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Research paper

Extractability of carcinogenic toxins in brewed beverages (tea) a study by hyphenated plasma mass spectrometry

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Abstract

Beryllium (Be) and arsenic (As) are noted for their potential carcinogenicity, and their elemental distribution in brewed teas was the subject of investigation. A study of this nature, where carcinogens are extracted by boiling, is underexplored. The study focused on brewed beverages and the experimentally determined concentrations were thus compared with drinking water levels. Tea is a favourite drink, is consumed several times a day and toxins could bio-accumulate in the body. Appreciable concentrations of carcinogens could therefore, affect the human body and restorative measures could be adopted to minimize such toxins in tea leaves. Eight brands of tea samples were procured and investigated for carcinogens after brewing in ultrapure Millipore water. Hyphenated plasma mass spectrometry (ICP-MS) was deployed to probe the levels of the elements of interest. The operation of the instrument was certified with reference standards. Aberrations in performance were reset by use of an internal standard. Beryllium levels occurred in the range 0.5-1.5 µg/L; As concentrations were between 1-3 µg/L. The distribution of the selected elements is discussed. These carcinogens could emerge from the ground and from the agricultural water used to cultivate the tea leaves, or alternatively, from the manufacturing processes associated with their production. It could be feasible to deploy the data to identify or "fingerprint" the country or region of origin of the corresponding tea sample. This study could definitely be considered a source of reference data, and could add to the growing knowledge on sustainable development.

Keywords: *Plasma Mass Spectrometry; Tea; Carcinogens; ICP-MS; Sustainability.*

1. Introduction

Beryllium and arsenic are potential carcinogens and their oral ingestion at appreciable doses could be harmful to the human body. The evaluation of the distribution of these carcinogens in brewed tea has not been previously documented and could form the source of a reference database. It is necessary to make the distinction between the presence of such carcinogens in brewed tea and in tea leaves. In brewed tea these toxic elements are extracted from the tea leaves [1], [2] and consumed orally. Therefore, pollutants and impurities in tea should be restricted to permissible levels, on par with drinking water to prevent harmful effects in the human body [3]. The introduction of noxious metal toxins in tea arises primarily from environmental sources. These unwanted impurities could be present in the soil and agricultural water that irrigate the tea plants, or they could originate from the manufacturing process [4]. The equipment, chemical additives and preservatives are linked to the mechanistic pathways by which these toxins enter the tea leaves and constitute a probable source of these impurities, and could be considered a salient factor in contributing to the levels of these impurities. The study focused on the trace elemental profiles of brewed tea products of commercial brands originating from various countries. Levels of the carcinogens were obtained numerically by ICP-MS (inductively coupled plasma mass spectrometry) [5], [6]. The study is novel from two aspects: (i) a comparative study of carcinogenic profiles in brewed tea is relatively underexplored; and (ii) the study makes a contribution to sustainable living. From this point of view the investigation could be considered a reference study to provide a guideline for typical levels of selected metal carcinogens in tea. At the outset it is important to underscore that features of our study are of considerable interest to sustainability and to food and nutrition organizations.
Many current analytical techniques are restricted in their ability to detect ultra-trace pollutants in environmental samples [7-14]. However, ICP-MS has a distinct advantage over its rivals. Popular analytical methods such as XRF (X-ray fluorescence), PIXE (particle-induced X-ray emission) and PIGE (particle induced gamma emission) lack the necessary sensitivity [7], [8], [10], [12]; whereas other existing techniques such as neutron activation and electro-thermal analysis have other drawbacks. ICP-MS can analyse a wide number of elements in a single run; it is facile, rapid, and highly favourable for detection at the ppt or ng/L level [15-19]. Prior to instrumental analysis the tea samples were subjected to preparation and treatment.

2. Materials and methods

Tea leaves of different brands, and from a variety of regions were obtained locally and brewed using Millipore water. The brews were subsequently processed in moderate aqueous acid media (1% HNO_3) and collected in customized vials for ICP-MS determination. The brewed samples were aspirated into a high-temperature argon plasma (6000-8000 K), and ions of the elements of interest were transported to a high-resolution mass spectrometer for detection. Individual mass-to-charge ratios (m/z) were filtered to produce discrete mass separation up to 2400 amu (atomic mass units). The analysis is rapid, ultra-sensitive and highly facile. It has been used for various analytical studies involving trace element detection.

