QUALITY AND ELEMENTAL CHARACTERIZATION OF COMMON SPICES OF BANGLADESH USING NUCLEAR REACTOR-BASED NAA AND GAMMA IRRADIATION TECHNIQUES

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MILITARY INSTITUTE OF SCIENCE SCIECE AND TECHNOLOGY

2020

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(*B.Sc Engg., HSTU*)

A THESIS SUBMITTED

FOR THE DEGREE OF MASTER OF ENGINEERING

DEPARTMENT OF NUCLEAR SCIENCE AND ENGINEERING

MILITARY INSTITUTE OF SCIENCE SCIECE AND TECHNOLOGY

2020

The thesis Titled "**Quality and elemental characterization of common spices of Bangladesh using nuclear reactor-based NAA and gamma irradiation techniques**" Submitted By: **Md. Mahfuzur Rahman**, Roll No: **1016280011** Session: April, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science in Nuclear Science and Engineering on 15 January2020.

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I do hereby declare that the thesis entitled "**Quality and elemental characterization of common spices of Bangladesh using nuclear reactor-based NAA and gamma irradiation techniques**" submitted by ID No: 1016280011, Department of Nuclear Science and Engineering, Military Institute of Science and Technology (MIST), Mirpur Cantonment, Dhaka-1216, Bangladesh in partial fulfillment of the requirement for the Degree of Masters of Science. This work has been carried out in the Institute of Nuclear Science and Technology and Institute of Radiation and Polymer Technology, Bangladesh Atomic Energy Research Establishment, Savar, Dhaka. No part of the research work presented in the thesis paper has been submitted for any other degree or qualification in any university or other institute of learning.

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ABSTRACT

In this study, elemental characterization and gamma irradiation technique for bacterial decontamination of common spices available in Bangladesh were evaluated. Total concentrations of 17 essential and toxic elements (Al, As, Br, Ca, Cd, Cl, Co, Cr, Fe, K, Mn, Na, Ni, Pb,Sc, V and Zn) in spices were determined by research reactor-based neutron activation analysis (NAA) and atomic absorption spectrometry (AAS) techniques. This study indicates that spices are a good source of a combination of the essential elements-Ca, Fe, K, Mn, Na and Zn. This study revealed that concentrations of some toxic elements As, Cd, Cr and Pb in some spices were higher than the WHO/ FAO permissible levels. However, health risks associated with these elements evaluated by dietary intake, target hazard quotient and target carcinogenic risk indices indicate that people would experience no potential risks due to consumption of the spices. This study also evaluated the effect of gamma radiation on the decontamination of the microbial population, physico-chemical, nutritional and sensory quality of spices during storage. Spices were irradiated with gamma doses of 0 (as control), 2, 4, 6, 8 and 10 kGy, packed in the glass vials and then stored at room temperature (22 ± 2^0C) and different properties were measured after 6 months. In this investigation, major food borne disease-producing organisms like *Bacillus, Salmonella* and *Listeria* species were identified in spice samples by molecular approaches. The results of this study indicated that gamma irradiations with increased doses significantly reduce the population of organisms compared to control and optimum gamma irradiation doses (6 kGy for red chili and turmeric; 4 kGy for cumin, coriander, garlic and black pepper; 2 kGy for ginger powder) were identified for decontamination of the microorganisms in the studied spices.

ACKNOWLEDGEMENTS

At first I express my entire admiration, devotion, greatest and deepest attitude to the almighty, the supreme of the universe, to whom all praise go for enabling me to complete my thesis work. I would like to express my gratitude, appreciation, deepest sense and indebtedness to my honorable supervisor Dr. Ruhul Amin Khan, Director, Institute of Radiation and Polymer Technology and co-supervisor Dr. Mohammad Amirul Islam, Principal Scientific Officer, Reactor and Neutron Physics Division, Institute of Nuclear Science and Technology, Atomic Energy Research Establishment (AERE), Savar, Dhaka for their enthusiastic guidance and instruction, constructive suggestion and consultant encouragement through this research work. I have nothing to say, simply because there is no way to estimate their kindness to me.

I am grateful to the Department of Nuclear Science and Engineering (NSE), Military Institute of Science and Technology (MIST) to permit me to carry on this research work. As my experiment has two parts one part done in INST and another part done in IRPT, AERE, Savar, Dhaka, I am extremely grateful to Director, INST and IRPT for kindly allowing me to use all the laboratory facilities that I required for research work. Moreover, the parts of my research have done in National Institute of Biotechnology, Institute of Food and Radiation Biology and Environmental Water Resources and Costal Engineering Department of MIST. I am really grateful for the staffs of those institutes. I want to special thanks to some parsons Eng. Md. BorhanUddin, Zahirul Islam Mollah, Md. Razzak and Mostafa Kamal scientific officer of the IRPT, Savar, Dhaka.

I also convey my gratitude to, Col Md Rosaidul Mawla, Col A Z M Salahuddin, Dr. M. A. Rashid Sarkar, Lt Col Dr. Md. Altab Hossain, Cap Md. Sifatul Muktadir and Md. Ghulam Zakir, teachers of the department of NSE, MIST, for their cordial love, spontaneous help, untiring efforts, unparalleled encouragement and deep assistance that played a significant role in completion of this thesis work. Without their indispensable help it was difficult to complete this thesis work. Finally, I want to dedicate the essence of my purest love to my parents who have made my existence possible. The greatest pleasure to me is to think about them.

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List of Symbols

Abbreviation	Term
AERE	Atomic Energy Research Establishment
IAEA	International Atomic Energy Agency
NAA	Neutron Activation Analysis
INAA	Instrumental Neutron Activation Analysis
AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectroscopy
XRF	X-Ray Fluorescence
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
RNAA	Radiochemical Neutron Activation Analysis
TNAA	Thermal Neutron Activation Analysis
ENAA	Epithermal Neutron Activation Analysis
PGNAA	Prompt Gamma-ray Neutron Activation Analysis
WHO	World Health Organization
HPGe	High Purity Germanium
ENAA	Epithermal Neutron Activation Analysis
PGNAA	Prompt Gamma-ray Neutron Activation Analysis
ICPS	Inductively Coupled Plasma Spectroscopy
ENAA	Epi-Thermal Neutron Activation Analysis
SRM	Standard Reference Material
CRM	Certified Reference Material
NIST	National Institute of Science and Technology
SD	Standard Deviation
PGNAA	Prompt Gamma-ray Neutron Activation Analysis
MNAA	Molecular Neutron Activation Analysis
DGNAA	Delayed Gamma-ray Neutron Activation Analysis
FNAA	Fast Neutron Activation Analysis
TRIGA	Training, Research, Isotopes, General Atomics
GM	Geiger-Mueller

IRPT	Institute of Radiation and Polymer Technology
FAO	Food and Agriculture Organization
INST	Institute of Nuclear Science and Technology
ADC	Analog to Digital Converter
DSA	Digital Spectrum Analyzer
TBC	Total Bacterial Count
XLD	Xylose Lysine Deoxycholate Agar
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose Agar
EMB	Eosin Methylene Blue Agar
MTDI	Maximum Tolerable Daily Intake
EDI	Estimated Daily Intake
THQ	Target Hazard Quotients
TCR	Target Carcinogenic Risk
GR	Gamma Radiation

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Chapter 1

Introduction

1.1 Background

Analytical science to develop the methodology for the investigation of properties and structure of matter at level of single nucleus, atom and molecule, and scientific analysis to determine either chemical composition or elemental contents in a sample are indispensable in basic research and development, as well as in industrial applications. Following the discovery of neutron by J. Chadwick in 1932 (Nobel prize, 1935) and the results of F. Joliot and I. Curie in 1934, neutron activation analysis was first developed by G. Hevesy and H. Levi [1, 2]. They used a neutron source (^{226}Ra +Be) and a radiation detector (ionization chamber) and promptly recognized that the element Dy (dysprosium) in the sample became highly radioactive after exposure to the neutron source. They showed that the nuclear reaction may be used to determine the elements present in unknown samples by measuring the induced radioactivity. Thereafter, the development of the nuclear reactors in the 1940s, the application of radiochemical techniques using low resolution scintillation detectors like NaI(Tl) in the 1950s, the development of semiconductor detectors (Ge, Si, etc.) and multichannel analyzer in the 1960s, and the advent of computers and relevant software in the 1970s, the nuclear technique has advanced to become an important analytical tool for determination of many elements at trace level. In spite of the developments in other chemical techniques, the simplicity and selectivity, the speed of operation, the sensitivity and accuracy of NAA have become a powerful analytical technique and a primary method of measurements [3]. Nowadays, there are many elemental analysis methods that use chemical, physical and nuclear

characteristics. However, a particular method may be favored for a specific task, depending on the purpose. Neutron activation analysis (NAA) is very useful as sensitive analytical technique for performing both qualitative and quantitative multi-elemental analysis of major, minor and traces components in variety of terrestrial samples and extra-terrestrial materials.

In addition, because of its accuracy and reliability, NAA is generally recognized as the"refereemethod"ofchoicewhennewproceduresarebeingdevelopedorwhenother methods yield results that do not agree. It is usually used as an important reference for other analysis methods. Worldwide application of NAA is so widespread it is estimated that approximately 100,000 samples undergo analysis each year. The method is based on conversion of stable atomic nuclei into radioactive nuclei by irradiation with neutrons and subsequent detection of the radiation emitted by the radioactive nuclei and its identification. The basic essentials required to carry out an analysis of samples by NAA are a source of neutrons, instrumentation suitable for detecting gamma rays, and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei. Brief descriptions of the NAA method, reactor neutron sources, and gamma ray detection are given below.

1.1.1 Neutron activation analysis

Neutron activation analysis (NAA) is a nuclear process used for determining the concentrations of elements in a vast amount of materials. NAA relies on excitation by neutrons so that the treated sample emits gamma-rays. It allows the precise identification and quantification of the elements, above all of the trace elements in the sample. NAA has applications in chemistry but also in other research fields, such as biological science, geology, archaeology, medicine, environmental monitoring and even in the forensic science.

1.1.2 Basic principles of NAA

The sequence of events occurring during the most common type of nuclear reaction used for NAA, namely the neutron capture or (n, gamma) reaction, is illustrated in (Figure 1.1) Creation of a compound nucleus forms in an excited state when a neutron interacts with the target nucleus via a non-elastic collision. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus.

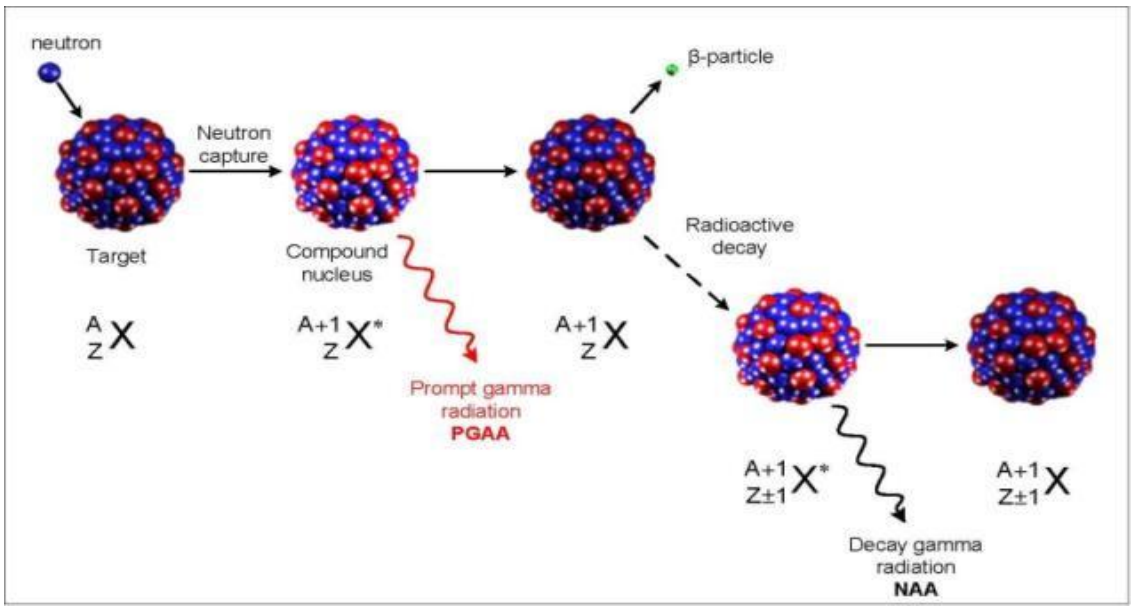


Figure 1.1: Diagram illustrating the process of neutron capture by a target nucleus followed by the emission of gamma rays [1, 2].

The compound nucleus will almost instantaneously de-excite into a more stable configuration through emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also de-excites (or decays) by emission of one or more characteristic delayed gamma rays, but at a much lower rate according to the unique half-life of the radioactive nucleus. Depending upon the particular radioactive species, half-lives can range from fractions of a second to several years.

1.1.3 Classification of NAA method

There are various forms of neutron activation analysis, which are as follows:

- Instrumental neutron activation analysis(INAA)
- Radiochemical neutron activation analysis(RNAA)
- Thermal neutron activation analysis(TNAA)
- Epithermal neutron activation analysis(ENAA)
- Prompt gamma-ray neutron activation analysis(PGNAA)
- Fast neutron activation analysis(FNAA)
- Delayed gamma-ray neutron activation analysis(DGNAA)
- Molecular neutron activation analysis(MNAA)
- Cyclic neutron activation analysis (CNAA)

Here an NAA method is used for this analysis.

1.1.4 Importance of NAA

We have seen that elemental analysis is a technique where inorganic or organic compounds were used to determine elements present in different samples such as soil, fruits, vegetables, human body parts (e.g. hair, nail, skin etc.). From ancient time to present, men are trying to invent techniques which are more accurate, precise, sensitive, reliable etc. Due to their hard and soul try they have invented various techniques to determine elements in samples. Now a day we have various techniques to determine elements. Through these techniques we have understood that how much the elements we have examined are polluted or safe. We have taken proper steps if any problem arises. It is easy to treat any disease in its initial stage. But proper steps are needed to take to

diagnose such disease. We can know the place where we live are safe or polluted by testing various elemental analyses. If it is polluted we can take proper steps to live there. Among different types of elemental analysis neutron activation analysis is more perfect and useable. Neutron Activation Analysis (NAA) has become very important due to its various advantages such as:

a) **Number of elements** - Sixty-seven common and rare earth elements become radioactive when exposed to the neutron flux in a reactor. Of these 67 elements, over 50 can be identified and measured quite readily. No other methods can determine such number of elements at a time.

b) **Multi-element** - By using different combinations of irradiation and decay times, it is possible to measure a large number of elements from isotopes of different activities and half- lives. A standard analysis package can routinely analyze for 32 elements in a single sample. NAA is the only procedure that can simultaneously measure so many elements.

c) **Highly sensitive** - The method permits measurement of all detectable elements with great sensitivity; many elemental concentrations are measurable in parts per million (ppm) or parts per billion (ppb).

d) **Elemental analysis** - Determines element regardless of their chemical form (ferric vs. ferrous). This can be either an advantage or a disadvantage.

e) **Non-destructive** - Unlike other techniques, the sample is not destroyed by the analysis, and can be re-analyzed if necessary.

Among all properties, non-destructiveness is most efficient property. Due to this property the samples we have analyzed do not losses any of its property. So we can re-use it in several times. If any confusion is occurred then we can re-analyze it. Among all techniques and all properties if NAA this is a referee method. Various types of analytical

tools have been implemented for studying the chemical composition of environmental geochemical samples, such as atomic absorption spectroscopy [4], inductively coupled plasma mass spectrometry [5, 6], inductively coupled plasma atomic emission spectrometry [7], inductively coupled plasma optical emission spectrometry etc. Unlike the above mentioned analytical methods, neutron activation analysis (NAA) is free from chemical digestion and is nondestructive as well as independent of chemical form [8]. NAA is considered to be a primary method of measurement and possesses a versatile applicability [8, 10]. So in this study we have used NAA for the chemical characterization of the spices samples.

1.1.5 Applications of NAA method

NAA can be applied to virtually all sample types without any pre-treatment of the samples this includes:

i. Archaeology such as pottery, obsidian, chart, basalt and limestone.
ii. Study the Redistribution of Uranium and Thorium due to Ore processing.
iii. The use of radiotracers to study the fit of hazardous elements in waste materials/ coal-char admixtures under gasification an emerging waste management technology.
iv. Selenium distribution in aquatic species in selenium-contaminated fresh water impoundments.
v. Nutrition Epidemiology– Nutritional and biochemical/genetic marks of cancer.
vi. Nutrition Epidemiology– A cohort study of the relationship between diet, molecular markers, and cancer risk.
vii. Nutrition Epidemiology– Non-melanoma skin cancer study.
viii. Nutrition Epidemiology– Molecular epidemiology of prostate cancer.
ix. Knock-out gene mouse model for cystic fibrosis.

x. Calcium metabolism study.

xi. Geological science.

xii. Semiconductor materials and other high-purity materials.

1.2 Gamma Radiation

Gamma radiation is a penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It consists of the shortest wavelength electromagnetic waves and so imparts the highest photon energy. Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900 while studying radiation emitted by radium. In 1903, Ernest Rutherford named this radiation gamma rays based on their relatively strong penetration of matter; he had previously discovered two less penetrating types of decay radiation, which he named alpha rays and beta rays in ascending order of penetrating power. Gamma rays from radioactive decay are in the energy range from a few kiloelectron volts (keV) to approximately 8 megaelectron volts (MeV), corresponding to the typical energy levels in nuclei with reasonably long lifetimes. The energy spectrum of gamma rays can be used to identify the decaying radionuclides using gamma spectroscopy [11, 12].

