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Relatively higher concentrations of trace elements to arsenic may have significantlyinfluenced the methylation process of Arsenic (As)in humans

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ABSTRACT

Trace elements (As, Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, and Hg) were analyzed in blood as well as urine of arsenic exposed people in Mexico by using ICP-MS to know the influence of trace elements for biotransformation process of inorganic arsenic.

The concentrations of Cu and Mn were significantly higher, and the concentrations of As, Zn, and Pb were significantly lower in blood for females compared to males. The correlations between the ratios of the concentrations of Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, or Hg to As in blood as well as urine and urinary arsenic metabolites were stronger than the correlations found between the concentrations of above elements and urinary arsenic metabolites for both females and males. The concentrations of trace elements (for examples: Se, Mn, Hg, etc.) relative to As in urine and blood were positively correlated with urinary % inorg As as well as % MMA, and negatively correlated with % DMA as well as the ratios of % DMA to % MMA.

On the other hand, due to low level of creatinine in urine, adjusted urinary trace elements concentrations express as µg/g cre were significantly higher than the unadjusted concentrations express as µg/L for the urine groups with arsenic concentrations ≤50 µg As/L. But there were no significant correlations observed between adjusted and unadjusted concentrations of trace elements for the urine groups with arsenic concentrations >50 µg As/L. Adjusted urinary Se, Mn, and Hg concentrations were negatively and significantly associated with urinary creatinine for both females and males.

The results of this study suggest that trace elements may have influenced for biotransformation process of inorganic arsenic in humans. The results also suggest that Se, Mn as well as Hg may decrease arsenic methylation with decreasing creatinine formation for both sexes, but it could be concentration dependent.

Abbreviation: SAM, S-adenosyl-L-methionine; SAHC, S-adenosyl-L-homocysteine.

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Introduction

The IARC (1987)[1] has classified arsenic as a group 1 human carcinogen. Chronic exposure to inorganic arsenic can cause cancerous[1-4] and non-cancerous health hazards[5,6] in humans. Arsenic can get entry into the human body via drinking water, eating food, inhaling dust, and/or ingesting soil.

With chronic and continuing exposure, steady-state concentrations of arsenic in blood and urine are achieved; these have been the potential to serve as biomarkers of arsenic exposure[7]. However, urinary arsenic is a reflection of As excretion and not actual tissue burden[8], and significant complexities are introduced when urinary arsenic concentrations are normalized for urinary creatinine in order to adjust for dilution factor[9,10]. Hall et al. (2006)[7] have been suggested that with chronic exposure, blood arsenic which receives inputs not only from recent exogenous exposure but also from tissue compartments – may better reflect an individual's total internal As burden.

The main organ for arsenic metabolism is the liver, but the metabolic pathway of inorganic arsenic is not yet fully

clarified[11,12]. Trivalent arsenic species are more ready to cross cell membrane and inorganic pentavalent arsenate in mostly reduced to trivalent arsenite in the blood stream before entering the cells for further metabolism[13,14]. Inorganic arsenic is metabolized in the body by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine as methyl donor[11,15] (Fig. 1).

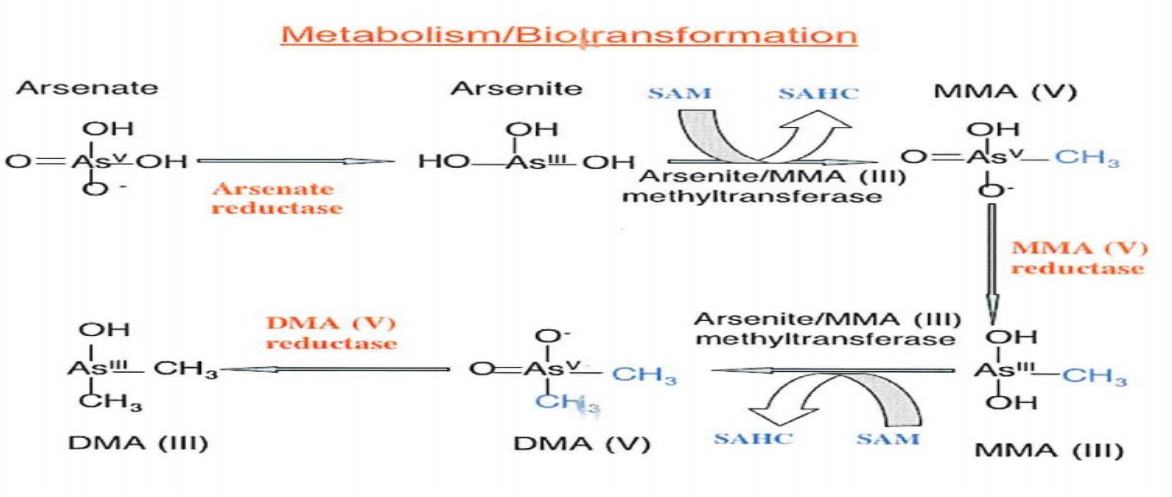

Figure 1. Metabolism of Inorg-As.

Determination of the concentrations and the proportional distribution of the various arsenic species including the inorganic arsenic and the methylated metabolites in urine can give a reflection of the capacity to methylate inorganic arsenic in human body. The ratio between MMA^V and inorganic arsenic (PMI, primary methylation index) and the ratio between DMA^V to MMA^V (SMI, secondary methylation index) are also used to assess the arsenic methylation capacity of the first and second methylation steps, respectively. Several studies have been shown an increasing prevalence of arsenic-related toxic effects with increasing % MMA in urine[5,16,17] and probably an increased concentrations of the highly toxic MMA^{III} at cellular level[18,19].

A number of studies have shown associations between the severity of arsenic related health effects and nutritional status[20-22]. Lower Se intake is associated with enhance As toxicity[20,23-25] and lower urinary Se levels were associated with increased % inorg As and decreased % DMA in urine[25]. Another study has been reported that subjects with higher intakes of Zn had lower % MMA and higher % DMA in urine[21]. Zinc (Zn) has been linked to decrease arsenic toxicity in some studies[2,26].

Trace elements are well known to play an important role in the maintenance of health[27]. Thus, monitoring the status of trace elements is of critical importance in human health. Today, biomonitoring of trace elements in human blood and urine have become an important tool for measuring trace elements status[28-31].

It is well known that the concentrations of pollutants in spot urine sample are highly dependent on the dilution of the sample caused by variation in the intake of fluids, physical activity, temperature, etc[32]. Commonly applied method to control for this variation is adjustment by the creatinine concentration in urine[9,32,33]. However, creatinine is a waste product formed by the spontaneous, essentially irreversible dehydration of body creatine and creatine phosphate from muscle metabolism and meat intake[9,34,35]. Thus, urinary creatinine (U-cre) varies by gender, age, body size, race/ethnicity, diet, renal function, etc[9,36,37]. Recent studies have been reported that urinary arsenic levels (µg/L) were found significantly correlated with urinary creatinine levels[10,38,39]. Gamble et al. (2005)[10,38] has found that higher urinary creatinine is associated with reduced risk for premalignant skin lesions among the arsenic exposed population in Bangladesh and folic acid supplementation significantly increased urinary creatinine. But Hindwood et al. (2002)[33] have been suggested that creatinine adjustment of urinary inorganic arsenic (Inorg-As) concentrations may not be required in population studies investigating environmental exposure.

To date, other metal or metalloids that may influence arsenic methylation are largely unknown. The aims of this study were to assess the influence of trace elements for biotransformation process of inorganic arsenic and the correlations between the concentrations of trace element in blood as well as urine and urinary arsenic metabolites among the population in Lagunera area of Mexico, who drunk water containing arsenic in range 38 to 116 µg/L. Our results suggest that trace elements had influenced arsenic methylation process in humans, but it was concentrations dependent. The results also suggested that urinary creatinine adjustment might be over-estimated of urinary trace element concentrations due to low concentrations of urinary creatinine for the people with low arsenic in urine.

Materials And Methods

Reagents. The chemicals used and there sources are as follows: Sodium arsenate (ACS reagent grade) from MCB Reagents

(Cincinnati, OH); dimethylarsinic acid (sodium salt), ammonium phosphate (dibasic), and glutathione (GSH) from Sigma Chemical Co. (St. Louis, MO); sodium m-arsenite and ammonium nitrate from Sigma-Aldrich Co. (St. Louis, MO); disodium methylarsenate from ChemService, Inc. (West Chester, PA). The arsenic and other elements standard solution was from SPEX Certiprep (Metuchen, NJ). Freeze-dried urine reference material for toxic elements (SRM 2670a) and frozen bovine blood reference material for toxic metals (SRM 966) from National Institute of Standards & Technology (NIST, Gaithersburg, MD 20899). Triton X-100 was from Pharmacia Biotech (Uppsala, Sweden). All other chemicals were analytical reagent grade or the highest quality obtainable. Water was doubly deionized and distilled.

Subjects. Urine and blood samples were collected from 191 subjects (98 females and 93 males), aged 18-77 years in the Lagunera area of Mexico. There were five groups, based on total arsenic concentration (38-116 µg/L) in their drinking water.

Urine and Blood Collection. All collecting containers were soaked overnight in 2% nitric acid (Baker analyzed for trace metal analysis) (J. T. Baker, Inc. Phillipsburg, NJ) and rinsed with double distilled and deionized water. All plastic measuring and collecting equipment were similarly washed, sealed in bags, placed in locked footlockers, and transported by air to the site of the study at the same time as the investigators. After collection, urine sample was immediately frozen in a portable icebox containing dry ice. Blood was collected by venous puncture, into vacutainers containing EDTA, transferred to the vial, and immediately frozen. The samples were kept frozen while being transported to the University of Arizona, Tucson where they were stored at -70° C before analysis.

Arsenic Species Analysis. Frozen urine samples were thawed at room temperature, filtered with a 0.45 µm filter (Nanosep MF Centrifugal Devices, Pall Life Sciences, Ann Arbor, MI), and diluted 5-fold using Milli-Q water before injection. An HPLC-ICP-MS (High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry) speciation method[40] was modified for the measurement of arsenic species including arsenodetaine (AsB) by author. The HPLC system consisted of a PerkinElmer Series 200 HPLC with an anion exchange column (PRP-X100, 10 µm, 250 x 4.6 mm, Hamilton Company, Nevada). The mobile phase (pH 8.5) contained 10 mM ammonium nitrate and 10 mM ammonium phosphate (dibasic) at a flow rate of 1 ml/min. The column temperature was maintained at 30° C. An ELAN DRCe ICP-MS (PerkinElmer) with a cyclonic quartz spray chamber and Meinhard nebulizer was used as a detector for the analysis of arsenic species [AsB, As^V, As^{III}, MMA^V, and DMA^V] in urine at 4° C. The operating parameters were as follows: R_f power, 1400 W; plasma gas flow, 15 L/min; nebulizer gas flow, 0.82 L/min; auxiliary gas flow, 1.2 L/min; oxygen flow for DRC, 0.87 mL/min; and arsenic was measured at m/z 91.

