表　　　　題　　パキスタン・イスラム共和国の都市農村部住民の鉛とヒ素曝露
Lead and arsenic exposure among the urban and rural population of Pakistan

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著　者　名　　Zafar FATMI（ザファー ファトミ）

所　　　　属　　Department of Community Health Sciences, Aga Khan University
自治医科大学　医学部　環境予防医学講座

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紹介教員　　医学部　環境予防医学講座
教授・市原佐保子

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Professor Syed Zafar Ahmed Fatmi

Supervisor

Professor Fujio Kayama

Overview

Lead (Pb) and arsenic (As) are among the top ten (10) chemicals of major public health concern worldwide which accrue death and disabilities. Pb causes 0.6% of global burden of disease leading to 143,000 deaths and 600,000 intellectual disabilities, mainly among young children of low- and middle-income countries. Pb is a man-made hazard pervasively present in the environment. Gasoline is the primary source of Pb. The control of Pb in primary source has increased the importance of secondary sources of Pb exposure globally including food, house dust and soil.

While As occurs naturally in earth crust (a natural hazard) and most of the exposure to population occurs through drinking groundwater. An estimated 140 million people are exposed to As above 10 ppb globally. The long-term exposure to As lead to development of cancers of liver, lung and bladder. The As is also associated with hypertension, diabetes, cognitive disabilities among children and adverse pregnancy outcomes such as abortion and premature births. The most frequent and typical adverse effects of arsenic appear as pigmentation of unexposed areas of the skin and the symmetrical hardening (hyperkeratosis) of palms of soles. The adverse health effects of As is still evolving and it may affect all organ and systems of the body.

Although Pb has been controlled in gasoline (primary source) in Pakistan since 2001, there has been consistent reports of exposure to Pb and high blood lead levels among vulnerable population (pregnant women, newborn and children), particularly urban population in Pakistan. One of the recent studies conducted in 2008 in the heart of the city of Karachi (megacity) reported that about 90% of newborn's cord blood had levels of Pb above 5 µg/dl. In scenarios where primary sources of exposure are controlled, the secondary sources such as food, dust and soil becomes more important source of exposure for several decades. Thus, there was a need to investigate the secondary sources of Pb exposure among the urban population of Pakistan.

Similarly, several studies have reported presence of As in groundwater along Indus river in Pakistan. Previous studies had shown low prevalence of arsenic skin lesions among population exposed to As. However, the policy makers were not convinced about the health burden of As in Pakistan. Therefore, I carried out an investigation of health burden of As in high exposed areas along River Indus to estimate the As associated health burden among these population.

I, therefore, conducted a health exposure assessment of Pb and As in urban and rural areas among the vulnerable population of Pakistan. In this respect, I present three linked studies:

The first study was carried out to identify the main sources of exposure to Pb among pregnant women, newborn and young children in an urban area (Karachi), Pakistan. The study assessed the Pb intake of pregnant women, newborn and one-to- three-year-old children from secondary sources including food, water, house dust, respirable dust, and soil around the house. We collected three-days food duplicates for the pregnant women and 1-3-year-old child from the same households. The exposure of Pb through cooking utensils were also tested. The house dust was collected using vacuum cleaners and the respirable dust. The inductive coupled plasma mass spectrometry (ICP-MS) was conducted to determine the Pb levels in food, water and blood samples. Energy dispersive x-ray fluorescence (EDXRF) method was used to determine Pb in house dust and respirable dust. We also conducted fingerprinting of the Pb isotopic ratios (LIR) of gasoline and secondary sources including food, house-dust, respirable dust, soil, *surma* (eye cosmetics) of exposure in the blood of pregnant women, newborns (cord blood), and children.

The eye cosmetics (*surma*) was considered a major source of exposure to Pb among the women and children in Pakistan. The second study determined the Pb levels in nails of rural women and possible contamination from external sources.

Previous studies have reported the presence of As in groundwater and associated health effects. However, these studies were not able to convince policy makers regarding the health burden of As among the population. One of the reason was that the study showed low prevalence of arsenic skin lesions, as the areas surveyed were both affected and non-affected by As. Therefore, the third study was conducted to determine the adverse health effects (typical arsenic skin lesions – pigmentation in unexposed areas and symmetrical hardening of palms and soles) among a population highly exposed (in villages within 18 km of the river as identified by previous study) to As through groundwater in rural areas along river Indus in Pakistan.

The first study found that the main sources of exposure to Pb for children were food and house-dust, and those for pregnant women were respirable dust and food. The LIR results suggests the same that food, house-dust, respirable dust are the main sources of exposure for blood lead levels. However, the LIR of *surma* and also gasoline was distinct from blood and have little contribution to blood lead levels.

The second study identified that *surma* was a potential external source of contamination for a commonly used biomarker of Pb i.e. nails. Of the 84 nail samples, 13 had Pb levels above which survival of human were not possible. The LIR of these nails showed that it had similarity to LIR of *surma*. Therefore, nails may not be suitable a biomarker in environments such as Pakistan where *surma* use is common.

The third study found higher prevalence of skin lesions among population exposed to high levels of As in the villages along river Indus. About 90% population in these villages were drinking water above 100ppb. The prevalence of skin lesions among population exposed to As 100ppb and above were between 12 to 14%.

Overall, findings of my studies suggested that urban women and children are exposed to high levels of Pb through secondary sources including food, house-dust and respirable dust. Also, the use of eye cosmetics makes nail biomarker ineffective in determining exposure levels in such scenarios as they may also be externally contaminated. The rural population living along river Indus are exposed to naturally occurring As through groundwater and have high prevalence of adverse arsenic skin health effects.

Therefore, regular monitoring of Pb in secondary sources is required. The simple measures of regular wet-mopping of living rooms may reduce the exposure to Pb. Furthermore, the food production, processing, and packaging needs to be monitored to identify the sources of exposure of Pb. Arsenic (As) in groundwater along river Indus require strategies such as switching of wells. The groundwater handpumps along River Indus have safe and unsafe wells lying usually close to each other. Population need to be made aware about the hazards of As and safe handpumps need to identified for safe use of drinking water. About 13 to 15 million people live along the length of River Indus within the high risk zones where As in groundwater is above 100 ppb. Thus, immediate measures need to be undertaken to protect these populations from hazards of As.

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Study I - Lead exposure assessment among pregnant women, newborns, and children: case study from Karachi, Pakistan.

Abstract

Background:

Lead (Pb) in gasoline has been banned in developed countries. Despite the control of Pb in gasoline since 2001, high levels were reported in the blood of pregnant women and children in Pakistan. However, the identification of sources of Pb has been elusive due to its pervasiveness.

Methods:

In this study, we assessed the lead intake of pregnant women and one-to-three-year-old children from food, water, house dust, respirable dust, and soil. In addition, we completed the fingerprinting of the Pb isotopic ratios (LIR) of gasoline and secondary sources (food, house-dust, respirable dust, soil, surma (eye cosmetics)) of exposure within the blood of pregnant women, newborns, and children. Eight families, with high (~50 g/dL), medium (~20 g/dL), and low blood levels (~10 g/dL), were selected from 60 families.

Results:

The main sources of exposure to lead for children were food and house-dust, and those for pregnant women were food, respirable dust, and house dust. LIR was determined by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) with a two sigma uncertainty of 0.03%. The LIR of mothers and newborns was similar. In contrast, surma, and to a larger extent gasoline, exhibited a negligible contribution to both the child's and mother's blood Pb.

Conclusions:

Household wet-mopping could be effective in reducing Pb exposure. This intake assessment could be replicated for other developing countries to identify sources of lead and the burden of lead exposure in the population.

1. Introduction

Lead (Pb) exposure causes an estimated 0.6% of the global burden of disease, predominantly occurring in developing countries (WHO 2016). Every year, 143,000 deaths and 600,000 new cases of intellectual disabilities occur due to lead exposure (WHO 2016). Lead may affect the health of an individual by injuring the kidney, liver, and the haematological and neurological systems (WHO 2016; IPCS 2017; IARC 2017).

Lead has contaminated the environment including food, soil, water, and air, mainly through its usage in gasoline. The decrease in the usage of lead in primary sources such as gasoline has substantially reduced the population exposure. However, lead exposure is still excessive in several developing countries (Kordas et al. 2010; Acosta-Saavedra et al. 2011; Linderholm et al. 2011; Islam et al. 2014; Obiri et al. 2016). The reduction of lead in primary sources has also enhanced the importance to ascertain the secondary sources of lead. Nonetheless, due to the pervasive use of lead, it is difficult to determine the major sources of lead exposure in these environments.

One of the modern methods used to ascertain the sources of lead exposure is lead isotope ratio (LIR) analysis. In the environment, lead exists as four main isotopes: ^{204}Pb, ^{206}Pb ^{207}Pb, and ^{208}Pb. The most common is ^{208}Pb (52%), followed by ^{206}Pb (24%), ^{207}Pb (23%), and ^{204}Pb (1%). Of these, three isotopes (^{206}Pb, ^{207}Pb, and ^{208}Pb) are produced by the radioactive decay of ^{238}U, ^{235}U, and ^{232}Th, respectively. ^{204}Pb is the only primordial stable isotope. Thus, the abundance of Pb isotopes in a sample depends on concentration of U, Th, and primordial Pb in the source and the time elapsed since their formation (Long et al. 1999; Kamenov et al. 2014). The composition of Pb isotopes is commonly expressed as a ratio. The ratios $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{207}Pb/^{204}Pb$, and $^{208}Pb/^{204}Pb$ are the most preferred because these can be determined more accurately. The isotopic compositions of Pb are not significantly affected by physico-chemical fractionation processes. Therefore, Pb isotopes are considered a proficient tool for determining the sources and pathways of Pb exposure (Kamenov et al. 2014).

Although few in number, all studies have reported high blood lead levels in Pakistan since 1989 (Manser et al. 1989a; Manser et al. 1989b; Manser et al. 1990; Janjua et al. 2008; Kadir et al. 2008; Rahbar et al. 2002; Kazi et al. 2014). Comparatively higher blood lead levels were reported from the megacity Karachi (range 7.2–38.2 g/dL) and Islamabad with less dense traffic (3.22–2.3 g/dL) (Manser et al. 1989a; Manser et al. 1989b; Manser et al. 1990; Janjua et al. 2008; Kadir et al. 2008; Rahbar et al. 2002; Kazi et al. 2014). Moreover, higher blood lead levels have also been reported among children of industrial workers (Khan et al. 2010).

2. Objectives

Therefore, the current study has empirically assessed the potential sources of lead exposure among pregnant women, newborns, and young children (one to three- years old) living in the same households in the city of Karachi, Pakistan. A comparison of lead of isotope ratios (LIR) among pregnant women's blood was done to estimate the exposure among newborns. The investigation of exposure among one to three year olds reflected environmental exposure of lead, particularly due to sources within the household. We analysed the common sources, including food (three-day food duplicate samples), gasoline, surma (eye cosmetics), soil, house dust, and respirable dust in the households. The study determined the percentage uptake of lead using LIR and the in-vitro bioaccesibility (source apportionment) for each source for pregnant women and the one- to three-year-old children.

3. Materials and Methods

This cross-sectional study was conducted during August 2014 to November 2015 in Karachi, which is the largest megacity of Pakistan with a population >20 million. Consent was taken from multiparous pregnant women visiting a tertiary care hospital (Qatar Hospital, Orangi Town, Pakistan) for prenatal care, who had at least one living child between one to three years of age and who had been a resident of the city of Karachi for the past four years.

A sample of peripheral venous blood from pregnant women and cord blood from the newborn were taken at delivery. Newborn clothes were provided as an incentive. Of the 66 women who came for delivery, 52 also agreed to give the blood of their one- to three-year-old child and were followed up at their homes after one month of delivery. We visited 66 homes for three consecutive days to collect several samples from the household for the determination of lead exposure for the women and child of the same family. These samples included separate food duplicate samples for the women and one- to three-year-old children, and those of house-dust, respirable dust (air sample), drinking water, and soil around the house. Additionally, we collected gasoline and engine lubricant samples from neighborhood gas stations. These samples were shipped to Japan for further analysis. Eight families were selected based on the blood lead levels of the pregnant women for a more detailed analysis: two with high levels (~50 g/dL), two with medium levels (~20 g/dL), and four with low levels (~10 g/dL).

3.1 Sample Collection and Preparation

The details of the samples and sampling strategy are as follows.

3.1.1 Blood Samples and Preparation

One mL of blood from the pregnant women, umbilical cord, and children was digested separately with 2 mL of nitric acid Ultrapur-100 (Kanto Chemical Co., Inc., Tokyo, Japan) in a microwave digestion system TOPwave (Analytik Jena Japan Co., Ltd., Kanagawa, Japan), according to the instruction manual, and were analyzed by inductive coupled plasma–mass spectrometry (ICP-MS).

3.1.2 Food Sample Collection and Preparation

Three-day, two weekdays, and one weekend food duplicate samples (i.e., the same amount of food and water, including snacks eaten) were obtained from the women and children. Nominal money was paid to the households for obtaining the food samples. The samples were collected in lead-free plastic bags or a stainless steel box (SUS302), and all liquid food and drinking water samples were individually stored in polypropylene bottles for each meal. The food items were also self-recorded by the women in a food diary for confirmation.

The entire three-day food samples were ground to make a paste by a food processor (Magimix Compact 3200XL; Magimix UK Ltd., Surrey, UK). The entire three-day sample was then mixed into a pooled sample for each subject. If the food was too solid for grinding, then a measured amount of deionized water was added. The ground food was further homogenized using a Polytron homogenizer PT10-35 GT (KINEMATICA AG., Luzern, Switzerland). The homogenized samples for the women and children were kept separately in plastic tubes. Homogenized food samples (2 g) were digested with 5 mL of nitric acid Ultrapur-100 and 1 mL of hydrogen peroxide for atomic absorption spectrometry (Wako

Pure Chemical Industries, Ltd., Osaka, Japan) using the microwave digestion system TOPwave. ICP-MS analysis was conducted by Japan Food Research Laboratories (JFRL; Tokyo, Japan), which is certified by ISO9001, ISO/IEC 17025 ISO9001, ISO/IEC 17025, and JAS, using Agilent 7500ce (Agilent Technologies Japan, Ltd., Tokyo, Japan),

3.1.3 Water Sample Collection and Preparation

Morning tap drinking water samples were collected in 25 mL centrifuge tubes (AGC TECHNO GLASS Co., Ltd., Shizuoka, Japan). In the case where more than one source of drinking was used, the most commonly used was sampled. Water samples were filtered with a 0.45 m cellulose acetate disk filter MILLEX-HA 33 mm diameter (Millipore Corporation, Billerica, MA, USA) and 1/100 volume of nitric acid was added to the filtered samples.

