Original Research Article

Heavy Metals Analysis in Human Body (Toenail, Fingernail and Hair Samples) and Drinking Waters of Santa Fe Region, Argentina

ABSTRACT

This study provides an insight into a province of Santa Fe region of a developing country, namely San Cristobal and Huanqueros, Argentina and possible link between toenail, fingernail and hair in arsenic, copper and iron of the population. Multivariate statistical tool known as Principal Component Analysis (PCA) was applied to explain the behaviour of the elements in toenails, fingernails, drinking waters and hair using multibase 2013 excel add ins. Correlation test, error bars, and a 2-factor ANOVA tools were employed. Results from one hundred and twenty nine (n=129) samples of tap well water (n=23), rain water (n=20), bottled water (n=6) and treated well water (n=80) and each of toenail, fingernail and hair (n=129) samples from the subjects were determined and the results compared with the previous works. Mean, standard deviation, covariance and maximum and minimum for each variable were reported. The hypothesis is to understand if there is correlation between fingernail and toenail metals levels and make comparison with previous researches. From the results, positive correlation exists between fingernail and toenail metals concentrations. Also the study reveals higher concentrations of arsenic, copper and iron in the samples tissues compare with the values available in the previous works. The elevated levels of these metals may be attributed to the drinking water sources. Since this study highlighted elevated levels of these metals, consumptions of contaminated drinking water should be constantly monitored. Finally, application of multivariate statistical techniques can provide powerful information on heavy metals bioaccumulation analysis in human and environment.

Keywords: Heavy Metals; Drinking Water; Human Nail; Human Hair; Principal Component Analysis.

1. INTRODUCTION

Heavy metals are naturally found on the earth crust, and they are generally regarded as environmental contaminant because they can neither be destroyed nor degraded. But to some point, significant amount of these metals enter into the human body system via drinking water, food and air and gradually accumulated [1]. Heavy metals exist in forms of their ores in various types of rocks, through which they are converted into minerals. Sulphides ores include arsenic, iron, cobalt, lead-zinc, nickel sulphides and gold-silver [2]. While the ore oxides are antimony, gold, aluminium, selenium and manganese. Iron, cobalt and copper can all exist in forms of oxide and sulphide ores [2]. Trace metals contamination of underground and surface water has resulted in considerable water pollution as the mining activities increases on the ground surface [3]. The generation of acid mine to air and rain is as a result of surface dumping. The presence of heavy metals in agricultural soils causes their accumulation in the plants tissues [4]. Animal grazing on such contaminated grass can also be affected [5]. Individuals have been poisoned through-exposure to these metals via taking contaminated animals and plants food sources, and a lot of these cases have been associated to cause numerous body systems disorders. Generally, Food chain along the ecosystem has been the major channel through which all living organisms are contaminated [6].

There has been constant research and progress made in the area of heavy metals analysis in human body from drinking water using different biomarkers and statistical methods. Environmental Scientists and Engineers continued to show much interest in hair and nail for heavy metals determinations [7]. Hair as one of the excretory organ gives and reflects good metabolic mineral activities in the body. Although, the results show a significant concentrations differences when compared to others [8]. It has been observed that, human hair as biological biomarker is so attractive due to its simplicity in handling, sampling, transport as well as providing more details interms of concentrations of heavy metals and easy analysis compared to other biological materials [9]. Human nails keep a good records of elemental composition and concentrations in human body, unlike other fluids in the body that gives a transient concentrations [10].The growth of human nails continues throughout life span ranging from 0.05 and 1.2 mm per week but the toenail growing is very slow which indicates metals integration is also slow normally at 30- 50 % rate [10]. Abdulrahman et al. [11] carried out a study to analyze the concentrations of arsenic, lead, manganese, copper, cobalt, nickel, chromium, cadmium, iron, and zinc in human toenail, hair and fingernail samples of different age and sex of people doing iron welding works, non-liquor and liquor users of Maiduguri town, northern part of Nigeria. The results indicated that Zn showed the highest concentrations while Cu showed the least levels. The results of the study also showed no contribution of liquor towards hair and nail metals concentrations [11]. The levels of all metals under considerations were higher in the toenail than fingernail samples [11]. Similarly, iron welding workers showed higher concentrations of heavy metals when compared to liquor users. The levels of all the metals studied were statistically higher in male than female subjects [11]. While the concentrations of heavy metals in nail samples were significantly higher when compared to hair samples. Therefore, these metals are said to be present in the individual work place as well as in the environment [11]. A similar work was conducted by Eman et al. [12] to investigate if the higher level exposure through drinking water containing high content of heavy metals contaminants in both the bottled sources and that of tap can have an adverse effect on consumers. The study highlighted the higher concentrations of heavy metals in tap water when compared to bottled water and how those heavy metals contaminants in both the two sources can adversely affects the functions of haemoglobin and kidney of consumers. About 28 different geothermal features from the Waikato region and Taupo volcanic zone (TVZ) New Zealand, were reported to content a significant concentrations of Total arsenic and four of its species; monomethylarsonic acid, arsenate, arsenite and dimethylarsonic acid [13]. In Tokaanu, three features were identified to contain the highest levels of arsenic concentrations (8.59, 8.70 and 9.08 mg/L As). Geothermal waters were predominantly inorganic arsenic species, with more than 70% arsenite of total arsenic in most of the samples [13].The study highlighted a serious risk to human health due to the high levels of arsenite as it linked to bathing pools [13]. Biomarkers that are often used to evaluate environmental exposure to arsenic and selenium (and other trace elements) in relation to human health and diseases are hair and nails [14, 15, 16]. These tissues provide a stable sample media for assessing chronic exposure of trace elements in the environment due to their slow growth; offer an exposure window of the past 6 – 12 months [14]. Many elements accumulate in the hair and nail following exposure, in particular arsenic due to the presence of keratin, a sulphydryl-rich protein [14, 15]. In contrast, blood serum is used to measure acute or short- term exposure to heavy metals in relation to human health disorders and diseases [14, 17]. This study was aimed at examining the levels of some heavy metals in drinking water sources of the studied area (San Cristobal and Huanqueros), the residents mostly depend on their individual source of drinking water such as tap, well, rain water rather than treated water due to lack of accessibility and therefore they are vulnerable. .

