**T.R.N.C.**
**NEAR EAST UNIVERSITY**
**INSTITUTE OF HEALTH SCIENCES**

# DETERMINATION OF IRON AND CADMIUM IN *MULLUS BARBATUS SPECIES* IN NORTHERN CYPRUS BY GRAPHITE FURNACE-ATOMIC ABSORPTION SPECTROMETRY

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**ANALYTICAL CHEMISTRY**

**MASTER OF SCIENCE THESIS**

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**NICOSIA**
**2018**

## APPROVAL

**To The Directorate of Health Sciences Institute,**

This study has been accepted by the Thesis Committee for the degree of Master of Science in Analytical Chemistry.

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**Approval:**

According to the relevant articles of the Near East University Postgraduate Study – Education and Examination Regulations, this thesis has been approved by the members of the Thesis Committee and the decision of the Board of Directors of the Institute.

Prof. Dr. K. Hüsnü Can BAŞER

Director of Institute of Health Sciences

## DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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**Signature** **:**

**Date** **:** 17 December 2018

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor Assist. Prof. Dr. Usama Alshana, you have shown that you are not only a supervisor but also a brother. Thanks for being patient with me even during my stubborn spells. I learnt so much from you during the course of this work and I hope to work with you in the future.

I also would like to thank my co-supervisor Assist. Prof. Dr. Hürmüs Refiker for being helpful and providing assistance whenever needed. Her resourcefulness and guidance were helpful towards the completion this work.

I want to thank Assoc. Prof. Dr. Hayati Çelik and Assist. Prof. Dr. Banu Keşanlı for giving their time, teaching and support during the course of my M.Sc.

My wonderful father who supported me morally and financially, I am grateful to you for the rest of my life for giving me the opportunity to chase my dream. I understand that it cost a lot of sacrifice in other to reach this level and I pray to Allah to bless you for this.

I would also appreciate my mother who has been my support since childhood, your prayers and word of encouragement have always spurred me on even in the darkest times. I would always hold you close to my heart no matter what happens.

I want to also appreciate my siblings, Afeez and Adebisi for their unwavering support. I also want to thank my sister in law and my beautiful nephew and niece, Majeed and Fawziyah for always giving me something to laugh about. You guys are the boat that rocks my world. I love you all very much

Last but not the least, my friends and colleagues at Near East University, it was pleasure to know you and spend time with you, I am proud to have you and feel grateful to you all.

To my family, who have been with me since the first day of my life till date, without having a doubt about my abilities. Everyone of you are the pillar upon which I exist. I also dedicate this work to my beloved late uncle who died few weeks after I arrived in North Cyprus. I will always remember you and I pray Allah grants you eternal rest.

## ABSTRACT

**Adegbenro, Adeyinka Moshood. Determination of Iron and Cadmium in *Mullus barbatus Species* in Northern Cyprus by Graphite Furnace-Atomic Absorption Spectrometry.**

**Near East University, Institute of Health Science, Analytical Chemistry Program, Master of Science Thesis, Nicosia, 2018.**

Heavy metals are a persistent source of pollution with several reported cases of contamination. Exposure to these toxic metals is still a major risk due to the increment in anthropogenic activities all over the world. As a result, there is a need to constantly monitor their concentration in food. In this study, a simple and effective method was developed using graphite furnace atomic absorption spectrometry (GFAAS) to determine the concentration of iron and cadmium in *Mullus barbatus species*, a known bioaccumulator of heavy metals, consumed in Northern Cyprus. Optimum digestion conditions were as follows: volume of acid used, 1.5 mL; digestion temperature, 100 °C; and digestion time, 120 min. Optimum GFAAS conditions were as follows: absorption wavelength, 248.3 and 229.4 nm; atomization temperature, 2200 °C and 1050; and atomization time, 3 and 4s, for iron and cadmium, respectively. Calibration graphs showed good linearity with coefficients of determination ($R^2$) higher than 0.9950. Accuracy of the developed method for both metals was checked by analyzing a fish certified reference material (CRM) using the conditions above and also a spike recovery method. Percentage recovery (%R) of 95.0% was recorded for iron, while the spike %R of the CRM for cadmium ranged between 89.0 and 92.0%. The results proved that the developed method can be used for the determination of iron and cadmium in *Mullus barbatus* fish species. To the best of our knowledge, this is the first study on the determination of iron and cadmium in *Mullus barbatus species* in Northern Cyprus.



**Keywords:** Acid digestion, Cadmium, Graphite furnace-atomic absorption spectrometry, Iron, *Mullus barbatus*, Northern Cyprus.

# ÖZET

**Adegbenro, Adeyinka Moshood. Kuzey Kıbrıs'ta Yakalanan *Mullus barbatus* Türlerindeki Demir ve Kadmiyumun Grafit Fırın-Atomik Absorpsiyon Spektrometresi ile Tayini.**

**Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Analitik Kimya Programı, Yüksek Lisans Tezi, Lefkoşa, 2018.**

Ağır metallerin kalıcı kirlilik kaynağı oldukları bilinmektedir. Tüm dünyadaki antropojenik faaliyetlerdeki artış nedeniyle bu toksik metallere maruz kalma hala büyük bir risk oluşturmaktadır. Sonuç olarak, yiyeceklerdeki konsantrasyonlarını sürekli olarak izlemek gerekiyor. Bu çalışmada, grafit fırın atomik absorpsiyon spektrometresi (GFAAS) kullanılarak Kuzey Kıbrıs'ta tüketilen ve ağır metal biyoakümülatörü olarak bilinen *Mullidae familyası*ndan *Mullus barbatus* ve *Mullus surmuletus* türlerindeki demir ve kadmiyum konsantrasyonunu belirlemek için basit ve etkili bir yöntem geliştirilmiştir. Optimum özümlenme şartları; kullanılan asit hacmi 1,5 mL, özümlenme sıcaklığı 100 °C ve özümlenme süresi 120 dakikadır. Demir ve Kadmiyum için ise optimum GFAAS şartları sırasıyle; absorplama dalgaboyu, 248,3 ve 229,4 nm; atomizasyon sıcaklığı, 2200 ve 1050 ºC; atomizasyon süresi 3 ve 4 saniyedir. Kalibrasyon grafikleri iyi doğrusallık gösterdi ($R^2 > 0.9950$). Her iki metal için de geliştirilen yöntemin doğruluğu, yukarıda belirtilen koşullar dahilinde CRM analizi ve zenginleştirme ve geri kazanma yöntemi kontrol edilmiştir. Demir için yüzdelik geri kazanım (%R) %95,0 kaydedilirken, kadmiyum için CRM'ın %R'si %89.0 ile %92.0 arasında yer almaktadır. Bildiğimiz kadarıyla bu çalışma gerçekleştirilen çalışma KKTC kıyılarına ait ilk metodolojik çalışmadır.

**Anahtar Kelimeler:** Asit parçalama, Kadmiyum, Grafit Fırın Atomik Absorpsiyon Spektrometri, Demir, *Mullidae Sp,* Kuzey Kıbrıs.

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## LIST OF ABBREVIATIONS

| ABBREVIATION | DEFINITION |
|---|---|
| **%R** | Percentage Recovery |
| **AAS** | Atomic Absorption Spectrometry |
| **DBP** | Disinfection By Products |
| **EDL** | Electrodeless Discharge Lamp |
| **EEA** | European Environmental Agency |
| **EPA** | Environmental Protection Agency |
| **FAAS** | Flame Atomic-Absorption Spectrometry |
| **GFAAS** | Graphite Furnace-Atomic Absorption Spectrometry |
| **HCL** | Hollow Cathode Lamp |
| **ICP** | Inductively Coupled Plasma |
| **LOD** | Limit of Detection |
| **LOQ** | Limit of Quantitation |
| **PMT** | Photomultiplier Tube |
| **PVC** | Polyvinyl Chloride |
| **RSD** | Relative Standard Deviation. |
| **TRNC** | Turkish Republic of Northern Cyprus |
| **USEPA** | United States Environmental Protection Agency |
| **WHO** | World Health Organization |

# CHAPTER 1
# INTRODUCTION

## 1.1 Heavy Metals

In recent years, the importance of environmental conservation has been on the rise due to the nature of contaminants that have been discovered and their effects on the environment. Heavy metals are one of the contaminants that have been of keen interest due to their dual capabilities of functioning as important nutrients in the body as well as being toxic to the extent of causing potential harm to both biotic and abiotic components of the environment. As a result, it has become very important to monitor these contaminants. Many researches have been carried out on heavy metals in different parts of the world in recent years, which have shown how important they are to the balance of the ecosystem. They have also shown that their toxicity is capable of causing adverse effects on different aspects of the environment and they need to be monitored on a regular basis.

Elements in the periodic table that have atomic masses between 63.5 and 200.6 with a specific gravity greater than 5.0 and are metals are generally regarded as heavy metals [1]. Some of these heavy metals include copper (Cu), lead (Pb), zinc (Zn), manganese (Mn), selenium (Se), arsenic (As), iron (Fe), among others.

Heavy metals can be categorized as either being non-essential/potentially toxic (e.g., arsenic, lead, mercury, etc.) or as essential metals (e.g., zinc, manganese, copper, iron, etc.) [2]. Essential metals which are often referred to as trace metals are important to living organisms but can also be potentially toxic when a significantly large concentration of it is ingested by the living organism. As a result, there are specified guidelines from different agencies regarding the concentrations, upon which the metal becomes toxic to the living organisms when ingested. However, non-essential metals when consumed by living organisms at very low concentrations are toxic over a particular period of time [3]. This process is known as bioaccumulation.

Furthermore, it is important to note that the toxicity of a non-essential metal depends on the chemical form, in which the toxic metals exist in the biological system. This is due to the fact that some of these metals exist in multiple compounds which can be in different forms and also with different oxidation states [2]. This implies that a heavy metal can be present at higher concentration in a compound and not be toxic to living or non-living organisms while the same heavy metal will be toxic in lower concentration in another compound.

## 1.2 Uses of Heavy Metals

Heavy metals have become very important for a long time due to their applications in various sectors. They have multiple uses in both the industry and to the existence of both the living and non-living component of the ecosystem.

Iron (Fe) is mainly used in the production of steel, which has multiple uses in households as utensils as well as in various manufacturing industries. It is also used in constructing bridges, fences, heavy machines, automobiles. Iron is also a very important essential metal to living organisms; it is a major component of hemoglobin in the red blood cells of the human body, whose main function is the transport of oxygen to the lungs which then circulates it to other parts of the body. Iron-based nanoparticles have recently been discovered and reported in cases to have been used for water treatment [4] and in improving the efficiency of some processes involved in effluent treatment [5].

Copper (Cu) is used generally for making wires which are used for electrical wiring and in generators as coils. It is also used in the making of coins of money. Copper is an important metal to humans as it functions as an antioxidant. Copper has been described as an antimicrobial agent, which has been adopted in the treatment of water. Copper nanoparticles have been employed for treating water and drinking water distribution due to its nature of inhibiting the growth of microorganisms. Copper has been reported by the Environmental Protection Agency (EPA) to be employed to inhibit the formation of microorganisms during the functioning of heating ventilation and air conditioning systems. The report concluded that the use of copper when compared to other metals employed for a

similar purpose produced the least amount of microorganisms [6]. Another study also supported the antimicrobial properties of copper by suggesting that its use in the clinical environment significantly reduces the number of infections that can occur in a healthcare environment [7]. Zinc oxide nanoparticles have recently been employed in water disinfection due to its high antimicrobial properties [8]. Aluminum (Al) has been employed in varieties of household applications such as cooking utensils, foil papers, cables, electrical wires and much more. A study reported the reaction of urine or wastewater and aluminum for high rate, clean and stable production of hydrogen which can be used as fuel [9]. Another study also reported that amorphous aluminum hydroxide catalyst when reacted with water can be used for hydrogen gas generation on demand, which can be employed for various applications [10].

Mercury (Hg) can exist in different forms, which means that it is not toxic in some forms. It is being used as a result for multiple applications such as in batteries, construction of thermometers, fluorescent lamps and some industrial chemicals.

Other metals such as gold (Au), silver (Ag), and platinum (Pt) are being used in different applications such as jewelry, sensing electrodes, which can be used for analyzing the presence of specific ions in solution. Gold nanoparticles are being extensively used in biomedical applications for the diagnosis and treatment of cancer. The study described them as theranostic agents [11].

Metals have been of great importance in all spheres of human activities in the past and more things can be expected from metals in the nearest future, which means more research on metals is needed in the future.

### 1.3 Occurrence and Release of Metals into the Environment

Metals are found in different compartments of the environment. There have been multiple studies describing different means through which metals occur in the environment. Some

heavy metals have been described to occur in different proportions in the earth crust mainly combining with other elements and existing as ores [12].

There are a few metals which also occur from natural emissions such as volcanic eruptions, weathering of rocks as well as forest fires [13]. Another study described some anthropogenic activities which induce heavy metals into the environment. The anthropogenic activities were classed into point and non-point sources. Some of these point sources include smelting and other mining activities that expose heavy metals into the environment. Non-point sources include others such as agricultural practices which use some chemicals containing metals such as pesticides, manures and many more [14].

There are many other different sources by which some specific metals are released into the environment, where they end up contaminating the ecosystem and leading to different environmental challenges that may pose a threat to both the biotic and abiotic components of the environment.

