

## APPLICATION OF HYPHENATED PLASMA MASS SPECTROMETRY TO THE STUDY OF ELEVATED METAL TOXINS IN BREWED BEVERAGES

A. E. PILLAY\*, S. STEPHEN

Department of Chemistry, Khalifa University of  
Science and Technology (KUST), Abu Dhabi, UAE,  
\*Corresponding Author: avinash.pillay@ku.ac.ae

### Abstract

Hyphenated plasma mass spectrometry is a powerful tool for ultra-sensitive measurements in aqueous solutions. Our team investigated elevated levels of aluminium, manganese, nickel and chromium in brewed beverage samples (tea). Tea is a popular beverage and is consumed on a regular basis by adults and children. Elevated concentrations of toxins could be detrimental to the human body and remediation measures are necessary to reduce the levels of these metal toxins in tea leaves. Eight brands of tea samples were analysed for toxic trace metals after brewing in ultrapure Millipore water. A study of this nature, where metal toxins are extracted by boiling, is relatively underexplored. High resolution Inductively Coupled Plasma Mass Spectrometer was employed to examine the levels of the elements of interest. The performance of the instrument was validated using certified reference standards. Minor perturbations in performance were adjusted by use of an internal standard. The selected elements produce elevated levels in excess of a factor of 20 in some cases, compared to European Union standards for drinking water. These toxins tend to originate from the soil and water used to cultivate the tea leaves, and possibly from the chemical processes linked to the production of the tea leaves. It may be possible to use these elevated elemental levels to identify or “fingerprint” the country of origin of the corresponding tea sample. Our work could be considered a valuable source of reference data, and could contribute to sustainable development.

Keywords: ICP-MS, Plasma mass spectrometry, Tea, Toxic trace metals.

## 1. Introduction

Tea is a widely popular beverage, consumed by adults and children in significant quantities. For this particular reason contaminants and toxins in tea should be minimised to acceptable levels on par with drinking water to prevent detrimental effects to the human body. Tea originating from various parts of the world are known to contain hazardous levels of heavy metals [1; 2]. The origin and mechanistic pathway of toxic elemental impurities in tea come mainly from environmental sources. These toxic elements could be characteristic of the soil and irrigation water that feed the tea plants, or they could originate from the tea-making process [3].

The machinery, additives and preservatives that are associated with the production of tea represent a highly feasible source of these impurities, and should be considered a significant factor in contributing to the levels of these impurities. Brewed tea products of different brands and from various countries were studied for trace toxic elements by ICP-MS (inductively coupled plasma mass spectrometry) [4]. The investigation is original from two perspectives: (i) that a comparative study of this nature is relatively underexplored; and (ii) that most studies of this type were limited to the tea leaves and not the actual brew. In this respect our research could be considered a baseline study to provide a guideline for typical concentrations of selected trace toxic elements in tea. At the outset it is important to emphasise that our study makes a marked contribution to sustainable development [5].

Some contemporary analytical techniques tend to be limited in their capability to detect ultra-trace metal toxins [6-14]. However, ICP-MS has demonstrated its advantage in this respect [15-18]. Well known instrumental techniques such as XRF (X-ray fluorescence), PIXE (particle-induced X-ray emission) [6; 7; 11] and PIGE (particle induced gamma emission) are not adequately sensitive; whereas other current techniques such neutron activation [9] and electro thermal analysis have distinct disadvantages which make them inadequate. ICP-MS is multi-elemental, rapid, and is capable of detection at the ppt or ng/L level [15; 17; 18]. The tea samples subjected to investigation were analysed for aluminium, manganese, nickel and chromium, all considered to be toxic at elevated levels [5]. Thus, from the standpoint of sustainability our work could be considered highly significant as toxins in tea are of universal importance. Food and forensic science could also benefit from this study.

