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Availability of CRM for validation of trace element speciation analysis
Disponibilitat de MRC per l'anàlisi d'espècies d'elements traça

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January 2021

UNIVERSITAT DE BARCELONA

B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

En primer lloc, m’agradaria agrair al meu tutor Dr. J. Fermín López, per la seva dedicació i suport que m’ha ofert durant la realització del treball.

També voldria agrair als meus amics i amigues pels bons i no tan bons moments passats durant la carrera.

Finalment, voldria donar les gràcies a la meva família, per recolzar-me sempre i animar-me quan més ho necessitava.

REPORT

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1. SUMMARY

Speciation analysis interest has been growing over the last years as it has been realised that it is necessary to determine the chemical form of an element to understand fully the effects or toxicity of it. The study of the contamination of food products, along with the pollution of the environment, are the most prominent applied fields on speciation analysis.

For this kind of analysis, it is necessary to combine a variety of techniques that alter the species in a sample, resulting in a loss of information. Therefore, quality control is required. Reference materials, especially certified reference materials, are among the most useful analytical tools as they provide a traceable value that can prove the reliability of a method. The CRM and the sample to be analysed must be as similar as possible with respect to matrix and analyte concentration.

For this reason, a review of the current available CRMs for this area will be made. The research will be focused on those articles that incorporate atomic spectrometry and molecular detection as the measurement techniques. The obtained results will be classified and discussed based on the trace element studied.

Keywords: speciation, reference material, certified reference material, atomic spectrometry, review

2. RESUM

L'interès per l'anàlisi d'especiació ha anat creixent en els últims anys, s'ha constatat que és necessari determinar la forma química d'un element per conèixer al màxim els efectes adversos o la toxicitat d'aquest. L'estudi de la contaminació de productes alimentaris i la pol·lució del medi ambient, són les àrees més prominents on s'aplica l'anàlisi d'especiació.

Per aquest tipus d'anàlisi, és necessari combinar diferents tècniques que poden alterar les espècies presents a la mostra, donant lloc a una pèrdua d'informació. Per tant, un control de qualitat és necessari. Els materials de referència, especialment els materials de referència certificats, es troben entre les eines analítiques més útils, ja que aporten un valor traçable que permet demostrar la fiabilitat d'un mètode. El MRC i la mostra a analitzar han de ser el més semblant possible pel que fa a la matriu i la concentració d'anàlit.

Per aquesta raó, es realitzarà una revisió dels MRCs disponibles actualment per aquesta àrea. La recerca estarà centrada en aquells articles que incorporen l'espectrometria atòmica i detecció molecular com a tècniques de mesura. Els resultats obtinguts seran classificats i discutits segons l'element traça estudiat.

Paraules clau: especiació, material de referència, material de referència certificat, espectrometria atòmica, revisió

3. INTRODUCTION

A reference material according to the IUPAC is “a substance or mixture of substances, the composition of which is known within specified limits, and one or more of the properties of which is sufficient well established to be used for the calibration of an apparatus, the assessment of a measuring method or for assigning values to materials”[1]. ISO Guide 30 differentiates between certified reference material and non-certified reference material, a CRM is defined as “reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability”[2].

A material in order to be considered a RM must meet three main requirements:[3,4]

- Homogeneity (uniformity of a specified property value throughout a defined portion of a reference material).
- Stability (characteristic of a reference material, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period of time).
- Compatible matrix and analyte concentrations close to real-life samples.

In the case of CRMs, the material must meet extra requirements, such as:

- Analyte content determined with the required precision and accuracy.
- The certified value must be accompanied by an uncertainty related to a specific confidence level.
- Certificate.

The fulfilment of these requirements makes the cost of production very high, resulting in a direct increment of their price.

The demand of certified reference materials has always been high as they are one of the most useful analytical tools for method validation among other uses but, although in the last ten years there has been an increased availability of these materials, there is still a shortage amount. The lack of availability of CRMs is due to its specific use, the matrix of these materials has to be compatible with the real-life samples and, as there are a lot of different types of matrices, covering everything is a difficult mission.

In the recent years, there has been a special interest in the speciation analysis of trace elements as it is been realized that it is crucial to know the chemical form of a specific element or the oxidation state to know the effects or toxicity of it. The availability of CRMs for speciation analysis is even lower.[5]

3.1. CLASSIFICATION OF REFERENCE MATERIALS

Apart from the differentiation between certified and non-certified reference materials, there are different approaches to classify reference materials. These can go from the material properties, uncertainty value, or intended use.

3.1.1 Based on material properties

Reference materials can be presented in various forms, a variety of RM can be found based on their properties.

- Physico-chemical material: the material represents physico-chemical quantities such as pH, viscosity, temperature, wavelength, mass (weight), etc.
- Pure substances, solutions, or mixtures of high purity.
- Matrix reference material: natural materials that contain major, minor and trace components. These include materials like soils, sediments, water, vegetables, animal tissues, food products, etc.
- Microbiological reference material: material containing microorganisms, mostly used in food quality control.

3.1.2 Based on uncertainty value

Reference materials can also be classified by the uncertainty of the estimated value. As RMs can be expensive, depending on the work done or for what is needed, laboratories do not need the material to have the highest quality and can work with one of less quality.

In figure 1, a division of three classes of reference materials is distinguished and ordered from having the lowest uncertainty value to the highest.

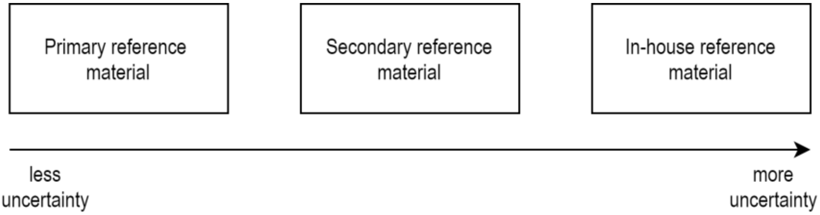

Figure 1. Scheme of the uncertainty of RMs.

ISO Guide 30 and IUPAC define these types of reference materials as[2,6]:

- Primary reference material (PRM): "a material of the highest metrological quality and whose value is accepted without reference to other standard of the same quantity. These are developed by national metrological institutes, approved by authorized national institutions, and verified during international comparative measurements." The availability of these materials is low because they are produced in small quantities and require rigorous studies.

- Secondary reference material (SecRM): "a material for which the value of the characteristic is determined by comparison with the appropriate value for the primary reference material of the same kind.". These are generally the ones produced by commercial organizations, the availability is higher than PRM. Most CRMs fall into this category, the certification is mostly carried out by a procedure traceable to a PRM.

- In-house reference material (LRM): also called working reference material or laboratory reference material. "Reference values are determined through tests conducted by means of at least one analytical procedure which has undergone a validation process. Such materials are accompanied by a description of the method of achieving measuring coherence and the value of the estimated measuring uncertainty. They are prepared mainly for use in inter-laboratory tests and for internal quality control.". The availability of these materials is higher as they are easier to produce and can easily have compatible matrix with the analyte. The specific product that is analysed and routine samples can be used as LRM.

3.1.3 Based on intended use

Reference materials can also be classified based on its intended use, when you are looking for a material one of the things you have to consider is the use you are going to give to that material. In figure 2, a scheme of the classification is shown.

The first distinction made is between certified and non-certified reference material, in some cases it is not needed to use a certified material to have the desired results.