Cadmium (Cd) is an example of a metal that poses a threat, especially to humans. In high concentrations, it is said to be carcinogenic. It is also reported to be found in ores of lead (Pb) and zinc (Zn) [15]. Another study explains that the use of phosphate-containing fertilizers for agricultural purposes exposes a high concentration of cadmium into the soil and food products produced from these fertilizers due to the high cadmium content in those fertilizers [16].

Other sources of cadmium in the environment includes its usage in rechargeable nickel (Ni)-cadmium batteries [17], as thermal stabilizers for polyvinyl chloride (PVC) in combination with other metals [18]. Cadmium is released into the aquatic environment mostly through discharges of effluents and also through runoffs of agrochemicals. This is taken up by the plants and animals and then redistributed into the food chain [19].

Iron is one of the most abundant metal in the environment. It occurs naturally in many aquatic bodies and also in the earth crust. It is released into these aquatic bodies through

anthropogenic activities, such as discharge of effluents and disposals of household wastes [20]. Another major source of release of iron into water bodies is through mining, untreated effluents from steel production industries, which are a high source of pollution and also from corrosion of metals [21]. Several other metals have different ways by which they are released into the environment. For instance, arsenic (As) is released into the environment through various human activities such as mining of other metals, smelting and also from the use of agricultural pesticides which contain small concentrations of arsenic [22,23]. Chromium (Cr) is another metal which is also released into through processes such as production of steel, wood shavings or dyes [24]. It is, however, important to note that the above-mentioned metals/ metalloids can exist in various oxidation states or forms and their toxicity depends on the form in which it exists in the environment.

There have been various studies that have reported the presence of heavy metals in food substances. A study reported the presence of cadmium in vegetables due to the usage of fertilizers containing cadmium in agricultural practices [25]. Another study also reported the bioaccumulation of metals in the tissues of a specific species of fish, where the report continued by emphasizing on the difference in the concentration of the metals due to the age and size of the fish [26].

### 1.4 Heavy Metal Contamination

Heavy metals have been classified as one of the traditional pollutants affecting the environment in a recent study describing it along with other pollutants such as persistent organic pollutants (POPs), polychlorinated biphenyls (PCPs) and much more. This means that they are also as important as emerging new contaminants such as nanomaterials, disinfection by-products (DBPs) and many more [27] because some of the sources of heavy metals mentioned previously have not been mitigated to a minimal extent.

There have been numerous studies that have reported effects of heavy metal contamination, which are of concern to human health and also pose a significant environmental risk to aquatic life since one of the routes of exposure is through the discharge of wastes into the

aquatic habitat. A study monitored the concentration of heavy metals in some water bodies and an aquatic organism and it reported the possibility of accumulation of heavy metals in the tissues and fish organs [28]. Another study carried out in Bangladesh found the concentration of some metals like nickel, iron, and aluminum to be above the acceptable limits recommended by regulating bodies such as the World Health Organization (WHO) [29]. A similar study was carried out in Iran to check heavy metal contamination and it also found a high concentration of heavy metals polluting the environment with arsenic and selenium being the highest pollutants [30].

Some studies have also been carried out to check the effect of heavy metals in some food samples such as mushrooms [31], fishes sold in the Persian Gulf with results indicating high concentrations of mercury and arsenic in the region [32]. Vegetables such as carrots, cabbage, and potatoes have also been studied with elevated cases of cadmium reported in some of the samples studied [33]. Rice and other food crops such as wheat were also studied along with the soil used for cultivation and high levels of contamination of cadmium, lead and nickel were reported in that study [34].

These various studies were also carried out in various countries such as Turkey [31], Bangladesh [29], China [34] and many more. It can be observed that heavy metal contamination is of high importance due to the environmental risks each pollutant possesses. It can also be observed from these studies that the most common source of these contaminations was from various human activities performed on the environment with very few resulting from natural emissions.

### 1.5 Risk Assessment and Health Risks to Humans

There are numerous potential health risks to humans from exposure to high concentration of heavy metals. There are various agencies that have developed means to determine the risk assessment involved with different pollutants.

The United States Environmental Protection Agency (USEPA) is one of such agencies dedicated to monitoring and suggesting various means to mitigate the effects of environmental pollutants and ensure healthy living conditions for humans and the ecosystem in the United States. The European Environmental Agency (EEA) is another of such bodies that are concerned with environmental challenges in Europe. In Turkey, the Ministry of Environment and Urban Planning is responsible for setting policies that will safeguard the country and minimize the risk involved with environmental pollutions.

There are so many potential health risks to humans as a result of exposure to high concentration of heavy metals in various environments. There have been various studies on the effect of exposure to a high concentration to some of the heavy metals. One of the most prominent and popular diseases caused by exposure of humans to cadmium is the Itai Itai diseases, which happened in Japan in 1912. It was caused by effluents from mining companies. Its effects on humans include weakening of bones as well as organ failures before eventually leading to death.

A study carried out on cadmium toxicity described it as an endocrine disruptor which is capable of causing infertility in human males who have been exposed to it [40]. Although the reports further indicated that there are controversies around, the results with reports that infertility cannot be attributed to just exposure of humans to cadmium but also other metals or substances. But, the report should not be discarded as it can be of vital importance to humans if proven beyond doubt.

Another metal that has been of great importance is iron due to its multiple functions in the human body. However, a recent study showed that iron toxicity can also be quite as detrimental as other heavy metals such as cadmium, lead and others. The study claimed that iron can be extremely toxic and can lead to severe conditions such as organ damage which affects the functions of the bone marrow in the formation of new blood cellular components [35].

Many other potential health risks to humans are associated with exposure to high concentrations of heavy metals. Mercury is another heavy metal with serious health risk to various humans all over the world. This is more serious than it looks because the means of exposure can be through consumption of fish. Mercury has been reported in various studies to bioaccumulate in the organs and tissues of fish [36]. This implies that there must be caution when consuming fish due to the effect of bioaccumulation of the metal in the human body.

It has become a necessity due to the risk involved with exposure to high concentration of these heavy metals that proper risk assessment be carried out in various locations where anthropogenic activities are carried out in order to determine the dangers those activities pose to humans and the ecosystem, while providing means on how they can be mitigated.

## 1.6 Analytical Techniques Used for the Determination of Heavy Metals

There have been multiple studies on various analytical techniques that have been employed for the determination of heavy metals in different samples and media due to the importance and dangers involved with having these heavy metals in the environment.

Spectrometric techniques have been used extensively for the determination of heavy metals. These involve atomic absorption spectrometry (AAS), which has been widely used for this purpose. Some techniques which have been reported under AAS include graphite furnace-atomic absorption spectrometry (GFAAS) [37, 38] and also in different samples such as rice [38], sugar [39], blood and urine [40] and many more, Flame atomic absorption spectrometry (FAAS) has also been employed for determining many metals including iron in different samples [41].

Chemical vapor generation in AAS is commonly employed in analysis of some metals due to their specific properties. Examples of metals that can be determined using these methods include arsenic, mercury, selenium and cadmium. These metals are volatile in nature and require additional steps to prevent analyte loss through volatilization. An example of these

methods is hydride generation, which has been employed in various samples including speciation studies of arsenic species in water [42].

Inductively coupled plasma-mass spectrometry (ICP-MS) is a common powerful technique that has been employed extensively in the analysis of metals in different samples. Nowadays, it is one of the most preferred methods for analysis of trace metals due to its higher atomization efficiency than in AAS, which is as a result of the high energy produced by the plasma and its sensitivity because of the mass spectrometer. Examples of different samples where this technique has been used include air and lake water [43], cocoa powder and chocolate [44], blood [45], mushroom [31], seawater [46] and many more.

## 1.7 Atomic Absorption Spectroscopy (AAS)

Absorption is one of the six methods of optical spectroscopic methods. The other methods include emission, phosphorescence, chemiluminescence, fluorescence, and scattering. These methods have something in common; they all make use of light and measurements are taken based on the interaction of the analyte with light.

Atomic absorption involves the measurement of the concentration of atoms of an element. This is done by passing the light emitted from a hollow-cathode lamp (HCL) through the sample and measuring the amount of light that is absorbed by the analyte. The reduction in the intensity of light coming from the HCL to the detector is proportional to the concentration of the element in the sample.

AAS is an analytical technique that is widely used to measure atoms of an element in multiple samples. It is currently being used to measure over 70 elements in the periodic table. Most of the elements being analyzed using this technique are mostly metals with only a few metalloids and non-metals appearing on the list. The popularity of this technique can simply be attributed to its simplicity and low cost when compared to other techniques.

### 1.7.1 Atomic Absorption Spectrophotometer

The main components of an AAS instrument are as follow: radiation source/ light source/ source lamp, sample holder/ atomization cell, wavelength selector, detection and the signal processor. These components are illustrated graphically in **Figure 1.1**.

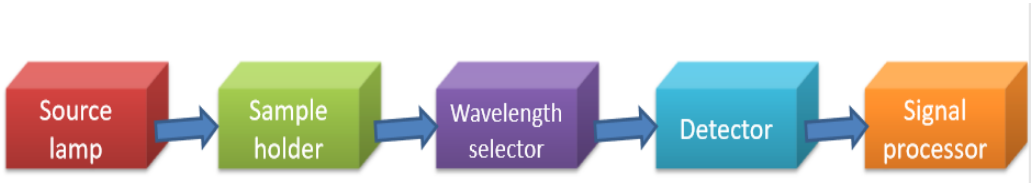


**Figure 1.1:** Graphical illustration of the components of an AAS.

### 1.7.2 Radiation Source

AAS employs the use of two major sources of radiation, which are line and continuum source. The line source is the most common source of radiation available. It is called a line source of radiation because it emits a narrow band of wavelength. A continuum source, on the other hand, emits a wide range of wavelength. The most commonly used examples of line source of radiation used in AAS are hollow cathode lamp (HCL) and electrodeless discharge lamp (EDL).

HCL is the most commonly used light source in AAS. It is an emission source of radiation since it emits radiation that is specific to a particular element. It is made up of a hollow cylindrical cathode, which is made up of the metal of interest or the metal to be analyzed, and an anode which is usually made of tungsten. Both electrodes are enclosed within a tube filled with neon or argon gas. A schematic diagram of an HCL is shown in **Figure 1.2**.

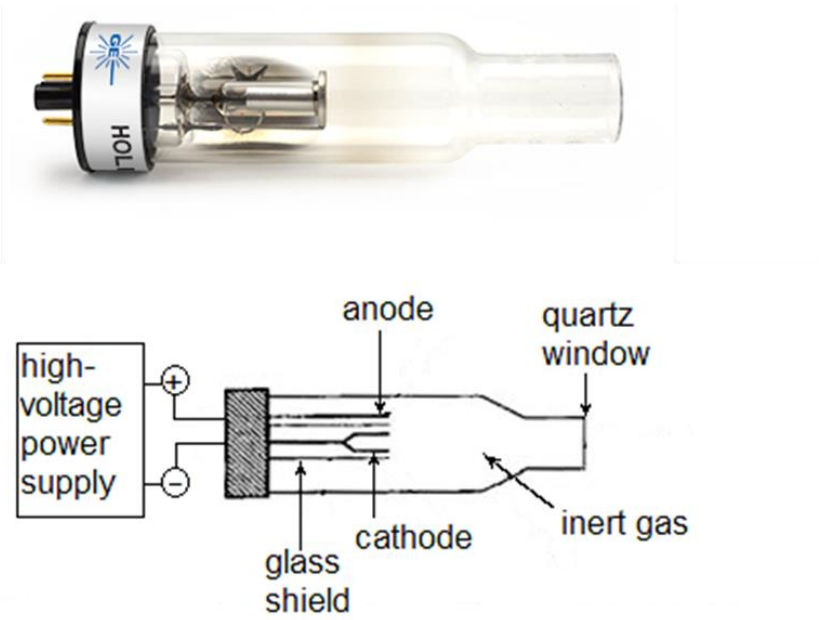


**Figure 1.2:** Hollow Cathode Lamp.

Radiation from the HCL is produced by passing a high voltage of electricity, which causes the gas to ionize, creating plasma. The gas ions accelerate towards the cathode, sputtering off atoms from the surface of the cathode. The gas and the sputtered cathode atoms will be excited by collisions with other atoms/particles in the plasma. As these excited atoms relax to lower states, they emit photons, which are then absorbed by the analyte in the sample.

### 1.7.3 Sample Holder/ Atomization Cell

The sample holder in AAS can be of different types which depend solely on the method of analysis employed for the analysis. The two most common methods of analysis in AAS are FAAS and GFAAS.

The sample holder used for analysis depends on the method is used for the analysis. Other methods used in AAS include hydride generation and cold vapor generation techniques, which are employed in special cases.

The atomization cell, as the name implies, means that apart from holding the sample, this is where molecules present in the sample are broken down into gaseous atoms. This process is known as atomization. The sample holder for FAAS is the flame, while the sample holder for the GFAAS is the sample cuvette/graphite tube. These two types of sample holders will be further discussed later.

### 1.7.4 Wavelength Selection

In spectroscopic analysis, a limited and narrow band of wavelength is desired to increase selectivity. However, radiation sources produce multiple wavelengths. The wavelength selector used in AAS is the monochromator. This functions by taking in polychromatic light (bands of multiple wavelengths) and producing a monochromatic light (a narrow band of wavelength). The narrower the band produced by the monochromator, the higher the sensitivity and selectivity of the instrument.

