Malaysian Journal of Chemistry, 2018, Vol. 20(2), 174 - 187

Chemometrics Approach to Identify Environmental Sources Contributing to Metals Exposure in Non-occupationally Exposed Pregnant Women of Western Australia

Ramalingam Malarvili[1*], McCafferty Peter[2] and Kittappa Shanmuga[3]
[1]Department of Chemistry Malaysia, Jalan Sultan, Petaling Jaya, 46661 Selangor, Malaysia.
[2]ChemCentre, Building 500, Chemistry and Resources Precinct, Manning Road, Bentley 6102, Western Australia, Australia
[3]Department of Civil Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur, Malaysia
*Corresponding author: (e-mail: malar@kimia.gov.my)

This study aimed to determine if chemometric techniques could be employed to identify the environmental sources contributing to metals exposure in non-occupationally exposed pregnant women living in Western Australia at background levels. Total metals (Al, Cd, Co, Cu, Pb, Mn, Hg, Ni, Se, U, V, Zn) concentrations were determined in 119 bloods and 109 urines, and environmental samples (104 house dust, 103 soil and 118 drinking water) collected from homes of the study population. Chemometric techniques, principal component analysis (PCA) and partial least squares (PLS) were used to identify the number of potential sources, and assess the percentage contribution of source signatures to the metal loadings in maternal blood and urine. Chemometric techniques were applied to the data generated from elemental analysis of blood, urine, drinking water, soil and dust samples to develop the signature. The use of PCA and PLS in establishing source signature using a suite of metals indicated that a mixed environmental source of metals contributed to the concentrations in maternal blood. Drinking water, soil and dust were identified as potential sources of metals in blood but not in urine. PLS analysis using the environmental data as the signature indicated 70-95% of the variance in the blood could be explained by the environmental signature for most blood samples.

Key words: Chemometrics; environmental sources; metals; non-occupationally exposed pregnant women

Received: June 2018; Accepted: September 2018

Human exposure to metals is traditionally assessed through monitoring metal concentrations in biological media such as blood and urine. However, this approach does not necessarily provide information about the source or route of exposure [1]. In order to identify the source of exposure, an understanding of the possible sources contributing to the exposure and the types of metal contaminants emitted from a potential source is required. Soil, dust, and water being ubiquitous are the most likely sources of metals exposure to human and this is supported by a number of studies [2-7]. However, the majority of these studies focussed on lead and high exposure concentrations in children. Studies to identify contributions from specific environmental sources to personal exposure, particularly at low levels, are limited. In studies, where this has been undertaken [6, 8-10], the task was often accomplished using isotopic analysis, which requires high-resolution instrumentation [10-12].

A conventional approach to determining source is to determine the metal concentrations in both biological and environmental samples, and a correlation is carried out for each metal independently to determine if there is an association. A more advanced method is to use regression analysis to identify relationships between a dependent variable and a set of independent variables [13]. Regression analysis is the commonly accepted approach in

many environmental sciences and epidemiological studies [6, 14-16] because it is often fairly robust and can be undertaken on smaller data sets; however, a major drawback is that it works under the law of probability. As a consequence, there is the possibility for relationships to go unnoticed in complicated environmental systems, especially when the metal concentrations dealt with are low [13]. An advanced multivariate statistical approach such as those employed in chemometrics may be required for the handling of such data [17].

Chemometric techniques use mathematical and statistical approaches, which are optimised and integrated into a user-friendly computational package to design experiments and provide maximum chemical information. This feature is employed in this study to enable the chemometric tools to distinguish the environmental sources and identify the metal pollutants (markers) that are characteristic of the biological samples, which can be accomplished by developing signatures. The use of the chemometric approach, which uses a fingerprinting concept, has been used successfully in the fields of environmental chemistry (e.g. oil spill identification and investigation of the fate of petroleum hydrocarbons) and environmental forensics [18, 19]. A general advantage of this approach over classical interpretation is the relative simplicity by which relationships between multiple variables (e.g. chemical concentrations) and samples can be determined and visualised using scores and loadings plots [18]. It makes interpretation easier and focuses attention on relationships that might go unnoticed when using traditional techniques such as regression analysis [13].

While the methods utilised to identify the sources of metals via chemometrics are many, principal component analysis (PCA) [13, 17, 20-22] and partial least squares (PLS) [19, 23-27] appear to be the common choices for many environmental investigations, principally due to their suitability in identifying the relationships between samples and the developing sources signature.

PCA is mainly used for pattern recognition and exploratory data analysis purposes. It is a fundamental approach of projection for other multivariate techniques as well [28]. PCA is an unsupervised technique that does not require input from the user on sample groupings. It is designed to achieve a new set of usually orthogonally arranged and uncorrelated reference variables or principal components by transforming the original data to smaller dimensions [17, 26, 28, 29]. Conversely, PLS is a supervised technique and is often known as "projections to latent structures" [28]. It is a special form of the more common multiple linear regression, which generalizes and combines the features of PCA and ordinary least square regression. PLS allows for the prediction of dependent variables (Y) from a large set of independent variables (X), or in other words, it allows the determination of the relationship between the two sets (X and Y) of variables. While PCA can be used to identify how many source types are present in a dataset, PLS can be used to explain the variance among the investigated variables and to assist in quantifying any one source is in a sample [19, 26].

A study involving human exposure assessment [30] enabled the determination of the significance of various factors including environmental (drinking water, soil and house dust) metal concentrations to measured biological metal concentrations. The analysis determined the statistical association between a single element in a biological matrix and these factors, but the contribution from each source could not be assessed because of the non-significant relationships [13, 30]. Either no correlation or weak correlation was observed between environmental metal concentrations and maternal biological metal concentrations in general and is likely due to the low recorded metal concentrations, which was indeed expected, making it difficult to identify relationships. The chemometric approach is expected to resolve this issue as source recognition is possible by the development of source signatures using several metal variables [25, 27, 31, 32].

The aim of this study was to employ PCA and PLS analysis on blood, soil, dust and drinking water samples. PCA was undertaken on environmental and blood samples to determine the possible origins of the metals found in maternal blood, whereas PLS was used to quantify the contribution made by the identified potential sources to the total metal content of the blood samples.

MATERIALS AND METHODS

Sample Collection

Biological (blood and urine) were obtained from a subset of participants of the 'Australian Maternal Exposure to Toxic Substances' (AMETS) study [30]. The participants were non-occupationally exposed pregnant women living in coastal, rural and urban areas of Western Australia. Participants were asked to complete a self-administered questionnaire and to provide a sample of drinking water, house dust and a composite surface soil sample taken from outside their home. Ethics approval was obtained for this study from Edith Cowan University Human Research Ethics Committee, WA Country Health Service, St John of God Health Care (Subiaco and Bunbury), Joondalup Health Campus and King Edward Memorial Hospital [33, 34]. All participants provided written informed consent.