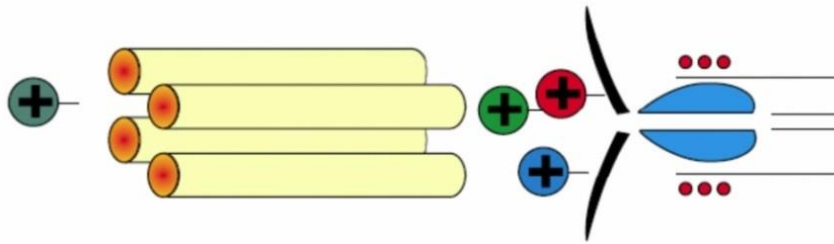
## 2. Materials and Methods

### Instrumentation / Sample treatment

Samples of tea leaves of different brands and from a variety of countries were procured from local retail outlets. The samples were brewed using Millipore water, treated in mild aqueous acid media (3% HNO<sub>3</sub>) and transferred to special vials for ICP-MS analysis. Each brewed tea solution thus prepared was introduced into the instrument via an aspirator/nebulizer unit and transported to a high-temperature argon plasma (6000-8000 K) where it was ionized, and the ions, characteristic of the elements of interest, were conveyed to a mass spectrometer for detection. The instrument is equipped with a high-performance mass filter composed of quadrupole rods that serve as a multi-elemental selector system (Fig. 1). A continuously varying

electric field applied across the quadrupole allows ions of individual mass-to-charge ratios ( $m/z$ ) to enter the filter so that at any given instant discrete mass separation up to 2400 amu (atomic mass units) can be attained. The technique is quick, multi-elemental, high-resolution and facile. It is particularly sensitive, capable of trace analysis, and well suited for environmental studies.

Spectra originating from the measurements were accumulated and recorded, and quantitative analysis was conducted by comparing sample data with suitable certified standards. Appropriate software was deployed to correct for sample interferences and matrix effects. Intermittent drift in the instrument and minor fluctuations in performance were monitored by the use of suitable internal standards. Linear calibration and background correction was achieved by the use of reference standards. The nebulizer gas flow in the system was 0.80 L/min. Prior to application the performance of the instrument was validated for repeatability.



**Fig. 1. The quadrupole mass filter responsible for highly discrete mass separation.**

### 3. Results and Discussion

#### 3.1. Repeatability

Certified reference standards (Fluka 70007) were used to establish the consistency of measurements and to evaluate the capability of the technique in attaining the prescribed detection limits. The analytical performance of the method was established by making measurements in triplicate ( $n = 3$ ) for each sample and standard. The instrument functions by aspirating three individual sample aliquots into the plasma in quick succession and the software subsequently calculates the standard deviation. If the relative standard deviation (RSD) exceeds 10% the sample is re-analysed to ascertain whether matrix effects are influencing the results.

For our study RSDs <5% were attained for certified material (Table 1) indicating that the instrumental performance of the system was acceptable. The ICP-MS system has proved to be superior in producing highly repeatable results. Several studies have established the capability of ICP-MS to perform multi-elemental, simultaneous measurements to high degrees of accuracy and reproducibility [19]. The software combined with exclusive technology minimises interferences and matrix effects for excellent accuracy and reproducibility. Subsequent to the standard calibration procedure, the mass spectrometer performance was checked using the Perkin Elmer provided quality control standard QC 2-1 (Lot No. 211125062-01) with different elements at 10  $\mu\text{g/L}$ .

**Table 1. Repeatability study using a multi-elemental aqueous standard (Fluka 70007), unit of measurement: ppb.**

Measurement	1	2	3	Mean $\pm$ RSD
Be	10.6	10.5	10.8	10.6 $\pm$ 0.93%
Mg	10.5	10.5	10.7	10.6 $\pm$ 1.7%
Co	9.9	10.5	9.9	10.1 $\pm$ 4.0%
Ni	9.9	10.2	10.1	10.0 $\pm$ 1.0%
In	9.6	9.5	9.7	9.6 $\pm$ 0.6%
Ce	9.7	9.2	9	9.4 $\pm$ 4.0%
Bi	9	9.5	9.2	9.2 $\pm$ 3.1%

