

In Vitro Assessment of Pulmonary Exposure of the Packing Workers with Arsenic in Cement Industry

Vahid Abolhasannejad¹, Mohammad Salehizadeh², Vahideh Abolhasannejad^{3*}, Hosein Akhlaghi Nasab⁴, Mahdi Tahernezhad Javazm⁵, Narjes Moasheri³

¹PhD Candidate of Transportation Engineering, Southeast University, China

²Assistant Professor, Occupational Health Department, Kerman University of Medical Sciences, Kerman, IR Iran

³Social Determinants of Health Research Center, Faculty of Health, Birjand University of Medical Sciences, Birjand, IR Iran

⁴BS of Lab Sciences, Occupational Health Department, Kerman University of Medical Sciences, Kerman, IR Iran

⁵MSc graduate of chemistry, Tarbiat Modares University, Tehran, IR Iran

*Corresponding author: Vahideh Abolhasannejad, Social Determinants of Health Research Center, Faculty of Health, Birjand University of Medical Sciences, Birjand, IR Iran Tel: +5632381653, Fax: +5631631651, Email: V.Abolhasannejad@bums.ac.ir

Received 2017 February 15; Revised 2017 February 27; Accepted 2017 March 13.

Abstract

Background: Exposure to chemicals and air pollutants is an important factor of health threat for workers. The present study was conducted with the aim to investigate the relationship between Arsenic metal ions in the air and that of extracted from simulated alveolar fluid.

Methods: In this cross-sectional study, personal and environmental sampling was performed according to OSHA standard and leaching testing was conducted in similar conditions of the lungs [pH=7.4, temp. 37°C, Co₂=5 %] to measure the rate of solubility. Measurement was done by means of the atomic absorption apparatus, Model Spec AA 220 with a graphite furnace Model GTA 110. Data processing was done by using the SPSS statistical software.

Results: The concentration of arsenic metal ion in the air was 0.0078±0.0014 mg/m³. That is lower than the allowable rate (0.01 mg/m³). The amount of metal ions in sustained particles of the environment air was 51.4±4.68 µg/g dust and that of extracted with simulated alveolar fluid in extra corporeal condition was 0.013±0.004 µg/g dust. Pearson correlation test did not reveal any significant relationship between them (p>0.05).

Conclusions: Since there was no significant relationship between arsenic metal ion in the air and that of extracted from alveolar fluid and also the solubility of this metal ion depends on many factors, therefore, these findings can be the basis of further deep and precise studies in future.

Keywords: Cement dust; Arsenic; Pulmonary exposure

1. Introduction

Exposure to chemicals and air pollutants is an essential factor for threatening people's health. The most important pollution in cement industry is associated with cement dust. Inhalation of cement dust and exposure to its particles carrying metal ions play a critical role in incidence and prevalence of respiratory problems and systemic toxicities. Therefore, measuring the rate of metal ions and performing toxicological investigations are very great importance. Long term exposures of workers to dusts and inhaling them can play an important role in histological changes of the lungs

(1). The greatest problem of contamination in cement industry is the dust that is disseminated from different parts of production process. The aerodynamic diameter of cement dust from 0.05 to 20 µm makes the air passage ways the target system for depositing of cement particles (2). On the one hand, the events associated with dissolution of dust or aerosols and inhaled particles are important physico-chemical factors in clarification of substances from the lower respiratory organ (3, 4). When particles containing metal ions are inhaled and deposited in different parts of the lungs, there may accrue a variety of mechanisms. Release of metal ions by dissolution of particles can cause various

systemic toxicities by transferring through blood circulation and reaching the target system. In addition, as several epidemiologic studies have also shown, there will be much relation between airborne metal ions and the incidence of respiratory diseases (5-7). Studies have shown that accumulated aerosols dissolve in a fluid with natural pH (natural pH of alveolar fluid=7.4). Regarding that the lungs' fluid has nearly a natural pH, it can be noted that a great number of elements contained in dust particles rapidly pass through the blood / air barrier in the form of soluble in water (8). Transfer of metal ions contained in aerosols can lead to developing free radicals and oxidation toxicity and change the flow of electron in the membrane and enzymes (9, 10). Arsenic, due to its toxicity and dangerous effect on human's health is more often taken into consideration and is among poisonous and dangerous metals whose concentration measurement in different samples has been of great concern for researchers. Occupational epidemiologic researches indicate the excess of lung cancer among workers exposed to arsenic (11, 12). Results of Arsenic nephrotoxicity Studies also have shown kidney failure caused by oxidative stress (13-15). Arsenic is absorbed through the lungs rapidly and distributed to most organs of the body. The rate of its absorption can be varied depending on the type and physical shape of the compound in different workplaces (16-19). Since few reports on the effects of arsenic accumulation in human body are available, the information indicating its presence in workplaces has shown that Arsenic concentration more than 54.6 µg/m³ increases the risk of cancer in exposed workers. Therefore, correct and precise measurement of trivial amounts of arsenic is of great importance and the measurement of arsenic concentration and its toxicological investigations is very necessary. In the lungs' conditions, different processes affect the rate of inhaled metal ions, one of which is the rate of solubility of elements that exist in the lungs' condition. The aim of this study was to investigate the relationship between Arsenic metal ions in the air and that of extracted from simulated alveolar fluid.