Sample Preparation

In total, 119 blood, 109 urine, 118 drinking water, 103 soil, and 104 dust samples were available for analysis.

Digestion of blood (1.0 ml) was undertaken with high purity concentrated nitric acid (HNO_3) (1.0 ml) (Merck) and hydrochloric acid (HCl) (0.40 ml) (Australian Chemical Reagents) using a 1000 W microwave digester (Milestone Ethos Touch Control Microwave, supplied by Milestone, Italy) set according to the European Standard (EN) 1380:2002. The digest was diluted to 10.0 ml with milli-Q (>18MOhm) water. Urine samples were diluted (1.0 ml + 9.0 ml) with a diluent solution containing 1% HNO_3 and 0.5% HCl for analysis of common metals. For mercury analysis, the urines were diluted (1.0 ml + 9.0 ml) with a diluent solution containing 1% HNO_3, 1% HCl and 1 mg/L gold (Au) [30]. Creatinine analysis was prepared using the Jaffe Reaction method [35]. The urine analyses were corrected for their creatinine content, as per the procedure. The water samples were acidified with concentrated HNO_3 and analysed directly. Soil and dust samples were air-dried and passed through sieves, 1000 µm and 600 µm respectively before to analysis, and digested following USEPA SW 846 test method 3050B [36]. Soil and dust samples of equal dry weight were weighed out and digested in a mixture of HNO_3, hydrogen peroxide (Rowe Scientific) and HCl in open beakers using a hot plate. The digested samples were cooled, filtered and diluted to a final volume of 100.0 ml with milli-Q water.

Instrumentation

The prepared blood, urine and drinking water solutions were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500cs-Octopole Reaction Cell, Agilent Technologies, USA). Analysis of creatinine was completed using a discrete analyser (Labmedics/ Thermo Fisher Aquakem 250). The analysis of soil and dust digests were performed with a Varian Vista Pro Inductively Coupled Atomic Emission Spectroscopy (ICP-AES) (Varian Analytical Instruments Australia).

Data Analysis

The data of biological and environmental samples were explored by developing PCA and PLS models using the statistical package SIMCA-P + 12.0 from Umetrics (version 9) [37]. Twelve elements were included: aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), uranium (U), vanadium (V) and zinc (Zn). PCA was used to analyse the dataset to determine the number of potential sources of the maternal metals, followed by projections to latent structures by means of PLS to determine how much of the variability in the Y block (biological data) could be explained or predicted from a signature in the X block (environmental data) [25]. PLS performs PCA on the source sample (drinking water, soil or dust) data, defined as the signature that is expected to contain metal contaminants and be responsible for metals exposure. The projections will allow the quantification of the amount of variance in Y block that is explained by each X block signature [25]. A high percentage of variance illustrates a strong fit or similar signature between the X and Y blocks, and poor fit if the variance is small [25]. An overview of the PCA and PLS mechanisms concerning source identification can be found in [25].

Prior to analysis, the raw metal concentrations data were converted into proportion, and then log-ratio transformation

was conducted. This was undertaken to obtain normally distributed data, enabling better separation and interpretation of data, while eliminating any concentration effect, if present [19]. The data were then standardized, which involves centering and scaling of the variables. The standardization is critical as it avoids the PCA being dominated by higher concentration range elements [25, 38]. During this stage, the variable averages are subtracted and divided by their standard deviation. By doing so, the variables are mean centered, and have a unit variance standardised to zero, giving equal importance to all components [19].

RESULTS

Principal Component Analysis

The concentrations of the investigated metals in the environmental and biological samples were relatively low, as reported in [30]. In general, maternal blood and urine had low metal concentrations, mostly within the previously reported literature ranges for similar populations. Drinking water and soil metal concentrations were below the relevant Australian and World Health Organisation (WHO) guidelines [39-41], while the dust metals concentrations were consistent with other findings from similar environmental settings. This was not surprising as the study dealt with a non-exposed population in a residential setting.

PCA was performed separately on both the elemental data of blood and environmental samples, and the elemental data of urine and environmental samples to determine if a pattern or correlation exists between the environmental and biological samples. Blood samples were observed grouped together and separated from environmental samples, with drinking water spread out in the middle (Figure 1). Many of the soil and dust samples were observed overlapping with each other suggesting that soil and dust may have similar chemical compositions attributable to their common origin. However, the soil and dust samples appeared to be isolated from blood samples indicating soil and dust samples may not have a similar elemental data pattern to the blood samples. The influence of a specific metal to the groupings of the samples is explained using the loadings plots (Figure 2). From the plots, there was evidence that the scores of the majority of soil and dust samples were influenced by elevated aluminium, lead, manganese, vanadium and uranium compositions, while elevated copper, selenium and zinc content might explain the blood scores.

Identification of sources at low metal concentrations can be very challenging and difficult. In order to establish a signature, choosing the correct suite of metals associated with source sample identification is critical. Therefore, it was decided to exclude non-diagnostic variables in the consecutive

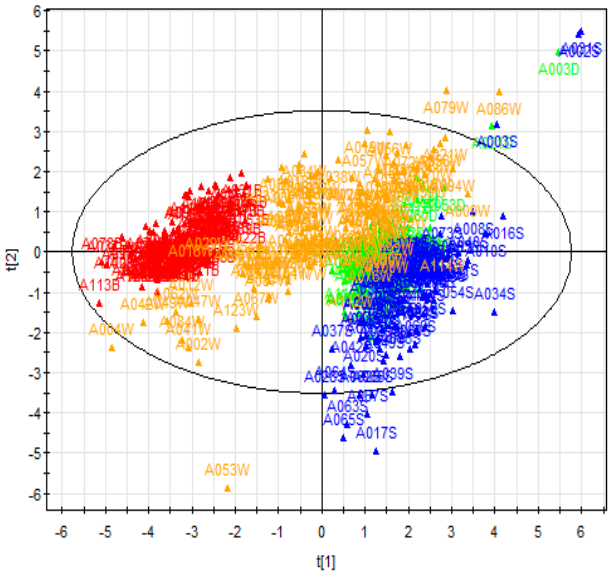

Figure 1. Scores plot for the elemental data of blood and environmental samples [blood (B) is red; drinking water (W) is orange; soil (S) is blue; and dust (D) is green].