Principal Component Analysis (PCA) is a method applied widely for multivariate data analysis. The new plots or graphs produced by PCA provide an insight into the structure of a data set and the relationship between the measured variables [18]. The dimensionality reduction of the data set is achieved by transforming original variables into a new set of variables in form of Principal Component (PCs). The PCs are then ordered such that the first few PCs retain most of the variation present in all of the original variables [19, 20]. These newly generated variables refer to as PCs explain the original correlated variables. Individual PCs describes different properties, samples with similar properties are grouped together in their respective PC score plots [19, 20]. To understand a more complex relationship between heavy metals, a multivariate statistical tool, principal component analysis was used [21]. Principal Component Analysis was further applied to investigate the behaviour of ten elements present in nails and hair [21]. There is no single, recommended procedure to conducting PCA [19, 22]. Choosing of the appropriate technique to employ requires proper examination of the original data and understanding of the computational impacts of applying different approaches.

2. MATERIALS AND METHODS

2.1 Study Region

San Cristobal and Huanqueros are situated in the province of Santa Fe and lie within the Chaco-Pampean plain. This region has naturally high-arsenic levels in the groundwater and a number of health problems, including diabetes, are observed within the population that may be associated with arsenic exposure from drinking water. San Cristobal is the main town in the region and Huanqueros is a smaller town situated about 35 km north of San Cristobal (Fig. 1). Both towns have no communal drinking water network or sewage system in place; instead each house has their own well and septic tank. There are 5 water treatment plants in San Cristobal and one in Huanqueros. All plants except one in San Cristobal town and the one in Huanqueros charge a fee for 25 L of the treated water. However, the free water is only available at certain times of the day, on certain days of the week. Generally well-water (groundwater) is used for cleaning and for drinking purposes only, though many lower socioeconomic families cannot afford the treated water, thus relies on their well-water for drinking purposes [13].

Fig. 1. Map of San Cristobal and Huanqueros Source [23]

2.2 The Data

This study consists of one hundred and twenty nine samples (n=129) each of water, hair, toenail and fingernail with three (3) main variables. Twenty three (23) of the samples are from tap well water drinking subjects, eighty (80) of treated well water, six (6) samples of bottled water and twenty (20) of rain water samples. These samples and their respective variables were all used for multivariate analysis. The three variables are Arsenic, Copper and Iron with sub variables of fingernail (FN), toenail (TN), drinking water (DW) and hair (HR). Some of the subject samples under considerations were reported to have been smokers, with varying age of six and eighty five years of both male and female. Others also have chronic diseases such as Sinusitis, Heart disease, Hepatitis, Sores, Blood pressure, Breast cancer, Rheumatism, Osteoarthritis, Asthma, Diabetes, Chronic renal failures, uterine cancer etc.

2.3 Overview of Multivariate Analysis

Multivariate analyses are techniques often used for investigating more complex relationship among the variables. The most commonly employed multivariate analyses are Cluster analysis (CA) and Principal component analysis (PCA), which have been employed to investigate the levels of heavy metals in human hair [24] in order to differentiate between cancer patients and healthy people.

2.4 Multibase 2013 Software

This is a statistical tool that provides a set of powerful graphical information for researchers, scientists and environmental engineers dealing with large data set [18]. Prior to perform the PCA, the data set was auto scaled to prevent the influence of variables with large values compared to those having small values. Data was analyzed using excel where variables and samples name area were defined. The categorization of samples was also performed, followed by the selection of appropriate method to be employed (Principal Components Analysis) and then run. Finally, the new plots or graphs showing different result were displayed in the new excel sheets [18].

2.5 Principal Components Analysis (PCA)

The Principal Component Analysis (PCA) is a sophisticated technique in which information are sorted out to determine the components required to explain the variability of the data [25]. Ideally, Principal Component Analysis requires a huge volume of data set with a relatively small number of samples groups [25]. PCA further explains the similarities and dissimilarities of samples using extracted factors in much easier than using unprocessed data [18]. PCA is a method applied widely for multivariate data analysis. The new plots or graphs produced by Principal Component Analysis provide an insight into the structure of a data set and the relationship between the measured variables. The dimensionality reduction of the data set is achieved by transforming of original variables into a new set of variables in form of Principal Component (PCs). The PCs are then ordered such that the first few PCs retain most of the variation present in all of the original variables [19, 20]. These newly generated variables are refer as PCs which explain the original correlated variables. Individual PCs describes different properties, samples with similar properties are grouped together in their respective PC score plots. Although Principal Component Analysis is a descriptive technique rather than inferential one, it is possible to test for the significance of discrimination of the objects on the PCs [20]. Therefore, scores of the objects saved for the retained Principal Components were used in ANOVA to investigate the significance differences between metals and conditions.

3. RESULTS AND DISCUSSION

3.1 Result Analysis

Principal Components Analysis results from the data are provided in the figure 2 and 3 respectively. These data are used to analyze the heavy metals concentrations relationships, comparison and their individual correlations.