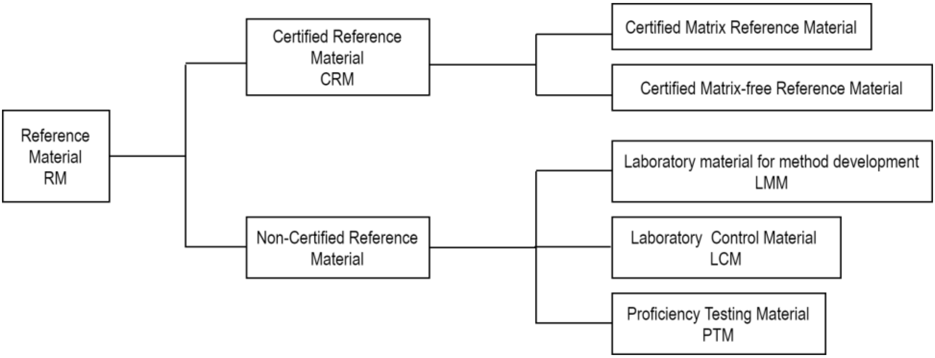

Figure 2. Diagram of the classification of RMs.

When talking about a CRM, two different kinds of materials are distinguished, certified matrix reference materials and certified matrix-free reference materials. The first one is a kind of material that is characteristic of a real sample, they can be obtained from biological, environmental, and industrial sources, or by spiking the component of interest into an existing material. These types of materials are intended to be used in conjunction with the analysis of real samples of similar matrix and have a critical role in analytical method validation, laboratory quality control and proficiency testing. The second one is a kind of material that is free of the influence of the matrix on the determination result, they include pure substances and standard solutions. These materials are mostly used for the calibration of analytic instruments.

Then, there are the non-certified reference materials. These are divided into three classes: laboratory material for method development, laboratory control material, and proficiency testing material. In the case of the first and third type of materials, the name implies the intended use they have. The second one, is used for statistical quality control.[7]

3.2. PRODUCTION OF REFERENCE MATERIALS

The production of reference materials is a difficult and time-consuming process as it requires a large series of steps, it can last up to 3 – 5 years. The process changes between the type of reference material that is produced as some require more monoecious work than others.[7]

The production, certification and storage of a CRM can exceed 1 million euros. In this case, a diagram with the most important steps of the production of a certified matrix reference material is shown in figure 3.

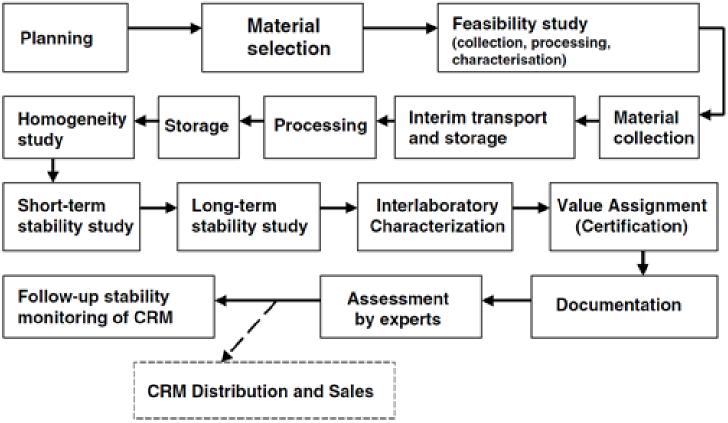

Figure 3. General procedure for the production of a CRM. (extracted from *Emons, et al*, ref. 7).

The first step of the production process is to estimate the demand for a reference material, it is important to know if the material is going to be profitable. Once a demand is found, a detailed plan for the production of the material must be elaborated as the production can last years. The process has to be correctly planned in order to control as much as possible every involved aspect, such as time, needed resources and cost.

Then, the material is selected, in order to do so, a feasibility study where all the relevant factors are considered is performed. Selecting the matrix, the source, and manner of acquisition is very important, also getting enough material for the whole process. The majority of materials require different processing, this can involve desiccation, crushing, milling, sieving, stirring, mixing, etc. Once the material has been processed, it is important to pack it into suitable containers so that the material will be preserved for a long period and maintain its physical stability.

Performing a homogeneity study is an essential step to ensure that it is homogeneous and that the result can be comparable with the values of the same parameter for a different sample of the same RM. Depending on the form of the produced RM, homogeneity study involves different tests. If the reference material is a solution, the study involves finding any irregularities or impurities, as solutions are inherently homogeneous. When the material is not homogeneous by nature, the study has to confirm whether its uniformity was preserved during its preparation. Two kinds of homogeneity are studied: within-bottle homogeneity (homogeneity inside one package) and between-bottle homogeneity (homogeneity within different units).[3,4]

Another essential step are the short-term and long-term stability studies, these are important because a valuable material should remain stable under the influence of deposition, storage, transportation, and use. These studies generally involve studying its resistance to light, humidity, and temperature. Samples are stored in different conditions and analysed over a period of time.

The next step would be certification of the reference material. In order to do so, an interlaboratory comparison is performed. A series of collaborating laboratories characterize the material intended to be certified, using one or more validated methods. When all the tests have been completed and the obtained results are congruous, the certificate report is prepared. ISO Guide 31 mentions the required content of a reference material certificate, which includes the description of the material and its certified values with their uncertainties. It also is required to contain other information; source of supply, storage conditions, instructions for proper use, report on the stability and homogeneity of the CRM, a list of the laboratories, and analysts that participated.

Even with all the study and tests, sometimes the desired results are not obtained and after working on a material for three years, at the end it is not possible to certify any value. This is mostly due to problems with stability or homogeneity.

The cost of a CRM can range from 60 to 1000 euros, which is considered expensive, but it can become even more expensive when the material must be stored under special conditions, like in cool conditions, or following special safety measures.

3.3. APPLICATIONS OF REFERENCE MATERIALS

Reference materials are one of the most useful tools in analytical chemistry, generally they are used to support measurements. They have a variety of applications, such as method development and validation, internal quality control and quality assurance, proficiency testing, calibration, estimation of measurement uncertainty, and training. The most common uses are discussed below.

3.3.1. Method development and validation

Method development requires different stages in which a material is used to define and optimize the method, then, the validation of the method is performed. The trueness of the method can be determined by participating in interlaboratory comparisons or by analysing appropriate CRMs. The RMs provide valuable information but only if the material used is the proper one, it must have compatible matrix and analyte concentration close to the routine sample. For method validation it is required that the assigned values with a stated uncertainty, can be traceable to the same reference as the analytical results of the method used, CRMs provide this traceable assigned value.

3.3.2. Verification of correct use of a method

Once a method has been developed and validated, it is important to determine if you are applicating it correctly. Sometimes, the desired results are not obtained when a method is applied because of problems with the availability of suitable equipment, reagents, or standards, or even the operator is not qualified. RMs are used to check whether there is a problem or not when unexpected results are obtained.

3.3.3. Calibration

The calibration in analytical chemistry is an important part when doing a measurement, it is important to have a material with whom you are able to compare the measurement values delivered by a device with the certified value. The CRMs used as calibrants tend to be matrix-free, that includes pure substances and standard solutions. The material has to be stable and homogeneous to ensure that the assigned property value and its uncertainty are valid, the uncertainty of the material will contribute to the total uncertainty of the measurement. In the case of trace analysis this does not affect much, but it can affect in other situations.

3.3.4. Quality control and quality assurance

Another major application of RMs is for quality control and quality assurance. There can be a differentiation of in-house QC and proficiency testing, also called interlaboratory comparison. The material is normally used for a long period of time to be able to make conclusions about the performance of the method and accuracy of the analysis. They are characterized with respect to homogeneity, stability, and the certified value(s), although this last requirement is not essential as usually non-certified materials are used.

4. OBJECTIVES

The aim of this project is to do a bibliographic research on current available certified reference materials for chemical speciation analysis, focusing on materials used for the validation of trace element analysis.

The objectives are:

- Study the applications and importance of CRMs.
- Determine the use of CRMs in the publications reviewed.
- Classify the available CRMs.