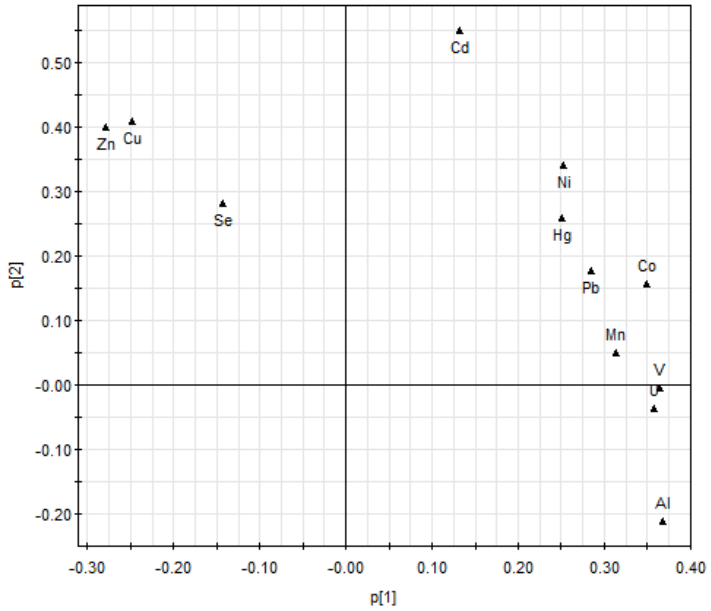

Figure 2. Loadings plot for the elemental data of blood and environmental samples.

analyses as their removal was anticipated to produce a better separation between sources. Given that copper, selenium and zinc are essential to humans and can be found naturally in the human body [42-45] their presence prevents the establishment of any correlation between the biological and environmental factors and thus were removed. Most blood and drinking water samples were found grouped indicating that they may have a similar pattern and thus, similar signatures (Figure 3). However, a blood sample with code A056B was found deviated from the normal grouping of samples (Figure 3), towards the right of the plot. The raw blood and questionnaire data of the particular woman was investigated to search any unusual behaviour, but no probable reason could be identified. Some water samples were seen separated from the blood samples as well, indicating they may not be relevant for source identification, and thus, participants with these sample codes were excluded during the defining of signatures in subsequent PLS analysis [25].

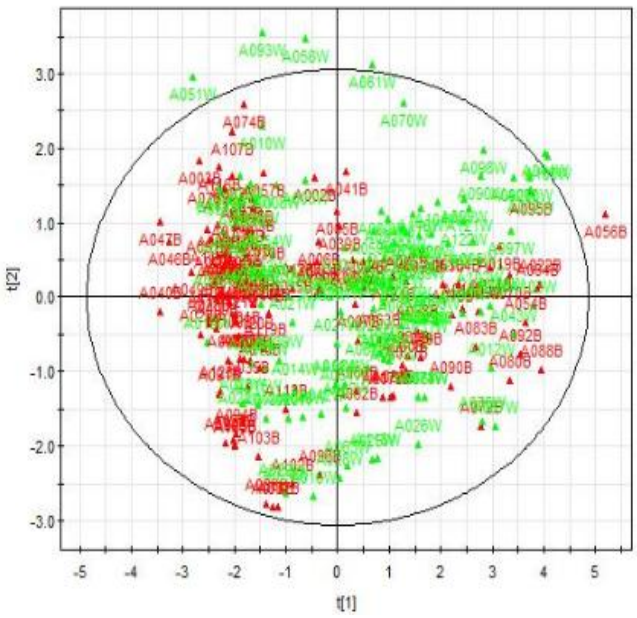

Figure 3. Scores plot for the elemental data of blood and drinking water samples [blood (B) is red; drinking water (W) is green].

Exclusion of aluminium, copper, selenium, and zinc yielded a model which exhibited a clearer relationship between blood and soil and dust samples (Figure 4). No definite explanation could be established for this observation, but an analysis of raw concentration data showed that aluminium was detected at elevated concentrations in soil and dust in general. This finding was in line with several other studies [46, 47]. This suggests that it is likely that the soil and dust sample loadings are mainly dominated by aluminium composition, which separated them from the blood samples. The overlapping of many soils and dust samples in Figure 4 may indicate the commonality between them. However, it should be noted that these associations do not necessarily represent a cause and effect [48, 49].

A PCA plot of urine and environmental samples shows that urine samples also isolated from the clustering of soils and dust, with drinking water samples spread out in between the two groups. Features that were apparent in the plots (data not shown) indicated that copper concentrations heavily influenced drinking water samples copper concentrations; soil and dust towards aluminium, lead, manganese, vanadium and uranium; and urine towards cadmium, selenium, and nickel. Although it seemed that there was no correlation between the urine and environmental samples, several attempts were again made to identify a possible source by removing non-diagnostic variables including copper, selenium, and zinc for the same reasons mentioned previously. However, no association could be established, with urine samples continuing to separate from the environmental samples. As PCA showed no association between the urine and environmental samples, the conclusion was that it would not be possible to define signatures through PLS analysis. This finding suggests two main possibilities. Either the source contributing to the exposure in urine is outside the matrices examined in this study e.g. food or the metals exposure is not reflected sufficiently in urine. Furthermore, blood, in general, is a better marker of exposure than urine, especially for assessing short-term exposure for many metals, with exceptions being nickel and uranium [50-52]. This is supported by the findings reported in [30], where more factors were found associated with blood metals in general when compared with urinary metals.

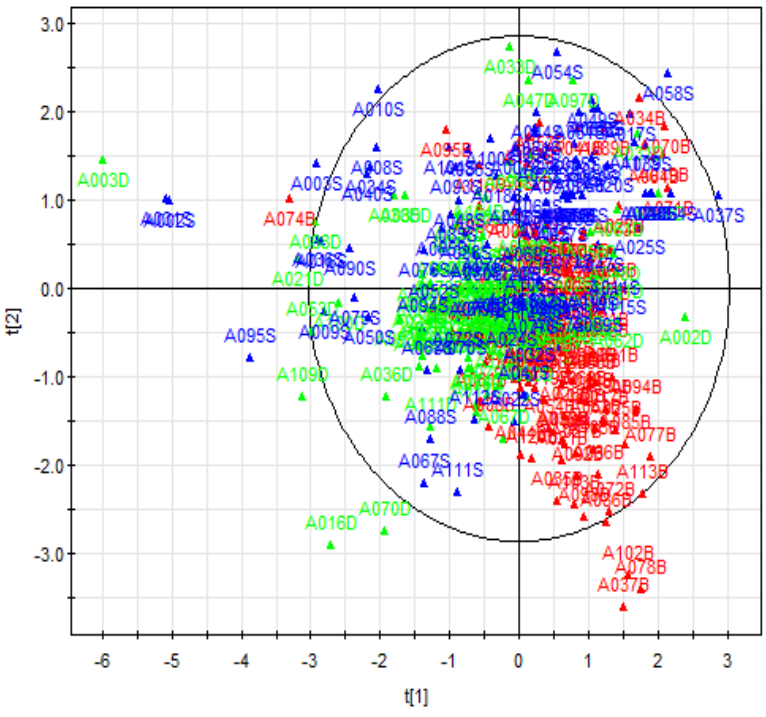

Figure 4. Scores plot for the elemental data of blood and soil and dust samples [blood (B) is red; soil (S) is blue; dust (D) is green].