There are two main types of monochromators, namely prism and grating monochromators with the latter mostly used in more modern instrumentation. A grating can be described as a transmissive or reflective surface with a series of closely spaced lines. The grating monochromator used in AAS uses the dispersive property of light in its functioning. It is also more preferred to prisms due to its characteristics which include;

- Higher ability to resolve adjacent wavelength
- Higher purity of its output
- Higher light gathering power

The grating monochromator in AAS uses the Czerny-Turner grating monochromator and the schematics are shown in **Figure 1.3**.

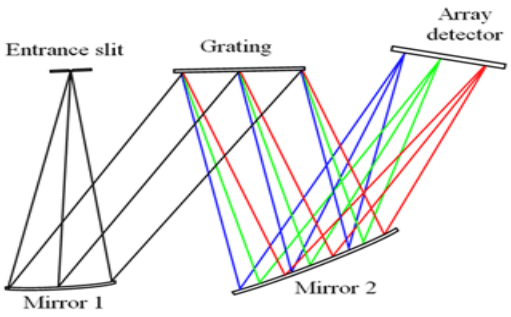


**Figure 1.3:** Czerny-Turner grating monochromator.

### 1.7.5 Detection

Detection in AAS is done after wavelength separation has been achieved. There are different types of detectors in AAS, with photomultiplier tube (PMT) detectors being the most common. PMT converts incident light into a current based on the photoelectric effect. PMT is a very sensitive detector, which is capable of multiplying the current produced by the incident light 100 million times, enabling even individual photons to be detected when the incident light is low. A diagram of the PMT is shown in **Figure 1.4**.

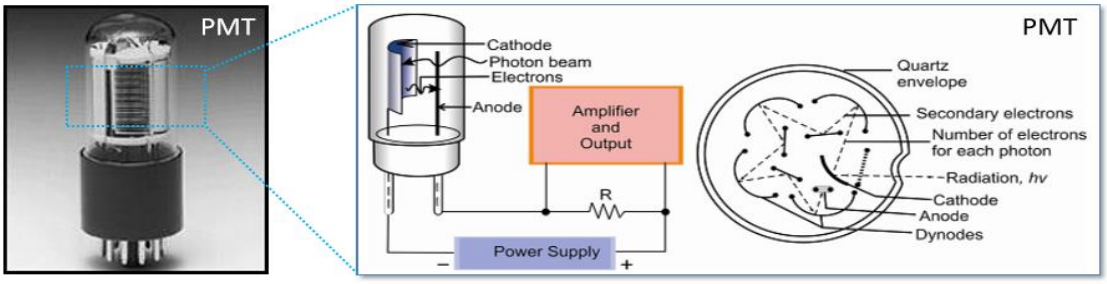


**Figure 1.4:** A diagram of a photomultiplier tube (PMT).

### 1.7.6 Flame-Atomic Absorption Spectrometry (FAAS)

The atomization cell is the part of the instrument which is responsible for producing ground state atoms. Many types of cells are available including those employed for special applications such as hydride generation and cold vapor techniques. The two most common

cells are the flame and the graphite furnace. In FAAS, the flame is the part of the instrument where atomization or the ground state atoms are produced. More than 70 elements in the periodic table can be analyzed using this technique.

### 1.7.7 Types of Flames Used in FAAS

The most common type of flame encountered in FAAS is the pre-mixed laminar flame (**Figure 1.5**). In this type of flame, the fuel and the oxidant gases are mixed before entering the laminar burner in an expansion chamber. The temperature (hotness) of the flame depends on the combination of the flame and oxidant used.

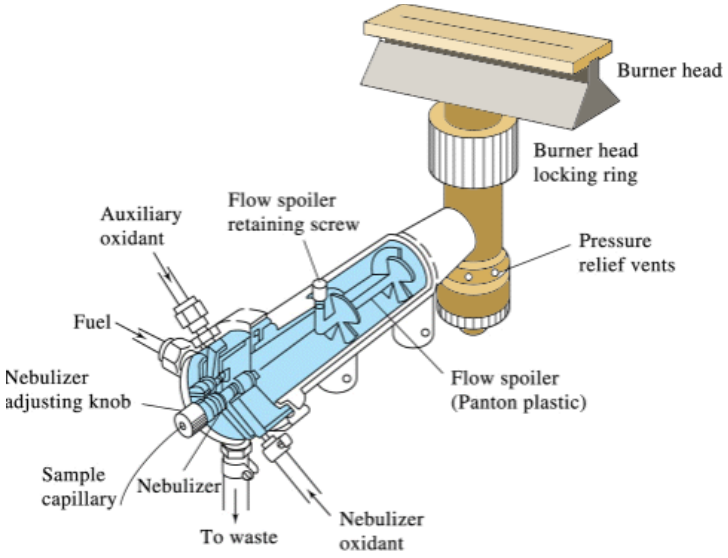


**Figure 1.5:** A laminar flow burner.

There are various types of flame mixtures used in AAS, each providing different properties. These are shown in **Table 1.1**.

**Table 1.1:** Flame Mixtures and Their Properties.

| Fuel | Oxidant | Temperature (°C) | Max. Burning Velocity (cm $s^{-1}$) |
|---|---|---|---|
| Natural gas | Air | 1700-1900 | 39-43 |
| Natural gas | Oxygen | 2700-2800 | 370-390 |
| Hydrogen | Air | 2000-2100 | 300-400 |
| Hydrogen | Oxygen | 2550-2700 | 900-1400 |
| Acetylene | Air | 2100-2400 | 158-266 |
| Acetylene | Oxygen | 3050-3150 | 1100-2480 |
| Acetylene | Nitrous oxide | 2600-2800 | 285 |

The acetylene-air and the acetylene-nitrous oxide flames are the most common flames used in AAS. The acetylene-nitrous oxide flame is reserved for refractory elements such as aluminum and tungsten which are known to be extraordinarily resistant to heat and wear.

### 1.7.8 The Flame Structure

There are different zones present in the flame produced using the fuel and oxidant combination. However, there are three main zones, which are of extreme importance during atomization. They include the primary combustion zone, interzonal region and the secondary combustion zone. The interzonal region is the most preferred region for atomization because it is the hottest region in the flame and also contains enough free atoms which can absorb wavelength from the source and then be excited. In addition, it is thermally stable. The other regions are unsuitable for effective atomization. Thermal equilibrium is not reached in the primary combustion zone. Therefore, it is seldom used. In the secondary combustion zone atoms are converted to stable oxides which escape into the surroundings and do not absorb that wavelength absorbed by the analyte.

A diagram of the flame used in AAS is shown in **Figure 1.6**.

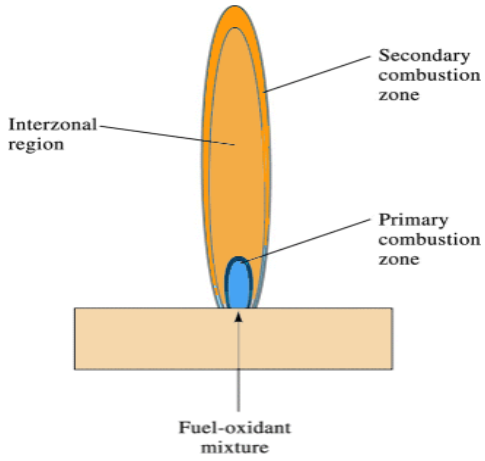


**Figure 1.6:** Diagram showing the different regions of a flame.

### 1.7.9 Graphite Furnace-Atomic Absorption Spectrometry (GFAAS)

This method was introduced by L'vov in 1961. In GFAAS, the atomizer is a graphite tube. The graphite tube atomizer replaces the pre-laminar which is the atomizer in FAAS. The operation of GFAAS includes the introduction of samples in discrete amounts (in μL) into a small opening in a graphite tube. The tube is arranged in such a way that radiation produced from the source can pass directly through the tube. Heating of the tube is done by electricity, and as a result, the GFAAS is also known as electrothermal AAS.

The design, compactness and more effective atomization of the graphite furnace lead to the higher sensitivity of the GFAAS when compared to flame with the latter reaching limits of detection in the parts per billion (μg $L^{-1}$) concentration range. However, the graphite furnace is more expensive when compared to the flame instrument. Longer analysis time is another drawback in GFAAS and the possibility of encountering chemical interferences is higher than in FAAS.

### 1.7.10 Graphite Tube

The graphite tube is the sample holder, or atomizer, in GFAAS. The tube is about 3-5 cm long and has a diameter of about 8 mm. There are different types of graphite tubes but the

most popular one is the pyrolytic graphite tube which is made by heating the graphite tube in a methane environment. This tube is constructed in a way that it allows the light coming from the HCL to pass directly the unit, allowing the atomized analyte to absorb the light from the HCL.

There are also other types of cuvette such as extended life cuvette, coated cuvette, among other. The selection is strongly dependent on the type of metal to be analyzed. A picture of a graphite tube is illustrated in **Figure 1.7**.

**Figure 1.7:** Graphite tube/ cuvette.

### 1.7.11 Temperature-Time Profile in GFAAS

There are four stages involved in an analysis involving the use of GFAAS. These are characterized by the temperature of the system during the analysis and hence, are called the temperature profile. The stages are as follows:

**Drying**: This is the first stage which involves removal of water from the prepared samples. The temperature at this stage is usually at 100 °C.

**Ashing**: This is also called the thermal pre-treatment stage, where organic matter present in the sample is broken down. This stage requires a higher temperature, which is dependent on the complexity of the sample and the analyte (metal) of interest. It can range from as low as 500 to about 950 °C.

**Atomization**: This involves the breaking down of the molecules or compounds into gaseous atomic form. This is the most important step since the absorption of radiation produced by the HCL is absorbed by the free atoms. The temperature at this stage can also

range from 1050 to about 2300 °C. It is highly dependent on the metal or analyte of interest.

**Cleaning**: This involves the use of temperature to clean the graphite tube after every analysis. This is a very important step as it helps to remove the residual materials present in the sample. Removal of these materials helps prevents memory effect on other analysis. The cleaning temperature is usually set by the manufacturer and its temperature is around 2500 °C, followed by cooling down of the instrument through the use of a water coolant system and an inert gas which is usually argon.

## 1.8 Sample Preparation Prior To the Determination of Heavy Metals

Before analysis can be carried out on different samples, it is important to convert the sample and/or analyte into a form that is compatible with the instrument. There are many methods of sample preparation and the choice to be employed during the analysis depends solely on the type of the instrument to be used.

In an analysis involving the use of FAAS and GFAAS, the analytes involved are mostly metals which exist in combined form. It is, therefore, necessary to break down the sample into a suitable aqueous form which can then be atomized by the flame or graphite furnace AAS. Some sample preparation methods employed in FAAS or GFAAS analysis are discussed below.

### 1.8.1 Acid Digestion

This involves the breaking down or decomposition of samples into a solution through the addition of oxidizing acids and heating them until the matrix present in the samples is completely destroyed and the analyte is freed into the solution.

The choice of acids employed for the digestion depends on the complexity of the matrix present in the sample; some samples require one acid, while others require a combination of oxidizing agents and acids for total destruction of the matrix to be achieved.

The most commonly used oxidizing agents and acids in digestion are $HNO_3$, $H_2SO_4$, HF and HCl due to their oxidizing strength. Additionally, these strengths can be further increased by using them with the addition of chlorates, permanganates or hydrogen peroxide. The combination of acids also increases the oxidizing strength but requires adequate care due to the possibility of a violent or explosive reaction.

Acid digestion can be carried out in three different methods based on the type of vessel used, which include the following:

**Microwave-Assisted Digestion**: This is the most commonly used means of digestion of solid samples; it employs the use of microwave radiation to provide the heat required for digestion and also cooling after digestion.

**Closed-Vessel Digestion**: This involves the use of a specially designed digestion block for providing heat to the samples. Depending on the manufacturer and model of the system, the closed-vessel digestion block can either be designed to provide heat to the system or be put into the oven as a source of heat for digestion. This is more preferred when the microwave digestion is not available due to its cost and also its ability to reduce contamination due to the materials used in its construction. The closed-vessel systems use a Teflon perfluoroalkyl vinyl ether (PFA), which greatly reduces the risk of contamination of the samples.

**Open-Vessel Digestion**: This method involves the use of open vessels for digestion; these can either be a beaker or any open container. Heat is applied mostly through a hot plate. This is one of the most classical methods of digestion. However, it comes with some disadvantages such as loss of some analytes due to volatilization and/or higher risk of contamination. This can be solved by the choice of acid used for the digestion and also the digestion apparatus used for the digestion. This problem is not encountered in closed-vessel and microwave-assisted digestion due to their superior set-up.

### 1.8.2 Dry Ashing

This method of sample preparation involves the use of heating the sample in a furnace in the presence of air at temperatures of about 350-800 °C. The residue after decomposition is then dissolved in an acid and then transferred into a volumetric flask before it is analyzed. The organic matter in the sample will be destroyed in the process. The disadvantage of this method is that it leads to loss of analyte when used for volatile elements such as mercury, arsenic, antimony, chromium etc.

## 1.9 AAS for the Determination of Heavy Metals in Fish

There have been multiple studies using AAS for the determination of heavy metals due to the importance of these studies and the possible effects of heavy metals contamination in the environment.

