

Whole Blood Characterization in School Teachers with Reference to Chalk Sticks

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Abstract

It is known that usage of chalk causes dust and exposure to chalk dust can cause health hazards in teachers. But the level of exposure to chalk dust in teachers were not known so far. Hence, present study aimed to investigate the level of exposure to chalk dust in teachers by studying the whole blood concentrations of trace elements (Al, Cr, Mn, Fe, Co, Ni, Si, Pb) that are associated with chalk. ICPMS method was adopted to conduct the trace element concentration in whole blood and due to lack of reference standards, the recovery percentages were analyzed. From the results, it is identified that except Si all the elements are noticed beyond the normal blood limits in teachers. For silicon, there is no normal blood limit. The relation between the trace elemental concentration and socio demographic variables like gender, age, Years of service was also studied. The age and gender did not show significant difference with the trace element concentration. The years of service revealed a positive association with trace element concentrations. The elements like Si (70847 µg/l), Iron (19499 µg/l), Al (1389.9 µg/l) were noticed with elevated concentrations in teachers. The same elements were reported with higher concentrations in past studies that are conducted on trace elements concentration of chalk. Hence, it is proved that the chalk is the prime reason for the elevated concentrations of trace elements in blood and it also concluded that usage of chalk causes health hazards in teachers.

Keywords: Trace elements; Whole blood; Chalk

Introduction

In addition to the occupational health hazards in teachers [1], few recent studies suggested that chalk can also cause occupational health hazards in teachers [2]. It is proved that chalk will behave like particulate matter (PM) which remains suspended in air for longer period of time [3]. The writing exercises on chalk also revealed that chalk produces large amount of dust [4]. Few epidemiological studies reported that metals in the chalk dust causes allergic contact dermatitis [5] respiratory ailments [6,7]. Recent Experimental studies also proved that metals in chalk dust causes oxidative damages to Alveolar Macrophages [8-9].

Black board concept of teaching is an ancient method of teaching and many schools in India are still following this concept because of its economic viability [10,11]. Chalk composes of CaCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, additives like CMC (carboxyl methyl cellulose), PVA (poly vinyl alcohol), and starch which were used as thickeners, and water binders ZnO, kaolinite, and colored pigments with standard grain size of these particles. The quality of chalk depends on the percentage of CaCO_3 and its controlling variables like binders. Chalks with less than 6% of CMC are dusty [12]. Initially, chalk was known to be dusted chalks composed of calcium sulfate (gypsum). The dust particles released from these chalks tend to remain in air for longer period of time. Later on, many industries launched dustless chalks. These dustless chalks are made up of calcium carbonate with the binder in the chalk that releases dust with large particles, which are not respirable and settle faster [13]. These settled dust particles, which settles on body parts of teachers can also causes allergic infections [14]. Even though it is dusted or dustless chalk it is fact that it will cause health hazards in teachers [15,16].

Predictable medium for assessment of exposure to toxic metals and essential trace elements are blood and urine. As an alternative substrates hair, Saliva, nails and in some rare cases even breast milk [17]. In the present study, blood is used as collective medium for exposure assessment.

In our previous studies, the concentration of trace elements in different brands on chalk was analyzed [18]. Later the trace elements

in chalk dust collected from classroom were examined [19]. In the present study, the concentration of trace elements in the blood samples of teachers was analyzed using ICPMS.

Materials and Methods

Sample collection

With the collaboration from the few medical laboratories samples of teachers were collected. Among the visitors who came for blood examination the teachers were identified separately based on the occupation. All the information related to this work was explained to the teachers and with the consent of the teachers; the sample was collected and stored for further studies.

Elemental analysis

Dilution process

The collected samples were diluted by following the alkali dilution method [20]. Aliquot (0.2 ml) of whole blood samples were diluted 1:25 with an alkali solution consisting of 2% (W/V) 1-butanol, 0.05% (W/V) EDTA, 0.05% (W/V) triton X-100, 1% (w/v) NH_4OH and internal standards 20 µg/L. The mixture was sonicated for 5 minutes and centrifuged at 1000 rpm for 2 minutes.

Experimental set up

The Agilent 7700s inductively coupled plasma mass-spectroscopy

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(ICP-MS) system (Agilent technologies, Tokyo, Japan) was engaged to assess the ion profile. Platinum sample cone and skimmer cone were utilized with an orifice diameter of 1.0 to 0.4 mm, respectively. Sample introduction was performed with a micro mist nebulizer combined with scott-type double pass spray chamber (Agilent technologies).

The instrument was tuned to optical conditions in terms of sensitivity (Li, Y, Co and Ti) and Ce0/Ce and Ce2+/Ce by using a tuning solution (Agilent technologies) containing 1 mg/L of Li, Y, Ti, Ce and Co in 2% HNO₃ (W/V). The instrument was operated in full quantitative mode, and typical operating conditions used in this study are summarized in the Table 1.