It is worth mentioning that instead of using all the elements to provide a signature, it is relevant to use those that have shown to be distinct in the loadings plot [27] and judgement from the aspect of theoretical knowledge is also important. For example, some metals such as copper can be naturally found in biological and environmental samples, and thus provide a naturally elevated background, which may not be useful in the identification of the potential sources or determining the signature. The use of such metals to determine the signature can be erratic. In general, when copper, selenium, and zinc were removed from the data set, the overlap between the blood and environmental samples increased. Also, clearer association was seen between blood and soil and dust samples, with the removal of aluminium. This supports the importance of using the relevant element to provide the signature. However, care needs to be exercised when choosing the elements, as a decrease in selectivity or total explained variance is possible [23, 25]. This explicates the reason for not excluding the variables observed close to the origin in loadings plot, although they seemed to have little effect.

Partial Least Squares

Preliminary investigation of PCA indicated that drinking water, soil, and dust are possible sources of metals in the blood. Consequently, the reduced dataset was utilised to develop a signature using a suite of metals identified earlier in the appropriate sections to see how much variance of each source signature can be predicted in blood in general. Partial least squares model of blood and drinking water samples was built using aluminium, cadmium, cobalt, lead, manganese, mercury, nickel, vanadium and uranium. PLS analysis showed that approximately 73% of the blood samples had 60-100% of the variance predicted by the drinking water signature (Figure 5). A few women (~18%), however, showed poor matching of this signature with the predicted variance between 10-40%, indicating that apart from drinking water, other factors might be relevant. The loadings diagram (Figure 6) has aluminium and manganese in the positive quadrant and cadmium, cobalt, mercury, nickel and vanadium in the negative quadrant. Metals on opposite sides of the graph behave inversely, so as one increase, the other decreases. For example, when the concentration of manganese increases, the concentration of cadmium decreases. Sometimes they may even be mutually exclusive. For instance, in the presence of manganese, cadmium is absent. Lead is close to the origin, indicating it has little influence in the signature [23].

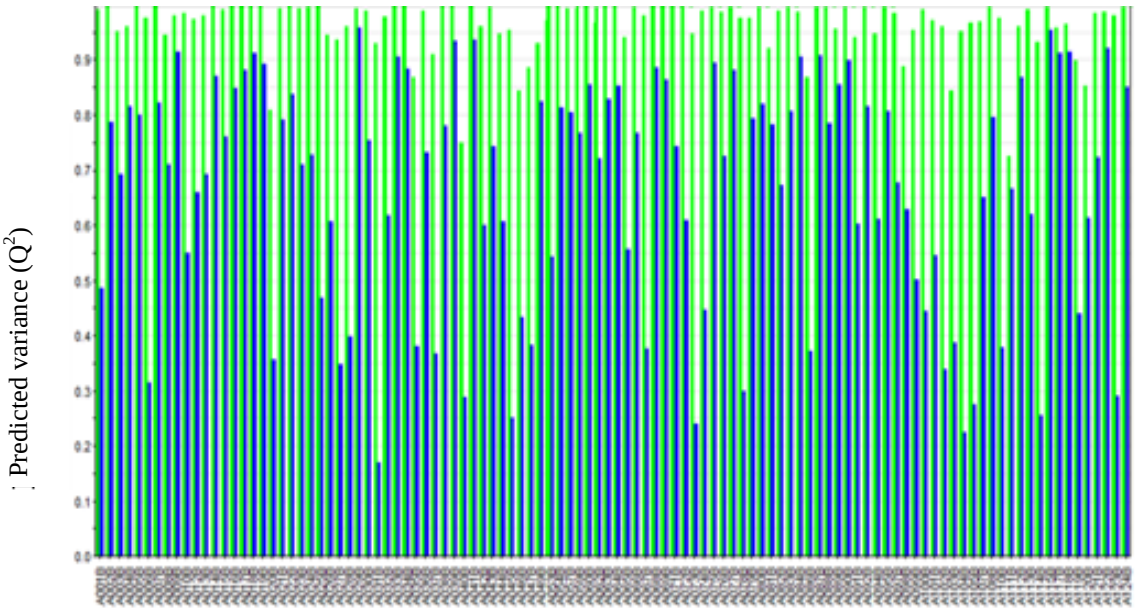

Figure 5. Predicted variance, Q^2 (blue) in blood using drinking water as the signature.

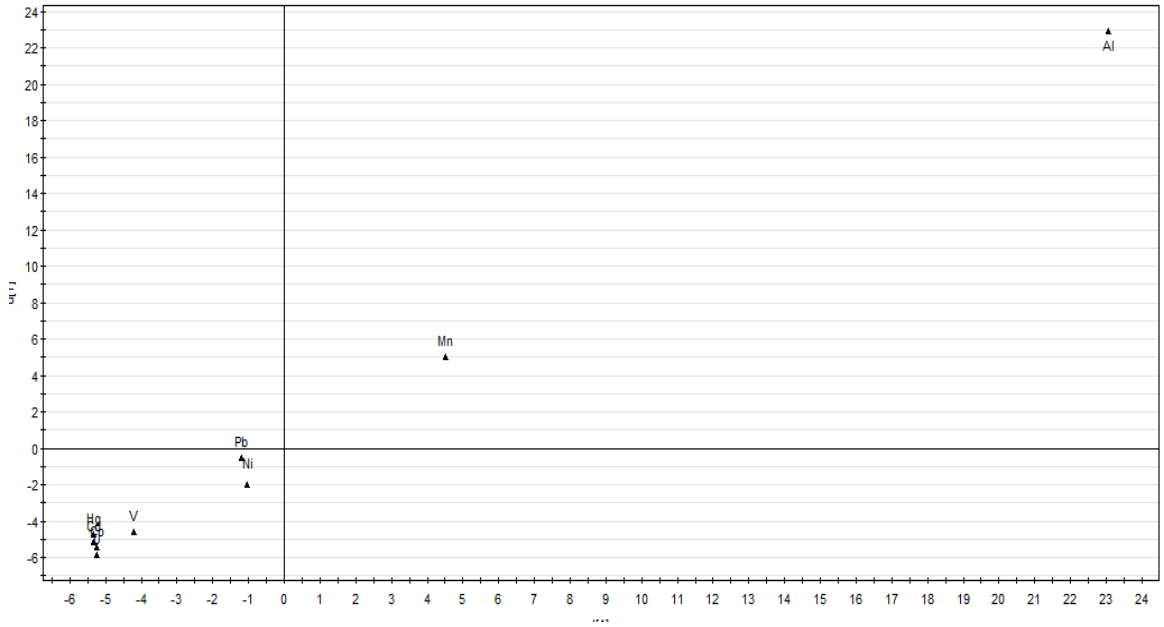

Figure 6. Loadings on the first principal component (t [1]) explains 70% of the variance in the drinking water signature and 30% in the blood, u [1].

