# Environmental Data Analysis: Heavy Metals from Drinking Water to Human Body

**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 toe nail, finger nail 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 toe nails, finger nails, drinking water and hair using  multibase 2013 excel add ins. Correlation test, error bars, and a 2-factor ANOVA tools were employed. Result 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) with varying age and gender of the population shows some samples similarities, differences and variables correlation. Mean, standard deviation, covariance and maximum and minimum for each variable were reported. The hypothesis is to understand if there is correlation between finger nail and toe nail metals levels and make comparison with previous researches. From the results, positive correlation exists between finger nail and toe nail 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: Environmental Data Analysis; Drinking Water; Human Body; Heavy Metals; 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

25  causes their accumulation in the plants tissues [4]. Animal grazing on such contaminated grass can

26  also be affected [5]. Individuals have been poisoned through-exposure to these metals via taking

27  contaminated animals and plants food sources, and a lot of these cases have been associated to

28  cause numerous body systems disorders. Generally, Food chain along the ecosystem has been the

29  major channel through which all living organisms are contaminated.

30  There has been constant research and progress made in the area of heavy metals analysis in human

31  body from drinking water using different biomarkers and statistical methods. Environmental Scientists

32  and Engineers continued to show much interest in hair and nail for heavy metals determinations [6].

33  Hair as one of the excretory organ gives and reflects good metabolic mineral activities in the body.

34  Although, the results show a significant concentrations differences when compared to others [7]. It

35  has been observed that, human hair as biological biomarker is so attractive due to its simplicity in

36  handling, sampling, transport as well as providing more details interms of concentrations of heavy

37  metals and easy analysis compare to other biological materials [8]. Human nails keep a good records

38  of elemental composition and concentrations in human body, unlike other fluids in the body that gives

39  a transient concentrations [9].The growth of human nails continues throughout life span ranging from

40  0.05 and 1.2 mm per week but the toe nail growing is very slow which indicates metals integration is

41  also slow normally at 30- 50 % rate [9]. Abdulrahman et al. [10]  carried out a study to analyze the

42  concentrations of arsenic, lead, manganese, copper, cobalt, nickel, chromium, cadmium, iron, and

43  zinc in human toe nail,  hair and finger nail samples of different age and sex of people doing iron

44  welding works, non-liquor and liquor users of Maiduguri town, northern part of  Nigeria. The results

45  indicated that Zn showed the highest concentrations while Cu showed the least levels. The results of

46  the study also showed that no contribution of liquor towards hair and nail metals concentrations [10].

47  The levels of all metals under considerations are higher in the toe nail than finger nail samples [10].

48  Similarly, iron welding workers showed higher concentrations of heavy metals when compared to

49  liquor users. The levels of all the metals studied were statistically higher in male than female subjects

50  [10]. While the concentrations of heavy metals in nail samples were significantly higher when

51  compared to hair samples. Therefore, these metals are said to be present in the individual work place

52  as well as in the environment [10]. A similar work was conducted by Eman et al. [11] to investigate if

53  the higher level exposure through drinking water containing high content of heavy metals

54  contaminants in both the bottled sources and that of tap can have an adverse effect on consumers.

55  The study highlighted the higher concentrations of heavy metals in tap water when compared to

56  bottled water and how those heavy metals contaminants in both the two sources can adversely

57  affects the functions of haemoglobin and kidney of consumers. About 28 different geothermal features

58  from the Waikato region and Taupo volcanic zone (TVZ) New Zealand, were reported to content a

59  significant concentrations of Total arsenic and four of its species; monomethylarsonic acid, arsenate,

60  arsenite and dimethylarsonic acid [12]. In Tokaanu, three features were identified to contain the

61  highest levels of arsenic concentrations (8.59, 8.70 and 9.08 mgL As). Geothermal waters were

62  predominantly inorganic arsenic species, with more than 70% arsenite of total arsenic in most of the

63  samples [12].The study highlighted a serious risk to human health due to the high levels of arsenite as

64  it linked to bathing pools [12]. Biomarkers that are often used to evaluate environmental exposure to