Validation of ICP-MS

To validate the method, ten randomly selected blood samples of 0.2 ml mixed together and spiked with known concentrations. From the results, we have calculated percentage recoveries based on the following equation.

$$\text{Percentage recovery} = [\text{CE}/\text{CM} \times 100]$$

Where CE is, the experimental concentration determined from the calibration curve and CM is the spiked concentration [21].

Elements analyzed

Based on the reference studies the elements like Al (Aluminium), Cr (Chromium), Mn (Manganese), Fe (Iron), Co (Cobalt), Ni (Nickel), Si (Silicon), and Pb (Lead) were analyzed in both settled and suspended chalk dust [2,18,19].

Statistical analysis

The statistical analyses were performed by using the SPSS 19.0 statistical software. Basic statistical analysis like mean, median and standard deviation were performed. Statistical associations between the variables were performed using chi square tests. The level of significance was set to be less than 0.05.

Results

Socio demographic characteristics

A total of 50 participants were included in this study, with a mean age of 39.82 ± 104.1 (Mean ± std) with an age group ranging from 20 to 57. Female participants are dominant in the group with a mean age group of 43.21 ± 1.87 (Mean ± std). Among all the participants, the participants from the age group 30-39 are overriding (Table 2).

Trace element concentration in whole blood samples of teachers

Due to lack of availability appropriate certified commercial reference materials of whole blood samples, the recovery rate was measured using known spiked concentrations of all the investigated elements (Al, Cr, Mn, Fe, Co, Ni, Si, Pb). The consistency of results was confirmed by measuring the recoveries of the spiked elements to the sample concentrations. The recovery percentages of the trace elements analyzed were in the range of 70 to 96% (Table 3) indicating good accuracy, precision and validity of the method.

The geometric mean of trace elements in whole blood samples of study participants (µg/l): blood aluminium 1398.9; blood chromium 39.04; blood manganese 249.6; blood iron 19499; blood cobalt 1.95; blood nickel 32.9; blood silicon 70847; blood pb 752.4. Among all the elements, the elements like Silicon (70847), Iron (19499) and Aluminum

Parameter	Value
Plasma conditions	Forward power 1500 W
Plasma glass flow	15.0 L/min
Carrier glass flow	0.75 L/min
Dilution glass flow	1 L/min
He gas flow	4-5 mL.min ⁻¹
QP bias	-15 v
Oct bias	-18 v
Cell entrance	-40 v
Cell exit	-60 v
Deflect	-0.8 v
Plate bias	-6.0 v
Nebulizer type	Miramist
Sample uptake rate	1.5 mL/min

Table1: IC-PMS operating parameters.

Variables	Distribution				
	Teachers (N=50)				
	Mean	STD	n	%	p-value
Age					
20-29	25.44	2.58	9	18	0
30-39	34.58	3.03	17	34	
40-49	44.18		11	22	
≥ 50	52.66		13	26	
Gender					
Male	37.65	2.09	19	38	0.034
Female	43.21	1.87	31	62	
Years of service					
0-10	27.7	3.4	13	26	0.15
11-20	37.92	6.7	23	46	
21-30	44.18	2.4	14	28	

Table 2: Socio Demographic characteristics of participants.

Elements	Added (µg/L)	Measured mass fraction		% Recovery
		Obtained value (µg/L)	Spiked value (µg/L)	
Al	1	15.4	16.1	70
Cr	1	0.7	1.6	90
Mn	1	7.5	8.3	80
Fe	10	237.7	238.4	70
Co	1	0.95	1.91	96
Ni	1	4.8	5.6	80
Si	10	497.2	506.7	95.5
Pb	10	3.51	10.88	73.7

Table 3: Recovery of the elements from the spiked whole blood samples (N=50).

(1398) are noticed with higher concentration in the whole blood samples of teachers. The normal value of silicon concentration in blood was not set so far [22,23]. The normal limit of Iron in the human blood [24] is 65 to 175 µg/dl in males and 50 to 170 µg/dl in females. This indicates that the concentration of iron is high in teachers. Few studies suggest that normal range of aluminum in human blood is 0-6 mcg/dl [25] which indicates that the aluminum concentration in the teachers was beyond the normal limit. The normal limit of manganese in human blood is

4-15 µg/l [26]. The concentration of manganese in teachers is 249 µg/l, which is beyond the blood normal limit. The normal limit of lead in blood [27] is 10 µg/dl. The concentrations of chromium [28] and Cobalt [29] in teachers are beyond the normal range i.e. 2-3 µg/l and <1 µg/l (Table 4).