5. METHODS

The bibliographic research has been done using two scientific databases, SciFinder and Web of Science.

Firstly, the research was focused on reviews published in the last 5 years, from 2015 to 2020 as reviews already summarize a lot of the information of interest. As there is a lot of information in this specific field, only reviews from 2017 to 2020 were finally studied. Also, as reviews did not include articles from 2019 to 2020, articles from these years were revised.

The research of the reviews was carried out in mid-October and the research of the articles was carried out in early November. To do the research keywords were used, different options were contemplated in order to get the best results and at the end the keywords used were reference material, speciation, atomic spectrometry, and element. The research was conducted via different paths, changing the order of the keywords. In Web of Science, the obtained references were close in every path but in SciFinder the obtained references did vary between the path followed.

Nowadays there are no reviews that only focus on certified reference materials for speciation, the reviews or articles dedicate one section to them and even none. To see if there were any focused reviews, the research was conducted without limitation of publication date and two interesting ones were found; *Trends in certified reference materials for the speciation of trace elements* from 2001 and *Certified reference materials for analytical mercury speciation in biological and environmental matrices: Do they meet user needs?; a review* from 2012. As they were too old, these were not included.

Web of Science has been very useful. A total of twenty-five reviews and fifteen articles were found, after the exploration twenty-nine were selected to be reviewed.

SciFinder has not conducted to as good results as Web of Science. The founded reviews talked about speciation or atomic spectrometry but did not mention reference materials, the ones that did talk about reference materials were in majority the ones already found through Web of Science. A total of twenty reviews and twelve articles were found, thirteen were chosen to be revised.

The obtained results vary a lot between the two databases, only five reviews and two articles were duplicated.

6. RESULTS AND DISCUSSION

As it has been introduced, there are two types of CRMs and depending on the needed characteristics of the material one is used instead of the other. There is a clear majority of studies reviewed that used certified matrix reference materials for the speciation of trace elements. When no CRM was available, most of the studies performed the validation by spike recovery, which is only admissible when there are no other accessible alternatives.[8]

Even though in recent years a lot of reference materials have been developed, a considerable amount of reviews concluded that there is still a lack of available materials.[8,9,18–23,10–17] For example, Han Wang claimed that only a few species of mercury, selenium, and arsenic were determined in the cited papers due to the unavailability of appropriate standards for elemental speciation in biological samples.[22]

6.1. PRESENT TRENDS OF ANALYTICAL SPECIATION

The most commented and studied elements in the publications are arsenic and mercury, there are reviews and articles explicitly dedicated to the speciation study of arsenic[12,20,24,25] or mercury.[17,26–28] Also, selenium and chromium are other elements that are mentioned and studied more throughout all the publications. This is directly related to the amount of available reference materials that will be shown.

In the last year, focusing on the articles selected to be reviewed, an increase in the speciation analysis of antimony is observed. But, the available CRMs is still very limited and the publications use the same ones or other alternatives.[29,30]

6.1.1. Distribution based on the type of study

The reviews chosen to be revised include a resume of all the relevant papers published of all types of studies. They are mainly dedicated to the description of new or optimized methods. However, there are some that focus on determined fields. For example, Wang, *et al.* focus on sample pretreatment techniques.[22]

A distribution related to the type of study of the revised articles was created (Fig. 4). It was divided between five types, sample pretreatment, environmental pollution analysis, method development and validation, development of reference materials and food contamination analysis.

Most of the studies focus on method development and validation, 47%. Among these articles, the methods developed imply the study of environmental and food related samples. The second main type of study performed is environmental pollution analysis, focusing on water. Two articles address the optimization of sample pretreatment, focusing on extraction techniques.[25,28] Then, two articles are devoted to the preparation and certification of reference materials.[26,31] Lastly, one article performed speciation analysis in food samples, including different types of flour, garlic, cranberries, goji berries, and raisins. [32]

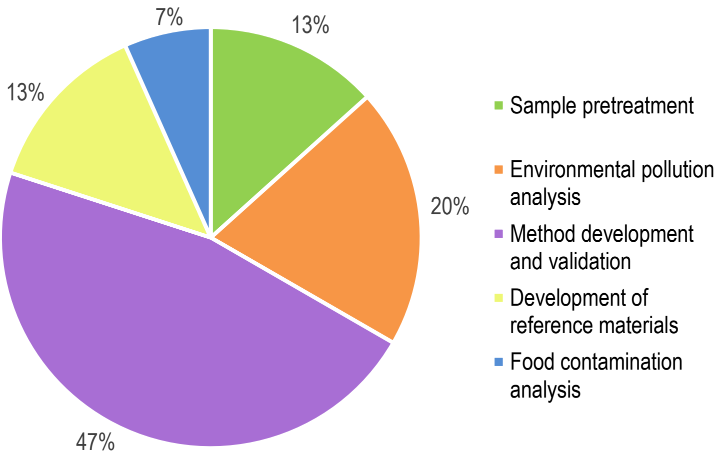

Figure 4. Distribution of type of study of the reviewed articles.

6.1.2. Distribution based on the type of matrix

From the data consulted, a distribution of these publications based on the type of matrix studied was elaborated (Fig. 5). It can be seen that most of the studies are related to environmental matrices, almost 50% and, especially water, which accounted for 22%. This is not surprising as the contamination by metals is a huge problem for the environment that needs to be addressed. So, it is essential to study the pollution of different aquatic systems (estuaries, groundwater, coastal water, drinking water, etc) to control the effects that this may cause[33]. The study of “sediment” matrices is also quite huge (14%), the analysis of the chemical forms of heavy metals has become important as they can be a source of heavy metals in the aquatic environment.[5]

Also, a bast majority of matrices studied are related to food, 32%. The study of the contamination of food is one of the main important areas in quality control, and as mentioned before, the toxicity of an element depends on its chemical form and concentration, so it is not surprising that the second most studied matrices are related to food. Rice is the main cereal-based sample studied, as it is part of the basic diet all around the world. The elements considered harmful for human health can be present at low concentrations and be a source of human exposure to toxic elements.[34]

Then, in less quantity, there are studies related to human biological matrices (6%), these include human hair, urine, and blood. These type of studies are performed to measure the exposure that suffer humans to toxic species, such as methylmercury[26,35].

Lastly, there is a 1% related to standard solutions, these are not really matrix reference materials but were included to show the small amount of non-matrix reference materials that are used in the speciation analysis.

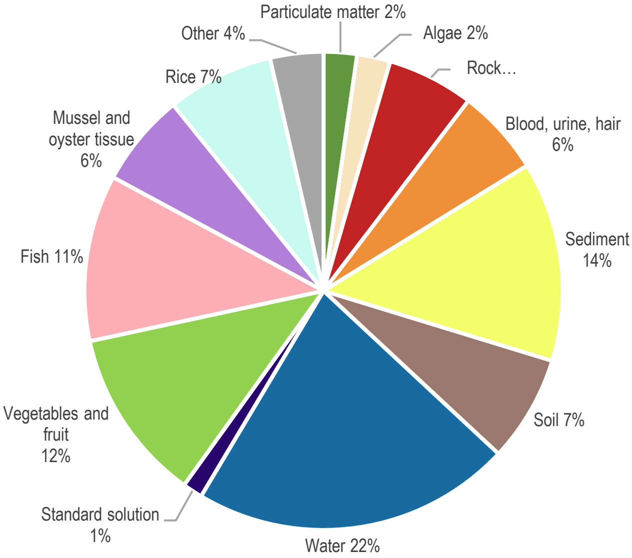

Figure 5. Distribution of types of matrices studied.

6.2. CURRENT AVAILABLE CRMS

Due to the huge amount of information available related to this topic, the current CRMs have been classified according to which analyte determination they have been used for in the publications. This means that some CRMs may be seen in various tables as some of them have certified values for different elements and species.