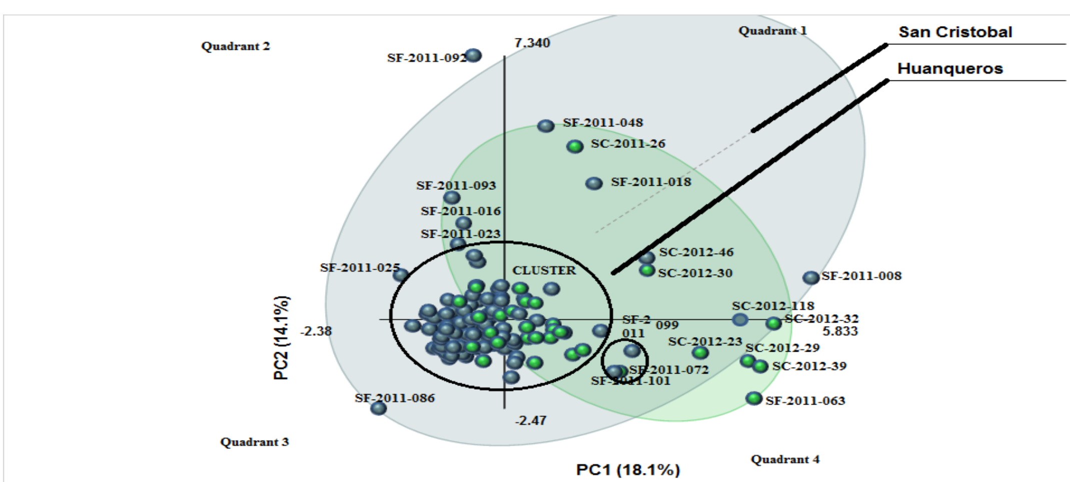

Fig. 2. Samples (Score) PC 1 vs PC 2

The score plot above (fig. 2) is a summary of the relationship among different samples of drinking water sources while the loading plots (fig. 3) represent variables summary of toenail (TN), fingernail (FN), hair (HR) and drinking water (DW) in arsenic, copper and iron respectively. The two plots can be superimposed and they complement each other. The direction of the plot corresponds to the same direction in the score plot. The samples graph shows how one hundred and twenty-nine (n=129) samples are related to each other. Samples close to each other have similar metals levels, whereas samples far from each other indicate dissimilar metals levels properties. The positioning of the samples points is determined based on samples scores for each retained principal component (PC). Samples from San Cristobal town are colour-coded in blue with a bigger eclipse indicating the majority of the samples are from San Cristobal while Huanqueros samples are colour-coded in green with a smaller eclipse. The eclipse was used to indicate the samples proportions from two different towns.

In the samples plot above (fig. 2), a number of clusters are evident. A bigger one was observed at the centre of the map which indicates the average metals levels of the respective samples. One of the most prominent is the clustering of San Cristobal samples in quadrant 4, due to close and high concentrations of heavy metals (Arsenic, Copper and Iron) among SF-2011-099, SF-2011-072 and SF-2011-101. This quadrant is also characterized by the existence of some samples outliers such as SF-2011-063 and SC-2012-23 indicating concentrations of heavy metals dissimilarities from Huanqueros compared to those from San Cristobal. But samples with code SC-2012-118 and SC-2012-32 positioned themselves along the PC 2 axis with average levels of metals. Another prominent cluster is illustrated by close grouping of most many San Cristobal samples in quadrant 2, reflecting levels of heavy metals concentrations. The only exception to this grouping is SF-2011-092 (San Cristobal sample). In quadrant 3, all samples are concentrated at the centre of the map except for SF-2011-086 from San Cristobal showing dissimilarity from other samples. Looking at quadrant 1, the samples dots are somewhat close to each other especially SC-2012-46 and SC-2012-30 even though they are from different locations. The most isolated points in the figure (i.e. samples that do not cluster with others) include SF-2011-092, SF-2011-086 and SF-2011-008 (figure 2). This reflects high levels or concentrations of the influential variables. For example, the positioning of SF-2011-092 in the uppermost portion of quadrant 2 reflects the high (outlier). Placement of SF-2011-008 in the lower rightmost of the first quadrant of the score plot also reflects the maximum samples level of arsenic concentrations in toenails with minimum concentrations recorded in SC-2012-115.

Maximum arsenic concentrations in drinking water (DW) was recorded in sample SF-2011-063 located at lower right of quadrant 4 while sample SC-2012-26 positioned in quadrant 1 possess the minimum concentrations of arsenic in drinking water (DW). Similarly, SF-2011-072 in quadrant 4 recorded the maximum concentrations of arsenic in fingernail (FN) and minimum concentrations of arsenic in fingernail (FN) was observed in sample SF-2011-054. Similar trend was noticed in SC-2012-32 with maximum concentrations of arsenic in hair positioned along the PC 2 axis. SF-2011-075 recorded the minimum concentrations of arsenic in hair (HR). Furthermore, maximum and minimum samples concentrations of copper in drinking water (DW) were identified in SC-2012-26 and SF-2011-077 respectively. In the case of concentrations of copper in fingernail (FN), SC-2012-30 in the quadrant 1, indicates it maximum level while SF-2011-082 possess minimum concentrations. SF-2011-076 has the highest concentrations of copper in hair (HR) and SF-2011-031 having the lowest. Likewise, SF-2011-018 in quadrant 1 recorded the highest concentrations of copper in toenail (TN) with minimum concentrations in SF-2011-081. SF-2011-086 in quadrant 3 and SF-2011-031 also recorded the maximum and minimum concentrations of iron in drinking water (DW) respectively. Sample with code SF-2011-092 in quadrant 2 has the highest concentrations of iron in fingernail (FN) but SF-2011-037 recorded the lowest concentrations of the same iron fingernail (FN). Subsequently, SC-2012-39 in quadrant 4 recorded the maximum concentrations of iron in hair (HR) while SF-2011-090 recorded the minimum. Finally, SF-2011-048 in quadrant 1, possess the highest concentrations of iron in toenail (TN) with SF-2011-101 recorded the least concentrations in toenail (TN). From figure 2 analysis, it can be concluded that most of the maximum samples are the outliers while the majority of the minimum samples are concentrated at the centre or very close to the centre of the map.