2. Methods

The present cross-sectional study was conducted in the packing saloon of Kerman cement factory by using ID-127(OSHA-2002) method. Participants were selected by random sampling considering the area of the plant. To collect air dust samples a sampling filter model S&S filter

paper cycles, and an environmental sampling SIBATA high volume pump model HVS-500-5 were used (20). The filters were put in laboratory condition and their primary weight was measured by using a digital scale Percisa gravimetric AG model 320-9802-005. To take the bulk sample regarding the pilot test performed on the sampling pump and the standard curve, the flow rate of the pump was considered 600 lit/min. The time length of the sampling for each sample noting the sampling condition was 3 to 77 minutes. A number of filters (n=15) that contained the collected dust samples from packing saloon were put under the conditions of acid digestion to measure the arsenic concentration, and some others (n=15) were put under the condition of simulated alveolar fluid to assess the rate of released metal ion. In order to measure the rate of released metal ion, the filters containing air dust were put inside a 12 cm diameter container holding the simulated fluid. The so-called fluid was made by dissolving several chemicals in one liter deionized water (21)(Table 1).

Table 1. Values of chemical parameters in simulated alveolar fluid

Chemical Material	Dose (gr)
MgCl ₂ . 6H ₂ O	0.2033
NaCl	6.0193
KCl	0.2982
Na ₂ HPO ₄	0.1420
Na ₂ SO ₄	0.0710
CaCl ₂ . 2H ₂ O	0.3676
CH ₃ COONa. 3H ₂ O	0.9526
NaHCO ₃	0.6043
C ₆ H ₅ Na ₃ O ₇ . 2H ₂ O	0.0970
Oil Extracted Lecithin Fraction	0.1

Since the pH of the buffer should be approximately 7.4, the pH was measured by a pH meter, and as much as needed, little amounts of hydrochloric acid or sodium hydroxide solution was added for more exact adjustment of the desired pH (4). To reduce the probable rate of error and obtaining more exact results, the dishes (containers) were washed and sterilized before use. Thus, the dishes (pipette, Petri- dishes, containers of polyethylene) were put inside 1 mol acid wash for 24 hours and then washed with water and cleaned with distilled water (22, 23). Because the rate of solubility of a metal depends on factors such as the chemical composition of the fluid used for its extraction and

conditions under which the extraction occurs (temperature, fluid volume, and time length) and also, due to the amount of collected samples, 60 ml of the fluid was used equally for all samples (23). In order that the filters containing the dust specimen (samples) rest at the simulation conditions, they must be soaked in the simulated alveolar fluid for a while until the extraction occurs. To determine the durability duration for keeping the dishes of the samples and the fluid inside the incubator (the duration for leaching test), and according to different solubility behaviors of different metals, with review of the literature, performing pilot tests, the desired duration was considered 48 hours (24). The dishes containing the samples and the fluid were put inside an incubator with 37 ± 2 °c, 5 % CO₂, 99.9 % humidity to pose the lungs' condition (6, 21, 25, 26). To help the metal ions release, stirring was done by shaking at 2 hour intervals. After 48 hours, the dishes were taken out of the incubator. To separate the suspended particles from the resulted fluid, 25 ml of the solution from the dishes containing the extracted metal ions was centrifuged. To analyze the metal ions, 5 ml, 5 Mol Nitric Acid was added to 20 ml of the centrifuged solution, and thus the result was a one mol acid solution. (The acidity of the solution prevents the deposition of metal ions in the form of hydrate). To reduce the rate of error and to omit the interference of potential contaminations in the extraction stage, according to the NIOSH-1998 guideline, two control samples per 10 samples were used (27). After some preparations, the resulted solutions were sent to the laboratory and were analyzed by using an atomic device model SPECT AA 220 equipped with an atomizer with graphite furnace model GTA 110, manufactured by Varian Company, Australia. After the analysis, the amounts of metal ions were measured. Data analysis was done using SPSS software (version 11.5) at the significant level of $P < 0.05$. To determine the relation between the rate of concentration of the so-called metal in the air and the simulated fluid, Spearman correlation coefficient test was used.