The advent of ICP-MS in the mid-1980s has revolutionized the field of inorganic analytical instrumentation [5], [6]. Having retained all the good characteristics of Atomic Absorption Spectrometry (AAS) and ICP Atomic Emission Spectrometry (ICP-AES), ICP-MS has emerged as a “super tool” among contemporary analytical instrumentation [15-17]. The superior detection limits stretching to ppt levels for several elements, comparatively higher throughput due to the sophisticated interface region (Fig. 1), and superfast sequential scanning has made it one of the most favored tools of the analyst [17]. The ability to detect all the elements in a single scan and the introduction of collision/ reaction cells to deal with matrix interferences has given the instrument unparalleled acceptance in the scientific world [16-18]. Unwanted drift in the instrument and minor shifts in performance were regulated by the use of certified internal standards. Calibration and noise suppression were accomplished with reference standards and suitable software. The nebulizer gas flow in the system was 0.80 L/min. Prior to application the performance of the instrument was validated for repeatability.

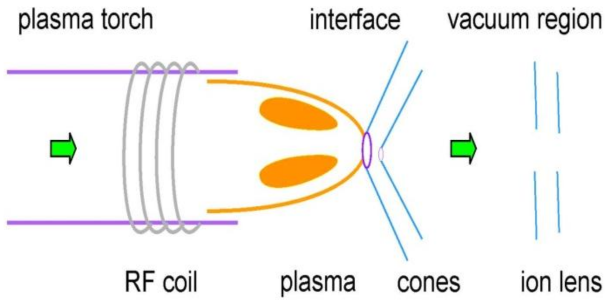

Fig. 1: Schematic of the Interface Region in the ICP-MS.

3. Results & discussion

3.1. Repeatability

The repeatability of the technique was established using certified reference standards (Fluka 70007). Measurements for each standard (and sample) in triplicate evaluated the capacity of the instrument in achieving the prescribed detection limits. The analytical capability of the method was thus validated by making such measurements (n = 3) and the relative standard deviation is automatically recorded. Briefly, the instrument operates by aspirating three separate sample fractions into the hot argon plasma in successive cycles and the embedded software computes the standard deviation. If the relative standard deviation (RSD) rises above 10% the sample is re-analyzed to determine the source of error, such as matrix effects or undesirable noise production. For our purposes RSDs <5% were attained for certified material (Table 1) indicating that the instrumental performance of the system was satisfactory.

3.2. Carcinogenic metal toxins

It is necessary to underscore that the toxins were monitored in the brew itself and not in the original tea leaves. The brew is prepared as an aqueous solution and therefore, the admissible limits associated with drinking water quality could be applied [3]. The tea bags were prepared in ultra-pure water and the carcinogenic toxins of interest were extracted from the tea bag into the brew during the process of boiling. Thus the constituents of the brew consisted of extracted beryllium and arsenic from the tea leaves in the bag. Our investigation entailed elemental profiling of the selected samples, and the concentrations of beryllium and arsenic were recorded. The results demonstrated that the distribution of these elements fluctuated. A point to note is that these contaminants from the brew add to the beryllium and arsenic present in the water used to brew the tea. It is likely that these impurities are embedded in the surrounding environment responsible for growth of the tea leaves such as soil, water, compost, peat and pesticides, which contribute to the observed levels of these toxins. Also minor concentrations of beryllium and arsenic could be introduced into the tea leaves when they came into contact with the machinery used to process the tea. Our study, therefore, deals with an environmental problem that could affect human health, and impact sustainable development, if restorative measures are not adopted.