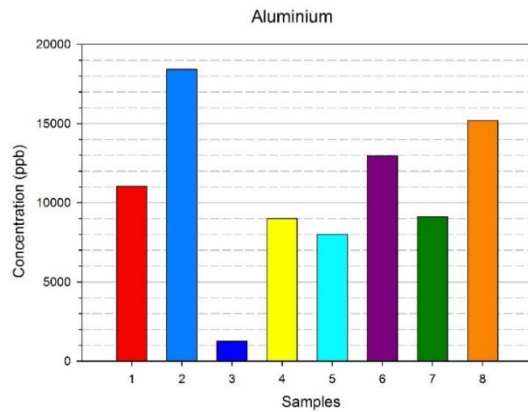
### 3.2. Elevated metal toxins

At the outset, it is important to emphasize that the toxins were measured in the brew itself and not in the tea leaves. The brew is consumed as an aqueous solution and the standards associated with drinking water quality could, therefore, be applied. Tea bags were brewed in ultra-pure water and the metal toxins under study were leached from the tea bag into the brew during the process of boiling. So the brew itself consisted of abstracted inorganic and organic chemicals from the tea leaves in the bag. Our study undertook systematic elemental profiling of these samples and discovered elevations in the concentrations of Al, Mn, Cr and Ni. We also found that these elements manifested toxicity at varying levels. These contaminants are entrenched in agricultural sources and components such as soil, water, manure, peat, and pesticides could all contribute to the elevated levels of these toxins. As aforementioned, the contamination could also have arisen during the processing of the tea leaves. We are, therefore, faced with an environmental problem that could be detrimental to human health, and could affect the development of sustainable living if it is not quickly addressed. An interesting point to note is that direct analysis of tea leaves would not produce an authentic reflection of toxicity compared to analysis of the brew for the simple reason that the brew is consumed, and the used tea leaves are discarded. Leaching of metals from tea leaves could take place at various rates because some metals could be entrapped in molecular arrangements that prevent rapid migration into solution.

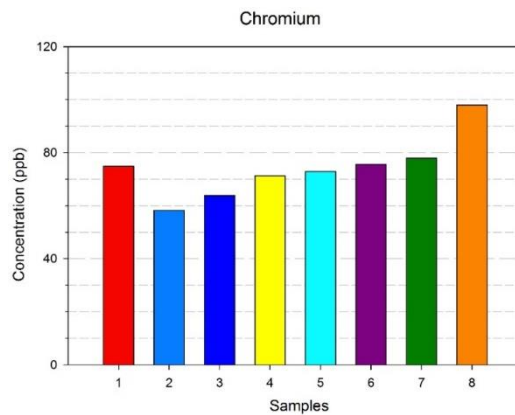
*Aluminium (Al)*: aluminium is the most abundant metal in the earth's crust and usually occurs as aluminium oxide in soils and rocks. A previous study reported that the levels of aluminium in tea leaves are generally higher than normal [20]. It is well known that elevated levels of aluminium are suspected of leading to Alzheimer's disorder [5; 21]. The European Union's (EU) [22] permissible level of Al in drinking water is 200  $\mu\text{g/L}$ . According to Fig. 2, most of the Al levels are in excess of 8000  $\mu\text{g/L}$  - a factor of 40 times higher. Only one sample (#3) registered a concentration of  $\sim 1200$   $\mu\text{g/L}$  - still 6 times higher than the EU acceptable limit. The trend in Fig. 2 shows that unusually high Al levels were observed for all samples. It is possible that the equipment employed to process the tea leaves could have contributed to these abnormal levels, and indicates that the general technological production of tea leaves could be revisited to establish some form of remediation to reduce the levels of this element.

*Chromium (Cr)*: the toxicology of chromium and its biological effects at elevated doses are well documented [5]. At abnormal levels, Cr is known to

produce chronic disorders such as lung cancer and recurring skin diseases. Our work shows (Fig. 3) that the range of Cr results was between 50-100  $\mu\text{g/L}$ .