The working detection limits and accuracy of this analytical method were as follows: The working detection limits were 0.80 - 1.75 µg/L for arsenic metabolites. Accuracy values were calculated by spiking standard compounds of all five species (5 µg/L) in urine samples. The recoveries of the added compounds were 98-103%. Standard samples (5 µg/L) containing all five arsenic species were also analyzed after analysis the urine samples each day. The values of mean ± SE for AsB, As^V, As^{III}, MMA^V, and DMA^V were found 4.86 ± 0.08, 5.09 ± 0.11, 5.16 ± 0.11, 5.02 ± 0.10, and 4.90 ± 0.05µg/L, respectively.

Trace elements analysis in urine. Urine samples in acid washed polypropylene tubes were digested with nitric acid (5: 1) while a water bath for 40 min at 70° C. Freeze-dried urine reference material for toxic elements containing arsenic at a level of 220 ± 10 µg As/L was used for quality control and to validate the assay. After acid digestion, analysis of this standard by ICP-MS yielded a range of 216.0 - 236.0 µg As/L with a range of recoveries of 98.18 - 107.27%. We also analyzed the spiking standard compounds of all the arsenic species [for AsB, As^V, As^{III}, MMA^V, and DMA^V] at levels of 10 µg total As /L and 20 µg total As /L. The recoveries of the spiking samples were 104.20 % (10.42 ± 0.13 µg As/L) and 97.70 % (19.54 ± 0.24 µg As/L), respectively. After acid digestion, analyzed trace elements in urine samples collected from the subjects and NIST reference urine samples. The recoveries of Se, Zn, Co, Cu, Mn, Ni, Cd, Pb, and Hg in NIST reference urine were 92.16 %, 93.01 %, 101.00 %, 94.77 %, 106.06 %, 100.84 %, 109.70 %, 100.72 %, and 94.28 %, respectively. The multi-element standard solutions were digested and diluted using the same procedure and dilution factors (as the samples) for preparation of the calibration curve. The calibration correlation coefficients (r^2) of the elements were greater than 0.999.

Trace element analysis in whole blood. Whole blood samples were analyzed for total As, Se, Zn, Co, Cu, Mn, Ni, Cd, Pb, and Hg concentrations using Perkin Elmer Elan DRCe ICP-MS. Inductively coupled plasma mass spectrometry method for elements in whole blood was developed (with modifications) based on published method[41]. Whole blood samples were thawed, thoroughly mixed, diluted 50 times with diluents containing 0.65% HNO_3 + 0.1% Triton X-100, and centrifuged for 10 min (3500 rpm at 4° C) with the supernatant reserved for analysis. The multi-element standard solutions were prepared from stock standard solution with 0.65% HNO_3 + 0.1% Triton X-100. Three working mercury standard solutions, viz., 1, 2.5, and 5 µg/L were prepared from stock standard solution with 0.65% HNO_3 + 0.1% Triton X-100, added gold (200 ppb), and mixed well. Five working other elements (As, Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn) standard solutions, viz., 5, 10, 20, 50, and 100 µg/L were prepared from stock standard solution with same diluents, added internal standards (Ga, In, & Re; 100 µg/L of each), and mixed well. The calibration correlation coefficients (r^2) of the elements were greater than 0.999.

Frozen bovine blood reference material for toxic metals was used for quality control and to validate the assay. The reference sample was thawed in ice, mixed thoroughly, and diluted 50 times with diluents containing 0.65% HNO_3 + 0.1% Triton X-100, and centrifuged for 10 min (3500 rpm at 4° C) with the supernatant reserved for analysis. The recoveries of the elements

in the reference bovine blood samples were very close to the certified values (Table 1). The recoveries of Pb, Cd, and Hg in the reference bovine blood samples were 92 %, 107%, and 97%,

Table 1. Recovery data for NIST SRM 'Bovine Blood' and spike recoveries for human blood. Values are the mean ± SE (n=3 for bovine blood and n = 8 for human blood).

Analyte	Bovine Blood (SRM 966) Cert. Value (µg/L)	% Recovery of Cert. Value	Low Spike Level (µg/L)	Low Spike % Recovery	High Spike Level (µg/L)	High Spike % Recovery
As	N.A.		10	125.21±4.04	50	115.80±3.51
Se	N.A.		10	128.96±5.15	50	116.25±4.07
Zn	N.A.		10	115.94±7.16	50	85.64±3.12
Co	N.A.		10	101.99±1.62	50	98.15±2.50
Cu	N.A.		10	97.94±3.43	50	92.75±1.57
Pb	252.70±2.20	91.93±0.90	10	94.41±1.51	50	90.06±2.87
Cd	5.22±0.16	107.44±2.04	10	98.55±1.49	50	93.05±1.30
Ni	N.A.		10	100.06±2.06	50	95.91±1.65
Mn	N.A.		10	99.68±1.94	50	93.62±2.15
Hg	31.40±1.70	97.32±1.87	1	98.00±2.61	2.5	97.72±0.74

N.A. = Not Available

-respectively. We also analyzed the spiking standard elements in the human blood samples and the quality control (QC) standard samples. The spiking and QC samples were prepared and analyzed using the same procedures as the human blood samples. The recoveries of the elements in the spiking and QC samples are shown in Table 1 and 2, respectively. The rinse solution contained 2% HNO_3 + 1% Triton X 100.

Table 2. Recovery data for trace elements in the quality control standard (QCS).Values are the mean ± SE (n=8).

Analyte	QCS Level 1 (µg/L)	QC Std 1 % Recovery	QCS Level 2 (µg/L)	QC Std 2 % Recovery
As	10	101.28±2.17	20	103.81±1.43
Se	10	103.50±0.98	20	100.38±1.05
Zn	10	105.04±5.06	20	106.88±1.99
Co	10	110.38±2.20	20	104.38±1.11
Cu	10	106.00±1.12	20	102.56±1.33
Pb	10	105.38±0.96	20	101.25±0.61
Cd	10	101.50±0.57	20	99.25±0.76
Ni	10	106.38±1.56	20	104.69±1.92
Mn	10	101.83±2.13	20	94.06±1.13
Hg	10	97.84±0.72	20	96.48±5.80

Creatinine Measurement. Creatinine concentration in urine was determined using the Randox Creatinine Colorimetric kit (San Diego, CA), which is based on the reaction of creatinine with picric acid in alkaline solution, forming a colored complex, measured at 492 nm[42].

Statistical Analysis: The means and standard error (SE) were calculated. The unpaired t test (GraphPad Software, Inc., 2005) was used to analyze the significance difference. The correlation coefficients for different variables were tested using the Spearman rank order correlation test (Richard Lowry, 1998-2009). *P* values less than 0.05 were considered significant.

Results

In this study, there were five groups (Gps) of participants based on total arsenic concentration in their drinking water (Gp1= 38.2, Gp2= 43.5,Gp3= 96.0,Gp4= 105.1, and Gp5= 116.3 µg As/L). The general characteristics of the study population have been previously described in detail (Manuscript submitted). Human blood samples were collected from 191 subjects. After collection, blood samples were transferred to the Nalgene vials (Nalge Nunc International, NY) and immediately frozen. We did not acid wash the Nalgene vials. But, we analyzed arsenic and other elements in rinse solution (2% HNO_3) of Nalgene vials. The concentrations of the trace elements in rinse solution of the

vials were below the MDL except Zn (2.42±0.79 µg/L).

Study population. In this study, out of 191 participants in Lagunera area of Mexico, 98 were females (F) and 93 were males (M). The average age of females versus males was not statistically significant.

Figure 2 shows the distribution of the concentrations of trace elements in urine of different arsenic exposure groups ((Gp1(n=38), Gp2(n=33),Gp3 (n=39),Gp4 (n=40), and Gp5 (n=41)) in Lagunera area of Mexico. The concentrations of trace elements in urine and blood are reported in Table 3. The element concentrations in urine expressed as ug/g cre were higher for females (F) compared to males (M). But the concentrations of most elements in blood were opposite for females compared to males. The mean concentrations of Cd, Hg, Se, Co, Cu, and Mn were significantly higher in urine for females than males ($p<0.01$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.01$, & $p<0.001$, respectively). The concentrations of As, Pb, Zn, and Ni in urine were not statistically different between females and males. A significant difference of the concentration between females and males was found with respect to As, Pb, Zn, Cu, and Mn in blood (Table 3 and Fig. 3). The mean concentrations of As, Pb, and Zn in blood were significantly lower for females compared to males ($p<0.0001$, $p<0.0001$, and $p<0.01$, respectively). But, the mean concentrations of Cu and Mn were significantly higher for females than males ($p<0.0001$ and $p<0.001$, respectively). The concentrations of Cd, Se, Co, and Ni in blood were not statistically significant between females and males. More than 92% and 88% of the blood samples contained below working MDL concentrations of Hg (<1.10 µg/L) for females and males, respectively.