3. Results

Comparison between the mean extracted concentration of arsenic in the air and its allowable threshold by using t-test showed that the mean concentration was lower than the allowable limit and this difference was statistically significant ($p < 0.05$) (table 2).

In another study that conducted to explore the exposure to metal ions of cadmium, cobalt, chromium and nickel in cement industry, the results indicated the lower level of these metal ions than the allowable limit (28). In addition, in other studies also the concurrent exposure to heavy metals was explored and genotoxic effects of heavy metals in case of concurrent exposure were established even when the concentration of each metal separately was lower than the allowable limit (29). Therefore, due to toxic behavior of these metals and their damaging effects in case of concurrent exposure, it is recommended that health and caution principles be considered obligatory. The amount of the studied metal ion in the particles of the packing saloon and the amount measured in the simulated fluid are illustrated in table 3. The results of Spearman correlation coefficient indicated that the relation between the concentration of the metal ion in the air and that obtained from the simulated fluid for the studied metal ion was not statistically significant ($p > 0.05$) (shown in Diagram 1)

Table 2. Comparison between The mean concentration of arsenic and its allowable threshold limit value(ACGIH)

Metal Ion	Occupational Exposure Limit (mg/m ³)	X±SD (mg/m ³)	P-value
As	0.01	0.0078±0.0014	0.000

Table 3. The amounts of Arsenic in the air and in the simulated fluid (µg/g)

Metal Ion	The amount of metal in the air (M±SD)	The extracted amount from the fluid (M±SD)
AS	51.4±4.6	0.013±0.004

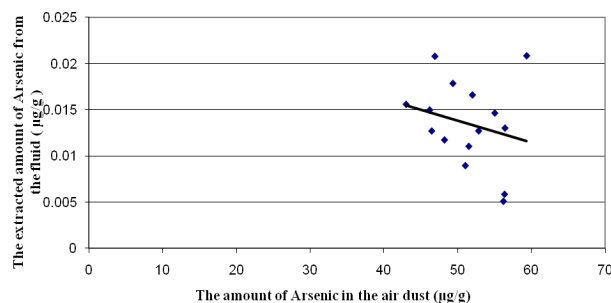


Diagram 1: Relationship between the amounts of Arsenic in suspending dust and the extracted amount (µg /g)

4. Discussion

Toxic properties of metals depend on factors such as solubility and oxidation state that strictly affects the intracellular bioavailability of the mentioned metal ions (30). Friberg researches in 1981 also showed that solving of metals deep in the lungs is carcinogenic and can create health hazards (31). According to the studies of Kyotani in 1998, metal sulphates have stronger solubility than metal oxides and, therefore, easily enter into systemic blood circulation in case of lung exposure (32). Elements whose solubility is lower may stay longer in the lungs (5, 33). Moreover, the results of other studies showed that the alveolar macrophages of inhaled particles specially make phagocyte the non soluble metal oxides and solve them in themselves (34, 35). The results of this study on the rate of metal ions under the lung alveolar condition indicated that there was no significant relation between the amount of released metal ion in environmental condition and its amount in the air dust of workplace. The results of previous studies on the rate of solubility of metal ions of Cadmium, Cobalt, Chromium and nickel also showed no significant relation (28). This result may be obtained because of other effective factors on the rate of released metal ion, that deposited particle behavior can be one of them. The size of particles not only affects the deposition but also clean up and attraction of the particles to the target cell (36). On the one hand, the concentration of protein and phosphate in the alveolar fluid also can affect the behavior of the digested particle (37). In this study, Arsenic had low level of solubility proportional to the existing amount in the air. However, the results of Ferguson and Kim's study (1990) showed that Arsenic was soluble within a wide range of pH (38, 39). Therefore, there exists the likelihood of its solubility in the lungs' condition. Low level solubility of Arsenic proportional to the amount existing in the air is probably due to the physical condition of the simulated fluid and the type of Arsenic particles in this research. The rate of solubility represents the availability, and availability is a good indicator for the limit of bioavailability, therefore, a lot of factors such as particle concentration, interference with other compositions of the suspending particles can affect the limit of bioavailability of a metal. It can be noted that the availability of metal ions in cement dust and following that, the limit of bioavailability of these metals increase the likelihood of systemic problems in exposed workers. The findings of this study showed that a lot of

factors can affect the metal ions after being inhaled. Despite the fact that this metal ion is widely used in different workplaces, not many studies have been conducted on its rate of solubility, pulmonary exposure and those factors that have considerable effect on it; therefore, it is suggested that more researches be done regarding the effects of different factors on the rate of solubility of this metal ion.