6.2.1. Arsenic

Arsenic is a metalloid very abundant in the environment and its compounds are widely used in a variety of applications, including medical agents, pesticides, herbicides, growth promoters, embalming fluids, pigments, alloy, and the most famous one, poison. As depending on the form of arsenic, its toxicity changes, the speciation studies of this element are of big concern for human health and the environment.[12] For this reason, the number of CRMs available for the speciation of arsenic is one of the highest.

Table 1 provides information on the type of matrices of the different CRMs and their certified values for two species, arsenobetaine (AB) and dimethylarsinic acid (DMA), and total content of arsenic. It also gives information about the producer institution and how to storage the material.

The organic species of arsenic are less toxic than the inorganic species, in fact, AB is not considered toxic at all, but DMA can be a carcinogen.[12] Arsenobetaine is widely studied, its special interest lies in the need to differentiate between the non-toxic arsenic from the toxic one. As observed in the table, the materials that have a certified value for this kind are the ones related to food, especially fish. Marine organisms are the main source of total arsenic exposure in humans and present naturally a variety of organic forms of arsenic, so an accurate speciation is necessary for food safety measures.[15] There are also a couple of CRMs of human urine, certified for both organic species which gives information to assess the risk from arsenic exposure and not overestimate it.

The most studied inorganic species are As(III) and As(VI), being As(III) more studied than As(V) due to its higher toxicity. The number of available materials with certified content is very limited. These species are more studied in environmental samples, including different types of water but also in food. Due to the toxic effects of inorganic arsenic forms, some CRMs also have a certified value for total inorganic arsenic content (iAs), measuring the total content of iAs helps to control the exposure that humans suffer from food or contaminated water.[20]

It can be observed that the certified values differ in order of magnitude between materials with the same or similar matrix. This is a fact that it is asked for a lot, in order to have more options when choosing the most representative material. However, there is still a need for more variety.

In addition, it can be observed that a great part of the materials are only certified for the total content which demonstrate the lack of certified values for arsenic species.

Table 1. List of CRMs used in the bibliography with certified values for total content of arsenic, arsenobetaine and dimethylarsinic acid.

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]		
				AB	**DMA**	**Total As**
Groundwater	ERM-CA615	ERM	13-23ºC, dark	-	-	9.9 ± 0.7(d)
Lichen powder	BCR-482	BCR	18ºC, dark	-	-	0.85 ± 0.07
Fish tissue	ERM-BB422	ERM	13 – 23 ºC	-	-	12.7 ± 0.7
Mussel tissue	ERM-CE278k	ERM	13-23 ºC	-	-	6.7 ± 0.4
Tuna fish tissue	BCR-627	BCR	4 ºC, dark	9.26 ± 0.53	0.43 ± 0.06	4.8 ± 0.3
Groundwater	BCR-610	BCR	Cool and dark	-	-	0.011 ± 0.004
Rice	ERM BC211	ERM	-20±5°C, dark	-	0.119 ± 0.013	0.260 ± 0.013
River prawn	PRON-1	NRCC	-20ºC	1.20 ± 0.06	-	1.73 ± 0.12
Cuttlefish	SQID-1	NRCC	-20ºC	13.96 ± 0.54	-	14.1 ± 2.2
Fish protein	DORM-4	NRCC	Cool	3.95 ± 0.36	-	6.87 ± 0.44
Estuarine water	SLEW-3	NRCC	Cool	-	-	$1.3\cdot10^{-3} \pm 9\cdot10^{-5}$
Seawater	NASS-6	NRCC	Cool	-	-	$1.4\cdot10^{-3}\pm1\cdot10^{-4}$
River water	SLRS-6	NRCC	+4ºC	-	-	$5.7\cdot10^{-3}\pm8\cdot10^{-5}$
Sea water	CASS-6	NRCC	+4ºC	-	-	0.001± 0.0001(a)
Dogfish liver	DOLT-5	NRCC	Cool	24.2 ± 0.8	-	34.6 ± 2.4
Marine sediment	PACS-3	NRCC	Cool	-	-	30.3 ± 2.4
Lobster hepatopancreas	TORT-3	NRCC	Cool and dark	54.9 ± 2.5	-	59.5 ± 3.8
Cod fish tissue	CRM 7402-a	NMIJ	5-35ºC, dark	35.5 ± 1.8	-	36.7 ± 1.8
Seaweed (Hijiki)	CRM 7405-b	NMIJ	5-35 ºC, dark	-	-	49.5 ± 1.0
Rice	CRM 7503-a	NMIJ	5-35 ºC, dark	-	0.0133 ± 0.0009	0.098 ± 0.007(b)
Rice flour	CRM 7503-b	NMIJ	5-35 ºC, dark	-	-	0.164 ± 0.005
Brown rice flour	CRM 7532-a	NMIJ	5-30 ºC, dark	-	0.019±0.001	0.320 ± 0.010

Table 1 (continued). List of CRMs used in the bibliography with certified values for total content of arsenic, arsenobetaine and dimethylarsinic acid.

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]		
				AB	**DMA**	**Total As**
DMA solution	CRM 7913-a	NMIJ	5-35 ºC, dark	-	25.11 ± 0.70	-
AB reference standard	CRM 7901-a	NMIJ	5-35 ºC, dark	19.98 ± 0.47	-	-
Swordfish tissue	CRM 7403-a	NMIJ	5-35 ºC, dark	6.23 ± 0.21	-	6.62 ± 0.21
Human urine	SRM 2669	NIST	<-80ºC	12.4 ± 1.9(d)	3.47 ± 0.42(d)	-
Human urine	SRM 3669	NIST	<-80ºC	51.0 ± 2.2(d)	77.1 ± 1.6(d)	185 ± 9.9(d)
Mussel tissue	SRM 2976	NIST	20-25ºC	-	-	13.3 ± 1.8
Apple juice	SRM 3035	NIST	<-80ºC	-	0.0052±0.0003	0.0249±0.0008
Peach leaves	SRM 1547	NIST	20-25 ºC, dark	-	-	0.062 ± 0.014
Rice flour	SRM 1568 b	NIST	20-25 ºC,	-	0.180 ± 0.012	0.285 ± 0.014
Spinach leaves	SRM 1570a	NIST	10-30ºC, dark	-	-	0.068 ± 0.012
Tomato leaves	SRM 1573a	NIST	20-25ºC, dark	-	-	0.112 ± 0.004
Bovine liver	SRM 1577	NIST	10-30ºC	-	-	0.062 ± 0.014
Oyster tissue	SRM 1566b	NIST	10-30ºC, dry	-	-	7.65 ± 0.65
Kelp powder	SRM 3232	NIST	20-25 ºC,	-	0.479 ± 0.077	38.3 ± 1.3
Natural water	SRM 1640a	NIST	Sealed inside an airtight container	-	-	$0.008 \pm 6.7\cdot10^{-5}$
Yellow croaker tissue	GBW 08573	NRCCRM	Keep dry	-	-	5.08 ± 0.39
Tea leaf	GBW 07605	NRCCRM	Cool and dry	-	-	0.28±0.04
Liaoning rice	GBW 10043	NRCCRM	<26ºC and dry	-	-	0.114±0.018
Hunan rice	GBW 10045	NRCCRM	<26ºC and dry	-	-	0.11 ± 0.02
Spinach	GBW 10015	NRCCRM	<26ºC and dry	-	-	0.23 ± 0.03
Carrot	GBW 10047	NRCCRM	<26ºC and dry	-	-	0.11 ± 0.02
Rock	GBW 07103	NRCCRM	Cool and dry	-	-	2.1 ± 0.4
Human urine	GBW 09115	NRCCRM	Cool and dark	-	$1.84\cdot10^{-4} \pm 1.28\cdot10^{-5}$(c)	-
Rock	GBW 07397	NRCCRM	Cool and dry	-	-	8.1 ± 0.4
Rock	GBW 07398	NRCCRM	Cool and dry	-	-	10.5 ± 1.0
Rock	GBW 07399	NRCCRM	Cool and dry	-	-	248 ± 22
Soil	GBW 07401	NRCCRM	Cool and dry	-	-	45 ± 4
Water	GSBZ07-3171-2014	NRCCRM	Cool and dark	-	-	43.9 ± 3.5

Table 1 (continued). List of CRMs used in the bibliography with certified values for total content of arsenic, arsenobetaine and dimethylarsinic acid.