Table 1: Repeatability Study Using A Multi-Elemental Aqueous Standard (Fluka 70007)

Measurement	Be	Mg	Co	Ni	In	Ce	Bi
1	10.7	10.5	10.2	9.9	9.6	9.7	9.1
2	10.6	10.4	10.5	10.2	9.5	9.3	9.5
3	10.8	10.4	10.1	9.9	9.6	9.1	9.1
Mean ± RSD	10.6 ± 0.93%	10.4 ± 1.7%	10.3 ± 4.0%	10.0 ± 1.0%	9.6 ± 0.6%	9.4 ± 4.0%	9.2±3.1%

Beryllium (Be): the detection of beryllium is uncommon mainly because it occurs at such low levels. The trend in Be concentrations in Fig. 2 indicates that levels in brewed tea could range between 0.3 to 1.4 ppb. The drinking water standard for Be is 4 ppb [3]. The cumulative effect of water and tea could, therefore, raise levels and pose a hazard. The relative standard deviation for Be concentrations in Fig. 2 is 44% demonstrating that Be uptake in tea leaves vary appreciably from region to region. As mentioned earlier this could be due to contaminated soil and water associated with the environment in which these tea plants are cultivated. The concentration of Be in soils is about 1.5 ppm. The carcinogenic toxicity of Be cannot be overstated. Oral ingestion of appreciable levels of Be is a risk and could lead to certain types of cancer. Children exposed to cancer are at greater risk because their organs are more sensitive. Beryllium replaces magnesium in enzymes and this displacement highlights its potential toxicity [2]. Since Be is a relatively rare element its presence in brewed beverages could be used for fingerprinting studies (see below).

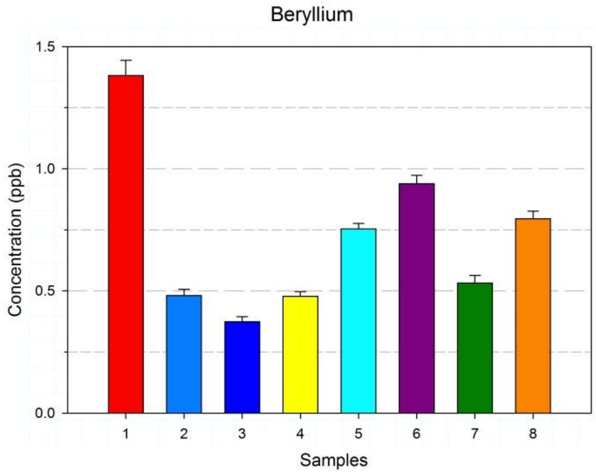

Fig. 2: Beryllium Levels in Tea-Brew.

Arsenic (As): the toxicological profile of arsenic is clearly delineated in Fig. 3. The drinking water standard for As is 10 ppb [3]. As with beryllium, the range of concentrations tends to fluctuate and occurs between 1 and 3 ppb, (standard deviation, 33%), which is within the admissible limit, however, the cumulative effect of the mixture of water and tea could elevate levels, which could pose a health threat. The adverse effects of arsenic on the human body are well documented. Appreciable levels of this element could affect the kidneys and liver; and cause lung and skin cancer [1, 2]. Tea is a favorite beverage and is consumed at all times of the day by adults, but the toxicity of this element could be more severe in children and minors who have sensitive organs. The profile of As in Fig. 3 could be due to a series or combination of factors. The appreciable level of this element in tea leaves originates from environmental sources, such as soil conditioners, pesticides, irrigation water and general pollution. Clearly, from the plot in Fig. 3 the fluctuation in concentrations of this element is wide, suggesting that the samples originated from diverse environmental sources. Remedial measures could minimize these levels.

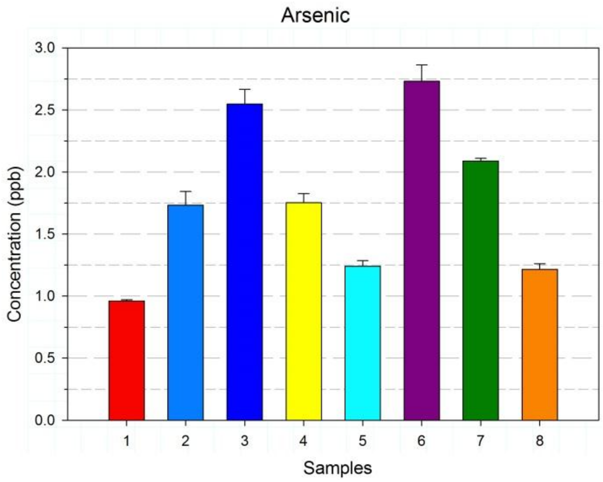

Fig. 3: Arsenic Levels in Tea-Brew.