5. Conclusion

Since there was no significant relationship between arsenic metal ion in the air and that of extracted from alveolar fluid and also the solubility of this metal ion depends on many factors, therefore, these findings can be the basis of further deep and precise studies in future.

6. Conflict of interests

The authors declared that there is no conflict of interest regarding the results of the present study. Also, no human participants or animals involved in this study.

7. Acknowledgements

The authors wish to acknowledge their indebtedness to the director general of Kerman cement factory for his kind support and continuous help in performing this research.

References

1. Vocaturo G, Colombo F, Zanoni M, Rodi F, Sabbioni E, Pietra R. Human exposure to heavy metals. Rare earth pneumoconiosis in occupational workers. *Chest* 1983; 83(5): 780-3.
2. Mwaeselage J, Moen B, Bratveit M. Acute respiratory health effects among cement factory workers in Tanzania: an evaluation of a simple health surveillance tool. *Int Arch Occup Environ Health* 2006; 79(1): 49-56.
3. Guyton AC, Hall JE. Textbook of Medical Physiology. 10th ed. Philadelphia: W.B. Saunders Company; 2000.
4. Kanapilly GM, Raabe OG, Goh CH, Chimenti RA. Measurement of in vitro dissolution of aerosol particles for comparison to in vivo dissolution in the lower respiratory tract after inhalation. *Health Phys* 1973; 24(5): 497-507.
5. Takaya M, Shinohara Y, Serita F, Ono-Ogasawara M, Otaki N, Toya T, et al. Dissolution of functional materials and rare earth oxides into pseudo alveolar fluid. *Ind Health* 2006; 44(4): 639-44.
6. Tong Y, Zhang G, Li Y, Tan M, Wang W, Chen J, et al. Synchrotron micro radiography study on acute lung injury of mouse caused by PM (2.5) aerosols. *Eur J Radiol* 2006; 58(2): 266-272.