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]		
				AB	**DMA**	**Total As**
Fortified lake water	TMDA-54.4	NWRI	Cool	-	-	43.6 ± 7.3(d)
Surface water	SPS-SW 2	LGC	<30ºC	-	-	50.0 ± 0.3
Lake water	TMDA-61	NWRI	Cool	-	-	33.6 ± 0.4(d)
Oyster tissue	CRM 108-04-003	KRISS	Cool and dark	-	-	10.39 ± 0.34
Tea leaves	INCT-TL-1	INCT	Cool and dry	-	-	0.106±0.021
Apple juice	HRM-2008A	HSA	-20ºC	-	0.060±0.005	0.185±0.015(b)
Spring water	CRM 1201	UME	(18±4)ºC	-	-	0.0065±0.0004
Rice flour	IMEP 107	IRMM	20-25ºC	-	-	0.172 ± 0.018(b)
Rice flour	NIES 106	NIES	Cool and dry	-	-	0.150(a)
Cabbage	NCS ZC73012	NACIS	Cool and dry	-	-	0.062 ± 0.014

(a) Reference value
(b) Also certified for total inorganic arsenic content (iAs)
(c) Also certified for As(III)
(d) Units: µg/L

6.2.2. Mercury

Mercury is considered by the World Health Organization one of the ten substances that threatens public health. Although inorganic Hg (Hg(II)) is not considered of high risk, it can easily convert to an organic form, which is more toxic, and can affect humans when they are exposed to it. In the European Union (EU) regulations, maximum levels of mercury have been established; 0.07 µg/L in surface water, 0.5 µg/g in fish with some exceptions which can have up to 1 µg/g. For this reason, the speciation of mercury has been an important topic for a long time leading to the production of more CRMs than for other elements or species where the interest is newer.[17,28]

Table 2 apart from providing information related to the producer, storage, and matrix, two certified values are given, for total Hg content and for methylmercury (MeHg). Methylmercury is the most toxic alkyl compound, it can cause blockage of enzyme binding sites, and obstruct protein synthesis. Therefore, is the most studied.[27]

Both inorganic and organic mercury tend to accumulate in sediments and animals, particularly those from marine origin. That is why there is a majority of CRMs matrices related to environmental samples. The speciation of sediments works as an indicator of how affected the environment is. A total of ten materials showed in table 2 have a sediment matrix, this shows the importance of it. In sediments, the predominant content of Hg is inorganic, being only 0.1-1% organic. This can be observed by the difference between total content of mercury and methylmercury values showed in the table, MeHg is only a small part of it. Hellmann, *et al.* claimed that there is a need of reference materials being certified for other species in sediments as MeHg is not representative.[17] The distribution of species in fish is very different from the previously commented in sediments and soil, being MeHg the predominant one. There are 22 CRMs that have a fish tissue matrix, meaning that it is the most studied area for mercury speciation.

Apart from the analysis of fish in the food industry, other types of samples are studied. Here, rice CRMs are also present. In this analysis it is important to determine the amount of MeHg present in a sample in order to do the correct assessment and determine if it is suitable for human consume.

There are some materials with matrices related to humans, including urine, hair, and blood. This kind of material is used to provide evidence of actual exposure to environmental chemicals and help to evaluate or assess the effects of exposure. Koichi Haracuchi, *et al*. claimed that although there are some available CRMs of human hair, they were not homogeneous enough for small samples. That is why his team developed and validated a human hair CRM, NIMD-01, to cover this necessity.[26]

Table 2. List of CRMs used in the bibliography with certified values for total mercury content and methylmercury.

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]	
				Total Hg	**MeHg**
Mussel tissue	SRM 2976	NIST	21-25 ºC	0.0610 ± 0.0036	0.02809 ± 0.00031
Oyster tissue	SRM 1566b	NIST	10-30 ºC dry	0.0371 ± 0.0013	0.0132 ± 0.0007
Mussel tissue	SRM 2976	NIST	21-25 ºC	0.061 ± 0.0036	0.0281 ± 0.0003
Lake Michigan fish tissue	SRM 1947	NIST	-80ºC (or lower)	0.254 ± 0.005	0.233 ± 0.010
Human blood	SRM 955d	NIST	-60ºC (or lower)	1.373 ± 0.081(b)	0.626 ± 0.020(b)
Human urine	SRM 3668	NIST	-70ºC (or lower)	0.910 ± 0.055(b)	-
Mussel tissue	SRM 2974a	NIST	Room temperature	0.195 ± 0.003	0.0691 ± 0.0008
Lake Superior fish tissue	SRM 1946	NIST	-80ºC (or lower)	0.433 ± 0.009	0.394 ± 0.015

Table 2 (continued). List of CRMs used in the bibliography with certified values for total mercury content and methylmercury.

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]	
				Total Hg	**MeHg**
Particulate matter	SRM 1648a	NIST	<30 ºC	1.323 ± 0.064	-
Peach leaves	SRM 1547	NIST	20-25ºC, dark	0.032 ± 0.004	-
San Joaquin soil	SRM 2709a	NIST	Dry	0.9 ± 0.2(a)	-
Dogfish liver tissue	DOLT-5	NRCC	Cool	0.44 ± 0.18	0.119 ± 0.058
Marine sediment	PACS-3	NRCC	Cool	2.98 ± 0.36	-
Fish protein	DORM-4	NRCC	Cool	0.412 ± 0.036	0.355 ± 0.028
Cuttlefish	SQID-1	NRCC	-20ºC	0.342 ± 0.054	0.324 ± 0.028
Lobster hepatopancreas	TORT-3	NRCC	Cool and dark	0.292 ± 0.022	0.137 ± 0.012
River prawn	PRON-1	NRCC	-20ºC	0.0124±0.008(a)	-
Human hair	NIMD-01	NIMD	Cool and dark	0.794 ± 0.050	0.634 ± 0.071
Fish tissue	GBW 10029	NRCCRM	-20 – 4ºC	0.85 ± 0.03	0.84 ± 0.03
Rice	GBW 10043	NRCCRM	<26ºC and dry	4.8 ± 0.8	-
Human hair	GBW 09101-b	NRCCRM	Cool and dry	2.16 ± 0.07	-
Lake sediment	GBW 08308	NRCCRM	Cool and dry	0.48 ± 0.03	-
Soil	GBW 07403	NRCCRM	Cool and dry	0.060±0.004	-
Water	GBW 08603	NRCCRM	Cool	0.0097 ± 0.0004	-
Codfish tissue	CRM 7402-a	NMIJ	5-35 ºC, dark	0.61 ± 0.02	0.58 ± 0.02
Swordfish tissue	CRM 7403-a	NMIJ	5-35 ºC, dark	5.34 ± 0.14	5.00 ± 0.22
Tuna tissue	ERM-CE464	ERM	+4 ºC, dark	5.24 ± 0.10	5.50 ± 0.17
Seaweed	ERM-CD200	ERM	13-23 ºC, dark	0.0186 ± 0.0016	-
Mussel tissue	ERM-CE278k	ERM	13-23 ºC	0.071 ± 0.007	-
Lichen powder	BCR-482	BCR	18ºC, dark	0.48 ± 0.02	-
Tuna tissue	BCR-463	BCR	Cool and dark	3.04 ± 0.16	2.85 ± 0.16
Estuarine sediment	BCR-580	BCR	Cool and dark	0.132 ± 0.003	0.075 ± 0.004
Coastal seawater	BCR-579	BCR	< 20 ºC, dark	$(1.9 \pm 0.5)\cdot 10^{-6}$	-
Channel sediment	BCR-320	BCR	20-25ºC	0.85 ± 0.09	-
Estuarine sediment	ERM-CC580	ERM	+4ºC, dark	132 ± 3	0.0755 ± 0.0037
Human hair	IAEA-085	IAEA	<20ºC, dark	23.2 ± 0.8	22.9 ± 1.0
Human hair	IAEA-086	IAEA	<20 ºC, dark	0.573 ± 0.039	0.258 ± 0.021
Marine sediment	IAEA-433	IAEA	4ºC, dark	0.168 ± 0.017	0.17 ± 0.07

Table 2 (continued). List of CRMs used in the bibliography with certified values for total mercury content and methylmercury.