Gamma rays are created by nuclear decay, while in the case of X-rays, the origin is outside the nucleus. In astrophysics, gamma rays are conventionally defined as having photon energies above 100 keV and are the subject of gamma ray astronomy, while radiation below 100 keV is classified as X-rays and is the subject of X-ray astronomy. This convention stems from the early man-made X-rays, which had energies only up to 100 keV, whereas many gamma rays could go to higher energies. A large fraction of astronomical gamma rays are screened by Earth's atmosphere. Gamma rays are ionizing radiation and are thus biologically hazardous. Due to their high penetration power, they can damage bone marrow and internal organs [13]. Unlike alpha and beta

rays, they pass easily through the body and thus pose a formidable radiation protection challenge, requiring shielding made from dense materials such as lead or concrete.

1.3 Applications of Gamma Radiation

Apart from the use of nuclear energy for the supply of electricity, the applications of radioactivity are numerous in many areas: medical physics, earth sciences, industry and preservation of cultural heritage. The properties used for these various applications are:

a) Irradiation of surgical and food materials

Irradiation is a privileged means to destroy micro-organisms (fungi, bacteria and virus). As a result, many applications radiation exists for sterilization of objects. For example, most medical-surgical equipment (disposable syringes, etc.) is today radio-sterilized by specialized industrialists. Similarly, the treatment by irradiation of food ingredients allows improve food hygiene: sterilization spices, elimination of salmonella from shrimp and frog legs [14]. This technique is also known as food ionization.

b) Medical physics applications

The major application of radiation in medicine is radiotherapy and/or treatment by ionizing radiation. A few months after the discovery of X-rays, there is over a century, it has become clear that biological action radiation could be used in the treatment of cancers. Cancers cells divided more quickly are more sensitive, than normal cells to ionizing radiation. By sending these cells a certain dose of radiation, it is possible to kill them and eliminate the tumor [15].

c) Irradiation of art objects

Treatment with gamma rays helps to eliminate larvae, insects or bacteria lived inside objects, to protect them from degradation. This technique is used in the treatment of conservation and restoration of arts objects, ethnology and archaeology. It is applicable to

different types of materials: wood, stone, leather [12].

d) Irradiation in food industry

Gamma irradiation is known as a very widespread application of nuclear technology for peaceful purposes, like physical agent of sterilization or decontamination due to the deep penetration power and low dose rate, with high efficiency in killing microorganisms by breaking the covalent bonds of bacterial DNA and viruses. Gamma radiation has been regarded as a safe, cost competitive methodology for the sterilization of healthcare products, extension of shelf-life quality improvement, and reduction of bio burden in food products [16]. Furthermore, exposure to gamma radiation can provide possible solutions for the treatment of contaminated waters (municipal, industrial waste waters) aiming at the improvement of their chemical and biological quality. Although there are various different sterilization methods correlated with the purpose of sterilization and the material that will be sterilized, there are some common practical features in using of gamma radiation including: precise dosing, rapid processing, uniform dose distribution, system flexibility, the immediate availability of product after processing. Water is known as an ubiquitous solvent highly benefic for the living world, but when it is subjected to ionizing radiation in the presence of oxygen it produces highly reactive oxygen species (ROS) able to induced chemical instability further responsible for organic compound destruction with molecular fragments release [17].

1.5 Motivation of This Study

Spices are considered as an essential elemental source for human body. But spices may contaminate by some toxic elements and pathogenic organisms. In this context, Neutron Activation Analysis (NAA) is one of important technique to determine the essential and toxic elemental concentration from the samples. Moreover, pathogenic organisms are one of vital treat for children and young health. Some recent study noticed that spices may

contaminate by pathogenic organisms. Currently, Gamma irradiation is widely used to decontaminate the pathogenic organisms. For measuring trace elements in a wide variety of biological samples, nuclear analytical technique are particularly suitable and for that reason, the techniques have made a significant contribution to biological research. Furthermore, NAA is particularly useful for biological studies. NAA works on the measurement of characteristic radiation from radionuclides formed directly or indirectly by neutron irradiation of the material [18]. On the other hand, the demands of irradiated foods are increased rapidly worldwide. Gamma irradiation is an effective method to damage DNA of organisms as a result microorganisms cannot grow and multiply in the food items [19]. The Joint FAO/IAEA/WHO Expert Committee reported that irradiation up to 10 kGy does not produce toxicological risks and nutritional problems in foodstuffs [20]. This technique will reduce the post-harvest loss, extend shelf-life and improved food safety without changing quality [21]. Among various element analysis, research reactor based NAA method is used to determine trace element concentrations in Bangladeshi spices this research and decontaminate the pathogenic organisms by gamma radiation.

1.6 Objectives of This Research

The main objectives of the research are:

a) To characterize essential and toxic elements in common spices of Bangladesh using research reactor based NAA technique.
b) To assess physicochemical and pathogenic organisms change in the studied spices due to different doses of gamma irradiation.
c) To assess different hazard indices as well as to compare elemental data of the studied spices with different international recommended values.

1.6 Structure of This Thesis

a) **Introduction:** In this chapter some basic information discussed about neutron activation analysis and gamma irradiation techniques and also listed down objectives of this study.

b) **Literature Review:** In this chapter some related journals on this regards which have been reviewed and written as summary.

c) **Experimental Procedure:** The research was done in atomic energy research establishment, Savar, Dhaka. This research has two parts one was neutron activation analysis which was done in INST, AERE and another part was gamma irradiation which was done in IRPT, AERE.

d) **Experimental Results and Observations:** Some results were showed in table and observation also.

e) **Discussion on Results and Relevance:** In this part, the results were briefly explained and experimental results compare with national and international values.

Chapter 2

Literature Review

Being a high accurate and precise method NAA is used globally for assessment of elemental contamination in biological samples. The concentrations of this element (Al, As, Br, Ca, Cd, Cl, Co, Cr, Fe, K, Mn, Na, Ni, Pb, Sc, V and Zn etc) may be determined by NAA techniques. NAA is multi-elemental concentration determine techniques. NAA techniques used to determine the concentration of biological, soil, sediment, archeological, hair, forensic and blood samples. The main purpose of this study is to reveal historical trace element contamination in spices samples of the Bangladeshi local market. A review of some related journals on this regards which have been done on biological sample at different part of the world are mentioned with proper information.

Messoudi *et al* [22] evaluated the elemental concentration obtained in two Algerian spices (*Coriandrum sativum* L. and *Cuminum cyminum* L.) by instrumental neutron activation analysis (INAA), in order to highlight the importance of these spices as a potential source of micronutrients. The daily intake of micronutrients and potentially toxic elements were determined and compared with the recommended values (RDA) and were found to be well below the tolerance limits. Twenty-two elements were assessed, eight essential chemicals were quantified with tendency K > Ca > Na > Fe > Zn > Cr > Co > Se, and three potential toxic elements were present in the descending content pattern Br > As > Sb.

Rahman and Islam *et al* [23] conducted to determine concentrations of five toxic trace elements (Cr, Ni, As, Cd, and Pb) in cereals, fruits, and vegetables of Bangladesh. The range of mass fractions (mg/kg) of Cr, Ni, As, Cd, and Pb in the foodstuffs was 0.090-

2.5, 0.03-2.6, 0.13-1.7, 0.010-0.74, and 0.37-2.2, respectively. This study indicates that concentration of Cr, and Pb in fruits; As, Cd, and Pb in vegetables were higher than WHO/FAO maximum allowable concentration levels. The hazard index (HI) values for adults and children of the toxic elements in studied cereals, fruits and vegetables were higher than 1.0, suggesting non-carcinogenic adverse health hazard to the consumers. The estimated target carcinogenic risk (TCR) values were greater than the threshold level of 1.0×10^{-4} for Cr and Cd in cereals and vegetables indicate potential cancer risk to both adults and children for consumption of the foodstuffs. The present study reveals that trace elements contamination in foodstuffs is a serious issue of concern in Bangladesh.

Massadeh *et al* [24] determined the concentrations of selected heavy metals including Pb, Zn, Cr, Ni, Cu, As, and Cd in different brands of canned vegetables and fruits including canned tomato sauce (ketchup), canned green beans, canned whole carrots, and canned juice (pineapple) imported to Jordanian market by acid digestion and atomic absorption spectroscopy. Samples were collected from popular Jordanian markets, Irbid city, Northern Jordan (11 samples of each type). The metal concentrations in the samples analyzed were found to be in the range of 2.6-3.0 mg/kg for Pb, 0.50-0.60 mg/kg for Cd, 2.50-5.10 mg/kg for As, 0.84-0.91 mg/kg for Cu, 0.32-3.02 mg/kg for Zn, 0.66-1.71 mg/kg for Cr, and 0.97-2.94 mg/kg for Ni. The results obtained showed that Pb and As have the highest concentrations in the most of samples analyzed, whereas, the lowest concentrations obtained were mainly in Cd. For example, in canned tomato sauce, the average concentrations of heavy metals are 3.50 mg/kg for As, 0.50 mg/kg for Cd, 0.66 mg/kg for Cr, 0.89 mg/kg for Cu, 1.15 mg/kg for Ni, 2.95 mg/kg for Pb, and 1.02 mg/kg for Zn. The results of this study reveal that the concentration of some toxic heavy metals (Pb, Cr, Ni, As, and Cd) in canned vegetable

and fruit samples being sold in Jordanian markets exceeded the permissible limits set by different health organizations.

Islam *et al* [25] checked the consumed food samples of cereals (rice, maize and wheat), vegetables (lentil, brinjal, carrot, bean, potato, tomato, onion and chili), fruits (banana, mango and jackfruit), fish (taki, rui, pangas and tilapia), egg (chicken and duck), milk (cow) and meat (chicken, duck, beef and mutton) were collected from some markets of Bogra district northern part of Bangladesh to evaluate the levels of arsenic (As) and associated health risk to the adult's and child inhabitants. Arsenic is a highly toxic element, and its presence in food composites is a matter of concern to the world scientists. Target hazard quotients (THQs) and target carcinogenic risk were calculated to evaluate the non-carcinogenic and carcinogenic health risk from ingested arsenic. The highest and the lowest mean concentrations of arsenic were noted in the Tilapia fish 0.94 mg/kg, and beef 0.012 mg/kg. The daily intakes of arsenic via foodstuffs were 1.92 and 3.30 µg/kg-bw/day for rural adults and children and 1.69 and 3.04 µg/kg-bw/day for urban adults and children, respectively. The result shows the highest THQs of arsenic in cereals and vegetables for both the rural and urban inhabitants which exceed the safe limit (>1) indicating that cereals and vegetables are the main food items contributing to the potential health risk.

Zhong *et a*l [26] assessed the health risk for the Chinese public when consuming vegetables grown in China, based on 1335 data records from 220 published papers during 2007–2016. The results showed that the average of Pb, Cd, and Hg concentration in vegetables was 0.106, 0.041, and 0.008 mg/kg, which were lower than the maximum allowable concentrations, respectively. Leaf vegetables contained higher heavy metals than root vegetables and fruit vegetables. On a provincial scale, the highest Pb, Cd, and Hg concentrations in vegetables were determined by those in soil and atmosphere. The

total health risk index showed that people in Guizhou, Yunnan, Guangxi, Hunan, Guangdong, Hubei provinces in southern China, and Liaoning Province in northeast China, faced a high risk of Pb, Cd, and Hg when consuming vegetables.

Islam *et al* [27] conducted to assess the concentration of six heavy metals ie, chromium (Cr), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd) and lead (Pb) in different foods associated with health hazard inference in Bangladesh. The range of Cr, Ni, Cu, As, Cd and Pb in food samples were 0.45-47.7, 0.22-38.6, 0.43-47.4, 0.72-6.05, 0.001-6.70 and 0.21-35.9 mg/kg, respectively. The estimated daily intake values of all the metals except Cu were higher than the maximum tolerable daily intake. The target hazard quotients of all metals were higher than 1 through consumption of cereal and vegetables, indicating significant health risks to both adult and children. The total carcinogenic risk (CR) of As (9.84E-01) was higher the threshold level (1.0E-06) and 9.84E-01 for Pb clearly revealed that consumption of these food items definitely poses cancer risks to the Bangladeshi population.

Singh *et al* [28] investigated Indian diet is primarily vegetarian and consists of various cereals and vegetables along with spices, often used in the preparation of curries. The nutritive potential of each ingredient, in terms of trace element contents, has been evaluated using instrumental neutron activation analysis (INAA). Four minor (Na, K, P and Cl) and 16 trace elements (Br, Co, Cr, Cs, Cu, Fe, Hg, Mn, Mo, Rb, Sb, Sc, Se, Sr, Th and Zn) have been determined in six cereals, nine vegetables and 20 spices and condiments, including two betel leaves. None of the carbohydrate-rich cereals or potato was rich in any of the essential elements but leafy vegetables showed higher contents of Fe and other nutrients. Fe/Zn is well correlated with Fe contents in cereals and spices. Out of various spices, cinnamon was most enriched in Fe, Co, Cr, Na, K, P and Zn, whereas turmeric and curry leaves were found to be particularly rich in Se. Cumin and mustard

seeds were rich in Cu. Some environmental contaminants, such as Hg, Cr, Br and Th, were also present in significant amounts. An attempt has been made to evaluate the contribution of essential elements (Cr, Cu, Fe, Mn, P, Se and Zn) in spices to the daily dietary intake (DDI) through an Indian vegetarian diet.

Garg *et a*l [29] investigated traditional Indian medicinal herbs used for strengthening the body immune system, are rich source of many essential nutrient elements in bioavailable form. Instrumental neutron activation analysis (INAA) employing short (5 minutes) and long (14 hours and 3 days) reactor irradiation followed by high resolution gamma-ray spectrometry has been used for the determination of Al, Au, Ba, Br, Ca, Ce, Cl, Co, Cr, Cu, Eu, Fe, K, La, Mg, Mn, Na, P, Rb, Sb, Sc, Sm, Th, V and Zn in 15 medicinal herbs commonly used in Indian household for treatment of various ailments. Several of herbs are enriched in Ca, Co, Cu, Mg, P, Fe, Mn and Zn, which play a vital role in biochemical and enzymatic processes. Jatamansi, often used as antibacterial, antipyretic and heart tonic is specially enriched in Co, Cr, Cu, Na, Mn, Fe, Rb and Zn. Also Guduchi and Laghu Haritaki are enriched in Ca and Mg, respectively. An attempt has been made to correlate elemental contents with the therapeutic importance of various herbs. Also our results for the participation in an Inter comparison Study of renewal of Pine Needles (SRM-1575a) from NIST, USA are presented.

Lalor *et al* [30] evaluated the biological standard reference materials Orchard Leaves SRM 1571 and Oyster Tissue SRM 1566a was analysed by instrumental neutron activation analysis (INAA) at the International Centre for Environmental and Nuclear Sciences, Jamaica at (ICEN) and at the Instituto de Pesquisas Energeticas Nucleares (IPEN-CNEN/SP), Brazil. The comparison of the results with those obtained with the more powerful reactor are used to evaluate the possibilities of INAA for the analysis of biological samples at ICENS. The detection limits, the precision and accuracy of the

results obtained in both laboratories are compared. The advantages and disadvantages of the different irradiation facilities are discussed. Some results obtained for Jamaican biological samples are also presented.

Zeisler *et al* [31] determination of arsenic at natural levels in biological materials remains difficult. Many analytical techniques cannot detect the low levels present in typical biological tissues and other techniques suffer from interferences. This paper reviews uses of neutron activation analysis (NAA) at NIST to determine nanogram amounts of arsenic in biological reference materials with radiochemical (RNAA) or instrumental (INAA) procedures. INAA is compromised by high activities from ^{24}Na, ^{82}Br, and ^{32}P that may be formed during irradiation of biological tissues, and result in detection limits as high as 0.1 mg. Lower detection limits have been achieved using state-of-the-art gamma-ray spectrometry systems in INAA and a variety of procedures in RNAA. These techniques and procedures were applied recently at NIST to the determination of arsenic in urine, nutritional supplements, and total diet samples.

Abou-Arab *et al* [32] determined the contamination of Egyptian spices and medicinal plants with heavy metals, a total of 303 samples, which represent 20 different types of spices and medicinal plants that were collected from areas of exportation in Egypt, were analyzed for heavy metals. Some of them have different growing seasons, and each has its own agricultural practices and several shipments. The results revealed that heavy metal contents in spice and medicinal plants dependon the plant species. The maximum levels of heavy metals in the analyzed samples were 14.4, 2.44, 33.75, 2.85, 0.10, 68.80, 343.0, 11.40, and 1046.25 μg/g for Pb, Cd, Cr, Ni, Sn, Zn, Mn, Cu, and Fe, respectively. Cobalt was not detected in any of the various samples under investigation. The levels of heavy metals determined in the analyzed samples were found to exceed the maximum allowable levels. The investigated medicinal plants were also processed by two different methods to

determine the behavior of their metal contents during processing. It has been found that boiling the plant in water leads to the extraction of higher amounts of the metal from the plant than immersing it in the hot water.

Sattar *et al* [33] determined the different spices dry fruits and plant nuts commonly consumed in Pakistan for the heavy metals cadmium, lead, copper, zinc, iron and manganese by the potentiometric stripping analysis and AAS. The results revealed wide variation in heavy metal content among different biological materials. Mixed spices generally exhibited higher value for trace metals specially lead (6.6-9.2 µg/g), cadmium (0.65-1.34 µg/g), iron (142.3-285.0 µg/g) and zinc (64.2-65.8 µg/g). Dry fruits contained relatively lesser amounts of heavy metals than plant nuts. Almonds contained higher levels of lead (1.02 µg/g) and cadmium (0.24 µg/g) than other nuts and dry fruits.