65 arsenic and selenium (and other trace elements) in relation to human health and diseases are hair
66 and nails [13, 14, 15]. These tissues provide a stable sample media for assessing chronic exposure
67 trace elements in the environment due to their slow growth; offer an exposure window of the past 6 –
68 12 months [13]. Many elements accumulate in the hair and nail following exposure, in particular
69 arsenic due to the presence of keratin, a sulphydryl-rich protein [13, 14]. In contrast, blood serum is
70 used to measure acute or short- term exposure to heavy metals in relation to human health disorders
71 and diseases [13, 16].  This study  was aimed at examining the levels of some heavy metals in
72 drinking water sources of the studied area (San Cristobel and Huanqueros),  the residents mostly
73 depend on their individual source of drinking water such as tap, well, rain water rather than treated
74 water due to lack of accessibility and therefore they are vulnerable .
75 Principal Component Analysis (PCA) is a method applied widely for multivariate data analysis. The
76 new plots or graphs produced by Principal Component Analysis provide an insight into the structure of
77 a data set and the relationship between the measured variables [17]. The dimensionality reduction of
78 the data set is achieved by transforming of original variables into a new set of variables in form of
79 Principal Component (PCs). The PCs are then ordered such the first few PCs retain most of the
80 variation present in all of the original variables [18, 19]. These newly generated variables are refer as
81 PCs which explains the original correlated variables. Individual PCs describes different properties,
82 samples with similar properties are grouped together in their respective PC score plots [18, 19]. To
83 understand a more complex relationship between heavy metals, a multivariate statistical tool, principal
84 component analysis was used [20]. Principal Component Analysis was further applied to investigate
85 the behaviour of ten elements present in nails and hair [20]. There is no single, recommended
86 procedure to conducting Principal Component Analysis [18, 21]. Choosing of the appropriate
87 technique to employ requires proper examination of the original data and understanding of the
88 computational impacts of applying different approaches.
89
90 **2. MATERIALS AND METHODS**
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92 **2.1 Study Region**
93 San Cristobal and Huanqueros are situated in the province of Santa Fe and lie within the Chaco-
94 Pampean plain. This region has naturally high-arsenic levels in the groundwater and a number of
95 health problems, including diabetes, are observed within the population that may be associated with
96 arsenic exposure from drinking water. San Cristobal is the main town in the region and Huanqueros is
97 a smaller town situated about 35 km north of San Cristobal (Fig. 1). Both towns have no communal
98 drinking water network or sewage system in place; instead each house has their own well and septic
99 tank. There are 5 water treatment plants in San Cristobal and one in Huanqueros. All plants except
100 one in San Cristobal town and the one in Huanqueros charge a fee for 25L of the treated water.
101 However, the free water is only available at certain times of the day, on certain days of the week.
102 Generally well-water (groundwater) is used for cleaning and for drinking purposes only, though many
103 lower socioeconomic families cannot afford the treated water, thus relies on their well-water for
104 drinking purposes [12].

105

**Fig. 1.  Map of San Cristobal and Huanqueros** Source [22]

**2.2 The Data**

This study consists of one hundred and twenty nine samples (n=129) of water, hair, toe nail 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 finger nail (FN), toe nail (TN), drinking water (DW) and hair. 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 [23] 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 [17]. Prior to perform the Principal Component Analysis, 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 personal windows 8 HP

130    laptop by opening the excel file where variables and samples name area were defined. The

131    categorization of samples was also performed, followed by the selection of appropriate method to be

132    employed (Principal Components Analysis) and then run. Finally, the new plots or graphs showing

133    different result were displayed in the new excel sheets [17].

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## 2.5 Principal Components Analysis (PCA)

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136    The Principal Component Analysis (PCA) is a sophisticated technique in which information are sorted

137    out to determine the components required to explain the variability of the data [24]. Ideally, Principal

138    Component Analysis requires a huge volume of data set with a relatively small number of samples

139    groups [24]. PCA further explains the similarities and dissimilarities of samples using extracted factors

140    in much easier than using unprocessed data [17]. Principal Component Analysis (PCA) is a method

141    applied widely for multivariate data analysis. The new plots or graphs produced by Principal

142    Component Analysis provide an insight into the structure of a data set and the relationship between

143    the measured variables. The dimensionality reduction of the data set is achieved by transforming of

144    original variables into a new set of variables in form of Principal Component (PCs). The PCs are then

145    ordered such the first few PCs retain most of the variation present in all of the original variables [18,

146    19]. These newly generated variables are refer as PCs which explains the original correlated

147    variables. Individual PCs describes different properties, samples with similar properties are grouped

148    together in their respective PC score plots. Although Principal Component Analysis is a descriptive

149    technique rather than inferential one, it is possible to test for the significance of discrimination of the

150    objects on the PCs [19]. Therefore, scores of the objects saved for the retained Principal Components

151    were used in ANOVA to investigate the significance differences between metals and conditions.

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## 3. RESULTS AND DISCUSSION

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## 3.1 Result Analysis

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156    Principal Components Analysis results from the data are provided in the figure 2 and 3 respectively.