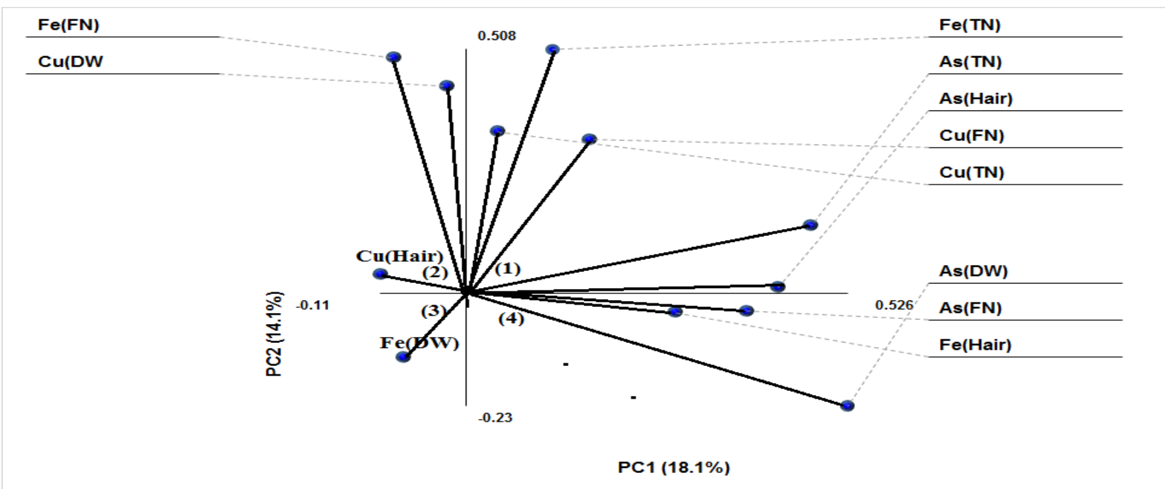

Fig. 3. Variables (Loading) PC 1 vs PC 2

The loading plot (fig. 3) above shows variables that are influential for the model and how these variables are correlated to each other. Figure 3 depicts correlations between the variables. For first principal component (horizontal axis), accounting for most (18.1%) of variation, As (DW), As (Hair), and As (TN) are well represented on PC 1 based on the small angle between the vectors and the PC 1 axis. For the second principal component (PC), Fe (FN) and Cu (DW) are the most influential. The dots of TN, HR and DW in arsenic are close to each other, which means these three variables have the most impact on the values of PC 1. When the value of DW increases there is corresponding increase in TN and HR values and vice versa. The dots that are far away from each other account for their lower values in all the elements. The strong association exist between the drinking water (DW) and toenail (TN) in arsenic for PC 1. In figure 3 each of the variables is represented by a vector (Black line from the centre). The angle of the vector relative to the two principal components (PCs) axes reflects the degree of association between the variables and the PCs. Similarly, the angle of each vector relative to other vector reflects the degree of correlation between the variables. For example, variables vectors lines separated by small angle are correlated whereas those separated by 90^{o} angle are perfect independence (i.e. signifying 0 correlation). Vectors oriented at 180º from each other are negatively correlated. Having this understanding in mind, figure 3 can be interpreted as follows; As (DW), As (Hair) and As (TN) are positively correlated with the first component, PC 1 (horizontal axis). Furthermore, the small angles between these variables indicate that they are positively correlated with each other. Cu (TN) and, to a lesser extent, Fe (TN) are positively correlated with the second principal component (vertical axis). The position of the variables vectors for Cu (HR), Fe (DW) and Cu (FN), at about 45º angles to both PC 1 and PC 2 axes shows that these variables are not well represented in the principal component analysis as other variables. PC 2 represents only 14.1% of contribution to the total variability in the data. The following loading and samples plots further explains the various relationship and correlation among the samples and variables respectively.

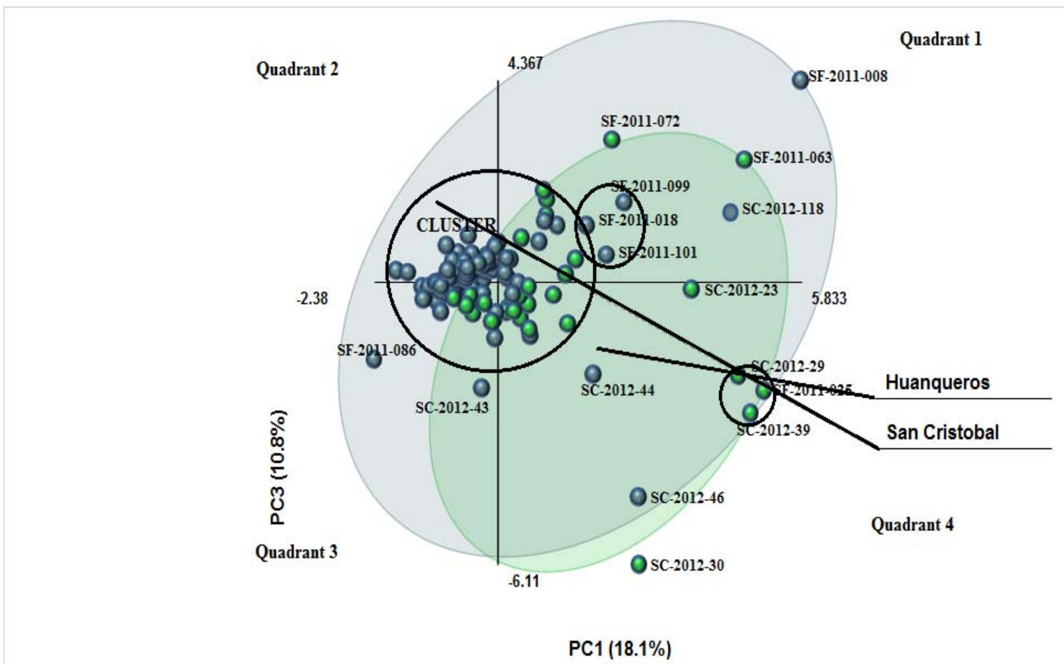

Fig. 4. Samples (Scores) PC 1 vs PC 3

In the above figure 4, similar approach was used to analyze the samples variables. The majority of the samples are concentrated at the centre of the map on both PC 1 and PC 3 indicating average heavy metals concentrations. In quadrant 1 a prominent cluster of San Cristobal samples showing similar concentrations of heavy metals among the samples. SF-2011-008 which is far away from the prominent cluster indicates metals concentrations dissimilarity. In the same quadrant, some outliers exist as in the case of SF-2011-072, SF-2011-063 and SC-2011-118 showing metals concentrations dissimilarities. In quadrant 2, no outlier was recorded. Similarly, quadrant 3 highlighted two moderately outliers (SF-2011-086 and SC-2012-43). Even though, SC-2012-43 aligned closely at PC 1 axis having close relation with a bigger cluster. In quadrant 4, grouping of samples SC-2012-29, SF-2011-025 and SC-2012-39 reflecting high concentrations of arsenic, copper and iron. The only exception to this grouping is SC-2012-44 with average concentrations of the metals.