The PLS model of blood and soil samples built using cadmium, cobalt, lead, manganese, mercury, nickel, vanadium and uranium showed that over 50% of blood samples had more than 60% of the variability predicted by soil signature (Figure 7). About 35% of samples had 90% of the variability predicted by soil signature. A few women (~27%) had relatively poor fit (<40%). The loadings diagram (Figure 8) shows lead and manganese in the positive quadrant, while cadmium, copper, mercury, and uranium are in the negative quadrant. Nickel and vanadium are near to origin and so are likely to have little effect on that PC.

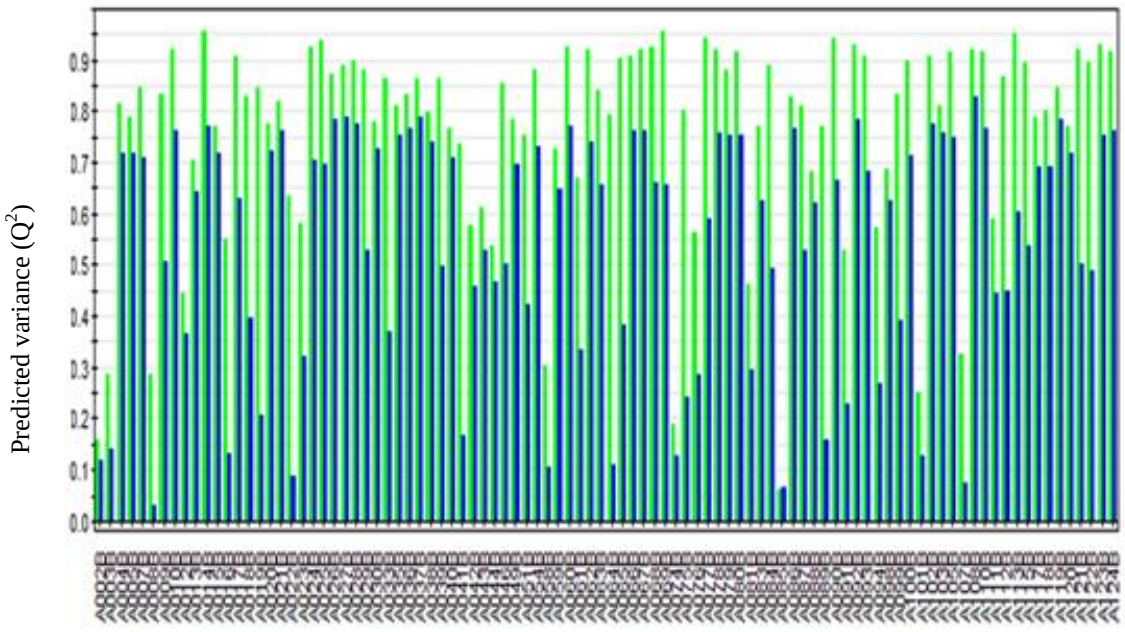

Figure 7. Predicted variance, Q^2 (blue) in blood using soil as the signature.

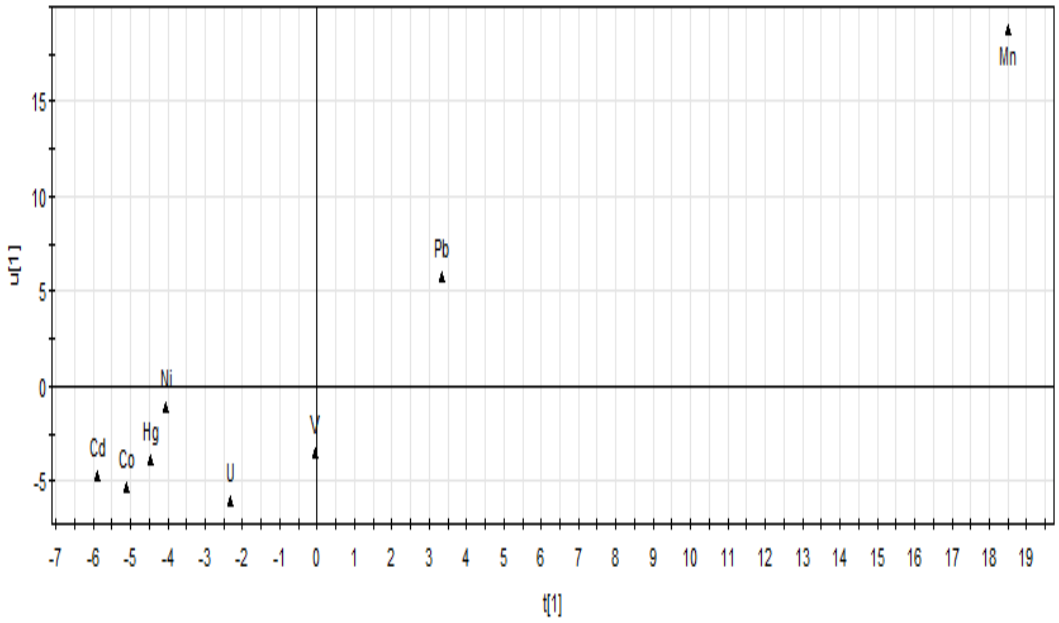

Figure 8. Loadings on the first principal component (t [1]) explains 76% of the variance in the soil signature and 54.7% in the blood, u [1].

The majority (~80%) of blood samples had 70% of the cadmium, cobalt, lead, manganese, mercury, nickel, vanadium and uranium variability predicted by dust the signature (Figure 9). Figure 10 shows a slightly better relationship between the loadings on PC1 from the X block (dust, t[1]) and those for the Y block (blood, u[1]) when compared with the data presented in Figures 8 and 6. Cadmium, cobalt, mercury, vanadium, and uranium were found negatively loaded to lead, manganese and nickel. As before, nickel was found close to the origin indicating that it may have little effect on the signature.