157    These data are used to analyze the heavy metals concentrations relationships, comparison and their

158    individual correlations.
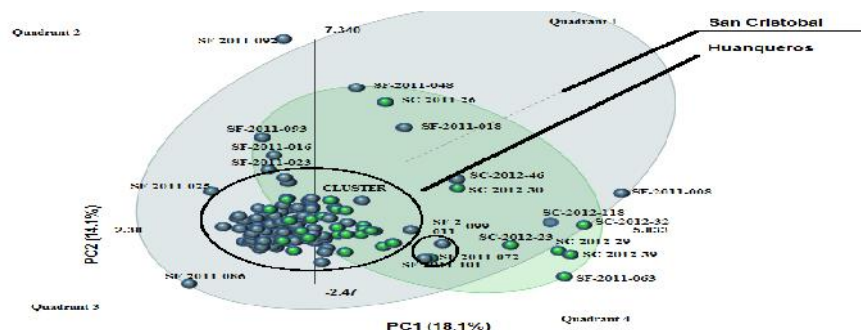


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160
161    ***Fig. 2. Samples (Score) PC 1 vs PC 2***

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163    The score plot above (fig. 2) is a summary of the relationship among different samples of drinking

164    water sources while the loading plots (fig. 3) represent variables summary of toenail (TN), fingernail

165    (FN), Hair and drinking water (DW) in arsenic, copper and iron respectively. The two plots can be

166    superimposed and they complement each other. The direction of the plot corresponds to the same

167    direction in the score plot. The samples graph shows how one hundred and twenty-nine (n=129)

168    samples are related to each other. Samples close to each other have similar metals levels, whereas

169    samples far from each other indicate dissimilar metals levels properties. The positioning of the

170    samples points is determined based on samples scores for each retained principal component (PC).

171    Samples from San Cristobal town are colour-coded in blue with a bigger eclipse indicating the majority

172    of the samples are from San Cristobal while Huanqueros samples are colour-coded in green with a

173    smaller eclipse. The eclipse was used to indicate the samples proportions from two different towns.

174    In the samples plot above (fig. 2), a number of clusters are evident. A bigger one was observed at the

175    centre of the map which indicates the average metals levels of the respective samples. One of the

176    most prominent is the clustering of San Cristobal samples in quadrant 4, due to close and high

177    concentrations of heavy metals (Arsenic, Copper and Iron) among SF-2011-099, SF-2011-072 and

178    SF-2011-101. This quadrant is also characterized by the existence of some samples outliers such as

179    SF-2011-063 and SC-2012-23 indicating concentrations of heavy metals dissimilarities from

180    Huanqueros compared to those from San Cristobal. But samples with code SC-2012-118 and SC-

181    2012-32 positioned themselves along the PC 2 axis with average levels of metals. Another prominent

182    cluster is illustrated by close grouping of most many San Cristobal samples in quadrant 2, reflecting

183    levels of heavy metals concentrations. The only exception to this grouping is SF-2011-092 (San

184    Cristobal sample). In quadrant 3, all samples are concentrated at the centre of the map except for SF-

185    2011-086 from San Cristobal showing dissimilarity from other samples. Looking at quadrant 1, the

186    samples dots are somewhat close to each other especially SC-2012-46 and SC-2012-30 even though

187    they are from different locations. The most isolated points in the figure (i.e. samples that do not cluster

188    with others) include SF-2011-092, SF-2011-086 and SF-2011-008 (figure 2). This reflects high levels

189    or concentrations of the influential variables. For example, the positioning of SF-2011-092 in the

190    uppermost portion of quadrant 2 reflects the high (outlier). Placement of SF-2011-008 in the lower

191    rightmost of the first quadrant of the score plot also reflects the maximum samples level of arsenic

192    concentrations in toe nails with minimum concentrations recorded in SC-2012-115.

193    Maximum arsenic concentrations in drinking water (DW) was recorded in sample SF-2011-063

194    located at lower right of quadrant 4 while sample SC-2012-26 positioned in quadrant 1 possess the

195    minimum concentrations of arsenic in drinking water (DW). Similarly, SF-2011-072 in quadrant 4

196    recorded the maximum concentrations of arsenic in finger nail (FN) and minimum concentrations of

197    arsenic in finger nail (FN) was observed in sample SF-2011-054. Similar trend was noticed in SC-

198    2012-32 with maximum concentrations of arsenic in hair positioned along the PC 2 axis. SF-2011-075

199    recorded the minimum concentrations of arsenic in hair. Furthermore, maximum and minimum

200    samples concentrations of copper in drinking water (DW) were identified in SC-2012-26 and SF-2011-

201    077 respectively. In the case of concentrations of copper in finger nail (FN), SC-2012-30 in the

202    quadrant 1, indicates it maximum level while SF-2011-082 possess minimum concentrations. SF-

203    2011-076 has the highest concentrations of copper in hair and SF-2011-031 having the lowest.

204    Likewise, SF-2011-018 in quadrant 1 recorded the highest concentrations of copper in toe nail (TN)

205    with minimum concentrations in SF-2011-081. SF-2011-086 in quadrant and SF-2011-031 also

206    recorded the maximum and minimum concentrations of iron in drinking water (DW) respectively.