A study, carried out in Brazil, employed GFAAS for the determination of mercury in fish obtained in the Amazon [47]. A recent study also monitored the concentration of arsenic and lead in shrimps from the Persian Gulf [48]. Different heavy metals such as cadmium, mercury, arsenic, lead and zinc were investigated in different fish samples, obtained from the Black Sea, which are highly consumed in Bulgaria [49]. A recent research in Turkey also suggested the use of GFAAS for health assessment of heavy metals in fish obtained from a large reservoir in the country [50]. Durali et al. [51] used FAAS for the determination of trace metals such as iron, zinc and others in about seven fishes in lakes of Tokat.

## 1.10 Literature Review

There have been multiple studies in the literature that employed the use of FAAS or GFAAS in the determination of iron and cadmium in fish. Durali et al. [51] analyzed about seven fish samples from different lakes in Tokat for different metals including iron and cadmium using FAAS and GFAAS. They found an average concentration of between 69.5

and 160 μg $g^{-1}$ of iron and between 0.1 and 1.2 μg $g^{-1}$ of cadmium in the fish samples investigated.

A recent study in China also investigated the bioaccumulation of some heavy metals including cadmium in *Cyprinus carpio* Linnaeus and *Pelteobagrus fluvidraco.* These fish samples were obtained from a lake and the trace concentration of metals was studied in two different seasons. The concentration of cadmium reported from this study ranged between 0.042 μg $g^{-1}$ in summer to 0.024 μg $g^{-1}$ in winter season [52]. Another study in Bulgaria investigated the concentration of cadmium in the muscles of different fish and reported average concentrations of less than 0.010 mg $kg^{-1}$ [49].

Mustafa Canlı [53] worked on the relationship between five trace metals, which included cadmium and iron using FAAS in six different species of fish obtained from the Mediterranean Sea. The study reported concentrations as high as 4.5 μg $g^{-1}$ in cadmium and about 885 μg $g^{-1}$ of iron in the gill of one of the fishes monitored. An assessment study carried out in İzmir, Turkey, on *Mullus barbatus* species for different metals including cadmium using GFAAS, respectively, reported concentrations as high as 0.82 μg $g^{-1}$ in cadmium [54].

### 1.11 Objectives of the Study

The aim of this study is to develop a method using GFAAS to investigate the concentration of trace metals such as iron and cadmium in *Mullus barbatus* species, a species of fish which is commercially available in Northern Cyprus market while also considering seasonal variations of the two metals in the fish.

To the best of our knowledge, this is the first time these two metals have been investigated in *Mullus barbatus* species of Northern Cyprus. This study would help to an assessment of the impact of anthropogenic activities in the region.

## 1.12 Future Work

In the nearest future, there should be a larger assessment of trace metals on more species of fish obtained from different regions of the Island in other to set legislation that regulate the impact of anthropogenic activities on the basin.

# CHAPTER 2
# EXPERIMENTAL

## 2.1 Instrumentation

Metal analysis was carried out using the Thermo Scientific iCE™ 3000 Series Atomic Absorption Spectrophotometer. This instrument is equipped with a graphite furnace system with normal cuvette/graphite tubes, an autosampler with capillary tips for sample introduction, a deuterium lamp for background correction, and hollow cathode lamps for iron and cadmium. The instrument is controlled by the Solaar software for atomic absorption. The instrument is shown in **Figure 2.1**.

A closed-vessel digestion block was used for digestion of the samples. An electronic weighing balance (Mettler Toledo) with ± 0.0001 g precision was used for weighing the mass of the homogenized *Mullus barbatus* species before digestion.

**Figure 2.1:** Thermo Scientific iCE™ 3000 Series AAS.

## 2.2 Reagents and Solutions

Analytical-grade concentrated $HNO_3$ 65-69% purity used for the acid digestion and preparation of the blank and diluent solutions obtained from Sigma-Aldrich (Germany), Stock solutions containing 1000 mg $L^{-1}$ standard solutions of iron and cadmium in 1% pure $HNO_3$ were used to prepare the calibration and spike standards for the standard addition and recovery experiments. Deionized water was used for the entire duration of this study for preparing solutions, diluting solutions and washing glassware before, during and after the analysis. 10% (v/v) $HNO_3$ was used for decontamination of glassware after daily usage.

## 2.3 Apparatus

Binder oven (USA) was used for providing the required temperature for digestion of the fish samples, Eppendorf micropipette of different volumes from Sigma-Aldrich (USA) and tips from ISOLAB laborgeräte GmbH was used for taking the accurate volume of reagents needed. Whatman filters (0.2 μm) were used for filtering solutions before injection into the GFAAS system. A refrigerator from Blomberg was used for preserving samples and standards until analysis.

## 2.4 Sample and Sample Pretreatment

The fishes collected from three different fishing areas in each season were collected in three different sizes as long as the weather conditions allowed. The fork length and age weights of the fish collected were recorded. The collected fishes were stored in the freezer until they were treated. The same season, the same fishing area and the same size of the possible renewable muscle tissue of three fish were taken from the part of the fish shown in **Figure 2.2**.

**Figure 2.2:** Cross-section of edible muscle tissue.

The fish samples were then dried under low temperature and high vacuum using a Labconco brand milling drying system. The dried fish samples were homogenized and transferred to plastic tubes and stored under -4°C until analysis.

## 2.5 Sample Preparation

100 mg of the homogenized *Mullus barbatus* species was weighed using the electronic balance and transferred into the Teflon cups inside the digestion block. Concentrated nitric acid (1.5 mL) was added to the fish sample after which the Teflon cups were covered for 30 min. The digestion block was put into the oven for 120 min at 100 °C. The digested sample was removed from the oven and allowed to cool for about 30 min at room temperature. The digest was filtered using the Whatman 0.22 μm filters and then transferred to a 100-mL volumetric flask. The flask was filled to the 100-mL mark with deionized water. The solutions were stored in the refrigerator until analysis. The procedure is shown graphically in **Figure 2.3**.

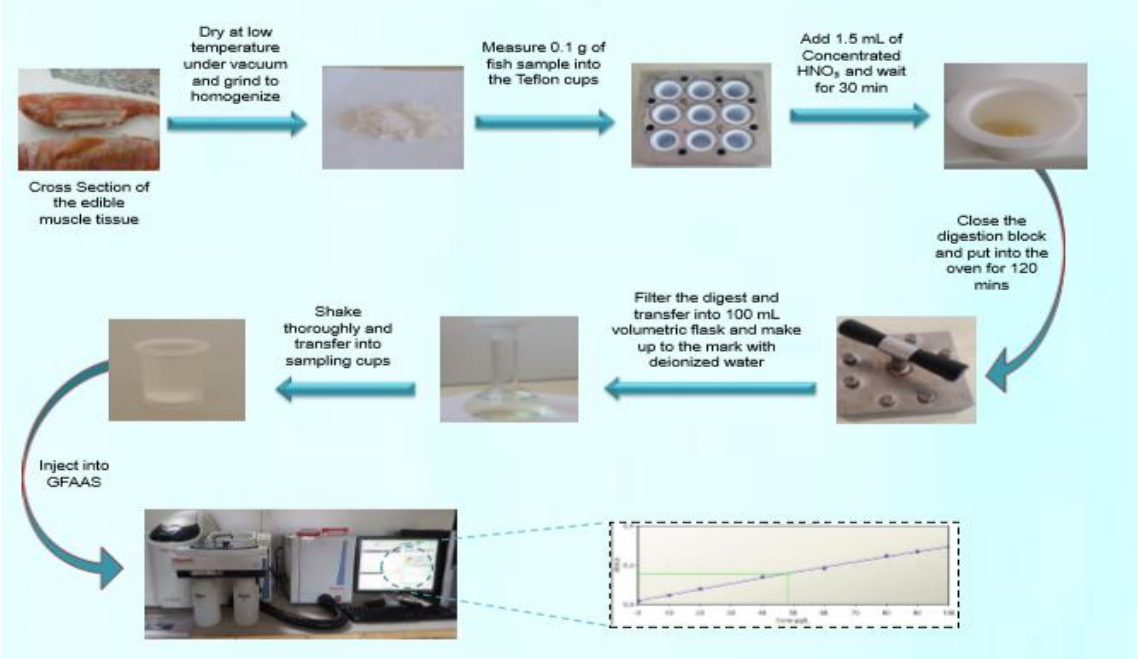


**Figure 2.3**: Procedures involved from sampling to analysis using GFAAS.

# CHAPTER 3
# RESULTS AND DISCUSSION

## 3.1 Selection of Absorption Wavelength for Iron and Cadmium

The absorption wavelength selected for analysis was 248.3 nm for iron and 228.4 nm for cadmium. This primary absorption wavelength was suggested by the instrument manufacturer for analysis and was expected to give the highest sensitivity, precision, and accuracy during the analysis of the metals of interest. There are also other secondary absorption wavelengths that can be produced from the specific HCLs of the metals, but that will amount to a reduction in sensitivity which is undesirable for this study.

## 3.2 Optimization of GFAAS Conditions

The graphite furnace system was optimized in order to obtain the highest possible sensitivity from the instrument. There are four stages in analysis involving the graphite furnace which can greatly affect the output of the signal obtained after an analysis. These stages are temperature and time controlled and are as follows; drying, ashing, atomization and the cleaning.

The drying and the cleaning temperatures are controlled by the software depending on the analyte/metal of interest. These two stages ensure that residual solvent is removed from the sample and also the removal of some matrix substances from the graphite tube after analysis, respectively. The ashing and atomization temperature along with time for both stages were optimized to ensure the best analytical sensitivity and precision from the analysis.

Tuna fish was used for the optimization of these conditions, which is due to the low mass of the *Mullus barbatus* species samples obtained for this study. It was dried at 80 °C and grounded to improve homogeneity. It was also used because it possesses a similar matrix to

the real samples, which is considered as matrix-matched for the purpose of minimizing matrix effect.

### 3.2.1 Optimization of Ashing Temperature for Iron

Ashing, or pyrolysis, is the breakdown of organic matter in the sample. This helps to reduce the amount of chemical interference and also matrix effect in the sample. Optimization started from 850 to 1100 ºC at 50 ºC intervals. It was necessary to optimize this variable because using a lower temperature can result in an observable matrix interference, while the use of higher temperature can in turn result in loss of analyte due to atomization, which is undesirable at this stage. In this experiment, 950 ºC was obtained as the optimum temperature.

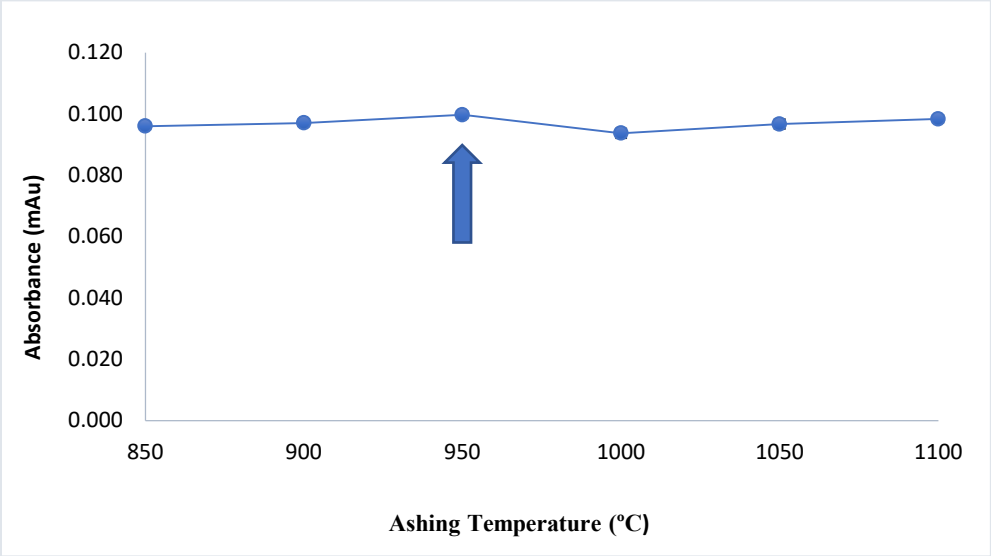


**Figure 3.1**: Optimization of ashing temperature.

### 3.2.2 Optimization of Ashing Time for Iron

The time required for pyrolysis was optimized between 14 and 24 s at 2s intervals. There was no noticeable change in the absorbance observed. The optimum time chosen in this

experiment was 18 s, which was also the same as the time recommended in the software. The effect of this variable on the recovery of analyte is depicted in **Figure 3.2**.

