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Determination of Trace Metals in Fruit Juices selected by ASAE using Atomic Absorption Spectroscopy

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Resumo

Os sumos de fruta são muito apreciados, não só em Portugal, onde são a segunda bebida não alcoólica mais consumida depois da água, mas também noutros países da União Europeia, onde o seu consumo tem vindo a aumentar nos últimos 20 anos.

Os sumos de fruta contêm várias substâncias, como nutrientes, minerais, elementos vestigiais, vitaminas e fitoquímicos, que são essenciais para uma vida saudável. No entanto, elevados níveis de metais podem também estar presentes na sua constituição, originando potenciais efeitos nocivos ao ser humano. As crianças constituem um grupo etário com um maior risco, pois os sumos de fruta são normalmente muito apreciados pelas crianças, e a sua dieta não é tão variada como a dos adultos. Outro aspeto importante é o facto da relação consumo/peso corporal ser muito maior nas crianças do que nos adultos. Esta dualidade em relação às consequências da ingestão de sumos de fruta tem feito com que estes sejam submetidos a um maior controlo, de maneira a garantir a segurança alimentar e a perceber até que ponto os sumos de fruta podem fazer parte de uma dieta equilibrada tendo em conta os seus aspetos nutricionais.

A presença de metais nos sumos de fruta é influenciada por diversos fatores, tais como: o tipo de fruto, a composição mineral e outras características do solo no qual o fruto é originado, a composição mineral da água de irrigação, as condições atmosféricas, as práticas agrícolas, a deposição atmosférica de metais com origem em atividades antropogénicas, os ingredientes usados na sua produção e as fases de embalamento e armazenamento.

Os metais, sendo o grupo de elementos químicos mais abundante na crosta terreste, estão normalmente presentes nos alimentos em pequenas concentrações. A sua importância toxicológica ou nutricional difere consoante o grupo a que pertencem e em que quantidades estão presentes. Elementos com o Ca, K, Mg e Na são necessários em quantidades mais elevadas para o bom funcionamento do nosso corpo. Outros, como o Cu, Fe, Ni, Zn, Cr e Mn, são importantes em vários processos biológicos, sendo necessários em concentrações vestigiais, pois quando presentes em maiores quantidades apresentam toxicidade, podendo provocar riscos para a saúde humana. Por outro lado, os elementos não essenciais, como Pb, Cd e As, são tóxicos mesmo quando presentes em baixas concentrações, dando origem, por vezes, a consequências graves para o ser humano. Os efeitos adversos causados pelos metais tóxicos dependem da via de exposição (inalação, oral ou dérmica) e na duração do período de exposição (agudo, subagudo, subcrónico e crónico).

O objetivo deste estudo centra-se na determinação da concentração de arsénio, cádmio, crómio, chumbo, manganês e níquel (metais mais estudados segundo a literatura consultada), em diferentes sumos de fruta disponíveis do mercado português. Os resultados obtidos foram comparados com os valores máximos admissíveis estipulados pela WHO (Organização Mundial de Saúde), USEPA (Agência de Proteção Ambiental dos Estados Unidos) e pela Legislação Portuguesa (Decreto-Lei 306/2007 de 27 de Agosto). Por último, os teores obtidos no presente estudo foram comparados com estudos semelhantes realizados por outros autores.

O conjunto de amostras deste estudo é constituído por 21 sumos de fruta de 4 marcas diferentes disponíveis no mercado português, previamente selecionados pela ASAE (Autoridade de Segurança Alimentar e Económica), de modo a englobarem as marcas mais consumidas em Portugal.

Com o propósito de demonstrar o grau de confiança dos resultados obtidos, foram efetuados os testes de validação indicados para este tipo de determinações. Assim, foi testada a linearidade, a gama de trabalho, os limites analíticos (limite de deteção e de quantificação), a precisão (repetibilidade, precisão

intermédia), a exatidão e a especificidade/seletividade. Também foram realizados testes para avaliar a repetibilidade do método de digestão.

Previamente à análise quantitativa dos metais, todas as amostras de sumos foram submetidas a uma digestão por micro-ondas com vasos fechados e pressão controlada. Este processo tem como objetivo destruir por completo a matéria orgânica no menor tempo possível, evitando perdas de metais por volatilização e minimizando a quantidade de ácido adicionado e o risco de possíveis contaminações. Um método de preparação de amostras adequado é muito importante para garantir a veracidade dos resultados obtidos nos subsequentes processos de análise quantitativa.

A quantificação dos metais foi realizada através da técnica de Espectrometria de Absorção Atómica (EAA). Esta é uma técnica muito usada para a determinação de elementos na sua forma atómica, pois apresenta uma configuração simples, baixo custo e boa sensibilidade. O fundamento deste método instrumental consiste na quantificação de um determinado elemento quando é emitida uma radiação que, sendo do mesmo comprimento de onda, é absorvida pelos átomos do elemento. Através da medição da quantidade de radiação absorvida, é possível determinar quantitativamente o elemento de interesse. Para a análise de As, utilizou-se a EAA com gerador de hidretos. Esta é considerada uma técnica analítica indicada para analisar metais que, sendo muito voláteis, dificilmente poderiam ser analisados utilizando outra técnica. Deste modo os hidretos formados, quando arrastados para a célula de quartzo aquecida, facilmente libertam o elemento na forma atómica. Para a quantificação dos restantes metais, foi usada a técnica de EAA com câmara de grafite, que é indicada para a análise de elementos vestigiais presentes em amostras alimentares ou biológicas devido a apresentar as seguintes características: rapidez, simplicidade na preparação de amostras, possibilidade de automatização, boa sensibilidade e consumo de pequenos limites de amostra.

Após análise dos diferentes sumos de fruta, os resultados obtidos demonstraram que os níveis de determinados elementos estão, em algumas amostras, acima dos valores admissíveis. Tendo em conta os limites máximos permitidos pelo Decreto-Lei 306/2007 de 27 de Agosto da Legislação Portuguesa, os níveis de As revelaram ser superiores ao valor máximo admissível (10 µg/L) em 4 amostras; a concentração de Ni é superior ao valor limite estipulado (20 µg/L) em 13 amostras; e a concentração de Mn está acima do valor máximo permitido (50 µg/L) em todas as amostras analisadas. No entanto, se considerarmos os valores máximos admissíveis estipulados pela WHO, a quantidade de Cd presente numa amostra é superior ao valor limite definido por esta organização (3 µg/L); e a concentração de Mn é superior ao valor máximo permitido (400 µg/L) apenas em 6 amostras. Em relação aos níveis de Ni, se considerarmos os valores máximos estabelecidos pela WHO (70 µg/L) e pela USEPA (100 µg/L), verificamos que estes valores não são excedidos em nenhuma das amostras analisadas.

Para além das comparações feitas com os valores estabelecidos pelas entidades acima referidas, foi também feito um estudo de comparação entre os resultados obtidos neste trabalho e outros valores publicados em trabalhos semelhantes presentes na literatura disponível e desenvolvidos, na maior parte dos casos, noutros países. Este estudo de comparação revelou que o intervalo de valores obtido para cada metal, correspondente a determinado tipo de sumo, é semelhante ao intervalo de valores encontrado noutros estudos equivalentes. No entanto, verificou-se uma grande variedade de resultados, o que é compreensível, visto que o nível dos elementos analisados nos sumos de fruta é influenciado por diversos fatores já referidos anteriormente. A contribuir para esta discrepância acresce o facto dos trabalhos analisados serem provenientes de outros países e com algum espaço temporal entre a sua realização.

A contaminação ambiental com metais pesados é um problema em algumas sociedades. É necessário criar programas e campanhas com o propósito de reduzir os níveis de contaminação, passando por

minimizar as descargas e libertações de metais provenientes de atividades antropogénicas (como exploração mineira e indústrias) e minimizar e/ou eliminar os contaminantes presentes na água potável, visto que esta é uma das possíveis principais fontes de contaminação dos sumos de fruta.

Apesar da toxicidade associada aos metais, é importante referir que se espera que o consumo diário de sumos de fruta seja muito inferior ao consumo de água. Visto que as concentrações de metais presentes nos sumos de fruta foram avaliadas tendo em conta os limites admissíveis estipulados, pelas diferentes entidades, para a água potável, a ingestão de sumos de fruta não implica necessariamente riscos para a saúde do ser humano. No entanto, este tipo de estudos tem uma grande importância, pois permite fazer uma estimativa da ingestão de metais através da alimentação, sendo possível fazer considerações nutricionais acerca dos alimentos em estudo.

Palavras-chave: Sumos de fruta, Análise de Metais, Espectrometria de Absorção Atómica, Digestão por micro-ondas

Abstract

Fruit juices are among the most appreciated and most consumed non-alcoholic beverages in European countries. These beverages contain minerals, nutrients, trace elements, vitamins and phytochemicals, which are essential for a healthy life. However, fruit juices may also contain high levels of metals, posing a health risk for humans, especially for children since they consume more fruit juice comparing to their weight and they have a less varied diet than adults. Thus, in order to guarantee food safety as well as to make nutritional considerations, fruit juices are a growing investigation topic.

Metal content in fruit juices may be influenced by several aspects, such as: fruit type, mineral composition and other characteristics of the soil from which it was originated, mineral composition of irrigation water, atmospheric conditions, agricultural practices, atmospheric deposition of metals from anthropogenic activities, ingredients used in its production and packaging and storage steps.

Metals are the most abundant group of chemical elements on the Earth's crust, being usually present in foods at low concentrations. Their toxicological or nutritional significance differs according to the group of metals and the amounts involved. Some elements are essential to life, being needed in higher contents (such as Ca, K, Mg, and Na) or in trace levels (such as Cu, Fe, Ni, Zn, Cr and Mn). Non-essential elements, such as Pb, Cd and As, are toxic even when present in low levels, posing a health risk. The adverse health effects caused by toxic metals depend on the route and on the duration of exposure.

The main purpose of this study was to determine arsenic, cadmium, chromium, lead, manganese and nickel concentrations in 21 fruit juices from 4 different brands, previously selected by ASAE (Portuguese Food and Economic Safety Authority) and available in the Portuguese market. Results obtained were compared to maximum levels set out by WHO (World Health Organization), USEPA (United States Environmental Protection Agency), by the Portuguese law, and to similar studies available in the literature.

A validation process, including linearity, working range, analytical thresholds, precision, accuracy and specificity/selectivity was conducted in order to guarantee reliable analytical data.

Before quantification steps, samples were prepared with a microwave digestion in closed vessels in order to complete digest them, avoiding losses of metals by volatilization and possible contamination. Hydride generation atomic absorption spectroscopy was used to quantify As while the other metals were quantified by graphite furnace atomic absorption spectroscopy.

Obtained results showed levels of As, Ni and Mn above the maximum limits specified by Decree-Law 306/2007 from 27th August of the Portuguese Legislation in some of the analyzed fruit juice samples. Other studies showed range values similar to the ones found in this study, despite the great variety of some, which is reasonable since the presence of trace metals in fruit juices is influenced by several aspects and studies are from different countries and with time gaps between them.

In order to avoid environmental contamination with heavy metals, which is recognized as a public health hazard worldwide, action is needed to minimize the metal releases and discharges from anthropogenic activities and its amount in drinking-water.

Besides the known toxicity associated with trace metals, it is important to refer that the amount of juice consumed per day is expected to be lower than the amount of consumed water. As metal concentrations in fruit juices were evaluated having in mind the maximum limits established for drinking-water, consuming fruit juices that exceed these values does not necessarily imply an increased risk for human

health. However, this type of studies is very important since it allows to quantify the dietary intakes of metals present in food, not only guaranteeing food safety but also making nutritional considerations.

Keywords: Fruit juices, Metal analysis, Atomic absorption spectroscopy, Microwave digestion

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List of Abbreviations

AIJN - European Fruit Juice Association

ASAE – Portuguese Food and Economic Safety Authority

ASS – Atomic Absorption Spectroscopy

CRM – Certified Reference Material

D.L. – Detection Limit

DMA – Dimethylarsinic acid

DNA – Deoxyribonucleic acid

DS^2 – Variance difference

EDL – Electrodeless-discharge Lamp

EFSA – European Food Safety Authority

EHQTA - externally heated quartztube atomizer

F-test – Tabulated value of Fisher-Snedecor distribution

GFAAS – Graphite furnace atomic absorption spectroscopy

HCL – Hollow-cathode lamp

HCl – Hydrochloric acid

HGAAS – Hydride generation atomic absorption spectroscopy

IARC – International Agency for Research on Cancer

iAs – Inorganic arsenic

ISO – International Organization for Standardization

ML – Maximum level

MMA – Monomethylarsonic acid

MMT – Methylcyclopentadienyl manganese tricarbonyl

PMT – Photomultiplier Tube

Q.L. – Quantification Limit

QTA – Quartztube atomizer

ROS – Reactive oxygen species

RSD – Relative Standard Deviation

USEPA – United States Environmental Protection Agency

WHO – World Health Organization

1. Introduction

1.1. Fruit juices

Among the different beverages commercially available, fruit juices are highly appreciated, being the most non-alcoholic consumed drink after water in Portugal. Its consumption has been growing for the last 20 years, which is in concordance to what is happening in the other countries of the European Union (Sardinha et al. 2014). In tropical countries, fruit juices are the most widely consumed beverages in the habitual diet (Bragança 2012). Commercial fruit juices usually contain nutrients, minerals, trace elements, vitamins and phytochemicals that have been shown to have many benefits. When consumed in moderation as part of a balanced diet, fruit juices have a positive effect on the human organism, promoting health and the reduction of risk disease (IFU 2013). However, they can be a potential source of toxic elements, some of them having a cumulative effect or leading to nutritional problems due to low concentration of essential elements (Dehelean and Magdas 2013).

Owing to recent heavy metal contamination of the environment, the analysis of trace elements in seasonal fruit samples as well as in their products has gained considerable importance because of health considerations (Cindrić et al. 2011). Trace element levels of fruit juices may be expected to be influenced by many factors including the nature of the fruit; the mineral composition of the soil from which it was originated and other characteristics that influence the availability of the element to be taken by the plant (such as soil's cation exchange capacity, soil pH and presence of fungi); the mineral composition of irrigation water; the weather conditions; the agricultural practices, such as the types and amounts of fertilizers used; the atmospheric deposition of metals from industrial activities and emissions from vehicles; the higher level of metals in water and other ingredients (such as added sugar) used by manufacturers in juice processing steps and the packing and storage stages (Tufuor et al. 2011; Bragança et al. 2012; Dehelean and Magdas 2013; USFDA 2013).

Industrial production of fruit juices includes several steps. Firstly, fruits have to be collected, transported and received in the manufacturing sites. There, fruits are washed to remove undesired substances, being the ones that do not present physical damages or contamination signs selected and then peeled. To obtain concentrated juice or paste, some extra steps are needed. Fruit juice processing steps are described in Figure 1.1. Fruit juices can be produced mixing pure water with concentrated juice/paste, fresh fruits or dry fruits, with or without the addition of different food ingredients, such as added sugar and acid ascorbic (E300). Before the mixing and preparation step, fresh fruits and dry fruits have to go through two different steps – pulping/juicing and cooking/extracting, respectively – and then both have to go through clarifying stage, which aims to clear the resulting product. After mixing and preparation step, the following stages are needed in order to get the final product: deaerating (removal of oxygen and other dissolved gases from the mixture) and homogenizing, ultra-high temperature processing (UHT) sterilizing (eliminate non sporulated pathogens and yeast), filling and capping (in controlled atmosphere in order to avoid a microbiologic re-contamination), tilting, tunnel cooling (cool the product without inducing detrimental changes to product quality), air drying and labelling and at last coding and casing (Guadalupe 2010; Sunshine 2016)

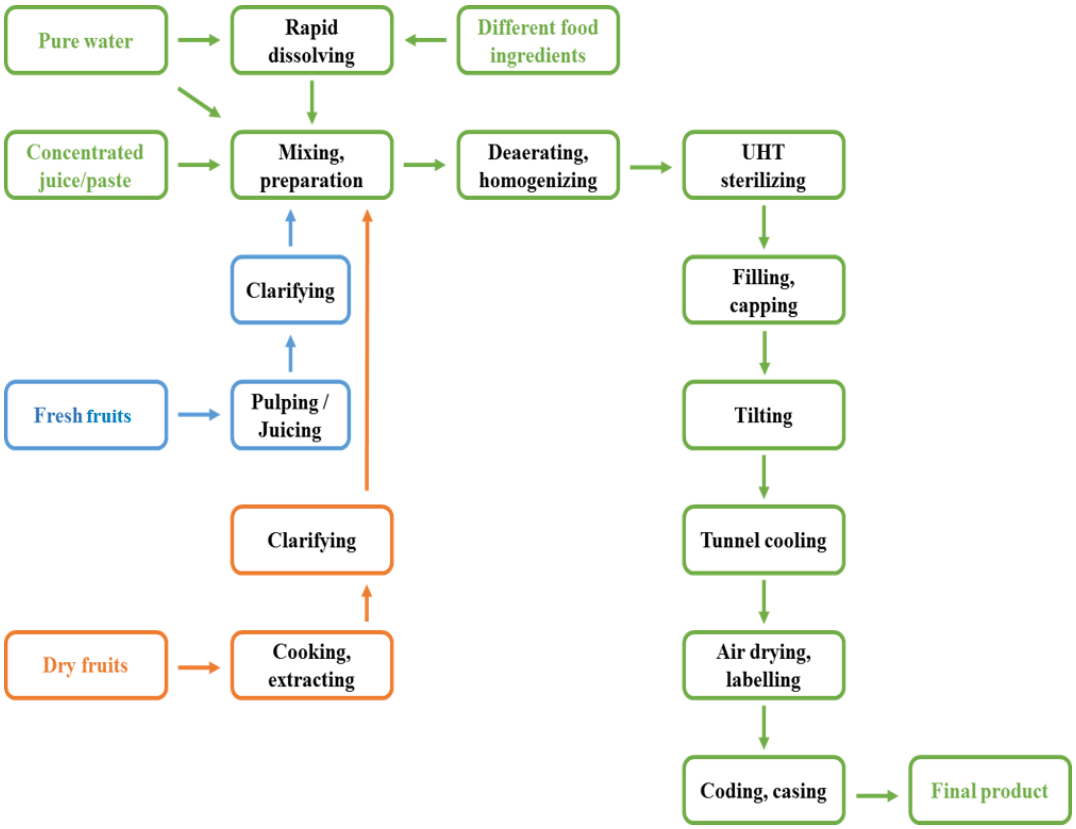

Figure 1.1. Fruit juice process (adapted from Sunshine 2016): Green, blue and orange colored words represent ingredients or products and black colored words represent fruit juice production steps. Blue and orange boxes represent steps needed for the pre-treatment of fresh fruits and dry fruits, respectively. Green boxes represent common steps of fruit juice production using concentrated juice or paste, fresh fruits or dry fruits, with or without the addition of different food ingredients.

Another issue that is of great concern is the fact that fruit juice is a greater potential source of dietary metal exposure to children than to adults, especially because children's dietary patterns are not only often less varied than those of adults, but also because fruit juice is among their favorite beverages. In addition, they consume more fruit juice relative to their body weight and they may have, for some metals, a higher susceptibility than adults (USNews 2012; USFDA 2013).

1.2. Metals

Metals are the most abundant group of chemical elements on the Earth's crust, being usually present in foods at low concentrations. Their toxicological or nutritional significance differs according to the group of metals and the amounts involved (Dehelean and Magdas 2013; Barone et al. 2015). Approximately 30 elements are recognized as essential to life. Whereas some are required in higher amounts, such as Ca, K, Mg, and Na, others occur in trace or ultra-trace levels. Metals as Cu, Fe, Ni, Zn, and Mn are at the top end of this trace scale, playing an important role in biological systems since they take part in numerous biochemical processes in the human body, preventing deficiency diseases when their intake

is adequate. However, in high concentrations these metals are toxic and can cause ill effects. Also Al, B, Co, Cr, Se and Sn are essential for normal development and function of human cells as long as the element intake is not excessively elevated, otherwise, when present at high amounts, these essential elements can be harmful and elicit toxic effects. On the other hand, metals like Pb, Cd and As, which are non-essential elements, are found to be toxic causing deleterious effects even when present at low levels (Cindrić et al. 2011; Tufuor et al. 2011; Dehelean and Magdas 2013; Ofori et al. 2013).

The adverse health effects caused by toxic metals depend on the route of exposure (inhalation, oral or dermal) and on the duration of exposure periods – acute, sub-acute, sub-chronic and chronic (Das et al. 2008, Klaassen 2008). After absorption, damages can be caused locally at their point of contact with the body or by systemic effects, i.e. when metals are transported within the body to various organs before exerting an adverse effect (Mohammadi and Ziarati 2015).

Metals' toxicity has two main aspects: the fact that they have no known metabolic function, but when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs; and the potential to accumulate in biological tissues, a process known as bioaccumulation. This occurs because, once taken up into the body, metals may be stored in particular organs, for example the liver or the kidney, and are excreted at a slow rate compared with its uptake (FSAI 2009).

Metals are not degradable in nature and will thus, once released to the environment, stay in circulation (NCM 2003). Environmental contamination through heavy metals is recognized as a public health hazard worldwide. Dietary intakes of metals present in food need to be monitored on a regular basis and rapidly updated to identify them in different countries in order to guarantee food safety as well as to make nutritional considerations (Millour et al. 2011; Dehelean and Magdas 2013).

According to the available literature, the most studied metals in fruit juices are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), manganese (Mn) and nickel (Ni). Therefore, these metals were selected for this study and in order to understand the importance of this selection, their main characteristics and properties will be summarized in the following paragraphs. However, it is important to refer that in several studies other metals are also analyzed, being Fe, Cu and Zn the most tested elements after the aforementioned ones.

