive correlation ( $r = 0.1922$ ) existed in Miner-II and office employees respectively (Fig. 3 & 4). It may be noted that zinc content when correlated with duration of exposure (in both Miner-II and office employees) it was found to be positively correlated ( $r = 0.1752$ ;  $r = 0.1160$ ) but insignificant (Fig. 5 & 6) .

**Table 1: Descriptive statistics for demographic characteristics according to study groups**

Characteristics	Study groups				P-value	Pair wise comparisons‡					
	Controls (n=30)	Office employees (n=47)	Miners-I (n=16)	Miners-II (n=71)		Control Vs Office employees	Control Vs Miners-I	Control Vs Miners-II	Office employees Vs Miners-I	Office employees Vs Miners-II	Miners-I vs Miners-II
Age (Yrs)	50.33 ± 6.81	50.42 ± 7.32	31.63 ± 5.26	51.52 ± 5.74	0.0001	0.9999	< 0.0001	0.8285	< 0.0001	0.7985	< 0.0001
BMI (Kg/m <sup>2</sup> )	25.23 ± 3.74	23.63 ± 3.45	20.36 ± 3.43	23.77 ± 3.63	0.0001	0.2243	0.0001	0.2406	0.0102	0.9968	0.0048
Exposure(Yrs)	-	25.08 ± 8.00	5.75 ± 2.84	27.89 ± 4.62	0.0001	-	-	-	< 0.0001	0.0339	< 0.0001

\* P-value estimated using *one-way ANOVA* Bold numbers indicate statistical significance; ‡ Tukey's post-hoc comparisons

\*denotes p value less than < 0.05 as considered statistically significant

\*\*denotes p value less than < 0.001 as considered highly significant

\*\*\* denotes p value less than < 0.0001 as considered extremely significant

**Table 2: Percentage wise distribution of Educational, dietary habits and Behavioral characteristics of the study groups**

Characteristics	Study groups				p Value
	Controls (n=30)	Office employees (n=47)	Miners-I (n=16)	Miners-II (n=71)	
<b>Education (%)</b>					
Illiterate	0	0	2 (12.5)	2 (2.82)	0.068
Literate	30 (100)	47 (100)	14 (87.5)	69 (97.18)	
<b>Diet (%)</b>					
Vegetarian	2 (6.67)	15 (31.91)	14 (87.5)	20 (28.17)	<b>0.0001</b>
Non-vegetarian	28 (93.33)	32 (68.09)	2 (12.5)	51 (71.83)	
<b>Habitual characteristics (%)</b>					
Smoking Habits	10 (33.33)	16 (34.04)	8 (50)	21 (29.58)	0.483†
Tobacco Chewers	11 (36.67)	19 (40.43)	11 (68.75)	43 (60.56)	0.017†
Alcohol Consumers	11 (36.67)	20 (42.56)	12 (75)	31 (43.66)	0.081†

†P-value estimated using *chi-square test*

**Table 3: Health and diseases status of subjects of the study groups**

Characteristics	Study groups			
	Controls (n=30)	Office employees (n=47)	Miners-I (n=16)	Miners-II (n=71)
Blood pressure (%)	3 (10)	12 (25.53)	1 (6.25)	24 (33.80)*
Skin Problem	0	2 (4.26)	1 (6.25)	2 (2.82)
Respiratory Discomfort (Asthma)	0	0	1 (6.25)	4 (5.63)
Bone and joint pain	0	4 (8.51)	5 (31.25)	29 (40.84)*
Chest pain	0	5 (10.64)	7 (43.75)*	16 (22.53)*
Headache	0	0	7 (43.75)*	14 (19.72)*
Backache Pain	0	3 (6.38)	6 (37.5)*	12 (16.90)*
Neurological disorder	0	0	1 (6.25)	1 (1.41)
Kidney disorder	0	0	0	2 (2.82)
Hair Colour change problem (Browning)	0	0	4 (25)*	13 (18.31)*
Jaundice	0	0	0	5 (7.04)
Abdominal pain	0	0	7 (43.75)*	8 (11.27)
Diabetes (%)	0	6 (12.77)	0	15 (21.13)*
Tuberculosis	0	0	1 (6.25)	2 (2.82)
IHD	0	1 (2.13)	0	11 (15.50)*

Note: Astic (\*) indicates high percentage of subjects suffered from Health and diseases problems.