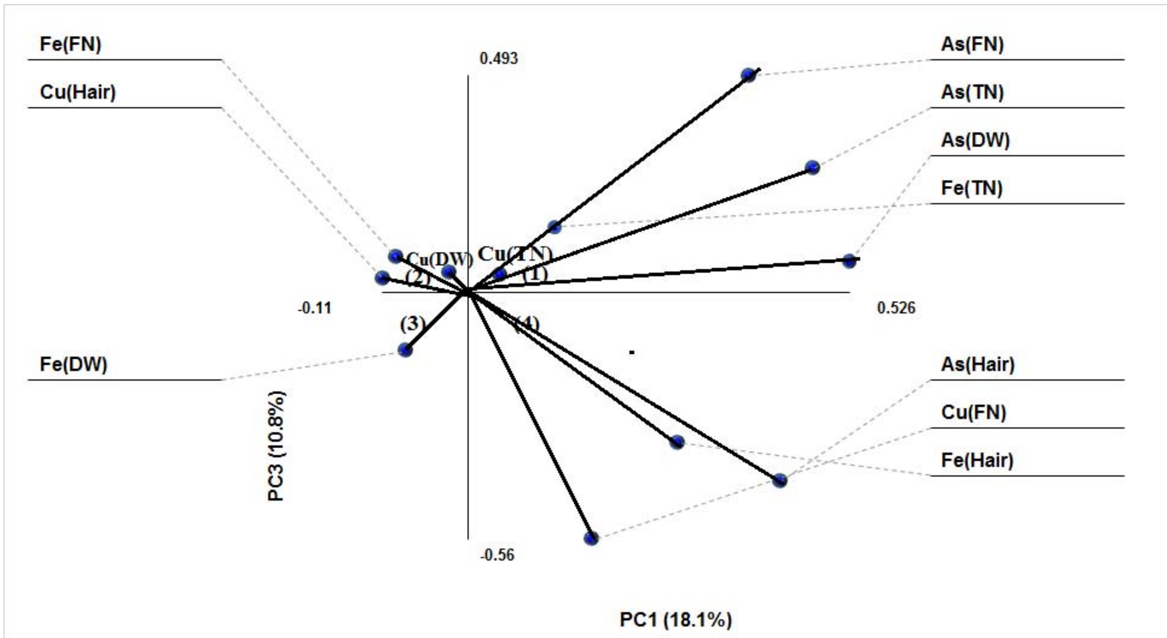

Fig. 5. Variables (Loading) PC 1 vs PC 3

Figure 5 illustrates the variables correlations, horizontal axis (First principal component) account for most of the variability contribution (18.1%) of the data. As (TN), As (HR) and As (DW) are well represented on PC 1 based on the same angle between the dots of variables and the PC 1 axis. For the third principal component (PC 3) which account for 10.8% of contribution to the total data variability, only As (DW) is well represented. Cu (FN) and As (HR) are negatively correlated along the PC 1 axis in quadrant 4. As (DW) is positively correlated with PC 1 axis due to the small angle from the centre of the map to the dot indicated by the vector line. Both Cu (TN), Cu (DW) and As (FN) inclined themselves at relatively 45º angle from the centre indicating that they are partially positively correlated to each other. As (TN) is also positively correlated with both PC 1 and PC 3 Axis. Moreover, Fe (FN) and Cu (HR) are positively correlated with PC 3 axis and, to a lesser extent with Cu (DW). Fe (DW) is negatively correlated with vertical axis (PC 3) due it small angle. Both As (HR) and Fe (HR) are negatively correlated with horizontal and vertical axes (PC 1 and PC 3) and, to a lesser extent with Cu (FN).

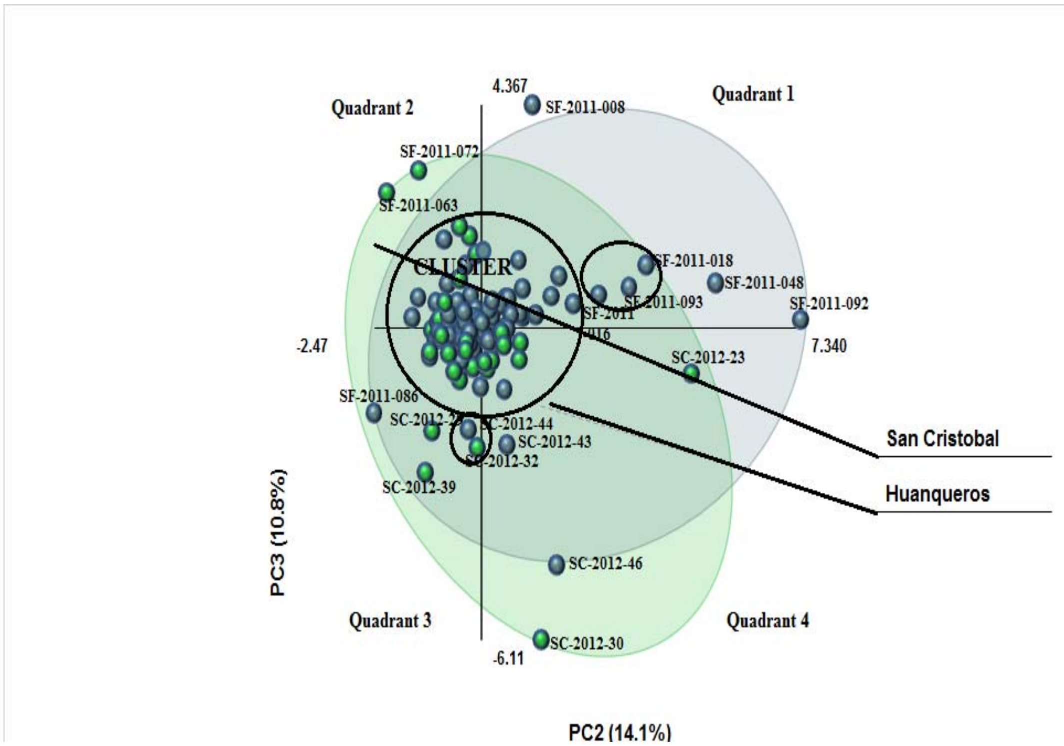

Fig. 6. Samples (Score) PC 2 vs PC 3

As demonstrated in the previous samples plots, similar major cluster occur at the centre of the map with average concentrations of all the metals in the samples (figure 6). San Cristobal samples been grouped together in quadrant 1 (SF-2011-018, SF-2011-093 and SF-2011-016) also have similar metals concentrations. SF-2011-008 which is far away from the main cluster has dissimilar metals concentrations. Samples SF-2011-048 and SF-2011-092 in quadrant 1 aligned relatively at 180º angle to the horizontal axis of PC 2 indicating high concentrations of the influential variables. In quadrant 2, reflects outliers (SF-2011-063 and SF-2011-072) which indicate that concentrations of heavy metals are high in these samples. Similar outliers was observed in quadrant 3 (SF-2011-086, SC-2012-29 and SC-2012-39) showing the samples concentrations of arsenic, copper and iron is high than those at the centre of the map. Another prominent cluster is illustrated by the close grouping of variables samples in the same quadrant 3. In quadrant 4, no cluster was evident; the separation of San Cristobal and Huanqueros samples from those representing the other samples is consistent with the distinctions between metals concentrations.