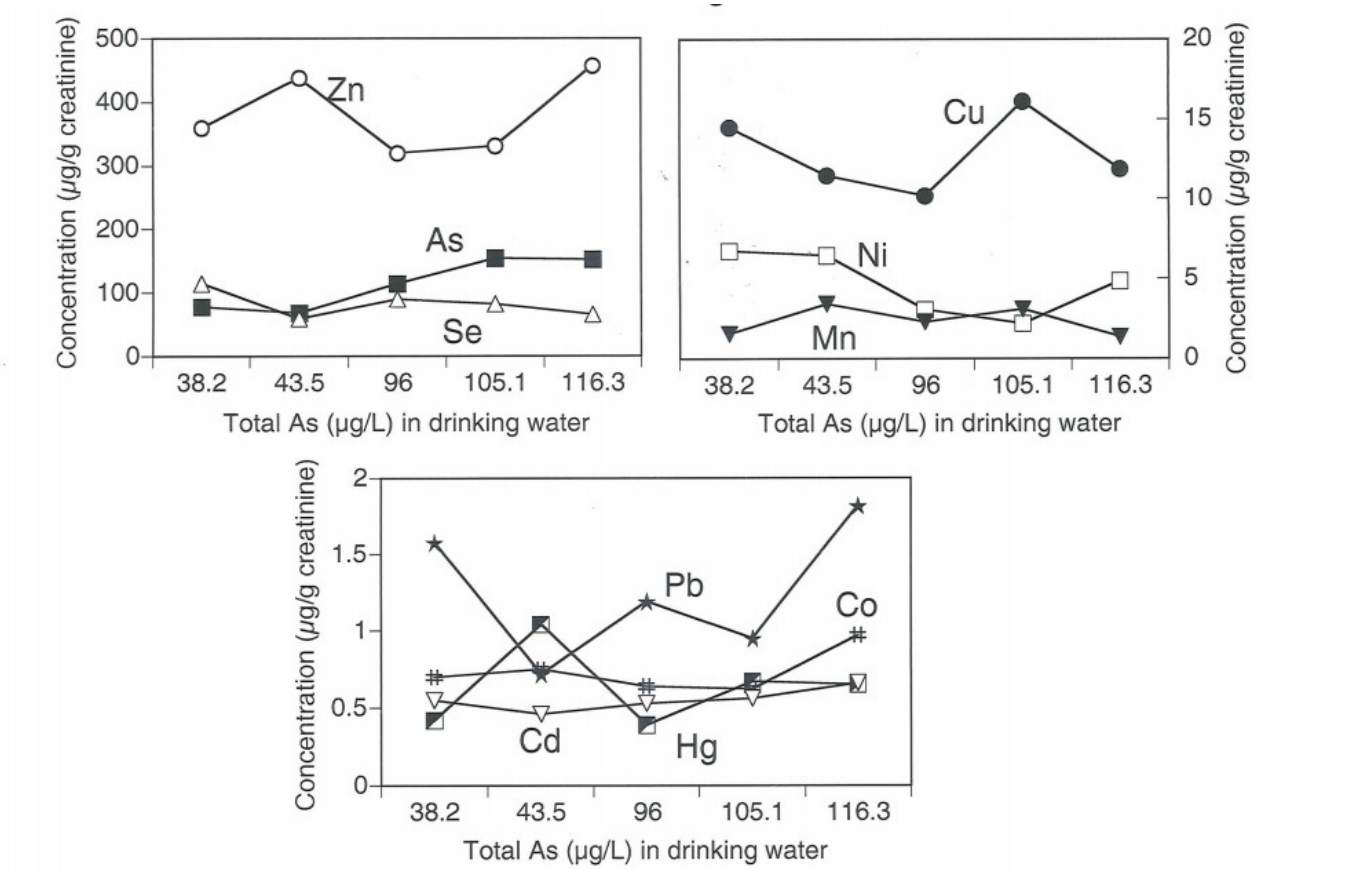

Figure 2. Distribution of the concentrations of trace elements in urine of different arsenic exposure groups.Values are the mean.

Table 3. Trace elements concentrations in urine and whole blood for females (F) and males (M). Values are the mean ± SE (F, n=98 and M, n=93).

	As	Cd	Pb	Hg	Se	Zn	Co	Cu	Ni	Mn
					A. In urine (µg/g cre)					
F	121.43±6.4	0.63±0.04	1.64±0.3	0.82±0.1	94.30±6.0	395.56±31.2	0.94±0.10	14.67±1.02	5.01±0.5	2.73±0.2
M	108.52±6.1	0.47±0.04	1.11±0.1	0.56±0.1	70.09±3.8	358.25±25	0.53±0.05	11.04±0.7	4.02±0.3	1.85±0.2
p values (F vs. M)	>0.05	<0.01*	>0.05	<0.05*	<0.001*	>0.05	<0.001*	<0.01*	>0.05	<0.001*
					B. In blood (µg/L)					
F	9.48±0.2	1.20±0.06	21.31±1.1	BDL	234.95±3.2	5452.65±60.8	0.87±0.04	945.13±15.6	3.58±0.3	16.29±0.3
M	11.18±0.4	1.30±0.06	28.06±1.2	BDL	232.77±3.0	5764.80±77.7	0.78±0.04	768.45±9.4	3.30±0.2	14.63±0.3
p values (F vs. M)	<0.0001*	>0.05	<0.0001*		>0.05	<0.01*	>0.05	<0.0001*	>0.05	<0.001*

BDL= Below Detection Limit; *Statistically significant

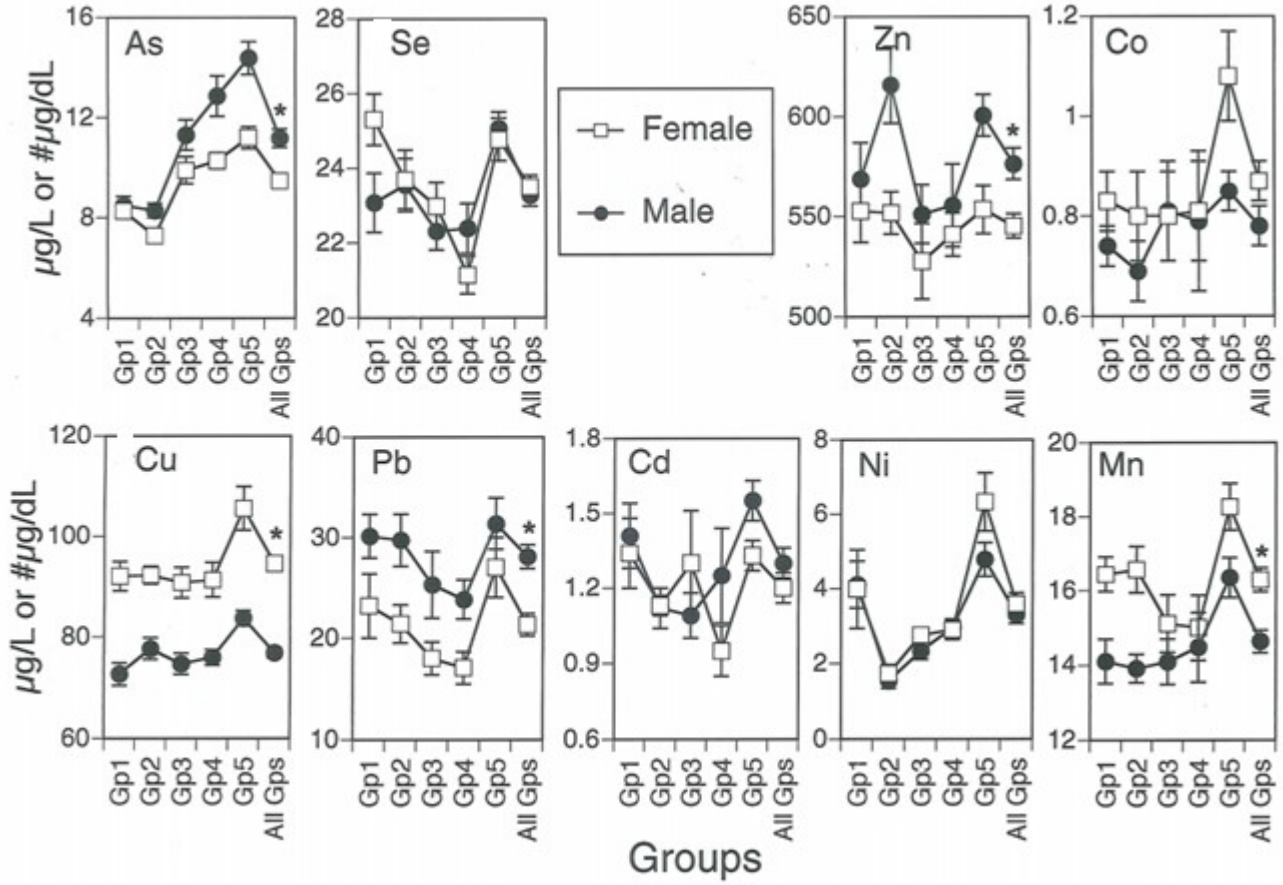

Figure 3. Concentrations of trace elements in whole blood of females and males. Values are the mean ± SE. (* statistically significant).

The mean concentrations of trace elements in urine followed the order: As> Pb> Hg> Cd (toxic elements) and Zn> Se> Cu> Ni> Mn>Co (essential elements) for both females and males. But in blood it was little different, for toxic elements: Pb> As> Cd, and essential elements: Zn> Cu> Se> Mn> Ni> Co for both females and males.

Correlations of the concentrations of elements in blood versus ages of the arsenic exposed people in Mexico. Zinc (Zn) and copper (Cu) concentrations in blood were positively and significantly correlated with age of females and males, respectively (Table 4). But manganese (Mn) concentrations were negatively and significantly correlated with age of females but not of males. The correlations between other elements and ages were not statistically significant for both females and males.

Table 4. Spearman correlation coefficients of bloodelements concentrations (µg/L) versus ages (years)for females (n=98) and males (n=93) of our study groups.

	Ages	
Analyte	Females	Males
As	0.169	0.028
Se	-0.060	0.039
Zn	0.222[a]	-0.038
Co	-0.016	0.082
Cu	-0.167	0.275[b]
Pb	0.157	0.135
Cd	0.003	0.101
Ni	0.037	0.041
Mn	-0.289[b]	-0.099

[a]p<0.05, [b]p<0.01

Correlations of the concentrations of As in blood versus As concentrations in drinking water or urine for females and males. Total arsenic concentrations in bloods expressed as µg/L were strongly and positively correlated with arsenic concentrations in drinking water expressed as µg/L and urinary arsenic concentrations expressed as µg/L or µg/g creatinine for both females and males (Table 5).

Table 5. Spearman correlation coefficients for As concentrations in bloods versus As concentrations in drinking water as well as urinesof females and males.

		As in blood (µg/L)		
		Females (F)	Males (M)	F + M
A.	As in drinking water (µg/L)	0.615[f]	0.731[e]	0.649[e]
B.	As in urine (µg/L, sum of Arsenic metabolites)	0.715[e]	0.549[f]	0.641[e]
C.	As in urine (µg/L, after acid digestion)	0.619[f]	0.471[e]	0.557[f]
D.	As in urine (µg/g creat.)	0.750[f]	0.756[e]	0.682[e]

[e]p<0.00001, [f]p<0.000001

Correlations of the concentrations of As and other elements in blood. Cobalt (Co) and nickle (Ni) concentrations in blood were positively and significantly correlated with Asconcentrations in blood of females (r_s= +0.32, p<0.01 and r_s= +0.57, p<0.000001, respectively) (Table 6). For males, blood As

Table 6. Spearman correlation coefficients (r_s) for As versus other elements concentrations in bloods of females and males.

Analyte	Females	Males
As vs. Se	0.074	0.227[a]
As vs. Zn	0.105	0.092
As vs. Co	0.323[b]	0.407[d]
As vs. Cu	0.128	0.393[d]
As vs. Pb	0.025	-0.176
As vs. Cd	0.089	0.187
As vs. Ni	0.566[f]	0.410[d]
As vs. Mn	-0.094	0.387[c]

[a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001, [f]p<0.000001

concentrations were positively and significantly correlated with blood Se, Co, Cu, Ni, and Mn concentrations (r_s= +0.23, p<0.05; r_s= +0.41, p<0.0001; r_s= +0.39, p<0.0001; r_s= +0.41, p<0.0001; and r_s= +0.39, p<0.001, respectively).