3.3. Sustainability factors

Our study is significant for two reasons: (i) extractability of ultra-trace inorganic metal carcinogens in tea is relatively underexplored; and (ii) it contributes to sustainable development. Sustainable development encompasses a broad perspective and essentially pertains to all activities relating to the welfare of humans [20]. This study, therefore, adds to the body of knowledge associated with toxins in brewed beverages. It is important to emphasize that because brewed beverages are consumed as aqueous oral drinks the levels of the carcinogenic toxins could be compared to levels in standard drinking water. The focus on sustainability, therefore, is influenced by this particular factor of oral ingestion, and the essential need to limit any such hazard in brewed tea. We showed in Fig.s 2 and 3 that the distributions of Be and As are well within the maximum permissible drinking water levels. However, as aforementioned, continuous consumption of tea could lead to some form of bio-accumulation in the human body, and this could affect health, especially of children. Another interesting feature of the study lies in the fluctuating distribution of these elements. In the case of Be we observed that sample#1 registered a concentration of about 3 times more beryllium than samples# 2,3,4 &5. The reason for this is unknown, but could be attributed to environmental factors such as contaminated fertilizer, soil and water used to cultivate the tea plants. On the other hand sample#1 contains the lowest level of arsenic compared to the rest of the samples. Here again this could be characteristic of the agricultural conditions under which the tea leaves were grown. Such marked differences in cross-concentrations could be useful in "fingerprinting" studies.

3.4. Impact of the study

The technique of fingerprinting is allied to pattern recognition and seeks to determine a pronounced pattern in the results, which could be used to identify the region or location of the sample. This particular exercise is also called "provenancing" and could be useful to pinpoint the origin of a certain brand of tea sample. Information of this nature is of interest to health and nutrition organizations and also to government food and beverage bodies who could assist to isolate brands containing appreciable levels of toxins, source their countries or regions of origin and request restorative measures to limit specific carcinogens in processing tea leaves. Such remedial exercises could be directed to imposing decontamination procedures to the environment in which the tea leaves are cultivated. This could entail close analysis of fertilizers, soil, irrigation water, and other soil conditioners. An inspection of Fig.s 2 and 3 reveals that simple ratios could be used to earmark samples that are distinct from the rest of the group. The As/Be ratio of each sample is given in Table 2. It is clear from this table that samples# 3 and 1 have the highest and lowest values of 6.3 and 0.71 and these parameters could be deployed (for example) to identify these particular brands of tea. It is interesting to note that sample# 5 and 8 have the same ratio, and suggest that they could originate from areas in close proximity. The remaining ratios fall between 3 and 4 and are not adequately pronounced to be of any particular use in "fingerprinting." Extension of this technique to other carcinogens could be useful.

3.5. Remediation

Extractability of ultra-trace metal carcinogens in brewed tea is an area of research that is not well documented. The primary impact of the study, therefore, is that the work is seminal and the information in this paper could be used to construct a reference database. Such a database could be highly useful to environmentalists, nutritionists and health organizations. The study is linked to sustainability and could spawn restorative measures. A baseline study of this nature could be correlated to investigations related to soil and water analysis. Extensions of this study could prompt remediation of the environment, with subsequent decontamination of soil and water; and limit the use of noxious pesticides that could contain such carcinogens. The variability of the results in Fig.s 2 and 3 suggests that such marked distributions could correspond to differences in uptake in different species of tea plants. For example pronounced uptake of beryllium in sample# 1 and corresponding limited uptake of arsenic in the same sample could be specific to certain species of tea plant. The study therefore, could have botanical connotations and special types of remediation could apply to different plant species.

Table 2: As/Be Ratios in Tea Samples

Sample#1	Sample#2	Sample#3	Sample#4	Sample#5	Sample#6	Sample#7	Sample#8
0.71	3.4	6.3	3.6	1.5	3.0	4.1	1.5

4. Conclusions

The thrust of this study involved profiling the extractability of metal carcinogens Be and As in brewed preparations of tea. The leaching out of these toxins in the brew has significant impacts, for example: (i) it forms the origin of a baseline study; (ii) it adds to the body of data relating to analysis of such beverages; and (iii) it contributes to sustainable living. We also found it could be feasible to link the data to fingerprinting techniques, which could potentially identify the location of the tea sample. Our study makes a salient contribution to sustainability. Future work would entail linking our study to environmental factors.

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