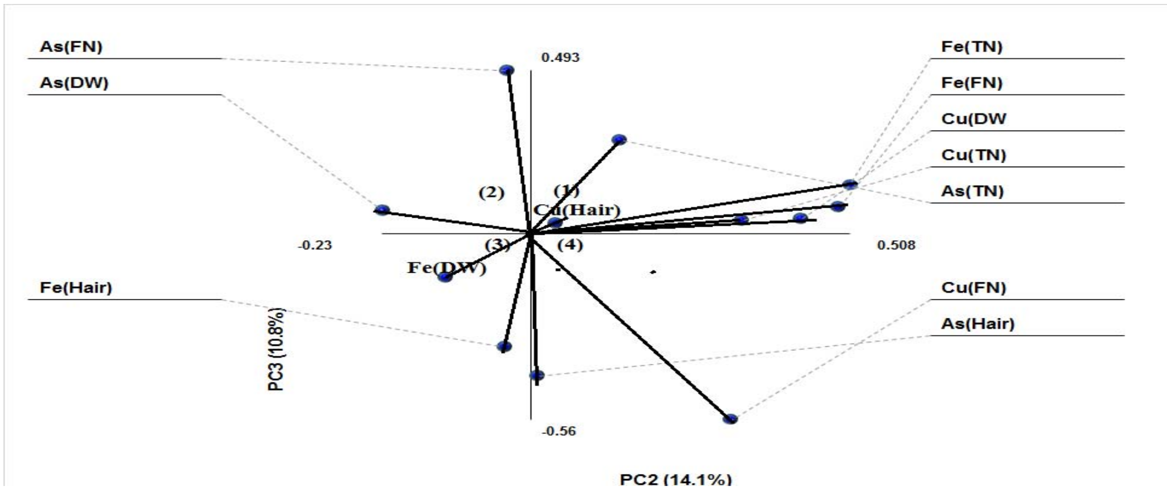

Fig. 7. Variables (Loading) PC 2 vs PC 3

The major positive correlation in first quadrant is between Cu (DW) and Cu (TN) with PC 2 (Figure 7). Cu (DW), Fe (TN) and Cu (TN) are well influential to PC 2 due small angle established between the variables and horizontal axis. Cu (Hair and As (TN) are not well represented in the second quadrant with both PC 2 and PC 3. As (FN) and As (DW) in the second quadrant are both positively correlated with PC 3. The angle between Fe (DW) and Fe (HR) in the third quadrant reflects the facts that they are negatively correlated to each other. Subsequently, in quadrant 4, As (Hair located at 90º angle vertical axis of PC 2 indicates no correlation. Cu (FN) vector line in the fourth quadrant oriented at 180º angle to both PC 2 and PC 3 indicates its negative correlation and degree of association between variables and the PCs.

<u>3.1.1 Metals Comparison and Conditions</u>

To compare the levels of metals and condition, an error bar is used to compare the variables condition for better understanding of common differences (Figure 8). Whiskers of bars indicating the confidence interval of 95%, the lower or smaller the bars the better the result, the higher the whiskers the more the concentrations of the metals in the samples variables. Whiskers with high bars confirmed the accumulation of heavy metals in the respective variables. The condition of the variable is evident by the colour used.

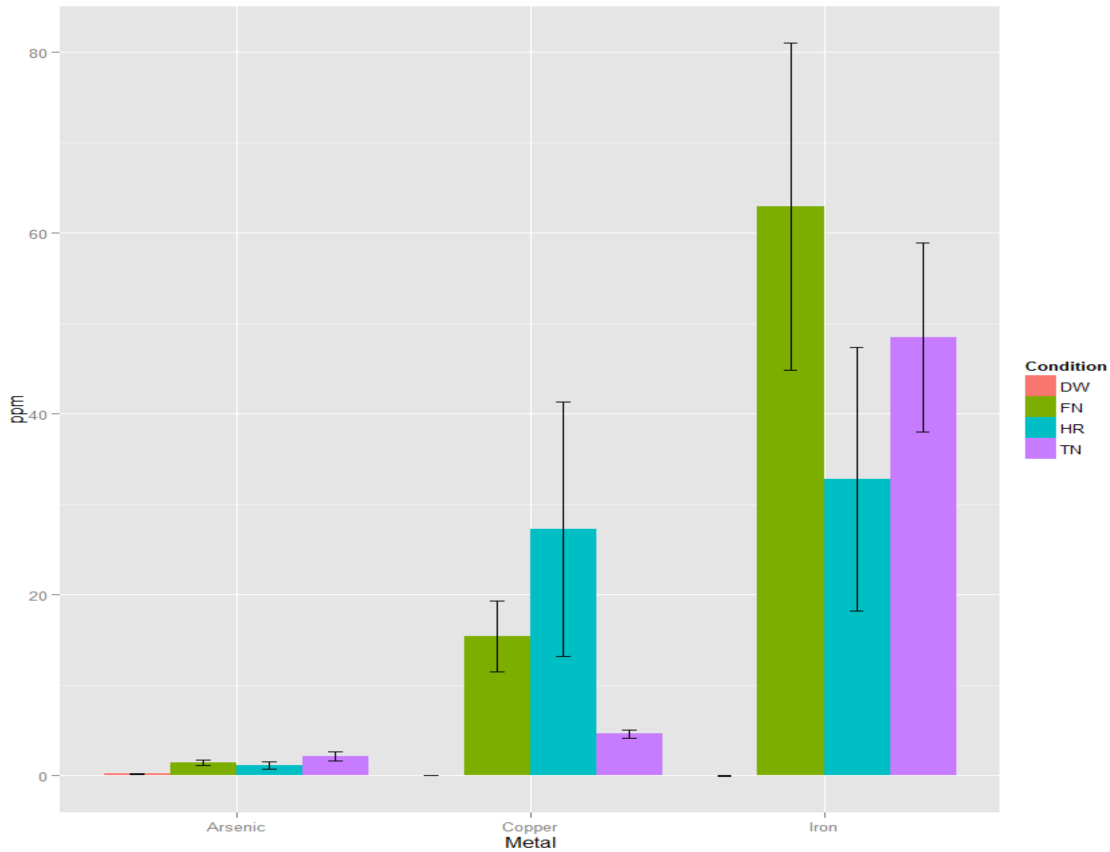

Fig. 8. Error bars for comparing all metals concentrations

As (DW) gets the lowest mean concentrations of 0.20 ppm among the arsenic FN, arsenic HR and arsenic TN variables. The highest concentrations in arsenic was recorded for Arsenic TN with arsenic FN and arsenic HR having a relatively close mean concentrations of 1.48 ppm and 1.15 ppm respectively. For copper, the highest mean concentrations were recorded in copper HR i.e. 27.28 ppm. It is interesting that DW of copper has the lowest mean concentrations of 0.05 ppm. Copper TN has 4.62 ppm average concentrations and copper FN mean value of 15.41 ppm. Similarly, the highest mean concentrations for metals were found in FN, TN and HR as 62.85 ppm, 48.48 ppm and 32.76 ppm respectively. But the same iron DW recorded 0.02 ppm mean concentrations. Looking at the whole variables, DW of arsenic has the highest value for elements in drinking water while DW of iron has the lowest.