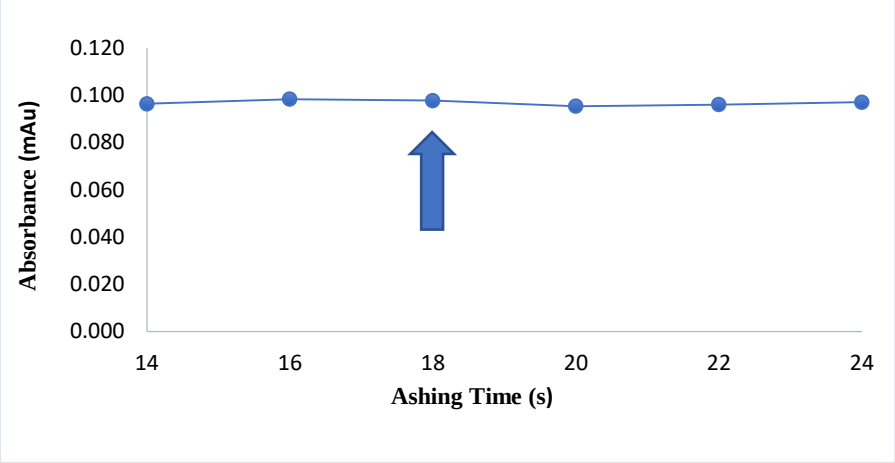


**Figure 3.2:** Optimization of ashing time for iron**.**

### 3.2.3 Optimization of Ashing Temperature for Cadmium

In this experiment, the ashing temperature was optimized for cadmium analysis. The tuna fish used for the optimization was spiked with 20 µL of 10.0 mg $L^{-1}$ of cadmium solution. This was done because the commercial tuna fish used was free of cadmium. The optimization was carried out from 500 to 800 ºC at 50 ºC intervals. The result obtained was as depicted in **Figure 3.3**. The optimum temperature observed was at 600 ºC, beyond which, there was a sharp decrease in analyte recovery when the temperature was increased. This can be due to loss of analyte during the step of atomization of the analyte or due to the volatility of the metal at a higher temperature.

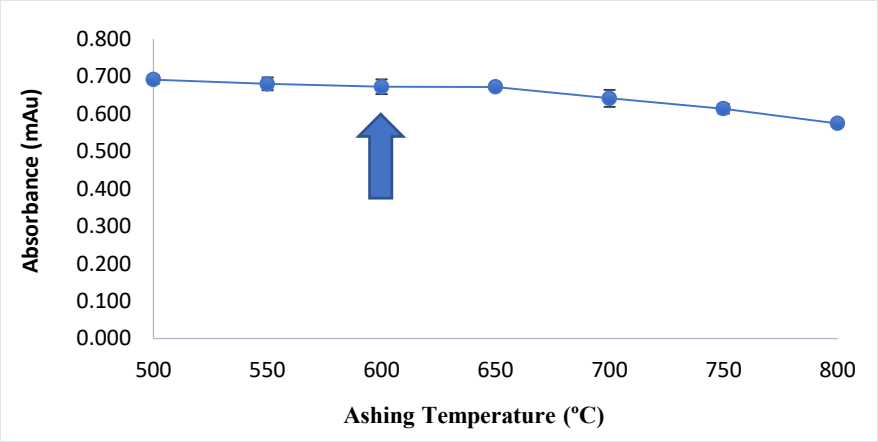


**Figure 3.3**: Optimization of ashing temperature for cadmium.

### 3.2.4 Optimization of Ashing Time for Cadmium

Ashing time was optimized for cadmium. The effect was studied from 14 to 28 s at 2s intervals. The optimum time for ashing was chosen as 20 s, since no signifacant effect was observed as illustrated in **Figure 3.4**. This time is 2s more than the specified time by the instrument (i.e., 16 s).

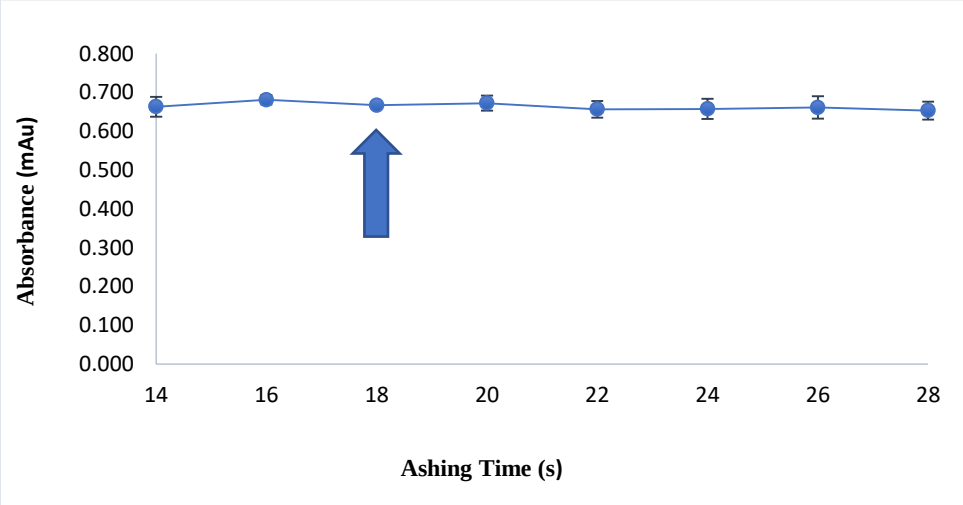


**Figure 3.4**: Optimization of ashing time for cadmium.

### 3.2.5 Optimization of Atomization Temperature for Iron

Atomization is another important parameter in AAS. It is expected that atomization of the analyte in a sample should be very effective to ensure that the analyte of interest should be, to a high degree, converted to gaseous atoms, which absorb incident light from the hollow cathode lamp. It is, therefore, imperative that the effect of this variable is studied. The experiment started from 2000 to 2400 ºC at 50 ºC intervals. The optimum temperature observed from the study as shown in **Figure 3.5** was 2200 ºC. After this temperature, the recovery of analyte had no significant increase when compared with the corresponding increase in temperature.

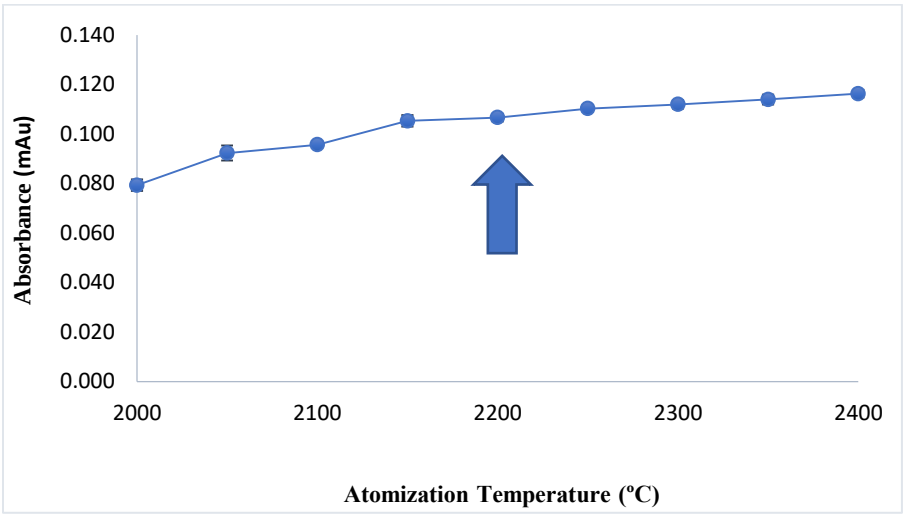


**Figure 3.5:** Optimization of atomization temperature for iron.

### 3.2.6 Optimization of Atomization Time for Iron

The atomization time in this experiment was examined from 2 to 6 s at 1s intervals. The results obtained from this study (**Figure 3.6**) showed that 3 s was the optimum time for atomization of the analyte, as a corresponding decrease in metal recovery was observed with an increase in time beyond this point.

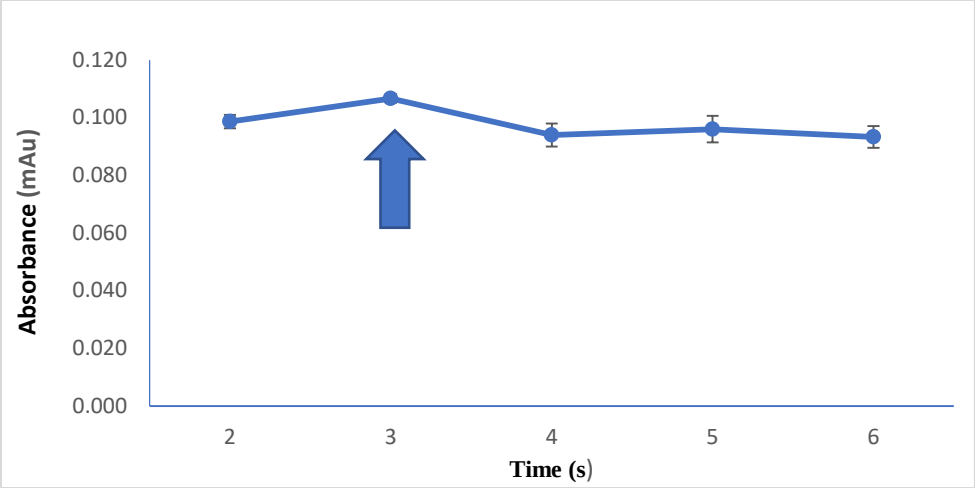


**Figure 3.6:** Optimization of atomization time for iron.

### 3.2.7 Optimization of Atomization Temperature for Cadmium

The atomization temperature optimization procedure was carried out from 950 to 1250 ºC at 50 ºC intervals. The optimum temperature was 1050 ºC as seen in the results depicted in **Figure 3.7**, with a decline in the absorbance of cadmium observed after this temperature. This could be as a result of the loss of cadmium due to its volatilization at higher temperatures.

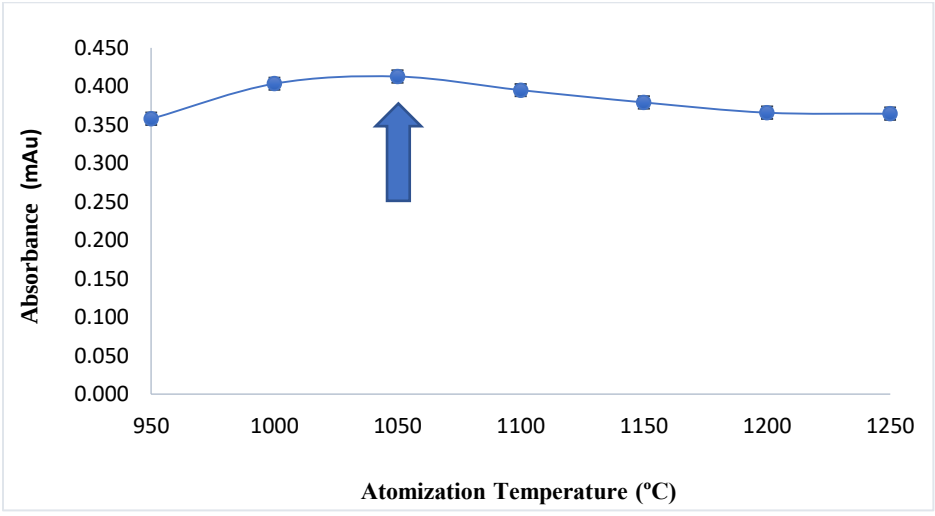


**Figure 3.7**: Optimization of atomization temperature.

### 3.2.8 Optimization of Atomization Time for Cadmium

The optimization time variable was studied from 2 to 6 s at 1s intervals. The optimum time was 4 s as illustrated in **Figure 3.8**. There was no considerable loss of analyte observed in this experiment, which can be attributed to the optimized temperature for this variable and reduced risk of contamination during sample preparation of cadmium.

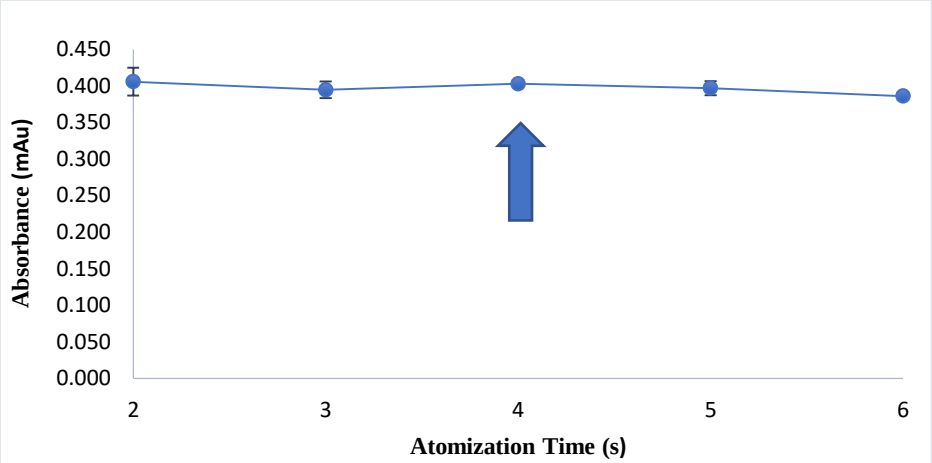


**Figure 3.8**: Optimization of atomization time for cadmium.

## 3.3 Optimized GFAAS Conditions for Analysis of Iron and Cadmium

The optimized GFAAS conditions used for the analysis of both iron and cadmium are given in **Table 3.1** and **Table 3.2**, respectively.