Influence of the relative concentrations of other trace elements to arsenic in urine on the percentage of urinary arsenic metabolites. There were better correlations between (a) the ratio of other element (Se, Zn, Mn, Ni, or Hg) to arsenic (As) in urine (µg/g cre) and the percentage of urinary arsenic metabolites than (b) the correlations found between the corresponding element concentrations expressed as µg/g cre in urine and percentage of urinary arsenic metabolites (Table 7).

Table 7. Comparison of spearman correlation coefficients between (a) the ratio of the concentrations of other element to As or (b) the concentration of element (µg/g creatinine) in urines and the percentage (%) of urinary As metabolitesof females and males.

	Ratio of Se to As	Se	Ratio of Zn to As	Zn	Ratio of Mn to As	Mn	Ratio of Ni to As	Ni	Ratio of Hg to As	Hg
Females:										
% Inorg As	0.290[b]	0.160	0.084	-0.080	0.229[a]	0.144	0.224[a]	0.151	0.413[d]	0.342[c]
% MMA	0.250[a]	-0.004	-0.027	-0.181	0.259[b]	0.129	0.214[a]	0.107	0.204[a]	0.093
% DMA	-0.337[c]	-0.168	-0.109	0.070	-0.225[a]	-0.108	-0.271[b]	-0.195	-0.385[d]	-0.332[c]
% MMA/% inorgAs	-0.026	-0.081	-0.124	-0.094	0.044	-0.072	-0.019	-0.012	-0.245[a]	-0.277[b]
% DMA/% MMA	-0.312[b]	-0.038	-0.014	0.197	-0.281[b]	-0.105	-0.252[a]	-0.153	-0.274[b]	-0.162
Males:										
% Inorg As	0.264[a]	0.174	0.223[a]	0.086	0.419[d]	0.323[b]	0.086	-0.077	0.471[e]	0.331[b]
% MMA	0.100	0.090	-0.028	-0.092	0.216[a]	0.204[a]	0.080	0.001	0.171	0.151
% DMA	-0.254[a]	-0.117	-0.189	-0.008	-0.423[d]	-0.290[b]	-0.133	-0.037	-0.441[d]	-0.270[b]
% MMA/% inorgAs	-0.163	-0.080	-0.242[a]	-0.115	-0.251[a]	-0.161	-0.065	0.039	-0.300[b]	-0.187
% DMA/% MMA	-0.145	-0.094	-0.034	0.081	-0.314[b]	-0.269[b]	-0.111	0.004	-0.288[b]	-0.233[a]

[a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001, [e]p<0.00001

Statistically significant correlations were not found between the concentrations (µg/g cre) of Se, Zn, Mn as well as Ni and % inorg As, % MMA, % DMA, as well as the ratios of % DMA to % MMA in urine for females. But, the ratios of the concentrations of Se, Mn, as well as Ni to As expressed as µg/gcrewerepositively and significantly correlated with % inorg As (r_s= +0.29, p<0.01; r_s= +0.23, p<0.05; and r_s= +0.22, p<0.05, respectively) as well as % MMA (r_s= +0.25, p<0.05; r_s= +0.26, p<0.01; and r_s= +0.21, p<0.05, respectively), and negatively correlated with % DMA (r_s= -0.34, p<0.001; r_s= -0.23, p<0.05; and r_s= - 0.27, p<0.01, respectively) as well as the ratios of % DMA to % MMA (r_s= -0.31, p<0.01; r_s= -0.28, p<0.01; and r_s= -0.25, p<0.05, respectively) in urine for females (Table 7).

For males, the ratios of the concentrations (µg/g cre)) of Se or Zn to As than the concentrations of Se or Zn were more strongly and positively correlated with % inorg As levels (r_s= +0.26, p<0.05 vs. r_s= +0.17 not significant and r_s= +0.22, p<0.05 vs. r_s= +0.086 not significant, respectively), but more strongly and negatively correlated with % DMA levels (r_s= -0.25, p<0.05 vs. r_s= -0.12 not significant and r_s= -0.19 not significant vs. r_s= -0.008 not significant, respectively) in urines (Table 7). The correlations between the ratio of the concentrations of Mn to As and the percentage of urinary arsenic metabolites were more significant than the correlation found between Mn concentrations and the percentage of arsenic metabolites (with % inorg As: r_s= +0.42, p<0.0001 vs. r_s= +0.32, p<0.01,

respectively; with % MMA: r_s= +0.22, p<0.05 vs. r_s= +0.20, p<0.05, respectively; with % DMA: r_s= -0.42, p<0.0001 vs. r_s= -0.29, p<0.01, respectively; with the ratios of %MMA to % inorg As: r_s= -0.25, p<0.05 vs r_s= -0.16 not significant, respectively; as well as with the ratios of % DMA to % MMA: r_s= -0.31, p<0.01 and r_s= -0.27, p<0.01, respectively) in urine for males. These relations were more significantly correlated in urine for males than females. It was also interesting that the correlation between (a) the ratio of Ni to As in urine (µg/g cre) and the percentage of urinary arsenic metabolites as well as(b) the correlation found between the Ni concentrations expressed as µg/g cre in urine and percentage of urinary arsenic metabolites were not statically significant for males.

The results show (Table 7) that the correlations were more significant between the percentage of arsenic metabolites and the ratios of the concentrations (µg/g cre) of Hg to As than the concentrations of Hg in urine for both females and males. The ratio of the concentrations of Hg to As expressed as µg/gcrewerepositively and significantly correlated with % inorg As (r_s= +0.41, p<0.0001 vs. r_s= +0.34, p<0.001, respectively) as well as % MMA (r_s= +0.20, p<0.05 and r_s= +0.09 not significant, respectively), and negatively correlated with % DMA (r_s= -0.39, p<0.0001 and r_s= -0.33, p<0.001), the ratios of %MMA to % inorgAs (r_s= -0.25, p<0.05 and r_s= -0.28, p<0.01, respectively) as well as the ratios of % DMA to % MMA (r_s= -0.27, p<0.01 and r_s= -0.16 not significant, respectively) in urine for females. For males, the ratio of the concentrations of Hg to As expressed as µg/gcrewere alsopositively and significantly correlated with % inorg As (r_s= +0.47, p<0.00001 vs. r_s= +0.33, p<0.01, respectively) as well as % MMA (r_s= +0.17 not significant vs. r_s= +0.15 not significant, respectively), and negatively correlated with % DMA (r_s= -0.44, p<0.0001 vs. r_s= -0.27, p<0.01, respectively), the ratios of %MMA to % inorgAs (r_s= -0.30, p<0.01, vs. r_s= -0.19 not significant, respectively) as well as the ratios of % DMA to % MMA (r_s= -0.29, p<0.01 vs. r_s= -0.23, p<0.05, respectively) in urine for males.

Influence of the relative concentrations of trace elements to arsenic in blood on the percentage of urinary arsenic metabolites. The ratios of the concentrations of Se, Zn, Mn, as well as Cu to As than the corresponding element concentrations in blood were more significantly and positively correlated with % inorg As (r_s= +0.36, p<0.001 vs. r_s= +0.11 not significant; r_s= +0.24, P<0.05 vs. r_s= -0.10 not significant; r_s= +0.41, p<0.0001 vs. r_s= +0.29, p<0.01; and r_s= +0.36, p<0.01 vs. r_s= +0.06 not significant, respectively), and negatively correlated with % DMA (r_s= -0.34, p<0.001 vs. r_s= -0.12 not significant; r_s= -0.24, p<0.05 vs. r_s=-0.005 not significant; r_s= - 0.28, p<0.01 vs. r_s=-0.15 not significant; and r_s= -0.26, p<0.05 vs. r_s= +0.05not

Table 8. Comparison of spearman correlation coefficients between (a) the ratio of the concentrations of other element to As or (b) the concentration of element (µg/L) in bloods and the percentage (%) of urinary As metabolitesof females and males.

	Ratio of Se to As	Se	Ratio of Zn to As	Zn	Ratio of Mn to As	Mn	Ratio of Cu to As	Cu	Ratio of Cd to As	Cd
Females:										
% Inorg As	0.361[c]	0.111	0.237[a]	-0.102	0.411[d]	0.290[b]	0.363[c]	0.062	0.214[a]	0.051
% MMA	0.267[b]	0.033	0.257[a]	0.050	0.198	0.044	0.183	-0.157	0.132	-0.030
% DMA	-0.335[c]	-0.124	-0.237[a]	-0.005	-0.283[b]	-0.150	-0.257[a]	0.051	-0.142	-0.003
% MMA/% inorgAs	-0.150	-0.007	-0.047	0.159	-0.264[b]	-0.240[a]	-0.243[a]	-0.167	-0.103	-0.061
% DMA/% MMA	-0.316[b]	-0.063	-0.290[b]	-0.049	-0.235[a]	-0.059	-0.222[a]	0.151	-0.147	0.023
Males:										
% Inorg As	0.250[a]	-0.043	0.259[a]	0.036	0.312[b]	0.015	0.181	-0.142	0.112	-0.137
% MMA	0.093	-0.018	0.060	-0.053	0.059	0.010	0.064	-0.025	-0.034	-0.128
% DMA	-0.296[b]	0.017	-0.313[b]	-0.050	-0.310[b]	-0.002	-0.248[a]	0.060	-0.123	0.132
% MMA/% inorgAs	-0.197	0.057	-0.229[a]	-0.065	-0.266[a]	0.016	-0.169	0.130	-0.150	0.038
% DMA/% MMA	-0.190	0.007	-0.159	0.013	-0.141	0.006	-0.140	0.048	-0.003	0.168

[a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001

significant, respectively), and with the ratios of % DMA to % MMA (r_s= -0.32, p<0.01 vs. r_s=-0.063 not significant; r_s= -0.29, p<0.01 vs. r_s=-0.049 not significant; r_s= -0.24, p<0.05 vs. r_s=-0.059 not significant; and r_s= -0.22, p<0.05 vs. r_s=+0.15 not significant, respectively) in urine for females (Table 8). The ratios of the concentrations of Se or Zn to As than the concentrations of Se or Zn in blood were also more positively and significantly correlated with % MMA (r_s= +0.27, p<0.01 vs. r_s= +0.033 not significant and r_s= +0.26, p<0.05 vs. r_s= +0.05 not significant, respectively) in urine for females. Strong correlations also found between the ratios of % MMA to % inorg As in urine and the ratios of the concentrations of Mn or Cu to As in blood (r_s= -0.264, p<0.01 and r_s= - 0.243, p<0.05, respectively) than the concentrations of Mn or Cu (r_s= -0.24, p<0.05 and r_s= -0.17 not significant, respectively) in blood for females.