207    Sample with code SF-2011-092 in quadrant 2 has the highest concentrations of iron in finger nail (FN)

208    but SF-2011-037 recorded the lowest concentrations of the same iron finger nail (FN). Subsequently,

209    SC-2012-39 in quadrant 4 recorded the maximum concentrations of iron in hair while SF-2011-090

210    recorded the minimum. Finally, SF-2011-048 in quadrant 1, possess the highest concentrations of

211    iron in toe nail (TN) with SF-2011-101 recorded the least concentrations in toe nail (TN). From figure 2

212    analysis, it can be concluded that most of the maximum samples are the outliers while the majority of

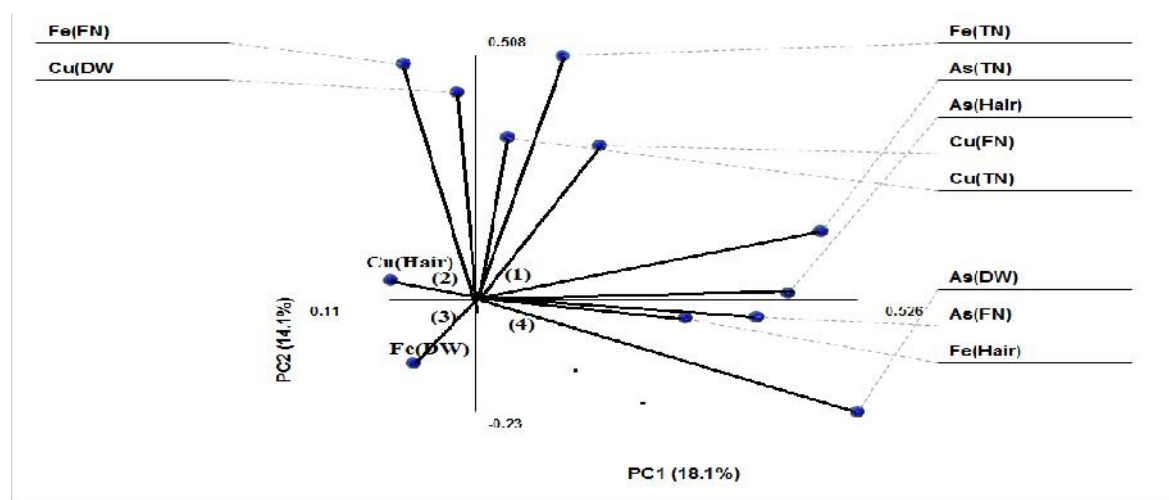213    the minimum samples are concentrated at the centre or very close to the centre of the map.

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215

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

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218    The loading plot (fig. 3) above shows which variables are influential for the model and how these

219    variables are correlated to each other. Figure 3 depicts correlations between the variables. For first

220    principal component (horizontal axis), accounting for most (18.1%) of variation, As (DW), As (Hair),

221    and As (TN) are well represented on PC 1 based on the small angle between the vectors and the PC

222    1 axis. For the second principal component (PC) Fe (TN), Fe (FN) and Cu (DW) are the most

223    influential. The dots of TN, Hair and DW in arsenic are close to each other, which mean these three

224    variables have the most impact on the values of PC 1. When the value of DW increase there is

225    corresponding increase in TN and Hair values and vice versa. The dots that are far away from each

226    other account for their lower values in all the elements. The strong association exist between the

227    drinking water (DW) and toe nail (TN) in arsenic for PC 1. In figure 3 each of the variables is

228    represented by a vector (Black line from the centre). The angle of the vector relative to the two

229    principal components (PCs) axes reflects the degree of association between the variables and the

230    PCs. Similarly, the angle of each vector relative to other vector reflects the degree of correlation

231    between the variables. For example, variables vectors lines separated by small angle are correlated

232    whereas those separated by 90° angle are perfect independence (i.e. signifying 0 correlation). Vectors

233     oriented at 180° from each other are negatively correlated. Having this understanding in mind, figure 3

234     can be interpreted as follows; As (DW), As (Hair) and As (TN) are positively correlated with the first

235     component, PC 1 (horizontal axis). Furthermore, the small angles between these variables indicate

236     that they are positively correlated with each other. Cu (TN) and, to a lesser extent, Fe (TN) are

237     positively correlated with the second principal component (vertical axis). The position of the variables

238     vectors for Cu (Hair), Fe (DW) and Cu (FN), at about 45° angles to both PC 1 and PC 2 axes shows

239     that these variables are not well represented in the principal component analysis as other variables.