1.2.1. Arsenic

Arsenic is one of the most analyzed elements in fruit juices and it was considered one of the ten chemicals of major public health concerns on the International Programme on Chemical Safety (IPCS) in 2010. Through this program, WHO (World Health Organization) works to establish the scientific basis for the sound management of chemicals, and to strengthen capabilities for chemical safety (WHO 2010a). ASAE, as the Focal Point of EFSA (European Food Safety Authority) in Portugal, has shown great concern with the possible As high levels in some brands of fruit juices available in the Portuguese market.

Chemical and physical information

Arsenic is a naturally-occurring steel gray and brittle solid metalloid, which means it shares properties with metals and non-metals. It is widely distributed throughout the Earth's crust, being the 20th most common element, generally as arsenic sulfide or as metal arsenates and arsenides. Arsenic can be released into the atmosphere and water mainly by high-temperature processes, natural activities

(volcanic activity and dissolution of minerals), remobilization of historic sources (mine drainage water) and mobilization into drinking-water from geological deposits by drilling of tube wells. In the atmosphere, it is mainly adsorbed on particles, which are dispersed by winds and deposited on land and water. Besides being present at low concentrations in rocks, soil and natural water, anthropogenic activity has also contributed to increase arsenic levels in the environment through mining activities, metal smelting, industrial emissions and combustion of fossil fuels (IARC 2004; USDHHS 2007a; WHO 2010b; Hughes et al. 2011; Davidowski and Sarojam 2012; WHO 2016).

Arsenic is found in the environment in both inorganic and organic forms (together referred as total arsenic) and in different valence or oxidation states (typically -3, +3 and +5) (USDHHS 2007; Hughes et al. 2011). Inorganic arsenic (iAs), the sum of arsenite (As^{+3}) and arsenate (As^{+5}), refers to arsenic combined with other elements such as oxygen, chlorine and sulfur; while arsenic combined with carbon and hydrogen is referred to as organic arsenic. Most inorganic and organic arsenic compounds are white or colorless powders, having no smell and no special taste in most cases, making it hard to tell if arsenic is present in food, water, or air (USDHHS 2007a). Inorganic arsenic can be naturally present in food and water because of geochemical conditions, consequently exposure varies significantly in different regions, primarily through the presence or absence of arsenic in groundwater sources for drinking-water (JEFCA 2010). Inorganic arsenic is generally considered more toxic than organic arsenic. As^{+3} is considered more harmful to human health than As^{+5} because of its reactivity with sulfur containing compounds and generation of reactive oxygen species (ROS). Organic arsenic compounds, which are abundant in seafood, are less harmful to health, being rapidly eliminated by the body (WHO 2010b; Hughes et al. 2011).

Uses

In the past, inorganic arsenic compounds were predominantly used as pesticides, but nowadays iAs compounds can no longer be used in agriculture. However, organic arsenic compounds are still used as pesticides and some of them are used as additives in animal feed (USDHHS 2007a). Although in the European Union the application of arsenic containing pesticides is not allowed, wood preservatives containing arsenic compounds are still used in some countries, including the United States of America (USA) (EFSA CONTAM Panel 2009). In industrial processes, arsenic is used in the pharmaceutical and glass industries, in the manufacture of alloys (greatly used in lead-acid batteries for automobiles), leather preservatives, metal adhesives, pigments, paper, textiles, ammunition, antifouling paints and poison baits. Arsenic compounds are also employed in limited amounts in the microelectronics (semiconductors and in light-emitting diodes production) and optical industries (USDHHS 2007a; WHO 2010b; WHO 2016).

Sources of exposure

Human exposure to arsenic can occur via different routes. Non-occupational human exposure to arsenic in the environment is primarily through the ingestion of food and water, being food the principal contributor. Fish, shellfish, meat, poultry, dairy products and cereals are the main sources of dietary intake. However, the arsenic content of fish and shellfish usually involves organic compounds, which are less toxic. There are cases where arsenic in drinking-water constitutes the principal contributor to the daily arsenic intake when arsenic in drinking-water is a significant source of exposure to inorganic arsenic. The consumption of groundwater containing naturally high levels of iAs, food prepared with this water and food crops irrigated with high-arsenic water sources are the principal routes of exposure

to arsenic. An estimated 200 million people worldwide are exposed to arsenic concentrations in drinking-water that exceed the recommended limit of 10 μg/L. Inorganic arsenic is naturally present at high levels in the groundwater of a number of countries, such as Bangladesh, India, Nepal, China, Argentina, Chile, Mexico and the USA (WHO 2001; EFSA CONTAM Panel 2009; WHO 2010a; George et al. 2014). High arsenic levels in air can be found in the working environment as well as the general environment around non-ferrous metal smelters and some coal-fired power plants. Smokers are more exposed to arsenic since tobacco plants essentially take up arsenic naturally present in the soil. In the past, this content was increased where tobacco plants have been treated with insecticide containing arsenic compounds (WHO 2001; USDHHS 2007a; WHO 2010b; WHO 2016).

Toxicokinetics

Arsenic toxicokinetics varies depending on the arsenic form and different factors such as life stage, gender, nutritional status and genetic polymorphisms. The absorption of inorganic arsenic is notably influenced by the solubility of the arsenical compound (As^{+3} and As^{+5} in drinking-water are almost completely and rapidly absorbed), the presence of other food constituents and nutrients in the gastrointestinal tract and by the food matrix itself (EFSA CONTAM Panel 2009). Once absorbed, iAs is extensively transformed and excreted via urine. The concentration of metabolites of inorganic arsenic in urine – iAs, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) - reflects the absorbed dose of inorganic arsenic on an individual level. It is recognized that organic arsenic forms are in general efficiently absorbed despite the limited human data (WHO 2001; EFSA 2014).

Health effects

Soluble inorganic arsenic is acutely toxic, being associated with vomiting, esophageal and abdominal pain and bloody "rice water" diarrhea in case of poisoning. These are followed by numbness and tingling of the extremities, muscle cramping and death, in extreme cases. Intake of inorganic arsenic over a long period can lead to arsenicosis (chronic arsenic poisoning). Effects, which can take years to develop depending on the level of exposure, include skin lesions (pigmentation changes and lesions and hard patches on the palms of the hands and soles of the feet), gastrointestinal symptoms, neurotoxicity, diabetes, developmental effects, conjunctivitis, renal system effects, enlarged liver, bone marrow depression, destruction of erythrocytes, high blood pressure, cardiovascular and respiratory diseases and cancer (WHO 2010a; USDHHS 2007a; WHO 2016). Pregnant women chronically exposed to arsenic-contaminated drinking-water are at increased risk for spontaneous abortion, stillbirth and preterm birth because arsenic can pass through the placenta. In utero and early-life exposures to arsenic have been linked to the development of lung cancer and bronchiectasis later in life (WHO 2010b). Chronic exposure to arsenic is also associated with deficits in children's cognitive and motor functions (George et al. 2014).

Arsenic has been evaluated on various occasions by the International Agency for Research on Cancer (IARC), which classified arsenic and iAs compounds as 'carcinogenic to humans' (Group 1). In 2010, IARC concluded arsenic in drinking-water causes urinary bladder, lung and skin cancers, being potentially associated with kidney, liver and prostate cancers. Evidence for arsenic-induced cancer in humans is based on epidemiological studies of oral arsenic exposure, primarily through inorganic arsenic in drinking-water (FSAI 2009; JEFCA 2010; USFDA 2013). The organic arsenic compounds MMA and DMA are classified as possibly carcinogenic to humans (Group 2B) (WHO 2010b).

Maximum levels

According to USEPA (United States Environmental Protection Agency), WHO and Decree-Law 306/2007 from 27th August of the Portuguese Legislation, the recognized arsenic standard value for drinking-water is 10 μg/L (Decree-Law 306/2007; EFSA CONTAM Panel 2009; EFSA 2014). However, this guideline value is considered to be provisional because of measurement difficulties and the practical difficulties in removing arsenic from drinking-water (WHO 2016).

1.2.2. Cadmium

Cadmium was also considered one of the ten chemicals of major public health concerns on the International Programme on Chemical Safety in 2010 (WHO 2010a).

Chemical and physical information

Cadmium, a soft, odorless, silver-white, blue-tinged malleable metal or grayish-white powder, occurs naturally in the Earth's crust, where it appears mainly in association with ores containing zinc, lead, and copper (in the form of complex oxides, sulfides, and carbonates) as well as in the ocean water. In addition, cadmium is recovered as a by-product of zinc mining and refining, and its production depends on the demand for zinc (NTP 2004; IARC 2012a; WHO 2011a). It is emitted to the environment as a result of both natural and anthropogenic activities. Natural sources of cadmium include volcanic activity, weathering and erosion of cadmium-containing rocks, sea spray, forest fires and mobilization of cadmium previously deposited in soils, sediments, landfills, etc. Anthropogenic sources of cadmium include the mining and smelting of zinc-bearing ores, metal production, fossil fuels combustion, waste incineration, cement production, phosphate fertilizers and releases from tailings piles or municipal landfills (IARC 2012a).

Uses

Production and consumption of cadmium, cadmium compounds, and cadmium-containing products are carried out on a global scale (CIEL 2008). Cadmium metal has specific properties (such as excellent corrosion resistance, low melting temperature, high ductility and high thermal and electrical conductivity) that make it suitable for a wide variety of industrial applications. The principal uses of cadmium are: nickel–cadmium batteries, pigments, coatings and plating, stabilizers for plastics, non-ferrous alloys, semiconductors and photovoltaic devices. However, in recent years, the use of cadmium in pigments, stabilizers and coatings has declined, mainly due to concerns over the toxicity of cadmium, and the introduction of regulations, particularly in the European Union, restricting its use (ATSDR 2012a; IARC 2012a).

Sources of exposure

The general population may be exposed to cadmium through consumption of food and drinking-water and through inhalation of cadmium-containing particles from ambient air, cigarette smoke or from

contaminated soil and dust (NTP 2004). For the smoking general population, since tobacco leaves naturally accumulate large amounts of cadmium, cigarettes are a significant source of cadmium exposure. Food is the main source of cadmium intake for non-occupationally exposed people and nonsmokers. Crops grown in polluted soil or irrigated with polluted water may contain increased concentrations, as well as meat from animals grazing on contaminated pastures, particularly animal kidneys and livers where cadmium concentrates. High concentrations of cadmium are found in leafy vegetables, starchy roots, cereals, grains, nuts and pulses (WHO 2011a; IARC 2012a). The main route of cadmium exposure in the occupational setting is through inhalation of dust and fumes, since cadmium and cadmium compounds are non-volatile, existing in air as fine particulates. Although levels vary widely among the different industries, occupational exposure generally have decreased since the 1970s (ATSDR 2012a; IARC 2012a).

Health effects

Cadmium is highly toxic and responsible for several cases of poisoning through ingestion of food and drinking-water, causing stomach irritation, vomiting, diarrhea and sometimes death. Although cadmium is relatively poorly absorbed into the body, once absorbed it is slowly excreted, and it accumulates in the kidney, which appears to be the most sensitive organ (WHO 2011a; ATSDR 2012a; IARC 2012a), possibly causing tubular proteinuria, aminoaciduria, glucosuria and phosphaturia. Disturbances in renal handling of phosphorus and calcium may cause resorption of minerals from bone, which can result in the development of kidney stones and osteomalacia (FSAI 2009; WHO 2011a; Dehelean and Magdas 2013; Mohammadi and Ziarati 2015). Oral exposure to Cd may determine adverse effects on a number of human tissues, including also the immune system, and the cardiovascular system (Barone et al. 2015). In occupational settings, where cadmium is present in high amounts, breathing air can severely damage the lungs, possibly leading to cancer (ATSDR 2012). The population at highest risk consists of women with nutritional deficiencies or low iron stores, people with kidney disorders and fetuses and children with low body iron stores. Maternal exposure to cadmium is associated with low birth weight and an increase of spontaneous abortion (CIEL 2008).

Animal studies have shown that cadmium and cadmium compounds induce tumor formation at various sites. It has been suggested that ionic cadmium, or compounds that release ionic cadmium, are the cause of genetic damage and thus the carcinogenic species. IARC has determined that cadmium is carcinogenic to humans (Group 1). Lung cancer has been found in some studies of workers exposed to cadmium in the air. Also, positive associations have been observed between exposure to cadmium and cadmium compounds and kidney and prostate cancer (Klaassen 2008; ATSDR 2012a; IARC 2012a).

Maximum levels

Cadmium guideline value for drinking-water is 3 µg/L according to WHO (WHO 2011b) and 5 µg/L according to USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation (Decree-Law 306/2007; ATSDR 2012a).

1.2.3. Chromium

Chemical and physical information

Chromium is a naturally occurring element widely distributed in the Earth's crust. It can be found in rocks, animals, plants and soil, where it exists in combination with other elements to form several compounds. It can exist in oxidation states from +2 to +6. The three main forms of chromium are chromium(0), chromium(III) – which is an essential element needed for human health in small amounts - and chromium(VI) (WHO 2011b; ATSDR 2012b). Chromium is released to the environment both from natural and anthropogenic sources, with the largest release occurring from industrial emissions and from the burning of natural gas, oil, and coal. In the air, chromium is present in the form of aerosols. However, chromium does not usually remain in the atmosphere, rather it is deposited into soil and water. Chromium has the capacity to change from one form to another in water and soil, according to the existing conditions, such as the redox potential and the pH (WHO 2003; ATSDR 2012b).

Uses

Chromium is widely used in industrial processes to make various metal alloys such as stainless steel welding and in industries involved in electroplating. Chromium can be found in many consumer products including wood treated with copper dichromate, leather tanned with chromic sulfate, textile, stainless steel cookware and metal-on-metal hip replacements (ATSDR 2012b). Chromium and its salts are used in the manufacture of catalysts, pigments, paints, fungicides, in the ceramic and glass industry, in photography, in chrome plating and in corrosion control (WHO 2003).

Sources of exposure

The general population is exposed to chromium by inhaling ambient air, ingesting food and drinking-water containing chromium. For the majority of the population, food appears to be the main source of chromium intake. Drinking-water intake can, however, contribute substantially when total chromium levels are above 25 μg/L. Chromium content in foods depends on its processing and preparation since some cooking utensils may contribute to increase chromium levels. Chromium highest concentrations are found in meat, fish, fruit, vegetables and nuts. Dermal exposure of the general public to chromium may also occur from skin contact with certain consumer products or soils that contain chromium. Workers in chromium-related industries can be exposed to greater chromium concentrations comparing to the general population. The main potential exposure occurs in the metallurgy and tanning industries, where workers may be exposed to high air concentrations (WHO 2003; WHO 2011b; ATSDR 2012b). Smokers are more exposed to chromium than the general population since tobacco smoke contains chromium (VI). Indoor air polluted by cigarette smoke can contain hundreds of times the amount of chromium (VI) found in outdoor air (IARC 2012b).

Toxicokinetics

In humans, the highest chromium concentrations are found in hilar lymph nodes and lungs, followed by spleen, liver, and kidneys. Tissue chromium levels decline with age. Absorption depends on chromium speciation: chromium(VI) appears to be absorbed from the gastrointestinal tract to a greater extent than chromium(III). The absorption of chromium(VI) is lowered by partial intragastric reduction to

chromium(III). Animal studies show that urine is the major elimination route of absorbed chromium (WHO 2003; ATSDR 2012b).

Health effects

Potential health effects caused by ingestion of chromium(VI) compounds can occur in stomach, small intestine (irritation and ulcer) and in the blood (anemia). Male reproductive system damage, including sperm damage, have also been observed in laboratory animals exposed to chromium(VI). Chromium(III) compounds are much less toxic and the only adverse effects noted in humans have been liver and kidney problems after ingestion of very high doses. The most common health problems in workers exposed to chromium involves the respiratory tract, including irritation of the lining of the nose, runny nose and breathing problems (asthma, cough, shortness of breath and wheezing). Workers may also develop allergies to chromium compounds, which can cause breathing difficulties and skin rashes. The concentrations of chromium in air that can cause these effects may be different according to the types of chromium compounds, with effects occurring at much lower concentrations for chromium(VI) compared to chromium(III). Actually, trivalent chromium, which is the form of chromium present in food, is essential for maintaining normal glucose metabolism. Impaired glucose tolerance, glycosuria, fasting hyperglycemia and elevated circulating insulin and glucagon are among the signs of chromium deficiency in humans. All of these symptoms are reversible upon chromium supplementation (Goldhaber 2003; ATSDR 2012b).

There is sufficient evidence in humans for the carcinogenicity of chromium(VI) compounds: chromium(VI) compounds can cause cancer of the lung. Also positive associations have been observed between exposure to chromium(VI) compounds and cancer of the nose and nasal sinuses. Thus, IARC has classified chromium(VI) in Group 1 (carcinogenic to humans) and metallic chromium and chromium(III) in Group 3 (not classifiable as to their carcinogenicity to humans) (IARC 2012b; WHO 2011b).

Maximum limits

Although different guideline values for chromium(III) and chromium(VI) should be fixed, current analytical methods and the variable speciation of chromium in water favor a guideline value for total chromium of 50 μg/L, set out by WHO and Decree-Law 306/2007 from 27th August of the Portuguese Legislation, which is designated as provisional because of uncertainties in the toxicological database (WHO 2003; Decree-Law 306/2007; WHO 2011b). On the other hand, USEPA established a maximum contaminant level of 100 μg/L (ATSDR 2012b).

1.2.4. Lead

Lead is also part of the 10 chemicals of major public health concerns on the International Programme on Chemical Safety in 2010 (WHO 2010a).

Chemical and physical information

Lead is a heavy, low melting, bluish-gray metal that occurs naturally in the Earth's crust. However, it is usually found combined with other elements to form lead compounds, such as lead sulfide, being rarely found naturally as a metal. Most of the high levels found throughout the environment come from human activities (mining and industries that use lead or lead compounds) which are globally widespread, causing extensive environmental contamination (ATSDR 2007; USDHHS 2007b; CIEL 2008; WHO 2010a). However, natural activities (such as volcanic activity, geochemical weathering and sea spray emissions) are also sources of lead in the environment. Concentrations in air depend on a number of factors, including proximity to roads and point sources. Lead may be present in tap water as a result of not only its dissolution from natural sources, but also mainly from household plumbing systems that contain lead, which usage has been declining. Sources of lead in dust, soil and water include lead that falls from the air, and weathering and chipping of lead-based paint from buildings or other structures. Past uses of lead, such as its use in gasoline, are a major contributor to lead in soil. Small amounts of lead may enter rivers, lakes, and streams when soil particles are moved by rainwater. As lead is immobile, it may remain stuck to soil particles or sediment in water for many years unless action is taken to decontaminate them (ATSDR 2007; WHO2010c; WHO 2011c).

Uses

Lead is used in smelting, refining, and in the production of lead-acid batteries, alloys (usually combined with other metals), cable sheathing, pigments, rust inhibitors, ammunition, ceramic glazes, plastic stabilizers, petrol additives and lead sheets used to protect us from radiation. The amount of lead used in these products has been reduced in the last years in order to minimize lead's harmful effects on people health (ATSDR 2007; WHO2010c; WHO 2011c).

Sources of exposure

People are exposed to Pb through inhalation route, through water and through ingestion of contaminated food, which is the major source of exposure for the non-smoking general population. Cereals, as well as spices, may have high levels of lead. The amount of lead in food plants is dependent on how close the soils are from mines and smelters. The lead content of food and beverage may be increased by the use of lead-soldered food and beverage cans, which is now becoming less common. The extent and rate of absorption of lead through the gastrointestinal tract depend on characteristics of the individual. Absorption is increased when the dietary intakes of iron or calcium and phosphorus are low. Moreover, smoking tobacco also contributes to lead intake. Human occupational exposure to lead can occur as well (FSAI 2009; WHO 2010c; WHO 2011c; Barone et al. 2015).

Toxicokinetics

The distribution of lead in the body is route-independent and, in adults, approximately 94% of the total body burden of lead is in the bones compared to approximately 73% in children. Several conditions, such as pregnancy, lactation, menopause and osteoporosis contribute to an increase of the bone resorption, which in turns also increase lead in blood, where it is primarily in red cells. Lead is excreted mostly in urine and feces regardless of the route of exposure (USDHHS 2007b).

Health effects

Lead is a cumulative toxicant, originating adverse effects at very low exposure levels, having acute and chronic effects on human health (CIEL 2008; WHO 2010a). Signs of acute intoxication include: dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, abdominal cramps, hallucinations and loss of memory. Signs of chronic lead toxicity include: tiredness, sleeplessness, irritability, headaches, joint pain, muscle weakness, impaired dental health, gastrointestinal symptoms, hematological effects (that can lead to anaemia), hepatitis, nephritic syndromes, paralysis (lead palsy), symptoms of peripheral neuropathy, kidney damage, cardiovascular diseases due to increased blood pressure, interference with calcium metabolism, injury on reproductive system (impaired fertility, adverse pregnancy outcomes and delayed sexual maturation) and on immune system and effects on the central and peripheral nervous system (subencephalopathic neurological and behavioural effects). The nervous system appears to be the principal target for lead toxicity (FSAI 2009; WHO 2011c; Ofori et al. 2013; Pramod and Devendra 2014). Regarding children in particularly, there is consistent evidence for an association of blood lead levels (generally in lower concentrations than those associated with the effects observed in other organ systems) with impaired neurodevelopment, specifically reduction of IQ that might be regarded as a marker for many other neurodevelopmental effects (such as attention deficit hyperactivity disorder, reading deficit, executive dysfunction, fine motor deficit). The most susceptible groups to lead adverse health effects are infants, children up to 6 years of age, the fetus and pregnant women and malnourished people, whose diets are deficient in proteins and calcium (CIEL 2008; WHO 2010a; WHO 2011c).