For males, better and significant correlations found between the ratios of the concentrations of Se, Zn, Mn, or Cu to As in blood and the percentage of urinary arsenic metabolites than the correlations found between the corresponding element concentrations in blood and the percentage of urinary arsenic metabolites (Table 8). The ratios of the concentrations of Se, Zn, or Mn to As in blood were more positively correlated with % inorg As in urine (r_s= +0.25, p<0.05 vs r_s= -0.043 not significant; r_s= +0.26, p<0.05 vs. r_s= +0.036 not significant; and r_s= +0.31, p<0.01 vs r_s= +0.015 not significant, respectively). The ratios of % MMA to % inorg As in urine were negatively correlated with the ratios of the concentrations of Se, Zn or Mn to As (r_s= -0.20 not significant; r_s= -0.23, p<0.05; and r_s= -0.27, p<0.05, respectively) in blood. But these correlations were not statistically significant with the concentrations of Se, Zn or Mn. The ratios of the concentrations of Se, Zn, Mn, or Cu to As in blood were negatively correlated with the ratios of % DMA to %MMA in blood for males, but these correlations were not statistically significant.

Concentrations of trace elements in urine expressed as µg/L versus µg/gcre. The mean concentrations of Cd, Pb, Hg, Se, Zn, Co, Cu, Ni, and Mn expressed as µg/L were not significantly difference between females and males with the exception of Zn concentration, which was significantly higher in urine for males compared to females (p<0.05) (Table 9A).

Table 9. Concentrations of trace elements in urine expressed as 'µg/L' and 'µg/g cre' for females (F) and males (M).

	As	Cd	Pb	Hg	Se	Zn	Co	Cu	Ni	Mn
				A.	Mean ± SE	(µg/L)				
F (n=98)	62.05±5.58	0.32±0.03	0.76±0.09	0.36±0.04	42.54±4.64	195.92±20	0.54±0.10	7.42±0.76	2.49±0.33	1.10±0.07
M (n=93)	90.57±8.38	0.44±0.07	0.91±0.09	0.36±0.04	52.33±4.01	301.85±41	0.44±0.05	10.18±1.5	3.59±0.51	1.06±0.09
F vs. M	p<0.01	p>0.05	p>0.05	p>0.05	p>0.05	p<0.05	p>0.05	p>0.05	p>0.05	p>0.05
				B.	Mean ± SE	(µg/g cre)				
F (n=98)	121.43±6.35	0.63±0.04	1.64±0.26	0.82±0.09	94.30±6.01	395.56±31	0.94±0.10	14.67±1.0	5.01±0.47	2.73±0.16
M (n=93)	108.52±6.11	0.47±0.04	1.11±0.08	0.56±0.07	70.09±3.74	358.25±24	0.53±0.05	11.04±0.7	4.02±0.33	1.85±0.17
F vs. M	p>0.05	p<0.01	p>0.05	p<0.05	p<0.001	p>0.05	p<0.001	p<0.01	p>0.05	p<0.001
				C.	'µg/L' vs. 'µg/g cre'					
F (A vs. B)	p<0.0001	p<0.0001	p<0.01	p<0.0001	p<0.0001	p<0.0001	p<0.01	p<0.0001	p<0.0001	p<0.0001
M (A vs. B)	p>0.05	p>0.05	p>0.05	p<0.01	p<0.01	p>0.05	p>0.05	p>0.05	p>0.05	p<0.0001

However, the mean concentrations of elements (Cd, Hg, Se, Co, Cu, and Mn) after creatinine adjustment expressed as ug/g cre were significantly higher in urine for females compared to males (p<0.01, p<0.05, p<0.001, p<0.001, p<0.01, and p<0.001, respectively) with the exception of Pb, Zn, and Ni concentrations (Table 9B). The mean concentrations (µg/g cre) of Pb, Zn, and Ni were also higher in urines for females compared to males but were not statistically significant. After adjustment, the above urinary elements concentrations expressed as µg/g cre were significantly higher than the concentrations of these elements without adjustment expressed as µg/L in urines for females (Table 9C). This was not so for males with the exception for the mean concentrations of Hg (p<0.01), Se (p<0.01), and Mn (<0.0001) after creatinine adjustment.

Concentrations of trace elements expressed as 'µg/L' versus 'µg/g cre' in urine for low concentrations (<= 50 µg As/L) and high concentrations (> 50 µg As/L) As groups. We found that adjusted urinary As and other trace element concentrations were significantly higher than the unadjusted concentrations in the case of the urine groups with arsenic concentrations less or equal to 50 µg As/L urine (Table 10A). These differences were not statistically significant for the urine groups with arsenic concentrations >50 µg As/L urine (Table 10B). The mean urinary creatinine concentrations were significantly low for the lower concentrations As (<= 50 µg/L) in urine groups compared to higher concentrations As (> 50 µg/L) in urine groups (0.32±0.02 g cre/L urine vs. 1.12±0.07 g cre/L urine (p<0.01), respectively). Ages were not significantly difference between these two groups (p=0.12).

Table 10. Difference of concentrations of trace elements expressed as 'µg/L' versus 'µg/g cre' in urine for low concentrations (<= 50 µg As/L urine) and high concentrations (> 50 µg As/L urine) As groups.

	As	Cd	Pb	Hg	Se	Zn	Co	Cu	Ni	Mn
A. <= 50 µg As/ L (females + males, n = 102)										
(I) µg/L urine	26.79±1.07	0.17±0.02	0.44±0.06	0.24±0.03	25.66±1.32	111.21±11.29	0.22±0.03	3.65±0.23	1.63±0.2	0.78±0.04
(II) µg/g cre	91.90±3.41	0.55±0.04	1.50±0.25	0.84±0.09	92.12±5.65	359.93±25.66	0.69±0.06	12.38±0.93	5.10±0.44	2.75±0.15
(I) vs. (II)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
B. > 50 µg/ L (females + males, n = 89)										
(I) µg/L urine	132.27±7.11	0.61±0.08	1.29±0.09	0.49±0.05	72.11±5.37	404.84±42.11	0.80±0.11	14.63±1.54	4.64±0.56	1.42±0.10
(II) µg/g cre	141.79±7.76	0.55±0.04	1.25±0.09	0.53±0.06	71.50±4.26	397.86±31.66	0.79±0.11	13.44±0.86	3.87±0.36	1.79±0.18
(I) vs. (II)	p=0.367	p=0.526	p=0.765	p=0.626	p=0.929	p=0.895	p=0.937	p=0.502	p=0.251	p=0.069

I**nfluence of creatinine adjustment on urinary trace elements concentrations.**The urinary As, Cd, Pb, Hg, Zn, Co, Cu, Ni, and Mn concentrations expressed as µg/L were positively and significantly correlated with the urinary concentrations of these elements expressed as ug/gcre for both females and males with the exception of Se concentrations (Table 11). The correlation between urinary Se concentrations expressed, as µg/L and µg/g cre was not statistically significant for both females and males.

Table 11. Spearman correlation coefficients (r_s) for urinaryelements concentrations, 'µg/L' versus 'µg/g cre' forfemales and males.

	As	Cd	Pb	Hg	Se	Zn	Co	Cu	Ni	Mn
	'µg/L' versus 'µg/g cre'									
Females	+0.532[f]	+0.485[f]	+0.717[e]	+0.611[f]	+0.193	+0.689[f]	+0.706[f]	+0.400[d]	+0.801[f]	+0.428[d]
Males	+0.463[e]	+0.493[f]	+0.560[f]	+0.501[f]	+0.071	+0.461[e]	+0.520[f]	+0.420[d]	+0.793[e]	+0.432[d]

[d]p<0.0001, [e]p<0.00001, [f]p<0.000001

Influence of arsenic and creatinine concentrations on the other trace elements concentrations in urine. Urinary total arsenic concentrations expressed as µg/L were positively and strongly correlated with U-Cre concentrations [r_s= +0.86, p<0.01 (n=191 including females and males both), r_s= +0.85, p<0.01 (n=98 for females), and r_s= +0.86, p<0.01 (n=93 for males)] (manuscript submitted). But after creatinine adjustment, total urinary arsenic concentrations expressed as µg/gcre were slightly and negatively correlated with U-cre (r_s= -0.04, p=0.62). U-cre concentrations (g/L) were also positively and significantly correlated with total arsenic concentrations in blood expressed as µg/L [r_s= +0.24, p<0.01 (n=191 including females and males both)]. Adjusted urinary total As concentrations were positively and significantly correlated with adjusted urinary Cd, Pb, Se, Co, and Cu concentrations for females only (r_s= +0.36, p<0.001; r_s= +0.31, p<0.01; r_s= +0.23, p<0.05; r_s= +0.26, p<0.05; and r_s= +0.30, p<0.01, respectively) (Table 12).

Table 12. Spearman correlation coefficients (r_s) for urinarytotal As or creatinine concentrations versus urinary traceelements concentrations (µg/g cre) for females and males.

	As	Cd	Pb	Hg	Se	Zn	Co	Cu	Ni	Mn
Total As (µg/g cre):										
Females	-	+0.363[c]	+0.307[b]	-0.124	+0.232[a]	+0.162	+0.257[a]	+0.299[b]	-0.173	+0.069
Males	-	+0.141	+0.186	+0.251[a]	+0.112	+0.131	+0.199	-0.031	-0.191	+0.116
Creatinine (g/L):										
Females	+0.053	+0.036	+0.042	-0.283[b]	-0.393[d]	+0.017	+0.117	-0.041	-0.167	-0.445[e]
Males	-0.024	+0.026	0.000	-0.460[e]	-0.404[e]	-0.121	-0.053	+0.056	+0.004	-0.612[f]

[a] p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001, [e]p<0.00001, [f]p<0.000001

On the other hand, urinary creatinine concentrations were negatively and significantly correlated with adjusted urinary Hg, Se, and Mn concentrations (µg/gcre) for both females and males [for females: r_s= -0.28, p<0.01; r_s= -0.39, p<0.0001; and r_s= -0.45, p<0.00001, respectively and for males: r_s= -0.46, p<0.00001; r_s= -0.40, p<0.00001; and r_s= -0.61, p<0.000001, respectively]. But the correlations were not statistically significant for other trace elements when adjusted for urinary creatinine.

Influence of ages on urinary and blood trace elements concentrations. Only urinary Cd concentrations expressed as ug/g cre were positively and significantly correlated with ages for both females and males participants (Table 13).

Table 13. Spearman correlation coefficients (r_s) for ages versus trace elements concentrations in urine samples (µg/L or µg/g cre) for females and males.