Matrix	**Code**	**Producer**	**Storage**	**Certified value [mg/kg]**	
				Total Hg	**MeHg**
Marine sediment	IAEA-158	IAEA	Dry	0.132 ± 0.014	0.00141 ± 0.0004[(a)]
Coastal sediment	IAEA-456	IAEA	Dry and dark	0.077 ± 0.005	0.000125 ± 0.000019
Clam tissue	IAEA-461	IAEA	Dry and dark	0.39 ± 0.04	0.0623 ± 0.007
Oyster tissue	IAEA-470	IAEA	Dry and dark	0.021 ± 0.002	0.00522 ± 0.0009
Estuarine sediment	IAEA-405	IAEA	Dry and dark	0.81 ± 0.040	0.00549 ± 0.0006
Human hair	NIES 13	NIES	-20ºC and dark	4.42 ± 0.20	3.8 ± 0.4
Rice plant	NCSZC73027	LGC	<25ºC	0.0048±0.0008	-
Bottom sediment	MODAS-2	LGC	20-25ºC, dark	0.884 ± 0.53	-
Herring tissue	MODAS-3	LGC	20-25ºC, dark	0.227 ±0.0 21	-
Cormorant tissue	MODAS-4	LGC	20-25ºC, dark	2.20 ± 0.14	-
Cod tissue	MODAS-5	LGC	20-25ºC, dark	0.310 ± 0.022	-

(a) Reference value
(b) Units: µg/L

6.2.3. Chromium

Chromium is a very toxic element, but the toxicity depends a lot on its oxidation state. For this reason, the differentiation between species is very important. Chromium mainly exists in two chemical forms, the ones observed in table 3, trivalent chromium and hexavalent chromium.

Cr(III) is considered an essential element, it is required in trace amounts for sugar and lipid metabolism. However, Cr(VI) is very toxic, it is considered a carcinogen, and it is highly irritant.[36] The majority of the publications focus on the determination of Cr(VI), but Cr(III) should be able to detect in order to monitor redox interconversions.[9]

The most studied type of matrices are the ones related to the environment, especially water.[37] There are 16 CRM that have a water matrix, being only one of them certified for Cr(VI). The Cr speciation analysis in water is performed to evaluate its ecotoxicological impact, but there is still a need for development of more reliable methods.

Another field of chromium speciation analysis is performed on soil. Chromium tends to accumulate in plant root tissues, affecting the soil-plant system that can lead to the contamination of human intake vegetables. Therefore, it is necessary to control the amount of chromium in soils to prevent humans from consuming contaminated food.[36]

Another aspect commented also in the other elements, are the CRMs related to human matrices used to control the level of exposure to Cr. For Cr only two materials were reported to be used for method validation and neither of them are certified for any species.

As it can be observed, there is a clear lack of materials certified for chromium species, most of them are only certified for total content of Cr. Then, among the materials that have a certified value for chromium species, three of the total four, are only certified for Cr(VI).

Table 3. List of CRMs used in the bibliography with certified values for total content of chromium and two oxidation states, Cr(III) and Cr(VI).

Matrix	**Code**	**Producer**	**Storage**	**Certified value** [mg/kg]		
				Cr(III)	**Cr(VI)**	**Total Cr**
Spring water	UME-1201	NMI-TUBITAK	20-25ºC, dark	-	-	0.035±0.001
Clay soil	CRM 041	Sigma-Aldrich	Dark and dry	-	216 ± 10	-
Clay soil	CRM 060	Sigma-Aldrich	Dark and dry	-	195 ± 9	-
Water	GSB07-3174-2014	IRMS	Cool and dark	-	-	503±15(a)
Environmental water	GSBZ50009-88	IRMS	Cool and dark	-	-	1.49±0.06(a)
Wheat	GBW 10011	NRCCRM	<26ºC, dry	-	-	0.096±0.014
Tea	GBW 07605	NRCCRM	Cool and dry	-	-	0.80±0.03
River sediment	GBW 08301	NRCCRM	10-30ºC	-	-	90 ±8
Farming soil	GBW 08303	NRCCRM	10-30ºC	-	-	112±11
Bovine serum	GBW(E)90006	NRCCRM	<-70ºC	-	-	0.216±0.013(a)
Water	GBW(E)080403	NRCCRM	Cool and dark	-	-	10.0±0.1(a)
Water	GBW(E)080404	NRCCRM	Cool and dark	-	-	20.0±0.8(a)
Human urine	SRM 2668	NIST	-70ºC	-	-	27.7±2.1(a)
Lake Ontario water	TM-23.4	NWRI	Cool and dark	-	-	6.80±0.63(a)
Lake Ontario water	TM-25.4	NWRI	Cool and dark	-	-	24.0 ± 1.73(a)
Natural water	SRM 1640a	NIST	Sealed inside an airtight container	-	-	0.040±0.003
Sediment	RM 8704	NIST	Dry	-	-	121.9±3.8(b)
Lyophilised water	BCR-544	BCR	Cool and dark	26.8 ± 1.0(a)	0.456 ± 0.020(a)	-

Table 3 (continued). List of CRMs used in the bibliography with certified values for total content of chromium and two oxidation states, Cr(III) and Cr(VI).

Matrix	Code	Producer	Storage	**Certified value** [mg/kg]		
				Cr(III)	**Cr(VI)**	**Total Cr**
Seawater	NASS-7	NRCC	+4ºC	-	-	0.11±0.02(a)
Near shore water	CASS-6	NRCC	Cool and dark	-	-	0.1±0.02(a)
Rain	TMRain-04	NWRI	Cool	-	-	0.87±0.16(a)
Mussel tissue	ERM-CE278k	ERM	Cool and dark	-	-	0.73±0.22
Industrial effluent	BCR-714	BCR	Cool and dark	-	-	123 ± 10(a)
Industrial effluent	BCR-715	BCR	Cool and dark	-	-	(1.00 ± 0.09) ·10^{3}(a)
Welding dust	BCR-545	BCR	+18ºC	-	40.2 ± 0.6(c)	39.5 ± 1.3(c)
Hard drinking water	ERM-CA011	ERM	<5ºC	-	-	48.2 ±1.6(a)
Human blood	SERO210105	LGC	2-8ºC	-	-	0.86±0.25(a)
Lake water	NWTM-27.2	NWRI	Cool	-	-	1.9 ± 0.1(a)

(a) Units: µg/L
(b) Reference value
(c) Units: g/kg

6.2.4. Selenium

The speciation study of selenium is and exception to the other elements studied as selenium is an essential element for humans and animals in low concentrations. It is important for many bodily processes; it contributes to thyroid hormone metabolism and DNA synthesis apart from helping to protect against different oxidative processes. The range of essential required and toxicity is pretty narrow, the recommended intake for adults is 55 µg per day.[26,38]

Selenium is found in a variety of forms, including inorganic Se(IV) and Se(VI), and organic compound, being selenomethione (SeMet) and selenocysteine (SeCys) the most studied. The inorganic forms of Se are the most toxic forms of selenium.[5]

The speciation analysis of selenium is focused on food, which can be observed through the number of matrices related to food of the CRMs used, eight of the total twenty-three. It is necessary to control the amount of selenium that humans consume, as it can lead to problems of deficiency or toxicity. Another important field is the environmental speciation, including sediments, rocks, and soil.