The amount of variance explained in the signature by the first PC is greater in soil (76%), followed by drinking water (70%) and finally dust (65%), indicating a greater degree of variability in the dust signature. The use of drinking water, soil, and dust as individual X-block signatures for blood metals dataset from Western Australian participants resulted in a wide variety of fits and the results are summarised

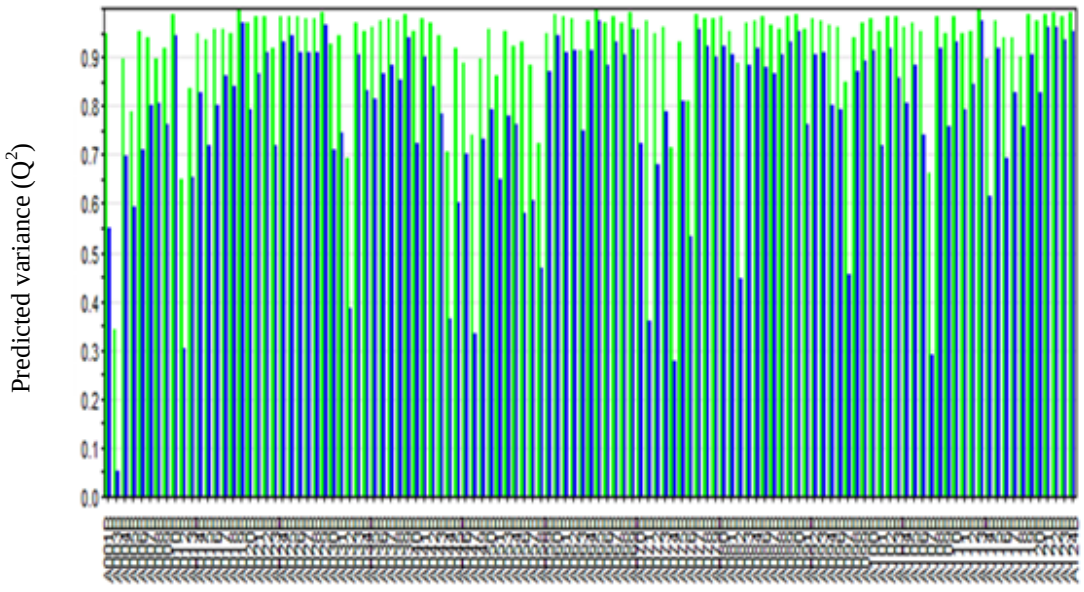

Figure 9. Predicted variance, Q^2 (blue) in blood using dust as the signature.

Table 1. Percentage of each predicted variance category by potential sources.

Explained variance	Drinking water (%)	Soil (%)	Dust (%)
0.0 – 0.2	0.8	13.4	0.8
0.2 – 0.4	17.1	13.4	6.0
0.4 – 0.6	9.4	17.5	6.8
0.6 – 0.8	34.2	54.7	29.1
0.8 – 1.0	38.5	1.0	57.3

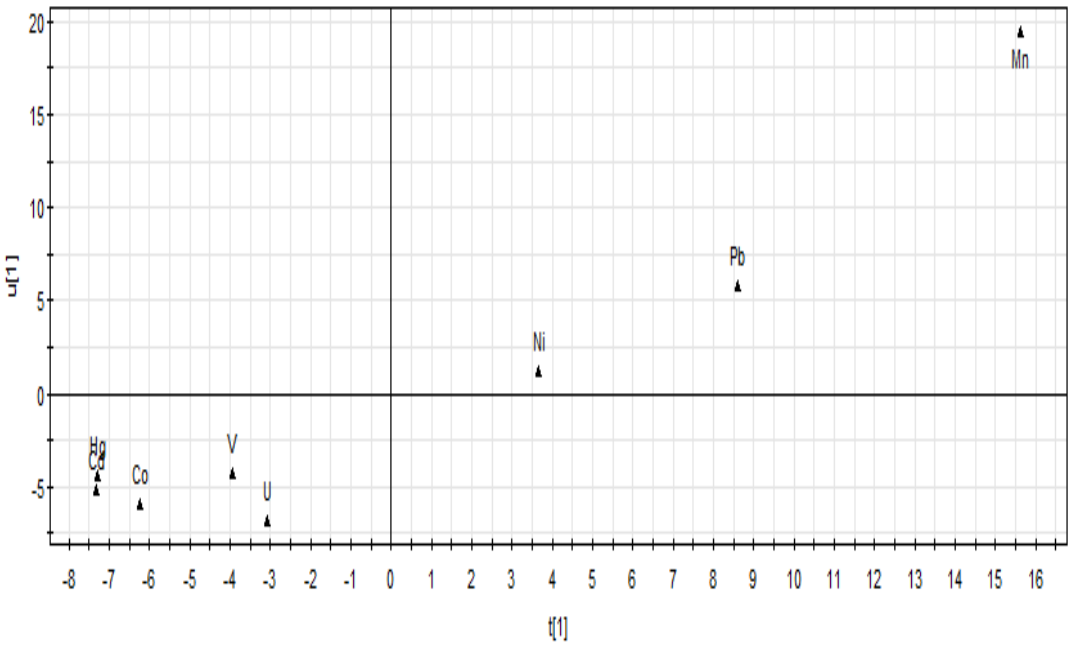

Figure 10. Loadings on the first principal component (t [1]) explains 64.8% of the variance in the dust signature and 43.3% in the blood, u [1].

in Table 1. There were relatively clear differ-rences between the fits. In general, the dust had a greater number of good fits (>60%) when compared with drinking water, while drinking water had a slightly larger number of good fits than soil. These data suggest that although drinking water, soil and dust signatures did not explain all the variance in blood samples when added together, these signatures make up the variance to some level, if not totally. This also suggests that in cases, where the total explained variance is poor, the main source contributing to exposure is outside the matrices examined here. A probable explanation concerning the poor fits obtained is that the women in this study were from various geographic locations with different environ-mental settings, so there may be the difference in localised sources at play depending on the location. The probability of being exposed to a direct food source is also suggested. It is also possible that the elements resulting in the variation are not included in the analysis.

DISCUSSION

Principal component analysis and PLS has revealed drinking water, soil, and dust as potential sources of metals exposure in blood. The fact that nickel was observed close to zero and uranium slightly off the regression line in Figures 8 and 10 suggests that their role in developing soil and dust signature to blood metals might be insignificant, indicating soil and

dust are less likely to be the main source contributing to nickel and uranium exposure in blood. Furthermore, in Figure 6, nickel and uranium were observed in the negative quadrant and close to the regression line. This indicates the nickel and uranium levels contribute to drinking water signature [27]. The solubility of nickel and uranium might be relevant here. A possible related explanation is that soluble nickel compounds are excreted rapidly through urine, whereas insoluble compounds of uranium are unlikely to enter the bloodstream, indicating the main component of nickel and uranium determined likely to be in soluble form, where main sources are usually diet-related factors or food [44, 53-55]. Based on published data [30], many blood samples had higher measurable concentrations when compared with urine samples. The high concentrations in blood as a well high percentage of samples with detectable levels of uranium in relative to urine suggest that the main component of uranium measured here could be in the form of soluble as insoluble compounds of uranium are unlikely to enter the bloodstream (e.g. uranium trioxide) [44]. As for nickel, many urine samples were reported to contain nickel, while only a few blood samples contained nickel. Also, nickel was detected in all water samples and uranium in many drinking water samples, again reflecting to their solubility in the media.