240     PC 2 represents only 14.1% of contribution to the total variability in the data. The following loading

241     and samples plots further explains the various relationship and correlation among the samples and
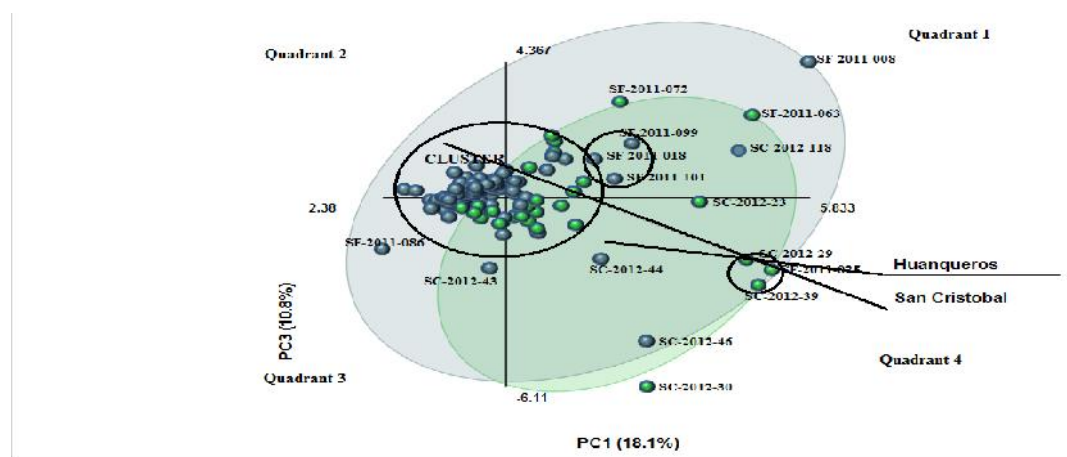
242     variables respectively.



243

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

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246     In the above figure 4, similar approach was used to analyze the samples variables. The majority of the

247     samples are concentrated at the centre of the map on both PC 1 and PC 3 indicating average heavy

248     metals concentrations. In quadrant 1 a prominent cluster of San Cristobal samples showing similar

249     concentrations of heavy metals among the samples. SF-2011-008 which is far away from the

250     prominent cluster indicates metals concentrations dissimilarity. In the same quadrant, some outliers

251     exist as in the case of SF-2011-072, SF-2011-063 and SC-2011-118 showing metals concentrations

252     dissimilarities. In quadrant 2, no outlier was recorded. Similarly, quadrant 3 highlighted two

253     moderately outliers (SF-2011-086 and SC-2012-43). Even though, SC-2012-43 aligned closely at PC

254     1 axis having close relation with a bigger cluster. In quadrant 4, grouping of samples SC-2012-29, SF-

255     2011-025 and SC-2012-39 reflecting high concentrations of arsenic, copper and iron. The only

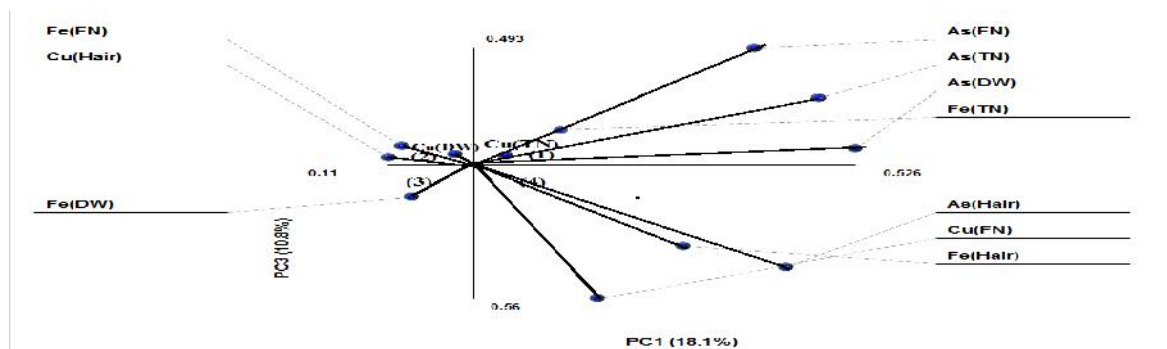256     exception to this grouping is SC-2012-44 with average concentrations of the metals.

**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 (Hair) 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 (Hair) 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 (Hair) 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 (Hair), and Fe (Hair) are negatively correlated with horizontal and vertical axes (PC 1 and PC 3) and, to a lesser extent with Cu (FN).
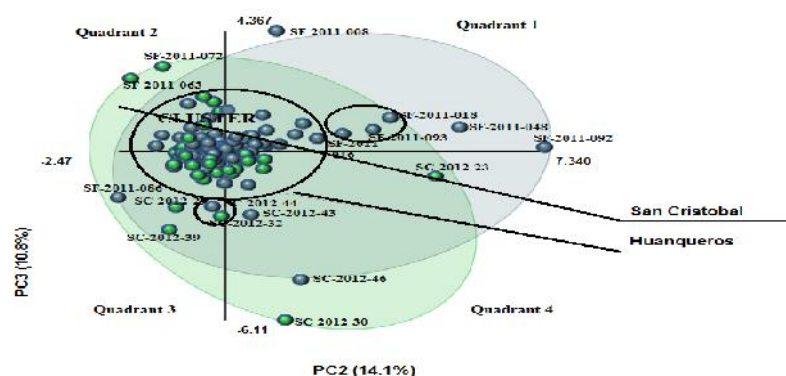