	As	Se	Zn	Co	Cu	Pb	Cd	Ni	Mn	Hg
					A. µg/L urine					
Age (years):										
Females	+0.135	+0.032	+0.150	-0.046	+0.058	+0.154	+0.240[a]	-0.083	+0.177	+0.035
Males	+0.088	+0.156	+0.148	+0.152	+0.171	+0.099	+0.322[b]	+0.204	+0.055	+0.021
					B. µg/gm creatinine					
Age (years):										
Females	+0.246[a]	+0.062	+0.168	-0.094	+0.133	+0.170	+0.490[f]	-0.058	+0.166	+0.011
Males	+0.045	+0.126	+0.095	+0.126	+0.263[a]	+0.086	+0.469[e]	+0.177	-0.029	-0.057

[a]p<0.05, [b]p<0.01, [e]p<0.00001, [f]p<0.000001

The correlations were stronger when urinary Cd concentrations were adjusted for urinary creatinine than unadjusted concentrations (females: r_s=+0.49, p<0.000001 vs. r_s= +0.24, p<0.05 and males: r_s=+0.47, p<0.00001 vs. r_s= +0.32, p<0.01, respectively). Adjusted urinary total As concentrations were positively and significantly correlated with ages for females only. There was not significant effect from the ages on blood trace element *(*As, Cd, Pb, Se, Co, or Ni) concentrations except Zn and Mn concentrations for females, and only Cu concentrations for males (Table 4). The concentrations of Zn as well as Cu were positively and the concentrations of Mn were negatively correlated with ages (r_s = +0.22, p<0.05; r_s = +0.28, p<0.01; and r_s = -0.29, p<0.01, respectively).

Discussion

This is the first study to provide the concentrations of trace elements (Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn) in whole blood of the population exposed to arsenic in drinking water living in the Lagunera area of Mexico. For this population in general, the mean concentrations of Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn were 233.89±2.17, 5604.64±50.18, 0.83±0.03, 859.10±1.20, 24.62±0.83, 1.25±0.04, 3.45±0.19, and 15.47±0.23 µg/L, respectively. The concentrations found for Zn, Co, Cu, Pb, Cd, Ni, and Mn in bloods are in the range as in other populations[43-47]. But the concentrations of Se in blood were higher than the other studies[43-45]. The main reason for higher concentrations of Se in blood is that the percentage of Se recovery in blood was high (129 %, for spike value 10 µg/L) and after correction the values are in the range observed in other regions of the world[44,46]. Selenium concentrations in body fluids (for example, blood) are known to be extremely susceptible to changes in dietary intake and reflect even short-term variations in input[44]. The major dietary source of selenium is plant foods, but some meats and seafood can also contribute dietary selenium.

The concentrations of Cu and Zn in blood of our study population were within the range reported from other countries for adults[44,48]. But blood selenium (BSe) and blood cobalt (BCo) concentrations were higher compared to other countries[45,49]and BMn concentrations were close to other[49]. The mean concentration of Cd was similar[49], but Pb level was lower to those reported from other countries for healthy adults[44,50].

The mean concentrations of the essential elements in blood follow the order: Zn> Cu> Se> Mn> Ni> Co in our present work. Rahil-Khazen et al (2002)[51] have been reported that the concentrations of the essential elements in most tissues (brain, cerebellum, heart, kidney, liver, pancreas, spleen, and ovary from normal organs) followed the order Fe> Zn> Cu> Mn> Se> Cr> Co (except in the ovary where Se was higher than Mn) which is almost similar with our study results in blood. Therefore, may be the concentrations of these essential elements in tissues of our study people in Mexico follow the order found in blood.

Sex differences in blood trace elements concentrations. In this study, the concentrations of trace elements As, Pb, Zn, Cu, and Mn in blood were all influenced by sex. The females had significantly lower concentrations of As, Pb, and Zn, but higher concentrations of Cu and Mn in blood compared to males. The findings could be explained by differences between females and males in both exposure levels and element absorption. The bioavailability of Cu, Zn, and Mn are markedly influenced by the amount of Cu, Zn, and Mn in the diet. May be due to higher As methylation and excretion capacity for females compared to males, the mean As level in blood was significantly lower for females. It is also known that the compositions of whole blood samples from female and male are different. Due to lower concentration of red cells in the female it is expected that the concentration of elements which are bounded to the red cells will be lower in blood samples from female than from male[44]. Previous report shows that females have higher blood Mn concentrations than males[52]. Lower iron levels increase the absorption of Mn, explaining the higher leves of Mn observed in females[53]. It was also reported that enhanced blood Mn levels have been observed in patients with liver failure[54]. Other studies reported that the mean Cu level was significantly higher, and Zn & Pd levels were lower in blood for females than from males[44,47,55,56].

Influence of ages on the concentrations of elements in blood. The concentrations of Zn in blood of females significantly increased and their Cu concentrations decreased with age, which has also been shown by Rodrigues et al. (2009)[47]. For males, these correlations were reversed and the concentrations of Cu in blood were positively and significantly correlated with age. We also found that the concentrations of Mn in blood of both females and males were negatively correlated with age and the correlation was statistically significant for females. The influence of age on blood Mn concentrations has not been demonstrated before as far as we know. The changes of lifestyle (food and drinking habits) could be a part of the explanation of the changes with age.

Blood or urinary trace elements concentrations could be a biomarker. Results show that drinking water As (WAs) concentrations (µg/L) were positively and strongly correlated with blood arsenic (BAs) concentrations (µg/L) for both females and males. BAs concentrations (µg/L) were also positively and strongly correlated with urinary arsenic (UAs) concentrations expressed as µg/L or µg/g creatinine of both females and males. Similarly to our findings, Hall et al. (2006)[57] found a strong positive correlation between BAs and UAs, and both were positively and significantly correlated with WAs. Therefore, blood arsenic could be use as a biomarker of arsenic exposure too and it would be a better biomarker than UAs. Major advantage of BAs measurements is that, unlike UAs, adjustments for creatinine are not necessary. Several demographic variables such as age, sex, food habits, race/ethnicity, and BMI are significantly predictors for urinary creatinine[9,10,38], thus confusing the expression of UAs as µg/g creatinine. We also found that urinary creatinine concentrations were significantly correlated with the percentage of urinary arsenic metabolites, % MMA^V as well as % DMA^V (Manuscript submitted). Elimination of the complexities introduced by adjustment of any urinary analytes for creatinine appears to offer a major advantage to the use of blood concentration as a biomarker.

Strong positive correlation found between BAs and BCo (blood cobalt) as well as BAs and BNi (blood nickel) of both females and males. These correlations have not been reported in humans before. Norwood et al. (2007)[58] has been observed that arsenic bioaccumulation in the amphipod *Hyalella azteca* was enhanced with increased the concentration of metals including Co and Ni in the mixture exposure. So, it is important to know that arsenic accumulation in the tissues of animal model would be enhanced(or would not be enhanced), with increasing the concentrations of Co and/or Ni in the mixture exposure, and this may help to know the mechanisms of arsenic toxicity in human tissues.

Influence of the relative concentrations of trace elements to arsenic on urinary arsenic metabolites Our results show that blood as well as urinary As concentrations were negatively correlated with urinary % inorgAs as well as % MMA and positively correlated with % DMA, the ratios of % MMA to % inorgAs as well as the ratios of % DMA to % MMA of both females and males (Manuscript submitted). We found remarkable results in our study that these correlations were reversed when correlated with the ratios of the concentrations of other element (Se, Zn, Mn, Cu, Cd, Ni, or Hg) to As instead of As concentrations in blood or urine and urinary arsenic metabolites. We also found that the correlations between the ratios of the concentrations of above element to As in blood as well as urine and urinary arsenic metabolites were stronger than the correlation found between the concentrations of the element (Se, Zn, Mn, Cu, Cd, Ni, or Hg) in blood as well as urine and urinary arsenic metabolites with the exception of Ni concentrations in blood. In blood, the correlation was stronger between the concentrations of Ni and urinary arsenic metabolites than the correlation between the ratio of the concentrations of Ni

to As and urinary arsenic metabolites. The results suggest that other element may have influenced for biotransformation process of inorganic arsenic and decrease the methylation process of inorg-arsenic with increasing values of the ratios of concentrations of above element to As i.e., this influence might be concentration dependent. The current literature suggests that reduced methylation capacity with increased MMA^V percentage, decreased DMA^V percentage, or decreased the ratios of % DMA to % MMA is associated with skin lesions, skin cancer, bladder cancer, peripheral vascular disease, muscle cramps and structural chromosomal aberrations in peripheral lymphocytes obtained from inorganic arsenic exposed subjects[59-63]. Results also suggest that more than one metylase may involve in the inorg-arsenic biotransformation pathway because the ratios of the concentrations of above element to As were positively correlated with % MMA, but negatively correlated with % DMA. Similarly to our findings, other researchers have also been suggested that more than one methylase are involved in the oxidative methylation of inorg As[11,64,65].

Sex differences urinary trace elements concentrations as µg/L vs. µg/g cre. In our study, U-Cre concentration was significantly less in urine of females (0.52±0.04 g/L) compared to males (0.88±0.08 g/L) which is similar to that observed in other studies[9,10,38,39]. The urinary creatinine concentrations in the USA, Europe, and Japan are about 1 g/L for females and 1.5 g/L for males[9,66-69]. This means that urinary arsenic and other elements concentrations in Mexican of our study area would be higher than those of similarly exposed people in the other population (For example, USA, Europe, and Japan) when adjusted with urinary creatinine.

The urinary concentrations of trace elements (Se, Co, Cu, Cd, Mn, and Hg) were significantly higher in urine for females compared to males when expressed as µg/g cre. Due to low concentrations of U-cre for females compared to males, urinary element concentrations expressed as µg/L were significantly lower than the concentrations of these elements adjusted for urinary creatinine (µg/g cre) for females, but not for males. The results indicate that may be we are measuring the concentrations of trace elements in urines more than the actual concentrations when adjusted for urinary creatinine. Specially, at low concentration of both element and creatinine in urine, the difference of the concentration expressed as µg/L vs.µg/g cre should be more significant.

In this study, we found that urinary As and other trace elements concentrations expressed as µg/L were positively and significantly correlated with the concentrations of the corresponding element after creatinine adjustment (µg/g cre) for both females and males. Other researcher has been reported that there was a positive and significant correlation between the adjusted and unadjusted UAs concentrations[33].

Urinary As versus urinary Se. The urinary total As concentrations expressed as µg/L were positively and significantly correlated with urinary other trace elements (Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, and Hg) concentrations expressed as µg/L for both females and males (data not shown). But, a strong and positive association between As and other elements expressed as µg/L could be biased by the variability in the dilution of the urines. However, urinary As concentrations were positively and significantly correlated with urinary Se concentrations, which is similar to that observed in other studies[70,71]. They reported the correlation between urinary As and urinary Se concentrations without adjustment for urinary creatinine. To our knowledge, this will be the first reporting that UAs concentrations are also positively and significantly correlated with urinary Se concentrations for females after creatinine adjustment. The results indicate that Se may work better to release As from the body for females compared to males.