Gupta *et al* [34] studied eight common spices for their mineral compositions using inductively coupled plasma optical emission spectrometry (ICP-OES) after acid digestion. The seeds of the following spices were used for analysis: coriander, cumin, anise, nigella, mustard, carum, black pepper, and fenugreek. Average elemental compositions of spices seeds were reported using robust Z-score statistic. Fourteen elements were considered, which include heavy metals like iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), nickel (Ni), molybdenum (Mo), lead (Pb), and chromium (Cr), alkaline earth metals like calcium (Ca) and magnesium (Mg), lighter element like aluminum (Al) and non-metals like silicon (Si) and phosphorus (P). A very strong linear correlation exists between Fe and Al contents in the spices. Zinc also correlates well with iron. This study provides a reliable account of the endogenic concentrations of a number of common elements including heavy metals present in these spices.

Divrikli *et al* [35] determined the trace metal levels in eleven different spice and herbal plant species from western Anatolia, Turkey by atomic absorption spectrometry. The contents of trace metals in the herbal plant samples were found in the ranges: 3.8-35.4 $\mu g g^{-1}$ for copper, 0.2-2.7 $\mu g g^{-1}$ for cadmium, 0.1-2.8 $\mu g g^{-1}$ for lead, 1.4-11.3 $\mu g g^{-1}$ for nickel, 0.1-9.7 $\mu g g^{-1}$ for chromium, 30.0–945.3 $\mu g g^{-1}$ for iron, 7.9-152.5 $\mu g g^{-1}$ for manganese and 5.2-83.7 $\mu g g^{-1}$ for zinc. Results obtained are in agreement with data reported in the literature.

Chizzola *et al* [36] monitored medicinal, aromatic and spice plants grown in different regions of Austria as to their Cd, Cu, Fe, Mn, Pb and Zn contents. Since the plants were grown under common field conditions, the essential elements were within the usual ranges for plant material. The contamination level with the toxic heavy metals, Pb and Cd, can be classified as normally low. Most samples contained less than 0.2 mg kg^{-1} Cd and less than 1.5 mg kg^{-1} Pb on a dry weight basis. Comparison with previous investigations suggests that contaminations with Pb occur rather by chance, whereas enhanced Cd values are restricted to some species having a tendency to accumulate this heavy metal. Some such species are St. John´s wort, poppy, yarrow, chamomile and absinth. Careful choice of growing site and appropriate soil management can reduce the Cd uptake of these critical species. These precautions are important when larger amounts of the product are consumed.

Byrne *et al* [37] used the neutron activation analysis (NAA) technique plays a very important role in the certification of reference materials (RMs) and their characterization, including homogeneity testing. This enables the derivation of essentially independent analytical information and the unique capacity of NAA for self-validation. The application of NAA to quantify natural or man-made radionuclides such as uranium, thorium, ^{237}Np, ^{129}I and ^{230}Th is discussed, including its advantages over

conventional radiometric methods, and its usefulness in providing independent data for nuclides where other confirmatory analyses are impossible, or are only recently becoming available through newer "atom counting" techniques. Certain additional, prospective uses of NAA in the study of RMs and potential RMs are mentioned, including transmutation reactions, creation of endogenously radiolabelled matrices for production and study of RMs (such as dissolution and leaching tests, use as incorporated radiotracers for chemical recovery correction), and the possibility of molecular activation analysis for speciation.

Muhamad *et al* [38] reported distribution of microorganisms in 15 samples of selected spices and the effects of irradiation of them were studied. The total aerobic bacteria in black pepper, white pepper, turmeric, rosemary and basil were determined to be 3×10^3 to 5×10^7 per gram. Coliforms were also determined in 8 samples to be 2×10^2 to 2×10^6 per gram. The main aerobic-spore-formers were identified as *Bacillus pumilus* and *B. subtilis*. Molds were determined in 10 samples to be 1×10^2 to 2×10^4 per gram which consisted mainly of the *Aspergillus glaucus, A. restrictus, A. flams, A. fumigatus, A. niger groups* and *Penicillium*. A study on the inactivation of microorganisms in spices showed that gamma-irradiation doses of 1.2 to 1.5 Mrad were required to reduce the total aerobic bacteria to below a detectable level, while doses of below 1.0 Mrad were required to decrease the spore-forming bacteria to below 10^3 per gram, the Japanese hygenic standard. Coliforms were eliminated with 0.4 to 1.0 Mrad irradiation. In the storage study, at humidity levels higher than 84% at 30 or 35°C, mold counts increased more than 10^6 per gram in many kinds of powdered spices in polyethylene pouches during 1 to 2 months of storage, while samples subjected to 0.4 Mrad irradiation were free from molds.

Ito *et al* [39] determined the total aerobic bacteria in spices range 1×10^6 to 6×10^7 per gram. A study on the inactivation of microorganisms in spices showed that doses of 6-

9kGy of EB (electron-beams) or γ-irradiation were required to reduce the total aerobic bacteria in many However, a little increase of resistance was observed on the inactivation of total aerobic bacteria in many spices in case of EB irradiation. These differences of radiation sensitivities between EB and γ-rays was explained by dose rate effect on oxidation damage to microorganisms from the results of radiation sensitivities of *Bacillus Pumilus* and *B. Megaterium* spores at dry conditions. On the other hand, these high dose rates of EB irradiation suppressed the increase of peroxide values in spices at high dose irradiation up to 80 kGy. However, components of essential oils in spices were not changed even irradiated up to 50 kGy with EB and γ-rays.

Sadecka [40] described about food irradiation is a process of exposing food to ionizing radiation such as gamma rays emitted from the radioisotopes ^{60}Co and ^{137}Cs, or high energy electrons and X-rays produced by machine sources. The use of ionizing radiation to destroy harmful biological organisms in food is considered a safe, well proven process that has found many applications. Depending on the absorbed dose of radiation, various effects can be achieved resulting in reduced storage losses, extended shelf life and/or improved microbiological and parasitological safety of foods. The most common irradiated commercial products are spices and vegetable seasonings. Spice irradiation is increasingly recognized as a method that reduces post-harvest losses, ensures hygienic quality, and facilitates trade with food products. This article reviews recent activities concerning food irradiation, focusing on the irradiation of spices and dried vegetable seasonings from the food safety aspect.

Alam *et al* [41] informed that spices such as coriander, cumin, turmeric, chilli collected from a local market were found to be highly contaminated with bacteria and fungi. A dose of 10 kGy was required to reduce the total bacterial count below detectable levels, while a dose of only 5 kGy was required to eliminate the fungal contamination. Coliforms were

totally eliminated at a radiation dose of 5 kGy. During 6 months storage of irradiated and non-irradiated spices, the irradiated spices were found to retain good microbiological quality.

MaKee [42] investigated that the popularity of highly spiced cuisine and consumer demand for more flavorful foods which are also low in sodium and fat have resulted in a continuing interest in the use of spices and herbs in food products. Although such condiments are generally used for the aesthetic properties they contribute to food products, spices and herbs can often be a major source of microbial contamination. Studies investigating bacterial and/or fungal contamination of spices and herbs are reviewed. The high levels of microbial contamination in spices and herbs reported by many of the studies reviewed suggests a need for better control in all aspects of the production, processing and usage of these products to prevent potential food spoilage and food-borne illnesses due to contaminated spices and herbs.

Farkas [43] despised the substantial efforts in avoidance of contamination, an upward trend in the number of outbreaks of foodborne illnesses caused by non-spore forming pathogenic bacteria are reported in many countries. Several decontamination methods exist but the most versatile treatment among them is the processing with ionizing radiation. Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process. Irradiation is particularly valuable as an end product decontamination procedure. Radiation treatment at doses of 2-7 kGy-depending on condition of irradiation and the food-can effectively eliminate potentially pathogenic non spore forming bacteria including both long-time recognized pathogens such as *Salmonella* and *Staphylococcus aureus* as well as emerging or "new" pathogens such as *Campylobacter, Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products without affecting sensory, nutritional and technical qualities.

Candidates of radiation decontamination are mainly poultry and red meat, egg products, and fishery products. Radiation decontamination of dry ingredients, herbs and enzyme preparations with doses of 3-10 kGy proved to be a viable alternative to fumigation with microbicidal gases. Radiation treatment at doses of 0.15-0.7 kGy under specific conditions appears to be feasible also for control of many foodborne parasites, thereby making infested foods safe for human consumption.

Alam and Abrahem [44] Irradiation is an effective method for food decontamination. Spices irradiation is a process of exposing spices to ionizing radiation such as gamma rays emitted from the radioisotopes ^{60}Co and ^{137}Cs, or high energy electrons and X-rays produced by machine sources. The use of ionizing radiation to destroy harmful biological organisms in food is considered a safe, well proven process that has found many applications. Depending on the absorbed dose of radiation, various effects can be achieved resulting in reduced storage losses, extended shelf life and/or improved microbiological and parasitological safety of foods. The most common irradiated commercial products are spices and vegetable seasonings. Spice irradiation is increasingly recognized as a method that reduces post-harvest losses, ensures hygienic quality, and facilitates trade with food products. This article reviews activities focusing on the irradiation of spices from the food safety aspect.

Emam *et al*[45] In this research, powdered black pepper from Egyptian markets was irradiated with different recommended doses of gamma rays (5.0 and 10.0 kGy) and with microwaves for different periods (20, 40 and 75s) to improve its hygienic quality. The most common bacterial isolates were of three genera *Bacillus, Clostridium* and *Micrococcus* (7.5×10^6), whereas the predominant fungi (7.8×10^4) were *Aspergillus* species, *A. glaucus, A. flavus, A. niger* and *A. ochraceus*. Doses of gamma irradiation used (5.0 and 10 kGy) were sufficient to decrease spore-forming bacteria

(SFB) and to inhibit the fungal flora and coliforms which contaminated the black pepper powder. In comparison, microwave treatments, particularly for 40s and 75s, increased the concentration of the same compounds. The results obtained indicate that microwave treatment, under these conditions, is a safe and suitable technique for decontamination of black pepper which does not result in a great loss of flavour compounds, as compared with recommended doses of gamma irradiation.

Rico *et al* [46] investigated the comparative effects of steaming and gamma irradiation on the physicochemical and microbiological properties of dried red pepper (*Capsicum annum* L.) during post-treatment storage at refrigerated (4 ± 2 °C) and room (20 ± 2 °C) (RT) temperatures for 6 months. Whole dried peppers were either steamed, hot air-dried and processed into powder form or powdered, packed in PE bags and gamma-irradiated at 10 kGy. The commercial steam treatment led to a 1-to2-log reduction in the initial microbial load (10^6 CFU/g) accompanied with changes in spice as indicated by low Hunter's colour values and reduced sensory scores in RT-stored samples. However, irradiation resulted in a 5-log reduction with minimal effects on the physicochemical properties, except for the decreased content of capsanthin in the irradiated samples. The functional components of spices were not apparently affected by both treatments. The refrigerated storage following irradiation is recommendable for powdered red pepper to minimize physicochemical changes.

Waje *et al* [47] investigated the effects of steam and irradiation treatments on the physicochemical properties (moisture content, pH, extractable yield, reducing sugar, soluble pigment, antioxidant activity, piperine, Hunter's color, and sensory attributes) and microbiological quality (total aerobic bacteria, coliforms, and yeasts and molds) of ground black pepper stored at refrigerated and room temperatures for 6 months were compared and evaluated. Irradiation resulted in a higher microbial reduction in pepper,

with minimal effects on the proximate composition,functional components, color, and sensory attributes of the spice. Steamed peppers appeared darker, and a considerable decrease in the piperine content was observed after treatment and storage. This study illustrates that irradiation is a better decontamination method than steam treatment in eliminating microorganisms without apparently affecting the quality of the powdered spice. Storage at 4 °C enhanced the microbial quality and minimized the loss of piperine content in ground black peppers.

Esmaeili *et al* [48] investigated the effect of gamma irradiation (0, 5, 10, and 15 kGy) under various atmospheres of packaging (air, N_2, and vacuum) on the microbial load and physicochemical properties of turmeric powder, including antioxidant activities, total phenolic content (TPC), color parameters, and curcuminoid content. The efficiency of irradiation in reducing microbial contamination in the samples was observed even at the lowest dose. By increasing the irradiation dose, the microbial load was not detectable. Irradiation in the presence of oxygen had synergistic effects on the extraction of curcuminoids and TPC, and increased the antioxidant activity of the methanolic extracts: highest activity was observed at 15 kGy.

Song *et al* [49] evaluated the effects of gamma irradiation on nutritional, physiological, physicochemical and sensory properties of the Korean lactic acid fermented vegetable, Kimchi, were investigated. The composition of amino acids and organic acids in Kimchi were not influenced by gamma irradiation less than 10 kGy. Angiotensine converting enzyme inhibitory, xanthin oxidase inhibitory, electron donating and antimicrobial activity of Kimchi extract were stable up to 10 kGy. There were no significant changes in pH and texture at less than 10 kGy. Color values were influenced at 10 kGy of gamma irradiation, and resulted in the increase of *L**- and reduction of *a**-value. About 90% of panelists identified a sensory difference between non-irradiated and 10 kGy-irradiated

sample, and Kimchi irradiated at 10 kGy had lower scores in acceptability than those of the control or irradiated at 2.5 and 5 kGy.

Thakur *et al* [50] studied food irradiation is one of the most effective methods of food preservation. Only 37 countries worldwide permit the use of this technology. If used to its full potential, food irradiation can save millions of human lives being lost annually due to food-borne diseases or starvation and can add billions of dollars to the world economy. This paper briefly reviews the history and chemistry of food irradiation along with its main applications, impediments to its adoption, and its role in improving food availability and health situation, particularly in developing countries of the world.

Latif *et al* [51] determined the concentrations of arsenic (As), chromium (Cr) and iron (Fe) in the foodstuffs, soils and sediments from various areas in Bangladesh and new data for these toxic trace elements were given. The arsenic pollution problems in the most of the areas of Bangladesh are of geological origin. The high level of As in foodstuffs, soils and sediments, except for tannery sediments is probably positively correlated to the Fe concentration. An excessive amount of chromium was found in the sediments from the tannery area of Bangladesh.

Islam *et al* [52] determined the concentration of mineral compositions of Japanese green tea leaves by using a combination of PGA and INAA. Due to the nondestructive, multi-element analytical capability and minimal sample preparation, these techniques can easily be used to determine a wide range of elemental contents (from 7.4% of H to 7.1 ng/g of Sc) in tea leaves. The extraction efficiencies of the elements in tea infusion were evaluated by comparing average elemental concentrations of the tea leaves before and after infusion, which show that Cl (93%), Br (80%), K (71%), Rb (66%), Cs (60%), Na

(59%) and Co (51%) are highly extracted, whereas Fe (9%), La (7%) and Mn (5%) are poorly extracted by a 6 min hot water infusion process.

2.1 Summary of Review of Previous Research Papers

From the reviewing the above literatures, elemental characterization of biological samples were done and toxic elements like Cr, Pb, As, Cd, Hg, Ni concentration determination mainly focused due to their toxic characteristics. Moreover, health risk assessment calculated and finally carcinogenic risk also determined as well as compared with international recommended WHO/FAO values. Gamma irradiation was treated decontamination of foodborne organisms from samples. Finally, optimum gamma doses were identified to kill the organisms.

Chapter 3

Experimental Procedure

Quality and elemental characterization of common spices are the main objectives of this study. After preparing properly, the samples are irradiated by the neutron flux of 3MW TRIGA MARK-II research reactor for NAA. Various types of trace elements are identified by the gamma ray peak energy and the concentrations of this element are obtained by gamma peak area. The experimental instruments for NAA are presented in Figure of 3.1. On the other hand, quality analysis and microbial decontamination were performed by gamma irradiation technique.

Experimental Instruments for NAA Technique:

Oven

Gamma counter

De-ionized water

Hot plate

Digital micro balance

Teflon (Plastic)

Impulse sealer

HPGe detector

3.1 Sample Collection

Eight types of spice samples in powder form were collected from local market of Bangladesh in May 2017. The samples are red chili (*Capsicum annuum*), turmeric (*Curcuma longa*), coriander (*Coriandrumsativum*), cumin (*Cuminumcyminum*), black pepper (*Piper nigrum*), garlic (*Allium sativum*), ginger (*Zingiberofficinale*) and green chili (*Capsicum annuum*) (Figure 3.2). All spice samples were stored in a refrigerator at 4°C till preparation for elemental analysis.

Figure 3.2: Different types of spices powder samples.

3.2 Sample Preparation for NAA

After weighing, the spices samples were made individual packet with individual identification number. The size and shape of packets were kept approximately same. The

packets were then preserved carefully for neutron irradiation. For this experiment Approximately 50 mg of each dried powder sample was weighted in polyethylene bag and heat sealed and presented in Figure 3.3. For relative standardization approach, two reference materials (RMs): NIST-1547 (Peach leaves), SRM-1515 (Apple leaves) and one standard reference material IAEA-336 (lichen powder) were used in this study.

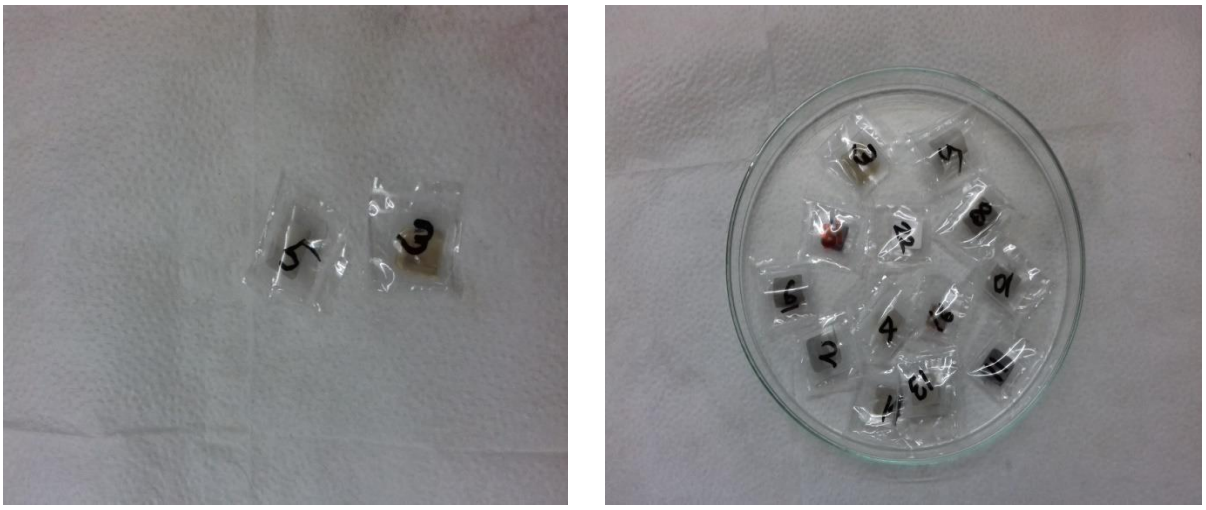


Figure 3.3: Ready samples for neutron irradiation.