Although lead compounds do not appear to cause genetic damage directly, they may do so through several indirect mechanisms, such as inhibition of DNA synthesis and repair, oxidative damage, and interaction with DNA-binding proteins and tumor-suppressor proteins (Klaassen 2008). Thus, the evidence for the carcinogenicity of lead in humans is not conclusive. Therefore, IARC has placed lead in Group 2B (possible human carcinogen – evidence inadequate in humans, sufficient in animals), whereas inorganic lead compounds are in Group 2A (probably carcinogenic to humans) and organic lead compounds are in Group 3 (not classifiable as to their carcinogenicity to humans) (WHO 2010c; WHO 2011c).

Maximum limits

WHO and Decree-Law 306/2007 from 27th August of the Portuguese Legislation set out a guideline value of 10 μg/L for lead in drinking-water (FSAI 2009; Decree-Law 306/2007), which is designated as provisional on the basis of treatment performance and analytical achievability (WHO 2011b). USEPA established an action level of 15 μg/L, which means actions must be taken to lower this level if 10% of tap water samples exceed it (ATSDR 2007).

1.2.5. Manganese

Chemical and physical information

Manganese is one of the most abundant metals in Earth's crust, usually occurring combined with other substances such as iron, oxygen, sulfur and chlorine. It is present in more than 100 minerals and its

elemental form is a silver-colored metal. Although it does not occur in the environment in its pure state, manganese is a naturally occurring substance found in many types of rocks and soil and in surface water and groundwater sources, particularly in anaerobic or low oxidation conditions. Manganese is released into the atmosphere via natural sources (such as soil erosion and volcanic emissions) and anthropogenic sources (industrial emissions, mining activities and the burning of methylcyclopentadienyl manganese tricarbonyl (MMT) containing petrol). It can bioaccumulate in lower organisms (like phytoplankton, algae, molluscs and some fish). Manganese occurs naturally in many food sources, being a trace element essential to the proper functioning of many living organisms, including humans. It is not only required for the proper functioning of many cellular enzymes (such as manganese superoxide dismutase and pyruvate carboxylase), but also some are activated by the element (e.g. kinases, decarboxylases, transferases and hydrolases). Manganese can exist in 11 oxidative states, being the most environmentally and biologically important manganese compounds the ones that contain Mn^{2+}, Mn^{4+} or Mn^{7+} (Goldhaber 2003; WHO 2011b; WHO 2011d; ATSDR 2012c).

Uses

Manganese is used principally in the manufacture of iron and steel alloys to improve hardness, stiffness and strength, and as an oxidant (potassium permanganate) for cleaning, bleaching and disinfection. Manganese dioxide and other manganese compounds are present in dry-cell batteries, glass, fireworks, fertilizers, leather textile, varnishes, fungicides, cosmetics, smoke inhibitors, livestock feeding supplements and in medical imaging. Manganese greensands are used in some locations for potable water treatment. More recently, an organic manganese compound (MMT) has been used as an additive in gasoline to improve the antiknock properties of fuel by the octane rating increase of the unleaded petrol in Canada, the USA, Europe, Asia and South America (WHO 2011b; WHO 2011d; ATSDR 2012c).

Sources of exposure

Manganese may enter our organism through different ways: inhalation of air containing manganese (which is the predominant route of occupational exposure); ingestion of contaminated food or drinking-water (which is the main route of exposure for the general population); dermal contact (only very small amounts of manganese can enter through skin when in contact with liquids containing it). Manganese occurs naturally in many food sources, such as leafy vegetables, nuts, beans, grains and animal products. Besides, consumption of manganese supplements is very common among the adult population of the USA. People who drink high quantities of tea may as well have a higher intake of manganese than an average individual. Moreover, people who smoke tobacco or inhale second-hand smoke are also typically exposed to manganese at levels higher than those who are not exposed to tobacco smoke (WHO 2011d; ATSDR 2012c).

Toxicokinetics

Manganese is present in all tissues of the body, being the highest levels usually found in the liver, kidney, pancreas, adrenals and in certain regions of the brain in infants. In general, the absorption extent of inhaled manganese is a function of particle size: manganese from smaller particles is mainly absorbed into blood and lymph fluids, while larger particles of manganese deposited in the nasal mucosa may be directly transported to the brain. The amount of manganese absorbed across the gastrointestinal tract is

variable, being regulated by normal physiological processes to help maintain manganese homeostasis. Manganese absorption may be higher in young infants because biliary excretion system, which is the primary route of manganese excretion, is not completely developed yet. The absorption of manganese is also influenced by iron absorption: iron-deficient diets lead to an increased absorption of both iron and manganese. Absorption is also related inversely to the level of calcium in the diet. Manganese is almost entirely excreted in the feces via hepatobiliary excretion, only a small proportion being eliminated in the urine. Besides, sweat, hair and the milk of lactating mothers also contribute to manganese excretion (WHO 2011d; ATSDR 2012c).

Health effects

Manganese adverse health effects can be caused either by its inadequate intake or overexposure to it. Manganese deficiency signs include infertility, congenital malformations in offspring, growth retardation, abnormal function of bone and cartilage, abnormal glucose tolerance and altered lipid and carbohydrate metabolism. However, manganese deficiency in humans appears to be rare as it is present in many common foods. Health problems in workers exposed to high levels of manganese dusts or fumes usually involve the nervous system. Harmful effects, such as slowed hand movements, in some workers are caused by manganese concentrations approximately twenty thousand times higher than the concentrations normally found in the environment. "Manganism" is characterized by a "Parkinson-like syndrome", generally with irreversible effects, such as behavioral changes, apathy, slow speech, monotonous tone of voice, emotionless facial expression, weakness, slow and clumsy movement of the limbs, muscle pain and anorexia. It has been found in some workers exposed to manganese concentrations about a million times higher than normal air concentrations. Moreover, other injurious effects resulting from occupational exposure include irritation of the lungs that could lead to pneumonia and loss of sex drive as well as sperm damage (Goldhaber 2003; Soldin and Aschner 2007; WHO 2011d; ATSDR 2012c). By the oral route, manganese is often regarded as one of the least toxic elements, despite the fact there is some controversy as to whether the neurological effects observed with inhalation exposure also occur with ingestion exposure. Although several case reports of oral exposure have described neurological impairment as an effect, the quantitative and qualitative details of exposure necessary to establish direct causation are still lacking (WHO 2011b; WHO 2011d). Studies in children have suggested that extremely high levels of manganese exposure may produce adverse effects on brain development, including behavioral changes, decreases in the ability to learn and remember and increased propensity for violence in adults (ATSDR 2012c; Pramod and Devedra 2014). Since manganese is an essential element and acts as a component of several enzymes, taking part in a number of physiological processes, the threat posed by overexposure to manganese must be weighed against the requirement for some minimum amount of manganese in the diet (WHO 2011d).

USEPA concluded the existing scientific information is not enough to determine whether or not excess manganese can cause cancer (ATSDR 2012c).

Maximum limits

For manganese, a drinking-water guideline value of 400 µg/L was set out by WHO (WHO 2008), while a guideline value of 50 µg/L was fixed by USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation (Decree-Law 306/2007; Dehelean and Magdas 2013).

1.2.6. Nickel

Chemical and physical information

Nickel, the 24th most abundant element, is a hard, silver-white ferromagnetic metal, having no characteristic odor or taste. It can be found in several oxidation states, ranging from -1 to + 4, with the +2 oxidation state being the most common form. Nickel is present in the Earth's crust usually combined with oxygen or sulfur as oxides or sulfides. It is found throughout nature (soil, meteorites and on the ocean floor in lumps of minerals called sea floor nodules) and is released into air and water both from natural sources (such as volcanic activity) and as a result of human activity (nickel mining and industries, oil-burning and coal-burning power plants and trash incinerators). High concentrations of nickel may occur in groundwater in areas with mafic or ultramafic rocks. Chemical and physical forces (e.g., erosion, leaching and precipitation) constantly redistribute nickel between land, water and air. Nickel can be highly mobile in soil depending on its type and pH. Nickel highest soil concentrations are found near industries that extract it from ores (WHO 2000; ATSDR 2005; Valko et al. 2005; WHO 2007; Das et al. 2008).

Uses

Nickel has properties that make it very desirable for combining with other metals (particularly iron, copper, chromium and zinc) to form mixtures called alloys. Nickel-containing materials offer better corrosion resistance, better toughness and strength at high and low temperatures, and a range of special magnetic and electronic properties, when compared to other materials. Thus, they are present in our lives in several ways, mainly as nickel alloys and stainless steel: food preparation equipment, mobile phones, medical equipment, transport, buildings, power generation, coinage, jewelry, marine engineering (ATSDR 2005; WHO 2011b; Nickel Institute 2016).

Sources of exposure

Like many environmental agents, the toxic effect of nickel is related to the way it gets into an organism. Nickel can enter body via inhalation, ingestion and dermal absorption, being absorbed by the lungs, gastrointestinal tract and skin, but the route by which nickel enters cells is determined by its chemical form. Occupational exposure occurs in mining, alloy production, electropainting, refining and welding and has been shown to give rise to elevated levels of nickel in blood, urine and body tissues, with inhalation as the main route of uptake. Non occupational sources of nickel exposure include food, air and water, but the levels found are usually several orders of magnitude lower than those typically found in occupational situations (WHO 2000; Valko et al. 2005; Das et al. 2008; Duda-Chodak and Blaszczyk 2008). Food is the dominant source of nickel exposure in the non-smoking, non-occupationally exposed population. Cocoa, oatmeal, spinach, dry legumes, nuts, chocolate and soybeans are among the products with high nickel contents. Water is generally a minor contributor to the total daily oral intake. However, it increases in vessels that contain corroded nickel plating or with the usage of some domestic appliances containing nickel alloys as well as in polluted areas or where there is mobilization of naturally occurring nickel in groundwater, making nickel contribution from water significant. Also administration of nickel-contaminated medications (e.g., albumin, radiocontrast media, hemodialysis fluids) leads to substantial exposure (ATSDR 2005; Duda-Chodak and Blaszczyk 2008; WHO 2011b).

Toxicokinetics

The amount of absorbed nickel by the gastrointestinal tract depends on the type of nickel species in the food, the content and the absorptive capacity (Valko et al. 2005). The amount of inhaled nickel that reaches lungs and enters blood depends on the size of the nickel particles: the smaller they are, the deeper they can enter the lungs. Moreover, nickel particles solubility also influences its absorbance: more nickel is absorbed when the nickel particles can dissolve easily in water. Once into the body, nickel can go to all organs, but it mainly goes to the kidneys (ATSDR 2005; Duda-Chodak and Blaszczyk 2008). Most of the nickel ingested is not absorbed, being eliminated in feces. The small amount that gets into blood is excreted in the urine. Nickel can also be eliminated through sweat and milk (ATSDR 2005; Das et al. 2008).

Health effects

Much of the toxicity of nickel may be associated with its interference with the physiological processes of manganese, zinc, calcium, and magnesium. Allergic reaction (either immediate or delayed hypersensitivity) is the most prevalent effect of nickel in the general population as well as in occupationally exposed groups. Approximately 10-20% of the population is sensitive to nickel. Although nickel is too small to be antigenic by itself, the metal can oxidize to a low molecular weight substance called a *hapten*, which can elicit an immune response when joined with a larger molecule. Allergic dermatitis, eczema and immunologic urticaria are common harmful health effects that may develop among people sensitized to nickel and can be seen in the area of contact as well as at distant sites. The respiratory tract is the target organ for allergic manifestations of occupational nickel exposure, causing irritation of the nose, sinuses, loss of the sense of smell, chronic bronchitis, reduced lung function, cancer of the lung and nasal sinus (WHO 2000; ATSDR 2005; Das et al. 2008; WHO 2011b). Generally, chronic inhalation exposure to nickel dusts and aerosols contribute to respiratory disorders such as asthma, bronchitis, rhinitis, lung fibrosis, sinusitis, and pneumoconiosis. Several symptoms manifest as other acute health effects, such as nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness and cough (Das et al. 2008; Duda-Chodak and Blaszczyk 2008). Possible reproductive and developmental effects in humans of occupational exposure to nickel have also been reported: spontaneous and threatening abortions, structural malformations in alive-born infants with nickel-exposed mothers and significant increased risks for total defects, cardiovascular defects and defects of the musculoskeletal system (Chashschin et al. 1994). Moreover, on a sub-chronic toxicity level, also in an occupational setting, it was reported an increase in airway and eye irritations, headaches, and tiredness (Das et al. 2008).

Many studies have shown that a variety of nickel compounds are genotoxic, producing DNA strand breaks, mutations, chromosomal damage, cell transformation and modulation of DNA repair (Klaassen 2008). IARC concluded inhaled nickel compounds are carcinogenic to humans (Group 1) and metallic nickel is possibly carcinogenic (Group 2B). However, there is a lack of evidence of a carcinogenic risk from oral exposure to nickel (WHO 2011b). Several cohort studies of nickel refinery workers demonstrated an increase in the incidence of pulmonary and nasal cavity cancers, specifically epidermoid, anaplastic, and pleomorphic cancers (Das et al. 2008; Klaassen 2008). Cancers of the throat and stomach have also been attributed to inhalation of nickel (Duda-Chodak and Blaszczyk 2008). Several theories have been suggested for the mechanisms of nickel tumorigenesis. All of these assume that the nickel ion is the ultimate active agent (WHO 2000).

Maximum limits

The guideline values in drinking-water for Nickel are 100 μg/L (set out by USEPA), 70 μg/L (set out by WHO) and 20 μg/L (set out by Decree-Law 306/2007 from 27th August of the Portuguese Legislation) (ATSDR 2005; Decree-Law 306/2007; Dehelean and Magdas 2013).

1.3. Methodology

The presence of certain metals in food is considered a Public Health problem due to the nutritional impact and toxicological effects they might have on the general public. Different atomic spectrophotometric techniques are often used to obtain reliable information about metal content in food, including beverages such as fruit juices (Korn et al. 2008).

In the following sections, it will be presented the most important features of each technique conducted in this work, as well as its advantages and disadvantages. Microwave pressure digestion was used for sample treatment, whereas atomic absorption spectroscopy (AAS) - graphite furnace atomic absorption spectroscopy (GFAAS) and hydride generation atomic absorption spectroscopy (HGAAS) - was used for metal quantification.

1.3.1. Microwave pressure digestion

Application of an adequate sample preparation method is extremely important to guarantee the veracity of chemical information obtained on subsequent elemental analysis steps. Sample treatment trends are to minimize sample handling and reagent consumption in order to reduce possible sample contamination, to avoid losses of target elements and to improve analytical throughput (Korn et al. 2008; Cindríc et al. 2011). In recent published studies, the method selected for the acid digestion of many different types of food samples have been the application of microwave ovens, as an alternative technique to other classical digestion procedures. A few years ago, wet and dry ashing were the two most widely used methodologies for digesting food samples. Since acquisition of microwave equipment involves investment by the laboratories, several still use the classic techniques, even with fewer advantages.

In this study, closed vessel microwave digestion with infrared controlled pressure was the technique selected for sample treatment. Its main aspects, advantages and disadvantages will be summarized in the following paragraphs.

Usually, although with some exceptions, samples should be introduced into the atomic absorption spectrophotometer (the equipment used in this study for metal quantification) in the liquid form, and without the presence of any organic matter. Acid digestion procedures are conducted in order to completely transfer the analytes into solution and decompose the matrix, avoiding loss or contamination of the analyte within the minimum handling and process time. For this purpose, a wide variety of reagents can be used, such as nitric and hydrochloric acids, hydrogen peroxide, potassium peroxide sulfate, boric acid. These specific reagents must be selected according to the sample to be digested.

Organic sample material is generally decomposed into carbon dioxide with the aid of oxidizing acids, primarily nitric acid and hydrogen peroxide, to obtain complete mineralization (Berghof 2014a).

Acid digestions can be carried out on either open or closed pressure systems. The advantage of the closed procedure used in this study in comparison with open digestion lies in the significantly higher working temperatures (in the range of 200-260ºC) that can be reached, whereas in open systems temperatures are limited by the boiling point of the acid solution. Moreover, microwave-assisted digestion in closed vessels under pressure has been considered to be a simple handling and fast dissolution technique that minimizes reagent consumption and, consequently, monetary costs, the risk of sample contamination and loss of volatile elements. Contrary to conventional heating on a hot plate where the energy is transferred by conduction, samples heated by microwaves are heated directly by the absorption of microwave radiation, allowing an extremely rapid, simultaneous heating of, typically, 8-12 sample solutions. Higher temperatures give rise to an increase in the reaction kinetics, which in turn decreases the digestion time, allowing it to be carried in less than an hour. On the other hand, greater temperatures lead to a pressure increase in the vessel, which can be a potential safety hazard. In order to monitor the pressure and temperature reaction parameters a variety of sensor systems have been developed to control the exothermic reactions (which are the greatest safety risk regarding digestion in closed systems) resultant from the speed with which the samples are heated during the digestion process. This feature should be taken into consideration for reasons of safety and to ensure optimum reproducibility since it varies according to the type of sample, sample quantity, reagent volumes, etc. (Korn et al. 2008; Berghof 2014a; Berghof 2014b).

The equipment used in this study (Berghof, Speedwave Two) allows the temperature of every single sample to be measured contact-free and directly in real time. Control of the microwave output is dependent on the temperatures of all the samples. Each time the turntable rotates, all of the sample temperatures are recorded separately and the microwave output is adjusted accordingly. This regulation concept guarantees absolute safety, reproducible heating curves and, as a result, reproducible digestion results (Berghof 2014b; Berghof 2014c). Once the digestion is completed, time required for cooling before the vessels can be opened may take hours, which is a limitation of this equipment (Korn et al. 2008). Nevertheless, the cooling process may be accelerated by immersion of the vessels in cold water.

1.3.2. Atomic absorption spectrometry

Atomic spectroscopy includes a set of methods used for determination of elemental composition of an analyte present in a sample by its decomposition into atoms in a flame, furnace or plasma. Each element is measured by absorption or emission of ultraviolet or visible radiation by the gaseous atoms. Several analytical techniques are available, therefore selecting the most appropriate one is essential to achieve accurate and reliable results (Harris 2007; Perkin Elmer 2013).

Atomic absorption spectrometry has been one of the most widely used technique for elemental determination owing to its simple setup, low running cost, robustness and good sensitivity (Pramod and Devendra 2014). The instrumentation for a single-beam atomic absorption spectrometer is shown in Figure 1.2. In AAS, an external source of radiation focuses on the analyte vapor in an atomizer. If the source radiation is of the appropriate frequency (wavelength), it can be absorbed by the analyte atoms and promote them to excited states. After a few nanoseconds, the excited atoms relax to their ground

state by transferring their excess energy to other atoms or molecules in the medium. The attenuated source radiation then enters a monochromator, which is responsible for isolating analytical lines' photons and to remove scattered light of other wavelengths. Then, the radiant power from the source, already attenuated by absorption, is converted by the photomultiplier tube (PMT) into an electrical signal, which is finally processed by a signal processor and directed to an output computer system for measure of the elements of interest (Chasteen 2000; Bolann et al. 2007; Skoog et al. 2014).

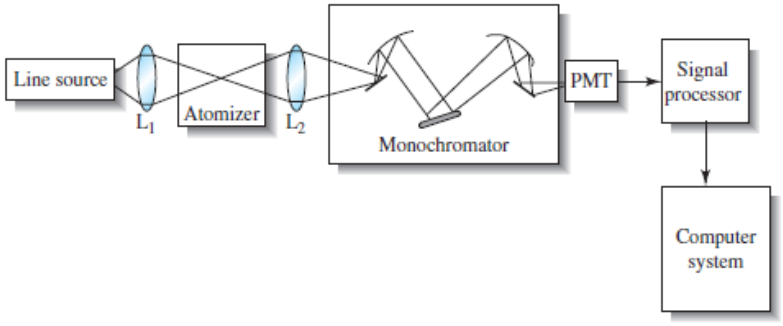

Figure 1.2. Block diagram of a single-beam atomic absorption spectrometer (Skoog et al. 2014): A line source emits a radiation that impinges on the atomic vapor in an atomizer. The attenuated radiation enters a monochromator that isolates the line of interest. Next, the photomultiplier tube (PMT) converts the radiant power into an electrical signal, which is processed and directed to a computer system for output.

Hollow-cathode lamp (HCL) is the most used radiation source for atomic absorption spectroscopy. It consists of a tungsten anode and a cylindrical cathode sealed in a glass tube containing an inert gas, usually argon. After turning on the lamp, Ar ions are formed and collide with the cathode, which leads to excitation of the analyte atoms. When these atoms return to a lower energy state, they emit a characteristic radiation. The aim of the hollow-cathode lamp is to provide an analytical light line for the element of interest and to provide a constant and intense beam of that analytical line (Chasteen 2000). This type of lamp was used, in this study, for the quantification of Cd, Cr, Pb, Mn and Ni.

In addition, electrodeless-discharge lamps (EDLs) are useful sources of atomic line spectra. Usually, an electrodeless-discharge lamp is constructed from a sealed quartz tube that contains an inert gas, such as argon, as well as a small amount of the metal to be analyzed. The lamp receives energy from an intense field of radio-frequency or microwave radiation. The argon ionizes in this field, and the ions are accelerated by the high-frequency component of the field until they have enough energy to excite (by collision) the atoms of the analyte. Electrodeless-discharge lamps are particularly useful for elements, such as arsenic, selenium and tellurium, where hollow-cathode lamp intensities are low (Skoog et al. 2014). Thus, for As quantification, this was the type of lamp used in this study.