3.1.2 Correlation Test

Correlation between variables is a measure of how well the variables are correlated. In order to investigate the correlation between the samples variables, Pearson's correlation test was used. Table 1 below shows how some of these variables are correlated with each other. The Pearson's correlation test was performed for each pair of variables say As (DW) and Cu (HR).

Table 1. Pearson's product moment correlation coefficient (Correlation test results)

	As(DW)	As(FN)	As(HR)	As(TN)	Cu(DW)	Cu(FN)	Cu(HR)	Cu(TN)	Fe(DW)	Fe(FN)	Fe(HR)	Fe(TN)
As(DW)		0.4424	0.3992	0.3942	-0.0088	0.0188	-0.0371	-0.0933	-0.0945	-0.1321	0.2823	-0.1089
As(FN)			0.0625	0.3895	-0.0684	-0.0675	-0.0828	-0.0439	-0.0488	-0.0528	0.0561	0.1150
As(HR)				0.2848	-0.1518	0.3319	-0.0660	0.0554	-0.0168	-0.1547	0.1944	-0.0151
As(TN)					-0.0758	0.0289	-0.0729	0.1057	-0.0421	-0.0394	0.0927	0.2559
Cu(DW)						-0.0406	0.1334	-0.1144	-0.0461	0.2305	-0.0973	-0.0680
Cu(FN)							-0.0337	0.0325	-0.0210	0.1662	0.1542	0.1878
Cu(HR)								-0.0142	-0.0278	0.0579	-0.0389	-0.0246
Cu(TN)									-0.0694	0.0317	-0.0087	0.2587
Fe(DW)										-0.0582	-0.0073	-0.0646
Fe(FN)											-0.0262	0.2036
Fe(HR)												0.0312
Fe(TN)												

**Those marked with green colour are the ones whose p value <0.05*

Table 1 above shows a statistics called correlation coefficient ranging between -1 and 1, with 1 indicating a perfect positive correlation, -1 indicating a perfect negative correlation, and 0 indicating perfect independence. The table represents a correlation matrix derived for the data set. From this table As (FN) is positively correlated with As (HR), and to a lesser extent with As (TN) and Fe (HR). As (TN) is positively correlated with Cu (FN) and a weak positive correlation with Fe (HR). This result is almost similar to the one obtained from principal components loadings.

3.1.3 ANOVA Test

When trying to compare more than two groups of data set (such as in-stream concentrations at different location along a river, each with multiple observations) either ANOVA test or variance analysis can be used [25]. Statistical approaches applied up to this point in the analysis of the result have been only restricted to descriptive and exploratory methods. This can be observed in the positioning of San Cristobal samples in the leftmost quadrants of figure 2. This indicated that there are some differences on the principal components. In order to justify this samples discrimination on the principal components. A 2-factor ANOVA test was performed. One factor is metals (Arsenic, copper and iron) each has four samples; the other factor is conditions (DW, FN, HR and TN) each has three samples. The result showed that there is no too much difference between them; one reason for this is that the samples size is too small. If significance level is set to 0.05, there are 11 samples showing significance difference between metals.

3.2 Discussion

For hundreds of years, people from Latin American countries have been drinking arsenic contaminated water. Despite all this no accurate data record on the extents of it health consequences and exposures. Latin America has been the region of arsenic investigation that leads to significant contribution to current research of arsenic consequences on human health. Most especially with respect to the development of lung, skin and bladder cancer. While development of and deaths from cancer may take up to 35 years long after exposure, respiratory diseases or deaths from cardiovascular in children may only occur during a period of chronic exposure.

The results of this analysis shows that heavy metals in drinking water, fingernails, hair and toenails samples from different subject accumulate differently based on exposure. It was observed that there is high value of arsenic in drinking water (DW= 0.20 ppm) compared to DW of iron and copper, such differences recorded might be as a result of the fact that higher levels of arsenic tend to be more in groundwater sources than in surface water sources (rivers and lakes) drinking water compared to copper and iron. This agreed with the previous studies of Wang and Mulligan [24] even though arsenic is found in both surface and groundwater but the levels are generally higher in groundwater. The average values of iron in FN (62.94 ppm) and copper in FN (15.41 ppm) is higher except for arsenic in FN (1.45 ppm). Cu, Fe, As, Cr, Ni, Cd and Mn has elevated levels in toenail than fingernail samples, the high levels of all the metals in toenails samples when compared with fingernails samples, might be attributed to the fact that fingernail grows continuous at a faster rate of 0.05 - 1.2mm per week while toenails grows at a slower rate and thus provide a longer integrated period for metals accumulation compared to fingernail [11]. It is also evident that both toenails and fingernails samples accumulate high concentrations of arsenic, copper and iron when compared with hair samples. Such differences might be as a result of incorporation of the metals into the structure of keratin in hair which take place by binding to the sulpurhydryl groups that are present in the follicular protein [11].

To further explains the complex relationship by considering the correlation among the samples variables, a positive correlation exists between fingernail and hair for arsenic as reported in table 1. A similar correlation was recorded between nail and hair concentrations for elements Zinc, Arsenic, Thallium and Cadmium [26]. There is limited knowledge of relationships between heavy metals in nails and hair in published literature, which has mainly focused on correlations between drinking water levels and other factors. Cecilie [26] carried out a study on relationships between nails and hair in which a significant positive correlation for Sb and As was reported. Controversially, it has been observed that individuals with high hair arsenic levels did not necessarily have elevated arsenic level in nail [26]. The study highlighted that individuals with relatively high arsenic level in hair had lower arsenic level in nail. Similar exposed subjects had significant correlation in nail and hair lead (Pb) level, whereas perfect independence correlation was found in unexposed subjects [26]. The work further observed that no relationship of cadmium concentration between nails and hair of both exposed and unexposed individuals.