Each of the standards were prepared as the same way as those of samples. Samples and standards were placed in a vial for irradiation. The samples, three standards and foils were packed in a plastic cylindrical vial for irradiation. IAEA-336 was used as the standard while; NIST-1547 and NIST-1515 were used as the control sample.

3.3 Irradiation

Two irradiation schemes were performed using pneumatic transfer (rabbit) system at the 3 MW TRIGA Mark-II research reactor of Bangladesh Atomic Energy Commission, Savar and presented in Figure 3.4. Two types of irradiation were performed for the determination of short and long-lived radionuclides.

(i) Long irradiation was performed simultaneously with all the samples and standards

with the thermal neutron flux of 2.11×10^{13} n $cm^{-2}sec^{-1}$ for 7 min at 2.4 MW. After long irradiation, samples were turned into highly radioactive [22]. For this reason, they usually were not handled immediately. They were in a shielded place for 2 days. Normally the used irradiation facility is G-ring.

(ii) Short irradiation was performed separately for each sample with the thermal neutron flux of 5.28×10^{12} n cm^{-2} sec^{-1} for 1min at 250 kW. To determine the neutron flux gradient within the sample stack, IRMM-530RAAl-0.1% Au (0.1 mm foil) monitor foils were also irradiated by placing them at the bottom, middle and top of the sample stack for the long irradiation scheme.

Figure 3.4: Sending and receiving center for sample vial at BAEC, Savar.

3.4 Gamma Ray Counting

After irradiation, gamma-ray counting was performed with a high purity germanium (HPGe) detector (CANBERRA, 25% relative efficiency, 1.8 keV resolution at1332.5 keV

of Co-60) coupled with a digital gamma spectrometer (ORTEC, DSPEC Jr™). For short irradiation, first counting was performed for 300 s after a decay time of about 300 s and second counting for 600 s after decay time of 2-3 h. For long irradiated samples, first counting was performed for 3600 sec after a decay time of 2-3 days while the second counting was performed for 7200 sec after a decay time of 7-10 days and third counting was performed for 8-12 hours after a decay time of 2-3 weeks. Short lived and long lived radio- nuclides were determined from the short and long irradiation separately. The gamma spectrometry of all the irradiated samples and certified reference materials was performed using a PC-based HPGe detector coupled with a digital gamma spectrometry system [53]. The data acquisition was performed using the software Genie-2000 (Canberra) and MAESTRO- 32 (ORTEC) and the gamma peak analysis was performed using the software Hypermet PC version 5.12. The gamma peak analysis is shown in Figure.3.5 and Figure. 3.6.

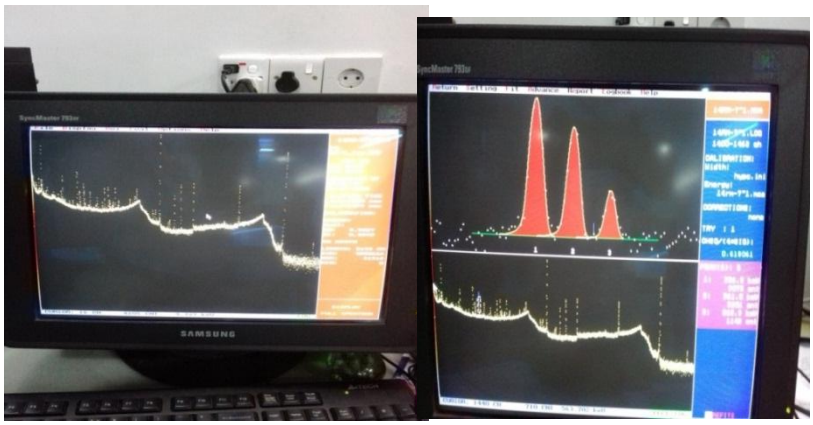


Figure 3.6: Counts the gamma peak area using Hypermet PC version

3.5 Fundamental Equations for NAA Method

There are two ways in which NAA can be treated mathematically such as:

- Absolute NAA method

- Comparative NAA method

3.5.1 Absolute NAA method

The basic equation used for NAA calculation in absolute method is,

$$A_{o=\frac{MN_A\theta}{W}\sigma\varphi\{1-e^{-\lambda t_i}\}} \quad \text{..... (3.1)}$$

Where, A_0 is the activity or the disintegration rate at the end of irradiation time t_i.

M is the mass of the element

$N_A = 6.673\times10^{23}$, is the Avogadro's number

σ is the cross-section, in barn

φ is the neutron flux, in neutrons $m^{-2}s^{-1}$

θ is the isotopic abundance

W is the atomic weight

Usually, in neutron activation analysis, the activity of the radionuclide is measured experimentally in a sample to deduce the unknown mass (M) of the element by the above equation.

Correction must be made for the decay period t_d and counting period t_c,

Where,

Decay factor, $F_d = e^{-\lambda t_d}$ and

Counting factor, $F_c = \frac{1-e^{-\lambda t_c}}{\lambda t_c}$

So, the basic equation for NAA calculation in absolute method becomes,

$$A_0 = \frac{MN_A\theta}{W}\sigma\varphi\{1 - e^{-\lambda t_i}\}\{e^{-\lambda t_d}\}\left\{\frac{1-e^{-\lambda t_c}}{\lambda t_c}\right\} \quad \text{..... (3.2)}$$

Hence,

$$M = \frac{A_o W}{N_A\theta\varphi\sigma\{1-e^{-\lambda t_i}\}\times F_d \times F_c} \quad \text{..... (3.3)}$$

All the factors on the right side of the above equation are, in principle, known or can be measured. Thus, it can be possible to calculate the mass of the element in a sample.

The difficulty of accurate measurement of σ leads to the difficulty of measuring neutron flux density φ and also the value of φ changes depending on time and the location in most powerful neutron sources like nuclear reactors, sample and its container cause perturbation of neutron flux density (flux depletion and self-shielding of neutrons), which is very difficult to evaluate precisely.

The activity A can be obtained from the following relationship,

$$A = R/ I_\gamma \epsilon$$

Where, R is the counting rate of full energy peak caused by the gamma rays used for the activity measurement, ϵ is the absolute counting efficiency of the gamma rays, and I_γ is the intensity of gamma rays.

3.5.2 Comparative NAA method

In the comparative NAA method, an element "x" in a sample and a known amount of the same element "x" as a standard are irradiated together and both sample and standard are counted under exactly the same conditions by the same radiation detector. This procedure

eliminates any uncertainty in the parameter σ, φ, λ and in the decay scheme and detection efficiency. The NAA equation by the comparative method is thus reduced to a simple form, as shown below

$$\frac{Mass\ of\ element\ \ \text{"X"}\ in\ sample}{Mass\ of\ element\ \ \text{"X"}\ in\ standard} = \frac{A_{X^*\ in\ sample\ \ \times (e^{\lambda t_d})_{sam.}}}{A_{X^*\ in\ standard\ \ \times (e^{\lambda t_d})_{std.}}} \quad \text{.....(3.4)}$$

Knowing the activities of x* in sample and standard, the sample and standard decay times and the mass of the element "x" in the standard, the mass of the element "x" in the sample is then calculated. In a multi elemental determination of 30 to 40 elements in the comparative method requires the use of several synthetic individual or mixed solutions, or certified reference material, whereas the absolute method requires only one standard [54].

3.6 Characteristics of NAA Method

There are many situations in which NAA has theoretically better analytical characteristics than other methods of elemental analysis such as atomic absorption spectroscopy (AAS), inductively coupled plasma spectroscopy (ICPS) and total reflection X-ray fluorescence spectroscopy (TR-XRE). So it is important to remain realistic in evaluating the role of NAA. Therefore, the most typical analytical characteristics of NAA are given as follows:

a) Relative freedom from matrix and interference effects
b) Virtual absence of an analytical blank.
c) Sensitivity and applicability for mirror and trace elements in a wide range of matrices.
d) An inherent potential for accuracy compared to other analytical technique. Since the theoretical basis of NAA is well understood, a complete uncertainty budget can be made.
e) The totally independent of nature of the method as a nuclear-based property in contrast to the electronic nature of most other analytical techniques.

f) The possibility of performing non-destructive analysis using neutron activation analysis.

g) High specificity based on the individual characteristics of the induced radionuclides.

h) The capability of NAA for multi-element determination, often allowing 30 to 40 elements to be determined in many matrices.

i) In case where the induced radionuclides of trace elements are marked by matrix activity, radiochemical separation provides interference free detection limits close to theoretical ones. Thus, in the radiochemical mode of NAA (RNAA) the technique has other advantages feature.

3.7 Requirement for NAA

The basic essentials required to carry out an analysis of samples by NAA are:

- A source of neutrons.
- Suitable instrumentation for detecting gamma rays.
- A detailed knowledge of the reactions that occur when neutrons interact with target nuclei.

3.8 Sensitivities Available by NAA

The sensitivities for NAA are dependent upon the irradiation parameters (i.e., neutron flux, irradiation and decay times), measurement conditions (i.e., measurement time, detector efficiency), nuclear parameters of the elements being measured (i.e., isotope abundance, neutron cross-section, half-life, and gamma-ray abundance). The accuracy of an individual NAA determination usually ranges between 1 to 10 percent of the reported value. Table 3.1 lists the approximate sensitivities for determination of elements assuming interference free spectra.

Table 3.1: Estimated detection limits for NAA using decay gamma rays. Assuming irradiation in a reactor neutron flux of $1x10^{13}$n $cm^{-2}s.^{-1}$

Sensitivity (pico-grams)	Elements
1	Dy, Eu
1 – 10	In, Lu, Mn
10 – 100	Au, Ho, Ir, Re, Sm, W
100 - 10^3	Ag, Ar, As, Br, Cl, Co, Cs, Cu, Er, Ga, Hf, I, La, Sb, Sc, Se, Ta, Tb, Th, Tm, U, V, Yb
10^3 - 10^4	Al, Ba, Cd, Ce, Cr, Hg, Kr, Gd, Ge, Mo, Na, Nd, Ni, Os, Pd, Rb, Rh, Ru, Sr, Te, Zn, Zr
10^4 - 10^5	Bi, Ca, K, Mg, P, Pt, Si, Sn, Ti, Tl, Xe, Y
10^5 - 10^6	F, Fe, Nb, Ne
10^7	Pb, S

3.9 Neutron Sources

Neutrons can be obtained from Reactors, Accelerators, and from Radio-isotopic neutron emitters.

(a) Isotopic sources

Isotopic neutron sources, like ^{226}Ra (Be), ^{124}Sb (Be), ^{241}Am (Be), ^{252}Cf. The neutrons have different energy distributions with a maximum in the order of 3–4 MeV; the total output is typically 10^5–10^7 s^{-1} GBq^{-1} or, for ^{252}Cf, 2.2 10^{12} $s^{-1}g^{-1}$.

(b) Particle accelerators or neutron generators

The most common types are based on the acceleration of deuterium ions towards a target containing either deuterium or tritium, resulting in the reactions ^{2}H (^{2}H, n) ^{3}He and ^{3}H (^{2}H, n) ^{4}He, respectively. The first reaction, often denoted as (D, D), yields mono-energetic neutrons of 2.5 MeV and typical outputs in the order of 10^8–10^{10} s^{-1}; the second reaction (D, T) results in mono-energetic neutrons of 14.7 MeV and outputs of 10^9–10^{11} s^{-1}.

(c) Nuclear research reactors

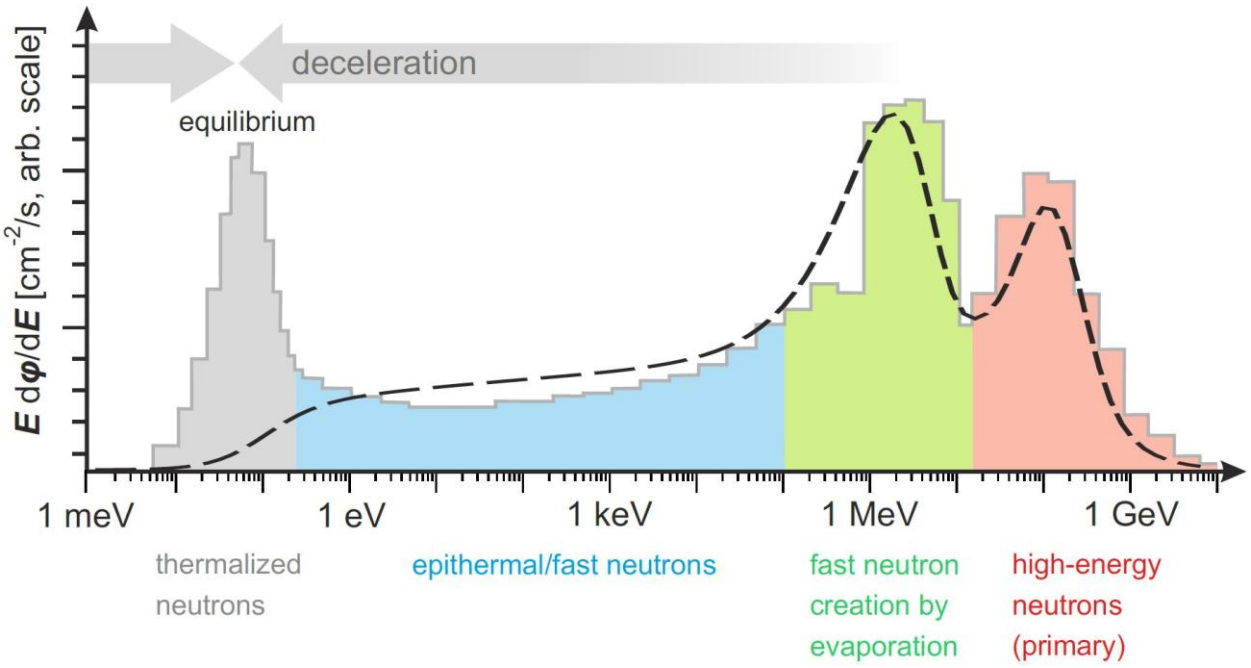
The neutron energy distribution depends on design of the reactor and its irradiation facilities. The neutron output of research reactors is often quoted as neutron fluence rate in an irradiation facility and varies, depending on reactor design and reactor power, between 10^{15} and 10^{18} $m^{-2}s^{-1}$.Different types of reactors and different positions within a reactor can vary considerably with regard to neutron energy distributions and fluxes due to the materials used to moderate the primary fission neutrons (Fig. 3.7). Most neutron energy distributions are quite broad and consist of three principal components:

i. **Thermalneutron:** The thermal neutron component consists of low-energy neutrons (energies below 0.5eV) in thermal equilibrium with atoms in the reactor's moderator. In most reactor irradiation positions, 90-95% of the neutrons that bombard a sample are thermal neutrons. In general, a one-megawatt reactor has a peak thermal neutron flux of approximately 10^{13} neutrons per square centimeter per second.

ii. **Epi-thermal neutron:** The epi-thermal neutron component consists of neutrons (energies from 0.5 eV to about 0.5 MeV) which have been only partially moderated. In a typical unshielded reactor irradiation position, the epi-thermal neutron flux represents about 2% the total neutron flux. Both thermal and epi-thermal neutrons induce (n, gamma) reactions on target nuclei. An NAA technique that employs only epi-thermal neutrons to induce (n, gamma) reactions by irradiating the samples being analyzed inside either cadmium or boron shields iscalled epi-thermal neutron activation analysis (ENAA).

iii. **Fastneutron:** The fast neutron component of the neutron spectrum (energies above 0.5 MeV) consistsof the primary fission yielding neutrons which still have much of their original energy followingFission. In a typical reactor irradiation position, about 5% of the total flux consists of fast neutrons. An NAA technique that employs nuclear

reactions induced by fast neutrons is called fast neutron activation analyses (FNAA).



The (n, γ) reactions of thermal neutrons and the (n, xγ) reaction of fast neutrons are quite complementary to each other. Whereas the (n, γ) cross-section is very low for most light elements, e.g. Li, Be, B, C, O, Na, Mg, Al, with notable exception of hydrogen, the (n, xγ) cross section for these elements is quite respectable. Furthermore, the yield and the specificity of gamma rays from (n, xγ) are very high.In nuclear research reactors,the neutron flux distribution is shown in Figure 3.7 below.

3.10 TRIGA MARK- II Research Reactor

In this study samples are irradiated by 3 MW TRIGA MARK- II research reactor at the Atomic Energy Research Establishment (AERE), Savar, Dhaka. A partial view of the 3 MW TRIGA MARK- II research reactors is shown in Fig.3.8. It is a multi-purpose reactor, capable of both steady state and pulsing operation, has been put into service in several disciplines since its commissioning. It is a light water cooled graphite reflected reactor designed for continuous operation at a steady-state power of 3 MW. The characteristic of this reactor is clear from the name "TRIGA" which is a combination of the words Training, Research, Isotope production and name of the manufacturing

company GA (General Atomic Company, USA). This reactor attained critically in the 14th September, 1986 is the only nuclear reactor in Bangladesh.