**Fig. 6. Samples (Score) PC 2 vs PC 3**

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

281 to the horizontal axis of PC 2 indicating high concentrations of the influential variables. In quadrant 2,
282 reflects outliers (SF-2011-063 and SF-2011-072) which indicate that concentrations of heavy metals
283 are high in these samples. Similar outliers was observed in quadrant 3 (SF-2011-086, SC-2012-29
284 and SC-2012-39) showing the samples concentrations of arsenic, copper and iron is high than those
285 at the centre of the map. Another prominent cluster is illustrated by the close grouping of variables
286 samples in the same quadrant 3. In quadrant 4, no cluster was evident; the separation of San
287 Cristobal and Huanqueros samples from those representing the other samples is consistent with the
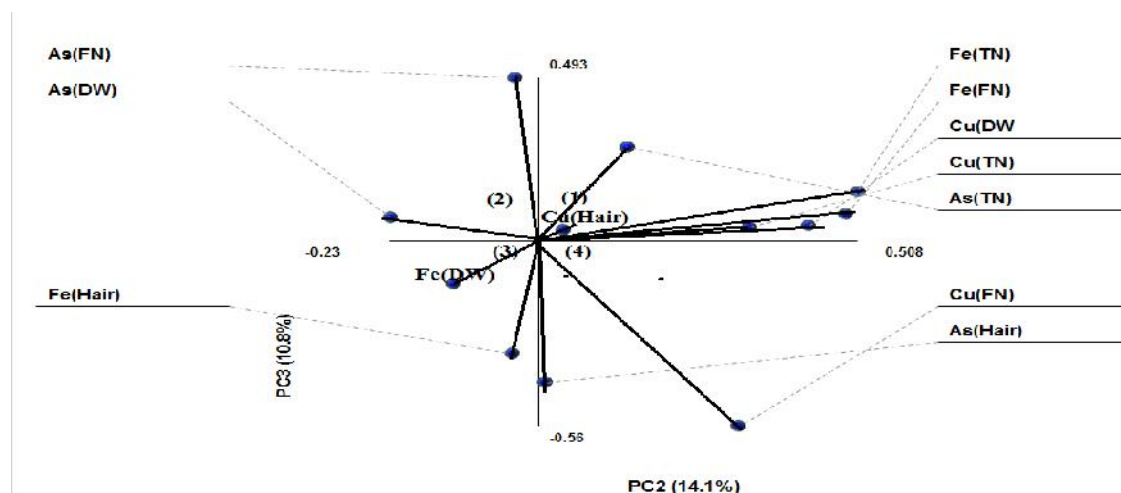288 distinctions between metals concentrations.



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

291

292 The major positive correlation in first quadrant is between Cu (DW) and Cu (TN) with PC 2 (Figure 7).
293 Cu (DW), Fe (TN) and Cu (TN) are well influential to PC 2 due small angle established between the
294 variables and horizontal axis. Cu (Hair and As (TN) are not well represented in the second quadrant
295 with both PC 2 and PC 3. As (FN) and As (DW) in the second quadrant are both positively correlated
296 with PC 3. The angle between Fe (DW) and Fe (Hair) in the third quadrant reflects the facts that they
297 are negatively correlated to each other. Subsequently, in quadrant 4, As (Hair located at 90° angle
298 vertical axis of PC 2 indicates no correlation. Cu (FN) vector line in the fourth quadrant oriented at
299 180° angle to both PC 2 and PC 3 indicates its negative correlation and degree of association
300 between variables and the PCs.