There is a need for more materials certified for selenium species, only one of the CRMs, SELM-1, is certified for a selenium species apart from the total content.

Table 4. List of CRMs used in the bibliography with certified values for total content of selenium.

Matrix	Code	Producer	Storage	Certified value of total Se [mg/kg]
Enriched yeast	SELM-1	NRCC	-20ºC	2031 ± 70
Milk powder	ERM-BD151	ERM	-20 ºC ± 5 ºC, dark	0.19 ± 0.04
Human serum	BCR-637	BCR	<70ºC	81±7(c)
Montana soil	SRM 2711a	NIST	Cool and dry	2(a)
Sediment	SRM 2709a	NIST	Cool and dry	1.5(a)
Tomato leaves	SRM 1573a	NIST	20-25ºC, dark	0.0543 ± 0.0020
Human plasma	SRM 1950	NIST	<-60ºC	0.1055 ± 0.0038(b)
Milk powder	SRM 1549	NIST	2-8ºC	0.242 ± 0.026
Wheat powder	SRM 1567	NIST	20-25ºC	1.14 ± 0.10
Sediment	SdAR-L2	USGS	Cool and dry	0.8(a)
Sediment	SdAR-M2	USGS	Cool and dry	2.7(a)
Sediment	SdAR-H1	USGS	Cool and dry	15(a)
Leaves	GBW 07602	NRCCRM	Dark and dry	0.184±0.013
Water	SRM 1643f	NIST	15-25ºC	0.012 ± $8 \cdot 10^{-5}$
Gabbro	GBW 07112	NRCCRM	Cold and dry	0.26 ± 0.09
Rock	GBW 07397	NRCCRM	Cold and dry	0.96 ± 0.05
Rock	GBW 07398	NRCCRM	Cold and dry	1.03 ± 0.05
Rock	GBW 07399	NRCCRM	Cold and dry	0.049 ± 0.004
Rock	GBW 07400	NRCCRM	Cold and dry	0,0385 ± 0.0019
Water	GBW(E) 080395	NRCCRM	Cool and dark	1000±90(c)
Rice	GBW 10010	NRCCRM	<26ºC, dry	0.061±0.015
Liaoning rice	GBW 10043	NRCCRM	<26ºC, dry	0.040 ± 0.013
Hunan rice	GBW 10045	NRCCRM	<26ºC, dry	0.053± 0.014

(a) Information value
(b) Reference value
(c) Units: µg/L
(d) Also certified for SeMet: 3190±260 mg/kg

6.2.5. Lead

Lead is a highly toxic element; it can have adverse effects for the environment. The most important forms of lead are the organolead compounds, especially tertraalkyl lead. The organometallic compounds of lead are more toxic than the inorganic forms.[5]

The most type of matrices studied for lead speciation are those related to the environment. The study of sediments, rocks and soil is clearly the prominent area of speciation. Although, it is also studied in water.[33]

Due to the lack of CRM related with food, Cerveira, *et al.* had to evaluate the accuracy of the proposed method for the determination of different elements and species in rice with the closest materials available, NIST materials with a matrix of peach and tomato leaves.[34]

However, as it can be observed in table 5, there is no reference material that is certified for any specie of lead yet. All the materials reported on the literature are only certified for total content of Pb.

Table 5. List of CRMs used in the bibliography with certified values for total content of lead.

Matrix	**Code**	**Producer**	**Storage**	**Certified value of total Pb** [mg/kg]
Cuttlefish	SQID-1	NRCC	-20ºC	0.1433 ± 0.0098
Apple leaves	SRM 1515	NIST	20-25 ºC, dark	0.470 ± 0.024
Particulate matter	SRM 2786	NIST	Room temperature	286 ± 3
Particulate matter	SRM 2787	NIST	Room temperature	240 ± 5
Soil	SRM 2586	NIST	<30ºC	432 ± 17
Soil	SRM 2587	NIST	<30ºC	3,242 ± 57
Oyster tissue	SRM 1566b	NIST	10-30 ºC dry	0.308 ± 0.009
Peach leaves	SRM 1547	NIST	20-25 ºC, dark	0.869 ± 0.018
Rock	GBW 07103	NRCCRM	Dry and dark	31 ± 4
Sediment	GBW 07397	NRCCRM	Dry and dark	16.1 ± 1.9
Sediment	GBW 07398	NRCCRM	Dry and dark	7.8 ± 0.5
Sediment	GBW 07399	NRCCRM	Dry and dark	37.0 ± 2.2
Sediment	GBW 07400	NRCCRM	Dry and dark	21.6 ± 1.2
Bovine liver	SRM 1577c	NIST	10-30 ºC, dark	0.063 ± 0.001
Sediment	IAEA-457	IAEA	Dry and dark	105 ± 7
Road dust	BCR-723	BCR	20-25ºC	866(b)
Soil	SRM 2586	NIST	<30ºC	432 ± 17
Lake Ontario water	TMDA-64.2	NWRI	Cool	288(a)
Fortified water	TMDA-53.3	NWRI	Cool	349(a)

(a) Units: µg/L
(b) Indicative value

6.2.6. Cadmium

Cadmium is a highly toxic environmental element, it usually accumulates in soil, which is then absorbed by plants. Exposure to cadmium and its compounds are known carcinogens, they can cause gastrointestinal, cardiovascular, and respiratory problems. The most studied forms of cadmium are free Cd(II), labile Cd and biosorbed Cd. [34]

In table 6, a list of the reported CRMs used in the publications is shown. It can be seen that most of the materials have an environmental related matrix, being four water and the other four soil or sediment. As the contamination of soil can affect plant-based food, it is important to study the levels of cadmium on this type of food to prevent the consumption of contaminated food and its adverse effects. The available food related matrices are rice, tomato, and peach leaves.

No CRM certified for any specie is currently available, the materials are only certified for total content of Cd.

Table 6. List of CRMs used in the bibliography with certified values for total content of cadmium.

Matrix	**Code**	**Producer**	**Storage**	**Certified value of total Cd** [mg/kg]
Oyster tissue	SRM 1566b	NIST	10-30 ºC dry	2.48 ± 0.08
Water	SRM 1640a	NIST	Sealed inside an airtight container	0.0040 ± 0.0007
Particulate matter	SRM 2786	NIST	Room temperature	4.34 ± 0.07
Particulate matter	SRM 2787	NIST	Room temperature	4.53 ± 0.12
Peach leaves	SRM 1547	NIST	20-25 ºC, dark	0.026 ± 0.002
Rice flour	NIES 10d	NIES	< 30ºC dry	0.401 ± 0.034
Tomato leaves	SRM 1573a	NIST	20-25 ºC, dark	1.517 ± 0.027
River water	SLRS-6	NRCC	+4ºC	$6.3 \cdot 10^{-6} \pm 1.4 \cdot 10^{-6}$
Soil	BCR-700	BCR	4ºC, dry	65.2±3.5
Sediment	GBW 07110	NRCCRM	Cold and dry	0.61 ±0.08
Soil	GBW 07401	NRCCRM	Cool and dry	4.3±0.4
Rice	GBW 10045	NRCCRM	Cool and dry	0.19±0.02
Lake Ontario water	TMDA-64.2	NWRI	Cool	288(a)
Fortified water	TMDA-53.3	NWRI	Cool	118(a)

(a) Units: µg/L

6.2.7. Others

Some other elements are studied throughout the publications, but are not as common as the presented before, this can be observed with the amount of CRM available. None of them have a certified value for any specie, only total content of the elements.