Table 3.2: Principal design parameters of the TRIGA MARK- II reactor.

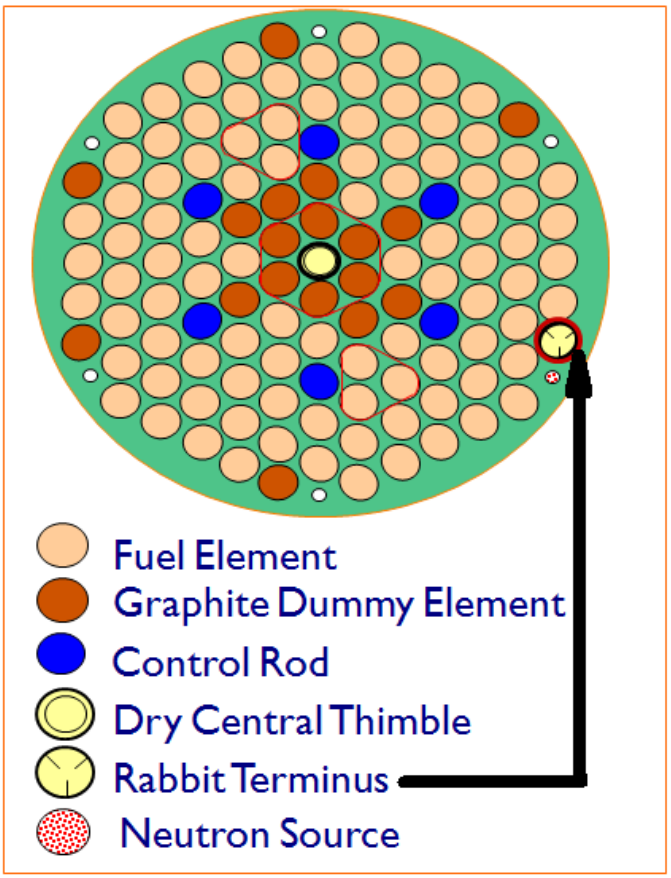
Maximum steady state power level	3 MW
Fuel element design	
Fuel- moderator material	U-ZrH
Uranium content	20 wt %
Uranium enrichment	19.7% U-235
Burnable poison	0.47 wt % Erbium
Shape	Cylindrical
Overall length of fuel	38 cm (15 inch)
Outside diameter of fuel	3.63 cm (1.43 inch)
Cladding material	Type 304 stainless steel

Number of fuel elements	100
Maximum excess reactivity	7.69 % Δk/k
Reactivity loss due to equilibrium Xe	2.5 % Δk/k
Number of control rods	
Shim / safety	4
Regulating	1
Safety /transient	1
Total reactivity worth of control rods	12.785Δk/k
Reactor cooling	Forced down flow of pool Water (above 500 kW)

3.11 Components of TRIGA MARK-II Reactor

Brief description of the components of TRIGA MARK-II research reactor is given below:

a) Reactor core: Any reactor consists of an active part in which fission chain reactions are sustained and most of the fission energy is released as heat. This active part is known as reactor core, which contains the nuclear fuel and moderator where required. The core of TRIGA MARK-II reactor has a 0.63 cm thick wall having an inside diameter of 2 m and a depth of 8.2 m. Figure 3.9 shows the cross-sectional view of TRIGA MARK- II reactor core. The reactor core and reflector assemble is a cylinder approximately 1.1 m in diameter and 0.89 meter high. [General Atomic Company, 1980]



The reactor core consists of a lattice of fuel-moderator elements, graphite dummy elements and control rods. A graphite reflector and a 5 cm thick lead gamma shield surround the core. The entire assembly is bolted to a stand that rest on the bottom of the reactor tank. The outer wall of the reflector housing extends 0.8 m above the top of the core to ensure retention of sufficient water for after-heat removed in the event of a tank drain accident. Cooling of the core is provided by natural circulation of up to 500 kW power level and by forced down flow circulation of tank water for higher powers, which is, in turn, cooled and purified in external coolant circuits. In case of loss of cooling water in the reactor tank there is a provision of emergency core cooling system with roof top backup system.

b) Fuel: There are total of 100 fuel elements in the reactor core. The fuel is solid,

homogeneous mixture of Eu-ZrH alloy containing 20% by weight of uranium enriched to about 19.7% ^{235}U and about 0.47% by weight of Erbium. The H/Zr ratio is approximately 1.6. Each element is clad with 0.051 cm thick stainless steel can. Two sections of graphite are inserted in the can, one above and one below the fuel, to serve as top and bottom reflectors for the core.

c) Moderator: Most of the fission in a reactor occurs from thermal neutrons. The function of the moderator is to slow down the high energy neutrons to thermal energy level through successive collisions. The best moderators are materials consist of elements of small mass numbers with small cross-sections for absorption of neutrons. Examples are hydrogen, water, heavy water, beryllium or its oxide, carbon as graphite, and hydrocarbons. In the BAEC TRIGA MARK-II $ZrH_{1.6}$ is mainly used as moderator. Light water is also used for this purpose.

d) Reflector: The reflector of TRIGA reactors is a ring shaped block of graphite that surrounds the core radially. It is 30.5 cm thick radially, with an inside diameter of 45.7 cm and height of 55.9 cm. The graphite is protected from water penetration by a leak-tight welded aluminum can. The purpose of the reflector is to decrease the loss of neutrons from the core by reflecting back some of the neutrons which tend to leak out or escape from the core.

e) Control rod: The heat generated in the reactor is proportional to the fission rate, which depends on the neutron density in the core. Control, including startup, operation to certain power, and shut down, is thus achieve by varying the neutron density in the core. This is done by moving rods of a material that has a very high neutron absorbing property. Insertion of a control rod like cadmium or boron results in a decrease in the neutron density and hence the reactor power. Withdrawal of the rod conversely increases

the neutron level and hence the reactor power. Boron and Cadmium, which have very high neutron absorption cross-sections, are mainly used as material for control rods.

For ^{10}B, σ_a = 3,800 barns (thermal) and

For ^{113}Cd, σ_a =20,000 barns (thermal).

The BAEC TRIGA MARK-II reactor is controlled by six boron carbide control rod. Each control rod is a sealed aluminum tube containing powdered boron carbide as a neutron poison. The control rods are approximately 51 cm long.

f) Reactor tank: The reactor tank consists of an aluminum vessel installed in the reactor shield structure. The tank has an inside diameter of approximately 1.98 m and a depth of 6.25 m. A 2 by 2 inch aluminum channel used for mounting the ion chambers and underwater lights is attached to the top of the tank.

g) Reactor shield: A reactor is a source of neutron and gamma radiation. To protect human and environment expose to these hazardous radiations, a reactor is always installed inside of some barriers capable of absorbing them. Such barriers are mainly known as reactor shielding. The reactor shield is a reinforced concrete structure standing 7.9 m above the reactor hall floor. The lower octagonal portion is 6.6 m across the flats. The beam ports are installed in the shield structure with tabular penetrations through the concrete shield and the reactor tank water, and they terminate either at the reflector assembly or at the edge of the reactor core. The radial shielding of the core is provided by a minimum of 2.29 m of concrete having a minimum density 2.75 g/cm3, 45.7 cm of water, 19 cm of graphite and 5 cm of lead. The heavy shield was made using locally available ilmenite and magnetite from beach sand of Cox's Bazar.

3.12 Irradiation Facilities of TRIGA Reactor

The TRIGA MARK- II reactor is designed to provide intense fluxes of ionizing radiation for research, training and isotope production. Experiments with the TRIGA reactor can be carried out using the following facilities:

1. Rotary specimen rack (Lazy Susan)
2. Pneumatic transfer system
3. Central thimble
4. Beam port facilities

a) Lazy Susan:

The Lazy Susan assembly consists of a stainless steel rack that holds specimens during irradiation and ring shaped, seal-welded aluminum housing. The rack supports forty-one (except one) evenly spaced tabular aluminum containers that serve as receptacles for the specimen containers. Each receptacle has an inside diameter of 1.25 inches (31.75 mm) and a height of 10.80 inches and can hold two specimen containers. The open bottom tube provides access for periodic testing of the bottom of the rotary specimen rack housing to determine the extent of accumulation of condensation as leaking water. The internal ring can be rotated around the core for the insertion or removal of samples. A single locking rod orients each tube with respect to the specimen removal tube. The internal rack is the rotated manually for equal irradiation of the samples from the drive mechanism on the center channel assembly.

b) Central thimble:

The central thimble in the center of the core provides space for the irradiation of the sample at the point of maximum flux. It also provides a highly collimated beam of neutrons and gamma radiation when the water is pneumatically expelled from the section of the thimble above the core.

c) Beam port facilities:

The beam ports provide tubular penetrations through the concrete shield and the reactor tank water, making beams of neutrons and gamma radiation available for a variety of experiments. There are four six-inch diameter beam ports divided into two categories- radial beam ports and tangential beam ports. There are three radial and one tangential beam ports in the TRIGA reactor.

Table 3.3: Values of Neutron flux (n/cm^2/cm) at TRIGA MARK – II research reactor, AERE, Savar, Dhaka.

Different position	Epithermal	Thermal
Average flux in rector core	1.1×10^{13}	5.3×10^{13}
Central tube	1.5×10^{13}	5.56×10^{13}
Rotary rack (at the bottom)	0.26×10^{13}	0.7×10^{13}
G- ring (the last circle of fuel center)	1.0×10^{13}	2.0×10^{13}

3.13 High Purity Germanium (HPGe) Detector System

Silicon semiconductor detectors have depletion depths less than 1 mm, which are sufficient for charged particle spectroscopy or soft X-ray detection. For photon spectroscopy in the energy region of hundred keV or several MeV, much thicker semiconductor detectors are required. It is obvious that a depletion depth of at least several cm is required. Due to its higher atomic number, Ge has a much larger linear attenuation coefficient, which leads to a shorter mean free path. Thus, Ge is preferred for hard X-ray or gamma-ray detection to achieve higher detection efficiency.HPGe crystal were first developed in the mid of 1970s. The starting material is bulk germanium intended for the semiconductor industry. Although already of very high purity, the material is further purified with the zone refining technique. The germanium is melted in a crucible using radio-frequency (RF) heating coils. The underlying principle is that

impurities concentrate in the liquid phase leaving the solid purer than the original melt as a liquid freezes and solid appears. HPGe gamma spectrometry system in the present experiment consists of the following parts:

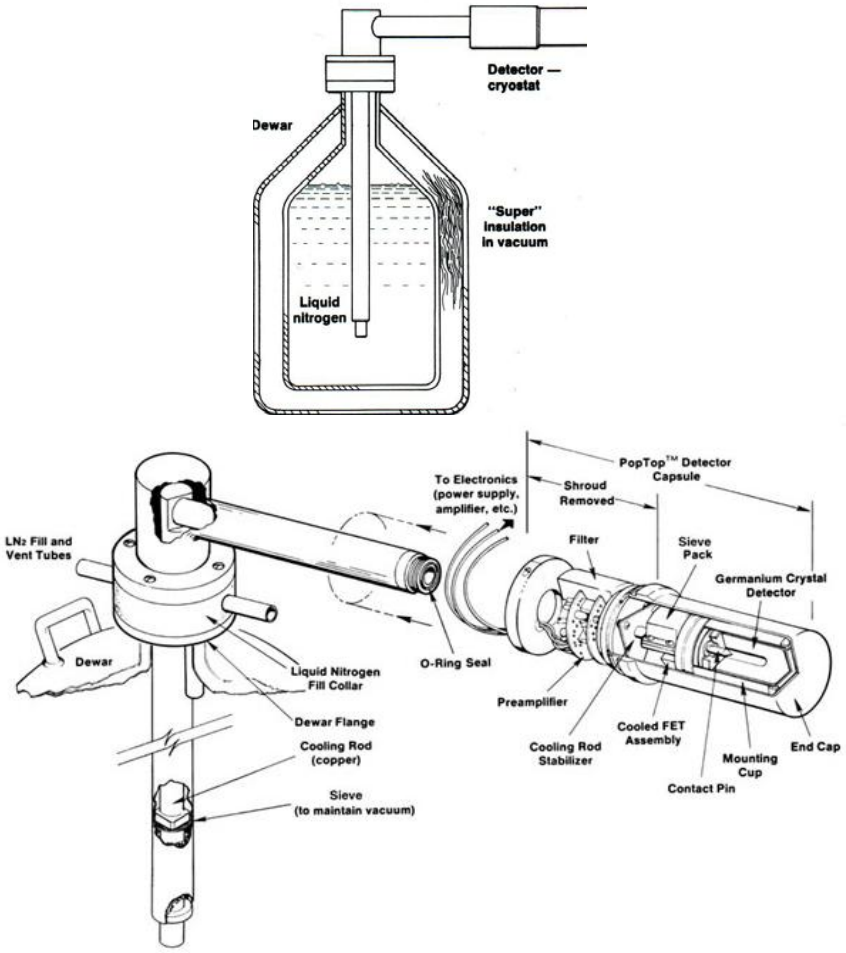
i. HPGe detector,

ii. Digital gamma spectrometer,

iii. Shielding arrangement.

3.13.1 Basic configuration of HPGe detector

The main parts of a HPGe detector are cryostat, dewar and pre-amplifier.Although silicon detectors can be operated at room temperature in principle, operating germanium detectors at room temperature is impossible due to the small band gap energy, which generates an intolerable leakage current. Therefore, germanium crystals must be cooled to reduce the leakage current. HPGe detectors are allowed to warm to room temperature when they are not in operation, however, they are always maintained at LN2 temperature (liquid nitrogen at -196° C or 77k) practically. The Ge crystal is encased in a vacuum tight container (cryostat) to reduce thermal conduction between the crystal and the air. The basic function of a cryostat is to cool the germanium detector to LN2 temperature (Fig 3.10 and Fig 3.11). The germanium detector preamp is normally included as part of the cryostat package. Since the preamp should be located as close as possible so that the overall capacitance can be minimized, the preamp is installed together. The input stages of the preamp are also cooled. The entire cold assembly is maintained by the cryostat under high vacuum for both thermal insulation and protection of the internal components from contamination.



All of the cryostat materials around the detector should be as low Z as possible to reduce photon scatter. Hence, aluminum, magnesium, beryllium, Teflon, and Mylar are used whenever possible. There are various types of dewars so that the detection geometry can be selected depending on the application. For general radioactive or neutron-activated samples, the vertical type is widely used. For in-beam spectroscopic purposes, either the horizontal or side-looking type is more convenient. Portable dewars are also available for outside field measurements.

3.13.2 Digital gamma spectrometer

Digital gamma spectrometer is consists of the following devices:

1. Amplifier.
2. High voltage unit,
3. Analog to digital converter (ADC).

These three items are integrated in a box. The product of Canberra is called DSA and the product of ORTEC is called DSPEC.

Digital spectrum analyzer (DSA): DSA-1000 is a full featured 16K channel integrated multi-channel analyzer constructed based on advanced digital signal processing techniques (DSP), which is operated through Genie-2000 spectroscopy software. The heart of DSA-1000 is the digital signal processing subsystem. DSA-1000 digitizes the preamplifier signals at the front of the signal processing chain. The DSA-1000 offers peak gain stability, in some cases a factor of two or three times better than the past generation analog products. It supports both the traditional Pulse Height Analysis (PHA) mode as well as Multi Channel Scaling (MCS) mode for time varying applications. The method of

host communication is the USB interface which provides fast host communication at 12Mbits/sec. DSA-1000 incorporates a high voltage power supply, preamplifier unit, amplifier unit, ADC, etc.

3.13.3 Shielding arrangement

All radiation detectors record some background signal due to cosmic radiation that continuously bombards the earth's atmosphere and the existence of natural radioactivity in the environment. The background effect is very important in the present type of work, in detecting gamma-rays by HPGe detector. The background radiation also may cause great harm to the devices of the gamma-ray spectrometry system. So, if these devices are not properly protected from such type of radiation, the radiation will affect the results of the experiment and also can change the physical properties of the devices. Therefore shielding of the detector is essential. The shielding provides a degree of isolation in laboratories where other radiation sources may be used or moved about during the course of a measurement. The shielding not only reduces the background resulting from the cosmic radiation and from natural radioactive traces in the building materials or in the surface of the earth, but also from nearby nuclear facilities and other radiation sources. Reduction of such type of radiation may be accomplished by putting a shied which will effectively absorb the undesired radiations before they reach the detector.For low background, the conventional shielding materials are lead, steel, mercury and concrete. Lead is the most widely used material for the construction of detector shield because of its high density (11.4gm/cc) and large atomic number (Z = 82). In the present experiment, lead was the shielding material of HPGe detector. A brief description of the shielding of the HPGe detector used in the present experiment is given in the Table 3.4.

Table 3.4: Shielding arrangement around the HPGe detector.