3.1.4 House Dust Collection and Preparation

Dust was obtained in bagless vacuum cleaners (Dyson DC50 upright vacuum cleaner; Dyson Inc., Chicago, IL, USA) during routine cleaning from the places in the house where the children spent the most amount of time.

The dust was then sieved through an opening size of 100 m (Tokyo Screen Co., Ltd., Tokyo, Japan). The hairs and fibrous materials which passed through the sieve were manually removed. The dust samples were dried at 60 C overnight in an oven, and kept in separate plastic bags in a cool and dry environment away from sunlight and fumes.

3.1.5 Particulate Matter (Respirable Dust) Collection and Preparation

Particulate matter of PM_4 (median aerodynamic diameter 4 m, 50% cut) was collected for 24-h from each household. PM_4 was considered appropriate for the determination of lead concentration in the air. The low volume air sampler with the dust separator model C-

30 (Sibata Scientific Technology Ltd., Saitama, Japan), with a suction flow rate of 9.6 L/min, at a height of 50 cm above the floor (to simulate child's respiratory zone), was used. The sampler was placed in the room where the children spent most of their time. Dust was collected on two glass-fiber filters of 55 mm diameter as supplied from the vendor for conformity with the separator. Filters with collected dust were kept in separate plastic bags in a cool and dry environment away from sunlight and fumes before analysis.

3.1.6 Gasoline, Engine Lubricant and Surma (Eye Cosmetics) Sample Collection and Preparation

A total of seven samples (six for gasoline and one for engine lubricant) were obtained from gasoline stations from the Orangi town neighborhood, from where all other household samples were collected. Also, several samples of surma/kohl (eye cosmetics) were bought from the open market in Karachi. These samples were kept in lead-free containers. $Pb(C_2H_5)_4$ and another alkyl lead in the samples were converted with trace metal grade concentrated hydrochloric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to $PbCl_2$, by mixing it overnight at room temperature. All of the lead in the mixture was extracted by ultrapure water for analysis.

3.1.7 Soil Collection

Soil samples were collected in the vicinity of each participating family. The first soil samples were discarded at Japanese Quarantine Office on arrival. We then collected more soil samples in the same places and prepared the samples using acidic extraction fluid in Karachi and the sample fluids were transferred to Japan.

3.2 Extraction of Bio accessible Lead

The extraction of bio accessible lead was carried out using the standard operating procedure for an in vitro bio accessibility (IVBA) assay for lead in soil (USEPA 2012). This method is validated by the United States Environmental Protection Agency (USEPA) for an in vitro assay used for estimating lead relative bioavailability (RBA) in environmental media (soil, dust, food, etc.).

The extraction fluid used was 0.4 M glycine (free base, reagent grade glycine in deionized water), adjusted to a pH of 1.50 0.05 using trace metal grade concentrated hydrochloric acid. Soil samples (200 mg) were mixed with the extraction fluid to a solid-to-fluid ratio of 1/100 (mass per unit volume) in a 25 mL lead-free tube. Samples were extracted at 37 C, at 30 rpm in a BR-40LF bio-shaker (TAITEC Corporation, Saitama, Japan) for one hour, ensuring that the pH was maintained at 1.5 0.5. The extracts were filtered with a 0.45 m cellulose acetate disk filter (33 mm diameter) and the filtered samples were stored at 4 C. The samples were analyzed by ICP-MS Agilent 7500cx in NIES (Agilent Technologies Japan, Ltd., Tokyo, Japan).

3.3 Analysis to Determine the Lead Concentration

The lead concentrations in blood, food, water, gasoline, and engine lubricant, as well as the extraction fluids for bio-accessible lead from environmental media (food, house dust, respirable dust and soil), were determined using inductively coupled plasma-mass spectrometry (ICP-MS). The ICP-MS Agilent 7500cx (Agilent Technologies Japan, Ltd., Tokyo, Japan) method was performed in The National Institute for Environmental Studies (NIES), Japan.

The measurement for lead was carried out by the calibration curve method using a lead standard solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and a thallium standard solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for an internal standard. The lower limit detection of lead was 0.001 ng/mL (ppb).

The test for quality control was performed by using commercial reference samples: National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 995c, Toxic Metals in Caprine Blood (NIST, Gaithersburg, MD, USA) for blood analysis; National Metrology Institute of Japan (NMIJ, Tsukuba, Japan) Certificated Reference Materials (CRM) 7202-b, Trace Elements in River Water (Elevated Level) (NMIJ, Tsukuba, Japan) for water analysis. The recovery of lead for the blood and water analysis methods was 95.7%, and 92.9%, respectively.

3.4. EDXRF Analysis for House Dust and Respirable Dust

Energy dispersive X-ray fluorescence spectrometry (EDXRF) was conducted by the Industrial Technology Center of Tochigi Prefecture, using an JSX-3100RII element analyzer (JEOL, Tokyo, Japan) to determine the lead concentrations of house dust and respirable dust.

For analysis, house dust was placed in a specific plastic cup with thin film sample supports of PROLENE 4.0 microns (Chemplex Industries, Inc., Palm City, FL, USA) and it was pressed by hand using a pestle. House dust samples were analyzed for 240 s (live time) under an air-condition using an X-ray lamp voltage of 50 kV, an auto lamp current, a 7 mm collimator, and a Pb filter. The measurement for lead was carried out by the calibration curve method equipped in the instrument. Samples for the calibration curve were prepared by cellulose, powder (Nacalai Tesque, Inc., Kyoto, Japan), and NIST SRM 2583.

For analysis, deposited respirable dust on the filter was placed on the measurement stage with the PROLENE film to prevent the contamination of the detecting element. Respirable dust samples were analyzed for 600 s (live time) under the same condition as for the house dust analysis. The measurement for lead was carried out by the calibration curve method. The standard filters for the calibration curve were prepared by the droplet method.

3.5. Lead Isotope Ratios Analysis

The acid digested solution of blood samples, food, and the extraction of bioaccessible lead from environmental media (house dust, respirable dust, soil, surma, gasoline and engine lubricant), was analyzed for a comparison of the lead isotope ratios (LIR). Measurements of the LIR ^{207}Pb/^{206}Pb versus ^{208}Pb/^{206}Pb were performed using ICP-QMS Agilent 7500cx in NIES. The instrumental conditions of ICP-QMS for LIR analysis are given in Table 1.1 (for details see Takagi et al.) (Takagi et al. 2008). The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 981, Common Lead Isotopic Standard, was used to correct for mass discrimination. The typical within-run RSD of the isotope ratio measurement of NIES SRM 981 was around 0.3% for both ^{207}Pb/^{206}Pb and ^{208}Pb/^{206}Pb. The extraction of NIST SRM 2583 was analysed for every ICP-QMS measurement for quality control. The value ($n = 5$) was 0.822 0.002 for ^{207}Pb/^{206}Pb and 2.027 0.003 for ^{208}Pb/^{206}Pb, which agreed with the value measured by the ICP-MS multi-collector (unpublished data, 0.8241 0.0000 for ^{207}Pb/^{206}Pb and 2.0279 0.0001 for ^{208}Pb/^{206}Pb) (Table 1.1).

Table 1.1: Instrumental conditions of ICP-QMS for lead isotope ratio (LIR) analysis.	
Parameters	**Conditions**
RF (W)	1600
Plasma gas (Ar) flow rate/L.min^{-1}	15.0
Carrier gas (Ar) flow rate/L.min^{-1}	0.90
Auxiliary gas (Ar) flow rate/ L.min^{-1}	0.90
Makeup gas (Ar) flow rate/L.min^{-1}	0.20
Sample uptake rate/rps	0.1
Acquisition time/point.mass^{-1}	3
Dwell time/s.points^{-1}	1
Integration time/s.points^{-1}	3
Number of measurements/time	10
Monitor mass/m.z^{-1}	206, 207, 208

The mean and standard error for LIR of $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ were separately determined for the blood of pregnant women, newborns, and children, as well as for all of the environmental media and food samples of pregnant women and children.

3.6. Lead Contamination Tests from Cooking Utensils

We also measured the lead concentration for raw (pre-cooked) and cooked food for common food items to ascertain the contribution of cooking utensils for increasing the lead levels in the food. Five different types of utensil, including commonly used alloys, steel, iron, non-stick utensils, and microwaves, were used for cooking the same food. Three common foods items including lentils (daal), potatoes, and chicken were assessed. The raw and cooked food items were processed in the same manner as the food duplicate samples and were measured for lead contents.

3.7. Calculation of Lead Uptake and Statistical Analysis

To compare the contribution of various lead intake sources, a calculation for the lead uptake from food, water, house-dust, respirable dust (PM_4), and soil [in g/kg body weight/week] was conducted with the following calculation formula, based on the USEPA Exposure Factors Handbook 2011 edition (USEPA, 2011).

$$\text{Food} = [C \times DI \div BW] \times 7 \qquad (1)$$

$$\text{Water} = [C \times DI \div BW] \times 7 \qquad (2)$$

$$\text{House dust} = [C \times IngR \div BW] \times 7 \qquad (3)$$

$$\text{Respirable dust} = [C \times InhR \div BW] \times 7 \qquad (4)$$

$$\text{Soil} = [C \times IngR \div BW] \times 7 \qquad (5)$$

where C is the concentration of lead in the respective media [food, g/g; water, g/mL; house dust, g/g; respirable dust, g/m^3; soil, g/g]; DI is the calculated daily intake of food (mg/day) and water (mL/day); IngR is the ingestion rate of house dust (for adults: 30 mg/day; child (1–6 years): 60 mg/day) and for soil (for adults: 20 mg/day; child (1–<6 years): 50 mg/day); InhR is the inhalation rate of respirable dust (for adults of normal weight between 23–<30 years, pregnancy 22nd week): 21.4 m^3/day and child (2–< 3 years) 8.9 m^3/day); BW is the body weight, kg; multiplied by seven to convert it into a weekly dose. The daily intake of food and water was calculated by the weighed value at sample preparation. For all of the ingestion and breathing rates, we used the data from the USEPA Exposure Factors Handbook 2011 edition (USEPA, 2011).

Environmental media including house-dust, respirable dust, soil, gasoline, surma, and water, as well as food, were extracted as bioaccessible lead. To assess the contributions of ingestion of these potential environmental lead sources, the calculation methods were used.

The study was given approval by the Ethics Review Committee of Aga Khan University and the Institutional Review Board of Jichi Medical University, Japan.

4. Results

The mean blood lead levels of the overall sample for pregnant women, one- to three-year-old children, and umbilical cord blood are provided in Table 1.2.

Table 1.2: Blood lead levels for pregnant women, newborns (umbilical cord) and child in Karachi.

	Age ±SD (children in months/women in years)	**n**	**Arithmetic mean (±SD) in µg/dl**	**Median (Interquartile range) in µg/dl**	**Range**	**≥5µg/dl n (%)**	**≥10µg/dl n (%)**
Pregnant women	25.24 (3.29)	66	16.18 (8.60)	14.73 (11.21- 18.16)	3.33-50.12	65 (98.48)	50 (79.37)
Newborn (umbilical cord)	At birth	61	14.08 (7.95)	12.69 (9.32-15.87)	4.44-42.91	59 (96.97)	41 (67.21)
Male newborn (umbilical cord)	At birth	37	15.54 (9.42)	12.87 (9.35-16.07)	6.37-43.00	37 (100.0)	25 (67.57)
Female newborn (umbilical cord)	At birth	24	11.69 (4.16)	11.81 (8.94-14.38)	4.44-19.10	22 (91.0)	15 (65.22)
Child	25.98 (6.42)	52	21.87 (9.37)	20.11 (14.51-25.36)	8.27-52.14	52 (100.0)	51 (98.08)
Male child	26.72 (6.65)	25	20.67 (8.50)	20.11 (13.97-24.59)	8.27-41.11	25 (100.0)	23 (95.83)
Female child	25.34 (6.24)	27	21.82 (8.45)	18.75 (14.67-25.39)	10.48-47.77	27 (100.0)	27 (100.0)

Among the selected eight households, based on the mothers' blood lead level, families (A–H) were categorized into three groups: two families with a high blood lead group [~50 g/dL], two for a medium blood lead group [~20 g/dL], and four families for a low blood lead group [~10 g/dL]. The cord blood lead levels were closer to the blood lead levels of pregnant women (~80%, ranged 46%–118%) (Table 1.3).

Table 1.3: Blood lead levels (μg/dl) of study participants (selected families) from Karachi, Pakistan.

Family ID	**Pregnant women**	**Cord blood (% mother's blood)**	**Child**	**Category**
A	50.12	43.00 (86)	NA	High
B	49.32	34.52 (70)	52.14	High
C	20.40	16.02 (79)	NA	Medium
D	24.42	18.06 (74)	24.52	Medium
E	12.09	5.54 (46)	14.05	Low
F	11.38	13.42 (118)	25.32	Low
G	11.21	8.94 (80)	11.85	Low
H	11.15	8.93 (80)	18.75	Low

NA: Refused to give consent for blood

The lead levels of the pregnant women's blood were highly correlated (spearman's ρ) with cord blood lead levels (rs = 0.88; p = <0.001). The cord blood levels were also correlated with the one-to three-year-old children (rs = 0.61; p = <0.001) (Table 1.4).

Table 1.4: Correlation coefficient between lead levels in blood of pregnant women, cord blood, young child and different sources of exposures in Karachi, Pakistan.

Correlation with blood lead level of pregnant women	Spearman's rho (ρ)	P value
Cord blood	0.88	<0.001
Young child blood	0.47	<0.001
Pregnant women food	0.29	0.03
Child food	0.32	0.01
House dust	0.38	0.35
Pregnant women water	- 0.04	0.76
Correlation with cord blood	**Spearman's rho (ρ)**	**P value**
Young child blood	0.61	<0.001
Pregnant women food	0.16	0.24
House dust	0.66	0.07
Correlation with 1-3 year old child blood	**Spearman's rho (ρ)**	**P value**
Pregnant women food	0.11	0.46
Young child food	0.38	0.007
Child water	0.006	0.96

The lead content levels of three common food items (i.e., chicken as meat, lentils, and potato as vegetables) were similar before and after cooking using four different utensils, suggesting a minimal contribution of utensils for increasing the lead content in the food. However, the lead concentrations for all food items were lower than the controlled limits (Table 1.5).