Influence of Se, Mn, or Hg on the formation of creatinine and inorg-As biotransformation process. Urinary creatinine concentrations were positively and significantly correlated with As concentrations expressed as µg/L in urine for both sexes. The results suggest that creatinine may influence to release arsenic from the body with unknown mechanisms. Recently, a study also reported that urinary creatinine concentrations were positively correlated with UAs concentrations expressed as µg/L[10,38]. In our study, we found important results that adjusted urinary Se, Mn, and Hg concentrations expressed as ug/g cre were significantly and negatively correlated with urinary creatinine for both sexes. We also found a statistically significant and positive correlation between Mn and Hg in urine for females and males (data not shown). Other studies reported that the combination of Mn and Hg might be more injurious to the brain, perhaps due to their synergistic effect[72]. The effect of Hg administered or exposed on renal dysfunction has been reported[73-75]. Selenium intoxication with selenite broth resulting in acute renal failure has also been reported[76]. Therefore, Se, Mn, and/ or Hg may have inhibitory effects in renal function and forming less creatinine when increasing Se, Mn, and Hg concentrations. Boeniger et al. (1993)[36] reported that 15-20% of the creatinine in urine could occur by active secretion from the blood through the renal tubules, i.e., urinary creatinine is influenced by renal function, which could have some unclear function for arsenic methylation process. We have already reported that urinary creatinine concentrations were strongly and positively correlated with % DMA in urines. Therefore, DMA formation should be decreased when creatinine formation will be decreased. On the other hand, creatinine concentrations will be decreased when Se, Mn, and Hg concentrations will be increased, i.e., DMA concentrations should be decreased. These results also confirmed our previous finding that % DMA and the ratio of % DMA to % MMA were decreased with increasing Se, Mn, and Hg concentrations expressed as ug/g cre in urine. The correlation was stronger for males compared to females. These results suggest that may be Mn and Hg are more potent inhibitor for MMA methyltransferase in males compared to females, and producing less % DMA in males compared to females. Due to that more MMA (highly toxic) accumulated in tissues of males compared to females. Other researchers have been reported that Se and Hg decreased As methylation[15,77-79]. They also suggested that the synthesis of DMA from MMA might be more

susceptible to inhibition by Se(IV)[79]as well as by Hg(II)[15,78]compared to the production of MMA from inorg-As(III).

A possible molecular link between As, Se, and Hg has been proposed by Korbas et al. (2008)[80]. The identifying complexes between the interaction of As and Se, Se and Hg as well as As, Se, and Hg in blood of rabbit has been reported[80,81]. The inhibitory effects of Se and Hg were concentration dependent[15,77-79].

Age differences urinary trace elements concentrations as µg/L vs. µg/g cre. The total urinary As concentrations expressed as µg/g cre were positively and significantly correlated with age for females, but the correlation was not statistically significant with As concentrations expressed as µg/L. We also found that Cd concentrations expressed as µg/L or µg/g cre, were positively and strongly correlated with ages for both sexes. The correlation between age and urinary As as well as Cd concentrations was more significant when adjusted for urinary creatinine, i.e., maybe there is a correlation in the mechanisms for excretion of As, Cd, and formation of creatinine as well as creatinine in urines for older people. Another study shown a significant positive correlation between age and urinary Cd concentrations, expressed as µg/g cre[82].

Conclusions.: The results of this study suggest to conclude the following: (a) The relative concentrations of other trace elements to arsenic may influence the biotransformation process of inorganic arsenic in humans, (b) Low As concentrations, in presence of comparatively higher concentrations of other trace elements (e.g., Se, Hg, Mn etc) may cause higher arsenic toxicity due to decreased methylation of highly toxic arsenic species to less toxic arsenic species (especially, MMA^{III} to DMA^{V}) causing subsequent increases in the tissue deposition of Inorg-As(III) *(*arsenite), and increased synthesis of highly toxic MMA^{III} (monomethylarsonous acid), (c) Urinary creatinine adjustment may highly over estimate of urinary trace elements concentrations, especially at low level of urinary creatinine due to low level of As in drinking water, (d) The results also suggest that Se, Mn as well as Hg may decrease arsenic methylation with decreasing creatinine formation for both sexes, but it could be concentration dependent.

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References

1. IARC (International Agency for Research on Cancer). 1987. In*IARC Monograph on the Evaluation of Carcinogenicity Risk to Humans. Overall Evaluation of Carcinogenicity:* An Update of IARC Monographs 1-42 (Suppl. 7). Lyon, France: International Agency for Research on Cancer, pp. 100-106.
2. NRC (National Research Council). 2001. *Arsenic in Drinking Water.* Update to the 1999 Arsenic in Drinking Water Report. Washington, DC: National Academy Press.
3. Chen, C.J., Chen, C.W., Wu, M.M., Kuo, T.L. 1992. Cancer potential in liver, lung, bladder, and kidney due to ingested inorganic arsenic in drinking water. Br. J. Cancer 66, 888-892.
4. Rossman, T.G., Uddin, A.N., Burns, F.J. 2004. Evidence that arsenite acts as a cocarcinogen in skin cancer. Toxicol. Appl. Pharmacol. 198, 394-404.
5. Huang, Y.K., Tseng, C.H., Huang, Y.L., Yang, M.H., Chen, C.J., Hsueh, Y.M. 2007. Arsenic methylation capacity and hypertension risk in subjects living in arseniasis-hyperendemic areas in southwestern Taiwan, Toxicol. Appl. Pharmacol. 218, 135-182.
6. Tseng, C.H. 2007. Metabolism of inorganic arsenic and non-cancerous health hazards associated with chronic exposure in humans. J. Environ. Biol. 28, 349-357.
7. Hall, M., Chen, Y., Ahsan, H., Slavkovich, V., van Geen, A., Parvez, F., Graziano, J. 2006. Blood arsenic as a biomarker of arsenic exposure: Results from a prospective study. Toxicology 225, 225-233.
8. Klassen, C.D. 2001. Casarett and Doull Toxicology: The Basic Science of Poisons (6th Edition).
9. Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L. 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192-200.
10. Gamble, M.V., Liu, X. 2005. Urinary creatinine and arsenic metabolism. Environ. Health Perspect. 113, A442-A443 (author reply).
11. Aposhian, H.V., Aposhian, M.M., 2006. Arsenic toxicology: Five questions. Chem. Res. Toxicol. 19, 1-15.
12. Tseng, C.H. 2009. A review on environmental factors regulating arsenic methylation in humans. Toxicol. Appl. Pharmacol. 235, 338-350.
13. Cohen, S.M., Arnold, L.L., Eldan, M., Lewis, A.S., Beck, B.D., 2006. Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. Crit. Rev. Toxicol. 36, 99-133.
14. Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology, 181-182, 211-217.
15. Zakharyan, R.A., Wu, Y., Bogdan, G.M., Aposhian, H.V., 1995. Enzymatic methylation of arsenic compounds: Assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem. Res. Toxicol. 8,

1029-1038.

16. Steinmaus, C., Bates, M.N., Yuan, Y., Kalman, D., Atallah, R., Rey, 0.A., Biggs. M.L., Hopenhayn, C., Moore, L.F., Hoang, B.K., Smith, A.H. 2006. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. J. Occup. Environ. Med. 48, 478-488.
17. Lindberg, A.-L., Rahman, M., Persson, L.-A., Vahter, M., 2008. The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. Toxicol. Appl. Pharmacol. 230, 9-16.
18. Petrick, J.S., Jagadish, B., Mash, E.A., Aposhian, H.V., 2001. Monomethylarsonous acid MMA(III) and arsenite: LD50 in hamsters and in vitro inhibition of pyruvate dehydrogenase. Chem. Res. Toxicol. 14, 651 656.
19. Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch. Toxicol., 74, 289-299.
20. Chen, Y., Hall M., Graziano, J.H., et al., 2007. A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions. Cancer Epidemiol. Biomark. Prev. 16, 207-213.
21. Steinmaus, C., Carrigan, K., Kalman, D., Atallah, R., Yuan, Y., Smith, A.H., 2005. Dietary intake and arsenic methylation in a U.S. population. Environ. Health Perspect. 113, 1153-1159.
22. Vahter, M., Akesson, A., Liden, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ. Res. 104, 85-95.
23. Wuyi, W., Linsheng, Y., Shaofan, H., Jian'an, T., Hairong, L., 2001. Prevention of endemic arsenism with selenium. Curr. Sci. 81, 1215-1218.
24. Yang, L., Wang, W., Hou, S., Peterson, P., Williams, W., 2002. Effects of selenium supplementation on arsenism: an intervention trial in Inner Mongolia. Environ. Geochem. Health 24, 359-374.
25. Huang, Z., Pei, Q., Sun, G., Zhang, S., Liang, J., Gao, Y., Zhang, X., 2008. Low selenium status affects arsenic metabolites in an arsenic exposed population with skin lesions. Clinica Chimica Acta 387, 139-144.
26. Milton, A., Zalewski, P., Ratnaike, R., 2004. Zinc protects against arsenic-induced apoptosis in a neuronal cell line, measured by DEVD capase activity. Biometals 17*,* 707-713.
27. Hambidge, M., 2003. Biomarkers of trace mineral intake and status. J. Nutr. 133, 9489-9555.
28. Angerer, J., Eweres, U., Wihelm, M., 2007. Human biomonitoring: state of art. Int. J. Hyg. Environ. Health 210. 201-228.
29. Bryan, C.E., Cristopher, S.J., Balmer, B.C., 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci. Total Environ. 388, 325-342.
30. Parsons, P.J., Barbosa, Jr.F., 2007. Atomic spectrometry and trends in clinical laboratory medicine. Spectrochim. Acta B 62, 992-1003.
31. CDC (Centers for Disease Control and Prevention), 2005. Third National Report on Human Exposure to Environmental Chemicals, Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.
32. Suwazono, Y., Akesson, A., Alfven, T., Jarup, L., *V*ahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117 126.
33. Hinwood, A.L., Sim, M.R., de Klerk, N., Drummer, O., Gerostamoulos, J., Bastone, E.B., 2002. Are 24-hour urine samples and creatinine adjustment required for analysis of inorganic arsenic in urine in population studies? Environ. Res. Section A 88, 219-224.
34. Davies, K.M., Heaney, R.P., Rafferty, K., 2002. Decline in muscle mass with age in women: a longitudinal study using an indirect measure. Metabolism 51, 935-939.
35. Hall Moran, V., Leathard, H.L., Coley, J., 2001. Urinary hormone levels during the natural menstrual cycle: the effect of age. J. Endocrinol. 170, 157-164.
36. Boeniger, M.F., Lowry, L.K., Rosenberg, J., 1993. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am. Ind. Hyg. Assoc. J. 54, 615-627.
37. Mage, D.T., Allen, R., Gondy, G., Smith, W., Barr, D.B., Needham, L.L., 2004. Estimating pesticide dose from urinary pesticide concentration data by creatinine correction in the Third National Health and Nutrition Examination Survey. J. Expo. Anal. Environ. Epidemiol. 14, 457-465.
38. Gamble, M.V., Liu, X., Ahsan, H., Pilsner, R., Ilievski, V., Slavkovich, V., Parvez, F., Levy, D., Factor-Litvak, P., Graziano, J.H., 2005. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ. Health Perspect. 113, 1683-1688.
39. Nermell, B., Lindberg, A.-L., Rahman, M., Berglund, M., Persson, L.A., Arifeen, S., Vahter, M., 2008. Urinary arsenic concentration adjustment factors and malnutrition. Environ. Res. 106, 212-218.
40. Reuter, W., Davidowski, L., Neubauer, K., 2003. Speciation of five arsenic compounds in urine by HPLC/ICP-MS. PerkinElmer Life &Analytical Sciences. Available: http://las.perkinelmer.com/content applicationnotes/appspeciationfivearseniccompounds.pdf
41. Goulle, J-P., Mahieu, L., Castermant, J., Ne*v*eu, N., Bonneau, L., Laine, G., Bouige, D., Lacroix, C. 2005. Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair reference values. Forensic Sci. Int. 153, 39-44.
42. Bartels, H., Bahmer, M., Heierli, C., 1972. Serum creatinine determination without protein precipitation. Clin. Chem. Acta 37, 193-197.
43. Barany, E., Bergdahl, I.A., Bratteby, L.E., Lundh, T., samuelson, A.G., Schutz, B.A., et al. 2002a. Trace elements in blood and serum of Swedish adolescents: relation to gender, age, residential area, and socioeconomic status. Environ. Res. 89, 72-84.