**Table 3.1**: Optimized graphite furnace conditions for iron

| | |
|---|---|
| Background Correction | Deuterium Lamp |
| Wavelength | 248.3 nm |
| Cuvette Type | Normal Graphite Tube |
| Drying Temperature | 100 ºC |
| Drying Time | 30 s |
| Ashing Temperature | 950 ºC |
| Ashing Time | 18 s |
| Atomization Temperature | 2200 ºC |
| Atomization Time | 3 s |
| Cleaning Temperature | 2500 ºC |
| Cleaning Time | 3 s |

**Table 3.2**: Optimized graphite furnace conditions for cadmium.

| | |
|---|---|
| Background Correction | Deuterium Lamp |
| Wavelength | 228.4 nm |
| Cuvette Type | Normal Graphite Tube |
| Drying Temperature | 100 ºC |
| Drying Time | 30 s |
| Ashing Temperature | 600 ºC |
| Ashing Time | 20 s |
| Atomization Temperature | 1050 ºC |
| Atomization Time | 4 s |
| Cleaning Temperature | 2500 ºC |
| Cleaning Time | 3 s |

## 3.4 Optimization of Digestion Conditions

Sample preparation is an important step in any analysis. It is essential that the sample is prepared into a form that is compatible with the instrument employed for the analysis. For

this study, the sample preparation method used was acid digestion. This method can be done using various types of strong oxidizing acids or a combination of these acids. In this study, concentrated $HNO_3$ was used for this purpose. The aim of this digestion procedure is to ensure complete solubilization of the sample.

Acid digestion can be carried out in different media such as in an open-vessel, closed-vessel by using a digestive block and by using a microwave-assisted digestion system. This study made use of the closed vessel system which is better than the open vessel system due to its lesser risk of contamination, loss of analyte when measuring volatile metals such as cadmium, arsenic, mercury and lower volume of acid required when compared to the open-vessel system. The microwave assisted digestion system would be the most preferred method but it was unavailable at the time of this study.

There are some variables which are important in a digestion procedure and they include the following; the digestion temperature, the time of digestion and the volume of acid used. These variables were optimized for this experiment for both analytes (iron and cadmium). The aim of optimizing these variables is to obtain the highest recovery, reproducibility and sensitivity for the metals of interest. Dried and grounded tuna fish was also used for the optimization of the digestion variables, which is because it possesses similar matrix to the real samples and also in much higher quantity than the real samples.

### 3.4.1 Optimization of Digestion Temperature for Iron

The temperatures used for this study started from 80 and ended at 120 ºC with 10 ºC intervals. The range was chosen based on various suggestions from related literature that have employed the use of acid digestion in the preparation of various samples for the determination of iron. 100 ºC was found as the optimum temperature for digestion of the samples as shown in **Figure 3.9**. Other variables such as time and volume of acid used were kept constant during this experiment.

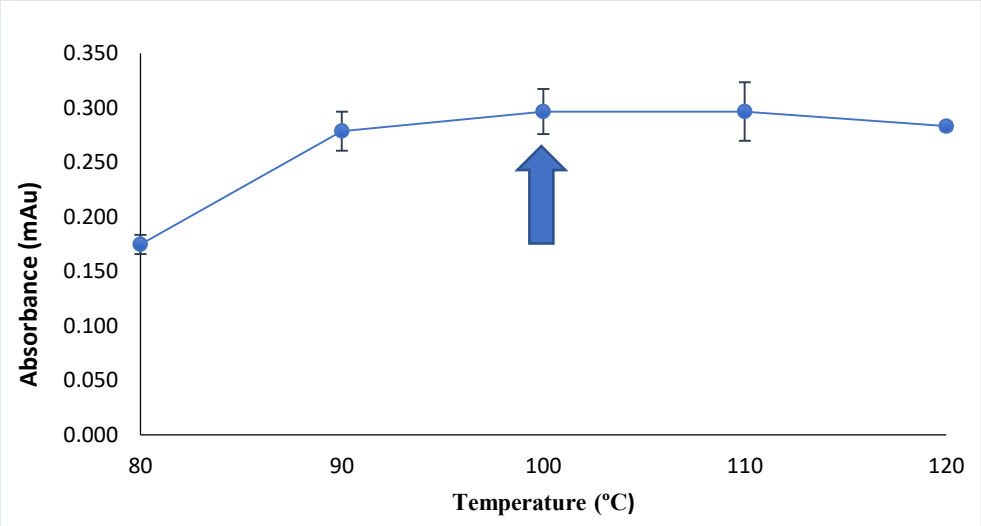


**Figure 3.9:** Optimization of the temperature of digestion for iron.

### 3.4.2 Optimization of Digestion Temperature for Cadmium

Optimization of this parameter started from 80 to 120 ºC at 10 ºC intervals. The optimum temperature, as represented in **Figure 3.10**, was found as 110 ºC.

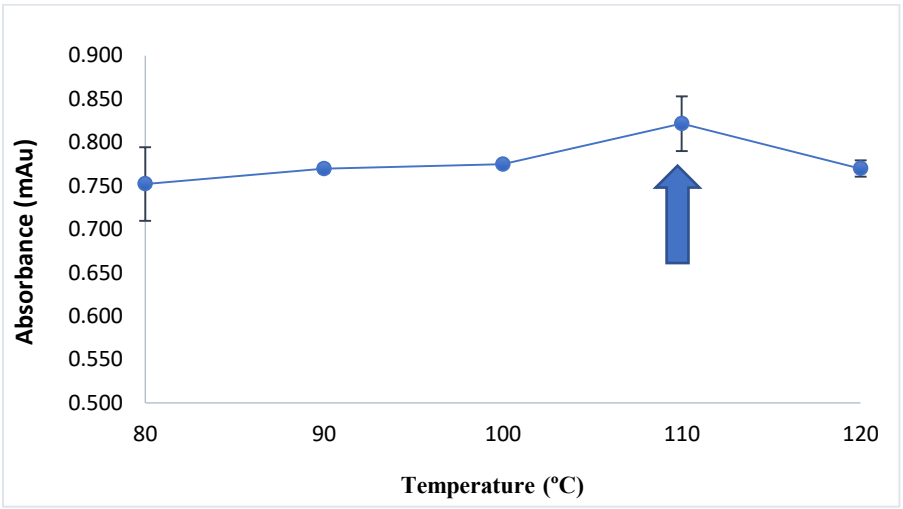


**Figure 3.10**: Optimization of temperature of digestion for cadmium.

### 3.4.3 Optimization of Digestion Time for Iron

The time taken for digestion is also very important in closed-vessel digestion. It is essential that the sample is decomposed completely and the analyte is leached/free from the matrix prior to introduction into the instrument. The time taken for various analytes to be extracted from different samples varies, which has made it important for this variable to be optimized in any digestion procedure. The time for digestion was studied from 0 to 120 min at 30 min intervals. The optimum time was found to be 120 min as shown in **Figure 3.11**.

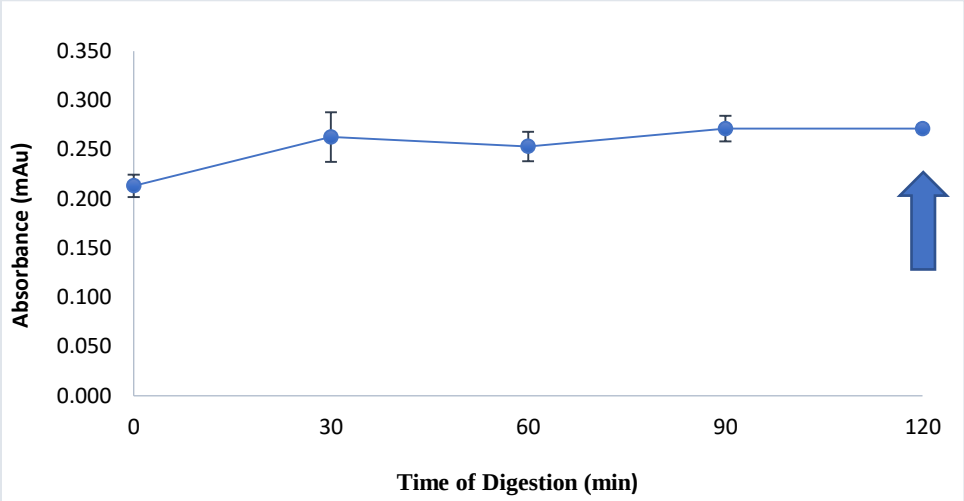


**Figure 3.11**: Optimization of digestion time for iron.

### 3.4.4 Optimization of Digestion Time for Cadmium

Optimization of digestion time for cadmium started from 0 to 120 min at 30 min intervals as carried out in for the optimization of the same parameter for iron. As shown in **Figure 3.12**, the optimum time was found to be 120 min. However, it was observed that this parameter had no effect on the extraction of analyte from the sample; the optimum time was chosen to allow determination of both cadmium and iron from the same sample.

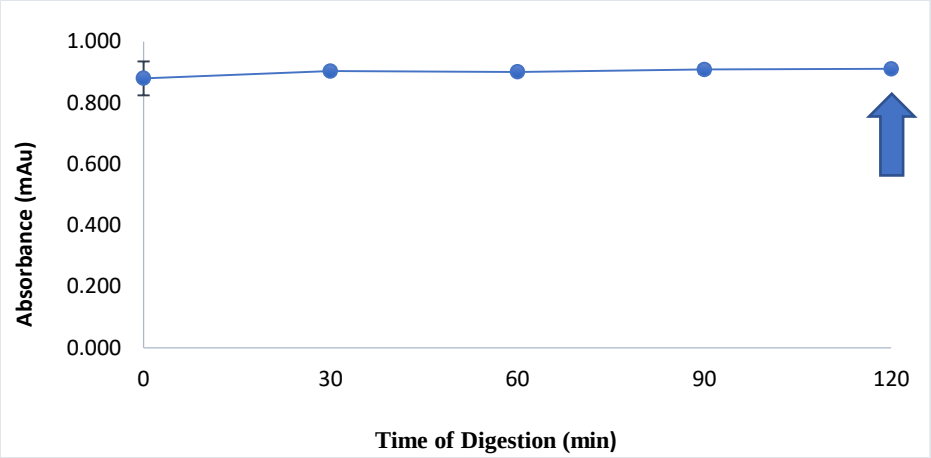


**Figure 3.12**: Optimization of digestion time for cadmium.

### 3.4.5 Optimization of Volume of $HNO_3$ used for Iron

The volume of acid used during the digestion procedure was also optimized. These started from 0.6 up to 1.4 mL at 0.2 mL intervals. This working range was chosen after a review of various literatures where acid digestion was employed in the preparation of fish samples. A volume of 1.4 mL was chosen as the optimum value as shown in **Figure 3.13**.

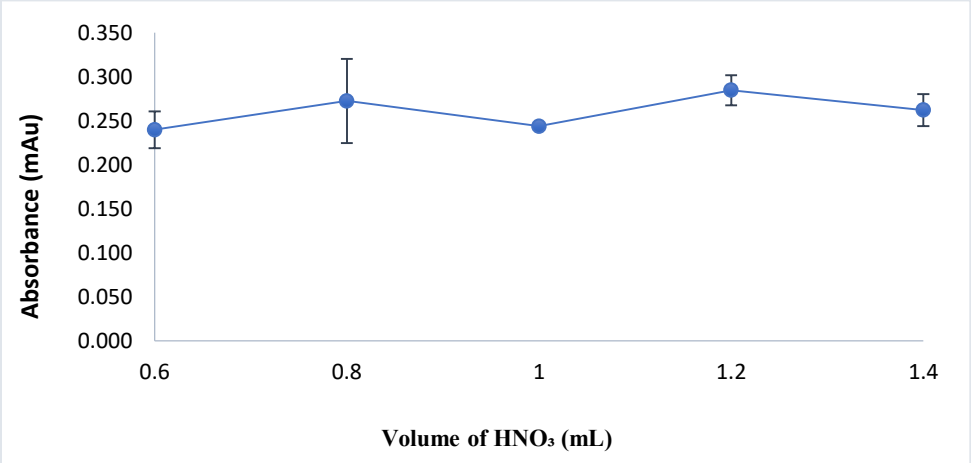


**Figure 3.13:** Optimization of the volume of acid used for iron.

### 3.4.6 Optimization of Volume of $HNO_3$ Used for Cadmium

This variable was optimized using the same range as used for the iron. As seen in **Figure 3.14**, the volume of nitric acid used did not have a significant effect on cadmium recovery as long as at least 0.6 mL is used per 100 mg of the fish. Hence, 1.5 mL of acid was used for both iron and cadmium because the instrument suggests analytical blank to be produced in 1.0% pure nitric acid solution. This meant that the total digest must also be in a 1.0% pure nitric acid solution. All the samples were digested in 1.5 mL of $HNO_3$ and diluted to 100 mL with deionized water to make 1% pure nitric acid solution.

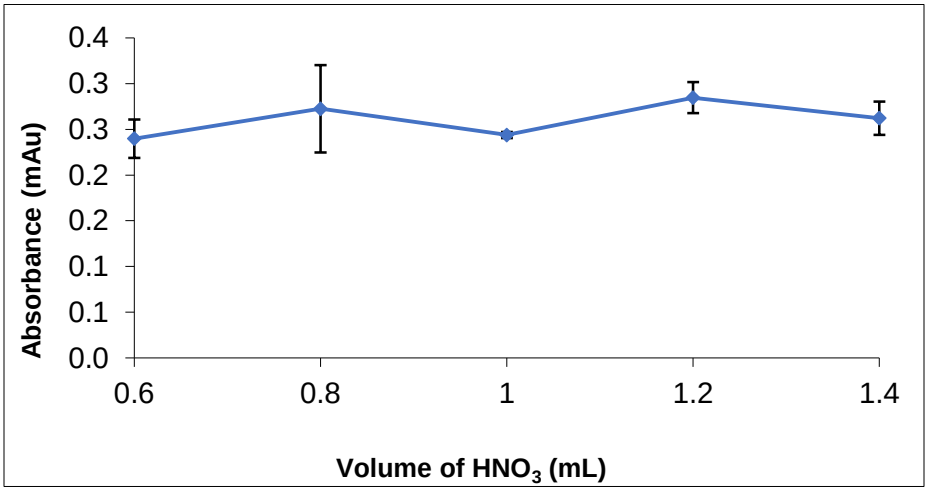


**Figure 3.14**: Optimization of the volume of acid used for cadmium.

## 3.5 Optimized Digestion Conditions

The optimized digestion conditions for iron and cadmium are given in **Table 3.3** and **Table 3.4**, respectively.