Hereafter, according to the metal to be analyzed, the techniques that were used for the atomization of samples are described below.

1.3.2.1. Graphite furnace atomic absorption spectroscopy

Graphite furnace atomic absorption spectroscopy is one of the suitable methods used to determinate trace elements in food and biological samples because of its favorable characteristics, such as: speed, minimum need for sample preparation, the possibility of automation, good sensitivity and low detection limit (Tüzen 2003). This technique was used to quantify cadmium, chromium, lead, manganese and nickel contents in this study.

With this technique, 1 to 100 µL of sample is introduced directly into a graphite tube (represented in Figure 1.3.). The first stage of this method is to dry the sample: light from a hollow-cathode lamp travels through windows at each end of the graphite tube, which is then heated in a programmed series of steps to remove the solvent. Major matrix components, mainly organic matter, are destroyed in the next phase, which is called charring or pyrolysis. Finally, the remaining sample is atomized and the atoms are retained within the tube for an extended period of time as well as the light path that passes through the tube. The analytical signal is the time-integrated absorbance during atomization. After atomization, the furnace is heated to 2500ºC during 3 seconds to clean out any remaining residue. To prevent oxidation of the graphite caused by the presence of oxygen, argon gas is passed over the furnace during each step (except atomization), being the maximum recommended temperature 2550ºC for not more than 7 seconds. Gas flow is halted during atomization to avoid blowing analyte out of the furnace (Harris 2007; Perkin Elmer 2013). Gas flow movements can be observed in Figure 1.4.

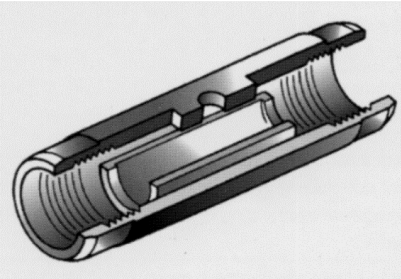

Figure 1.3. Representation of a graphite tube with L'vov platform (Lab-training 2013): graphite tube is where atomization takes place.

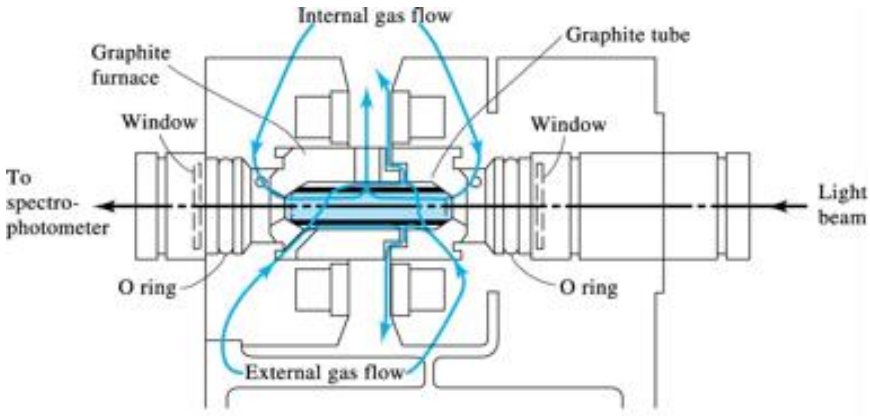

Figure 1.4. Schematic diagram of a graphite furnace (adapted from Chemical Instrumentation 2016): Radiation from a light beam passes through the graphite tube, heating it. Gas flow protects the tube from atmospheric oxidation. External gas flow surrounds the outside of the tube while internal gas flow purges the tube. Quartz windows at each end of the tube help to seal the tube and allow light to pass through.

When compared to flame spectroscopy, which is another technique of atomic methods, graphite furnace AAS has some advantages and disadvantages. On one hand, GF involves more operator skills to achieve the best conditions for each type of sample, takes more time during sample analysis and fewer elements can be determined. On the other hand, due to the longer time of atomization, GFAAS is a more sensitive technique and is able to analyze very small samples, allowing the determination of over 40 elements in microliter sample volumes contrary to 1-2mL minimum for flame analysis, if the optimization had already been done. Detection limits are typically 100 to 1000 times better than those of flame AA systems, significantly expanding the capabilities of atomic absorption in comparison with flame spectroscopy (Harris 2007; Perkin Elmer 2013).

However, quantification of heavy metals in some samples by GFAAS may be difficult sometimes since the influence of a complex matrix seriously affects the analytical results. Therefore, different matrix modifiers are used to control interferences during the measurement process. Matrix modifiers are chemical substances added to the sample to reduce the loss of the analyte by promoting the separation of the analyte from the matrix prior to atomization such that the interference is not present during atomization. For volatile elements, a chemical modifier reduces the volatility of the analyte or increases the volatility of the matrix. Higher pyrolysis temperature can be used to evaporate more matrix components and therefore, minimize scatter and molecule formation in the atomization step. However, modifiers may introduce some problems for real sample analysis, causing spectral interference. They may require the use of a higher atomization temperature, which may reduce the characteristic mass due to higher rate of diffusion from the tube. In addition, the analyte may condense in cool regions of the graphite tube. Most common matrix modifiers are magnesium nitrate and ammonium dihydrogen phosphate (which were the ones used in this study), diammonium hydrogen phosphate, nickel nitrate, and palladium nitrate alone or in combinations (Bader 2001; Tüzen 2003; Harris 2007)

1.3.2.2. Hydride generation atomic absorption spectroscopy

Hydride generation atomic absorption spectroscopy is one of the available techniques for many modern AAS instruments and, in this work, it was used to determine total arsenic levels. Hydride generation is considered to be a very effective analytical method designed to separate hydride forming metals, such as As, from a range of matrices and acid concentrations (Hineman 2012).

Many of the main parts of the HGAAS system are identical to that of AAS, so the two different techniques can be installed in the same equipment. There are essentially four steps during HG system mechanism coupled to an AAS: generation of the hydride; collection of the hydride (if necessary); transfer of the hydride to the quartz tube atomizer; and atomization of the hydride. HG can be performed in batch mode, continuous-flow and flow-injection systems, which is the case in this study. Hydride generation system is responsible for aspirating liquid sample and for mixing sample with sodium borohydride and HCl, creating a volatile hydride (H_3As in this case since arsenic is the analyte). The liquid mixture flows through a tube, being ultimately flowed into a gas/liquid separator where the hydride and some gaseous hydrogen (which is required to give rise to free atoms needed for hydride atomization) are purged into the optical cell via a gas transfer line. Optical cell is placed in the horizontal arm of the T-shaped quartztube atomizer (QTA) used in this study, being aligned in the optical path of the AAS spectrometer. The central arm of the T-tube is designed to receive a flow of gas (argon) containing hydrides from a hydride generator. In turn, the job of the optical cell is to decompose the hydride form of the metalloid from the hydride generation module, creating atoms of the element of

interest (which is arsenic). The monochromator only allows the light not absorbed by the analyte atoms in the optical cell to reach the PMT. When atoms are present in the cell from hydride decomposition, while the sample is aspirated, some of that light is absorbed by those atoms. This causes a decrease in PMT signal that is proportional to the amount of analyte. Conventional QTA can be classified into two basic types according to the way oxygen is introduced in the atomizer: flame-in-tube atomizer and conventional externally heated quartztube atomizer (conventional EHQTA), being the one used in this study and the most common. This type of atomizer does not have a specific tube for oxygen introduction, but a certain oxygen content in the gas mixture is required for accomplish a better sensitivity. Conventional EHQTAs employ either an electrical resistance device or the acetylene-air flame to heat the atomizer optical tube to a temperature between 700°C and 1100°C (900ºC was the temperature used in this study). Most of the reagents introduced into the system flow to a waste container (Bye 1989; Campbell 1992; Chasteen 2000; Dědina 2007; Kumar and Riyazuddin 2010). A schematic diagram of HGAAS system can be seen in Figure 1.5.

Coupling hydride generation to AAS allows low detection limits using low operating costs, making it an affordable investment. In addition, flow-injection system is particularly useful as it enables hydride generation, avoids the offline manipulations and, consequently, the risk of contamination and/or loss of the analyte and improves precision and the sample throughput. Moreover, the heated quartz tube atomizer is especially advantageous for the determination of arsenic because the absorption wavelengths for this element are below 200 nm, which represents an area subject to intense interference from flame radicals that can significantly affect detection limits. Furthermore, separating the analyte from the matrix is probably the main advantage of HG since it enables not only sensitivity of the atomic absorption technique to be improved and physical, matrix and spectral interferences to be reduced or even eliminated, but also preconcentration of the analyte. However, non-spectral interferences may still occur during the chemical reaction, throughout formation of the hydrides, in separation of the volatile species from the liquid phase and in atomization. The magnitude of interferences depends on several factors, such as the type of HG system and atomizer, the concentrations of acid and reducing agent and the mixing order of the reagents (Campbell 1992; Kumar and Riyazuddin 2010; Hineman 2012; Sigrist et al. 2016).

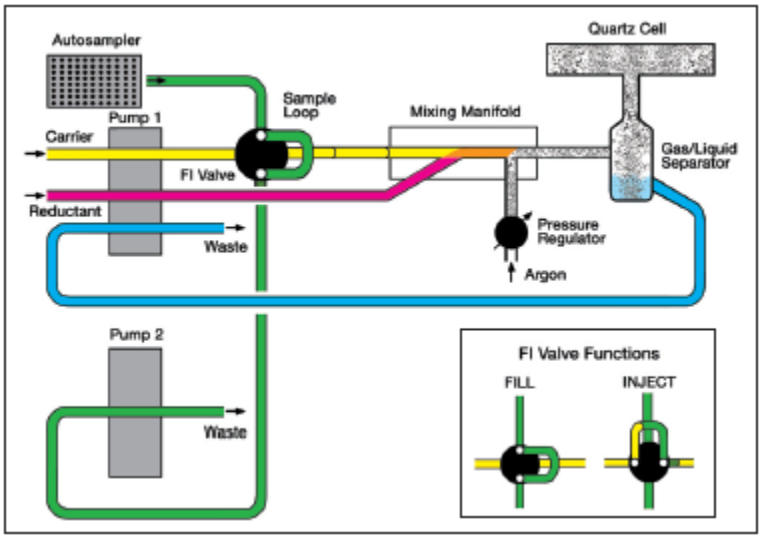

Figure 1.5. Schematic diagram of HGAAS automated system (Hineman 2012): Samples and reagents (reductant and carrier) are pumped separately till they are blended in the mixing manifold, generating an hydride. The liquid mixture is ultimately flowed into a gas/liquid separator where the hydride is purged into the quartz cell via an argon transfer line. Hydride in gaseous form vaporizes and analyte free atoms can absorb radiation. Most of the reagents introduced into the system flow to a waste container.

2. Objectives

The main purpose of this study was to determine the content of some selected heavy metals in fruit juices available in the Portuguese market.

All objectives of this work are described with more detail in the next points:

1 – Optimization of the operating microwave digestion conditions (reagent volumes, temperature program and time) for a complete sample digestion, all in the shortest possible time and with the smallest reagent consumption.

2 – Validation of microwave digestion method, in order to demonstrate results repeatability in different digestion vessels and different positions.

3 – Selection of the best conditions to analyze arsenic by HGAAS and the other metals mentioned above by GFAAS, in order to obtain results with good precision and accuracy.

4 – Method validation for all metals analyzed, including: study of linearity, working range, analytical thresholds, precision (repeatability, intermediate precision), specificity/selectivity and accuracy. To ascertain the repeatability of the microwave digestion method, aliquots of the same fruit juice package were analyzed at equal conditions and simultaneously.

5 – Determination of the content of arsenic, cadmium, chromium, lead, manganese and nickel in fruit juices available in the Portuguese market. Portuguese Food and Economic Safety Authority (ASAE) had the responsibility to select and collect the samples to be analyzed in this study. This collaboration with ASAE was very important since it allowed us to analyze the most interesting brands regarding metal quantification, and also the most consumed by Portuguese population.

6 – Comparison of obtained laboratory results with reference levels to drinking-water established by WHO, USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation, and also with similar published data by other authors available in the literature.

3. Material and Methods

3.1. Samples characterization

Twenty-one varieties of packaged fruit juices available on the Portuguese market were selected and obtained by ASAE from July to December 2015 for this study. Samples including several juice types from the four most consumed brands are described in Table 3.1.

Table 3.1. Fruit juice types selected for this study: Each sample was designated with a letter from A to U; samples are grouped according to its type of fruit juice; numbers from 1 to 4 represent a different fruit juice brand.

Samples	Juice types	Brand
A	Red fruits	1
B, P	Pear	1, 3
C	Plum	1
D, K, N	Peach	1, 2, 3
E, L, M	Multifruit	1, 2, 4
F	Passion fruit	1
G, J, Q, S	Orange	1, 2, 3, 4
H	Apple	1
I, T	Mango	1, 4
O	Apricot	3
R	Strawberry	4
U	Pineapple	4

Samples were maintained with the seal of ASAE until analysis and conserved at -20ºC in a duplicate containers, after opening to obtain aliquots for analysis.

3.2. Reagents and standard solutions

Reagents and standard solutions used in this study are represented in Table 3.2.

Table 3.2. Reagents used in this study: Reagents used for sample preparation, analytical methods and material treatment.

Reagents		**Brand**
Standard Solutions	Arsenic (1000 µg/mL)	SCP Science (Quebec, Canada)
	Cadmium (1000 µg/mL)	SCP Science (Quebec, Canada)
	Chromium (1000 µg/mL)	SCP Science (Quebec, Canada)
	Lead (1000 µg/mL)	SCP Science (Quebec, Canada)
	Manganese (1000 µg/mL)	SCP Science (Quebec, Canada)
	Nickel (1000 µg/mL)	SCP Science (Quebec, Canada)
Ascorbic acid		Merck (Darmstadt, Germany)
Certified reference material (TM-24.3; TM-26.3)		Environment Canada
Chemical modifier ($Mg(NO_3)_2$) 2% Mg		SCP Science (Quebec, Canada)
Chemical modifier ($NH_4H_2PO_4$) 100±2 g/L		Merck (Darmstadt, Germany)
Hydrochloric acid 37%		Sigma-Aldrich (Steinhen, Germany)
Hydrogen peroxide 30%		Merck (Fluka Analytical, Switzerland)
Nitric acid 65% (for metal analysis)		Panreac (Barcelona, Spain)
Nitric acid 67%		Prolabo
Potassium iodide		Panreac (Barcelona, Spain)
Sodium borohydride		Merck (Darmstadt, Germany)
Sodium hydroxide		Merck (Darmstadt, Germany)

Standard solutions were prepared with HNO_3 5% (for metal analysis) and, whenever available, the reagents used were appropriate for metal analysis.

3.3. Materials and equipments

In order to avoid any possible extra metal contamination, all used material were left submersed for 24 hours under a HNO_3 15% solution. After this time, material was washed 3 times with deionized water 18.2 MΩcm, dried and stored in a place protected from dust. Table 3.3. presents all materials and equipment used in this study.

Table 3.3. Equipment and material used in this study: Material used for sample preparation and analytical methods.

Equipment		Brand/model
Microwave digestor		Berghof Speedwave Two
Heating plate		SBS
Water purification system		Direct-Q UV3, Millipore - Bedford
Balance		Mettler Toledo
Atomic Absorption Spectrophotometer		PerkinElmer Instruments Analyst 700 equipped with deuterium background corrector
Graphite chambers with L'vov platform		PerkinElmer
Graphite Furnace Automatic Sampler		Perkin Elmer AS800
Hydride Generation System		PerkinElmer FIAS 100 Flow Injection System
Lamps	Arsenic (λ = 193,7 nm)	(EDL) Sys 2 - PerkinElmer
	Cadmium (λ = 228,8 nm)	Cathode Lamp - PerkinElmer
	Chromium (λ = 357,9nm)	Cathode Lamp - PerkinElmer
	Lead (λ = 283,3 nm)	Cathode Lamp - PerkinElmer
	Manganese (λ = 279,5nm)	Cathode Lamp - PerkinElmer
	Nickel (λ = 299,44 nm)	Cathode Lamp - PerkinElmer

3.4. Sample preparation

A microwave digestion procedure was carried out in a Berghof microwave digestion system (Speedwave Two, represented in Figure 3.1.) in order to achieve a total digestion in a shorter time, avoiding loss of metals by volatilization and minimizing the amount of added acid. This device can digest ten samples simultaneously.

The procedure specified by the equipment manufacturer for digesting fruit juices was to weigh 5 mL of the sample into the digestion vessel and add 5 mL of nitric acid and 1 mL of hydrogen peroxide, followed by microwave oven heating with a specific temperature program. However, this method appeared not to be successful in completely digesting the sample. For this reason, several digestion procedures were performed in order to achieve a total sample digestion by varying the amount of sample, acid and hydrogen peroxide, as well as time and temperature program. Considering all the experiences made, the

best digestion procedure was the following: weigh 3 mL of the sample into the digestion vessel, add 1,5 mL of nitric acid and 2 mL of hydrogen peroxide. After this procedure, the samples were kept at room temperature during 6 hours to guarantee its homogenization as well as a slow digestion. After this time, the vessels were closed and the digestion program was carried out with the temperature program presented in Table 3.4.

Table 3.4. Microwave digestion temperature program: Ramp expresses the minutes necessary to achieve the temperature of the next step; time represents the minutes at the same temperature.

Step	**1**	**2**	**3**	**4**	**5**
Ramp (min)	10	5	0	2	0
Time (min)	10	15	10	15	0
T (°C)	170	200	200	100	75

All Teflon vessels were left closed overnight for cooling. The day after, vessel content was transferred into a glass put on a hot plate to slowly evaporate the majority of the acid solvent. To ensure minimal losses, vessel was washed with deionized water. This aspect is very important since concentrations greater than 10% of nitric acid should not be used when the sample is analyzed by GFAAS. After this step, the resulting colourless solutions were diluted and made up to 10 mL with nitric acid 5%. To confirm complete digestion of the sample a rapid test was performed.

Figure 3.1. Microwave digestor (Berghof Speedwave Two): Equipment used for samples' digestion. This model has the capacity for 10 vessels.

3.5. Metal quantification

Metal quantification was performed using atomic absorption spectrometry. Hydride generation atomic absorption spectrometry was used for arsenic, while graphite furnace atomic absorption spectrometry was used for the other elements quantification.

3.5.1. Graphite furnace atomic absorption spectroscopy

After turning on the equipment (represented in Figure 3.2.) and the intensity of the cathode lamp (for the concerned metal) was stable, calibration curve could start being created followed by sample analysis. HNO_3 5% and matrix modifier ($NH_4H_2PO_4$ or $Mg(NO_3)_2$) in its correct amounts previously specified in the analysis software were automatically added to the standard solutions and samples during analysis. The temperature program used for the quantification of each metal is presented in Table 3.5.

Table 3.5. Cadmium, chromium, lead, manganese and nickel analytic conditions: Selected lines, pretreatment and atomization temperatures for metals quantified by GFAAS.

Element	Wavelength (nm)	Ashing temperature (ºC)	Atomization temperature (ºC)
Cd	228,8	850	1650
Cr	357,9	1650	2500
Pb	283,3	1100	1600
Mn	279,5	1400	2200
Ni	299,44	1400	2500

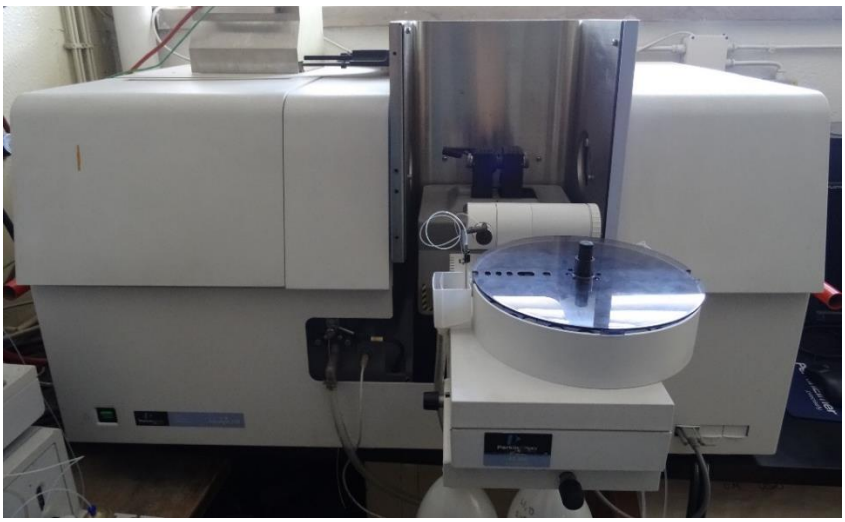

Figure 3.2. AA Spectrophotometer (PerkinElmer Instruments Analyst 700) with GF Automatic Sampler (Perkin Elmer AS800): Equipment used for Cd, Cr, Pb, Mn and Ni quantification.

3.5.2. Hydride generation atomic absorption spectroscopy

Before sample analysis, a reduction reaction must be carried out, as described hereinafter. 1 mL of sample, 1 mL of hydrochloric acid, 1 mL of ascorbic acid and 1mL of potassium iodide were added to a 10 mL volumetric flask and the volume was completed with deionized water. Standard solutions were prepared with the same reagents and proportions. Solutions were left at room temperature for at least 45 minutes before analysis. The preparation of HCl 10% and $NaBH_4$ 0,2% and NaOH 0,05% solutions is required for this type of technique used in this study for arsenic quantification. After turning on the equipment (represented in Figure 3.3.) and the quartz cell was hot enough (900ºC), calibration curve could be done followed by sample analysis.