**Table 4: Blood metal analysis and serum ceruloplasmin in various study groups**

Metal	Study groups				Pair wise comparisons‡		
	Controls (n=30)	Office workers (n=47)	Miners- II (n=71)	P-value*	Control vs Office workers	Control vs Miners- II	Office workers vs Miners- II
<b>Copper (mg/l)</b>	0.54 ± 0.18	2.13 ± 0.84	2.27 ± 0.82	< .0001***	< 0.0001***	< .0001***	0.5651
<b>Manganese (mg/l)</b>	0.94 ± 0.55	1.86 ± 1.39	2.18 ± 1.87	0.0015**	0.0312*	0.0009**	0.5153
<b>Zinc (mg/l)</b>	7.16 ± 1.19	8.39 ± 2.42	8.2 ± 3.11	0.1098	0.1147	0.1636	0.9264
<b>serum Ceruloplasmin</b>	26.05 ± 9.12	42.38 ± 10.4	46.16 ± 14.49	< 0.0001	< 0.0001***	< 0.0001***	0.125

\*P-value estimated using *one-way ANOVA*; ‡ Tukey's post-hoc comparisons

\*denotes p value less than < 0.05 as considered statistically significant

\*\*denotes p value less than < 0.001 as considered highly significant

\*\*\* denotes p value less than < 0.0001 as considered extremely significant

Table 5: Blood Metal Analysis and Serum Ceruloplasmin in various study groups post-adjustment with confounder

Biochemical Parameters	Controls (n=30)	Office workers (n =47)	Miners- II (n=71)	P-value*	Pair wise comparisons‡		
					Control Vs Office workers	Control Vs Miners- II	Office workers Vs Miners- II
Copper (mg/l)	0.66 + 0.34	2.14 + 0.84	2.22 + 0.79	<b>0.0001***</b>	<b>0.0001***</b>	<b>0.0001***</b>	0.8206
Manganese (mg/l)	1.09 + 0.65	1.89 + 1.35	2.09 + 1.85	<b>0.0118*</b>	0.0674	<b>0.0087*</b>	0.7686
Zinc (mg/l)	7.31 + 1.19	8.44 + 2.46	8.1 + 3.01	0.1699	0.1482	0.3385	0.7618
Serum Ceruloplasmin	27.81 + 9.09	44.09 + 11.49	46.26 + 14.87	<b>0.0001***</b>	<b>0.0001***</b>	<b>0.0001***</b>	0.412

†Adjusted for age, bmi, smoking, tobacco and alcohol; \*P-value obtained using one way ANOVA; ‡Tukey's post hoc comparisons Bold numbers indicate statistical significance