**Table 3.3**: Optimized conditions for digestion of iron.

| | |
|---|---|
| Acid Used | Concentrated $HNO_3$ |
| The volume of Acid Used | 1.5 mL |
| Temperature of Digestion | 100 ºC |
| Time of Digestion | 120 min |

**Table 3.4**: Optimized conditions for digestion of cadmium.

| | |
|---|---|
| Acid Used | Concentrated $HNO_3$ |
| Volume of acid used | 1.5 mL |
| Temperature of digestion | 110 ºC |
| Time of digestion | 120 min |

The temperature used for analysis was 100 ºC to enable the simultaneous determination of iron and cadmium using the same digest. Any change in absorbance or concentration of cadmium in the real sample as a result of this would be corrected by performing any of two accuracy checks, which are the standard addition calibration method and the use of a standard reference material.

## 3.6 Calibration and Quantitation

Aqueous calibration curves were prepared in by preparing different standard solutions from 1000 mg $L^{-1}$ solutions of iron and cadmium. The master standards prepared for plotting the aqueous calibration curves were 100 µg $L^{-1}$ for iron and 5.0 µg $L^{-1}$ for cadmium in the instrument. The calibrations obtained are shown below in **Figure 3.15** and **Figure 3.16**.

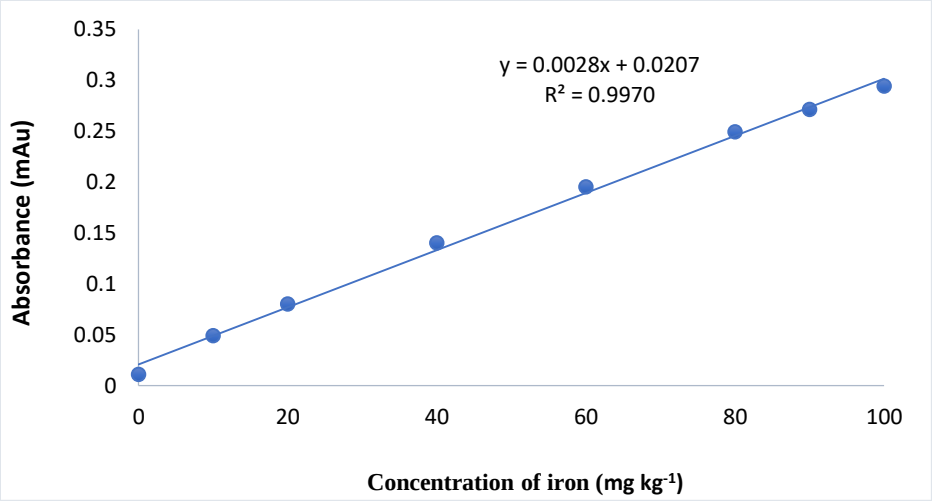


**Figure 3.15**: Aqueous calibration for iron.

The coefficient of determination ($R^2$) as shown in **Figure 3.15** was 0.9970 within a linear dynamic range (LDR) between 10.0 and 100.0 mg $kg^{-1}$, indicating a good linearity.

The aqueous calibration plot for cadmium was also plotted using the prepared master standard, which is 5.0 mg $kg^{-1}$. The LDR was between 1.0 and 5.0 mg $kg^{-1}$. The graph is shown in **Figure 3.16**. However, it should be note that in atomic absorption, the calibration curve is not always linear, with possibility of a quadratic curve as illustrated in **Figure 3.16**. This is due to chemical interferences which is common in graphite furnace atomic absorption spectrophotometry. However, since the software gives the quadratic equation for the curve, it is possible to use it for calculating concentration.

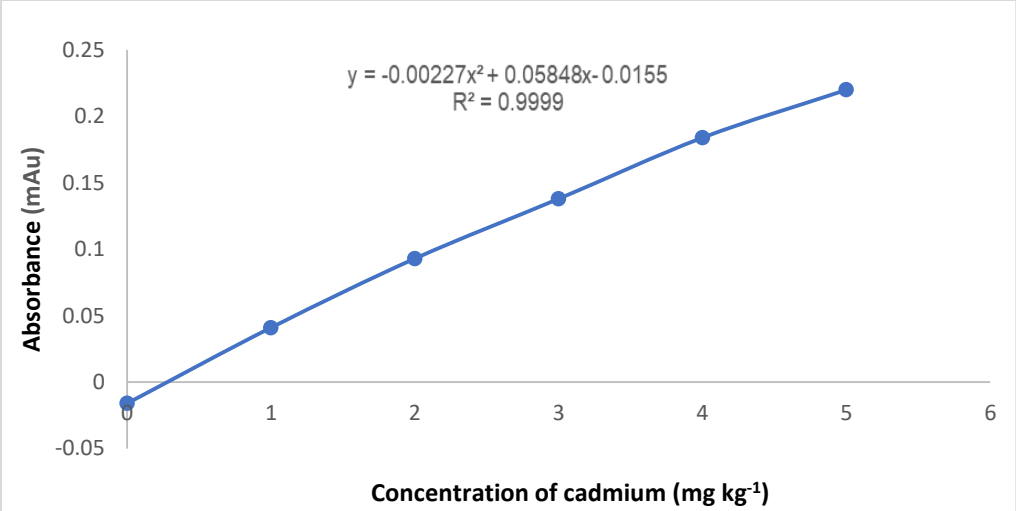


**Figure 3.16**: Aqueous calibration of cadmium.

As seen in **Figure 3.16**, the regression equation and the fit are provided by the software. The coefficient of determination ($R^2$) is 0.9999 within a LDR between 1.0–5.0 mg $kg^{-1}$, indicating a good linearity. In cases such as this, where the calibration curve is not linear, it is important to carry out recovery studies to determine the extent of the interferences. Interference studies can be carried out using methods such as standard addition calibration or the use of a certified reference material (CRM) or a combination of both if they are available.

### 3.6.1 Standard-Addition Calibration

For analysis involving using an analytical instrument for the determination of analyte in a sample or involving the development of a method for quantifying an analyte in a sample, it is essential that an accuracy check is performed on the method to determine the concentration of the analyte present in the sample. Standard-addition calibration was carried out in this study to determine the following;

- Matrix effect and extent of chemical interferences in the analysis
- To calculate the relative recovery of analytes
- To check the accuracy of the method

Standard-addition calibration is normally performed on every sample to determine the matrix effect. However, to minimize matrix effect, the calibration was carried out on pooled samples obtained by mixing the individual samples. Spiking of the pooled sample with standard solutions of iron and cadmium were done at five different concentrations as shown in **Table 3.5** and **Table 3.6**, respectively. Both iron and cadmium were spiked on the same samples to enable determination of both metals in one analysis. Spiking concentration for iron and cadmium were 10 mg $kg^{-1}$, respectively.

**Table 3.5**: Spike volume for standard addition calibration of iron (10 mg $kg^{-1}$).

| **Spiking Volume (µL)** | **Concentration in Final Solution (µg $kg^{-1}$)** |
|---|---|
| 100 | 10 |
| 200 | 20 |
| 300 | 30 |
| 400 | 40 |
| 500 | 50 |

**Table 3.6**: Spike volume for standard addition calibration of cadmium (10 mg $kg^{-1}$).

| **Spiking Volume (µL)** | **Concentration in Final Solution (µg $kg^{-1}$)** |
|---|---|
| 10 | 1.0 |
| 20 | 2.0 |
| 30 | 3.0 |
| 40 | 4.0 |
| 45 | 4.5 |

The standard-addition calibration plots obtained for iron and cadmium are shown in **Figure 3.17** and **Figure 3.18**, respectively.

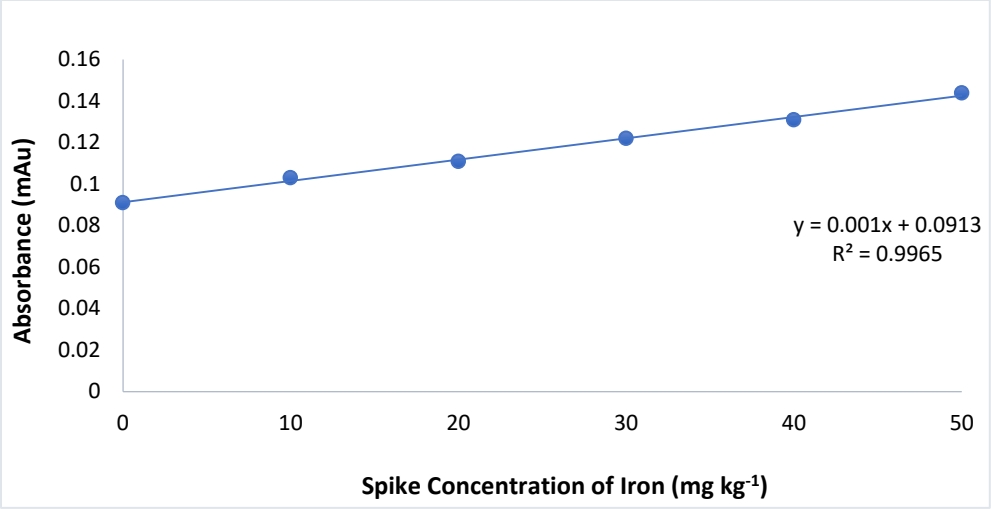


**Figure 3.17:** Standard addition calibration of iron in pooled *Mullus barbatus species*.

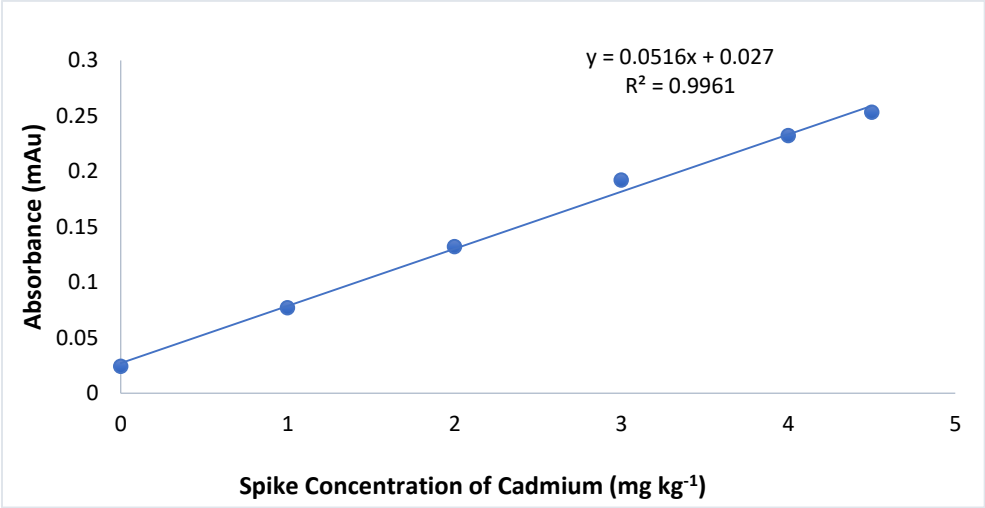


**Figure 3.18:** Standard addition calibration of cadmium in pooled *Mullus barbatus* species.

### 3.6.2 Accuracy Check

Analysis of the certified reference material (CRM, IAEA 436-Tuna Fish homogenate) was done to validate the developed method for accuracy (Reference sheet is shown in **Figure 3.19**). The CRM was digested using the optimized conditions and measured in triplicates. The accuracy of the proposed method for iron obtained percentage recovery (%R) of 95.0%. The concentration of cadmium in the CRM was below LOD. Hence, the

%R was calculated by spiking the CRM at two levels, 2.0 and 3.0 μg $L^{-1}$, and were found to be between 89.0 and 92.5%. The calibration used for calculating the standard and the results of the CRM analysis are given in **Figure 3.20** and **Figure 3.21**, respectively.