**Fig. 2. Aluminium levels in tea-brew.**

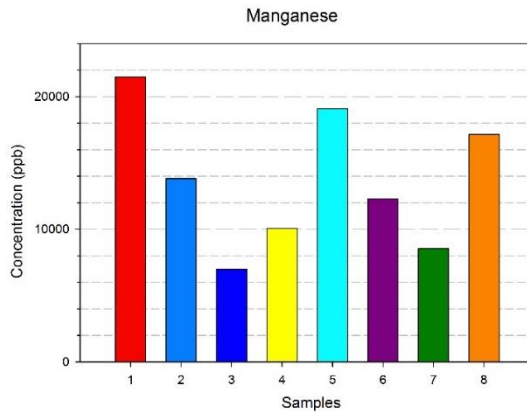


**Fig. 3. Chromium levels in tea-brew.**

The EU permissible level is 50  $\mu\text{g/L}$  [22]. The most elevated level was by a factor of about 2 in sample #8, which was not as elevated as the aluminium levels but nevertheless sufficiently abnormal to create a cause for concern. Tea is usually consumed in large quantities by adults and children and even slightly elevated levels could be hazardous and calls for measures to be adopted to de-metallise tea leaves prior to public consumption. Here again, it is suggestive that these elevated levels could have originated from the metal equipment associated with tea production and it is recommended that in the interest of sustainable living such processes should be overhauled to eradicate toxic elements. Future work could be linked to toxic speciation studies of this metal.

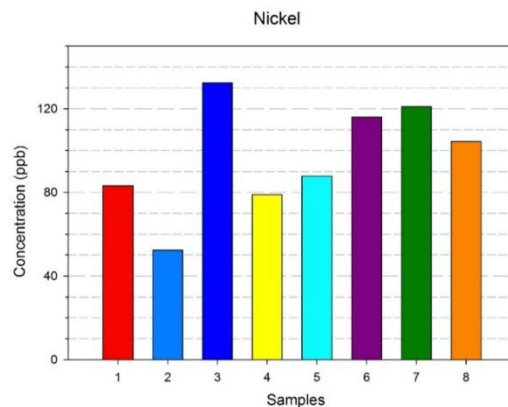
*Manganese (Mn)*: the biological effects of manganese are not as well documented as chromium and aluminium. However, Mn in excessive doses could lead to detrimental effects in the human body [5]. It has been reported that neurological effects are discernable at consistent low-level doses. The EU acceptable level in drinking water is 50  $\mu\text{g/L}$  [22]. Our research detected levels

above 400 times higher in some brews. Figure 4 delineates a range between 7,000 to 20,000  $\mu\text{g/L}$ . These levels are highly abnormal and regular consumption of tea with such levels could lead to chronic disorders. There is therefore, a critical need to reduce the concentration of Mn in tea leaves. Such concentrations could stem from agricultural sources such as soil and water, so there is a need to examine the irrigation water and soil for contamination of Mn, prior to planting the tea [23].



**Fig. 4. Manganese levels in tea-brew.**

*Nickel (Ni)*: nickel occurs in the earth's crust and in soils mainly in the inorganic form as *nickel* sulphide. Nickel-poisoning in the human body results in neuro and renal disorders [5]. The EU standard for Ni in potable water is 20  $\mu\text{g/mL}$  [22]. The results in Fig. 5 denote that the Ni levels are elevated by factors between 5-6 in samples #3, 6, 7, 8. The remaining samples reflect elevations by factors between 2 to 4. The source of Ni could be traced back to the environment and possibly linked to contamination from soil, irrigation water, manure and pesticides. The adverse biological effects of Ni necessitate some cost-effective chemical treatment for its removal. Alternatively, it is possible that nickel could occur at minor levels in the equipment employed for processing the tea. Contamination from such equipment could lead to elevated levels.



**Fig. 5. Nickel levels in tea-brew.**

### **3.3. “Fingerprinting”/Mapping**

The feasibility of employing trace toxic metals in tea-brew for use in “fingerprinting” or mapping studies was examined. Such methods could be employed to identify tea produced from a specific region or country based on trace elemental levels. Pronounced levels in the observed concentrations tend to make the task of “fingerprinting” more facile. Updated fingerprinting techniques could include pattern recognition methods that deploy intricate software. Therefore, this particular area of study could be complex and the subject of a more detailed future investigation.

The tea products investigated in this work originated from various regions and fingerprinting or mapping studies could be based on metal content or ratios. For example parameters such Ni/Al, Cr/Mn, Mn/Ni or Al/Cr could be used to “fingerprint” specific tea products and the regions of their origin. Such parameters could be helpful in food studies and forensic science, in tracing tea products to certain outlets that retail tea from specific areas and countries. Such ratios and similar parameters could be “fine-tuned” or narrowed down and used for “mapping” tea farms in a specific locality or district.