Table 1.5: Lead concentration in common food items before and after cooking in different cooking utensils.

Uncooked (ng/g)		Cooked (ng/g)			
		Steel	**Alloy**	**Iron**	**Non-Stick**
Potato	10.3	9.4	8.9	13.4	10.1
Lentil (daal)	-	34.6	55.1	8.6	9.1
Chicken	-	13.0	19.3	23.0	13.3

The lead concentrations of the gasoline and engine lubricant from gas stations in the neighborhood of the study participants ranged from 0.013 to 0.083 ppm, well below the control levels of less than 20 ppm (Table 1.6).

Table 1.6: Lead content in gasoline and engine lubricant in Karachi, Pakistan.

No.	Type	Pb concentration (ppm)
1	Gasoline	0.083
2	Gasoline	0.042
3	Gasoline	0.025
4	Gasoline	0.018
5	Gasoline	0.013
6	Gasoline	0.015
7	Lubricant	0.022

Figure 1.1A shows the mothers' lead intake from food, water, house dust, and respirable dust, calculated by the equation in Section 2.7. In this calculation, we used the lead values of house dust and respirable dust measured by EDXRF analysis and food and water

determined by ICP-MS after total acid digestion by the microwave. As the soil in Karachi could not be transported to Japan, the soil data is missing in this figure. Figure 1.1B depicts a mother's in-vitro bioaccesibility (IVBA). All of the bioaccessible lead values used were measured after the extraction described in Section 3.2. The body intake of lead among pregnant women from different families (A to H) ranged from 8.9 to 22.6 µg/kg body weight/week. The food was the most important source of lead intake among pregnant women. The IVBA of lead from food ranged between 29%–83% (mean = 62.37%). The contribution of lead by food was higher for families with a higher exposure to lead.

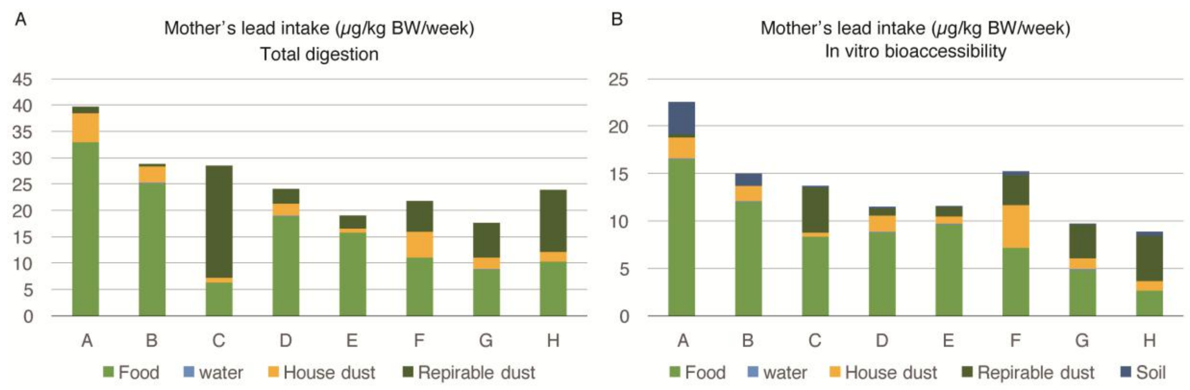


Figure 1.1: Lead intake by multiple sources among pregnant women of eight families (A–H) in Karachi, Pakistan: (A) Lead intake (total acid digestion) from sources in µg/kg BW/week; (B) Lead intake measured as in-vitro bio-accessible lead in µg/kg/week.

The second most important source of lead exposure among pregnant women was respirable dust (PM_4) intake, which ranged from 2% to 75% (mean = 27.12%), while IVBA ranged from 0% to 54% (mean = 20.37%). The percentage contribution of lead by respirable dust was higher for families exposed to lower levels of lead. House dust was also an important source; however, water was contributing a negligible amount of lead intake among pregnant women.

The lead intake by various sources among the one- to three-year-old child of the family is described in Figure 1.2 A, B. The intake of lead seen for each child was almost three times higher compared to that of pregnant women, and ranged from 24.4 to 87.3 µg/kg body weight/week. Both the food and house-dust equally contributed to the body burden of each child's lead levels. The proportion of lead intake by food ranged between 15%–67% (mean = 39.75%), while it ranged between 11%–68% (mean = 38.12%) due to house-dust. The IVBA of lead from food ranged between 13%–55% (mean = 34.50%), while it ranged between 12%–69% (mean = 36.75%) due to house-dust. Respirable dust (PM_4) and soil were also important sources of exposure for one- to three-year-old children, as opposed to pregnant women. However, lead from water was contributing a negligible amount to the body burden of lead in the young child similar to the pregnant women. There were no marked differences in the sources of exposure (percentage contribution of lead) for young children among families exposed to high and low lead levels. The children of all selected families were similarly exposed to lead from food, house dust, respirable dust, and soil.

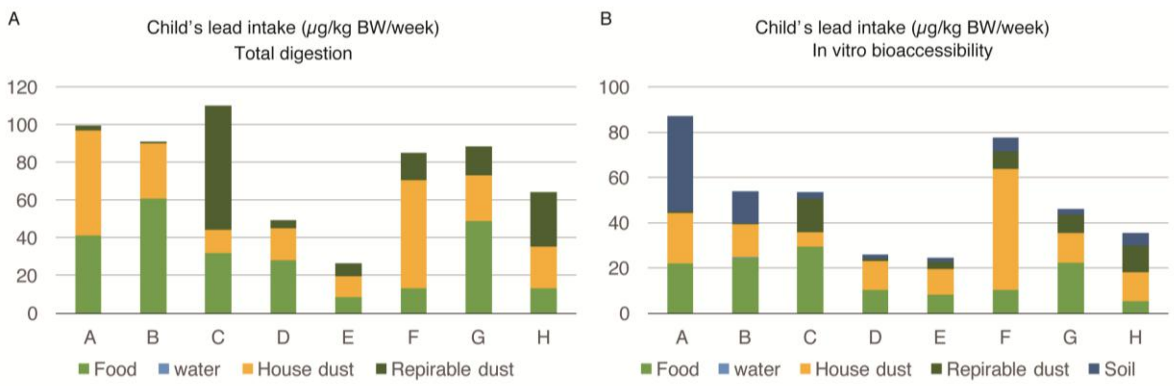


Figure 1.2: Lead intake (in-vitro bio-accessibility) from multiple sources among one- to three-year-old children of eight families (A–H) in Karachi, Pakistan: (A) Lead intake from sources in µg/kg BW/week; (B) Percentage contribution from each source.

The bio-accessibility of lead in different sources was calculated in the data presented in Figure 1.1A, B and 1.2A, B (Table 1.7). The average values show that approximately 60% of the lead contents were extracted from food and house dust samples, but a lower percentage of lead originated from the respirable dust.

Table 1.7. Bio accessibility of lead from various sources.

Family ID	**Mother's Food**	**Child's Food**	**House Dust**	**Respirable Dust**
A	50%	53%	40%	30%
B	48%	41%	50%	13%
C	131%	92%	52%	23%
D	47%	37%	73%	26%
E	61%	95%	103%	43%
F	65%	80%	93%	54%
G	55%	46%	54%	53%
H	25%	41%	57%	41%
Average	60%	61%	65%	35%

Figure 1.3 is a graphical representation of the mean LIR of mother's, cord, and child's blood, and the environmental samples for all eight families. The LIR of pregnant women, cord blood, and children's blood were very similar based on their error bars. These LIRs were also close to those of house dust and respirable dust. However, the LIR of 208/206 of the children in Figure 1.3B is lower than the values seen for the mothers' and cord blood, which is rather close to the value of soil. The LIR of gasoline was not related to the LIR of the blood of pregnant women or cord blood, but slightly overlapped with that of the young children's blood. Additionally, the LIR of gasoline is rather close to that of soil. Moreover, the LIR of surma had no similarity to pregnant women, cord blood, or the children's blood lead level.

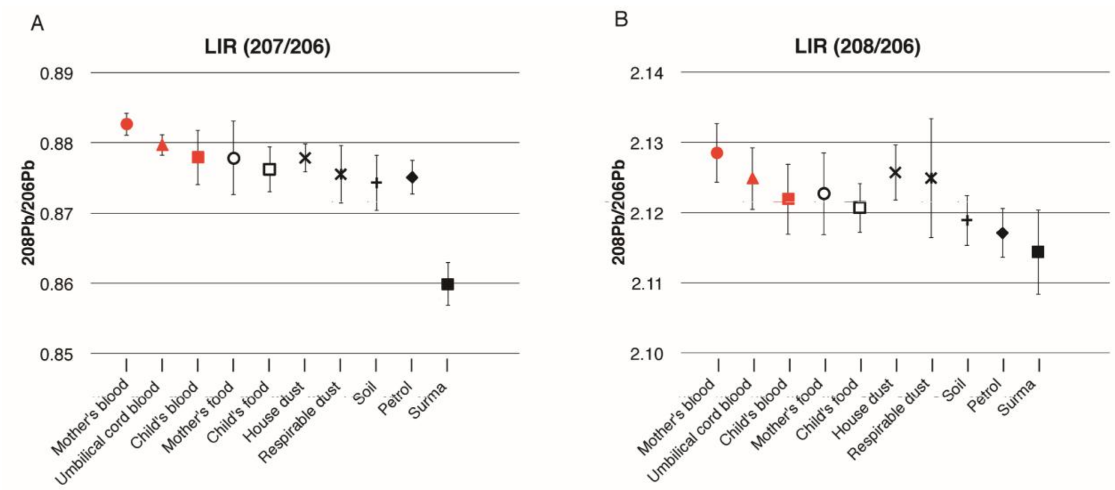


Figure 1.3: Lead isotopes ratios (LIR) for eight families (combined) in Karachi: (A) LIR 207/206; (B) LIR 208/207. Legends: Mother's blood (●), Umbilical cord blood (▲), Child blood (■), House dust (X), Respirable dust (✳), Soil (+), Mother food (o), Child food (□), Gasoline (◆), Surma (■).

5. Discussion

Recent studies in Pakistan have shown high blood lead levels in the vulnerable population, including newborns and children (Janjua et al. 2008; Kadir et al. 2008; Kazi et al. 2014). The current study empirically ascertained the main sources of Pb exposure and the proportion contribution for blood from potential sources using IVBA extraction among pregnant women/newborns and the one- to three-year-old children in the megacity of Karachi, Pakistan. In this regard, few studies are available regarding the source apportionment of lead exposure using food duplicate studies and other potential sources from developing countries (Yu et al. 2016).

Besides validating the findings of high lead exposure among this population, the study identified that food, house-dust, and respirable dust were the main sources contributing to the lead level in the blood of pregnant women, and food and house-dust contributed the most to the lead level seen among young children. The contribution of dust to the blood lead level is most critical for children aged one to three years, typically with the highest lead levels and greater hand-to-mouth activity (Clark et al. 1985). For the individuals older than four years, hand-to-mouth activity is minimal and diet assumes a greater importance as a source of lead (Bolger et al. 1996).

Previous available studies in Pakistan had limitations, as these were purely epidemiological in nature and had identified behavioral and subjective factors (Janjua et al. 2008; Kadir et al. 2008). Some of the previous investigations had also misled the researchers and policy makers. For example, water has been implicated as a major source of exposure for taking countermeasures against lead (Ul-Haq et al. 2011). This study clearly identified that water was not major source of exposure among the pregnant women and children. Similarly, surma (eye cosmetic) was not a major contributor to the body burden of lead. A previous study has implicated surma as a major source of lead exposure in the same population (Janjua et al. 2008).

Studies used to identify lead sources are so far based on behavioural studies and the determination of the lead levels in samples gasoline, paint, dust, soil, and water, separately. These measurements have been made without objectively linking them to determine the proportion contribution of these sources for blood lead levels. A study in several geographically different locations in Karachi suggested that high blood lead levels were related to vicinity to the main street and intersection, surma/kohl use (eye cosmetic), father's occupational lead exposure, a parent's illiteracy, and a child's habit of hand-to-mouth activity (Rahbar et al. 2002). Another study conducted in Karachi city found that umbilical cord blood levels were higher among mothers living in houses with windows open, those using surma daily, and in households where the mothers took no calcium or less iron supplements during pregnancy (Janjua et al. 2008). In one study, water has also been found as a major source of lead in Karachi (Ul-Haq et al. 2011). All of these studies point to one or the other source of lead exposure. However, the information from these studies does not provide the exposure contribution from sources for pregnant women, newborns, and small children.

This study is the first food duplicate study in Pakistan and provides information about the oral intake of lead in food. Few studies have conducted the measurement of lead exposure through food. Since the implementation of unleaded gasoline in developed and many developing countries, food may be considered as a major source of secondary exposure. However, due to the unavailability of reliable methods and laboratories, it has not been studied, particularly in developing countries. The proportion of bioaccessible lead from multiple sources and source apportionment using LIR were estimated for pregnant women and young children in Karachi, to identify the important contributors of lead in these vulnerable populations. Food, house-dust and respirable dust were identified as major sources of exposure among pregnant women. Besides food, house dust was identified to contribute to blood lead levels among young children in Pakistan. This investigation informs that regular wet-mopping in the households could be an important intervention for the prevention of exposure to lead. Also, further investigations are needed to identify the contamination sources of food and major foods contributing to lead exposure in this population.

We used isotopic analysis by ICP-QMS validated by a ICP-MS multi-collector. Our analysis of ILR was not precise enough to determine the percentage contribution of lead from individual sources. However, LIR of pregnant women's blood and cord blood were closely related in most families and the child's blood was more closely related with current environmental sources of exposure such as food, house-dust, and respirable dust. The LIR of gasoline (largely) and surma (particularly) was distinct from the blood LIR of pregnant women, newborns, and young children in most families, indicating that these are not the primary major sources of exposure.

Pregnant women's LIR was relatively higher and distinct from all other current sources of lead exposure, suggesting past exposure and the mobilization of lead deposited in bone tissue. Alternatively, pregnant women's exposure might relate to some other environmental sources which have not been studied in this investigation. However, a strong relation of a newborn's and child's blood LIR with current sources clearly indicate that the lead level of pregnant women could only be due to past exposure. The lead deposits in the bone tissues remain in constant exchange with blood and that might be a larger contributor to pregnant women's blood lead levels, particularly during pregnancy and breast feeding (Gulson et al. 2003; Manton et al. 2003).