301
302 **3.1.1 Metals Comparison and Conditions**
303 To compare the levels of metals and condition, an error bar is used to compare the variables
304 condition for better understanding of common differences (Figure 8). Whiskers of bars indicating the
305 confidence interval of 95%, the lower or smaller the bars the better the result, the higher the whiskers
306 the more the concentrations of the metals in the samples variables. Whiskers with high bars
307 confirmed the accumulation of heavy metals in the respective variables. The condition of the variable
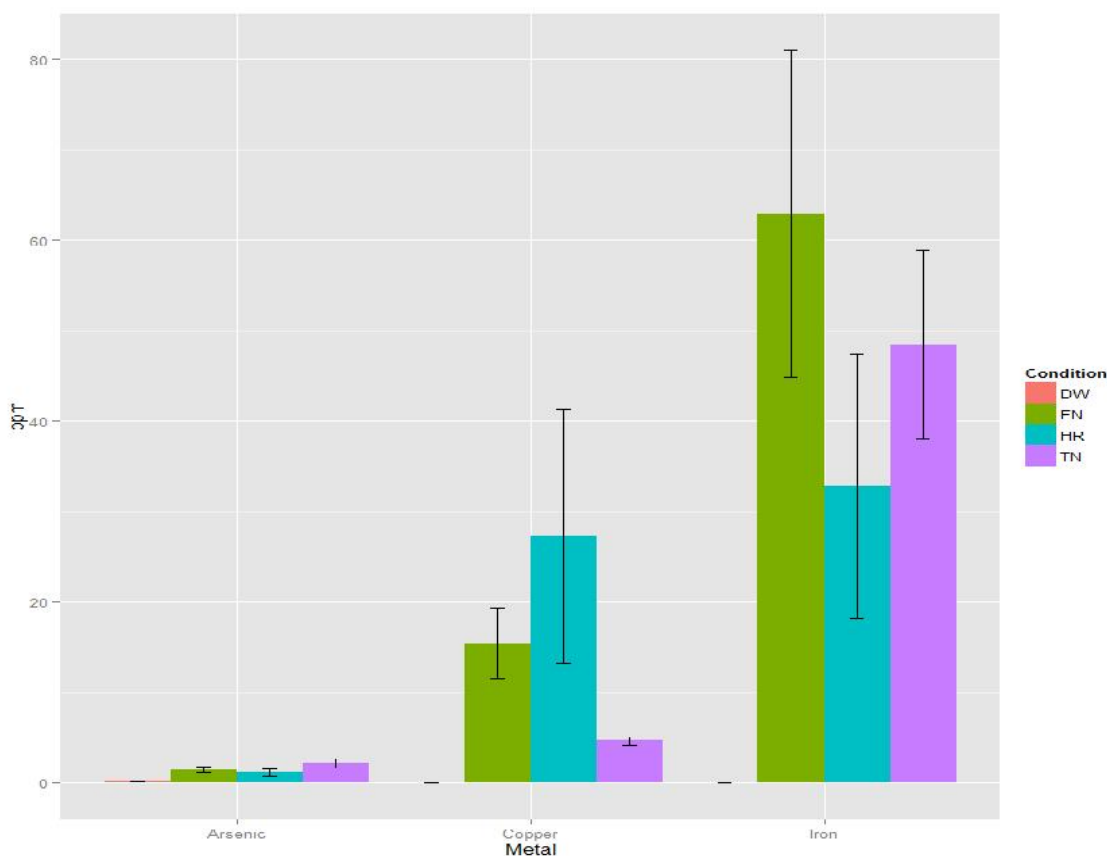308 is evident by the colour used.

309

310    ***Fig. 8.  Error bars for comparing all metals and condition***

311

312    As (DW) gets the lowest mean concentrations of 0.20 ppm among the arsenic FN, arsenic HR and

313    arsenic TN variables. The highest concentrations in arsenic was recorded for Arsenic TN with arsenic

314    FN and arsenic HR having a relatively close mean concentrations of 1.48 ppm and 1.15 ppm

315    respectively. For copper, the highest mean concentrations were recorded in cupper HR i.e. 27.28

316    ppm. It is interesting that DW of copper has the lowest mean concentrations of 0.05 ppm. Copper TN

317    has 4.62 ppm average concentrations and copper FN mean value of 15.41 ppm. Similarly, the highest

318    mean concentrations for metals were found in FN, TN and HR as 62.85 ppm, 48.48 ppm and 32.76

319    ppm respectively. But the same iron DW recorded 0.02 ppm mean concentrations. Looking at the

320    whole variables, DW of arsenic has the highest value for elements in drinking water while DW of iron

321    has the lowest.

322    **3.1.2 Correlation Test**

323    Correlation between variables is a measure of how well the variables are correlated. In order to

324    investigate the correlation between the samples variables, Pearson's correlation test was used. Table

325    1 below shows how some of these variables are correlated with each other. The Pearson's correlation

326    test was performed for each pair of variables say As (DW) and Cu (Hair).

327

328 **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) | | | | | | | | | | | | |

329

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

331

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

338

339 ### 3.1.3 ANOVA Test
340 When trying to compare more than two groups of data set (such as in-stream concentrations at
341 different location along a river, each with multiple observations) either ANOVA test or variance
342 analysis can be used [24]. Statistical approaches applied up to this point in the analysis of the result
343 have been only restricted to descriptive and exploratory methods. This can be observed in the
344 positioning of San Cristobal samples in the leftmost quadrants of figure 2. This indicated that there are
345 some differences on the principal components. In order to justify this samples discrimination on the
346 principal components. A 2-factor ANOVA test was performed. One factor is metals (Arsenic, copper
347 and iron) each has four samples; the other factor is conditions (DW, FN, HR and TN) each has three
348 samples. The result showed that there is no too much difference between them; one reason for this is
349 that the samples size is too small. If significance level is set to 0.05, there are 11 samples showing
350 significance difference between metals.

351

352 ## 3.2 Discussion
353 For hundreds of years, people from Latin American countries have been drinking arsenic
354 contaminated water. Despite all this no accurate data record on the extents of it health consequences
355 and exposures. Latin America has been the region of arsenic investigation that leads to significant
356 contribution to current research of arsenic consequences on human health. Most especially with
357 respect to the development of lung, skin and bladder cancer. While development of and deaths from

358  cancer may take up to 35 years long after exposure, respiratory diseases or deaths from
359  cardiovascular in children may only occur during a period of chronic exposure.