Vance *et al*. (1988) cited in [26] showed that concentrations of non-essential heavy metals were positively correlated in nail and hair, whereas essential heavy metals were not correlated. However, the current study about nail and hair relationships for heavy metals is confusing. Looking at the correlation table, it can be concluded that more than half of the correlation analyses for iron and copper did not shows any significant matched relationship. The results highlighted that incorporation of heavy metals is somewhat different for nails and hair, even though they possess some similar chemical composition.

4. CONCLUSION AND RECOMMENDATIONS

The findings of current research indicate that San Cristobal and Huanqueros population were exposed to higher levels of arsenic than copper and iron. The differences were most clearly observed for toenails and fingernails but apparent for both tissues. Values of drinking water were significantly higher compared to toenails, fingernails and hair in arsenic, copper and iron. One reason for this may be attributed to the fact that drinking water is not a biomarker and therefore no long term accumulation of metals. Likewise, it might be as a result of spatial variations of the two towns. Correlations values were somewhat similar to previous studies. Some differences in values of fingernails and toenails exist. There is risk of health impairment in some of the subject based on comparison with arsenic, copper and iron in hair, toenails and fingernails. Despite the statistical analysis conducted on San Cristobal and Huanqueros data for toenail, drinking water, fingernail and hair heavy metals concentrations. There is need to analyze the data by Cluster Analysis for samples categorization base on two different towns and see if such spatial variations have influence on the levels of heavy metals. Furthermore, it is important to analyze other heavy metals such as zinc, chromium, cadmium etc alongside with arsenic in drinking water of arsenic affected areas of Province of Santa Fe. Future statistical analysis is required to have a better knowledge and understanding of how specific factors influence heavy metals bioaccumulation. Smoking habit, age and gender should be put into consideration to identify interactions among toxic heavy metals. It is also recommended to statistically analyze several heavy metals in human exposure studies as a deficiency of some metals; e.g. Manganese and Zinc could modulate and possibly increase the toxicity of other metals such as arsenic.

REFERENCES

1. United Nations Environmental Protection/Global program of Action. Why the Marine Environment need Protection from Heavy Metals 2004, UNEP/GPA Coordination office. 2004.

2. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences. 2007; 2 (5):112-118.

3. Institute of Environmental Conservation and Research INECAR. Position Paper against Mining in Rapu-Rapu, published by INECAR: Ateneo de Naga University, Philippines.2000. Available at: www.adnu.edu.ph/institutes /inecar/pospaper1.asp. (Accessed: 12 August 2013).

4. Peplow D. Environmental Impacts of Mining in Eastern Washington. Center for Water and Watershed studies Fact Sheet, University of Washington, Seattle.1999.

5. Trueby P. Impact of Heavy Metals on Forest Trees from Mining Areas. In: 3rd International Conference on Mining and the Environment, Sudbury, Ontario, Canada. 2003. Available at: **www.x-cd.com/sudbury03/prof156.html**. (Accessed: 12 August 2013).

6. Reilly C. Metal contamination in Food. The metals are consume. 2nd Ed. Elsevier Science Publishers Limited.1991:1-20.

7. Arnold W, Sachs H. Hair analysis for medicaments – the best proof for a drug career, Freseniu. Journal of Analytical Chemistry.1994; 348: 484-489.

8. Kaluza J, Jeruszka M, Brzozowska A. Iron, Zinc and Copper status of elderly people living in warsaw district determined by hair analysis. Roczniki Panstwowego Zak adu Higieny. 2000. 52: 111-118.

9. Zhunk LI, Kist AA. Human hair instrumental neutron activation analysis and medicine. Journal of Radio analytical and Nuclear Chemistry.1995; 195:75-81.

10. VCH Publishers. New York. 1988; 224-254.

11. Abdulrahman FI, Akan ZM, Chellube ZM, Waziri M. Levels of Heavy Metals in Human Hair and Nail Samples from Maiduguri Metropolis, Borno State, Nigeria. World Environment.2012; 2(4): 81-89.

12. Eman AE Badr, Asmaa AE, Agrama, Safaa AE Badr. Heavy metals in drinking water and human health, Egypt. Nutrition and Food Science.2011; 41(3):210-217.

13. Gillian L, Nick K, Neil IW. Arsenic speciation of geothermal waters in New Zealand. Journal of Environmental Monitoring. 2012. DOI: 10.1039/c2em30486d.

14. Slotnick MJ, Nriagu JO. Validity of human nails as a biomarker of arsenic and selenium exposure: a review. Environmental research. 2006; 102(1):125-139.

15. Raab A, Feldmann J. Arsenic speciation in hair extracts. Analytical and bioanalytical chemistry.2005; 381(2): 332-338.

16. Rodrigues JL, Batista BL, Nunes JA, Passos CJ, Barbosa Jr F. Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. Science of the Total Environment.2008; 405(1):370-376.

17. Lin SM, Yang MH. Arsenic, selenium, and zinc in patients with Blackfoot disease. In Selenium. Humana Press.1988:213-221.

18. Numerical dynamics. 2013. Available at: http://www.numericaldynamics.com (Accessed: 6 August 2013).

19. Jolliffe IT. Principal Component Analysis. Spring- Verlag, New York.1986.

20. United State for Department of Energy. "Environmental Sciences Laboratory" Multivariate Statistical Analysis of Water Chemistry in Evaluating the Origin of Contamination in Many Devils Wash, Shiprock, New Mexico. 2012. LMS/SHP/S09257-ESL-RPT-2012-03.

21. Samanta G, Sharma R, Roychowdhury T, Chakraborti D. Arsenic and other elements in hair, nails, and skin-scales of arsenic victims in West Bengal, India. Science of the total environment. 2004; 326(1):33-47.

22. Schuenemeyer JH, Drew LJ. Statistics for Earth and Environmental Scientists, John Wiley & Sons, Inc.2011.

23. Department de San Cristobal, Santa Fe Province, Argentina. Sancristobal and Huanqueros local map. 2013. Available at: www.weather-forecast.com/locations/San-Cristobal-3 (Accessed: 25 September, 2013).

24. Wang S, Mulligan CN. Occurrence of arsenic contamination in Canada: sources, behaviour and distribution. Science Total Environment. 2006; 366(3): 701-721.

25. Pitt, R. Module 5: Statistical Analyses.2007.

26. Cecilie E. Arsenic and trace metals in hair, nails and blood of villagers from the vicinity of a gold mine in Tanzania. Published Msc. Dissertation. University of Oslo.2011.