The first element observed in table 7 is vanadium. Vanadium is an element that is widely distributed in the earth's crust but in low concentration. It exists in multiple oxidation states in the environment, being V(IV) and V(V) the most common. It is necessary to determine and differentiate V(V) from the other species, as it is the most toxic form of V, it acts as an inhibitor of enzyme function.[9] The main field of speciation of vanadium is environmental pollution, especially water. Therefore, it is not surprising that five of the eight CRM used for vanadium speciation are water.

The second element present in table 7 is antimony. The interest for the speciation of antimony has grown in the last couple of years as the application of it has spread. Sb is mainly found in inorganic forms, Sb(III) and Sb(V), but some methyl species can be found in water. These are reported to have adverse health effects on human health, the European Union set a limit of 5.0 µg/l in drinking water. The recent published articles that are dedicated to the study of antimony species, focus mostly in environmental matrices.[29,30,39] There is also an article that focuses in the speciation of Sb and As in urban dust.[40] Although the concern against antimony toxicity is high, there is still a clear lack of CRMs.

Lastly, there are tellurium and germanium. The study of tellurium is relatively new, its environmental behaviour is still not fully understood. Because of its use as a semiconductor, the interest in its measurement has increased. It mainly exists in the environment in the form of Te(IV) and Te(VI), both of which are toxic and related to impaired neurotransmission that affects the secretion of human saliva and sweat. There is also an increasing interest in the analysis of germanium due to its increasing use in modern technologies. It mainly exists in the environment as Ge(IV) but it can also be found in some organic forms, including methyl germanium and dimethyl germanium. These materials are mostly studied in environmental samples, including rocks, soil, and sediments. The availability of CRM for these elements is very limited, and especially for tellurium as some materials used for method validation are not even certified.

Table 7. List of CRMs used in the bibliography with certified values of total content of vanadium, antimony, tellurium, and germanium.

Matrix	**Code**	**Producer**	**Storage**	**Certified value of total V** [mg/kg]
River water	SLRS-6	NRCC	+4ºC	$3.5 \cdot 10^{-4} \pm 6 \cdot 10^{-6}$
Apple leaf	SRM 1515	NIST	20-25ºC, dark	0.254 ± 0.027
Rock	GBW 07103	NRCCRM	Cool and dry	24 ± 3
Gabbro	GBW 07112	NRCCRM	Cold and dry	768 ± 42
Water	SRM 1640a	NIST	Sealed inside an airtight container	0.015 ± 0.0002
Seawater	NASS 6	NRCC	Cool	0.0014±0.0002
Hard drinking water	ERM-CAO11	ERM	<5ºC	4.75 ± 0.34(a)
Fortified water	TMDA-54.2	NWRI	Cool	349±25(a)
Matrix	**Code**	**Producer**	**Storage**	**Certified value of total Sb** [mg/kg]
Water	SRM 1640a	NIST	Sealed inside an airtight container	$0.0051 \pm 4.5 \cdot 10^{-5}$
Coal fly ash	SRM 1633c	NIST	Dry	8.56 ± 0.29
Lobster tissue	LUTS-1	NRCC	Cool	2.46 ± 0.28
Tea leaves	GBW 07605	NRCCRM	Cool and dry	0.056±0.006
Citrus leaves	GSB-11	Geostats	Dark	11.66 ± 0.25
Matrix	**Code**	**Producer**	**Storage**	**Certified value of total Te** [mg/kg]
Water	SRM 1634f	NIST	15-25ºC	0.997 ± 0.08 (a)
Rock – anorthosite	CH-4	CANMET	Cool and dry	0.414 ± 0.073
Rock – diabase	TDB-1	CANMET	Cool and dry	0.2 (b)
Stream sediment	GBW 07303	NRCCRM	Cool and dry	0.16±0.03
Pond sediment	GBW 07305	NRCCRM	Cool and dry	0.14±0.04
Matrix	**Code**	**Producer**	**Storage**	**Certified value of total Ge** [mg/kg]
Soil	GBW 07401	NRCCRM	Cool and dry	1.34±0.20
Soil	GBW 07404	NRCCRM	Cool and dry	1.9±0.3
Soil	GBW 07407	NRCCRM	Cool and dry	1.6±0.3
Rock	BHVO-2	USGS	Dark and dry	1.623±0.039
Rock	AGV-2	USGS	Dark and dry	1.202±0.083
Rock	BCR-2	USGS	Dark and dry	1.46±0.26

(a) Units: µg/L
(b) Informational value

In the publications revised, a couple of CRMs for the speciation of thallium were used in various publications but these are no longer available, they have been replaced. These materials were both produced by NIST, one having a matrix of water (SRM 1643e) and the other one having a matrix of a river sediment (SRM 2704). No other materials were commented, this means that the study of thallium is not very common at the moment.

Other CRMs are needed to be developed for other element species, such as iron. Iron speciation is widely used for the identification of the redox conditions in aquatic environments but still no speciation reference material had been developed before. Alcott, *et al.,* developed four RMs that can be used for this determination, but no certification was accomplished. [41]

7. CONCLUSIONS

The results obtained from the bibliographic research from both databases are sufficiently different to say that for this kind of research it is necessary to use more than one database in order to obtain a broader amount of information.

The interest on speciation analysis has only been growing towards the years, everyday more and more concern arises from the need to control the pollution levels on the environment or assess food safety. As more limits have been established, researchers have to develop more accurate methods to meet the requirements. Although some advances have been accomplished and the availability has increased, this work indicates that there is still a lack of available materials certified for trace element species. It has been shown that the variety of matrices and concentration ranges are not sufficient to meet current needs.

In addition, it has been shown that arsenic and mercury continue to be the most prominent fields of speciation and that there is a linearity relation to the number of available materials. The interest regarding other elements, including antimony, selenium, and chromium, has grown recently, more CRMs are expected to be available soon.

The institution that has produced the most used CRMs is NIST, followed by NRCC, JRC (ERM, IRMM, and BCR), and NRCCRM.

To conclude, as the need of CRMs is still high, more research towards the development and certification of these materials is necessary to meet the amount of demand. Due to the high interest on the development of these materials, it is expected that the number of initiatives that moves towards the development of them will grow in the following years.

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12. ACRONYMS

AB - Arsenobetaine

BCR – Bureau for Reference Materials

CANMET - Canadian Centre for Mineral and Energy Technology

CRM - Certified reference material

DMA - Dimethylarsinic acid

ERM - European Reference Materials

HSA - Health Sciences Authority (Singapore)

INCT - Institute of Nuclear Chemistry and Technology

IRMM - Institute for Reference Materials and Measurements (EU)

JRC - Joint Research Centre

KRISS - Korea Research Institute of Standard and Science

LGC - Laboratory of the Government Chemist (UK)

LRM – Laboratory Reference Material

MeHg - Methylmercury

NACIS – National Analysis Center for Iron and Steel

NIES - National Institute of Environmental Studies (Japan)

NIMD – National Institute for Minamata Disease (Japan)

NIST - National Institute of Standards and Technology (USA)

NMIJ - National Metrology Institute of Japan

NRCC - National Research Council Canada

NRCCRM - National Research Centre for Certified Reference Materials (China)

NWRI - National Water Research Institute (Canada)

PRM – Primary Reference Material

QC - Quality control

RM - Reference material

SecRM – Secondary Reference Material

UME – TUBITAK National Metrology Institute (Turkey)

USGS – United States Geological Survey