360  The results of this analysis shows that heavy metals in drinking water, fingernails, hair and toenails
361  samples from different subject accumulate differently based on exposure. It was observed that there
362  is high value of arsenic in drinking water (DW= 0.20 ppm) compared to DW of iron and copper, such
363  differences recorded might be as a result of the fact that higher levels of arsenic tend to be more in
364  groundwater sources than in surface water sources (rivers and lakes) drinking water compared to
365  copper and iron. This agreed with the previous studies of Wang and Mulligan [23] even though
366  arsenic is found in both surface and groundwater but the levels are generally higher in groundwater.
367  The average values of iron in FN (62.94 ppm) and copper in FN (15.41 ppm) is higher except for
368  arsenic in FN (1.45 ppm). Cu, Fe, As, Cr, Ni, Cd and Mn has elevated levels in toenail than fingernail
369  samples, the high levels of all the metals in toenails samples when compared with fingernails
370  samples, might be attributed to the fact that fingernail grows continuous at a faster rate of 0.05 -
371  1.2mm per week while toenails grows at a slower rate and thus provide a longer integrated period for
372  metals accumulation compared to fingernail [10]. It is also evident that both toenails and finger nails
373  samples accumulate high concentrations of arsenic, copper and iron when compared with hair
374  samples. Such differences might be as a result of incorporation of the metals into the structure of
375  keratin in hair which take place by binding to the sulpurhydryl groups that are present in the follicular
376  protein [10].

377

378  To further explains the complex relationship by considering the correlation among the samples
379  variables, a positive correlation exists between finger nail and hair for arsenic as reported in table 1. A
380  similar correlation was recorded between nail and hair concentrations for elements Zinc, Arsenic,
381  Thallium and Cadmium [25]. There is limited knowledge of relationships between heavy metals in
382  nails and hair in published literature, which has mainly focused on correlations between drinking water
383  levels and other factors. Cecilie [25] carried out a study on relationships between nails and hair in
384  which a significant positive correlation for Sb and As was reported. Controversially, it has been
385  observed that individuals with high hair arsenic levels did not necessarily have elevated arsenic level
386  in nail [25]. The study highlighted that individuals with relatively high arsenic level in hair had lower
387  arsenic level in nail. Similar exposed subjects had significant correlation in nail and hair lead (Pb)
388  level, whereas perfect independence correlation was found in unexposed subjects [25]. The work
389  further observed that no relationship of cadmium concentration between nails and hair of both
390  exposed and unexposed individuals.

391

392  Vance *et al*. (1988) cited in [25] showed that concentrations of non-essential heavy metals were
393  positively correlated in nail and hair, whereas essential heavy metals were not correlated. However,
394  the current study about nail and hair relationships for heavy metals is confusing. Looking at the
395  correlation table, it can be concluded that more than half of the correlation analyses for iron and
396  copper did not shows any significant matched relationship. The results highlighted that incorporation

397    of heavy metals is somewhat different for nails and hair, even though they possess some similar

398    chemical composition.

399

400    **4. CONCLUSION**

401    The findings of current research indicate that San Cristobal and Huanqueros population were

402    exposed to higher levels of arsenic than copper and iron. The differences were most clearly observed

403    for toenails and fingernails but apparent for both tissues. Values of drinking water were significantly

404    higher compared to toenails, fingernails and hair in arsenic, copper and iron. One reason for this may

405    be attributed to the fact that drinking water is not a biomarker and therefore no long term

406    accumulation of metals. Likewise, it might be as a result of spatial variations of the two towns.

407    Correlations values were somewhat similar to previous studies. Some differences in values of

408    fingernails and toenails exist. There is risk of health impairment in some of the subject based on

409    comparison with arsenic, copper and iron in hair, toenails and fingernails. Despite the statistical

410    analysis conducted on San Cristobal and Huanqueros data for toenail, drinking water, fingernail and

411    hair heavy metals concentrations. There is need to analyze the data by Cluster Analysis for samples

412    categorization base on two different towns and see if such spatial variations have influence on the

413    levels of heavy metals. Furthermore, it is important to analyze other heavy metals such as zinc,

414    chromium, cadmium etc alongside with arsenic in drinking water of arsenic affected areas of Province

415    of San Fe. Future statistical analysis is required to have a better knowledge and understanding of how

416    specific factors influence heavy metals bioaccumulation. Smoking habit, age and gender should be

417    put into consideration to identify interactions among toxic heavy metals. It is also recommended to

418    statistically analyze several heavy metals in human exposure studies as a deficiency of some metals;

419    e.g. Manganese and Zinc could modulate and possibly increase the toxicity of other metals such as

420    arsenic.

421

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