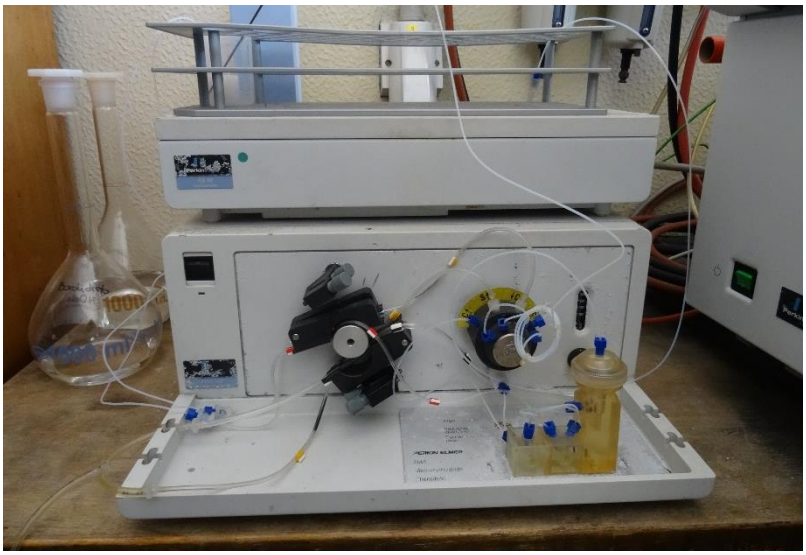

Figure 3.3. Hydride generation system (PerkinElmer FIAS 100 Flow Injection System): Equipment used for As quantification.

3.6. Method Validation

Validation is an important step in every analytical procedure since it is a way of laboratories to demonstrate their internal assay methods lead to reliable results. Since analytical procedures are usually associated with susceptible cumulative handling errors (systematic or random) that can significantly influence the final results, it is essential that laboratories have objective and uniformed criteria that lead to credible data according to the intended quality (RELACRE 2000).

Validation process involves the study of parameters through direct and indirect evaluation. In this study, determination of such parameters for each metal was performed according to “Guia Relacre 13 – Validação de métodos internos de ensaio em análise química”.

3.6.1. Indirect evaluation

This type of validation is done by determination and evidence of their characteristic parameters.

3.6.1.1. Linearity

Linearity can be evaluated using a statistical model, according to ISO 8466-1. In quantitative analysis, calibration indicates a process by which a response of a measurement system relates with a known concentration substance.

In this study, calibration curves were performed daily for each series of analysis. A number of standard solutions with known concentrations of the element to be measured were prepared. This standard calibration solutions were measured in the same conditions as the samples to be analyzed. A calibration curve was established (signal *versus* concentration) and element concentration in the samples was determined by interpolation.

From a calibration curve, linear and nonlinear calibration functions can be calculated, using an ordered set of pairs, as well as the respective residual standard deviations, $S_{y/x}$ e S_y^2.

Variance difference (DS^2) is given by the equation:

$$3.1.\quad DS^2 = (N-2)\,.\,S^2_{y/x} - (N-3)\,.\,S^2_{y2}$$

where N is the number of calibration standards.

Then, test value PG is calculated:

$$3.2.\quad PG = \frac{DS^2}{S^2_{y2}}$$

This PG value is then compared with the tabulated value of the Fisher-Snedecor distribution:

- If PG ≤ F : calibration function is linear.
- If PG > F : calibration function is not linear.

3.6.1.2. Working range

Calibration standard values should be comprised in the working range. According to ISO 8466-1, five to ten calibration points are recommended, equally distributed on the concentration range. The first and last standards were analyzed 10 times independently.

Working range can be evaluated through variance homogeneity test, calculating variances associated to first and last standards (S^2_1 and S^2_{10}):

$$3.3.\quad S_i^2 = \frac{\sum_{j=1}^{10}(y_{i,j} - \bar{y}_i)^2}{n_i - 1}$$

where:

$$3.4.\ \overline{y_i} = \frac{\sum_{j=1}^{10} y_{i,j}}{n_i}$$

for i=1 and i=10.

Where:

i – standard number (in this case ranging from 1 to 10)

j – number of repetitions for each standard

Variances were tested to examine if there were significant differences between them, calculating the PG test value:

$$3.6.\ PG = \frac{S_{10}^2}{S_1^2}$$

when $S^2_{10} > S^2_1$

$$3.7.\ PG = \frac{S_1^2}{S_{10}^2}$$

when $S^2_1 > S^2_{10}$

PG test value was then compared with the tabulated value of Fisher-Snedecor distribution, for n-1 degrees of freedom:

- If PG ≤ F: variance differences are not significant and the working range is well adjusted.

- If PG > F: variance differences are significant and the working range should be reduced till the difference between variances relative to first and last standard allow to have PG ≤ F.

3.6.1.3. Analytical thresholds

Detection limit (D.L.) is the smallest measured amount beyond which it is possible to detect the analyte presence with some statistical certainty. It corresponds to the lower substance quantity to be analyzed that can be detected on a sample, but not quantified as an exact value. It is important to refer that a result lower than the detection limit does not mean the element is absent. Instead, we can say its concentration is lower than a particular value. In qualitative terms, D.L. corresponds to the minimum concentration that is possible to distinguish from blank (a sample with the same matrix and analyte free). Because the quantification method involves a linear calibration in the present study, the detection limit can be obtained through the following equation:

$$3.7.\ D.L. = \frac{\left[3{,}3\ .\ S_{y/x}\right]}{b}$$

where:

- $S_{y/x}$ is the residual standard deviation of the calibration curve;

- b is its slope.

Another way of calculating D.L. is through the following equation:

$$3.8.\ D.L. = X_0 + 3{,}3\sigma_0$$

where:

- X_0 is the arithmetic average of the measured content of a series of blanks (between 10 and 20 readings), prepared independently and analyzed throughout several working days, mimicking a routine situation as far as possible;
- σ_0 represents the standard deviation associated with X_0.

Quantification limit (Q.L.) corresponds to the lowest measured concentration beyond which it is possible to quantify the analyte, with some accuracy and precision. In practical terms, it usually corresponds to the standard calibration with the lowest concentration (excluding blank). Since the quantification method involves a linear calibration in this study, Q.L. can obtained through the following equation:

$$3.9.\ Q.L. = \frac{\left[10 \cdot Sy_{/x}\right]}{b}$$

Another way of calculating Q.L. is through the following equation:

$$3.10.\ Q.L. = X_0 + 10\sigma_0$$

3.6.1.4. Precision

Precision aims to evaluate the dispersion of the results among independent trials repeated on the same sample, similar samples or standards, under defined conditions. Precision usually differs according to the concentration range. There are two extreme methods to evaluate this dispersion: repeatability and reproducibility. Between them, there are an in-between method, which is called intermediate precision.

Repeatability expresses the precision of a trial method performed in identical conditions. In other words, it refers to trials on the same sample, in the most stable conditions, such as: same laboratory, same analyst, same equipment, same reagents, and short time periods. In order to define the repeatability of a method in the laboratory itself, a series of analysis of the same sample or standard is performed ($n \geq 10$), in repeatability conditions.

In this study, the repeatability was studied analyzing 10 times the first and the last calibration point of each metal calibration curve. Selection of both these points allowed us to work with the extreme concentrations.

Reproducibility refers to the precision of a method performed in different conditions (such as different laboratories, different operators, different equipment and longer time periods), using the same technique and the same sample.

Intermediate precision refers to the evaluated precision of the same sample, identical samples or standards, using the same method, in the same laboratory or in different ones, but defining exactly which are the conditions to vary, such as: different analysts, different equipment, different period times, with/without calibration verification. This precision measurement is recognized as the most representative of the results variability in a laboratory. Therefore, it is the most recommended used measurement. In order to determine intermediate precision, n sample measurements are made in replicate, duplicate or in a single test, with pre-defined conditions.

In this study, intermediate precision was calculated analyzing 10 times the first calibration standard of each metal calibration curve, on 3 nonconsecutive days. Lowest concentration was also selected for this calculation because it is more sensitive to slight variations. Thus, the results obtained for all other concentrations must have a lower relative standard deviation (RSD).

3.6.1.5. Specificity/Selectivity

Selectivity is the ability of a method to identify and distinguish an analyte in particular within a complex mixture without interferences from other components.

A method is considered to be specific when it alloys to discriminate the analyte among other substances eventually present on the analyzed sample. Hence, it is necessary to analyze a complex sample with more than one component in order to prove there are no interferences from other substances eventually present on the sample.

In this study, the selectivity of the method was evaluated through the analysis of certified reference material (CRM), which contains one or more features with values well established by a process technically valid.

3.6.1.6. Study of method digestion variation

In order to estimate if there were possible interferences in the metal quantification caused by the different vessels used for the microwave digestion procedure, it was conducted a study to understand if this parameter introduces variations in the digestion method. The same digestion temperature program performed in this study (presented in Table 3.4.) was carried out with five vessels with one sample (fruit juice B). All conditions (reagent volumes, time and temperature) were exactly the same, and the vessels were located at the same time in the apparatus.

3.6.2. Direct evaluation

Direct evaluation aims to determinate the accuracy of assay methods. It is defined as the concordance between a test result and the accepted reference value as conventionally true. The term accuracy, when applied to a series of test results, implies a combination of components of random errors and systematic errors.

Certified reference materials are often used to evaluate a method accuracy, as well as inter-laboratory assays and comparative tests. It has a concentration value for each element and an associated uncertainty. The acquisition of a certified reference material should be done through a recognized and reliable provider entity (Environment Canada in this study).

The correct use of certified reference materials lies on its analysis to evaluate the laboratory performance. The experimentally obtained CRM result must be compared with the certified value, calculating the analysis error and accuracy. When the obtained value is not on the uncertainty range indicated for the certified value, laboratory should search the causes of that deviation and try to eliminate or accept them. According to the defined rigor to the results, laboratory can adopt different criteria to accept CRM results. Some methodologies used to evaluate CRM results are: relative error, hypothesis test (t-test), Z-score factor and standard error.

Z-score was the test used in the present study, which is calculated through the following equation:

$$3.11.\quad Z = \frac{(Xlab - Xv)}{S}$$

where:

Xlab – Laboratory obtained value;
Xv – CRM certified value;
S – Deviation unit

Evaluation can be made according to the following classification scale:

- $|Z| \leq 2$ – Satisfactory
- $2 < |Z| \leq 3$ – Questionable
- $|Z| > 3$ – Incorrect

3.7. Statistical Treatment

All graphics and statistical treatment including one-way ANOVA with $\alpha = 0.01$ significance level were done using the tools from Microsoft Excel 2013 Version 15.0.4849.1003.

4. Results and discussion

4.1. Validation

As mentioned before, validation is an important step in every analytical procedure to ensure results' reliability. In this study, method validation was conducted according to "Guia Relacre 13 - Validação de métodos internos de ensaio em análise química".

In the following paragraphs, data regarding the evaluated parameters will be presented.

4.1.1. Indirect evaluation

4.1.1.1. Linearity

Linearity was verified using calibration curves that were performed daily for each series of analysis. Each result is the mean of at least one duplicate.

To facilitate the presentation of the results, in the following graphics (from Figure 4.1. to Figure 4.6.) it is displayed a calibration curve for every metal which was made from the average of all calibration curves for the referred metal. Each curve was performed with a minimum of six points including the zero.

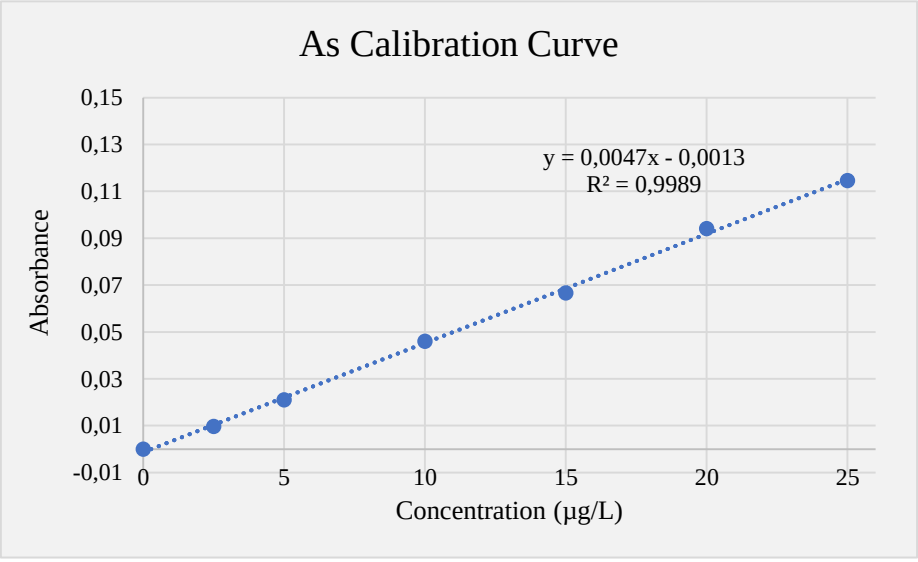

Figure 4.1. Arsenic calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=7).

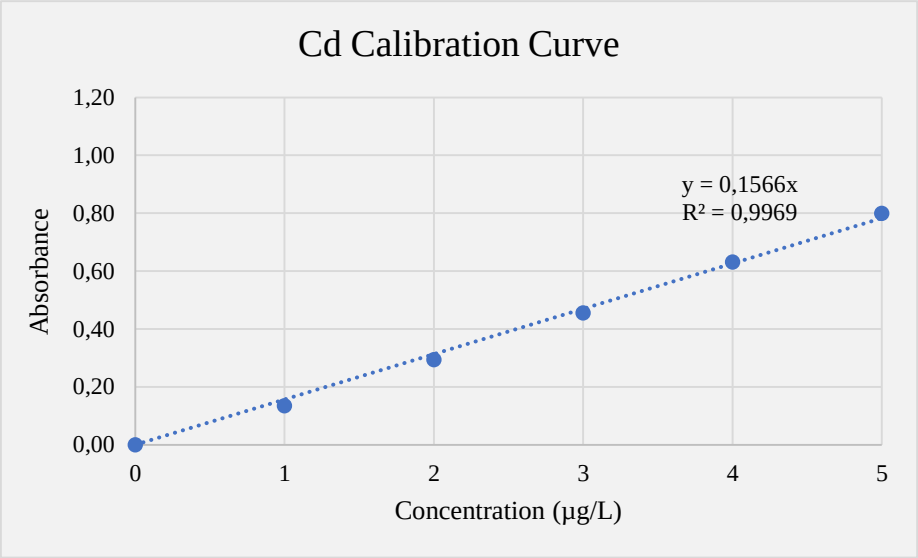

Figure 4.2. Cadmium calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=4).

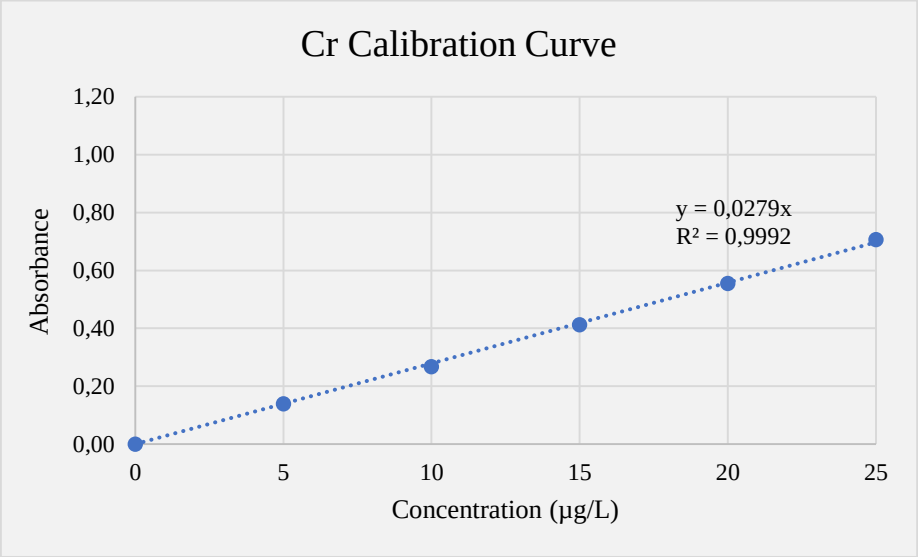

Figure 4.3. Chromium calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=4).

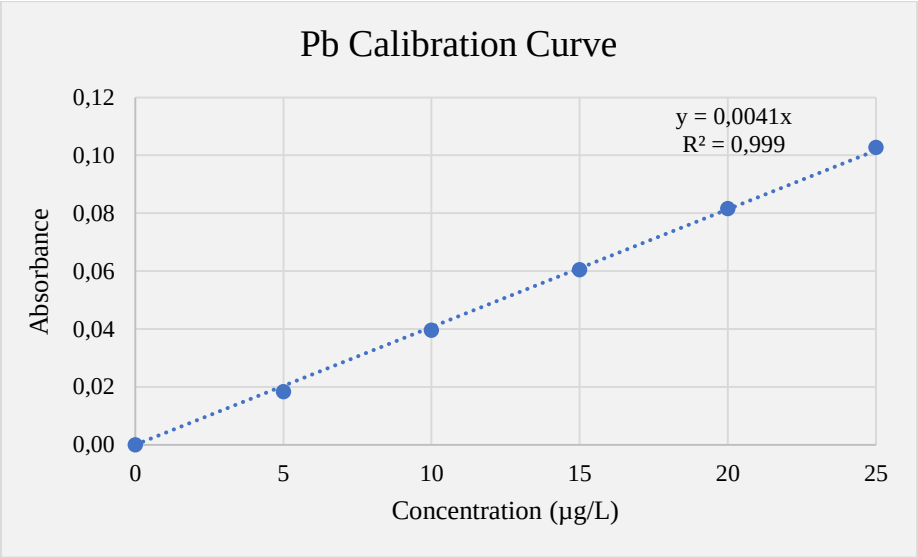

Figure 4.4. Lead calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=6).

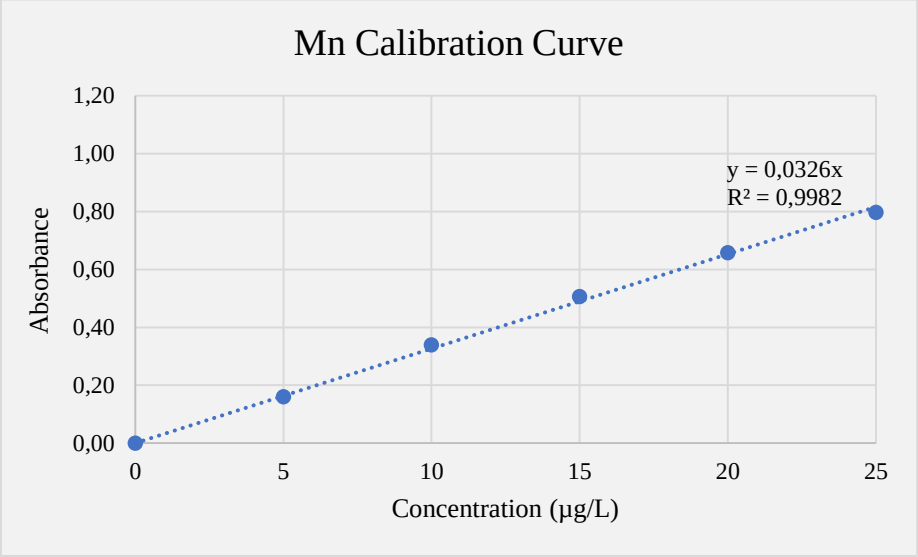

Figure 4.5. Manganese calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=5).

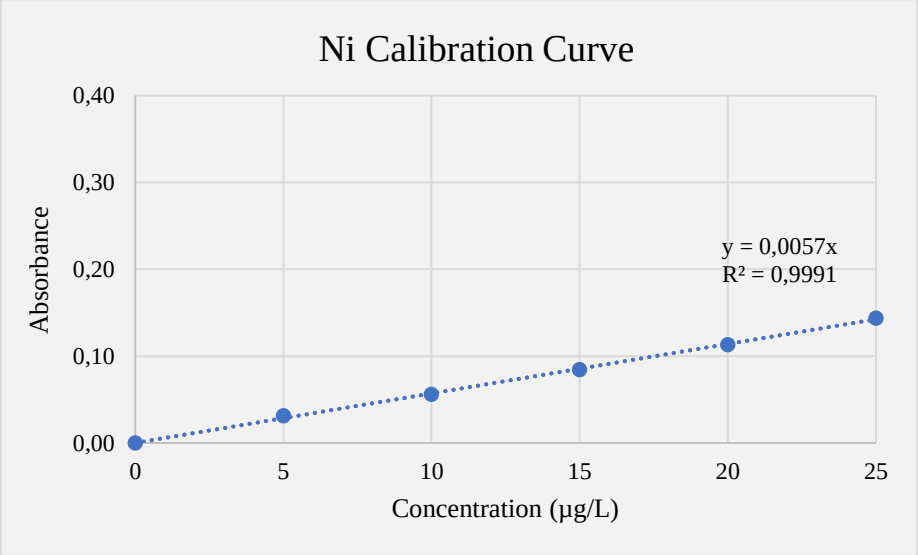

Figure 4.6. Nickel calibration curve (Concentration *vs* Absorbance): Concentrations are presented in µg/L. Each point of the curve represents an average, (n=8).

Table 4.1. presents the linear parameters of each analyzed metal.

Table 4.1. Linear parameters of arsenic, cadmium, chromium, lead, nickel and manganese: a - slope; b - intercept; r^2 - coefficient of determination; $S_{x/y}$ - residual standard deviation; S_y^2 - standard error; N - number of calibration curve points; DS^2 - variance difference; PG - test value; F-test - tabulated value of Fisher-Snedecor distribution with a $\alpha = 0.01$ significance level. All calibration curves are linear.