44. Lyengar, C.V. 1987. Reference values for the concentrations of As, Cd, Co, Cr, Cu, Fe, I, Hg, Mn, Mo, Ni, Pb, Se, and Zn in selected human tissue and body fluids. Biol. Trace. Elem. Res. 12, 263-295.
45. Minoia, C., Sabbioni E., Apostoli, P., Pietra, R., Pozzoli, L., Gallorini, M., et al. 1990. Trace element reference values in tissues from inhabitants of the European community I. A study of 46 elements in urine, blood and serum of Italian subjects. Sci. Total Environ. 95, 89-105.
46. Moreno, M.A., Marin, C., Vinagre, F., Ostapczuk, P. 1999. Trace elements in whole blood samples from residents of the city Badajoz, Spain. Sci. Total Environ. 229, 209-215.
47. Rodrigues, J.L., Batista, B.L., Fillion, M., Passos, C.J.S., Mergler, D., Barbosa Jr. F. 2009. Trace element levels in whole blood of riparian villagers of the Brazilian Amazon. Sci. Total Environ. (**In press**).**???**
48. Bonforte, G., Surian, M., Dozio, B., Scanziani, R., Baj1, A., Colombo1, S., ToVoletto1, F., 1998. Plasma or whole blood concentrations of trace elements in patients treated by haemodiafiltration with on-line prepared substitution fluid. Nephrol Dial Transplant 13 [Suppl 5], 29–33.
49. Goulle, J-P., Mahieu, L., Castermant, J., Neveu, N., Bonneau, L., Laine, G., Bouige, D., Lacroix, C. 2005. Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair reference values. Forensic Sci. Int. 153, 39-44.
50. Kristiansen, J., Christensen, J.M., Iversen, B.S., Sabbioni, E. 1997. Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors. Sci. Total Environ. 204, 147-160.
51. Rahil-Khazen, R.R., Bolann, B.J., Mykinh, A., Ulvik, R.J., 2002. Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). J. Trace Elem. Med Biol. 16, 15-25.
52. Baldwin, M., Mergler, D., Larribe, F., Belanger, S., Tardif, R., Bilodeau, L., Hudnell, K., 1999. Bioindicator and exposure data for a population based study of manganese. Neurotoxicology 20, 343-353.
53. Finley, J.W., 1999. Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr. 70, 37-43.
54. Krieger, D., Krieger, S., Jansen, O., Gass, P., Theilman, L., Lichtnecker, H., 1995. Manganese and chronic hepatic encephalopathy. Lancet 346, 270-274.
55. Pirkle, J.L., Kaufmann, R.B., Brody, D.J., Hickman, T., Gunter, E.W., Paschal, D.C., 1998. Exposure of the U.S. population to lead, 1991-1994. Environ. Health Perspect. 106, 745-750.
56. Vahter, M., Akesson, A., Liden, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ. Res. 104, 85-95.
57. Hall, M., Chen, Y., Ahsan, H., Slavkovich, V., van Geen, A., Parvez, F., Graziano, J. 2006. Blood arsenic as a biomarker of arsenic exposure: Results from a prospective study. Toxicology 225, 225-233.
58. Norwood, W.P., Borgmann, U., Dixon, D.G. 2007. Interactive effects of metals in mixtures on bioaccumulation in the amphipod *Hyalella azteca.* Aquatic Toxicol. 84, 255-267.
59. Tseng, C.H. 2007. Metabolism of inorganic arsenic and non-cancerous health hazards associated with chronic exposure in humans. J. Environ. Biol. 28, 349-357.
60. Del Razo, L.M., Garcia-Vargas, G.G., Vargas, H., Albores, A., Gonsebatt, M.E., Montero, R., Ostrosky-Wegman, P., Kelsh, M., Cebrian, M.E., 1997. Altered profile of urinary arsenic metabolites in adults with chronic arsenicism: A pilot study. Arch. Toxicol. 71, 211-217.
61. Yu, R.C., Hsu, K.H., Chen, C.J., Froines, J.R. 2000. Arsenic methylation capacity and skin cancer. Cancer Epidemiol. Biomarkers Prev. 9, 1259 1262.
62. Chen, Y.C., Guo, Y.L., Su, H.J., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Chao, S.C., et al. 2003. Arsenic methylation and skin cancer risk in southwestwen Taiwan. J. Occup. Environ. Med. 45, 241-248.
63. Maki-Paakkanen, J., Kurttio, P., Paldy, A., Pekkanen, J. 1998. Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. Environ. Mol. Mut. 32, 301-313.
64. Chowdhury, U.K., Zakharyan, R.A., Hernandez, A., Avram, M.D., Kopplin, M.J., Aposhian, H.V., 2006. Glutathione-S-transferase-omega [MMA(V) reductase] knockout mice: Enzyme and arsenic species concentrations in tissues after arsenate administration. Toxicol. Appl. Pharmacol. 216, 446-457.
65. Loffredo, C.A., Aposhian, H.V., Cebrian, M.E., Yamauchi, H., Silbergeld, E.K., 2003. Variability in human metabolism of arsenic. Environ. Res. 92, 85-91.
66. Heavner, D.L., Morgan, W.T., Sears, S.B., Richardson, J.D., Byrd, G.D., Ogden, M.W., 2006. Effects of creatinine and specific gravity normalization techniques on xenobiotic biomarkers in smokers' spot and 24-h urines. J. Pharm. Biomed. Anal. 40, 928-942.
67. Moriguchi, J., Ezaki, T., Tsukahara, T., Fukui, T., Ukai, H., Okamoto, S., Shimbo, S., Sakurai, H., Ikeda, M., 2005. Decreases in urinary specific gravity and urinary creatinine in elderly women. Int. Arch. Occup. Environ. Health 78,438-445.
68. Poortmans, J.R., Boisseau, N., Moraine, J.J., Moreno-Reyes, R., Goldman, S., 2005. Estimation of total-body skeletal muscle mass in children and adolescents. Med. Sci. Sports Exerc. 37, 316-322.
69. Suwazono, Y., Akesson, A., Alfven, T., Jarup, L., Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117 126.
70. Christian, W.J., Hopenhayn, C., Centeno, J.A., Todorov, T., 2006. Distribution of urinary selenium and arsenic among pregnant women exposed to arsenic in drinking water. Environ. Res. 100, 115-122.
71. Hsueh, Y-M., Ko, Y-F., Huang, Y-K., Chen, H-W., Chiou, H-Y., Huang, Y-L., Yang, M-H., Chen, C-J., 2003. Determinates of inorganic arsenic methylation capacity among residents of the Lanyang Basin, Taiwan: arsenic and

selenium exposure and alcohol consumption. Toxicol. Lett. 137, 49-63.

72. Shukla, G. S., Chandra, S. V., 1982. Effect of interaction of Mn2+ with Zn2+, Hg2+, and Cd2+ on some neurochemicals in rats. Toxicol. Lett. 10, 163-168.
73. Flamenbaum, W., 1973. Pathophysiology of acute renal failure. Arch. Intern. Med. 131, 911-928.
74. Jarosinska, D., Horvat, M., Sallsten, G., Mazzolai, B., Dabkowska, B., Prokopowicz, A., Biesiada, M., Barregard, L., 2008. Urinary mercury and biomarkers of early renal dysfunction in environmentally and occupationally exposed adults: A three-country study. Environ. Res. 108, 224-232.
75. Tian, L., Guo, H. F., Gao, A., Lu, X. T., Li, Q. Y., 2009. Effects of mercury released from gold extraction by amalgamation on renal function and environment in Shanxi, China. Bull Environ. Contam. Toxicol. 83, 71-74.
76. Kamble, P., Mohsin, N., Jha, A., Date, A., Upadhaya, A., Mohammad, E., Khalil, M., Pakkyara, A., Budruddin, M., 2009. Selenium intoxication with selenite broth resulting in acute renal failure and severe gastritis. Saudi J. Kidney Dis. Transplant. 20, 106-111.
77. Kenyon, E. M., Hughes, M. K., Levander, O. A., 1997. Influence of dietary selenium on the disposition of arsenate in the female B6C3F1 mouse. J. Toxicol. Environ. Health 51, 279-299.
78. Siyblo, M., Delnomdedieu, M., Thomas, D. J., 1996. Mono- and dimethylation of arsenic in rat liver cytosol in vitro. Chem. Biol. Interact. 99, 147-164.
79. Styblo, M., Thomas, D, J., 2001. Selenium modifies the metabolism and toxicity of arsenic in primary rat hepatocytes. Toxicol Appl. Pharmacol. 172, 52-61.
80. Korbas, M., Percy, A. J., Gailer, J., George, G. N. (2008). A possible molecular link between the toxicological effects of arsenic, selenium and methylmercury: methylmercury (II) seleno bis (S glutathionyl) arsenic (III). *J. Biol. Inorg. Chem*. 13, 461-470.
81. Gailer, J. (2007). Arsenic-selenium and mercury-selenium bonds in biology. *Coord. Chem. Rev.* 251, 234-254.
82. Suwazono, Y., Akesson, A., Alfven, T., Jarup, L.; Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117 126.