Furthermore, apart from the drinking water source system and diet in general, there were no other specific factors found influencing nickel and uranium exposure in this population [30]. All this suggests that diet could be the main source contributing to the nickel and uranium exposure. The fact that no source (of the investigated environmental samples) signature could be defined from PCA of urine, (data not shown) though urine is a reliable biomarker for nickel and uranium, also supports the above assertion. However, as speciation studies were not conducted, it is difficult to critique the results here.

No doubt, the use of several metals assisted in distinguishing the sources and provided signatures that were helpful in identifying the sources; however, the signatures were not very definitive. A likely explanation is that the suite of elements used might be inadequate and there was a lack of pure source materials. Pure elemental sources would not occur. For any one source the elements would be mixtures, hence multiple elements in multiple sources would confound any one source being attributed as the sole contribution. An adequate number of elements and source materials will aid in raising the total explained variance due to the commonality of the source compounds [25].

CONCLUSION

The results outline the application of the different methods to investigate sources of human exposure when concentrations were low and suggested that a chemometric approach could be useful to identify the environmental sources contributing to human exposure at background levels. The chemometric analysis of this data showed that drinking water, soil, and dust were potential sources of metals in maternal blood, but not in urine.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the researchers of ‘Maternal Exposure to PTS’ (AMETS) for the samples and Australian Research Council, Edith Cowan University (ECU), and the Arctic Monitoring and Assessment Programme for financial support. The author would also like to thank the staff of ChemCentre and ECU for their analytical assistance. Special appreciation goes to Department of Chemistry Malaysia for the study opportunity; Public Service Department of Malaysia for providing the research scholarship.

REFERENCES

1. Lynch, R. A., Malcoe, L. H., Skaggs, V. J. and Kegler, M. C. (2000) The relationship between residential lead exposures and elevated blood lead levels in a rural mining community, *Journal of Environmental Health*, **63(3)**, 9-15.

2. Gulson, B., Mizon, K., Taylor, A., Korsch, M., Stauber, J. and Davis, J. M. (2006) Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentadienyl manganese tricarbonyl in gasoline-preliminary results, *Environmental Research*, **100**, 100-114.

3. Vimercati, L., Baldassarre, A., Gatti, M. F., Gagliardi, T., Serinelli, M. and De Maria, L.

(2016) Non-occupational exposure to heavy metals of the residents of an industrial area and biomonitoring, *Environmental Monitoring and Assessment*, **188(12)**, 673. http://doi.org/10.1007/s10661-016-5693-5.

4. Gulson, B. L., Jameson, C. W., Mahaffey, K. R., Mizon, K. J., Korsch, M. J. and Vimpani, G. (1997) Pregnancy increases mobilization of lead from maternal skeleton, *The Journal of Laboratory and Clinical Medicine*, **130(1)**, 51-62.

5. Dewailly, E., Levesque, B., Duchesne, J., Dumas, P., Scheuhammer, A. and Gariepy, C. (2000) Lead shot as a source of lead poisoning in the Canadian Arctic, *Epidemiology*, **11(4)**, S146.

6. Gwiazda, R. H. and Smith, D. R. (2000) Lead isotopes as a supplementary tool in the routine evaluation of household lead hazards, *Enviromental Health Perspective.*, **108**, 1091.

7. Gulson, B. (2008) Stable lead isotopes in environmental health with emphasis on human investigations, *Science of the Total Environment*, **400(1-3)**, 75-92.

8. Komarek, M., Ettler, V., Chrastny, V. and Martin, M. (2008) Lead isotopes in environmental sciences: a review, *Environment International*, **34**, 562-577.

9. Wiechula, D., Fischer, A., Kwapulinski, J., Loska, K., Fischer, T. and Kurpas, P. (2006) Multivariate statistical analysis of metal concentrations in teeth of residents of Silesian Region, Southern Poland, *Archives of Environmental Contamination and Toxicology*, **51**, 314-320.

10. Santos-Burgoa, C., Rios, C., Mercado, L. A., Arechiga-Serrano, R., Cano-Valle, F. and Eden-Wynter, R. A. (2001) Exposure to manganese: health effects on the general population, a pilot study in Central Mexico, *Environmental Research Section A*, **85(2)**, 90-104.

11. Diez, S., Delgado, S., Aguilera, I., Astray, J., Perez-Gomez, B., and Torrent, M. (2009) Prenatal and early childhood exposure to mercury and methylmercury in Spain, a high-fish-consumer country, *Archives of Environmental Contamination and Toxicology*, **56**, 615-622.

12. Al-Saleh, I., Shinwari, N., Mashhour, A., Mohamed, G. E. D. and Rabah, A. (2011) Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women, *International Journal of Hygiene and Environmental Health*, **214(2)**, 79-101.

13. Koki, I. B.,Taqui, S. N. and Dhaif-Allah, M. A. H. (2018) A chemometric approach for the distribution and source identification of heavy metals in Tannery contaminated soil, *International Journal of Theoretical & Applied Sciences*, **10(1)**, 01-08.

14. Christensen, J. H. (2005) *Chemometrics as a tool to analyse complex chemical mixtures: environmental forensics and fate of oil spills*, Ph. D. Thesis, Roskilde University, Denmark.

15. Mudge, S. M. and Duce, C. E. (2005) Identifying the source, transport path and sinks of sewage derived organic matter, *Environmental Pollution*, **136**, 209-220.

16. Loska, K. and Wiechula, D. (2003) Application of principal component analysis for the estimation of source of heavy metal contamination in surface sediments from the Rybnik Reservoir, *Chemosphere*, **51(8)**, 723-733.

17. Yongming, H., Peixuan, D., Junji, C. and Posmentier, E. S. (2006) Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China, *Science of the Total Environment*, **355**, 176-186.

18. May, C. D. (2009) Development of novel analytical and interpretational protocols to facilitate the provenance establishment of glass and plastic evidence, Doctoral Dissertation, the University of Western Australia.

19. Boehm, P. D., Douglas, G. S., Brown, J. S., Page, D. S., Bence, A. E. and Burns, W. A. (2000) Comment on "natural hydrocarbon background in Benthic sediments of Prince

William Sound, Alaska: oil vs coal, *Environmental Science and Technology*, **34(10)**, 2064-2065.

20. Wingfors, H., Sjodin, A., Haglund, P. and Lunden-Brorstrom, E. (2001) Characterisation and determination of profiles of polycyclic aromatic hydrocarbons in a traffic tunnel in Gothenburg, Sweden, *Atmospheric Environment*, **35**, 6361-6369.