\*denotes p value less than < 0.05 as considered statistically significant

\*\*denotes p value less than < 0.001 as considered highly significant

\*\*\* denotes p value less than < 0.0001 as considered extremely significant

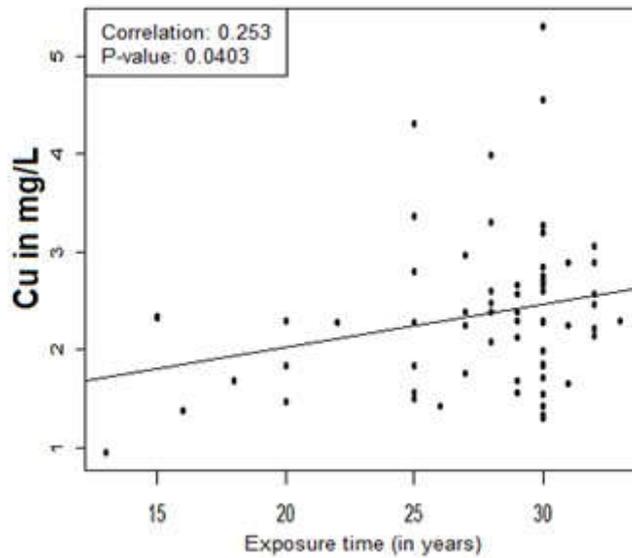


Fig. 1: Miners-II Group: Copper Vs Total Exposure

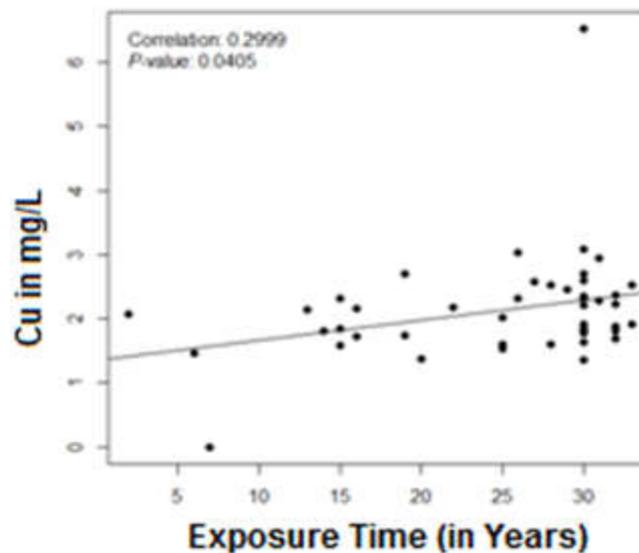


Fig. 2 : Office Employees Group: Copper Vs Total Exposure

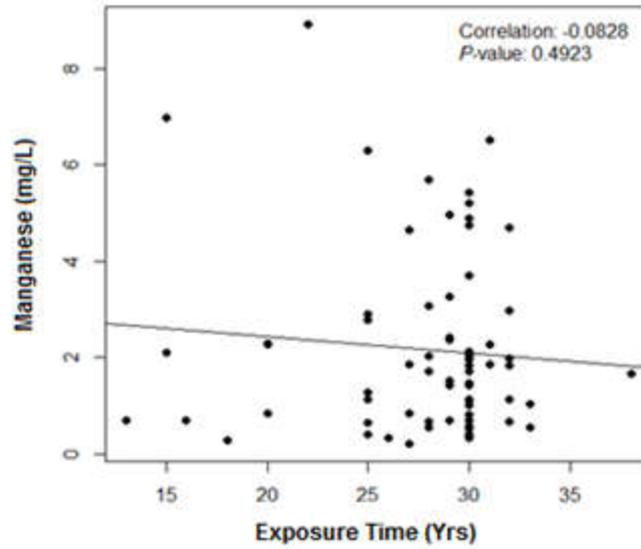


Fig. 3 : Miners-II Group: Manganese Vs Total Exposure

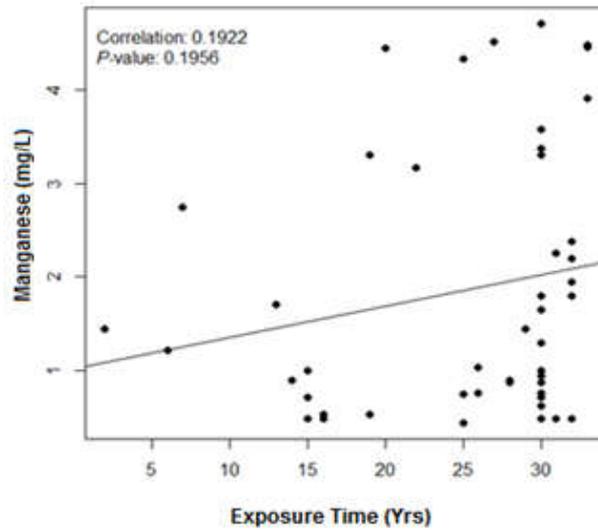


Fig. 4 : Office Employees Group: Manganese Vs Total Exposure

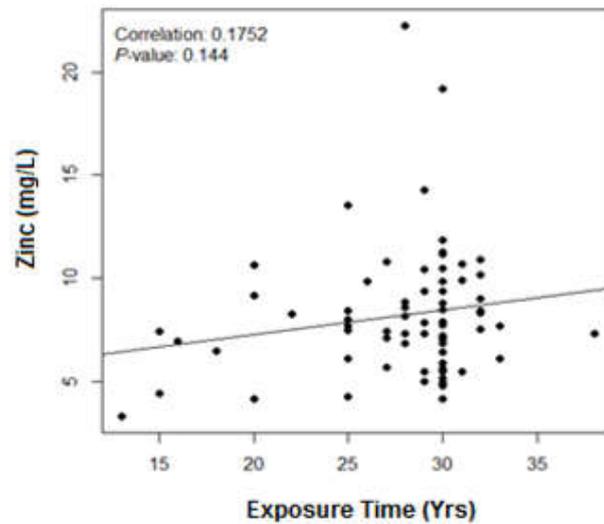
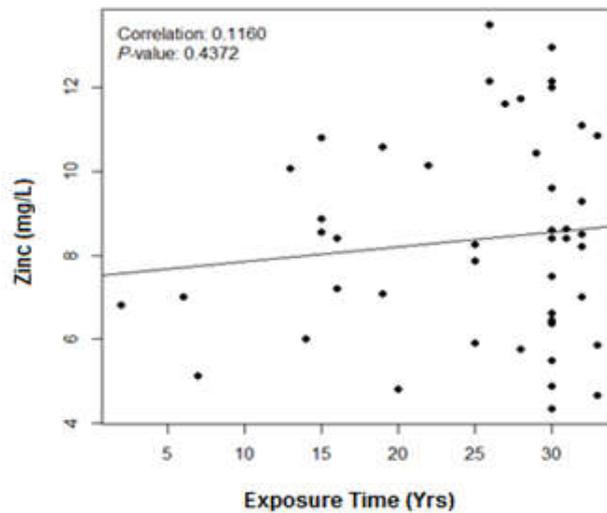


Fig. 5 : Miners-II Group: Zinc Vs Total Exposure



**Fig. 6 : Office Employees Group: Zinc Vs Total Exposure**

## DISCUSSION

Mining operation is one of the hazardous occupations in the world [21,22]. In fact major environmental alterations have been perceived due to mining operations [23,24]. Release of heavy metal in soil and water from mining operations of such origins as ferrous (manganese, Chromium, and iron) and nonferrous (aluminum, copper, lead, silver, cadmium & Zinc etc) groups and their toxic effects on occupational workers have been widely recognized [25]. Systematic epidemiological reports of occupationally exposed workers with respect to certain metals such as lead, Manganese, gold, Iron ore, Limestone, uranium and aluminum are available [26, 27, 28, 29, 20 31 and 32]. Few reports regarding health and disease status of workers exposed to such copper industries as smelting, brassing, welding, cutting, polishing and wire production are also available [33, 34, 35, 36 and 37]. However, no reports have so far been made to provide a systematic epidemiological profiling of workers in a copper mine without having any industrial setting. This is probably the first ever attempt that has been made to screen the general health status of copper miners in the world in general and India in specific.