The uptake of lead per body weight, as determined by IVBA, by young children, was almost three times higher compared to pregnant women (Figures 1.1B and 1.2B). This is an alarming level of exposure for the vulnerable population. It means that the exposure of young children after they were born tended to increase and would have severe detrimental effects on their developing brains. A marked improvement in the overall environment of the children is required in Pakistan and developing countries, to reduce lead exposure. Household cleaning practices and behavioral interventions are needed to decrease the lead exposure among young children in the households in Pakistan. Wet-mopping of household could be a key intervention to reduce lead exposure.

We further investigated the main source of exposure, i.e., food, for possible sources of contamination. First, we investigated lead contamination through cooking with several cooking utensils in a laboratory in Aga Khan University. The increment of lead after cooking was eligible and the lead concentrations in the food ingredients examined were generally low (Table 1.5). It suggests that food samples might be contaminated with lead from house dust during cooking in the kitchen, and during the sampling and processing process. Manton et al. (2005) revealed that in the LIR study in Omaha and Nebraska during the period of 1990 to 1997, most of the dietary collection contained a large component of house dust.

Therefore, we suggest that, first, a more systematic surveillance for lead contamination in food and the environment is required in Pakistan. Second, we must delineate possible contamination sources during agricultural and animal farming practices and the processing of various food items in Pakistan.

The currently available gasoline contains lead levels much lower than the recommended guidelines (<20 mg/L). It is evident that current automobile exhaust gas is not a major lead contamination source. We could not obtain gasoline or alkyl lead used in the past. However, we speculate that similar alkyl lead was added to gasoline in the past, which produced ubiquitous lead contamination that is sustained in the environment. This is supported by studies conducted in western countries which show that emitted lead remains a source of exposure for a longer duration, maybe decades (Manton et al. 2005). The lead content in gasoline has gradually decreased in Pakistan from 1.5–2.0 g/L in 1991, to 0.4 g/L in 1993–1996, and then to 0.36 g/L in 1999. Lead has been controlled in gasoline sources since 2001 to less than 0.02 g/L (Parekh et al 2002; ATSDR 2017). Nonetheless, there are some unanswered questions regarding whether food was contaminated with lead by the absorption from farmland soil or deposited from fallout dusts during transportation and cooking. This needs further investigation.

There were certain limitations in this analysis which need to be considered. As the samples were collected from one megacity, the study findings can only be applied to this city. However, Karachi is a megacity where approximately 10% of the population of Pakistan resides. Also, being the main harbor of the country, most of the gasoline and food are processed and transported up-country from Karachi, so we consider that a similar LIR would be prevalent in other parts of the country. Due to the high cost and time required to conduct the laboratory analysis, for several matrices of triad (pregnant women, newborns, and young children), as well as for four isotopes, we limited the analysis to eight families. However, the samples were selected from a larger study based on the blood lead levels among pregnant women. The samples chosen were from both high and low levels of lead exposure among the same population exposure range.

Nevertheless, the study methodology can be used for determining the sources of lead exposure in similar situations. To the best of our knowledge, it is among the first few studies of this nature which has comprehensively determined the source apportionment and utilized LIR analysis to compare patterns of Pb exposure in blood specimens, food duplicates, and environmental samples in a developing country. The information would provide management strategies for public health action.

The study capitalized on the strong collaboration between developing and developed country and we feel that this has been an important strength of this study. The methodology required several sophisticated advanced analyses, which are generally not available in a developing country like Pakistan. The limited capacity has been the major limitations for such studies to be replicated in developing countries.

6. Conclusions

High levels of blood lead were present among pregnant mothers and young children and may induce adverse developmental effects in the newborns and young children. Food, house-dust, and respirable dust among pregnant women and young children were the main contributor of blood Pb in this population. Surma, and to a large extent gasoline, are not major contributors of the blood lead levels of mothers and children in Pakistan. Behavioral interventions such as wet-mopping and clean cooking practices may help to control the lead exposure among this population. Therefore, a surveillance of lead contamination is urgently needed to devise countermeasures to reduce environmental lead contaminations.

References

Acosta-Saavedra, L.C.; Moreno, M.E.; Rodríguez-Kessler, T.; Luna, A.; Arias-Salvatierra, D.; Gómez, R.; Calderon-Aranda, E.S. Environmental exposure to lead and mercury in Mexican children: A real health problem. Toxicol. Mech. Methods 2011, 21, 656–666.

Agency for Toxic Substances and Disease Registry. Center for Disease Control. Department of Health and Human Services, Public Health Service, Division of Toxicology and Environmental Medicine. Public Health Statement. Lead (CAS#: 7439-92-1). Available online: http://www.webcitation.org/6pe3RUxnu (accessed on 11 April 2017).

Bolger, P.M.; Yess, N.J.; Gunderson, E.L.; Troxell, T.C.; Carrington, C.D. Identification and reduction of sources of dietary lead in the United States. Food Addit. Contam. 1996, 3, 53–60.

Clark, C.S.; Bornschein, R.L.; Succop, P.; Que Hee, S.S.; Hammond, P.B.; Peace, B. Condition and type of housing as an indicator of potential environmental lead exposure and pediatric blood lead levels. Environ. Res. 1985, 38, 46–53.

Gulson, B.L.; Mizon, K.J.; Korsch, M.J.; Palmer, J.M.; Donnelly, J.B. Mobilization of lead from human bone tissue during pregnancy and lactation—A summary of long-term research. Sci. Total Environ. 2003, 15, 79–104.

International Agency for Research on Cancer. Summaries & evaluations: Inorganic and organic lead compounds. Lyon, IARC Monographs for the Evaluation of Carcinogenic

Risks to Humans, Vol. 87. Available online: http://www.webcitation.org/6pe1SljIL (accessed on 22 March 2017).

International Program on Chemical Safety. Inorganic Lead. Geneva, World Health Organization, IPCS (Environmental Health Criteria 165). Available online: http://www.webcitation.org/6pe0iaN2p (accessed on 11 April 2017).

Islam, M.S.; Ahmed, M.K.; Habibullah-Al-Mamun, M.; Islam, K.N.; Ibrahim, M.; Masunaga, S. Arsenic and lead in foods: A potential threat to human health in Bangladesh. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 2014, 31, 1982–1992.

Janjua, N.Z.; Delzell, E.; Larson, R.R.; Meleth, S.; Kabagambe, E.K.; Kristensen, S.; Sathiakumar, N. Maternal nutritional status during pregnancy and surma use determine cord lead levels in Karachi, Pakistan. Environ. Res. 2008, 108, 69–79.

Kadir, M.M.; Janjua, N.Z.; Kristensen, S.; Fatmi, Z.; Sathiakumar, N. Status of children's blood lead levels in Pakistan: Implications for research and policy. Public Health 2008, 122, 708–715.

Kamenov, G.D.; Gulson, B.L. The Pb isotopic record of historical to modern human lead exposure. Sci. Total Environ. 2014, 490, 861–870.

Kazi, T.G.; Shah, F.; Shaikh, H.R.; Afridi, H.I.; Shah, A.; Arain, S.S. Exposure of lead to mothers and their new born infants, residents of industrial and domestic areas of Pakistan. Environ. Sci. Pollut. Res. Int. 2014, 21, 3021–3030.

Khan, D.A.; Qayyum, S.; Saleem, S.; Ansari, W.M.; Khan, F.A. Lead exposure and its adverse health effects among occupational worker's children. Toxicol. Ind. Health 2010, 26, 497–504.

Kordas, K.; Queirolo, E.I.; Ettinger, A.S.; Wright, R.O.; Stoltzfus, R.J. Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay. Sci. Total Environ. 2010, 408, 4488–4494.

Linderholm, L.; Jakobsson, K.; Lundh, T.; Zamir, R.; Shoeb, M.; Nahar, N.; Bergman, Å. Environmental exposure to POPs and heavy metals in urban children from Dhaka, Bangladesh. J. Environ. Monit. 2011, 13, 2728–2734.

Long, L. Lead isotopes. In Encyclopedia of Geochemistry; Marshall, C.P., Fairbridge, R.W., Eds.; Springer: New York, NY, USA, 1999; pp. 223–245.

Manser, W.W.; Khan, M.A.; Hasan, K.Z. Trace element studies on Karachi population. Part IV: Blood copper, zinc, magnesium and lead levels in psychiatric patients with depression, mental retardation and seizure disorders. J. Pak. Med. Assoc. 1989, 39, 269–274.

Manser, W.W.; Khan, M.A.; Hasan, Z. Trace element studies in Karachi populations. Part III: Blood copper, zinc, magnesium and lead levels in psychiatric patients with disturbed behaviour. J. Pak. Med. Assoc. 1989, 39, 235–238.

Manser, W.W.; Lalani, R.; Haider, S.; Khan, M.A. Trace element studies on Karachi populations. Part V: Blood lead levels in normal healthy adults and grammar school children. J. Pak. Med. Assoc. 1990, 40, 150–154.

Manton, W.I.; Angle, C.R.; Krogstrand, K.L. Origin of lead in the United States diet. Environ. Sci. Technol. 2005, 39, 8995–9000.

Manton, W.I.; Angle, C.R.; Stanek, K.L.; Kuntzelman, D.; Reese, Y.R.; Kuehnemann, T.J. Release of lead from bone in pregnancy and lactation. Environ. Res. 2003, 92, 139–151.

Obiri, S.; Yeboah, P.O.; Osae, S.; Adu-Kumi, S. Levels of arsenic, mercury, cadmium, copper, lead, zinc and manganese in serum and whole blood of resident adults from mining and non-mining communities in Ghana. Environ. Sci. Pollut. Res. Int. 2016, 23, 16589–16597.

Parekh, P.P.; Khwaja, H.A.; Khan, A.R.; Naqvi, R.R.; Malik, A.; Khan, K.; Hussain, G. Lead content of gasoline and diesel and its assessment in an urban environment. Environ. Monit. Assess. 2002, 74, 255–262.

Rahbar, M.H.; White, F.; Agboatwalla, M.; Hozhabri, S.; Luby, S. Factors associated with elevated blood lead concentrations in children in Karachi, Pakistan. Bull. World Health Organ. 2002, 80, 769–775.

Takagi, M.; Tamiya, S.; Yoshinaga, J.; Utagawa, H.; Tanaka, A.; Seyama, H.; Shibata, Y.; Uematsu, A.; Kaji, M. Source apportionment of lead in Japanese Children using isotope ratio. J. Environ. Chem. 2008, 18, 521–531. (In Japanese).

Ul-Haq, N.; Arain, M.A.; Badar, N.; Rasheed, M.; Haque, Z. Drinking water: A major source of lead exposure in Karachi, Pakistan. East Mediterr. Health J. 2011, 17, 882–886.

U.S. EPA. Exposure Factors Handbook 2011 Edition (Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011.

World Health Organization. Assessing the Environmental Burden of Disease at National and Local Levels. Available online: www.who.int/quantifying_ehimpacts/publications/en/leadebd2.pdf (accessed on 5 December 2016).

World Health Organization. Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. Geneva, Switzerland. Available online: www.who.int/healthinfo/global_burden_disease/ GlobalHealthRisks_report_full.pdf (accessed on 5 December 2016).

Yu, Y.; Li, Y.; Li, B.; Shen, Z.; Stenstrom, M.K. Metal enrichment and lead isotope analysis for source apportionment in the urban dust and rural surface soil. Environ. Pollut. 2016, 216, 764–772.

Study II - External lead contamination of women's nails by *surma* in Pakistan: Is the biomarker reliable?

Abstract

Introduction:

Adverse health effects of heavy metals are a public health concern and lead may cause negative health impacts to fetal and infantile development. The lead concentrations in Pakistani pregnant women's nails, used as a biomarker, were measured to estimate the lead exposure. Thirteen samples out of 84 nails analyzed contained lead higher than the concentration (13.6 mg/g) of the fatal level of lead poisoning, raising the possibility of an external contamination. Eye cosmetics such as *surma* are recognized as one of the important sources of lead exposure in Pakistan.

Methods:

We collected 30 eye cosmetics made in Pakistan, Saudi Arabia and Western countries. The metal composition analysis by energy dispersive X-ray fluorescence spectrometry revealed that some *surma* samples were made of more than 96% of lead. Therefore, we hypothesized that the *surma* might have contaminated the nail specimen.

Results:

Scanning electron microscopy observations showed that lead-containing *surma* consisted of fine particle of galena (ore of lead sulfide) of respirable dust range (less than 10 µm). In addition, relative in vitro bioavailability of lead in the *surma* was determined as 5.2%. Thus, lead-containing surma consists of inhalable and bioavailable particles, and it contributes an increased risk of lead exposure. Moreover, the relationship between the *surma* and the lead-contaminated nails by lead isotope ratios analysis indicated the potential of lead contamination in nails by *surma*.

Conclusions:

These results suggest that lead in the nails was derived both from body burden of lead and external contamination by lead-containing *surma*. Therefore, nail is not suited as a biomarker for lead exposure in the countries where *surma* is used, because we may overestimate lead exposure by surface lead contamination in the nail by *surma*.

1. Introduction:

The negative health effects of heavy metal elements such as lead are public health concerns. Joint FAO/WHO Expert Committee on Food Additives (JECFA) reported that exposure to lead has been shown to be associated with a wide range of effects, including various neurological and behavioral effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, delayed sexual maturation and impaired dental health (JECFA, 2011). And women with a blood lead level greater than 10 mg/dL during pregnancy were at increased risk of delivering preterm or small for gestational age infants. Moreover, prenatal and postnatal exposure to lead even at low concentration could impair neurodevelopment in children, e.g. impediments of cognitive development and intelligence (JECFA, 2011). Sources of lead exposure have been investigated in many environmental media. Some sources of lead exposure are specific to particular regions or cultures (JECFA, 2011). In Pakistan, the many different kinds of objects e.g. leaded gasoline, lead-based paints, lead water pipes, lead-acid batteries, lead food cans, traditional remedies and lead containing cosmetics, etc. were identified as the sources of lead exposure (Farooq et al., 2008; Kadir et al., 2008).

Surma (also known as Kohl and Kajal) is commonly used as cosmetics of eye makeup. It is widely used by women and children in South Asia, the Middle East and parts of Africa for the purpose of religious and traditional beautification and preventive medicine (Parry and Eaton, 1991; USFDA, 2006). In the United States, surma cannot be imported and is not permitted by regulation. An import alert about eye area cosmetics containing kohl, kajal, or surma was published by United States Food and Drug Administration (USFDA) for detention without physical examination of the product (USFDA, 2014). In contrast, manufacturing of surma is not regulated in Pakistan and estimated lead content varies greatly, from 16 to 70 percent (NIH, 2010). Some surma are also traditionally made at home (Hardy et al., 2008).