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# REFERENCE SHEET

## REFERENCE MATERIAL

# IAEA-436

## TRACE ELEMENTS AND METHYLMERCURY IN TUNA FISH FLESH HOMOGENATE

**Date of issue: 17 February 2006**

**Recommended values: Trace Elements**
***(Based on dry weight)***

| Element | Concentration [1] (mg $kg^{-1}$) | Std Deviation [2] (mg $kg^{-1}$) | 95% Confidence Interval [3] (mg $kg^{-1}$) | N [4] |
|---|---|---|---|---|
| Aluminium | 3.06 | 0.42 | 2.68 – 3.44 | 7 |
| Arsenic | 1.98 | 0.17 | 1.91 – 2.06 | 22 |
| Cadmium | 0.052 | 0.007 | 0.050 - 0.054 | 35 |
| Cobalt | 0.042 | 0.006 | 0.039 – 0.045 | 16 |
| Chromium | 0.194 | 0.058 | 0.168 – 0.219 | 23 |
| Copper | 1.73 | 0.19 | 1.66 – 1.79 | 38 |
| Iron | 89.3 | 4.2 | 87.8 – 90.9 | 30 |

**Figure 3.19:** Reference sheet for certified reference material IAEA 436.

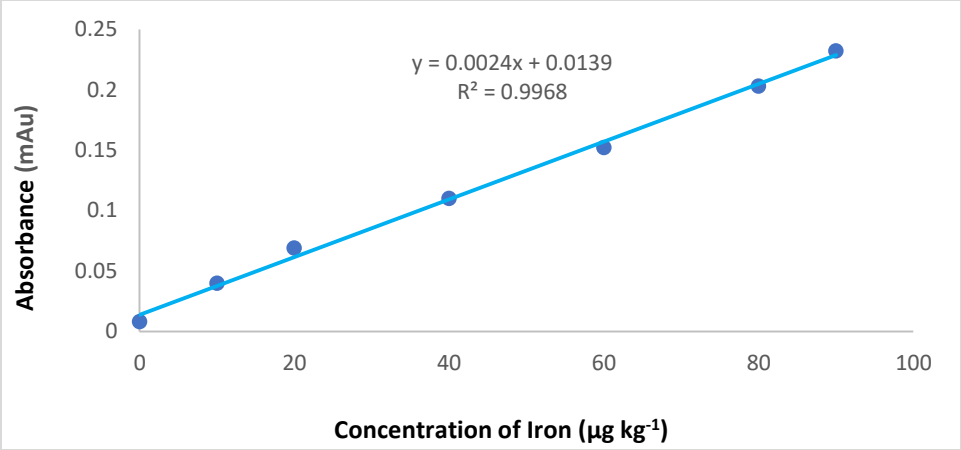


**Figure 3.20:** Aqueous calibration of iron for analysis of certified reference material.

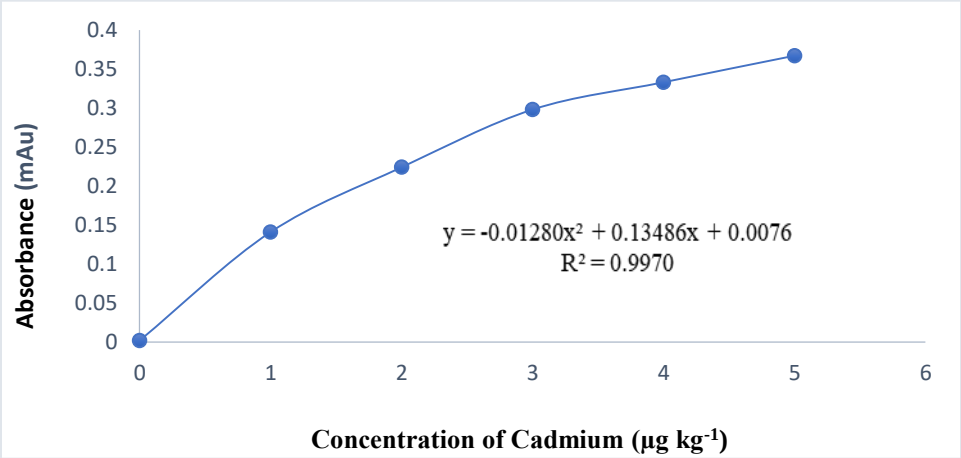


**Figure 3.21**: Aqueous calibration of cadmium for analysis of certified reference material**.**

The certified value for cadmium in the CRM is 0.052 mg $kg^{-1}$. This value is below the LOD of the developed method for this study. Hence, an additional spike recovery experiment was needed for cadmium to validate the accuracy of the developed method. The results are shown in **Table 3.7** and **Table 3.8**.

**Table 3.7**: Determination of iron and cadmium in tuna fish homogenate certified reference material.

| Analyte | Certified values (mg $kg^{-1}$) | Found value (mg $kg^{-1}$) | %RSD | Recovery (%) |
|---|---|---|---|---|
| Fe | 89.3 | 85.6 | 5.0 | 95.6 |
| Cd | 0.052 | <LOD | - | - |

**Table 3.8**: Addition and recovery of cadmium from IAEA 436 (Tuna Fish Homogenate CRM).

| Analyte | Spiked Concentration (µg $kg^{-1}$) | Found value (µg $kg^{-1}$) | %RSD | Recovery (%) |
|---|---|---|---|---|
| Cd | 0.00 | - | - | - |
| | 2.00 | 1.85 | 4.9 | 92.5 |
| | 3.00 | 2.67 | 2.0 | 89.0 |

## 3.7 Results of Sample Analysis

After completing optimization of the conditions, the optimized method was applied to analyze *Mullus barbatus species* samples.

These samples were obtained from different locations for spring and summer seasons from the Island and their concentrations were obtained from the regression equation from the calibration plots shown in **Figure 3.15** and **Figure 3.16**, respectively. The results obtained and the relative standard deviation of the analysis for both iron and cadmium are shown in **Table 3.9** and **Table 3.10**, respectively. The samples obtained in winter and fall seasons were also analyzed. The results obtained from the analysis are shown in **Table 3.11** and **Table 3.12** respectively.

**Table 3.9:** Results for analysis of iron in samples obtained in spring and summer.

| Sample | Sample Location/ Season | Concentration ($mg\ kg^{-1}$)* | %RSD |
|---|---|---|---|
| WLS 2 | Spring Lefke | 12.2 | 5.9 |
| WLS 2 | Spring Lefke | 7.6 | 1.0 |
| WLS 2 | Spring Lefke | 10.5 | 1.9 |
| YLS 2 | Summer Magusa | 19.8 | 2.5 |
| YLS 2 | Summer Magusa | 17.9 | 0.0 |
| YLS 2 | Summer Magusa | 18.3 | 3.1 |
| YLS 3 | Summer Lefke | 11.9 | 3.6 |
| YLS 3 | Summer Lefke | 8.36 | 5.4 |
| YLS 3 | Summer Lefke | 9.36 | 10.3 |
| WMS 3 | Spring Magusa | 25.03 | 1.8 |
| WMS 3 | Spring Magusa | 17.22 | 13.7 |
| WMS 3 | Spring Magusa | 16.68 | 4.7 |
| WMS 1 | Spring Magusa | 10.11 | 1.6 |
| WMS 1 | Spring Magusa | 12.24 | 6.3 |
| WMS 1 | Spring Magusa | 14.02 | 2.2 |
| WYES 3 | Spring Yenierenkoy | 20.07 | 0.0 |
| WYES 3 | Spring Yenierenkoy | 20.78 | 1.7 |
| WYES 3 | Spring Yenierenkoy | 18.29 | 1.2 |
| YMS 3 | Summer Magusa | 17.62 | 10.0 |
| YMS 3 | Summer Magusa | 102.63 | 1.8 |
| YMS 3 | Summer Magusa | 108.32 | 3.7 |
| YLS 1 | Summer Lefke | 15.09 | 2.0 |
| YLS 1 | Summer Lefke | 18.64 | 2.2 |
| YLS 1 | Summer Lefke | 20.48 | 0.6 |
| WLS 4 | Spring Yeniernekoy | 95.51 | 9.5 |
| WLS 4 | Spring Yeniernekoy | 16.15 | 4.3 |
| WLS 4 | Spring Yeniernekoy | 20.78 | 0.7 |

*LOD $= 3.0\ \mu g\ kg^{-1}$

**Table 3.10**: Results of cadmium analysis for spring and summer season.

| Sample | Sample Location/ Season | Concentration ($mg\ kg^{-1}$)* | %RSD |
|---|---|---|---|
| WLS 2 | Spring Lefke | <LOD | - |
| WLS 2 | Spring Lefke | <LOD | - |
| WLS 2 | Spring Lefke | <LOD | - |
| YLS 2 | Summer Magusa | <LOD | - |
| YLS 2 | Summer Magusa | <LOD | - |
| YLS 2 | Summer Magusa | <LOD | - |
| YLS 3 | Summer Lefke | <LOD | - |
| YLS 3 | Summer Lefke | <LOD | - |
| YLS 3 | Summer Lefke | <LOD | - |
| WMS 3 | Spring Magusa | <LOD | - |
| WMS 3 | Spring Magusa | <LOD | - |
| WMS 3 | Spring Magusa | <LOD | - |
| WMS 1 | Spring Magusa | <LOD | - |
| WMS 1 | Spring Magusa | <LOD | - |
| WMS 1 | Spring Magusa | <LOD | - |
| WYES 3 | Spring Yenierenkoy | <LOD | - |
| WYES 3 | Spring Yenierenkoy | <LOD | - |
| WYES 3 | Spring Yenierenkoy | <LOD | - |
| YMS 3 | Summer Magusa | <LOD | 9.4 |
| YMS 3 | Summer Magusa | <LOD | - |
| YMS 3 | Summer Magusa | <LOD | - |
| YLS 1 | Summer Lefke | <LOD | - |
| YLS 1 | Summer Lefke | <LOD | - |
| YLS 1 | Summer Lefke | <LOD | - |
| WLS 4 | Spring Yeniernekoy | 0.49 | 2.3 |
| WLS 4 | Spring Yeniernekoy | 0.45 | 8.2 |
| WLS 4 | Spring Yeniernekoy | 0.47 | 9.1 |

*LOD $= 0.3\ \mu g\ L^{-1}$

The samples measured in winter and fall are shown in **Table 3.11**.

**Table 3.11**: Analysis of *mullus barbatus species* for iron in samples obtained during winter and fall season.

| **Sample** | **Sample Season/ Location** | **Measured Concentration (mg $kg^{-1}$)** | **%RSD** |
|---|---|---|---|
| MEV 1 | Winter Yenierenkoy | 73.1 | 2.8 |
| MEV 4 | Winter Yenierenkoy | 43.1 | 5.0 |
| MEV 6 | Winter Lefke | 66.2 | 7.9 |
| MEV 3 | Winter Yenierenkoy | 42.5 | 7.9 |
| SS 7 | Fall Yenierenkoy | 41.0 | 0.9 |
| SS 5 | Fall Lefke | 8.6 | 8.5 |
| SS 4 | Fall Lefke | 35.8 | 11.1 |
| SS 3 | Fall Lefke | 86.3 | 4.2 |
| MEV 7 | Winter Lefke | 116.8 | 4.3 |
| SS 1 | Fall Magusa | 54.2 | 3.4 |
| SS 10 | Fall Magusa | 38.9 | 9.1 |
| MEV 2 | Winter Yenierenkoy | 40.8 | 4.2 |

**Table 3.12***:* Analysis of *Mullus barbatus species* For Cadmium in Samples Obtained During Winter and Fall Season.

| **Sample** | **Sample Season/Location** | **Measured Concentration (mg $kg^{-1}$)** | **%RSD** |
|---|---|---|---|
| MEV 1 | Winter Yenierenkoy | 0.14 | 63.4 |
| MEV 4 | Winter Yenierenkoy | 0.26 | 17.5 |
| MEV 6 | Winter Lefke | 0.16 | 2.3 |
| MEV 3 | Winter Yenierenkoy | 0.33 | 2.3 |
| SS 7 | Fall Yenierenkoy | 0.23 | 5.8 |
| SS 5 | Fall Lefke | 0.25 | 4.9 |
| SS 4 | Fall Lefke | 0.37 | 14.1 |
| SS 3 | Fall Lefke | 0.29 | 7.4 |
| MEV 7 | Winter Lefke | 0.30 | 10.6 |
| SS 1 | Fall Magusa | 0.52 | 1.4 |
| SS 10 | Fall Magusa | 0.65 | 4.7 |
| MEV 2 | Winter Yenierenkoy | | |

# CHAPTER 4

# CONCLUSION AND RECOMMENDATION

The aim of the study was to develop a simple and reliable method that would have high efficiency using graphite furnace-atomic absorption spectrometry for the determination of iron and cadmium in fish samples.

To the best of our knowledge, this is the first study to determine the concentration of iron and cadmium using graphite furnace atomic absorption spectrometry in TRNC.

Other objectives set for the experiments included extraction of the analytes from the sample matrix while ensuring the highest recovery expected and eliminating interferences. The use of a closed-vessel digestion block for this study reduced the risk of contamination and chemical interferences that is common with the open digestion system.

The method was used to determine the concentration of iron and cadmium in *Mullus barbatus* species, a known bioaccumulator of heavy metals in fish, to help assess the impact of anthropogenic activities on fish consumed in TRNC.

Optimized instrumental and digestion parameters were applied to plot standard-addition calibration graphs through spiking of pooled *Mullus barbatus* species samples with known concentrations of iron and cadmium to minimize matrix effect in the samples.

The accuracy of the proposed method was checked and carried out by analysis of IAEA 436, a certified reference material (CRM) under optimized instrumental and extraction conditions. The recoveries of the analyte obtained from the experiment was 95% for iron. Spike experiments at two levels was carried out on the CRM to check the accuracy of the method for cadmium and was found to fall between 89 and 92%. The relative standard deviation for this experiment was less than 7%.

The analysis of the samples obtained for this study during the spring and summer season had concentrations between 7.0 and 109 mg $kg^{-1}$ for iron and between 0.3 and 0.47 mg $kg^{-1}$ for cadmium.

It is recommended that a more comprehensive study be carried out to study the effect of other heavy metals in order to set legislations and control human activities on the Mediterranean Sea shores of the TRNC.

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