The present study documents that all employees of the mine under study were sufficiently literate and obtained healthy vegetarian and non vegetarian diet due to one time meal sponsored by mining authority. It was seen that most of the miners were non vegetarian and possessed proportional BMI with age. It may be noticed that both pit workers as well as office employees of the mine were habituated to such addiction as smoking, tobacco chewing and alcoholism. Earlier, Sahaa *et al* [34] also reported smoking addiction in workers from copper based industries these habit forming characteristics may be attributed to the monotonous and strenuous work situation as well as to affluent wage pattern.

Reports are available regarding respiratory, nervous, skin, musculoskeletal disorders as well as hypertension and diabetes in copper wire factory, iron ore mine and gypsum mine workers [35, 30 and 36]. The present study reveals the morbidity pattern of open pit workers with chronic exposure to copper dust. It was found that these workers suffered predominantly from hypertension, diabetes and IHD accompanied with bone and joint pain, chest pain along with complaints of frequent backache, abdominal pain and browning of hair problems. However, the observation of lesser morbidity in mildly exposed miners indicates that chronicity of exposure is an essential factor for health morbidity. Whether long term copper dust exposure to pit workers was solely responsible for such health morbidity is not yet clear, since these subjects had an age factor along with them. This is validated by the fact that pit workers with shorter period of exposure who were relatively young (Miner-I) lesser sign of morbidity. It may be also noticed that office employees with lesser physical activities also suffered from such morbidity as high blood pressure and proneness to diabetes. In fact, Howard D and Sesso *et al* [38] showed a relationship between physical activity and hypertension and some sort of ischemic heart disease. Hence, the above mentioned aberration observed in the health status of the pit workers in general as well as of the office employees residing in the vicinity of mining area in particular could be attributed to either age, physical activity and or duration of exposure or all. It may be recalled that aberrated health status observed in gypsum and iron ore mines was also attributed to age, physical activity and stressful environment [30,36].

Serum Ceruloplasmin levels can act as a reliable biomarker for the systemic contents of copper in human [34]. Ehsanollah *et al* [37] reported high levels of serum Ceruloplasmin and copper in subclinical copper poisoning in people from residential area near copper smelting complex revealing that health hazards increase with closeness of copper mining industry and smelting complex.

Evaluation of the effects of long term exposure to copper dust (Miner-II) after adjustment for other predictor like age, BMI and smoking, tobacco chewing and alcoholism on systemic level of copper and other accompanying metals like zinc and manganese revealed that there were significant enhancement in the contents of copper, manganese and serum Ceruloplasmin when compared to unexposed control. Almost the same picture emerged, post adjustment with other covariates in office employees except for insignificant raise in the contents of manganese. Thus, although the contents of blood manganese and zinc were not found to be correlated with duration of exposure, sole elevation in the content of copper seems to show positive relationship with duration of exposure. Thus, it may be inferred that long term exposure to copper dust from the environment may precipitate into copper toxicity and related health morbidity in both miners as well as office employees residing in the vicinity of the copper mine.

## CONCLUSION

Most of the miners under study were non vegetarian and possessed proportional BMI with age. Chronic and mildly exposed pit workers as well as office employees of the mine were habituated to such addiction as, smoking, tobacco chewing and alcoholism. Hypertension, diabetes and IHD accompanied with bone and joint pain, chest pain along with complaints of frequent backache, abdominal pain and hair depigmentation problems were witnessed in those pit workers who were chronically exposed to copper dust. Office employees with lesser physical activity also suffered from such morbidity as high blood pressure and proneness to diabetes. Significant enhancement in the blood contents of copper and manganese along serum Ceruloplasmin were noticed in chronically exposed pit workers as well as office employees. The elevation in the content of copper found in chronically exposed miners and office employees were found to be positively correlated with the tenure of exposure. Sole responsible factor seems to be copper since no relationship of manganese with duration of exposure could be observed. Thus, it may be inferred that long term exposure to copper dust from the environment precipitates into copper toxicity and observed health morbidity in both chronically exposed miners as well as office employees.

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## CONFLICT OF INTEREST

The authors state no conflict of interest.

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