Metals	**As**	**Cd**	**Cr**	**Pb**	**Mn**	**Ni**
a	0,0047	0,1566	0,0279	0,0041	0,0326	0,0057
b	-0,0013	0,0000	0,0000	0,0000	0,0000	0,0000
r^2	0,9989	0,9969	0,9992	0,9991	0,9982	0,9991
$S_{x/y}$	0,0016	0,0140	0,0076	0,0010	0,0136	0,0018
S_y^2	0,0153	0,0061	0,0042	0,0007	0,0079	0,0019
N	7	6	6	6	6	6
DS^2	0,0009	0,0007	0,0002	0,000002	0,0005	0,000002
PG	-3,9475	17,9402	10,0818	5,5069	8,7072	0,5482
F-test (1;0,99;n-3)	21,1977	34,1162	34,1162	34,1162	34,1162	34,1162
Linearity	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted

Since PG test value is always lower than the tabulated value of Fisher-Snedecor distribution, linearity for the calibration curves of all the studied elements is proved.

4.1.1.2. Working range

Calibration standard values should be comprised on the working range. The first and last standards were analyzed 10 times independently. Its results as well as the relative standard deviation are presented in Table 4.2.

Table 4.2. Arsenic, cadmium, chromium, lead, manganese and nickel working range: The first and last calibration curve standards were analyzed. Each mean value is the result of ten measurements (n=10).

Metals	Calibration curve standards	Absorbance		Calculated Concentration (µg/L)	
		Mean	RSD (%)	Mean	RSD (%)
As	**2,5 µg/L**	0,016	4,869	2,465	4,807
	25 µg/L	0,126	1,851	26,560	1,794
Cd	**1 µg/L**	0,135	5,400	0,884	5,526
	5 µg/L	0,887	2,099	5,651	2,112
Cr	**5 µg/L**	0,127	4,794	4,719	4,732
	25 µg/L	0,804	0,727	24,865	0,714
Pb	**5 µg/L**	0,019	2,606	4,156	2,632
	25 µg/L	0,088	1,738	25,432	1,733
Mn	**5 µg/L**	0,132	3,368	5,136	3,317
	25 µg/L	0,861	1,927	22,405	1,929
Ni	**5 µg/L**	0,041	6,507	5,415	6,365
	25 µg/L	0,183	4,356	27,389	4,355

Every analysis series had a relative standard deviation lower than 5%, except for cadmium and nickel first calibration standard.

In order to check if working range was well adjusted, variance homogeneity test was performed for each metal, where PG test value was compared with the tabulated value of Fisher-Snedecor distribution. Results can be consulted in Table 4.3.

Table 4.3. Variance homogeneity test for arsenic, cadmium, chromium, lead, manganese and nickel: PG - test value; F-test - tabulated value of Fisher-Snedecor distribution with a $\alpha = 0.01$ significance level. Working range is well adjusted for all metals.

Metals	As	Cd	Cr	Pb	Mn	Ni
PG	0,115	0,153	1,078	0,108	0,071	0,110
F-test	0,187	0,187	0,187	0,187	0,187	0,187
Working range	Well adjusted	Well adjusted	Well adjusted	Well adjusted	Well adjusted	Well adjusted

As indicated in table 4.3., PG test value is always lower than the tabulated value of Fisher-Snedecor distribution, which means the working range is well adjusted for all analyzed metals.

4.1.1.3. Analytical thresholds

Detection and quantification limits for each analyzed metal are presented in Table 4.4., which were calculated through both calibration curve method (Equations 3.7. and 3.9.) and general case (Equations 3.8. and 3.10.).

Table 4.4. Detection limit and quantification limit for arsenic, cadmium, chromium, lead, nickel and manganese: D.L. and Q.L. were both calculated through calibration curve method and general case.

Metals		As	Cd	Cr	Pb	Mn	Ni
D.L. (µg/L)	**Calibration curve method**	1,108	0,294	0,893	0,767	1,375	1,029
	General case	0,941	0,465	0,607	0,163	1,411	1,164
Q.L. (µg/L)	**Calibration curve method**	3,358	0,892	2,706	2,325	4,166	3,117
	General case	1,867	0,689	1,246	0,308	3,647	2,142

For every analyzed metal, except for arsenic, Q.L. is lower than the corresponding calibration curve first point, when calculated through calibration curve method. However, when calculated through general case, Q.L. is below the corresponding calibration curve first point, for all the analyzed metals. By this determination it can be demonstrated that all lowest standard concentrations are quantifiable.

4.1.1.4. Repeatability

In order to define the repeatability, the first and last calibration standards of each metal calibration curve were analyzed 10 times. Results are presented in Table 4.5., which is presented hereafter.

Table 4.5. RSD (%) of first and last calibration points of arsenic, cadmium, chromium, lead, manganese and nickel calibration curves: Repeatability is proved if RSD (%) is below 10%.

Metals	Calibration standards	RSD (%)
As	2,5 (µg/L)	4,807
	25 (µg/L)	1,794
Cd	1 (µg/L)	5,526
	5 (µg/L)	2,112
Cr	5 (µg/L)	4,732
	25 (µg/L)	0,714
Pb	5 (µg/L)	2,632
	25 (µg/L)	1,733
Mn	5 (µg/L)	3,317
	25 (µg/L)	1,929
Ni	5 (µg/L)	6,365
	25 (µg/L)	4,355

Since for every metal calibration curve, the RSD (%) of first and last calibration standards is below 10% (which is the value defined internally by the laboratory), it is possible to affirm this study has acceptable repeatability conditions.

4.1.1.5. Intermediate precision

In this study, intermediate precision was calculated analyzing 10 times the first calibration standard of each metal calibration curve, on 3 nonconsecutive days. Lowest concentration was selected because it is the worst case of all concentrations. Analysis results are presented in Table 4.6.

Table 4.6. Intermediate precision mean values for three different days and its RSD (%) for arsenic, chromium, lead, manganese and nickel: Each mean value from different days is the result of 10 measurements (n=10).

Metals	As (µg/L)	Cd (µg/L)	Cr (µg/L)	Pb (µg/L)	Mn (µg/L)	Ni (µg/L)
Mean Day 1	2,465	0,884	4,471	4,293	5,281	5,415
Mean Day 2	2,644	0,776	4,719	4,156	5,136	4,753
Mean Day 3	2,677	0,850	4,832	4,318	4,819	5,388
Mean	2,596	0,837	4,674	4,256	5,079	5,185
RSD (%)	4,400	6,586	3,958	2,047	4,658	7,231

Relative standard deviation is below 10% for each element, which indicates intermediate precision is acceptable for every analyzed element.

4.1.1.6. Specificity/Selectivity

A method is considered to be specific when it alloys to discriminate an analyte among other substances eventually present on the analyzed sample. Since CRMs contain a complex mixture of substances and it was possible to distinguish and quantify the analyte of interest from that mixture, (as it can be observed in Table 4.8.) the selectivity/specificity of the method can be affirmed.

4.1.1.7. Study of method digestion variation

A study of the variation within the digestion method was performed with the same digestion temperature program (presented in Table 3.4.) using five vessels with one sample (B) and the other reagents needed for this technique in the exact same amounts. Results are presented in Table 4.7.

Table 4.7. Study of the digestion method variation for arsenic, cadmium, chromium, lead, manganese and nickel quantification: Each mean value is the result of 5 measurements (n=5).

Metals	As	Cd	Cr	Pb	Mn	Ni
Mean (µg/L)	9,808	1,837	8,186	3,921	158,838	54,389
RSD (%)	4,526	2,503	3,602	25,764	1,546	13,841

Analyzing the data presented above, it is possible to say As, Cd, Cr and Mn quantification did not suffered from possible interferences caused by the vessels used during the microwave digestion method, since RSD (%) for the referred metals is lower than 5. However, Pb and Ni quantification might have been influenced by interferences caused by the microwave vessels since their RSD (%) is higher than 5.

4.1.2. Direct evaluation

Direct evaluation aims to know assay methods accuracy, which was evaluated through certified reference materials purchased from a recognized and reliable provider entity (Environment Canada in this study).

In Table 4.8., which is presented hereafter, CRM analysis and its evaluation is presented for each metal.

Table 4.8. Certified reference material analysis for arsenic, cadmium, chromium, lead, manganese and nickel: TM-24.3 and TM-26.3 were the CRMs used.

Metals	Certified value (µg/L)	Experimental value (µg/L)	Z-score
As	5,21 ± 0,53 (TM-24.3)	5,79	1,09
Cd	3,97 ± 0,37 (TM-24.3)	4,22	0,68
Cr	12,3 ± 1,3 (TM-26.3)	13,15	0,65
Pb	5,82 ± 0,45 (TM-24.3)	6,48	1,47
Mn	17 ± 1,4 (TM-26.3)	14,26	1,95
Ni	10,2 ± 1,3 (TM-26.3)	14,45	3,27

Analyzing table 4.8., it can be assumed that the observed values experimentally obtained are within the range of expected values since Z-score is lower than 2, which is considered to be satisfactory, except for nickel that presents a Z-score somewhat higher than 3.

4.2. Metal quantification in fruit juices

After optimization of the conditions for sample digestion and metal quantification, all samples were analyzed by GFAAS and HGAAS, according to the type of metal, as it was explained in sections 1.2.2.

In Table 4.9. is presented metal average concentrations (µg/L) for each analyzed sample, which is the result of 2 readings of each duplicate. Whenever a value was below quantification limit, it is indicated as < Q.L.

Table 4.9. Metal quantification (µg/L) in fruit juice samples (n=21): Values are mean (n=2). < Q.L. - below the quantification limit calculated through general mode. Values in bold are above the limit set out by Decree-Law 306/2007 from 27th August of the Portuguese Legislation.

Samples	Metals (µg/L)					
	As	Cd	Cr	Pb	Ni	Mn
A	**10,727**	1,772	12,859	4,109	**52,431**	**833,000**
B	9,487	1,783	7,927	4,316	**46,520**	**158,626**
C	5,485	0,942	5,701	< Q.L.	17,542	**249,473**
D	4,519	1,141	13,260	8,510	**34,316**	**296,903**
E	**11,210**	< Q.L.	15,673	5,691	**44,023**	**3502,731**
F	6,749	3,442	14,682	< Q.L.	**42,208**	**137,657**
G	5,650	< Q.L.	6,818	< Q.L.	7,581	**54,850**
H	8,779	< Q.L.	9,126	6,106	**22,815**	**122,885**
I	9,822	< Q.L.	6,732	0,742	11,923	**442,690**
J	2,757	< Q.L.	5,831	0,470	9,242	**125,162**
K	3,710	< Q.L.	8,094	3,591	**30,166**	**93,182**
L	5,616	1,121	7,263	5,221	**46,753**	**644,539**
M	**13,380**	1,415	5,578	7,051	**48,493**	**337,662**
N	< Q.L.	0,949	10,387	0,963	**37,113**	**210,664**
O	2,355	0,721	26,520	6,210	**43,956**	**142,724**
P	< Q.L.	< Q.L.	6,787	< Q.L.	**20,992**	**110,323**
Q	< Q.L.	< Q.L.	8,054	< Q.L.	15,435	**153,717**
R	6,749	< Q.L.	8,690	2,292	14,724	**440,093**
S	**10,236**	< Q.L.	6,956	2,494	18,763	**342,922**
T	9,156	< Q.L.	6,627	3,726	15,501	**391,009**
U	8,036	< Q.L.	10,546	< Q.L.	**31,104**	**1949,382**

According to the quantification limits calculated through general case, and as it is possible to see in Table 4.9., all samples were quantifiable for chromium, nickel and manganese. Arsenic content was able to be quantified in eighteen samples, while cadmium content was possible to be quantified in nine samples and lead content in fifteen samples.

Since the analyzed samples are commercialized in the Portuguese market, results were evaluated having in mind the established values for drinking-water set out by Decree-Law 306/2007 from 27th August of the Portuguese Legislation. Besides arsenic concentration is above maximum limit (10 µg/L) in four samples (A, E, M and S), there are some samples (B, H, I and T), which content is very close to maximum level. Nickel content is higher than the maximum limit (20 µg/L) in thirteen samples (A, B, D, E, F, H, K, L, M, N, O, P and U). Manganese concentration is beyond the maximum limit (50 µg/L) in all analyzed samples. Cadmium, chromium and lead content are below the maximum values (5 µg/L, 50 µg/L and 10 µg/L, respectively) in all the analyzed samples. However, when considering the maximum levels specified by WHO, cadmium concentration in sample F is higher than the maximum value (3 µg/L), and manganese concentration is above the maximum limit (400 µg/L) only in six samples (A, E, I, L, R and U). Moreover, nickel content is below the maximum limit specified by WHO (70 µg/L) and USEPA (100 µg/L) in all the analyzed metals.

Samples E and M (both multifruit juice) present the highest levels for some of the elements. In fact, sample M presented the highest arsenic level and the second highest lead and nickel values. In turn, sample E presented the highest manganese level and the second highest arsenic level. However, sample L, which is also a multifruit juice, does not present metal levels as high as samples E and M, except for nickel, being the third sample with the highest level of this element.

For a better visualization, in the following graphics (from Figure 4.7. to Figure 4.12), it is possible to observe metals quantification as well as the corresponding maximum limits. Whenever samples revealed a metal concentration below quantification limit calculated through general case, no value is presented.

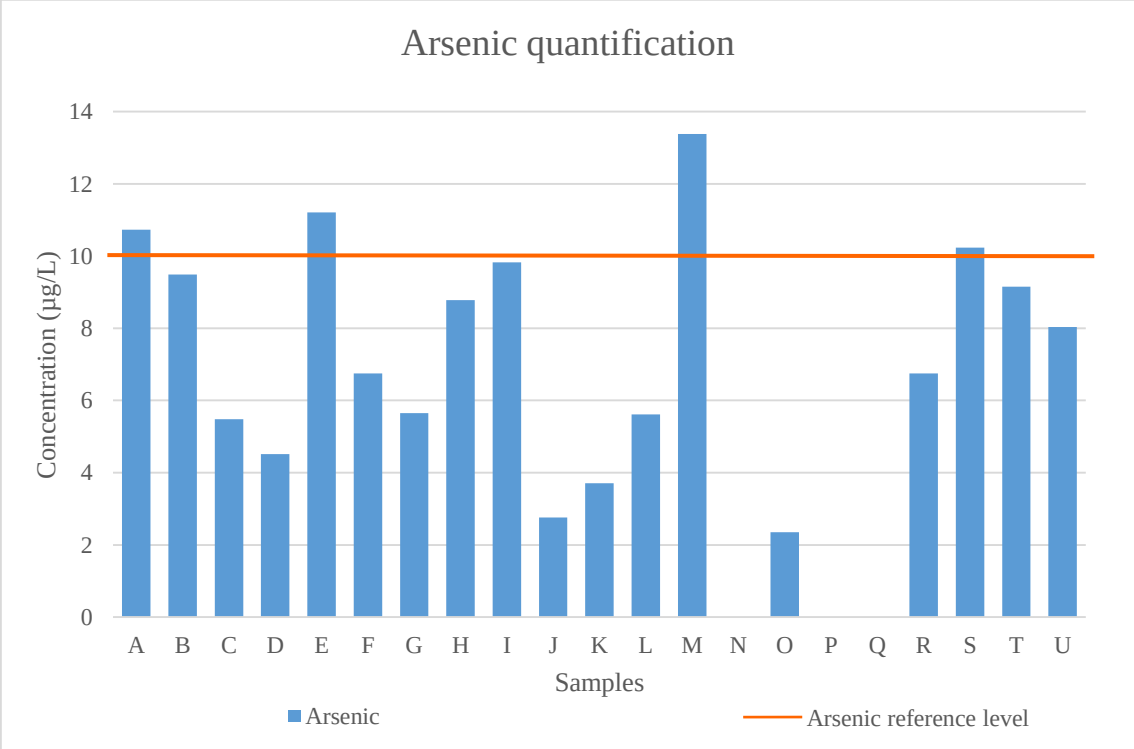

Figure 4.7. Arsenic quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. Samples N, P and Q are below QL. Samples A, E, M and S are above maximum level (ML). As ML is 10 µg/L, established by WHO, USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation.

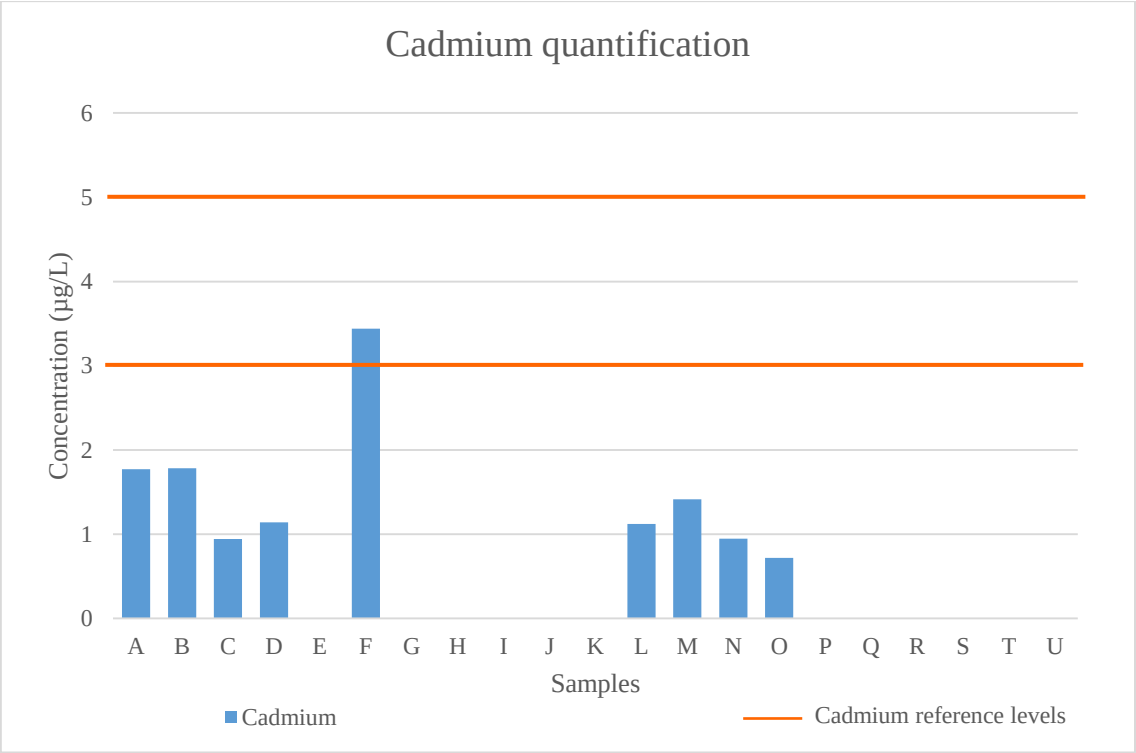

Figure 4.8. Cadmium quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. Samples E, G, H, I, J, K, P, Q, R, S, T and U are below QL. Sample F is above ML. Cd ML is 3 µg/L, established by WHO, and 5 µg/L, established by USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation.

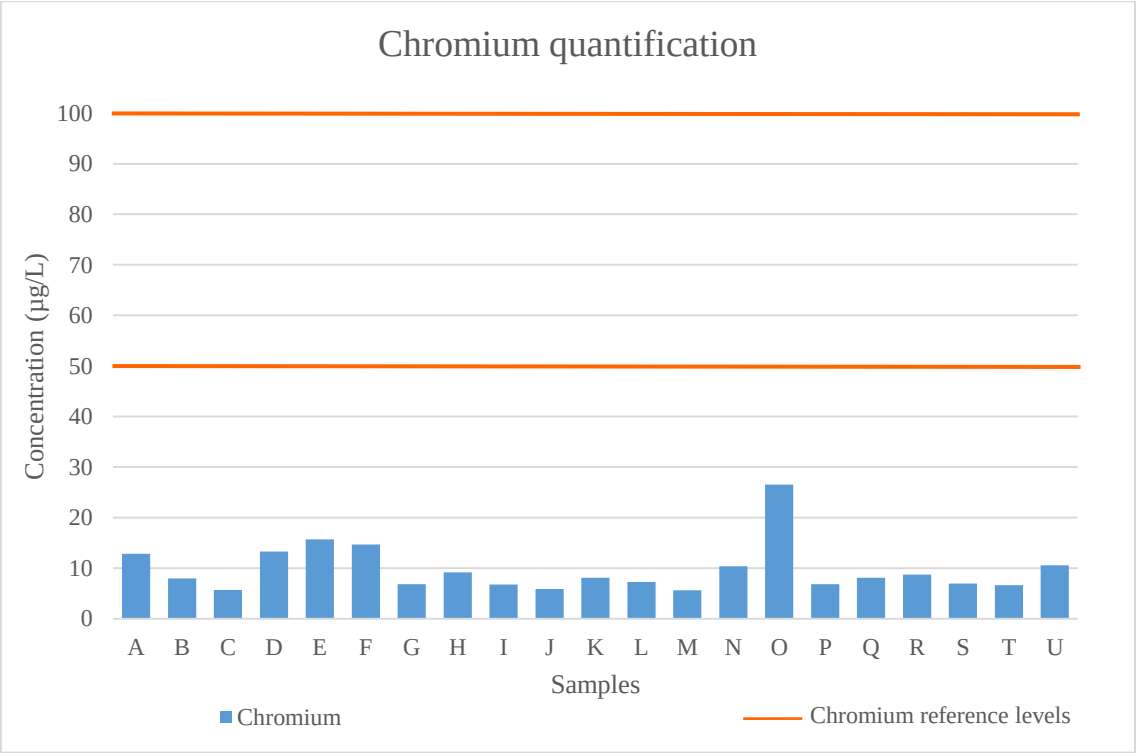

Figure 4.9. Chromium quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. Samples E, G, H, I, J, K, P, Q, R, S, T and U are below QL. Cr ML is 50 µg/L, established by WHO and Decree-Law 306/2007 from 27th August of the Portuguese Legislation, and 100 µg/L, established by USEPA.