Material	Lead (Pb)
Form	Square
Length	46 cm
Height	66 cm
Thickness	6 cm

3.14 Full Specification of HPGe Detector

As a whole, the following are the specification of the HPGe detector, which is used in the present study:

Detector	:	HPGe
Detector model	:	GC 4020
Serial number	:	07037658
Crystal geometry	:	Closed-end coaxial
Crystal diameter	:	6.2 cm
Crystal length	:	5.7 cm
Crystal active volume	:	172 cm^3
Crystal/window distance	:	0.5 cm
Dewar capacity	:	30 liters
Cooling temperature	:	77K
Energy resolution of the detector	:	2keV (specified by manufacturer)
at 1332keV of ^{60}Co γ-rays		
Relative efficiency	:	25%
Peak to Compton ratio	:	54: 1

3.15 Background of Gamma Radiation

Within the electromagnetic radiation spectrum, gamma radiation is located near the high energy end along with X rays. The energy associated with gamma radiation (for example, gamma rays emitted by cobalt-60) is high enough to break the molecular bonds and ionize atoms, but not high enough to affect structure of the atomic nucleus (avoiding induction of radioactivity). Gamma radiation may, therefore, modify chemical, physical or biological properties of the irradiated material/product; however, the irradiated product does not become radioactive. Radiation with such high energy is referred to as ionizing radiation. All radiation processing is performed with ionizing radiation, which includes-besides gamma radiation-high energy electrons (generally >80 keV) and X rays generated from high energy electrons (e.g. 5-10 MeV). Cobalt-60 and caesium-137 are the most suitable gamma radiation sources for radiation processing because of the relatively high energy of their gamma rays and fairly long half-life (5.27 years for cobalt-60 and 30.1 years for caesium-137). However, the use of caesium-137 has been limited to small self-contained, dry-storage irradiators, used primarily for the irradiation of blood and for insect sterilization. Currently, all industrial radiation processing facilities employ cobalt-60 as the gamma radiation source.

3.16 Sample Preparation for Gamma Irradiation

a) Irradiation of samples and dosimetry

The experiment was done in the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Saver, Dhaka, Bangladesh. Samples were irradiated using a gamma irradiator with cobalt-60 source (Gamma beam 650, AECL, Canada). The activity of gamma irradiator was 50 kCi that was used for spices irradiation. The absorbed dose was measured using ferrous sulphate (Fricke) and red perspex dosemeters. Red

perspex dosemeters (Type 4034 AF, AERE, Harwell, UK, and dose range 5-50 kGy) were standardized against Fricke dosimeters.

b) Radiation Process

In a radiation process, a product or material is intentionally irradiated to preserve, modify or improve its characteristics. This process is carried out by placing the product in the vicinity of a radiation source (such as cobalt-60) for a fixed time interval whereby the product is exposed to radiation emanating from the source. A fraction of the radiation energy that reaches the product is absorbed by the product; the amount depending on its mass and composition, and time of exposure. For each type of product, a certain amount of radiation energy is needed to realize the desired effect in the product; the exact value is determined through research. Radioactive material, such as a cobalt-60 source, emits radiation. However, the product that is irradiated with gamma rays does not become radioactive, and thus it can be handled normally. This is similar to X ray examination in a hospital for diagnostic purposes; the patient is exposed to radiation (X rays) but he/she does not become radioactive.

3.17 Cobalt-60 Radiation Source

The radionuclide cobalt-60 (Co-60 or $^{60}Co_{27}$) is the most commonly used source of gamma radiation for radiation technology, both for industrial and medical purposes. Production of radioactive cobalt starts with natural cobalt (metal), which is an element with 100% abundance of the stable isotope cobalt-59. Cobalt-rich ore is rare and this metal makes up only about 0.001% of the earth's crust. Slugs (small cylinders) or pellets made out of 99.9% pure cobalt sintered powder and generally welded in Zircaloy capsules are placed in a nuclear power reactor, where they stay for a limited period (about 18–24 months) depending on the neutron flux at the location. While in the reactor, a cobalt-59

atom absorbs a neutron and is converted into a cobalt-60 atom. During the two years in the reactor, a small percentage of the atoms in the cobalt slug are converted into cobalt-60 atoms.

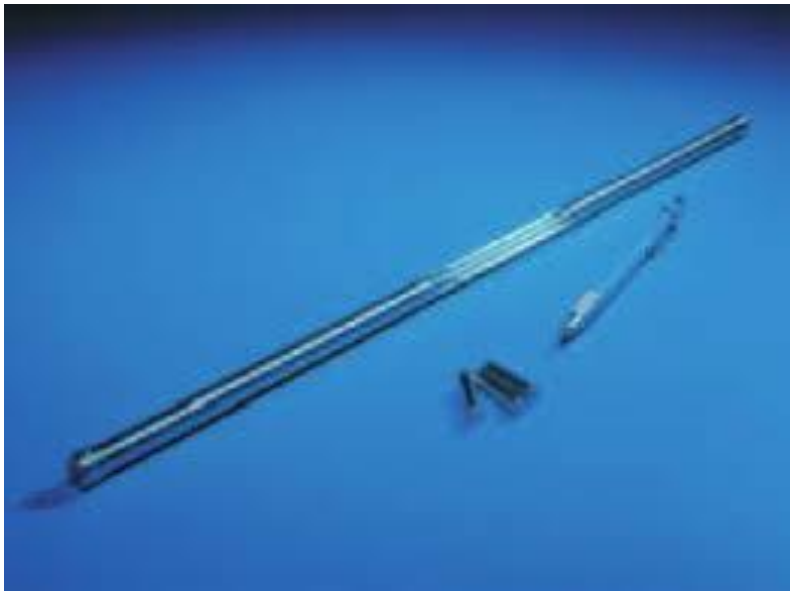


Figure 3.12: Slugs and pencil of cobalt-60, which are the building blocks of a radiation source rack (courtesy of MDS Nordion, Canada) [39, 40].

Specific activity is usually limited to about 120 Ci/g of cobalt (about 4×10^{12} Bq/g). After irradiation, the capsules containing the cobalt slugs are further encapsulated in corrosion resistant stainless steel to finally produce the finished source pencils in a form such that gamma radiation can come through but not the radioactive material (cobalt-60) itself (see Fig. 3.13).

Gamma irradiation facility

In a large irradiation facility, the irradiation room where the product is treated with radiation is the focal point of the facility (see Fig. 3.14). Other major components of an industrial facility include:

- shielded storage room (dry or wet) for the radiation source rack (cobalt-60),

- source hoist mechanism,
- radiation shield surrounding the irradiation room,
- control console (room),
- product transport system through the shielding maze,
- control and safety interlock system,
- areas for loading and unloading of products, and

The radiation source is either in the irradiation room (during irradiation of the product) or in its shielded storage room (generally located under the irradiation room), which could be dry or wet. There is enough shielding provided by solid wall (dry storage) or water (wet storage) so that the personnel can work in the irradiation room, e.g. for maintenance, when the source is in the storage room. Water has several desirable characteristics as a shielding material; it is an easily available liquid that is convenient to circulate for heat transfer, and is transparent. For a wet-storage facility, nearly all materials used to construct source rack, guide system, and source containers are stainless steel, to eliminate galvanic corrosion. Surrounding the irradiation room is the radiation shield, which is also referred to as biological shield, generally consisting of a concrete wall thick enough (normally 2 m in thickness) to attenuate the radiation emanating from the source, so as to maintain the radiation level at the location of the control console close to natural background. The concrete wall is constructed as a maze (labyrinth) so as to permit movement of the product and yet significantly reduce the scattered radiation reaching the control console, from where the operator can control or monitor the movement of the source and the product. The transport mechanism for the product can be simple or can be quite elaborate depending on the irradiator design. For continuous irradiation (as shown in Fig. 3.13), the product containers are moved around the radiation source on a conveyor bed that passes through the maze.

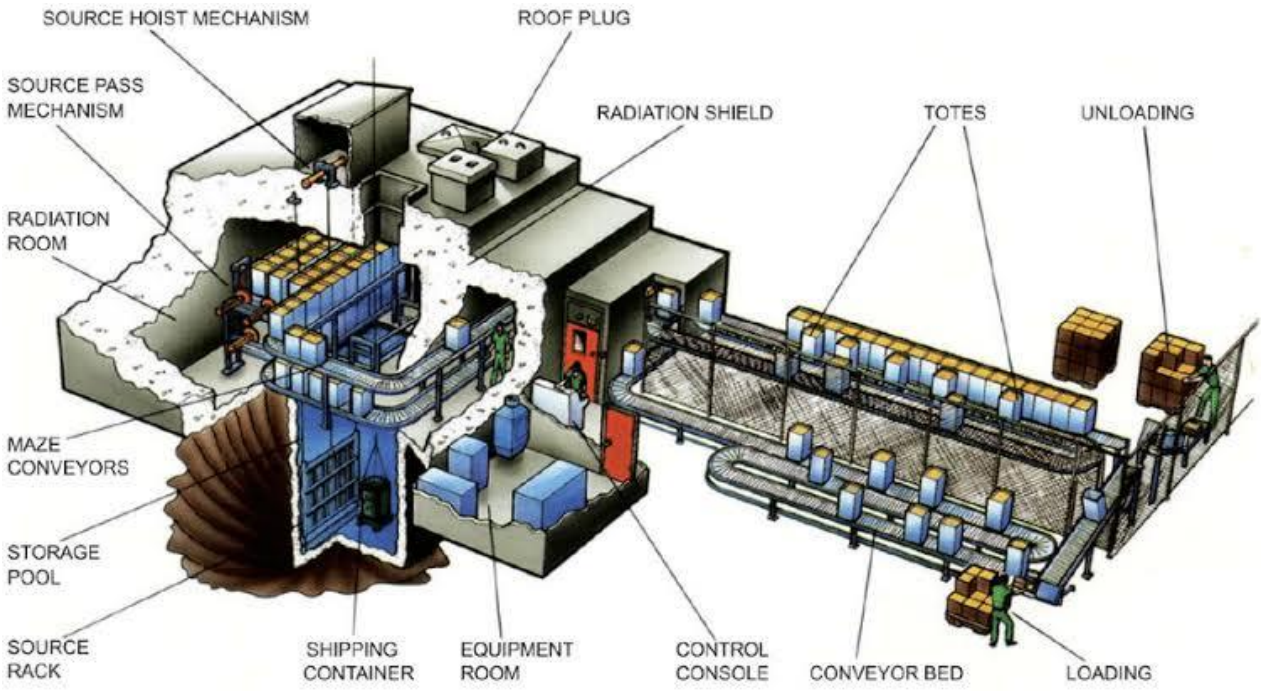


Figure 3.13: Schematic diagram of a typical panoramic, wet storage gamma irradiation facility [39].

For stationary irradiation, the radiation source is moved into the irradiation room *after* the product containers have been arranged there for irradiation. The irradiation facility also provides areas for the storage of the un-processed product and the processed product. It is a regulatory requirement that the design of the facility is such that these two types of product cannot be mixed inadvertently (Fig. 3.14). Also, all facilities have laboratories suitable for carrying out dosimetry measurements. Some facilities also have a microbiology laboratory or a materials testing laboratory.

3.18 Analysis of Microbial Loads

All samples were analyzed for the total bacterial count (TBC) by a standard plate count method. Five grams of each spices powder was mixed with 45 ml sterilized water. Subsequent dilutions were prepared and plated on plate count agar for determining the

bacterial count. Microbial counting was performed 24-48 h after incubation at 37^0c for TBC [41].

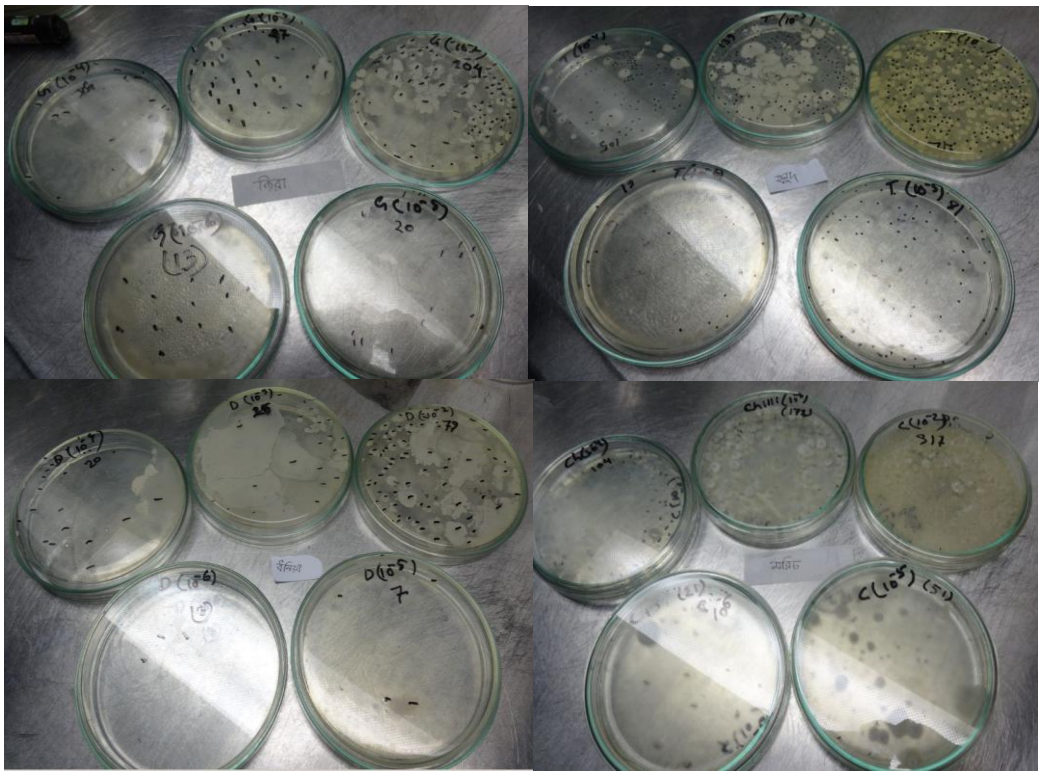


Figure 3.14: Investigation of bacterial growth in the spices samples.

3.18.1 Bacteria identification

Bacterial isolation was carried out according to the protocol suggested by Fawole & Oso [55]. About 5g samples dissolved in about 45ml of autoclaved distilled water and incubated at 37°C for 24-48 h. About 5 ml of the incubated this culture was selectively enriched into the *HiCrome Bacillus* agar (Hi Media), and incubated again at 37°C for overnight (Fig. 3.14). Then the presumptive colonies of *Bacillus spp.* were sub-cultured onto nutrient media to get pure culture. The isolates were identified as *Bacillus spp.* on the basis of Gram staining, colony morphology on *HiCrome Bacillus* agar, SS agar biochemical characterization of the isolates (amylase, lipase, protease, glucose, lactose, mannitol, galactose, oxidase, citrate, indole, catalase and VP) followed by standard procedure [56, 57]. Five selective media xylose lysine deoxycholate agar/XLD (liofilchem, Italy), pseudomonas selective agar (liofilchem, Italy), listeria selective agar

(Oxoid, Basingstoke, UK), Thiosulfate-citrate-bile salts-sucrose agar/TCBS (liofilchem, Italy), and Eosin methylene blue agar/EMB (Oxoid, Basingstoke, UK) were used to determine the attendance of selective types organisms at 37^0C for 24-48 h [58, 59].

3.19 Physicochemical Properties of Spices

i. **Moisture:** Spices powder was mixed properly to determine the moisture content of samples. 1g of sample was heated at 105^oC for 2 h by automatic moisture analyzer Kern and Sohn GmbH, D-72336, Germany [60].

ii. **pH:** To determine the pH values, spices: distilled water=1:9 suspensions for each sample was prepared and stirred for 2h in 200 ml beaker [47]. The pH of the suspension was measured using edge® digital pH meter.

iii. **Nutritional properties (Fat, protein and ash):**Protein, fat and ash content were determined using official methods

Figure 3.15: a) Moisture analyzer b) pH meter c) Digestion samples d) Sample preparation for pH

3.20 Atomic Absorption Spectrometry (AAS)

One gram of each spice samples were taken into the 250-ml digestion vessels. Fifteen milliliters of a tri-acid mixture (concentrated HNO_3, HClO4, H_2SO4; 5:1:1) was added to the vessel and heated at 80°C until the solution becomes transparent [62]. The digested spice samples were cooled and filtered through whatman No. 42 filter papers, and the filtrates were diluted to 50 ml with double distilled water. All samples were stored at ambient temperature before analysis. Concentrations of Pb, Ni, Cd, and As in the filtrate of digested spice samples were estimated using an atomic absorption spectrophotometer (model AA-6800, Shimadzu Corporation, Japan) (Figure 3.16). The blank reagents and standard reference materials as used in NAA were also used to verify the accuracy and precision of AAS. The spike recoveries were from 85 to 99%.