Many people may be unaware of the lead poisoning risk of surma. The study shows that most mothers who apply surma to their children (54%) did not have any formal education in Pakistan (Rahbar et al., 2002). Consequently, children exposed to surma have increased levels of lead in their blood (USFDA, 2006). Furthermore, researchers have found association between high lead levels in the umbilical cord and the use of surma by mothers in a study of prenatal lead exposure in Pakistan (NIH, 2010).

2. Objectives:

Investigations of the heavy metal contamination, such as lead and arsenic, of foods and living environments in Pakistan and Japan are ongoing in our laboratory. More recently, the multi-element analysis of keratinized matrices like hair or nail by ICP-MS is commonly used as a biomarker for heavy metal exposure (Goullé et al., 2009). It is considered a useful laboratory method for epidemiological studies, because of its non-invasive nature. In this study, we analyzed lead concentrations in the Pakistani pregnant women's nails to estimate the lead exposure. Unexpectedly some of the results showed high lead concentrations above the concentration in finger nails of the fatal lead poisoning case (Lech, 2006). With this high level, we anticipated a possibility of an external contamination of the nails by lead. We were focused on the traditional eye cosmetic "surma" and confirmed lead-containing surma consists of inhalable and bioavailable particles. Moreover, we were determined the relationship between lead-containing surma and lead-contaminated nails using the lead isotope ratios analysis to confirm a potential of lead contamination in the nails by surma. Therefore, this study was undertaken to estimate the risk of lead-containing surma and determine the reliability of nail as a biomarker for lead exposure.

3. Materials and methods:

A total of 84 nail samples (from both hands and feet) were collected from pregnant women in Gambat, Khairpur district, Pakistan. In addition, 30 eye cosmetics including surma, kohl, kajal and similar products were purchased at local markets and handmade ones were also collected in Karachi, Pakistan. All samples were kept in separate plastic bags in cool and dry environment away from sunlight and fumes before analysis.

2.1. ICP-MS analysis for nail

Nail samples were washed by 70% EtOH, acetone, 2% Triton X100 and water according to a protocol for element determinations in human nail clippings (Sanches and Saiki, 2011). After decontamination process, samples were digested with 1.45 ml of nitric acid Ultrapur-100 (Kanto Chemical Co., Inc., Tokyo, Japan) using microwave digestion system TOPwave (Analytik Jena Japan Co., Ltd, Kanagawa, Japan) according to the instruction manual. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was performed to determine the lead concentrations in nail samples. ICP-MS analysis was conducted by The National Institute for Environmental Studies (NIES) using Agilent 7500cx (Agilent Technologies Japan, Ltd, Tokyo, Japan). The measurement for lead was carried out by calibration curve method using a lead standard solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and a thallium standard solution (Wako Pure Chemical Industries, Ltd.) for an internal standard. Linearity measured as the correlation coefficient was 1.000 for lead. Low limit of detection of lead was 0.001 ng/g (ppb). Quality control test was performed using NIES Certificated Reference Material No.13, Human Hair, instead of nail. The recovery of lead for the analysis method was 94.2%.

2.2. Observation of surma by scanning electron microscopy

A portion of surma was fixed on a holder with carbon adhesive tape for (Nisshin EM Corporation, Tokyo, Japan). Observations of the morphologies of surma were made with JSM-6510LA (JEOL Ltd., Tokyo, Japan).

2.3. In vitro bioaccessibility assay for lead in surma

Determination of lead bioaccessibility in surma was carried out using the standard operating procedure (SOP) for an in vitro bioaccessibility (IVBA) assay for lead in soil (USEPA, 2012). This method is a United States Environmental Protection Agency (USEPA) validated in vitro assay for estimating relative bioavailability (RBA) in environmental media (soil, dust, water, food, air, paint, etc.).

The extraction fluid was used 0.4 M glycine (free base, reagent grade glycine in deionized water), adjusted to a pH of 1.50 ± 0.05 using trace metal grade concentrated hydrochloric acid. Samples (200 mg) were mixed with the extraction fluid to a solid-to-fluid ratio of 1/100 (mass per unit volume) in a 25 ml lead-free tube.

Samples were extracted at 37º C, 30 rpm in Bio-shaker BR-40LF (TAITEC Corporation, Saitama, Japan) for 1 h ensuring the pH was maintained at 1.5 ± 0.5. The extracts were filtered with a 0.45 mm cellulose acetate disk filter (33 mm diameter) and stored the filtered samples at 4 _C. The samples were analyzed by ICP-MS Agilent 7500cx in NIES. IVBA and RBA were calculated using the equations on the standard operating procedure manual.

2.4. Lead isotope ratios analysis

Nail samples that had high lead contamination (n=13) were selected (>13.6 mg/g). A total of four (n=4) surma samples were used for the extraction of lead bioaccessibility for comparison of isotopes. Measurement of the lead isotope ratios 207Pb/206Pb and 208Pb/206Pb was performed using ICP-MS Agilent 7500cx in NIES, details can be found in Takagi et al. (Takagi et al., 2008). National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 981, Common Lead Isotopic Standard, was used to correct for mass discrimination. Quality control tests were performed using NIST SRM 2583, Trace Elements in Indoor Dust.

4. Results and Discussion:

Lead concentrations in nail samples of Pakistani pregnant women ranged from 0.002 to 405 mg/g, geometric mean 0.309 mg/g, arithmetic mean (sd) 11.7 (±45.6) mg/g, median value 1.77 mg/g. The level of lead in 15% (13 of 84) samples was above the concentration in finger nails (13.6 mg/g) of the fatal lead poisoning case, and 43% of samples (36 of 84) had levels below the lower limit of detection of ICP-MS (Table 2.1).

Table 2.1: Summary of lead concentrations in Pakistani pregnant women's nail samples (n=84).

		Lead concentration (µg/g)
Geometric mean		0.309
Arithmetic mean		11.7±45.6
Percentile	5	0.002
	25	0.004
	50 (median)	1.77
	75	8.01
	85	13.5
	95	32.8
Minimum		0.02
Maximum		405
Samples >fatal case*		15.5% (n=13)
Sample <LLD**		43% (n=36)

*Fatal case=13.6 µg/g in finger nails of the fatal lead poisoning case (Lech, 2006).

**LLD=lower limit of detection.

Reference ranges of lead in finger nail samples from 130 healthy volunteers were reported by Goullé et al. the values of median, 5th and 95th percentiles were 0.52, 0.10 and 3.71 mg/g, respectively (Goull_e et al., 2009). Median lead concentration in nails of Pakistani pregnant women was at least 3 times higher compared to the reference value of healthy volunteers. Furthermore, 95th percentile lead concentration was 32.8 mg/g in four sample, which was about 9 times higher than the reference value. Moreover, the fatal lead concentration reported by Lech et al., in finger nailswas 13.6 mg/g (Lech, 2006). It was an equivalent value of 85th percentile lead concentration 13.5 mg/g in our sample. All the Pakistani pregnant women were apparently healthy and had no known health problem. These results suggest that the nails may had external contamination of lead. The latest multi-element analysis of nail by ICP-MS is commonly used as a biomarker for heavy metal exposure (Goullé et al., 2009). But, we suspected that women's nails in Pakistan is not suited as a biomarker.

In Pakistan, eye cosmetics such as surma, kohl and kajal is widely used by women and children. USFDA reported that eye cosmetics such as surma, kohl, or kajal are one of the important sources of lead exposure. In this respect, some firms and their products in Pakistan were warned by USFDA in 2014 (USFDA, 2014). In addition, these cosmetics have a chance of direct physical contact with women's nails, especially while use. Therefore, we collected a total of 30 eye cosmetics samples from Karachi, Pakistan and Saudi Arabia. The eyes cosmetics (specially surma) are widely used in Middle East, and ones brought from Saudia Arabia have religious values.

The eye cosmetics collected were analyzed their metallic composition by energy dispersive X-ray fluorescence spectrometry (EDXRF) and revealed four surma products contained more than 96% lead (A: 98.7%, B: 97.6%, C: 97.1%, D: 96.8%), shown in Table 2.2 (unpublished data, Naeem et al.).

Table 2.2: Surma products containing lead in this study.

Product	Country (made in)	Lead content (%)	IVBA[a] (%)	RBA[b] (%)
A	Pakistan	98.7	9.1	5.2
B	Saudi Arabia	97.6	10.5	6.4
C	Saudi Arabia	97.1	7.2	3.6
D	Pakistan	96.8	9.8	5.8
Mean			9.2	5.2

[a]IVBA = in vitro bioaccessibility.

[b]RBA = Relative bioavailability.

The elemental composition of local surma sample in Tunisia was analyzed by EDXRF and the lead composition was 94.09% (Nouioui et al., 2016). It was almost same value as surma products in this study. Surma alias kohl containing lead was traditionally made by grinding an ore of galena (Hardy et al., 2008). Galena is the natural mineral form of lead sulfide (PbS). EDXRF cannot analyze element with atomic mass less than sodium. Therefore, it was possible that surma might contain other chemical components of light elements such as sulfur (S) and/or oxygen (O) for the constitution of the ore.

Crystal habit of galena is typically cube, octahedron and combinations of the two. From the observations of surma containing lead, these were observed as the characteristic structure of galena (Fig. 4).

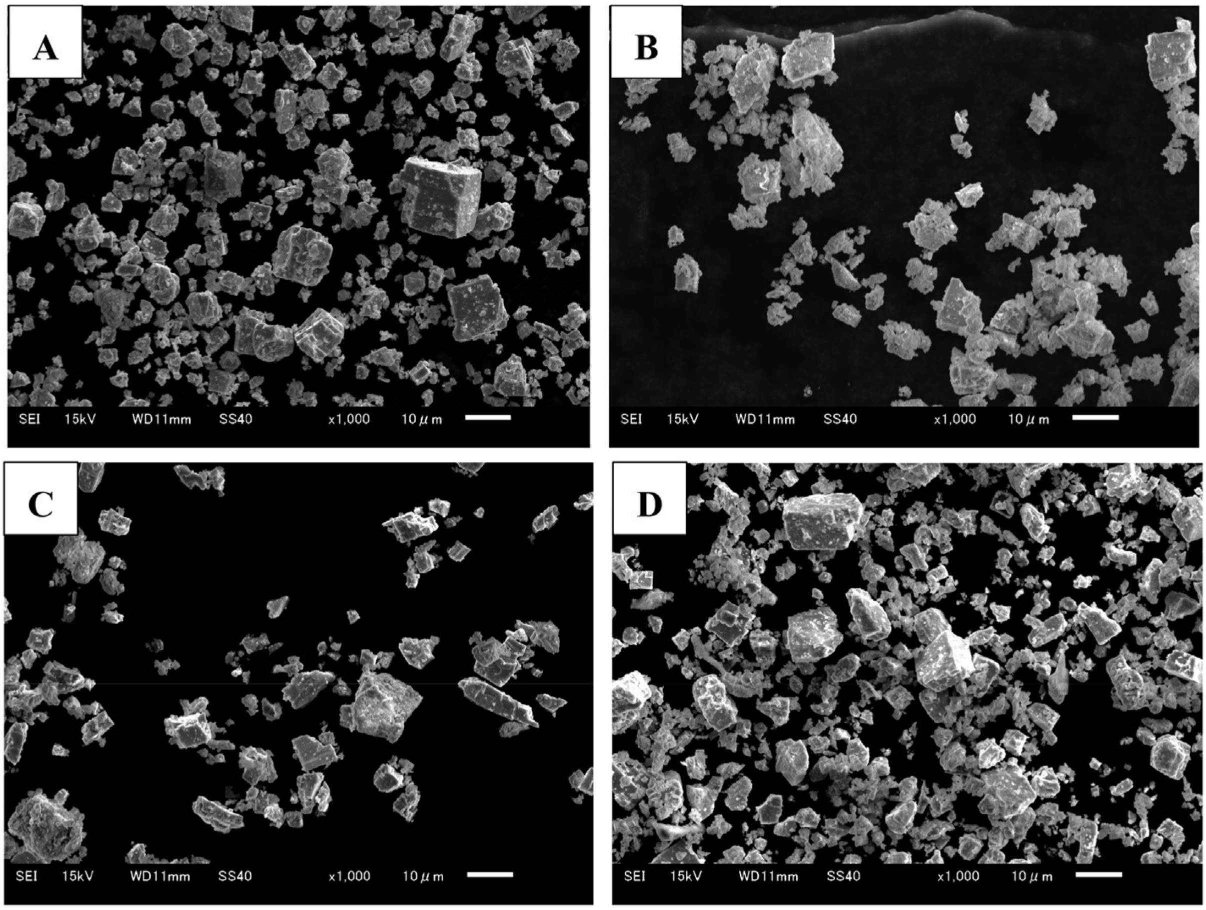


Fig. 2.1: Observations of surma products. Particles of surma (product A, B, C and D in Table 2.2) were observed at magnification (x1000). The size of bar at the bottom of pictures is 10 μm.

In addition, these were ground into a powder, it mainly consists of less than 10 mm particles. Moreover, a portion of the surma was also nanometer-size particles, it may tightly adhere to the nail surface and can easily penetrate into the nail cracks. External lead contamination in Pakistani women's nails may be derived from the nanometer-size particles of lead-containing surma.

These results also suggested that lead-containing surma in this study are inhalable particles and hazardous. Most of the surma were classified as PM10, particles with a diameter of 10 mm or less, also known as respirable suspended particles. Therefore, the particles of surma may deposit in the respiratory system. When women and children use lead-containing surma, they get exposed to the lead not only via eyes and mouth but also via the respiratory system. USEPA reported that absorption of lead deposited in the respiratory tract is influenced by particle size and solubility, as well as by the pattern of regional deposition within the respiratory tract (USEPA, 2006). Lead-containing surma in this study was mainly larger particles (>2.5 mm) that are primarily deposited in the airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed (USEPA, 2006).