Relationship between trace element concentrations among different age groups

The trace elemental concentration of whole blood samples based on different age groups was mentioned in Table 5. In the present study age groups did not noticed any statistical difference.

Comparison of trace elements concentrations between the genders

The trace elemental concentration of whole blood samples in teachers were mentioned in Table 6. There no significant differences

were noticed between the genders. Almost all the elements were noticed at similar concentrations in genders.

Relationship between the trace element concentration and years of service

The influence of trace element concentration on years of service was mentioned in Table 7. The trace element concentration revealed a positive association between the years of service. The trace elemental concentration was increased with the years of service, which clearly indicates the chalk is the prime reason for the higher concentration of trace elements in whole blood samples in teachers.

Discussion

Till today there is no proper standard method for accurate assessment of trace elements in human body. As per the literature review to assess the low dose chronic exposure of heavy metals, blood sample is considered as good indicator and it can reveal the recent comprehensive information of various elements in the body. In the present random sampling method was followed for the collection of blood samples. Basing on the literature studies alkali dilution method was chosen as best suitable method for dilution of blood samples [20]. Due to lack of proper standard reference materials the recovery percentages was analyzed for the qualitative data [22]. From the report a total of eight elements were observed in the blood samples of teachers. Among the eight elements the Silicon, Aluminum, Iron was noticed with higher concentrations in teachers. The reason for the higher concentration of silicon in blood is respirable silica dust, which occurs from the chalk dust. Zhang et al. reported that silicon exposure from the chalk dust causing oxidative damages to Alveolar macrophages, He also reported that deposition of the silicon in lungs might cause silicosis [8,9]. Unfortunately there is no standard limit of Silicon in blood was reported so far. The normal limit of Iron in the human blood is 65 to

Elements	Teachers				
	Min (µg/l)	Max (µg/l)	Mean (µg/l)	Median (µg/l)	SD (µg/l)
Al	852.3	1811.3	1398.9	1472.8	315.8
Cr	25.8	48.03	39.04	39.8	5.04
Mn	201.9	262.4	249.6	257.6	15.6
Fe	16267	20594	19499	20213	1143
Co	0.02	3.67	1.95	2.11	1.08
Ni	14.8	40.8	32.9	36.4	7.9
Si	16245	92182	70847	81397	24385
Pb	101.8	996.6	752.4	838.9	233

Table 4: Trace elemental concentrations in whole blood samples of teachers.

Elements	Age groups of teachers				
	20-29 (N=9)	30-39 (N=17)	40-49 (N=11)	≥ 50 (N=13)	P-value
Al	912.7 ± 49.4	1693.7 ± 101.3	1585.4 ± 98.1	1308.1 ± 199.4	0.028
Cr	31.1 ± 3.6	43.1 ± 2.1	40.5 ± 2.0	39.1 ± 1.5	0.034
Mn	224.3 ± 11.1	260 ± 1.5	257.1 ± 5.2	252.2 ± 7.0	0.175
Fe	17446 ± 815.3	20352 ± 176.7	20183.5 ± 150.5	19619 ± 524.9	0.086
Co	0.34 ± 0.27	3.1 ± 0.3	2.53 ± 0.2	1.53 ± 0.57	0.023
Ni	17.9 ± 1.6	39.6 ± 0.8	37.4 ± 1.2	33.3 ± 3.4	0.045
Si	25616 ± 7713	89967 ± 1088	86325 ± 3540	72113 ± 12467	0.098
Pb	320.3 ± 160.4	941 ± 31.2	880.9 ± 33.4	777.9 ± 65.7	0.136

Table 5: Trace element concentration in different age groups.

Elements	Gender		P-value
	Male	Female	
Al	1411.3 ± 303.1	1411.7 ± 313.8	0.045
Cr	38.6 ± 4.8	39.6 ± 4.3	0.027
Mn	251.2 ± 13.9	250.3 ± 14.2	0.016
Fe	19514 ± 1181.7	19634 ± 1076	0.011
Co	1.9 ± 1.0	2.0 ± 1.0	0.034
Ni	33.5 ± 7.6	33.2 ± 7.9	0.037
Si	72824 ± 24304	71768 ± 23646	0.157
Pb	761.1 ± 240.6	772.2 ± 218.7	0.05

Table 6: Trace elements in different gender groups.