21. Mudge, S. M. (2002) Reassessment of the hydrocarbon in Prince William Sound and the Gulf of Alaska: identifying the source using partial least-squares, *Environmental Science Technology*, **36**, 2354-2360.

22. Mudge, S. M. (2002) Aspects of hydrocarbon fingerprinting using PLS-new data from Prince William Sound, *Environmental Forensics*, **3**, 323-329.

23. Mudge, S. M., Birch, G. F. and Matthai, C. (2003) The effect of grain size and element concentration in identifying contaminant sources, *Environmental Forensics*, **4(4)**, 305-312.

24. Maitra, S. and Yan, J. (2008) Principle component analysis and partial least squares: two dimension reduction techniques for regression, *Discussion Paper Program: Casuality Actuarial Society*.

25. Quinn, G. and Keough, M. J. (2002) Design and power analysis, *Experimental Design and Data Analysis for Biologists*, University Press, Cambridge.

26. Ramalingam, M. (2011) Metal exposure in non-occupationally exposed pregnant women of Western Australia and contribution of environmental sources, Ph.D. Thesis, Edith Cowan University, Australia

27. Burns, W. A., Mankiewicz, P. J., Bence, A. E., Page, D. S. and Parker, K. R. (1997) A principal-component and least-squares method for allocating polycyclic aromatic hydrocarbons in sediment to multiple sources, *Environmental Toxicology and Chemistry*, **16(6)**, 1119-1131. doi: 10.1002/etc.5620160605.

28. Paladino, O., Moranda, A. and Seyedsalehi, M. (2017) A method for identifying pollution sources of heavy metals and PAH for a risk-based management of a Mediterranean Harbour, *Scientifica*, 2017, 4690715. http://doi.org/10.1155/2017/4690715

29. Callan, A. C., Hinwood, A. L., Ramalingam, M., Boyce, M., Heyworth, J. and Mc Cafferty, P. (2013) Maternal exposure to metals - concentrations and predictors of exposure, *Environmental Research*, **126**, 111-117. http://dx.doi.org/10.1016/j.envres.2013.07.004

30. Hinwood, A. L., Callan, A. C., Ramalingam, M., Boyce, M., Heyworth, J. and McCafferty, P. (2013) Cadmium, lead and mercury exposure in non smoking pregnant women, *Environmental Research*, **126**, 118–124. http://dx.doi.org/10.1016/j.envres. 2013.07.005

31. Jaffe (1965) Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method, *Scandinavian Journal of Clinical and Laboratory Investigation*, **17**, 381-387.

32. *US EPA* (2002) *United States Environmental Protection Agency. SW-846 on-line. Update IIIB of SW-846 on-line [electronic resource]*, Retrieved from http://www.epa.gov/epawaste/hazard/test methods/sw846/pdfs/3050b.pdf

33. Umetrics (2009) *www.umetrics.com*, Retrieved 27 September, 2011

34. Suhaila, R. (2004) Significance of blood trace metals in hypertension and diseases eelated to hypertension in comparison with normotensives, Ph.D. Thesis., University of the Punjab Lahore.

35. *NEPC* (1999) National Environment Protection Council, *Schedule B(1) guidelines on the investigation levels for soil and groundwater, National Environmental Protection (Assessment of Site Contamination) Measure*, Retrieved 15 June 2011.

36. *NHMRC* (2004) *Australian drinking water guidelines 6, 2004 /* Australian Government National Health and Medical Research Council, Natural Resource Management

Ministerial Council, National Water Quality Management Strategy.

37. *WHO* (2006) *Guidelines for drinking water quality: incorporating first Addendum to vol. 1, Recommendations* (3 edn.).

38. Ysart, G., Miller, P., Croasdale, M., Crews, H. and Robb, P. (2000) 1997 UK total diet study-dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead mercury, nickel, selenium, tin and zinc, *Food Additives and Contaminants*, **17(9)**, 775-786.

39. Fisher, C. D. (2001) Chapter 76: Zinc. Section V: Specific health hazards and toxins, in *Clinical and Environmental Health and Toxic Exposures*, J. B. Sullivan Jr & G. R. Krieger (eds.), 2 edn., pp. 902, Philadelphia, USA.: Lippincott Williams & Wilkins.

40. *ATSDR* (2011) Agency for Toxic Substances and Disease Registry, *Toxicological Profile for Uranium*, Atlanta: US Department of Health and Human services, Public Health Service.

41. Brodkin, E., Copes, R., Mattman, A., Kennedy, J., Kling, R. and Yassi, A. (2007) Lead and mercury exposures: interpretation and action, *Canadian Medical Association Journal*, **176(1)**, 59-63.

42. Rasmussen, P. E., Subramaniam, K. S. and Jessiman, B. J. (2001) A multi-element profile of house dust in relation to exterior dust and soils in the city of Ottawa, Canada, *Science of the Total Environment*, **267(1-3)**, 125-140.

43. Turner, A. and Simmonds, L. (2006) Elemental concentrations and metal bioaccessibility in UK household dust, *Science of the Total Environment*, **371**, 74-81.

44. Norusis, M. J. (2000) *SPSS 10.0 Guide to data analysis*, Upper Saddle River, New Jersey: Prentice-Hall, Inc.

45. Pallant, J. (2007) *SPSS Survival Manual: a step-by-step guide to data analysis using SPSS for Windows (Version 15)* (3 edn.), Crows Nest, NSW: Allen & Unwin.

46. Lauwerys, R. R. and Hoet, P. (eds.) (2001) *Industrial chemical exposure: guidelines for biological monitoring (3 edn.)*, Boca Raton, London, New York, Washington DC: Lewis.

47. Barbosa Jr, F., Tanus-Santos, E., Gerlach, F. R. and Parson, P. J. (2005) A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations and future needs, *Environmental Health Perspectives*, **113**, 1669-1674.

48. Rao, A. N. (2005) Trace element estimation-methods & clinical context, *Online Journal of Health and Allied Sciences*, **4(1)**.

49. Sunderman Jr, F. W. (1993) Biological monitoring of nickel in humans. *Scandinavian Journal of Work, Environment and Health*, **19(suppl -1)**, 34-38.

50. *ATSDR* (2005) Agency for Toxic Substances and Disease Registry, *Toxicological Profile for Nickel*, Atlanta: US Department of Health and Human services, Public Health Service.

51. Klein, C. and Costa, M. (2007) Nickel,in *Handbook on the toxicology of metals*, G. F. Nordberg, B. A. Fowler, M. Nordberg & L. Friberg (eds.). http://www.ecu.eblib.com.au/patron/FullRecord.aspx?p=299461 & tstamp=1301912292 (3 ed.): Academic Press.