### **3.4. Impact of the study**

As mentioned earlier a toxicology study of this nature, where the brew of different brands of tea products originating from various regions are investigated for elevated metal toxins, has not been widely reported. Documentation of our experimental data is significant from two points of view: (i) wide public consumption of tea makes such studies essential; and (ii) it contributes to sustainable living. It should be stressed that toxins in tea tend to originate largely from the environment. Therefore, to examine toxins in tea it is essential to examine the environmental sources. Soil and water are the main sources for contaminants in plants such as tea leaves. And it is these sources that have to be de-contaminated to reduce the hazard.

Alternative theories for some of these abnormal experimental results are possible. For example, it is possible that the equipment used to process the tea leaves could have been partially responsible for the elevated levels of metal toxins studied here. Use of equipment composed of aluminium, manganese, chromium and nickel could have contributed to such contamination. In addition, instrumental error could have also been responsible for these pronounced levels, but the random variation within each range of measurements debunks this particular theory.

The principal trend observed in Figs. 2-5 is that the levels of the metal toxins of interest are consistently elevated, to various degrees. This particular feature alone could be further developed as a topic for further study and used for fingerprinting purposes. The most serious hazard is posed by aluminium in the range 1200-20000 µg/L (6-100 times higher than the acceptable EU level in potable water). It is not clear why Al is present at such abnormal levels but further studies are expected to be instituted to evaluate Al concentrations in other tea brands to establish if equivalent levels exist. The biomedical character of Al is widely reported and since this element is suspected of causing Alzheimer’s disease its levels should be restricted.

In the case of manganese, the range recorded is abnormally higher than the tolerable EU level in drinking water. The biological effects of Mn toxicity have not been widely documented, but it has emerged that toxic concentrations of Mn could lead to specific disorders, which predicts early hazards for children who consume appreciable quantities of tea.

Chromium was present at moderately elevated levels, which is still in excess of the EU permissible level of 50 µg/L in potable water. The detrimental effects of high doses of Cr to the human body have been seriously investigated by the medical fraternity and are linked to chronic disorders.

Nickel poisoning of the human organism also leads to acute and chronic disorders. The trend in Ni results observed in Fig. 5 clearly denotes consistency in the range 50-100 µg/L - all above the EU tolerable level of 20 µg/L in drinking water. In the interest of sustainable development certain remedial measures should be undertaken to limit these abnormal levels of metal toxins in tea leaves. This is clearly a mission for health authorities who need to be alerted on this subject.

An interesting point is that some of these toxins could have originated from the tea-making process. Chemicals and reagents used in this process could have embedded impurities in them, which, in turn, could enter the tea product and contaminate it. In addition, the machinery and technical equipment used to produce the tea could be contaminated and could be responsible for the impurities of interest. The system of processing tea leaves should therefore, be revisited and decontaminated. Thus the primary impact of this study is to make both tea manufacturers and consumers aware that certain impurities or toxins could be introduced into these products and remedial measures could be adopted to limit the presence of toxic elements in tea.

#### **4. Conclusion**

This study is an exercise in sustainable development. To maintain sustainability and reduce environmental threats it is essential to investigate and restrict all toxins in products that are consumed by the public. Our research has detected above average levels of Al, Mn, Cr and Ni in the brew of eight brands of tea. It is essential for remedial measures to be put in place to curb these elevated levels either by demetallisation or some other suitable chemical process prior to marketing the tea to the public.

Our study could be extended to other popular beverages such as coffee and cocoa, and additional brands of tea. The kinetics of leaching of these metals from tea leaves into the brew would also form an interesting future study. The food and beverage industry would be equally interested in research of this nature, especially as tea is widely consumed by adults and children. Future work could also include the application of trace/toxic elements in tea in mapping or fingerprinting studies for purposes of locating the regions of origin of the tea leaves.

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**Nomenclatures**

$m/z$       Mass-to-charge ratio

**Abbreviations**

EU	European Union
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
KUST	Khalifa University of Science and Technology
PIGE	Proton Induced Gamma-ray Emission
PIXE	Proton Induced X-ray Emission
RSD	Relative Standard Deviation
XRF	X-ray Fluorescence

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