175 µg/dl. The concentration of Iron in blood samples of teachers were beyond the normal limit. The Iron toxicity causes the local and systemic effects. 10-20 mg/kg of iron causes the local irritation of gastrointestinal tracts and which triggers symptoms like nausea, vomiting, Diarrhea and abdominal pain. The chronic exposure to Iron i.e. more than 60 mg/kg can cause the organ damage and tissue malfunction. Even a lethal dose of Iron (200-250 mg/kg) can cause death. The daily intake of aluminum is 0-0.6 mg/kg [30]. The normal limit of aluminum in blood is 0-6 mcg/dl. The previous studies reported that aluminum can damage the human brain [31]. The normal limit of manganese in human blood is 4-15 µg/l. The concentration of manganese in teachers is 249 µg/l which is beyond the normal limit. The exposure to manganese causes the behavioral changes and nervous system disorders [32]. The concentrations of chromium and cobalt were also beyond the normal limit. The relation of trace element concentration with socio demographic variables like gender, age and years was also studied. There is no statistical difference between the gender was noticed with trace element concentration. As per literature, it is noticed that gender did not influence the concentration in metals [33]. The same was repeated in the present study. About the influence of age on trace element concentration, there were more reports Bohnen reported that Mn, Cu, Zn showed differences with age and he also mentioned that the elements like Pd, Cd, Cr, Ni, Se and Al did not showed any differences with age [34]. Kristiansen et al. reported that only arsenic showed significant difference with the age group [35]. Jiqiang et al. reported that age did not show any difference in trace element concentration [36]. In the present study also the age did not showed any difference in trace element concentration. The years of service has shown a significant difference in all the elements. The concentrations of elements were increased with the increase in years

of service. The elements like Silicon, Iron, Aluminium were noticed in elevated concentrations in teachers. So far there is no studies were conducted on blood samples of teachers.

This study is first of its kind to report the toxicity of usage of chalk sticks in teachers. The results of the present study were compared with the trace element concentrations of chalks (Table 8). From the past studies, it is observed that the elements like Iron, silicon and Aluminum are dominant. The results of the present study also showed the higher concentrations of Iron, Silicon, and Aluminum. Therefore, it is confirmed that the chalk is the prime reason for the higher concentrations of these elements in teacher. This study did not attempt in the control group to correlate with the teachers. Even with this limitation, it is proved that chalk is main reason for the trace elements concentrations in teachers.

Conclusion

The concentrations of trace elements like Aluminum (Al), Chromium (Cr), Manganese (Mn), Iron (Fe) Cobalt (Co), Nickel (Ni), Silicon (Si) and Lead (Pb) were analyzed using ICPMS. The overall concentrations of all the elements except silicon were beyond the normal limits of blood. For silicon there is so standard normal limit. Among all the elements Silicon, Iron and Aluminum were noticed with higher concentrations. Relation between the trace elements concentration and demographic variables like age, gender, and years of service was also studied. The gender and age did not show any significant difference in trace element concentration. The positive association was noticed between the trace element concentration and years of service. Almost all the elements noticed with increasing concentration with increasing

Elements	Years of service			P-value
	0-10 (N=13)	11-20 (N=23)	21-30 (N=14)	
	Mean ± STD	Mean ± STD	Mean ± STD	
Al	957.5 ± 97.6	1472.8 ± 170.2	1683.2 ± 93.1	0.092
Cr	32.9 ± 4.0	40.10 ± 1.6	43.2 ± 1.67	0.035
Mn	229.7 ± 12.4	255.9 ± 5.0	260.3 ± 1.39	0.046
Fe	17879.6 ± 945.3	19981.2 ± 301.3	20367.5 ± 176.5	0.025
Co	0.480 ± 0.35	2.0 ± 0.5	3.12 ± 0.35	0.179
Ni	21.0 ± 4.8	35.87 ± 1.91	39.7 ± 0.72	0.057
Si	33844.8 ± 14858.6	81610.0 ± 5603.7	89828.4 ± 1255.6	0.026
Pb	435.2 ± 218.6	834.77 ± 55.5	941.48 ± 28.78	0.075

Table 7: Trace elemental concentration in teachers with different years of service.

S.No	Elements (Mean values of chalk samples in µg/kg)								Ref
	Al	Cr	Mn	Fe	Co	Ni	Si	Pb	
1	2177	138.4	801.8	334.8	223.7	31.4	516.2	197.8	[18]
2	3596	230.5	1061	516	213.9	57.8	360.6	217	[18]
3	32514285	88500	3938000	1590000	-	34000	94985000	48000	[37]
4	2100000	-	-	100000	-	-	2000079	-	[2]
5	252700	8300	-	740000	-	22700	4454000	-	[19]
6	-	2430	-	-	685	2000	-	12262	[5]
7	-	110300	85500	-	10000	22200	-	37500	[12]

Table 8: Comparison of results found in this study with previous studies.

in years of service. The results of the study were compared with studies on trace element concentrations in chalk. The similarity in elevated concentrations of Silicon, Iron and Aluminum proved that chalk is the prime reason for the elevated concentrations of trace elements in teachers. Hence, it is concluded that exposure to chalk causes health hazards in teachers and it is suggested that teachers should follow safety measures to reduce chalk dust related occupational health hazards in teachers.

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