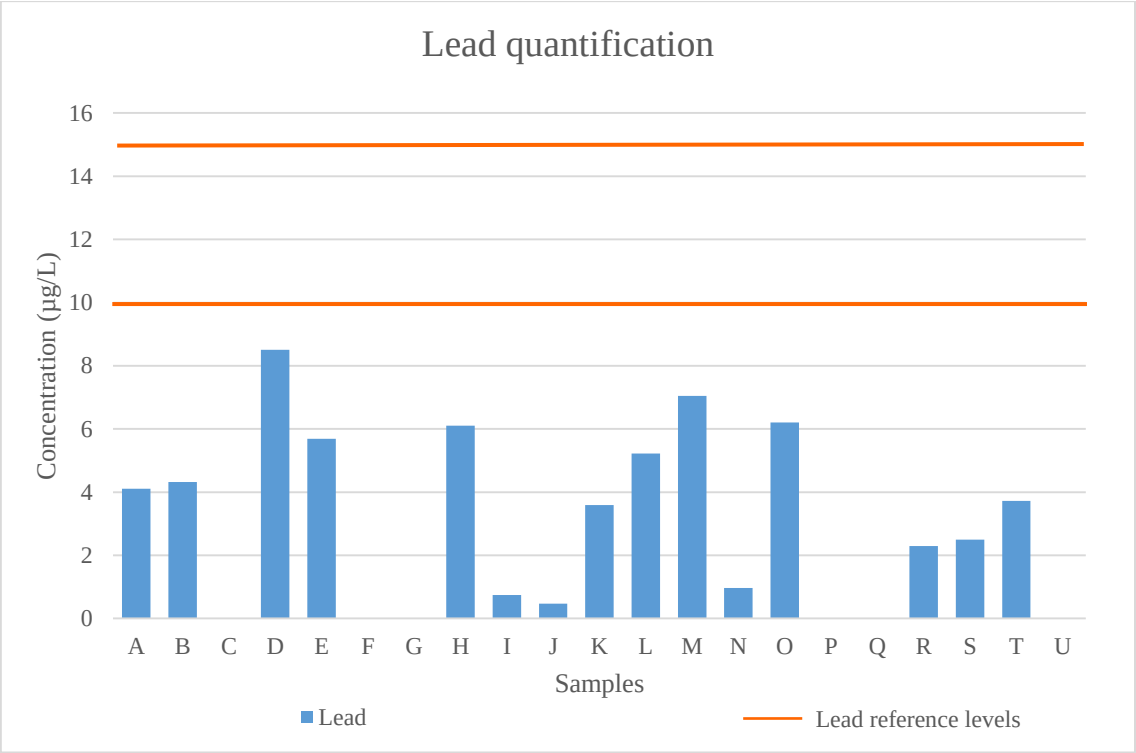

Figure 4.10. Lead quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. Samples C, F, G, P, Q and U are below QL. Pb ML level is 10 µg/L, established by WHO and Decree-Law 306/2007 from 27th August of the Portuguese Legislation, and 15 µg/L, established by USEPA.

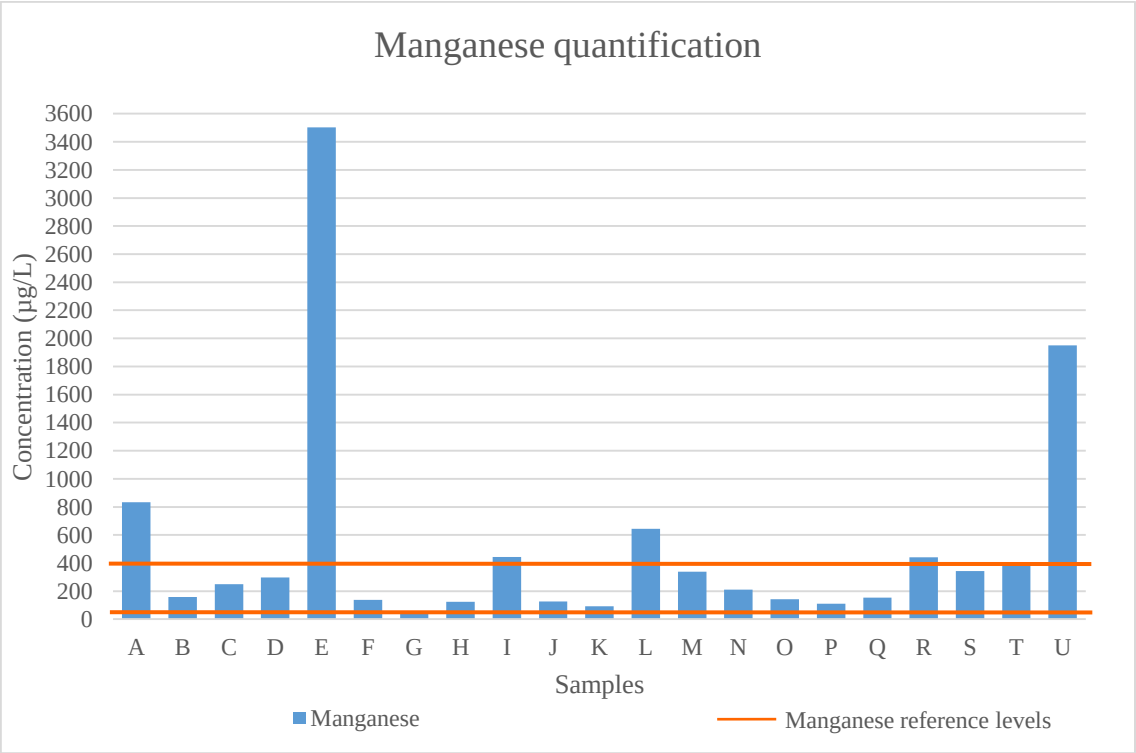

Figure 4.11. Manganese quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. All samples are above ML. Mn ML is 400 µg/L, established by WHO, and 50 µg/L, established by USEPA and Decree-Law 306/2007 from 27th August of the Portuguese Legislation.

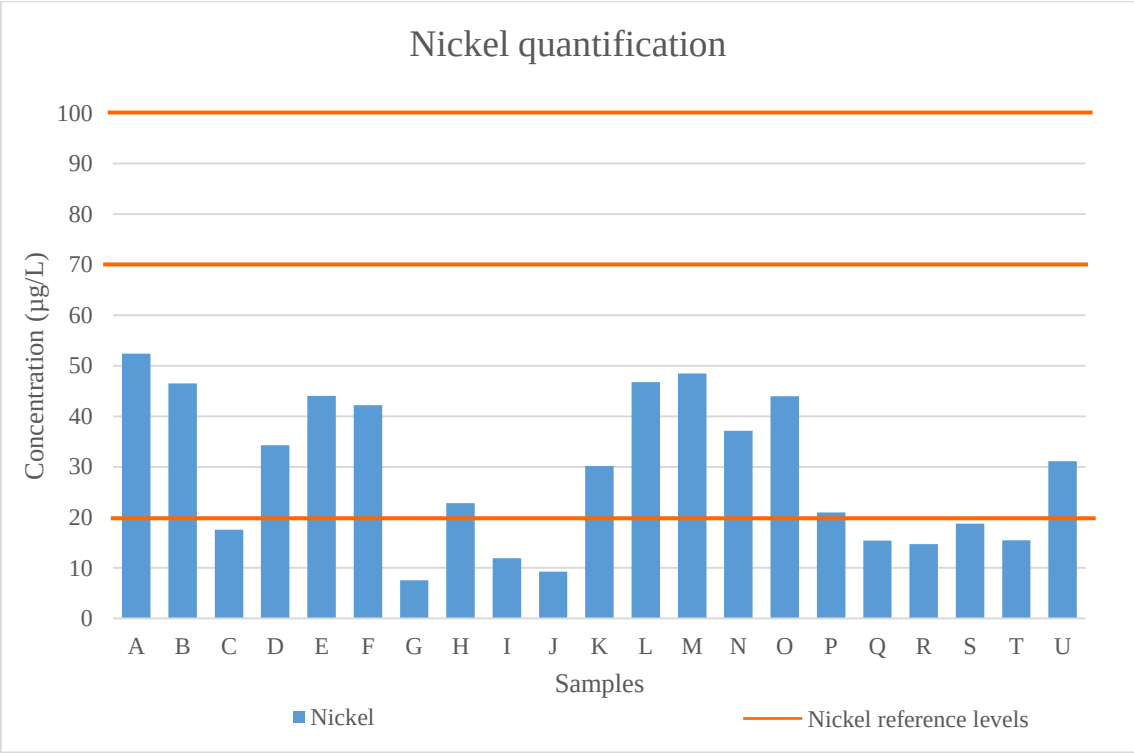

Figure 4.12. Nickel quantification in fruit juice samples (n=21): Values are mean (n=2). Concentrations are presented in µg/L. Samples A, B, D, E, F, H, K, L, M, N, O, P and U are above ML. Ni ML is 70 µg/L established by WHO, 100 µg/L established by USEPA and 20 µg/L established by Decree-Law 306/2007 from 27th August of the Portuguese Legislation.

4.3. Comparison study between obtained and published metal values

The variation range of each type of fruit juice for every metal analyzed in this study was compared with values reported in similar studies in the available literature. Results are present in the following tables (Table 4.10. to Table 4.19.).

Table 4.10. Metal quantification range (µg/L) in pear juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Pear juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	< Q.L. - 9,487	< Q.L. - 1,783	6,787 - 7,927	< Q.L. - 4,316	110,323 - 158,626	20,992 - 46,520
Sardinha et al. 2014	2	< 2	15	< 4	376	N.A.
Dehelean and Magdas 2013	< 0,001	< 0,001	7,56	1,62	169,56	31,52
Tormen et al. 2011	N.A.	0.3 ± 0.1 - 0.4 ± 0.1	N.A.	0.7 ± 0.1	140 ± 6 - 330 ± 5	15.3 ± 0.6 - 27.0 ± 0.3

Table 4.11. Metal quantification range (µg/L) in passion fruit juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Passion fruit juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	6,749	3,442	14,682	< Q.L.	137,657	42,208
Sardinha et al. 2014	< 2	< 2	N.A.	< 4	170	N.A.
Bragança et al. 2012	N.A.	< 10 ± 0,3 - < 10 ± 1	< 5 ± 0.1 - < 5 ± 0.2	< 10 ± 0.2 - < 10 ± 0.6	20 ± 0.1 – 70 ± 0.3	N.A.
Tormen et al. 2011	N.A.	< 0,07 - 0.5 ± 0.1	N.A.	0.5 ± 0.1 - 1.6 ± 0.1	130 ± 1 - 290 ± 9	12.4 ± 0.2 - 21.8 ± 0.5

Table 4.12. Metal quantification range (µg/L) in plum juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Plum juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	5,485	< Q.L.	5,701	< Q.L.	249,473	17,542
Ashraf et al. 2000	868	9	77	264	140	68

Table 4.13. Metal quantification range (µg/L) in apricot juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Apricot juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	< Q.L.	< Q.L.	26,52	6,21	142,724	43,956
Kıhç et al. 2015	2.2 ± 0.6 - 31 ± 8.1	< 0.05 - 0.8 ± 0.1	N.A.	3.9 ± 0.6 - 42 ± 6.9	N.A.	N.A.
Dehelean and Magdas 2013	1,52	0,46 - 0,78	26,98 - 55,45	3,36 - 5,36	206,98-244,46	35,04-146,60
Harmankaya et al. 2012	N.A.	6 ± 0,8 - 9,2 ± 0,8	N.A.	N.A.	128 ± 4 - 311 ± 3	72,5 ± 6,9 - 136 ± 7,2

Table 4.14. Metal quantification range (µg/L) in mango juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Mango juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	9,156 - 9,822	< Q.L.	6,627 - 6,732	< Q.L.	391,009 - 442,690	11,923 - 15,501
Bragança et al. 2012	N.A.	<10 ±0,3 - <10±0,5	<5 ± 0.1 – 5 ± 0.1	<10 ± 0.2 - <10 ± 0.3	80 ± 0.2 – 190 ± 0.7	N.A.
Tormen et al. 2011	N.A.	0.3 ± 0.1	N.A.	0.3 ± 0.2	140 ± 8	15.6 ± 0.7
Farid and Enani 2010	N.A.	N.A.	7.64 ± 1.02	N.A.	21.85 ± 2.58	5.93 ± 0.96
Chukwujindu et al. 2008	N.A.	380 - 600	<1 - 2	2 - 6	2 - 10	850-1150
Ashraf et al. 2000	1113 - 1399	9 - 13	27 - 95	65 -338	159 - 220	103 - 137

Table 4.15. Metal quantification range (µg/L) in peach juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Peach juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	< Q.L. - 4,519	< Q.L. - 1,141	8,094 - 13,260	< Q.L. - 8,510	93,182 - 296,903	30,166 - 37,113
Kıhç et al. 2015	0.7 ± 0.2 - 20 ± 5.2	0.6 ± 0.06 - 1.1 ± 0.1	N.A.	4.0 ± 0.7 - 10 ± 1.6	N.A.	N.A.
Sardinha et al. 2014	< 2	< 2	17	< 4	206	N.A.
Dehelean and Magdas 2013	< 0,001 - 3,78	0,52 - 1,38	5,61 - 39,64	1,94 - 18,58	59,60 - 229,80	25,42 - 53,12
Bragança et al. 2012	N.A.	< 10 ± 0,2 - < 10 ± 0,9	< 5 ± 0.1 – 6 ± 0.1	< 10 ± 0.1 - < 10 ± 0.4	10 ± 0.5 – 80 ± 0.2	N.A.
Harmankaya et al. 2012	N.A.	7,2 ± 0,3 - 11,3 ± 1,6	N.A.	N.A.	197 ± 13 - 349 ± 16	86,9 ± 10 - 174,6 ± 22,2
Tormen et al. 2011	N.A.	0.6 ± 0.1	N.A.	0.6 ± 0.1	210 ± 5	21.4 ± 0.8

Table 4.16. Metal quantification range (µg/L) in pineapple juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Pineapple juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	8,036	< Q.L.	10,546	< Q.L.	1949,382	31,104
Sardinha et al. 2014	< 2	< 2	40	< 4	13800	N.A.
Dehelean and Magdas 2013	2,84	0,64	27.05	0.64	320.8	208.96
Tormen et al. 2011	N.A.	1.1 ± 0.1	N.A.	1.6 ± 0.1	1360 ± 70	43.5 ± 2.0
Williams et al. 2009	N.A.	N.D.	N.D.	90	15000	N.A.
Chukwujindu et al. 2008	N.A.	< 1 - 2	430 - 1370	500 - 660	400 - 410	840 - 1030

Table 4.17. Metal quantification range (µg/L) in orange juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Oranje juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	< Q.L. - 10,236	< Q.L.	5,831 - 8,054	< Q.L. - 2,494	54,850 - 342,922	7,581 - 18,763
Kıhç et al. 2015	1.5±0.4 - 21±5.5	0.1±0.01 - 1.2±0.1	N.A.	3.7±0.6 - 10±1.6	N.A.	N.A.
Sardinha et al. 2014	< 2 - 3,5	< 2	10 - 15	< 4	197 - 379	N.A.
Dehelean and Magdas 2013	< 0,001 - 1,70	< 0,001 - 0,64	5,28-28,21	1,02-10,05	64,14-307,88	31,24-145
Harmankaya et al. 2012	N.A.	6,4 ± 0,1 - 9,2 ± 1,1	N.A.	N.A.	100 ± 5 - 466 ± 23	66,6 ± 6,5 - 34,2 ± 4,9
Tormen et al. 2011	N.A.	< 0,07	N.A.	2.0±0.1	180±5	20.5± 0.1
Farid and Enani 2010	N.A.	N.A.	5.93 ± 0.92	N.A.	20.93 ± 2.36	5.73 ± 0.91
Williams et al. 2009	N.A.	N.D.	N.D.	80	450	N.A.
Chukwujindu et al. 2008	N.A.	10 - 70	<1-20	520-1320	<1-1650	40-1020
Krejpcio et al. 2005	N.A.	4 - 40	N.A.	46-251	N.A.	N.A.
Ashraf et al. 2000	146	170	7	119	198	276

Table 4.18. Metal quantification range (µg/L) in apple juice samples in present study compared with published values: < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Apple juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	8,779	< Q.L.	9,126	6,106	122,885	22,815
Kıhç et al. 2015	< 0,08 - 36 ± 9.4	0.01 ± 0.001 - 0.6±0.06	N.A.	1.1 ± 0.2 - 12 ± 2.0	N.A.	N.A.
Sardinha et al. 2014	2,8	< 2	7,5	< 4	289	N.A.
Tvermoes et al. 2014	2,1 – 8,5	0,15 – 0,70	5,2 - 18	0.84 – 9,1	200 - 1200	N.A.
Dehelean and Magdas 2013	< 0,001 - 4,36	0,14-1,42	4 – 55,6	4,66-75,68	127,08-342,92	18,96-204,4
Tormen et al. 2011	N.A.	<0,07	N.A.	0.2 ± 0.1	75 ± 2	3.9 ± 0.3
Farid and Enani 2010	N.A.	N.A.	6.36 ± 0.94	N.A.	23.48 ± 2.23	6.21 ± 0.90
Williams et al. 2009	N.A.	N.D.	N.D.	80	530	N.A.
Chukwujindu et al. 2008	N.A.	<1 - 30	<1 - 840	100 - 3720	<1 -1 0	<1 - 710
Krejpcio et al. 2005	N.A.	10 - 60	N.A.	51-460	N.A.	N.A.
Ashraf et al. 2000	238 - 2920	10 - 14	10 - 45	101 - 376	90-168	154-204

Table 4.19. Metal quantification range (µg/L) in multifruit juice samples in present study compared with published values : < Q.L. – below quantification limit; N.A. - not analyzed in the referred study.

Multifruit juice analysis	Metals (µg/L)					
	As	Cd	Cr	Pb	Mn	Ni
Present study	5,616 - 13,380	< Q.L. – 1,415	5,578 - 15,673	5,221 - 7,051	337,662 - 3502,731	44,023 - 48,493
Kıhç et al. 2015	4.7 ± 1.2 - 16 ± 4.2	0.1 ± 0.01 - 1.0 ± 0.1	N.A.	6.1 ± 1.0 - 14 ± 2.3	N.A.	N.A.
Sardinha et al. 2014	2,3	< 2	30	< 4	439	N.A.
Dehelean and Magdas 2013	0,38	< 0,001	4,46	1,88	131,64	32,2
Chukwujindu et al. 2008	N.A.	<1 - 430	<1 - 1320	390 - 1680	<1 - 1020	<1 - 1370
Ashraf et al. 2000	787 - 2342	10 - 205	12 - 159	163 - 2039	103 - 222	53 - 368

Soil conditions, pesticides, additives, water, processing and storage steps are potential sources of metal contamination. Although most of the obtained values are within the ranges observed in other studies, the wide variation range of reported data in literature could be explained both by the variability of used raw materials in the fruit juices production and by different manufacturing processes applied. Because studies, and consequently fruit juices, are from different countries and with a great time gap between them, the metal content reflects differences in soil composition where the fruits were grown as well as atmospheric conditions and agricultural practices during its growing time. When elements are less mobile in the soil-plant system, such as lead, sources of contamination in fruit juices are most probably originated from processing steps, such as sugar addition or fruit juice reconstruction with water. In addition, packing quality is another factor that influences metal content in fruit juices. The analyzed samples in this study were all contained in the same type of packaging, which is not the case for the other studies referred (Garcia et al. 1999; Harmankaya et al. 2012; Dehelean and Magdas 2013; Kıhç et al. 2015).

High levels of manganese are present in most of the fruit juices analyzed in all studies reported. Levels of manganese seem to be particularly influenced by acidic soil conditions in which the fruits were produced. However, further investigation is needed to properly correlate the concentration of this metal with the soil characteristics of the many growing locations (Bragança et al. 2012). Pineapple juice seems to concentrate manganese to a greater extent than do other types of fruit juices, as it presents high levels of this metal in the majority of the reported studies (Beattie and Quoc 2000).

As it can be observed in the previously presented tables, orange and apple juices are the most analyzed type of fruit juices, possibly because they are the most favorite ones in the majority of the countries and, as a result, the most consumed worldwide. On the other hand, fruit juices like plum, red fruits and strawberry juices are not included in many analysis. Indeed, it was not possible to find similar studies analyzing red fruits juice or strawberry juice. Consequently, there are no data available to compare with the results obtained in this study.

According to European Fruit Juice Association (AIJN), who has done a country profile of fruit juice and nectars by flavors for some European countries, orange juice is the most consumed fruit juice in Portugal (17,4%), followed by peach juice (16,8%), flavor mixes juices, such as multifruit or red fruit juice (15,9%), mango juice (15%), apple juice (10,1%). Other type of fruit juices account for 24,8% of the total consumption (AIJN 2016).

Metal releases and discharges from industry and other anthropogenic activities such as mining should be minimized in order to avoid environmental contamination. In addition, action is needed to reduce the concentration of heavy metals from drinking-water, since this is possibly one of the main sources of metal contamination in fruit juices, in areas with naturally high levels in the groundwater. Screening of drinking-water supplies for metal levels, and informing both the general public and the health sector of the results is essential, as well as making awareness-raising campaigns about the harmful effects, and the early signs of metal poisoning, of high metal intake and how to avoid it (WHO 2010a; WHO 2010b).