Figure 3.16: Atomic Absorption Spectroscopy Instrument (AAS)

Chapter 4

Experimental Results and Observations

In this study, research reactor-based Neutron Activation Analysis (NAA) technique is applied to determine an elemental concentration in Bangladeshi spices samples. A typical gamma-ray spectrum of an irradiated red chili sample analyzed in this study is shown in Figure 4.1. The gamma-ray peaks from the radioisotopes of Ti, Br, Na and K are observed in the spectrum. Data of radionuclides with their Half-lives and gamma-ray energies for NAA determination of the elements are given in Table 4.1

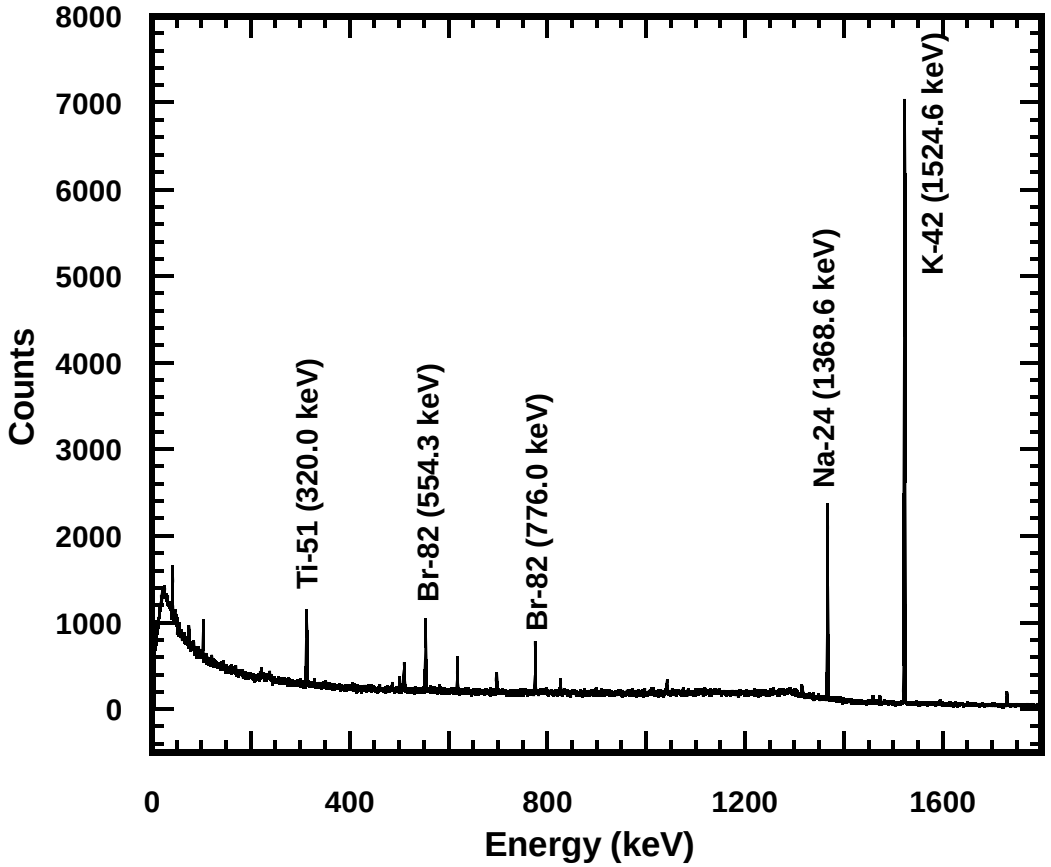


Figure 4.1: Typical gamma-ray spectrum of an irradiated spices sample (Irradiation time: 1 min.; decay time: 10 mins; counting time: 300 s).

Table 4.1: Radionuclides with their half- lives and gamma-ray energies for NAA determination of the elements [63]

Elements	Radionuclide	Half-life	Gamma-ray energy (keV)
Al[a]	^{28}Al	2.24 min	1779.0
Br	^{82}Br	35.3 h	554.3
Ca[a]	^{49}Ca	8.72 min	3084.4
Cl[a]	^{38}Cl	37.3 min	1642.4
Co	^{60}Co	5.27 y	1173.2,1332.5
Cr	^{51}Cr	27.7 d	320.1
Fe	^{59}Fe	44.5 d	1099.2,1291.6
K[a]	^{42}K	12.4 h	1524.6
Mn[a]	^{56}Mn	2.58 h	1810.7
Na[a]	^{24}Na	14.7 h	1368.6
Sc	^{46}Sc	83.8 d	889.3,1120.5
V[a]	^{52}V	3.74 min	1434.1
Zn	^{65}Zn	244 d	1115.5

[a] Elements are determined by short irradiation of NAA.

4.1 Accuracy of the Analysis

To determine elemental concentration in spices samples by comparative NAA method, Two standard reference materials (SRMs): SRM-1547 (Peach leaves) and SRM-1515 (Apple leaves) and one certified reference material: IAEA-336 (lichen powder), along with the spice samples, were analyzed in this study. For relative standardization approach of NAA, IAEA-336 was used as a standard, while NIST-1515 and NIST-1547 were used as the control samples. The consistency and accuracy of the results were checked by analyzing SRMs NIST-1547 (peach leaves) and NIST-1515 (apple leaves). To determine the laboratory performance, we determine the parameter of Z-score and the ratio of determine value to the certified/non-certified value. Z-score was calculated according to the following equation [22]:

$$Z_{score} = \frac{X_{lab} - X_{ref}}{\sigma_{ref}}$$

where X_{Lab}, X_{Ref} and σ_{Ref} are the laboratory result, the reference value and uncertainty with the reference value, respectively. Based on the Z-score value, the laboratory performance is evaluated as satisfactory if |Z|-score ≤ 2, questionable for $2 <$ |Z|-score < 3 and unsatisfactory for |Z|-score ≥ 3.

The mass fractions of total seventeen elements were determined by NAA and AAS techniques. To study the accuracy and precision of the analytical data, the results of the replicate analyses (n=3) of NIST-1545 and NIST-1515 by NAA and AAS are tabulated in Table 4.2. The certified/non-certified values, statistical values of Z-scores and the ratio of determined to certified values for the studied elements are also given in Table 4.2. It is observed that absolute Z-score values are ranged from 0.24 to 2.33 for the reference materials. For some elements, Z-score values are not reported due to unavailability of the uncertainty values with their non-certified values. According to the criteria of Z-score value, the values for the studied elements are within 2.0, except Mn (2.33), which indicates laboratory performance as satisfactory. Moreover, for the studied elements, the ratio of determined values to certified values ranged from 0.91 to 1.13 (Table 4.2). All these observations support the high accuracy of the analytical data obtained in this work.

Table 4.2: Comparison of measured values with certified values (mg/kg) in the standard reference materials of SRM-1547 (peach leaves) and SRM-1515 (apple leaves).

	SRM-1547				SRM-1515			
Elements	This work value (n=3)[b]	Certified value	Z-score	Determined /Certified values	This work value (n=3)[b]	Certified value	Z-score	Determined /Certified values
Al	262±26	248.9±6.5	2.02	1.05	275±12	284.5±5.8	-1.64	0.97
As[a]	0.055±0.003	(0.06)[c]	-	0.92	0.035±0.02	(0.038)[c]	-0.43	0.92
Br	10.0±0.4	(11.0)	-	0.91	2.00±0.10	(1.8)	-	1.11
Ca	15300±646	15590±160	-1.81	0.98	15100±105	15250±100	-1.50	0.99
Cd[a]	0.024±0.010	0.026±0.002	-0.95	0.92	0.015±0.003	0.013±0.001	1.20	1.14
Cl	390±4	361±14	2.07	1.08	594±35	582±15	0.80	1.02
Co	0.068±0.006	(0.07)	-	0.97	-	(0.09)	-	-
Cr	1.06±0.21	(1)	-	1.06	-	(0.3)	-	-
Fe	225±36	219.8±6.8	0.77	1.02	87.1±1.1	82.7±2.6	1.69	1.05
K	24800±1415	24330±380	1.24	1.02	16400±1027	16080±210	1.52	1.02

Mn	102±4	97.8±1.8	2.33	1.04	56.00±2.60	54.1±1.1	1.73	1.04
Na	26.1±1.5	23.8±1.6	1.44	1.10	27.4±2.1	24.4±2.1	1.43	1.12
Ni[a]	0.712±0.030	0.689±0.095	0.24	1.03	0.874±0.110	0.936±0.094	-0.66	0.93
Pb[a]	0.820±0.041	0.869±0.018	-2.72	0.94	0.510±0.110	0.470±0.024	1.67	1.09
Sc	0.045±0.006	(0.04)	-	1.13	-	(0.03)	-	-
V	0.405±0.070	0.367±0.038	1.00	1.10	0.230±0.050	0.254±0.027	-0.89	0.91
Zn	18.8±0.04	17.97±0.53	1.57	1.05	13.2±2.0	12.45±0.43	1.74	1.06

[a] Values were determined by AAS technique. [b]This work values are mean values (n=3) and uncertainties are due to standard deviation (1σ); Values in parenthesis are non-certified.

[c]Compiled value from GeoRem.

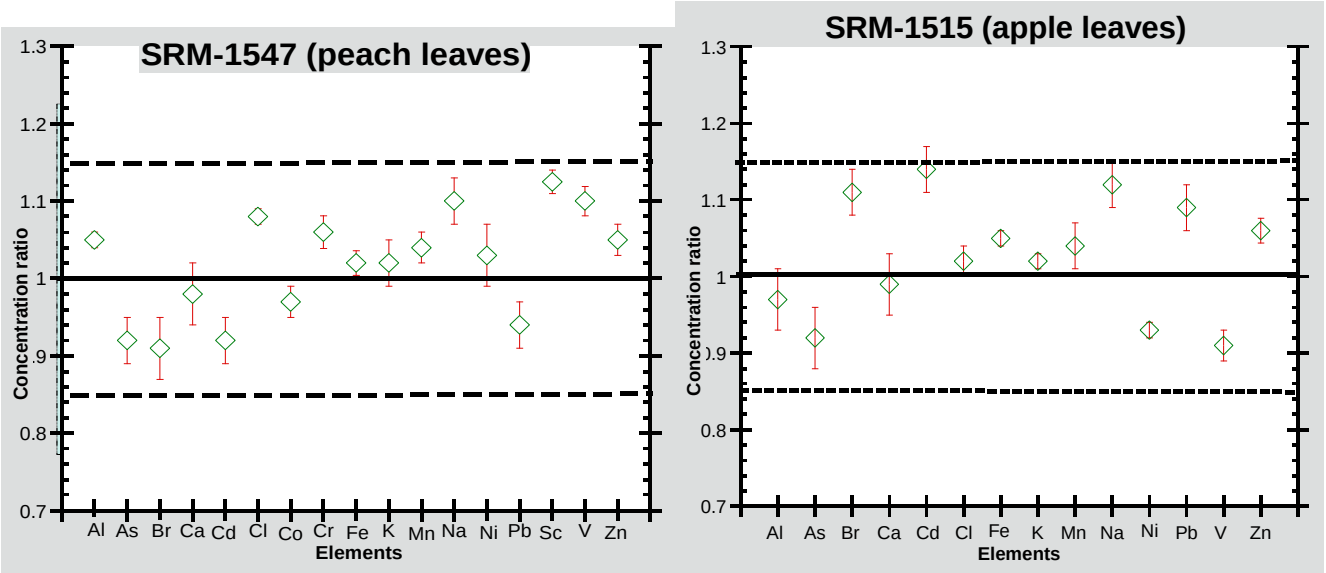


Figure 4.2: Quality Control of the analysis for standard SRM-1547 and SRM-1515.

4.2 Detection Limits of NAA

The ability of a given procedure to determine the minimum amounts of an element reliably is presented by the detection limit. The detection limit depends on the amount of material to be irradiated and to be counted, neutron flux, irradiation time, decay time and the counting condition. The detection limits are presented in the Table 4.3.

Table 4.3: Detection limits of the determined elements in the spices samples under experimental conditions.

Element	Detection limit (mg/kg)	Element	Detection limit (mg/kg)
Al	60.5	Co	0.03
As	0.001	Cr	0.02
Br	1.50	Fe	50.0
Ca	1200	K	250
Cd	0.001	Mn	8.00
Cl	755	Na	22.0
Ni	0.001	V	0.05
Pb	0.002	Zn	4.50
Sc	0.027		

4.3 Elemental Concentrations in the Spices

The total concentrations of the elements (Al, As, Br, Ca, Cd, Cl, Co, Cr, Fe, K, Mn, Na,Ni, Pb, Sc, V and Zn) in common spices used in Bangladesh were determined and listed in Table 4.4. In the studied spices, the variation of element concentrations was observed and the levels of the elements can be attributed to variable capabilities of absorption and accumulation of spice trees [68, 69], dissimilarities in growth rates and phases of foodstuffs and climatic condition changes of the producing areas [70]. The highest concentration of Potassium (K) was found 25300 mg/kg in red chili and lowest 11300 mg/kg in garlic. The order of K concentrations in spices are as follows, red chili> cumin> turmeric> green chili> coriander> ginger> black pepper> garlic. The recommended average intake of K is 2300 mg/day for adult women and 3100 mg/day for adult men [71]. It has been mentioned that high intake of K is useful for reduction of Hypertension and diabetes and better cell membrane in the body. Calcium (Ca) is another essential macro-nutrient like K on the basis of bodybuilding, strength to bones and teeth formation [72]. In this study, the maximum concentration of Ca12000 mg/kg in ginger and minimum concentration of 2330 mg/kg in turmeric powder was detected (Table 3).

From this study, it is observed that Bangladeshi spices are a potential source for K and Ca. Chlorine (Cl) is related to different body function to regulate acid-alkali balance, stimulate the liver function and helps to distribute hormones [70]. The maximum concentration of Cl (7300 mg/kg) was found in Cumin.

Potassium, Chlorine and Sodium together form a part of blood plasma. Biologically, without sodium, cells cannot get the nutrients to survive and lack of Na in human body can lead to dehydration and weakness [73]. Vanadium (V) is present in all studied spices in minor concentration except in green chili powder. In this study, the maximum concentration of V was found in garlic powder (7.78 mg/kg) and a minimum in green chili (<0.470 mg/kg). Vanadium is a vital nutrient for a body which is found in many foods [74]. Zinc is also a vital element for growth and development for plants and humans [75]. The lowest and highest Zn concentration in spices was detected in ginger powder 11.6 mg/kg and coriander powder 66.1 mg/kg, respectively (Table 4.4). In this study, mean Zn concentration in spices followed the descending order of: coriander> cumin> greenchili>garlic>red chili>blackpepper>turmeric>ginger. According to WHO/FAO, the recommended limit for Zn is 100 mg/kg [76]. Iron (Fe) is not only important for blood production and human growth but also important for metabolic processes, including oxygen transport, DNA synthesis, and electron transport [77]. The highest and lowest mean concentration of Fe was observed in garlic (2070 mg/kg) and green chili (<151 mg/kg), respectively. According to WHO, the permissible limit of Fe is 300 mg/kg [78]. In this study, the Fe concentrations in coriander (535 mg/kg), black pepper (1160 mg/kg) and garlic powder (2070 mg/kg) were above the WHO limit. Therefore, this study is very much important to create public awareness based on concentration of essential elements in the spices.

Arsenic (As) is a highly toxic element for animal when it exceeds the tolerable limit. In the present study, the mean highest concentration of As was found in black pepper (0.143 mg/kg) and lowest in coriander, cumin and ginger (0.060 mg/kg) as shown in Table 4.4. According to FAO/WHO, 2011, the concentration of As in vegetables should not exceed 0.10 mg/kg. In this study, As concentration in black pepper was slightly higher than the permissible limit.Previous studies also reported that As concentration in Bangladeshi green chili and red chili were 0.004 mg/kg and 0.34 mg/kg, respectively [79, 68]. In the studied spice samples, the maximum concentration of Chromium (Cr) was observed in garlic (7.83 mg/kg) and the minimum was found in turmeric (0.450 mg/kg), (Table 3). The mean Cr concentration in spices followed the descending order of: garlic>green chili>black pepper>cumin>coriander>red chili> ginger> turmeric. A previous study showed that the mean Cr concentration in chili of Bangladesh was 0.70 mg/kg [80]. Similarly, a study in India reported that Cr concentration in turmeric, cumin, coriander seeds, black pepper, chili, ginger and garlic were 0.22, 0.23, 0.33, ND, 5.54, 0.46 0.70 mg/kg, respectively [28]. However, the permissible limit of Cr in fruits and vegetables recommended by Codex is 2.3 mg/kg [81]. In this study, average Cr concentrations (mg/kg) in four spices like cumin, black pepper, garlic, green chili powder were higher than the WHO recommended value (3.71 mg/kg). The highest and lowest Cd levels in spices were found in red chili (0.090mg/kg) and in green chili (0.050 mg/kg), respectively (Table 4.4). Al-Rmalli reported that Cd concentrations in Bangladeshi coriander, cumin, chili, and curry powder were 37.6, 49.5, 61.2 and 48.9 μg/kg [82], respectively. If anyone consumes the excess amount of Cd, he will affect in many diseases such as kidney, lung damage and skeletal changes [83]. Codex maximum permissible limit of Cd in vegetables is 0.05 mg/kg [84]. According to results of this study, the concentrations of Cd in spices were slightly higher the Codex permissible limit except for garlic and green chili powder.

Lead **(**Pb) is one of the major toxic elements for the human body that enter into the body with the aid of air, water and food [85]. In this study, the highest and lowest concentrations of Pb were found in ginger (1.61 mg/kg) and coriander (0.320 mg/kg), respectively (Table 4.4). For fruits and vegetables, the maximum/permissible concentration level of Pb is 0.10 mg/kg, according to report FAO/WHO, 2011 [85]. According to the results of this study, Pb concentration in ginger, cumin and turmeric were found to be sixteen times, eleven times and ten times higher than the safe limit of WHO. High concentrations of Pb were also reported in Bangladeshi chili (1.8 mg/kg) [80], vegetables (3.7 mg/kg)[86] and marine fish(<0.06-8.92 mg/kg) [87].The mean Ni concentration in spices following the descending order of: black pepper>coriander>garlic>turmeric>red chili>green chili>cumin>ginger. The highest Ni was determined in black pepper (0.271 mg/kg), whereas the lowest level was found in ginger (0.132 mg/kg). According to Codex, the maximum intake of Ni for fruit samples was 0.8mg/kg [88], vegetable samples were 10 mg/kg [80]. The determined concentration of Ni in spices showed below the risk level of consumption.

Table 4.4: The mass fractions (mg/kg) of the chemical elements in common spices of Bangladesh.

	Red chili (n=4)			Turmeric (n=4)			Coriander (n=4)		
Elements	**Conc.**		**SD**	**Conc.**		**SD**	**Conc.**		**SD**
Al	795	±	342	1310	±	423	2320	±	834
As	0.0713	±	0.0201	0.080	±	0.020	0.061	±	0.021
Br	7.53	±	2.81	3.67	±	0.30	5.07	±	1.58
Ca	2440	±	416	2330	±	176	8180	±	803
Cd	0.090	±	0.003	0.064	±	0.006	0.062	±	0.022
Cl	3060	±	300	2890	±	902	4280	±	539
Co	0.296	±	0.030	0.072	±	0.041	0.368	±	0.024
Cr	1.82	±	0.63	0.450	±	0.024	2.19	±	1.08
Fe	164	±	17	251	±	98	535	±	209
K	25300	±	2076	22800	±	1248	19500	±	1081
Mn	15.4	±	2.1	34.9	±	15.4	15.6	±	3.9
Na	77.6	±	45.2	154	±	31	600	±	100
Ni	0.241	±	0.040	0.243	±	0.061	0.270	±	0.040
Pb	0.420	±	0.084	0.982	±	0.071	0.320	±	0.015

Sc	0.0812	±	0.0053	0.113	±	0.012	0.253	±	0.020
V	0.584	±	0.034	1.15	±	0.25	1.83	±	0.49
Zn	20.4	±	3.1	13.1	±	8.8	66.1	±	3.7

Table 4.4: The mass fractions (mg/kg) of the chemical elements in common spices of Bangladesh.