The lead bioaccessibility of lead-containing surma was determined by USEPA validated the in vitro bioaccessibility assay for estimating RBA. The values of IVBA and RBA were calculated that lead-containing surma made from pure galena (lead content rate 86.6%). The values of IVBA and RBA of lead-containing surma products were shown in Table 2.2, and the mean values of IVBA and RBA were 9.2% and 5.2%. This result reflects that if pregnant woman (assuming a body weight of 60 kg) take only 29 mg lead-containing surma (RBA ¼ 5.2%) in a week, it will exceed the former Provisional Tolerable Weekly Intake (PTWI) of lead 25 mg/kg body weight/week. Besides, JECFA has withdrawn the PTWI levels in 2010 and considering setting up a lower limit for health protection JECFA, 2011). Thus, lead-containing surma consists of inhalable and bioavailable particles are an important source of lead exposure for women and children in Pakistan.

To confirm the relationship between lead-containing surma used by the locals and high lead level nails of Pakistani pregnant women, lead isotope ratios (LIR: 207Pb/206Pb versus 208Pb/206Pb) analysis was carried out. The distribution of LIR of four lead containing surma products, made in Pakistan or Saudi Arabia, and 13 high lead level nail samples was shown in Fig. 5.

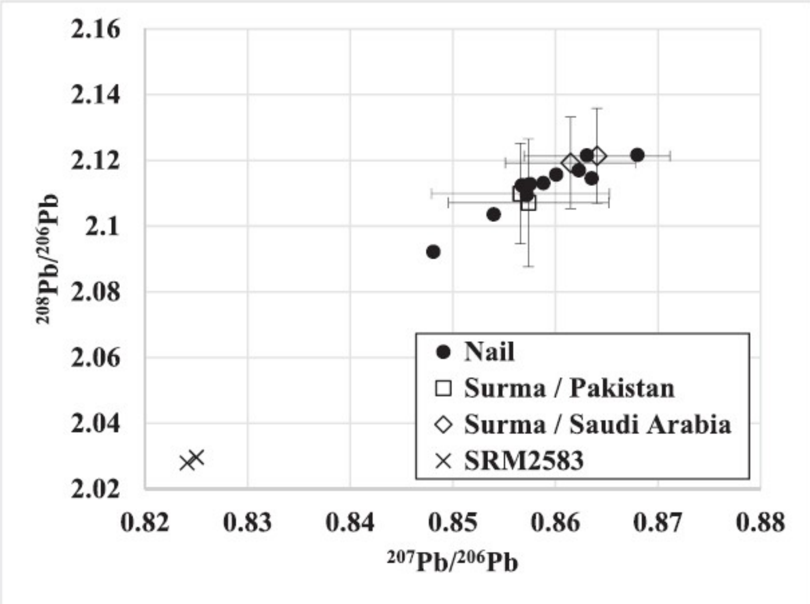


Figure 2.2: Lead isotope ratios of Pakistani pregnant women's nails and surma made in Pakistan or Saudi Arabia. SRM2583 for quality control tests of the analysis. Error bars represent typical error (2SD).

The LIR of extracted lead in SRM2583, indoor dust, agreed with the value measured by multicollector ICP-MS (unpublished data, 0.8241 ± 0.0000 for 207Pb/206Pb and 2.0279 ± 0.0001 for 208Pb/206Pb). All 13 LIR spots of the nails were distributed within 2 standard deviations (2SD) of LIR of four lead-containing surma products. These results indicate that lead-containing surma was involved with high lead level nails of Pakistani pregnant women. However, 85th percentile concentration of lead in the nails (13.5 mg/g) was same as the concentration of lead in finger nails of fatal lead poisoning (13.6 mg/g). Three samples had lead level in nails (82, 88, 405 mg/g), which were more than 6 times higher than fatal lead poisoning. This suggests that the surface of high lead level nails in Pakistani women were probably contaminated by lead-containing surma.

This study revealed that we may overestimate lead body burden from the concentration of lead in Pakistani pregnant women's nail, due to combination of an external contamination by the surma and the real internal body burden. Therefore, without further developments and refinement of the analytical method, nail is not suited as a biomarker for the lead exposure in the countries where surma and similar products used. Although the blood lead level during pregnancy changes by pregnancy related physiological changes. For instance, blood lead level decreases during the first half of pregnancy and increase during the second half of it (JECFA, 2011). Nevertheless, the measurement of blood lead level is still more reliable method of analysis to estimate the lead exposure in the countries where surma used. Furthermore, analysis of the lead contamination in cosmetics such as surma is also important to identify the source of lead exposure.

5. Conclusions:

The lead concentrations in Pakistani pregnant women's nails indicate unexpectedly high lead concentrations above the concentration in finger nails of the fatal lead poisoning case. It was supposed the possibility of an external contamination of the nails by lead. We focused on the traditional eye cosmetic "surma" and confirmed lead-containing surma consists of inhalable and bioavailable particles. Moreover, the relationship between the lead containing surma and the lead contaminated nails in the Pakistani pregnant women by lead isotope ratios analysis indicated the potential of lead contamination in the nails by surma. These results suggest that lead in the nails were derived from exposure to lead and external contamination by lead-containing surma. Therefore, nail is not suited as a biomarker for lead exposure in the countries where surma used, as it may lead to overestimate the lead exposure.

References

Farooq, Y., Hussain, M.M., Aleem, S.B., Farooq, M.A., 2008. Lead intoxication: the extent of problem and its management. Pak. J. Physiol. 4, 36-41.

Goullé, J.P., Saussereau, E., Mahieu, L., Bouige, D., Groenwont, S., Guerbet, M., Lacroix, C., 2009. Application of inductively coupled plasma mass spectrometry multielement analysis in fingernail and toenail as a biomarker of metal exposure. J. Anal. Toxicol. 33, 92-98.

Hardy, A.D., Farrant, A.J., Rollinson, G., Barss, P., Vaishnav, R., 2008. A study of the chemical composition of traditional eye cosmetics ("kohls") used in Qatar and Yemen. J. Cosmet. Sci. 59, 99-418.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2011. Safety evaluation of certain food additives and contaminants. WHO Food Addit. Ser. 64, 381-497.

Kadir, M.M., Janjua, N.Z., Kristensen, S., Fatmi, Z., Sathiakumar, N., 2008. Status of children's blood lead levels in Pakistan: implications for research and policy. Public Health 122, 708-715. http://dx.doi.org/10.1016/j.puhe.2007.08.012.

Lech, T., 2006. Exhumation examination to confirm suspicion of fatal lead poisoning. Forensic Sci. Int. 158, 219-223.

NIH (National Institutes of Health), 2010. Traditional surma eye makeup poses lead risk. Glob. Health Matters 9, 7.

Nouioui, M.A., Mahjoubi, S., Ghorbel, A., Yahia, M.B.H., Amira, D., Ghorbel, H., Hedhili, A., 2016. Health risk assessment of heavy metals in traditional cosmetics sold in Tunisian local markets. Int. Sch. Res. Notices 2016, 1e12. http://dx.doi.org/10.1155/2016/6296458.

Parry, C., Eaton, J., 1991. Kohl: a lead-hazardous eye makeup from the third world to the first world. Environ. Health Perspect. 94, 121-123.

Rahbar, M.H., White, F., Agboatwalla, M., Hozhbari, S., Luby, S., 2002. Factors associated with elevated blood lead concentrations in children in Karachi, Pakistan. Bull. World Health Organ. 2002 80 (10), 769-775.

Sanches, T.P., Saiki, M., 2011. Establishing a protocol for element determinations in human nail clippings by neutron activation analysis. In: 2011 International Nuclear Atlantic Conference (INAC 2011), ISBN 978-85-99141-04-5.

Takagi, M., Tamiya, S., Yoshinaga, J., Utagawa, H., Tanaka, A., Seyama, H., Shibata, Y., Uematsu, A., Kaji, M., 2008. Source apportionment of lead in Japanese Children using isotope ratio. J. Environ. Chem. 18, 521e531 in Japanese.

USEPA (U.S. Environmental Protection Agency), 2006. Air Quality Criteria for Lead. EPA/600/R-05/144aF.

USEPA (U.S. Environmental Protection Agency), 2012. Standard Operating Procedure for an In vitro Bioaccessibility Assay for Lead in Soil. EPA 9200.2-86.

USFDA (U.S. Food and Drug Administration), 2006. Kohl, Kajal, Al-Kahal, or Surma: by Any Name, a Source of Lead Poisoning. Available at:

http://www.fda.gov/Cosmetics/ProductandIngredientSafety/ProductInformation/ucm137250.htm
(Updated October 16, 2006).

USFDA (U.S. Food and Drug Administration), 2014. Detention without Physical Examination of Eye Area Cosmetics Containing Kohl, Kajal, or Surma. Import Alert # 53-15. Available at: http://www.accessdata.fda.gov/cms_ia/importalert_134. html (Published Date: 02/07/2014).

Study III - Burden of skin lesions of arsenicosis at higher exposure through groundwater of taluka Gambat district Khairpur, Pakistan: a cross-sectional survey

Abstract

Background:

Prior surveys conducted have found higher proportion of arsenic-contaminated wells in villages along river Indus in Pakistan. This study aimed to determine the prevalence of arsenicosis skin lesions among population exposed to higher exposure in taluka Gambat district Khairpur in Sindh, Pakistan.

Methods:

The cross-sectional survey was conducted from August 2008 to January 2009 among 610 households. A total of 707 water sources (hand pumps/wells) were tested from the villages of Agra and Jado Wahan for arsenic levels with Quick rapid arsenic field test kits. A total of 110 households exposed to arsenic levels >50 ppb were identified. Case screening for arsenic skin lesions was performed for 610 individuals residing in these 110 high-risk households. Information regarding household and socio-demographic characteristics, height and weight measurements and arsenic exposure assessment were collected. Physical examinations by trained physicians were carried out to diagnose the arsenic skin lesions. After data cleaning, 534 individuals from all age groups were included in the final analysis which had complete exposure and outcome information.

Results:

Overall prevalence of arsenicosis skin lesions was 13.5% (72 cases). Of the 534 individuals, 490 (91.8 %) were exposed to arsenic levels of ≥100 ppb in drinking water (8.2% to >50–99 ppb, 58.6% to 100–299 ppb, 14.6% to 300–399 ppb and 18% to ≥400 ppb). Prevalence rate

(per 100 population) of arsenicosis was highest at arsenic levels of 100–199 ppb (15.2 cases) followed by ≥400 ppb (13.5 cases) and 300–399 (12.8 cases). Prevalence rate was higher among females (15.2) compared to males (11.3).

Discussions:

Our study reports arsenicosis burden due to exposure to higher arsenic levels in drinking water in Pakistan. Exposure to very high levels of arsenic in drinking water calls for urgent action along river Indus. Prevalence of skin lesions increases with increasing arsenic levels in drinking groundwater. Provision of arsenic-free drinking water is essential to avoid current and future burden of arsenicosis in Pakistan.

1. Introduction:

High levels of arsenic in drinking groundwater have been detected in both developed and developing countries (Mazumder et al. 2010). Inorganic arsenic, predominantly found in groundwater, is mainly responsible for adverse health effects (Arsenic Contamination in Ground Water 2006). Acceptable limit for arsenic in drinking water is ≤10 ppb as recommended by WHO (Water Sanitation and Health 2001). However, due to limited capacity to improve the environmental conditions, some worst arsenic affected developing countries including India (Chowdhury et al. 2000), Pakistan (Mazumder et al. 2010) and Bangladesh (Smith 2000) have set this limit to ≤50 ppb (Water Sanitation and Health 2001).

Skin is the first site for manifestation of arsenic toxicity (Yoshida et al. 2004). There is risk of skin lesions at arsenic levels >10 ppb in drinking water (Ahsan et al. 2006). Arsenic skin lesions have strong tendency to convert into cancerous lesions at arsenic levels of >50 ppb (Morales et al. 2000; Chen and Ahsan 2004). Increase dose of arsenic levels in groundwater leads to increase prevalence of arsenicosis (Mazumder et al. 1998; Tondel et al. 1999). Arsenic exposure in drinking water also leads to increase in cardiovascular diseases such as hypertension, diabetes mellitus, decrease in lung function and impaired cognitive function (Agency for Toxic Substances and Disease Registry 2007; Rosado et al. 2007; Rahman et al. 1999a, b).

In Pakistan, national arsenic screening of groundwater samples was done in 2004 from randomly selected districts. Approximately 9% had arsenic levels >10 ppb (0.7 % had >50 ppb) (Ahmad et al. 2004). Selected districts in Pakistan ascertained arsenic skin lesions among arsenic exposed individuals through groundwater, and greater arsenic exposure was observed among those living closer to Indus River (Fatmi et al. 2004). However, observed low prevalence was not able to convince policy makers to take mitigation measures against arsenic.

People living closer to Indus River are consuming higher concentration of arsenic-contaminated drinking water and therefore are at increased risk of arsenic toxicity. This study was conducted in order to estimate the prevalence of arsenic skin lesions among selected villages exposed to high levels of arsenic in drinking water in two union councils of taluka Gambat, district Khairpur, Sindh Pakistan.

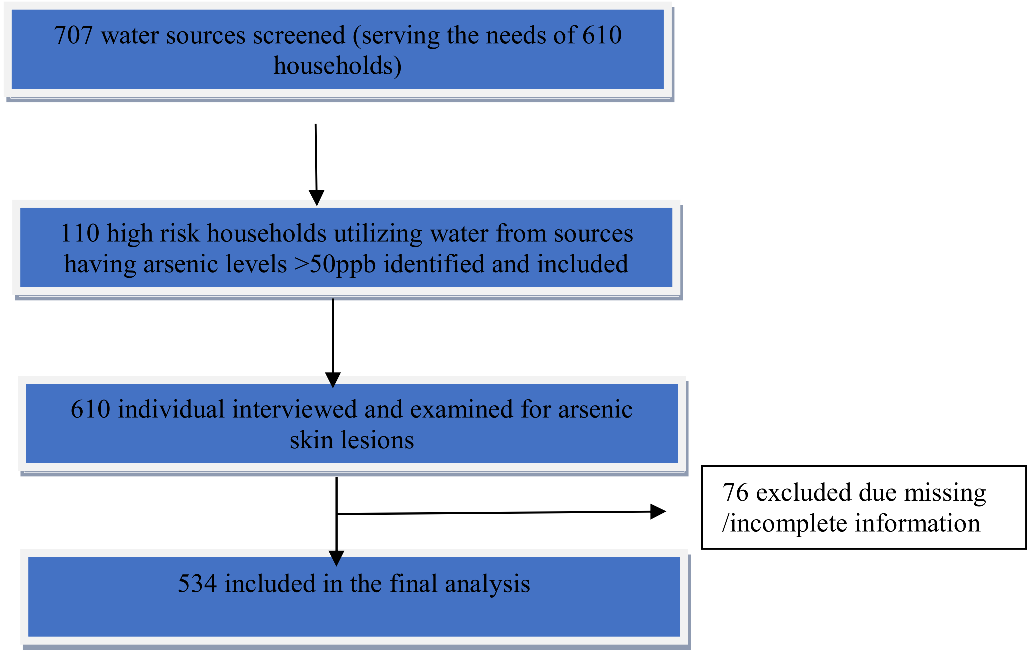
2. Methods and materials:

Gambat is taluka (subdistrict) of district Khairpur, Sindh province. The Indus River flows through Gambat taluka. Population of Gambat is approximately 0.25 million (Fatmi et al. 2004). Gambat taluka is administratively divided into nine units (union councils). The majority of people are Sindhi speaking and their main occupation is agriculture. This cross-sectional study was conducted between August 2008 to January 2009 in highly arsenic-contaminated villages of two union councils (Agra and Jado Wahan) of Gambat.