Possible mitigation measures to make available drinking-water proper for human intake include the use of alternative groundwater sources, the use of microbiologically safe surface water (e.g. rainwater harvesting) and the installation of metal removal systems, either centralized or domestic, ensuring appropriate disposal of the removed metals (WHO 2010a;WHO 2010b). Lead is a different case as most of its content in drinking-water arises from plumbing in buildings. Thus, solutions to be implemented consist mainly on removing plumbing and fittings containing lead as well as corrosion control. In recent decades, especially in developed countries, blood lead levels have shown substantial reductions since

programs such as those that have eliminated the use of leaded petrol and discontinued the use of lead solder in food cans have been carried out (WHO 2010a; WHO 2011c).

Although arsenic, cadmium, nickel and manganese exceed the maximum limit values set out either by WHO, USEPA or Decree-Law 306/2007 from 27th August of the Portuguese Legislation for drinking-water in some of the analyzed fruit juice samples in this study, there are at least one of the reviewed studies in which levels are above the maximum settled values for every element quantified in this study. Besides the known toxicity associated with these metals, it is important to note that most drinking-water standards are set as a fraction of the tolerable or acceptable daily intake value for a given contaminant divided by the daily drinking-water consumption rate. Therefore, this comparison can be considered as a conservative approach since the amount of juice consumed per day is expected to be considerably less than the amount of water ingested per day. As such, consuming fruit juices that exceed the maximum values does not necessarily imply an increased risk for human health (Tvermoes et al. 2014).

5. Conclusions

In this study, trace metal analysis was conducted on twenty-one fruit juice samples, selected by ASAE, from four different brands available on the Portuguese market. Arsenic, cadmium, chromium, lead, nickel and manganese were the elements selected since their quantification have recognized importance and interest both from toxicological and nutritional points of view.

Regarding sample treatment, conducted in a closed vessel microwave digester with infrared controlled pressure, optimization of an adequate sample preparation method was performed since it is an important step to ensure the veracity of element analysis.

Metal quantification was performed by atomic absorption spectrometry, where different methods were validated and used according to the metal to be analyzed. Parameters, such as linearity, working range, analytical thresholds, precision (repeatability, intermediate precision), accuracy and specificity/selectivity were studied for each method, and good confidence levels were obtained. To ascertain the repeatability of microwave digestion method, aliquots of the same fruit juice package were analyzed at equal conditions and a good confidence level was also obtained.

Results of metal quantification showed high levels of arsenic, nickel and manganese in some of the analyzed samples. Comparing our results with the maximum level (ML) for drinking-water established by Decree-Law 306/2007 from 27th August of the Portuguese Legislation, arsenic concentration is above maximum level (10 µg/L) in four samples (A, E, M and S); Ni content is higher than the maximum limit (20 µg/L) in thirteen samples (A, B, D, E, F, H, K, L, M, N, O, P and U) and Mn concentration is beyond the maximum level (50 µg/L) in all analyzed samples. On the other hand, concentrations of all other metals (cadmium, chromium and lead) were below the maximum levels (5 µg/L, 50 µg/L and 10 µg/L, respectively) established by the Portuguese Legislation in all samples, not representing concerns about them.

After comparing the obtained metal values in the examined fruit juices with results published in similar studies, it is possible to affirm most of the obtained values are within the ranges observed in the analyzed studies; however, there is at least one of the reviewed study in which the levels are above the maximum settled values for every element quantified in this study. There are several aspects that influence the presence of trace metals in fruit juices, such as soil conditions, agricultural practices, as well as processing and storage steps. Therefore, the wide variation range of metal concentrations reported in the literature is reasonable since studies, and consequently fruit juices, are from different countries and with a great time gap between them.

In order to avoid environmental contamination with heavy metals, which is recognized as a public health hazard worldwide, it is suggested a minimization of metal releases and discharges from anthropogenic activities aiming the decrease of metal amount in drinking-water, since this is one of the possible major sources of contamination in fruit juices.

Besides the known toxicity associated with trace metals, it is important to refer that the amount of fruit juice consumed per day is expected to be lower than the amount of water ingested per day. As metal concentrations in fruit juices were evaluated having in mind the maximum limits established for drinking-water, consuming fruit juices that exceed these values does not necessarily imply an increased risk for human health. Nevertheless, this type of studies is very important since it enables to quantify the dietary intakes of metals present in food, not only guaranteeing food safety but also allowing to understand if fruit juices can be part of a balanced diet due to its nutritional importance.

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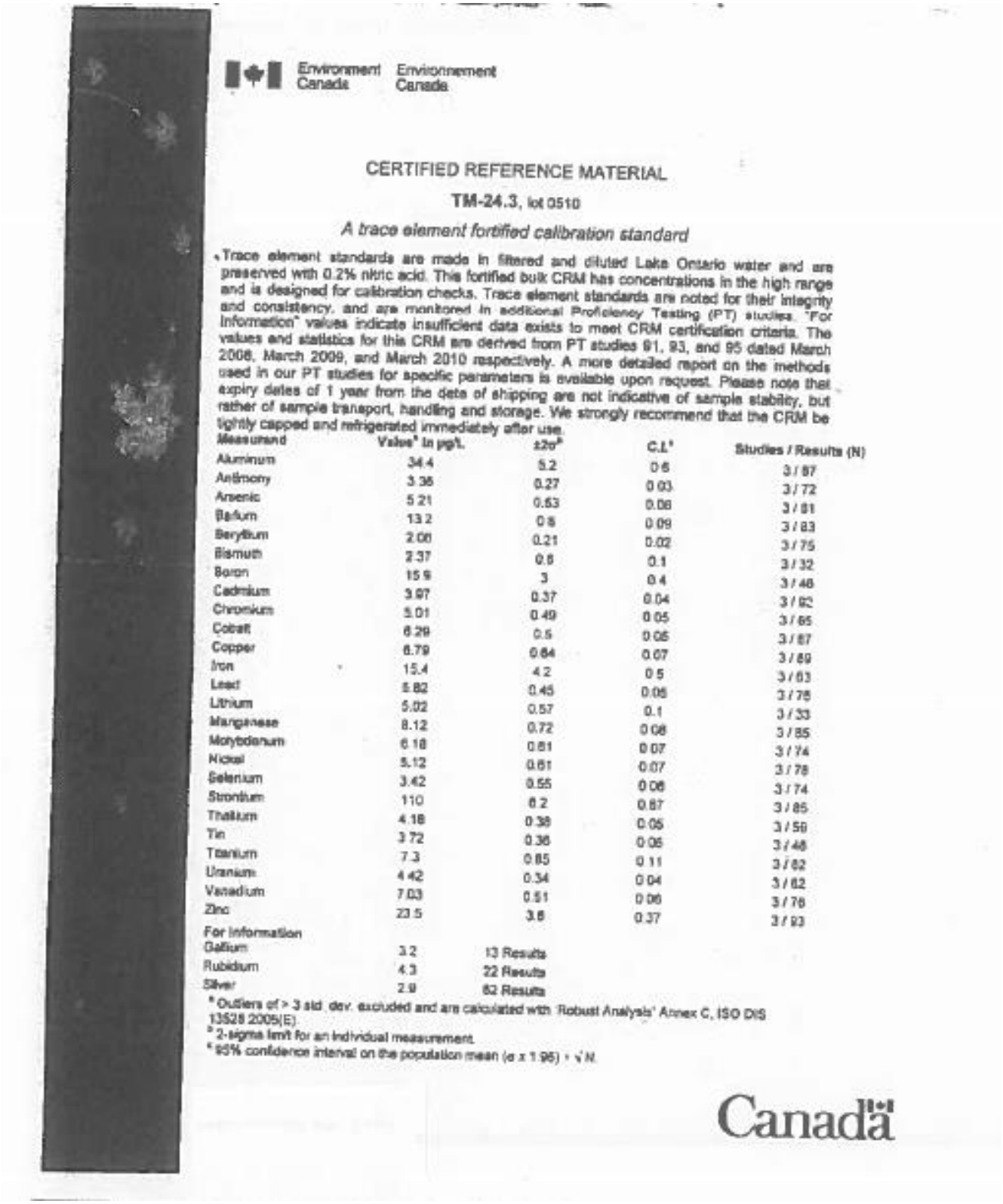
7. Annexes

Table 7.1. Fisher-Snedecor distribution with a $\alpha = 0.01$

Denominator Degrees of Freedom (df_2) \ Numerator Degrees of Freedom (df_1)	1	2	3	4	5	6	7	8	9
1	4052.2	4999.5	5403.4	5624.6	5763.6	5859.0	5928.4	5981.1	6022.5
2	98.503	99.000	99.166	99.249	99.299	99.333	99.356	99.374	99.388
3	34.116	30.817	29.457	28.710	28.237	27.911	27.672	27.489	27.345
4	21.198	18.000	16.694	15.977	15.522	15.207	14.976	14.799	14.659
5	16.258	13.274	12.060	11.392	10.967	10.672	10.456	10.289	10.158
6	13.745	10.925	9.7795	9.1483	8.7459	8.4661	8.2600	8.1017	7.9761
7	12.246	9.5466	8.4513	7.8466	7.4604	7.1914	6.9928	6.8400	6.7188
8	11.259	8.6491	7.5910	7.0061	6.6318	6.3707	6.1776	6.0289	5.9106
9	10.561	8.0215	6.9919	6.4221	6.0569	5.8018	5.6129	5.4671	5.3511
10	10.044	7.5594	6.5523	5.9943	5.6363	5.3858	5.2001	5.0567	4.9424
11	9.6460	7.2057	6.2167	5.6683	5.3160	5.0692	4.8861	4.7445	4.6315
12	9.3302	6.9266	5.9525	5.4120	5.0643	4.8206	4.6395	4.4994	4.3875
13	9.0738	6.7010	5.7394	5.2053	4.8616	4.6204	4.4410	4.3021	4.1911
14	8.8616	6.5149	5.5639	5.0354	4.6950	4.4558	4.2779	4.1399	4.0297
15	8.6831	6.3589	5.4170	4.8932	4.5556	4.3183	4.1415	4.0045	3.8948
16	8.5310	6.2262	5.2922	4.7726	4.4374	4.2016	4.0259	3.8896	3.7804
17	8.3997	6.1121	5.1850	4.6690	4.3359	4.1015	3.9267	3.7910	3.6822
18	8.2854	6.0129	5.0919	4.5790	4.2479	4.0146	3.8406	3.7054	3.5971
19	8.1849	5.9259	5.0103	4.5003	4.1708	3.9386	3.7653	3.6305	3.5225
20	8.0960	5.8489	4.9382	4.4307	4.1027	3.8714	3.6987	3.5644	3.4567
21	8.0166	5.7804	4.8740	4.3688	4.0421	3.8117	3.6396	3.5056	3.3981
22	7.9454	5.7190	4.8166	4.3134	3.9880	3.7583	3.5867	3.4530	3.3458
23	7.8811	5.6637	4.7649	4.2636	3.9392	3.7102	3.5390	3.4057	3.2986
24	7.8229	5.6136	4.7181	4.2184	3.8951	3.6667	3.4959	3.3629	3.2560
25	7.7698	5.5680	4.6755	4.1774	3.8550	3.6272	3.4568	3.3239	3.2172
26	7.7213	5.5263	4.6366	4.1400	3.8183	3.5911	3.4210	3.2884	3.1818
27	7.6767	5.4881	4.6009	4.1056	3.7848	3.5580	3.3882	3.2558	3.1494
28	7.6356	5.4529	4.5681	4.0740	3.7539	3.5276	3.3581	3.2259	3.1195
29	7.5977	5.4204	4.5378	4.0449	3.7254	3.4995	3.3303	3.1982	3.0920
30	7.5625	5.3903	4.5097	4.0179	3.6990	3.4735	3.3045	3.1726	3.0665
40	7.3141	5.1785	4.3126	3.8283	3.5138	3.2910	3.1238	2.9930	2.8876
60	7.0771	4.9774	4.1259	3.6490	3.3389	3.1187	2.9530	2.8233	2.7185
120	6.8509	4.7865	3.9491	3.4795	3.1735	2.9559	2.7918	2.6629	2.5586
∞	6.6349	4.6052	3.7816	3.3192	3.0173	2.8020	2.6393	2.5113	2.4073



Environment Canada Environnement Canada

CERTIFIED REFERENCE MATERIAL

TM-24.3, lot 0510

A trace element fortified calibration standard

Trace element standards are made in filtered and diluted Lake Ontario water and are preserved with 0.2% nitric acid. This fortified bulk CRM has concentrations in the high range and is designed for calibration checks. Trace element standards are noted for their integrity and consistency, and are monitored in additional Proficiency Testing (PT) studies. "For Information" values indicate insufficient data exists to meet CRM certification criteria. The values and statistics for this CRM are derived from PT studies 91, 93, and 95 dated March 2008, March 2009, and March 2010 respectively. A more detailed report on the methods used in our PT studies for specific parameters is available upon request. Please note that expiry dates of 1 year from the date of shipping are not indicative of sample stability, but rather of sample transport, handling and storage. We strongly recommend that the CRM be tightly capped and refrigerated immediately after use.

Measurand	Value[a] in µg/L	±2σ[b]	C.L.[c]	Studies / Results (N)
Aluminum	34.4	5.2	0.6	3 / 87
Antimony	3.36	0.27	0.03	3 / 72
Arsenic	5.21	0.53	0.06	3 / 81
Barium	13.2	0.8	0.09	3 / 83
Beryllium	2.06	0.21	0.02	3 / 75
Bismuth	2.37	0.8	0.1	3 / 32
Boron	15.9	3	0.4	3 / 48
Cadmium	3.97	0.37	0.04	3 / 82
Chromium	5.01	0.49	0.05	3 / 85
Cobalt	6.29	0.5	0.05	3 / 87
Copper	6.79	0.64	0.07	3 / 89
Iron	15.4	4.2	0.5	3 / 83
Lead	5.82	0.45	0.05	3 / 78
Lithium	5.02	0.57	0.1	3 / 33
Manganese	8.12	0.72	0.08	3 / 85
Molybdenum	6.18	0.61	0.07	3 / 74
Nickel	5.12	0.61	0.07	3 / 78
Selenium	3.42	0.55	0.06	3 / 74
Strontium	110	6.2	0.67	3 / 85
Thallium	4.18	0.38	0.05	3 / 59
Tin	3.72	0.36	0.05	3 / 48
Titanium	7.3	0.85	0.11	3 / 82
Uranium	4.42	0.34	0.04	3 / 82
Vanadium	7.03	0.51	0.06	3 / 76
Zinc	23.5	3.6	0.37	3 / 93
For Information				
Gallium	3.2	13 Results		
Rubidium	4.3	22 Results		
Silver	2.9	62 Results		

[a] Outliers of > 3 std. dev. excluded and are calculated with 'Robust Analysis' Annex C, ISO DIS 13528:2005(E).
[b] 2-sigma limit for an individual measurement.
[c] 95% confidence interval on the population mean ($\sigma \times 1.96$) ÷ $\sqrt{N}$.

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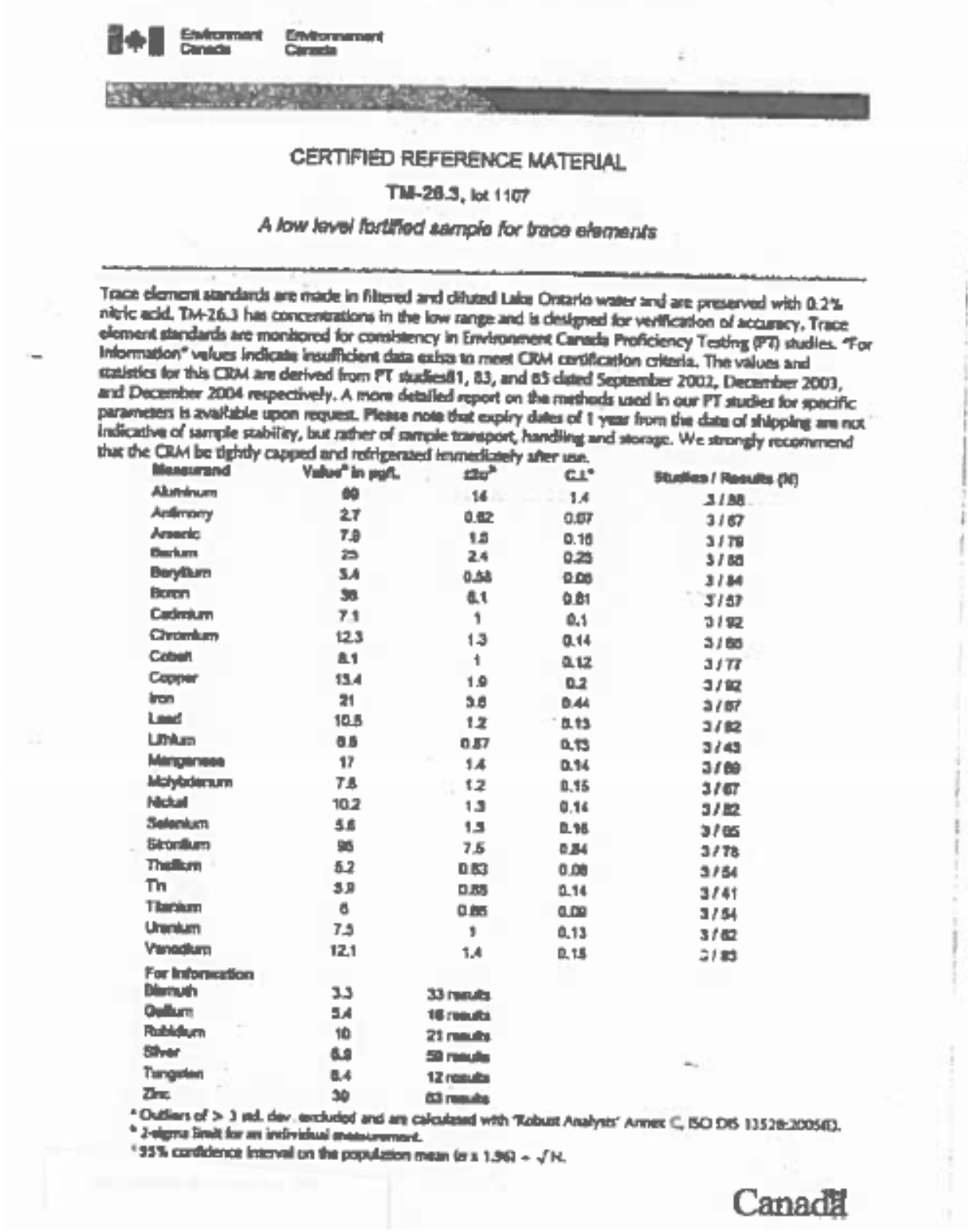
Figure 7.1. Certified Reference Material TM-24.3



Environment Canada Environnement Canada

CERTIFIED REFERENCE MATERIAL

TM-26.3, lot 1107

A low level fortified sample for trace elements

Trace element standards are made in filtered and diluted Lake Ontario water and are preserved with 0.2% nitric acid. TM-26.3 has concentrations in the low range and is designed for verification of accuracy. Trace element standards are monitored for consistency in Environment Canada Proficiency Testing (PT) studies. "For Information" values indicate insufficient data exists to meet CRM certification criteria. The values and statistics for this CRM are derived from PT studies81, 83, and 85 dated September 2002, December 2003, and December 2004 respectively. A more detailed report on the methods used in our PT studies for specific parameters is available upon request. Please note that expiry dates of 1 year from the date of shipping are not indicative of sample stability, but rather of sample transport, handling and storage. We strongly recommend that the CRM be tightly capped and refrigerated immediately after use.

Measurand	Value[a] in pg/L	±2σ[b]	C.I.[c]	Studies / Results (N)
Aluminum	89	14	1.4	3 / 88
Antimony	2.7	0.62	0.07	3 / 87
Arsenic	7.9	1.5	0.16	3 / 79
Barium	25	2.4	0.25	3 / 88
Beryllium	5.4	0.58	0.06	3 / 84
Boron	38	8.1	0.81	3 / 57
Cadmium	7.1	1	0.1	3 / 92
Chromium	12.3	1.3	0.14	3 / 86
Cobalt	8.1	1	0.12	3 / 77
Copper	13.4	1.9	0.2	3 / 92
Iron	21	3.6	0.44	3 / 87
Lead	10.5	1.2	0.13	3 / 82
Lithium	8.8	0.87	0.13	3 / 43
Manganese	17	1.4	0.14	3 / 89
Molybdenum	7.8	1.2	0.15	3 / 87
Nickel	10.2	1.3	0.14	3 / 82
Selenium	5.6	1.3	0.16	3 / 65
Strontium	95	7.5	0.84	3 / 78
Thallium	5.2	0.83	0.08	3 / 54
Tin	3.9	0.85	0.14	3 / 41
Titanium	6	0.85	0.09	3 / 54
Uranium	7.5	1	0.13	3 / 82
Vanadium	12.1	1.4	0.15	3 / 83
For Information				
Bismuth	3.3	33 results		
Cesium	5.4	16 results		
Rubidium	10	21 results		
Silver	6.8	59 results		
Tungsten	8.4	12 results		
Zinc	30	83 results		

[a] Outliers of > 3 std. dev. excluded and are calculated with 'Robust Analysis' Annex C, ISO DIS 13528:2005(E).

[b] 2-sigma limit for an individual measurement.

[c] 95% confidence interval on the population mean ($\bar{x} \pm 1.96) \div \sqrt{N}$.

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Figure 7.2. Certified Reference Material TM-26.3