7. Graham JA, Gardner DE, Waters MD, Coffin DL. Effect of trace metals on phagocytosis by alveolar macrophages. *Infect Immun* 1975; 11(6): 1278-1283.
8. Graney JR, Landis MS, Norris GA. Concentrations and solubility of metals from indoor and personal exposure PM2.5 samples. *Atmos Environ* 2004; 38(2): 237-247.
9. Choi JH, Kim JS, Kim YC, Kim YS, Chung NH, Cho MH. Comparative study of PM2.5 - and PM10 - induced oxidative stress in rat lung epithelial cells. *J Vet Sci* 2004; 5(1): 11-8.
10. Johansson A, Curstedt T, Jarstrand C, Camner P. Alveolar macrophages and lung lesions after combined exposure to nickel, cobalt, and trivalent chromium. *Environ Health Perspect* 1992; 97: 215-219.
11. Yoshikawa M, Aoki K, Ebine N, Kusunoki M, Okamoto A. Correlation between the arsenic concentrations in the air and the SMR of lung cancer. *Environ Health Prev Med* 2008; 13(4): 207-218.
12. Welch K, Higgins I, Oh M, Burchfield C. Arsenic exposure, smoking, and respiratory cancer in copper smelter workers. *Arch Environ Health* 1982; 37(6), 325-335.
13. Sinha M, Manna P, Sil PC. Arjunolic acid attenuates arsenic-induced nephrotoxicity. *Pathophysiology* 2008; 15(3):147-156.
14. Walvekar R, Kane SV, Nadkarni MS, Bagwan IN, Chaukar DA, D'Cruz AK. Chronic arsenic poisoning: a global health issue—a report of multiple primary cancers. *J Cutan Pathol* 2007; 34(2): p. 203-206.
15. Yuan Y, Marshall G, Ferreccio C, et al. Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. *Am J Epidemiol* 2007 ;166(12): 1381- 1391.
16. Pinto SS, Varmer MO, Nelson KW, Labbe AL, White LD. Arsenic trioxide absorption and excretion in industry. *J Occup Med* 1976; 18: 677-680.
17. Hakala E, Pyy L. Assessment of exposure to inorganic arsenic by determining the arsenic species excreted in urine. *Toxicol Lett* 1995; 77(1-3): p. 249-258.
18. Blessing Ebele O. Mechanisms of arsenic toxicity and carcinogenesis. *Afr J Biochem Res* 2009; 3(5): p. 232-237.
19. Schlesinger RB, Künzli N, Hidy GM, Gotschi T, Jerrett M. The health relevance of ambient particulate matter characteristics: coherence of toxicological and epidemiological inferences. *Inhal Toxicol* 2006; 18: 95-125.
20. Metal & metalloid particulates in workplace atmospheres. Available from: <http://www.osha.gov/dts/sltc/methods/inorganic/id121/id121.html>
21. Stopford W, Turner J, Cappellini D, Brock T. Bioaccessibility testing of cobalt compounds. *J Environ Monit* 2003; 5(4): 675-680.
22. Midander K, Pan J, Leygraf C. Elaboration of a test method for the study of metal release from stainless steel particles in artificial biological media. *Corros Sci* 2006; 48(9): 2855-2866.
23. Eller PM, Cassinelli ME. *NIOSH manual of analytical methods*. 4th ed. DIANE Publishing company, 1994.
24. Desboeufs KV, Sofikitis A, Losno R, Colin JL, Ausset P. Dissolution and solubility of trace metals from natural and anthropogenic aerosol particulate matter. *Chemosphere* 2005; 58(2): 195-203.
25. Kabe I, Omae K, Nakashima H, et al. In vitro solubility and in vivo toxicity of indium phosphide. *J Occup Health* 1996; 38(1): 6-12.
26. Forde S, Hynes MJ, Jonson B. Dissolution of glass compositions containing no added lead in simulated lung fluid. *Int J Hyg Environ Health* 2008; 211(3-4): 357-66.
27. Ashton I, Gill F. *Monitoring for health hazard at work*. 3rd ed. London: Wiley-Blackwell; 2000.
28. Abolhasannejad V, Salehizadeh M, Ghotbi Ravandi M.R, Mehrbani M, Sabetjahromi M, Nakhe'ee Amroudi N. Evaluating the solubility of metal ions in cement dust within lung alveoli through in vitro method. *J Birjand Univ Med Sci* 2010. 17(2): p. 107-117.
29. Hengstler J, Bolm-Audorff U, Faldum A, et al. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis* 2003; 24(1): p. 63-73.
30. Frías M, Sánchez de Rojas MI. Total and soluble chromium, nickel and cobalt content in the main materials used in the manufacturing of Spanish commercial cements. *Cem Concre Res* 2002; 32(3): 435-40.
31. Friberg, L, Integrated exposure monitoring for health risk assessment. *Environ Monit Assess* 1982; 2(1): 7-13.
32. Kyotani T, Iwatsuki M. Determination of water and acid soluble components in atmospheric dust by inductively coupled plasma atomic emission spectrometry, ion chromatography and ion-selective electrode method. *Anal Sci* 1998; 14(4): 741-748.
33. Ghio AJ, Bennett WD. Metal particles are inappropriate for testing a postulate of extra pulmonary transport. *Environ Health Perspect* 2007; 115(2): A70.
34. Lundborg M, Lind B, Camner P. Ability of rabbit alveolar macrophages to dissolve metals. *Exp Lung Res* 1984; 7: 11-22.
35. Marafante E, Lundborg M, Vahter M, Camner p. Dissolution of two arsenic compounds by rabbit alveolar macrophages in vitro. *Fundam Appl Toxicol* 1987; 8(3): 382-8.
36. Abbracchio MP, Simmons-Hansen JS, Costa M. Cytoplasmic dissolution of phagocytized crystalline metal sulfide particles: a prerequisite for nuclear uptake of nickel. *J Toxicol Environ Health* 1982; 9: 663-76.

37. Kanapilly GM. Alveolar microenvironment and its relationship to the retention and transport into blood of aerosols deposited in the alveoli. *Health Phys* 1977; 32(2):89–100.
38. Fergusson JE. *The heavy elements: Chemistry, environmental impact and health effects*. Oxford, UK: Pergamon.1990.
39. Kim JY, Kim KW, Lee JU, Lee JS, Cook J. Assessment of As and heavy metal contamination in the vicinity of Duckum Au-Ag mine, Korea. *Environ Geochem Health* 2002; 24, 215-27.

Archive of SID