Water sampling and selection of participants

Figure 1 shows the selection procedure of the study participants. A consecutive sample of 610 households was identified in the two union councils. These households were using 707 water sources for drinking purposes. Of them 98.5 % were using groundwater for drinking. The majority of water sources (97 %) were privately owned, whereas 3 % were public water sources.

Figure 3.1: Sample selection



Duration of drinking (in years) from the source was inquired based on recall. After collection, samples were marked with an identification number. Screening was performed using Quick rapid arsenic field test kits (Arsenic Quick Test Kits 2011). The tests were performed in the field where the samples were collected to avoid misclassification. The test kits results were measured as categories: [50–99 ppb, 100–299 ppb, 300–399 ppb and >C400 ppb. Results of these tests were compared and were found consistent with those of a survey conducted by UNICEF (Ahmad et al. 2004).

Total of 110 households (271 water sources), consuming water from high-risk water sources (arsenic >50 ppb), were included in this study. Water sources having arsenic levels >50 ppb were painted in 'red' and those having arsenic levels ≤50 ppb were painted in 'green' color.

Case definition

A manual of diagnosis and mitigation was developed after consultations with dermatologists in a prior study conducted by the same authors. Study staff was trained regarding differential diagnosis of skin lesions using this manual. Details of the manual are given elsewhere (Fatmi et al. 2004). A case of arsenicosis was defined as having characteristic arsenic skin lesions, that is pigmentation change on the sun unexposed parts of the body (Melanosis) and/or thickening of the skin of palms and soles (Keratosis).

Case screening for arsenic skin lesions was performed for 610 individuals present in the house at the time of data collection. Information regarding household and socio-demographic characteristics, height and weight measurements and arsenic exposure was collected using a structured questionnaire. Physical examinations were carried out by trained physicians to diagnose the arsenic skin lesions. A total of 76 individuals were excluded due to incomplete and/or missing information, and final analysis was performed on 534 individuals (Fig. 1).

Quality control

Project team leaders made periodic visits to observe the process of data collection. Some of the high-risk water sources were also randomly checked for arsenic levels. All diagnosed cases were re-assessed and validated by examination of the digital images of skin lesions and random field visits to the houses.

Ethical considerations

Verbal and written informed consent was taken from the participants. Those found with arsenicosis lesions were given symptomatic treatment and were referred to taluka/district hospital for further evaluation. Health education brochures containing information about arsenic in local language (Sindhi) were distributed among the residents of the area. People were advised to avoid utilization of water from the contaminated sources (sources were painted red). The study was approved by ethics review committee of the Aga Khan University Karachi.

Statistical analysis

Data on variables of age, sex, education and body mass index (BMI) were collected as potential confounders. Data were entered into Epidata 3.1 and analyzed using SPSS 16 for windows. Descriptive statistics were calculated for socio-demographic variables and household characteristics.

Prevalence of arsenicosis cases was calculated within categories of age, gender, BMI and smoking status.

3. Results:

All recruited households (0 % refusal) participated in the survey. All individuals present (610) in the house at the time of survey were included in the study.

Table 3.1 shows the socio-demographic characteristics of the study population and distribution of arsenic levels in water sources. Mean age of study population was 23.4 years (SD ± 19.4). Predominant type of occupation was farmers (44.4 %) and laborers (36.7 %). Monthly household income was 5,000 Pakistani rupees (US $ 55.6). Mean number of individuals per household was 9.2 (±4.45). Proportion of undernourished, that is BMI B18.5, was 55.4 % and 15.1 % had history of smoking (Table 3.1). Mean duration of using current water source was 8 years.

Of the 534 individuals, 490 (91.8 %) were consuming water that had arsenic levels of ≥100 ppb. On further sub-categorization, 8.2% of the population were exposed to >50–99 ppb, 58.6% to 100–299 ppb, 14.6 % to 300–399 ppb and 18% were consuming water containing arsenic ≥400 ppb. Overall prevalence of arsenicosis was 13.5% (72 cases). Prevalence rate (per 100 population) of arsenicosis was highest at arsenic levels of 100–199 ppb (15.2 cases) followed by >400 ppb (13.5 cases) and 300–399 (12.8 cases).

Table 3.1: Showing socio-demographic characteristics of the study population, BMI, smoking status and exposure to arsenic levels (ppb) in drinking water in Agra and Jado Wahan (taluka Gambat district Khairpur) (534 subjects)

Characteristics	Percentage	(n)
Age (years)		
≤15	43.1	(230)
16-30	29.8	(159)
31-45	12.5	(67)
>45	14.6	(78)
Gender		
Male	44.6	(238)
Female	55.4	(296)
Education (n=315[a])		
No education	64.1	(201)
Primary and secondary	20.0	(62)
Matriculation and above	15.9	(52)
Occupational status (n=315[a])		
House wife	61.9	(195)
Working	28.9	(91)
Retired and unemployed	9.1	(29)
Body mass index (kg/m^2)		
≤14.67	23.8	(127)
14.68-17.68	24.0	(128)
17.69-20.71	24.0	(128)
>20.71	28.3	(151)
Ever smoker (n=304[a])		
Yes	15.1	(46)
No	84.9	(258)
Arsenic levels		
>50-99	8.2	(44)
100-299	58.6	(315)
300-399	14.6	(78)
≥400	18.0	(97)

Prevalence rate was higher among females (15.2) compared to males (11.3) (Table 3.2).

Table 3.2: Prevalence of arsenic skin lesions (arsenicosis) among study population of Agra and Jado Wahan (taluka Gambat, district Khairpur) exposed to high arsenic levels (n=534)

Variable	No. of cases	Prevalence rate per 100 (95% confidence interval)
Arsenic levels		
>50-99	2	4.5 (2.74-6.26)
100-299	47	14.8 (10.88-18.72)
300-399	10	11.7 (13.85-20.23)
≥400	13	12.8 (9.24-14.76)

Distribution of arsenicosis by age groups was: 11.7 % (95 % CI: 7.55–15.85) among ≤15 years, 16.9% (95% CI: 11.16–22.84) among 16–30 years, 13.4% (95% CI: 5.24–21.56) among 31–45 and 11.5% (95% CI: 4.42–18.58) among >45 years; by gender: 15% (95 %CI: 11.11–19.29) among females, 11.3% (95 % CI: 7.28–15.32) among males; by smoking status: 17.4% (95 % CI: 6.44–28.36) among smokers, 13.1 % (95 % CI: 10.11–16.09) among nonsmokers; by BMI: 11.5% (95 % CI: 8.49–15.71) among those with BMI ≤18.5 and 16.5% (95 % CI: 10.72–20.28) among those with BMI >18.5.

4. Discussion:

Our study reports arsenicosis prevalence at high arsenic exposure in Pakistan. Prior studies conducted locally have evaluated the respiratory effects of arsenic exposure (Arain et al. 2009) as well as risk of arsenic toxicity among adults and children by determining arsenic content in scalp hair at various levels of arsenic exposure (Baig et al. 2010; Kazi et al. 2010).

Prevalence reported in this study is higher compared to that reported in previous study (Fatmi et al. 2004). Similar studies from neighboring countries conducted in populations exposed to high arsenic have reported increased prevalence of arsenicosis (Mazumder et al. 1998; Tondel et al. 1999). High arsenic content in drinking water of the study areas is due to their proximity to river Indus. National survey also suggested that arsenic contamination is higher in the proximity of Indus plain (Ahmad et al. 2004).

Our study showed that females had higher prevalence of arsenicosis compared to males. Different studies have found varied results for role of gender in prevalence of arsenicosis with some reporting higher prevalence among males (Ahsan et al. 2006), whereas some have found higher prevalence among females (Ahmad et al. 1999). Due to increased sun exposure, males are considered to be more at risk for arsenic skin lesions, but these variations point out toward some unknown environmental and/or genetic factors that require further research. Our study found higher prevalence of arsenicosis among smokers. Similar findings have been reported by other studies (Lindberg et al. 2009). Studies have yielded mixed results regarding effect of smoking on arsenic methylation. In some studies, smoking appears to impair the methylation of bodily arsenic (Lindberg et al. 2009), whereas in other studies, smoking did not appear to have any such effect (Wu et al. 2001). Malnutrition is another modifiable risk factor for arsenicosis which lowers body immunity. Studies have reported important role of malnutrition in causing arsenic skin lesions among populations chronically exposed to arsenic in drinking water (Milton et al. 2004). People of older age have been found to be at higher risk of skin lesions possibly due to longer period of exposure (Rahman et al. 2006). We estimated the duration of drinking from current water source based on recall of the participants using local events, that is floods, droughts and other significant local events. We did not use the calendar years as they are not widely observed by rural population in the study area.

The identification of arsenic skin lesions was a two step process. First, a manual of diagnosis for arsenicosis was developed in consultation with dermatologists. Physicians were rigorously trained in diagnosis and differential diagnosis of skin problems. Development of manual of diagnosis is the main strength of our study. By using the manual, physicians were able to diagnose the arsenic lesions with accuracy while also maintaining uniformity of diagnosis. Therefore, chances of misdiagnosis were minimal. Secondly, experts reviewed the photographs of skin lesions. Lastly, random periodic field visits were also made by the experts in order to cross check the process of diagnosis.

Our study used rapid method for measuring arsenic levels in drinking water. However, arsenic categories identified by quick method are not mutually exclusive. This may have led to misclassification of exposure status. For that reason, we did not perform multivariate analysis because such misclassification can lead to spurious associations.

Limitations

Our study had some limitations. Arsenic levels of hair and nail which are a reliable indicator of chronic arsenic toxicity were not measured in this study. Nevertheless, two-stage process of identifying the arsenic skin lesions was rigorous enough for accurate diagnosis. Present study was conducted in the area with high level of arsenic. Populations of the nearby areas exposed to low levels were not included thus limiting the generalizability of the study.

5. **Conclusion:**

Estimated several millions people, living at same distance parallel to Indus River as this study population, are exposed to detrimental levels of arsenic in groundwater. Exposure of more than 90% of the population to arsenic levels of >100 ppb requires mitigation measures to be implemented with immediate effect. Raising awareness about health effects of consuming arsenic-contaminated water can be an important intervention to prevent current and future burden of arsenic toxicity.

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References

Agency for Toxic Substances and Disease Registry. (2007). Toxicological profile for Arsenic. Georgia.

Ahmad, T., Kahlown, M. A., Tahir, A., & Rashid, H. (2004 October 25–29). Arsenic an emerging issue: experience from Pakistan. People-centered approaches to water and environmental sanitation. In: Thirtieth WEDC international conference. Vientiane, Lao PDR, WEDC, pp. 459–466.

Ahmad, S., Sayed, M. H., Faruquee, M. H., Khan, M. H., Jalil, M. A., Ahmed, R., et al. (1999). Arsenicosis: Sex differentials. Journal of Preventive and Social Medicine, 18, 35–40.

Ahsan, H., Chen, Y., Parvez, F., Zablotska, L., Argos, M., Hussain, I., et al. (2006). Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: Baseline results from the Health Effects of Arsenic Longitudinal Study. American Journal of Epidemioloy, 163, 1138–1148.

Arain, M. B., Kazi, T. G., Baig, J. A., Jamali, M. K., Afridi, H. I., Jalbani, N., et al. (2009). Respiratory effects in people exposed to arsenic via the drinking water and tobacco smoking in southern part of Pakistan. The Science of the Total Environment, 407, 5524–5530.

Arsenic Contamination in Ground Water. (2006). http://www.pcrwr.gov.pk/Arsenic_CS/ACS_TOC.htm. Accessed 6 June 2012.

Arsenic Quick Test Kits. (2011). http://www.hometestkits.name/arsenic-quick-100-test-kit.html. Accessed 6 June 2012.

Baig, J. A., Kazi, T. G., Shah, A. Q., Afridi, H. I., Khan, S., Kolachi, N. F., et al. (2010). Evaluation of toxic risk assessment of arsenic in male subjects through drinking water in southern Sindh Pakistan. Biological Trace Element Research, 143, 772–786.

Chen, Y., & Ahsan, H. (2004). Cancer burden from arsenic in drinking water in Bangladesh. American Journal of Public Health, 94, 741–744.

Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., et al. (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. Environmental Health Perspectives, 108, 393–397.

Fatmi, Z., Azam, I., Ahmed, F., Kazi, A., Gill, A. B., Kadir, M. M., et al. (2004). Health burden of skin lesions at low arsenic exposure through groundwater in Pakistan. Is river the source? Environmental Research, 109, 575–581.

Kazi, T. G., Baig, J. A., Shah, A. Q., Arain, M. B., Jamali, M. K., Kandhro, G. A., et al. (2010). Determination of arsenic in scalp hair of children and its correlation with drinking water in exposed areas of Sindh Pakistan. Biological Trace Element Research, 143, 153–162.

Lindberg, A. L., Sohel, N., Rahman, M., Persson, L. A., & Vahter, M. (2009). Impact of smoking and chewing tobacco on arsenic-induced skin lesions. Environ Health Perspectives, 118, 533–538.

Mazumder, D. N., Ghosh, A., Majumdar, K. K., Ghosh, N., Saha, C., & Mazumder, R. N. (2010). Arsenic contamination of ground water and its health impact on population of district of Nadia, west Bengal, India. Indian Journal Community Medicine, 35, 331–338.

Mazumder, D. N., Haque, R., Ghosh, N., De, B. K., Santra, A., Chakraborty, D., et al. (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. International Journal of Epidemiology, 27, 871–877.

Milton, A. H., Hasan, Z., Shahidullah, S. M., Sharmin, S., Jakariya, M. D., Rahman, M., et al. (2004). Association between nutritional status and arsenicosis due to chronic arsenic exposure in Bangladesh. International Journal of Environmental Health Research, 14(2), 99–108.

Morales, K. H., Ryan, L., Kuo, T. L., Wu, M. M., & Chen, C. J. (2000). Risk of internal cancers from arsenic in drinking water. Environmental Health Perspectives, 108, 655–661.

Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H.,& Axelson, O. (1999a). Hypertension and arsenic exposure in Bangladesh. Hypertension, 33, 74–78.

Rahman, M., Tondel, M., Chowdhury, I. A., & Axelson, O. (1999b). Relations between exposure to arsenic, skin lesions and glucosuria. British Medical Journal, 56, 277–281.

Rahman, M., Vahter, M., Sohel, N., Yunus, M., Wahed, M. A., Streatfield, P. K., et al. (2006). Arsenic exposure and age and sex specific risk for skin lesions: A population based case referent study in Bangladesh. Environmental Health Perspectives, 114(12), 1847–1852.

Rosado, J. L., Ronquillo, D., Kordas, K., Rojas, O., Alatorre, J., Lopez, P., et al. (2007). Arsenic exposure and cognitive performance in Mexican school children. Environmental Health Perspectives, 115, 1371–1375.

Smith, A. H., Lingas, E. O., & Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: A public health emergency. Bulletin of the World Health Organization, 78, 1093–1103.

Tondel, M., Rahman, M., Magnuson, A., Chowdhury, I. A., Faruquee, M. H., & Ahmad, S. A. (1999). The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. Environmental Health Perspectives, 107, 727–729.

Water Sanitation and Health (WSH). (2001).
http://www.who.int/water_sanitation_health/diseases/arsenicosis/en/. Accessed 6 June 2012).

Wu,M.M., Chiou, H. Y.,Wang, T.W.,Hsueh,Y.M.,Wang, I.H., Chen, C. J., et al. (2001). Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. Environmental Health Perspectives, 109, 1011–1017.

Yoshida, T., Yamauchi, H., & Fan Sun, G. (2004). Chronic health effects in people exposed to arsenic via the drinking water: Dose-response relationships in review. Toxicology and Applied Pharmacology, 198, 243–252.

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Publications related to fulfilment of PhD at Jichi Medical University

Fatmi Z, Sahito A, Ikegami A, Mizuno A, Cui X, Mise N, Takagi M, Kobayashi Y, Kayama F. Lead exposure assessment among pregnant women, newborns, and children: case study from Karachi, Pakistan. *Int J Environ Res Public Health.* 2017 Apr 13;14(4). pii: E413.

Ikegami A, Takagi M, **Fatmi Z**, Kobayashi Y, Ohtsu M, Cui X, Mise N, Mizuno A, Sahito A, Khoso A, Kayama F. External lead contamination of women's nails by surma in Pakistan: Is the biomarker reliable? *Environ Pollut.* 2016 Nov; 218:723-727.

Fatmi Z, Abbasi IN, Ahmed M, Kazi A, Kayama F. Burden of skin lesions of arsenicosis at higher exposure through groundwater of taluka Gambat district Khairpur, Pakistan: a cross-sectional survey. Environ Geochem Health. 2013 Jun;35(3):341-6.

Curriculum Vitae

Name: Syed Zafar Ahmed Fatmi

Birth date: January 01, 1971

Nationality: Pakistani

E-mail address: zafar.fatmi@aku.edu

Summary: I am a public health academician and researcher with a fellowship in public health. I currently work as Professor and Head, Unit Environmental Occupational Health & Injuries at the Department of Community Health Sciences of Aga Khan University, Karachi, Pakistan. I was Director for Community Medicine Residency Program from 2007-2016 and served as coordinator since 2003. I am also supervisor in Community Medicine for College of Physicians & Surgeons, Pakistan since 2008. I have supervised thesis/dissertation work for more than 25 students of FCPS (Community Medicine) and MSc graduates (Epidemiology & Biostatistics and Health Policy & Management) at Aga Khan University. I direct research and academic activities of a group 10 public health faculty pertaining to environmental health and non-communicable diseases. I have more than 100 peer-reviewed publications and authored a chapter on a book by APHA (2011) on "Megacities and Global Health". I collaborated for research with several international institutes and organizations for example Institute for Health and the Environment, University at Albany New York and Wadsworth Center, New York State Department of Health, University of Alabama at Birmingham, Emory University, United States, University of Southampton UK, King Abdul Aziz University, Saudi Arabia. I am also a member of 'WHO Global Platform on Air Quality and Health' to represent the region.

Education

Fellowship (FCPS)	2003	Community Medicine	CPSP (AKU), Pakistan.
Graduation (MBBS)	1994	Medicine and Surgery	University of Karachi (Dow Medical College), Pakistan.

Scholarly Interests

Biological and Medical Sciences: Public Health and Epidemiology; Environmental and Occupational Health; Non-communicable Diseases.

Selected Publications

Book Chapter & Monographs:

1. Book Chapter: **Fatmi Z**, Pappas G. Chapter 6: Environmental Health and Megacities. In: Megacities and Global Health. Editors: Khan O, Pappas G. **American Public Health Association (APHA)** Press, 2011. [ISBN: 978-0-87553-003-1].
2. **Fatmi Z**, Kadir MM, Ahmed W, Kazi A. Situation analysis of household energy use and indoor air pollution in Pakistan. Department of Child and Adolescent Health and Development, **World Health Organization. WHO/FCH/CAH/05.06**.
3. Khan T, Zaidi A. Contributors: Kadir MM, **Fatmi Z**, Siddiqui R. Indoor Air Pollution and Child Health in Pakistan: report of a seminar held at the Aga Khan University, Karachi, Pakistan. 29 September 2005. **World Health Organization 2006. (NLM classification: WA 754) ISBN 978 92 4 159416 5.**

Journal publications

Pubmed publication list: https://www.ncbi.nlm.nih.gov/pubmed/?term=Fatmi+Z

4. **Fatmi Z**, Coggon D. Coronary heart disease and household air pollution from use of solid fuel: a systematic review. Br Med Bull. 2016 Jun;118(1):91-109.

5. **Fatmi Z**, Coggon D, Kazi A, Naeem I, Kadir MM, Sathiakumar N. Solid fuel use is a major risk factor for acute coronary syndromes among rural women: a matched case control study. Public Health. 2014 Jan;128(1):77-82.
6. Jamali T, **Fatmi Z**, Shahid A, Khoso A, Kadir MM, Sathiakumar N. Evaluation of short-term health effects among rural women and reduction in household air pollution due to improved cooking stoves: quasi experimental study. Air Qual Atmos Health 2017; 10(7):809-819.
7. Rabbani U, Sahito A, Nafees AA, Kazi A, **Fatmi Z**. Pulmonary tuberculosis is associated with biomass fuel use among rural women in Pakistan: an age- and residence-matched case-control study. Asia Pac J Public Health. 2017;29(3):211-218.
8. **Fatmi Z**, White F. A comparison of 'cough and cold' and pneumonia: Risk factors for pneumonia in children under-5 years revisited. Int J Infect Dis. 2002 Dec;6(4):294-301.
9. Rabbani U, Mahar G, Siddique A, **Fatmi Z**. Risk assessment for arsenic-contaminated groundwater along River Indus in Pakistan. Environ Geochem Health. 2017;39(1):179-190.
10. Deepa M, Grace M, Binukumar B, Pradeepa R, Roopa S, Khan HM, **Fatmi Z**, Kadir MM, Naeem I, Ajay VS, Anjana RM, Ali MK, Prabhakaran D, Tandon N, Mohan V, Venkat Narayan KM; CARRS Surveillance Research Group. High burden of prediabetes and diabetes in three large cities in South Asia: The Center for cArdio-metabolic Risk Reduction in South Asia (CARRS) Study. Diabetes Res Clin Pract. 2015 Sep 25. pii: S0168-8227(15)00378-2.
11. Berg CJ, Ajay VS, Ali MK, Kondal D, Khan HM, Shivashankar R, Pradeepa R, Mohan D, **Fatmi Z**, Kadir MM, Tandon N, Mohan V, Narayan KM, Prabhakaran D. A cross-sectional study of the prevalence and correlates of tobacco Use in Chennai, Delhi, and Karachi: data from the CARRS study. BMC Public Health. 2015 May 11;15:483.

12. Ali MK, Bhaskarapillai B, Shivashankar R, Mohan D, **Fatmi Z**, Pradeepa R, Masood Kadir M, Mohan V, Tandon N, Venkat Narayan KM, Prabhakaran D; CARRS investigators. Socioeconomic status and cardiovascular risk in urban South Asia: The CARRS Study. Eur J Prev Cardiol. 2015 Apr 27. pii: 2047487315580891.
13. Khan AA, Tanzil S, Jamali T, Shahid A, Naeem S, Sahito A, Siddiqui FA, Nafees AA, **Fatmi Z**. Burden of asthma among children in a developing megacity: childhood asthma study, Pakistan. J Asthma. 2014 Nov;51(9):891-9.
14. Ahmed M, **Fatmi Z**, Ali A. Correlation of arsenic exposure through drinking groundwater and urinary arsenic excretion among adults in Pakistan. J Environ Health. 2014;76(6):48-54.
15. Khwaja HA, **Fatmi Z**, Malashock D, Aminov Z, Kazi A, Siddique A, Qureshi J, Carpenter DO. Effect of air pollution on daily morbidity in Karachi, Pakistan, Journal of Local and Global Health Science, 2012:3.
16. Sabzwari SR, **Fatmi Z**. Comparison of exhaled carbon monoxide levels among commuters and roadside vendors in an urban and a suburban population in Pakistan. Environ Monit Assess. 2011 Sep;180(1-4):399-408.
17. Nafees AA, **Fatmi Z**, Kadir MM, Sathiakumar N. Pattern and predictors for respiratory illnesses and symptoms and lung function among textile workers in Karachi, Pakistan. Occup Environ Med. 2013 Feb;70(2):99-107.
18. Nafees AA, Taj T, Kadir MM, **Fatmi Z**, Lee K, Sathiakumar N. Indoor air pollution (PM2.5) due to secondhand smoke in selected hospitality and entertainment venues of Karachi, Pakistan. 2012 Sep;21(5):460-4.
19. **Fatmi Z**, Abbasi IN, Ahmed M, Kazi A, Kayama F. Burden of skin lesions of arsenicosis at higher exposure through groundwater of taluka Gambat district Khairpur, Pakistan: a cross-sectional survey. Environ Geochem Health. 2013 Jun;35(3):341-6. doi: 10.1007/s10653-012-9498-3.

20. **Fatmi Z**, Rahman A, Kazi A, Kadir MM, Sathiakumar N. Situational analysis of household energy and biomass use and associated health burden of indoor air pollution and mitigation efforts in Pakistan. Int J Environ Res Public Health. 2010 Jul;7(7):2940-52. Epub 2010 Jun 20. Review.
21. Nafees AA, Kazi A, **Fatmi Z**, Irfan M, Ali A, Kayama F. Lung function decrement with arsenic exposure to drinking groundwater along River Indus: a comparative cross-sectional study. Environ Geochem Health. 2011 Apr;33(2):203-16.
22. **Fatmi Z**, Azam I, Ahmed F, Kazi A, Gill AB, Kadir MM, Ahmed M, Ara N, Janjua NZ; Core Group for Arsenic Mitigation in Pakistan. Health burden of skin lesions at low arsenic exposure through groundwater in Pakistan. Is river the source? Environ Res. 2009 Jul;109(5):575-81.
23. Kadir MM, Janjua NZ, Kristensen S, **Fatmi Z**, Sathiakumar N. Status of children's blood lead levels in Pakistan: implications for research and policy. Public Health. 2008 Jul;122(7):708-15. Epub 2008 Mar 21.
24. Khushk WA, **Fatmi Z**, White F, Kadir MM. Health and social impacts of improved stoves on rural women: a pilot intervention in Sindh, Pakistan. Indoor Air. 2005 Oct;15(5):311-6.

Technical reports

25. **Fatmi Z**, Jamali T, Kadir MM, Sathiakumar N. Impact of improved stoves on household air pollution and illnesses among rural women. (Report submitted to University of Alabama, US, Dec, 2014).
26. **Fatmi Z**, Raheel H, Kazi A, Kadir MM. Evaluation of Nutrition Support Program (NSP), Sindh (Evaluation report submitted to UNICEF, Karachi March 2003).
27. **Fatmi Z**, Ali S, Agha A, Saani AJ. Baseline survey for Health Situation Analysis for Integrated Mother and Child Health Care Programme in Gambat, Khairpur, Sindh (Report

submitted to Patient Welfare Society and Trust for Voluntary Organization, Sindh, September 2003).

28. Altaf A, **Fatmi Z**, Kazi A, Hussain T, Ahmed W. Evaluation of Reproductive Health Program of Save the Children USA in District Haripur, NWFP, Pakistan. (Evaluation report submitted to Save the Children, Pakistan Program in May 2003).
29. Altaf A, Agboatwalla M, **Fatmi Z**, Ajmal A, Memon E, Dahar N, Arshad M, Hussain T. In-depth interviews of healthcare providers, patients and home care givers to identify determinants of therapeutic injections in Sindh, Pakistan (Report submitted to WHO – SIGN – May 2001).
30. Altaf A, Shah SA, Agboatwalla M, Hussain T, **Fatmi Z,** Arshad M, Ajmal A, Dahar N, Memon A, Baig A. Determinants of therapeutic injection practices among communities in Sindh province, Pakistan. (Report submitted to WHO – SIGN – July 2001).
31. White F, Kadir MM, **Fatmi Z** and Community Medicine Residents. Assessment of Health Status and Trend in Pakistan – 2001. Community Medicine Residency Program, Department of Community Health Sciences, Aga Khan University.
32. **Fatmi Z**, Ahmed F, Kazi A, Azam I, Janjua N, Kadir MM. Health Effects of Drinking Arsenic Contaminated Underground Water in District Khairpur, Sindh, Pakistan. (Report submitted to Water and Sanitation (WES), UNICEF, Islamabad, Pakistan, December, 2006).
33. **Fatmi Z,** Ahmed W, White F, Kadir MM. Preliminary assessment of Smoke-Free Stoves (SFS) in rural Sindh: a pilot study [Acceptability, Health and Social Impact]. (Evaluation report submitted to Aga Khan Planning and Building Services, Pakistan in June 2002).