	Cumin (n=3)			Black pepper (n=3)			Garlic (n=3)		
Elements	**Conc.**		**SD**	**Conc.**		**SD**	**Conc.**		**SD**
Al	1010	±	117	1770	±	17	4350	±	145
As	0.060	±	0.012	0.143	±	0.004	0.080	±	0.041
Br	29.8	±	8.4	25.8	±	0.8	32.1	±	1.1
Ca	9610	±	917	5570	±	330	8290	±	427
Cd	0.071	±	0.015	0.060	±	0.031	0.053	±	0.012
Cl	7300	±	871	5240	±	197	1520	±	637
Co	0.451	±	0.110	0.492	±	0.018	0.974	±	0.036
Cr	2.87	±	1.02	3.04	±	0.29	7.83	±	0.52
Fe	258	±	15	1160	±	132	2070	±	222
K	22800	±	811	18800	±	959	11300	±	580
Mn	31.4	±	0.5	253	±	9	33.2	±	1.4
Na	2980	±	955	777	±	25	1210	±	39
Ni	0.161	±	0.061	0.271	±	0.051	0.260	±	0.036
Pb	1.09	±	0.69	0.602	±	0.143	0.510	±	0.012
Sc	0.118	±	0.051	0.248	±	0.006	0.809	±	0.029
V	1.16	±	0.19	2.59	±	0.18	7.78	±	0.46
Zn	37.5	±	13.2	13.8	±	1.7	29.6	±	2.8

Table 4.4: The mass fractions (mg/kg) of the chemical elements in common spices of Bangladesh.

	Ginger (n=3)			Green chili (n=3)			Range	MAC [64]
Elements	**Conc.**		**SD**	**Conc.**		**SD**		**-**
Al	605	±	29	221	±	9	221-4350	0.1
As	0.061	±	0.022	0.080	±	0.010	0.060-0.080	-
Br	2.04	±	0.09	0.661	±	0.041	0.661-32.1	-
Ca	12000	±	919	2530	±	169	2330-12000	0.05
Cd	0.071	±	0.014	0.050	±	0.004	0.050-0.090	-
Cl	1470	±	621	3060	±	116	1470-7300	-
Co	0.206	±	0.061	0.166	±	0.022	0.166-0.974	2.3
Cr	1.10	±	0.17	7.80	±	0.51	1.10-7.83	-
Fe	201	±	34	<151	±	-	<151-2070	-
K	18800	±	960	21800	±	915	11300-25300	-
Mn	171	±	6	13.6	±	0.6	13.6-171	-
Na	1000	±	452	227	±	4	141-2720	10

Ni	0.132	±	0.022	0.171	±	0.091	0.132-0.271	0.1
Pb	1.61	±	0.42	0.322	±	0.016	0.320-1.09	-
Sc	0.042	±	0.003	<0.0133	±	-	<0.042-0.81	-
V	0.925	±	0.106	<0.470	±	-	<0.470-7.78	-
Zn	11.6	±	1.4	30.7	±	2.9	11.6-66.1	-

4.4 Assessment of Dietary Intake and Health Risk of the Elements

4.4.1 Daily intake of heavy elements

The estimated daily intake (EDI) of element plays a vital role in population diet about the potential nutritional deficiencies or exposure to food contaminants. The EDI values of seven elements (As, Cd, Cr, Mn, Ni, Pb and Zn) are given in Table 4.5, which were evaluated according to the mean concentration of elements in spices and consumption rates. Total daily intake of As, Cr, Ni, Cd, Mn, Pb, and Zn were 0.0583, 2.76, 0.156, 0.0474, 36.6, 0.428 and 18.8 mg/day, respectively [67, 66, 89]. In this study, the maximum contribution of dietary intake of elements came from red chili due to the highest consumption rate. Maximum tolerable daily intake (MTDI) and EDI in spices are given in Table 4.5. The descending order of EDI in spices was: Mn>Zn>Cr>Pb>Ni>As>Cd. From this study, it is observed that total intake (mg/day) of Cr, Cd, Mn and Pb are higher than the MTDI values.

Table 4.5: Comparison of daily intake of elements from spice samples with maximum tolerable daily intake (MTDI) in the Bangladeshi population.

Spices	Consumption rate (g/day)	Estimated daily intake (EDI) (mg/day)						
	Adult	**As**	**Cd**	**Cr**	**Mn**	**Ni**	**Pb**	**Zn**
Red chili	10.5	0.0124	0.0157	0.322	2.70	0.0420	0.0735	3.48
Turmeric	5.25	0.00702	0.00528	0.0367	2.04	0.0215	0.0576	0.758
Coriander	3.5	0.00356	0.00350	0.127	0.908	0.0155	0.0183	3.86
Cumin	3.5	0.00350	0.00414	0.167	1.83	0.00939	0.0639	2.19
Black pepper	3.5	0.00834	0.00351	0.177	14.8	0.0157	0.0350	0.807
Garlic	3.5	0.00469	0.00294	0.467	1.93	0.0156	0.0297	1.73

Ginger	3.5	0.00357	0.00414	0.0641	10.0	0.00770	0.0939	0.673
Green chili	10.5	0.0152	0.00878	1.39	2.37	0.0299	0.0560	5.37
Total intake		0.0583	0.0474	2.76	36.6	0.156	0.428	18.8
MTDI		0.126[c]	0.046[c]	0.2[a]	2-5[b]	0.3[b]	0.21[c]	60[b]

MTDI=Maximum tolerable daily intake [a]RDA, 1989 [66]. [b]WHO, 1996 [67]. [c]JECFA, 2003 [65].

4.4.2 Non-carcinogenic risk

Risk assessment is the practice that estimates the potential health effects for different concentration of elements to the body by receiving through one or more exposure ways. The health risks from ingestion of spices by adult people were measured based on the target hazard quotients (THQs). The estimated THQs of studied elements are given in Table 4.6, indicating that THQ values of seven elements (As, Cd, Cr,Mn, Ni,Pb and Zn) were far less than 1.0. Therefore, the consumption of these spices was considered to be safe.

Table 4.6: Non-carcinogenic risk due to the consumption of some elements through spices.

Spices	Target hazard quotient (THQ)						
	As	**Cd**	**Cr**	**Mn**	**Ni**	**Pb**	**Zn**
Red chili	4.14×10^{-2}	5.19×10^{-3}	2.14×10^{-4}	1.92×10^{-2}	2.10×10^{-3}	2.10×10^{-2}	1.15×10^{-2}
Turmeric	2.36×10^{-4}	1.72×10^{-3}	2.45×10^{-5}	2.18×10^{-2}	1.07×10^{-3}	2.47×10^{-3}	3.78×10^{-3}
Coriander	1.28×10^{-2}	1.22×10^{-3}	1.09×10^{-4}	6.02×10^{-3}	7.75×10^{-4}	5.25×10^{-3}	1.19×10^{-2}
Cumin	1.16×10^{-2}	1.38×10^{-3}	1.11×10^{-4}	1.21×10^{-2}	4.75×10^{-4}	1.69×10^{-2}	6.76×10^{-3}
Black pepper	2.72×10^{-2}	1.16×10^{-3}	1.17×10^{-4}	9.81×10^{-2}	7.87×10^{-4}	9.28×10^{-3}	2.49×10^{-3}
Garlic	1.55×10^{-2}	9.72×10^{-4}	3.11×10^{-4}	1.28×10^{-2}	7.58×10^{-4}	7.89×10^{-3}	5.33×10^{-3}
Ginger	1.24×10^{-2}	1.36×10^{-3}	4.27×10^{-5}	6.63×10^{-2}	3.79×10^{-4}	2.49×10^{-2}	2.08×10^{-3}
Green chili	5.07×10^{-2}	2.91×10^{-3}	9.29×10^{-4}	1.69×10^{-2}	1.48×10^{-3}	1.60×10^{-2}	1.79×10^{-2}
Total	0.172	0.0159	0.00186	0.253	0.00782	0.104	0.0617

4.4.3 Target carcinogenic risk (TCR) of As and Pb

The TCR values for adults based on As and Pb contents in spice items were calculated and also given in Table 4.7. For other elements having toxic effects, TCR values could not be assessed due to unavailability of the oral cancer slope factor values. The TCR values for As and Pb ranges from 1.57E-7 to 7.99E-7 and 2.10E-9 to 1.84E-8, respectively (Table 4.7). The target carcinogenic risk is greater than 10^{-4} to be considered intolerable [90]. From this study, the calculated TCR for studied spices were found to be lower than the risk level. Therefore, TCR valuesofAs and Pb for the studied spices indicates that there is no carcinogenic risk to consume the spices by the people.

Table 4.7: Carcinogenic risk due to the consumption of some elements through spices.

Spices	Target carcinogenic risk (TCR)	
	As[a]	**Pb**
Red chili	6.25×10^{-7}	1.84×10^{-8}
Turmeric	7.29×10^{-7}	1.05×10^{-8}
Coriander	1.57×10^{-7}	5.25×10^{-9}
Cumin	5.40×10^{-7}	5.25×10^{-9}
Black pepper	2.98×10^{-7}	1.22×10^{-8}
Garlic	2.52×10^{-7}	7.00×10^{-9}
Ginger	7.99×10^{-7}	5.25×10^{-9}
Green chili	4.76×10^{-7}	2.10×10^{-9}
Total	3.88×10^{-6}	6.60×10^{-8}

[a] Considering 50% inorganic As in spices [32].

4.5 Effects of Gamma Irradiation on Microbial Load of Spices

The total microbial load determined for irradiated and un-irradiated spice powder samples are presented in Table 4.8. In this study, total aerobic bacteria in un-irradiated spice samples were found in the range from 1.37×10^{3} to 2.46×10^{5} cfu/gm. The maximum level

of contamination was found in red chili and minimum was found in ginger powder (Table 4.8). All samples were stored in room temperature (about 22 ± 2^0C) and continuously monitored. The presence of bacterial load was detected in red chili powder 2.46×10^5 cfu/gm;after 6 months bacterial load was found 2.9 5×10^5 cfu/gm. Similarly, the rate of contaminations was increased rapidly for all un-irradiated spices during storage. Thus, proper irradiation treatment was needed to reduce bacterial contaminations in the samples. No bacterial contaminations were found by treated 6, 8 and 10 kGy gamma irradiation doses in samples. Acceptable limit of microorganisms was set by codex alimentarius in spices below 10^3-10^4 cfu/gm [91]. The level of contaminations by organisms in red chili, turmeric, cumin, garlic and black pepper powder were exceeded the safe level compared to codex report. The limit of total coliforms considered as satisfactory $<10^4$ and unsatisfactory $\geq 10^4$ according to ICMSF [92].

Table 4.8: Microbial means for control (0 kGy) and irradiated (2, 4, 6 kGy) in spices powder during room temperature storage.

Doses	Days	Red chili	Turmeric	Cumin	Coriander	Garlic	Ginger	Black pepper
0	0	2.46×10^5	1.62×10^5	1.54×10^4	4.75×10^3	3.09×10^4	1.37×10^3	3.59×10^4
	180	2.95×10^5	2.38×10^5	2.38×10^4	5.18×10^3	4.72×10^4	4.07×10^3	4.05×10^4
2	0	4.47×10^3	1.37×10^3	9.53×10^2	2.65×10^2	2.45×10^2	ND	2.55×10^2
	180	3.73×10^2	6.16×10^2	5.06×10^2	2.05×10^2	1.25×10^2	ND	2.05×10^2
4	0	6.25×10^2	4.23×10^2	ND	ND	ND	-	ND
	180	5.00×10^2	2.30×10^2	ND	ND	ND	-	ND
6	0	-	-	-	-	-	-	-
	180	-	-	-	-	-	-	-

[a]ND= Not detectable (the minimum detection level as 10 CFU/g).
[b]Values are means of triplicate experiments (n=3).

4.6 Physico-Chemical and Nutritional Properties of Spices for Different Doses

The determined properties (moisture, fat, protein, ash and pH) in spices samples during storage are presented in Table 4.9. The maximum moisture content in cumin (7.74%) and minimum in red chili (5.84%) were found among the seven types of un-irradiated spices.

In this investigation, irradiated spices presented lower moisture content compared with that of the un-irradiated samples. The amount of moisture content was a little increased in un-irradiated samples; on the other hand, storage for 6 months moisture content was decreased in irradiated samples. From the literature study, similar results were reported for black pepper powder [47]. Other properties of the spice samples like contents of fat (%), protein (%) and ash (%) were found in un-irradiated samples ranged from 1.27-9.04, 2.79-10.20 and 7.03- 11.10, respectively for 0 month storage time and 1.19-8.94, 2.70-10.03 and 6.99-10.90, respectively for 6 months storage. The result indicated that fat, protein and ash contents were gradually reduces in the un-irradiated and irradiated samples. Similar observations were found in red ginseng powder [93, 94].Another study reported that the moisture (%), ash (%), protein (%) and fat (%) contents were found in turmeric samples as 8.92, 2.85, 9.40 and 6.85, respectively [95]. The previous study also reported that moisture content (%) and pH were found 13.76 and 4.7 in red chili, 9.89 and 6.1 in black pepper, 12.70 and 6.6 in turmeric, 13.0 and 7.1 in ginger. Fat and protein are important components of the body; protein provides amino acids and vital nitrogen for human for body growth and development [96]. This study reveals that percentage of fat and protein were not affected by low gamma doses in spices. The pH values were also determined before and after gamma irradiation of the spice samples. The pH values were found in un-irradiated and irradiated samples range 5.71-6.47 for 0 month storage; 5.64-6.44 for 6 months storage period. The maximum and minimum pH values were found in coriander and red chili powder respectively. In this investigation pH value slightly decreases after storage in control and irradiated samples because of the increased amount of organic acids released during gamma irradiation. Overall, this study provides information before and after treatment of gamma irradiation with different doses in spices (0, 2, 4 and 6 kGy). After low irradiation gamma doses up to 6 kGy, there are no

significant changes in physico-chemical and nutritional properties in the samples. Other properties like color, flavor, aroma, phenolic and sensory properties does not change after treated low irradiation gamma doses (below 10 kGy) were also found in the previous studies [97, 98].

Table 4.9: Physico-chemical and nutritional properties of spices (Red chili, Turmeric, Cumin and Coriander, Garlic, Ginger and Black pepper powder) during storage.

Dose (kGy)	parameters	Red chili						Turmeric		
		0 month		**SD**	**6 months**		**SD**	**0 month**		**SD**
0	Moisture (%)	5.84	±	0.03^{a}	6.05	±	0.02^{a}	7.73	±	0.02^{a}
	Fat (%)	6.52	±	0.03^{a}	6.47	±	0.02^{a}	9.04	±	0.05^{a}
	Protein (%)	10.2	±	0.3^{a}	10.0	±	0.2^{a}	6.62	±	0.03^{a}
	Ash (%)	10.1	±	0.1^{a}	9.97	±	0.25^{a}	11.1	±	0.1^{a}
	pH	5.71	±	0.02^{a}	5.64	±	0.02^{a}	6.36	±	0.01^{a}
2	Moisture (%)	5.81	±	0.01^{a}	5.80	±	0.03^{b}	7.68	±	0.03^{b}
	Fat (%)	6.50	±	0.02^{a}	6.43	±	0.05^{ab}	9.08	±	0.02^{a}
	Protein (%)	10.1	±	0.6^{a}	10.0	±	0.6^{a}	6.63	±	0.07^{a}
	Ash (%)	10.2	±	0.2^{a}	9.90	±	0.05^{a}	11.2	±	0.2^{a}
	pH	5.62	±	0.02^{b}	5.61	±	0.02^{a}	6.26	±	0.01^{b}
4	Moisture (%)	5.84	±	0.02^{a}	5.81	±	0.01^{b}	7.69	±	0.01^{ab}
	Fat (%)	6.52	±	0.03^{a}	6.43	±	0.06^{ab}	9.08	±	0.03^{a}
	Protein (%)	10.2	±	0.1^{a}	9.97	±	0.16^{a}	6.70	±	0.10^{a}
	Ash (%)	10.1	±	0.2^{a}	9.86	±	0.15^{a}	11.0	±	0.1^{a}
	pH	5.53	±	0.07^{c}	5.51	±	0.04^{b}	6.20	±	0.02^{c}
6	Moisture (%)	5.76	±	0.04^{b}	5.78	±	0.04^{b}	7.68	±	0.03^{b}
	Fat (%)	6.35	±	0.07^{b}	6.34	±	0.08^{b}	8.95	±	0.15^{a}
	Protein (%)	10.2	±	0.3^{a}	10.0	±	0.4^{a}	6.64	±	0.07^{a}
	Ash (%)	10.1	±	0.1^{a}	9.97	±	0.15^{a}	10.1	±	0.1^{a}
	pH	5.52	±	0.02^{c}	6.50	±	0.03^{b}	6.19	±	0.01^{c}

Table 4.9: Physico-chemical and nutritional properties of spices (Red chili, Turmeric, Cumin and Coriander, Garlic, Ginger and Black pepper powder) during storage.

Dose (kGy)	parameters	Turmeric			Cumin					
		6 months		**SD**	**0 month**		**SD**	**6 months**		**SD**
0	Moisture (%)	7.93	±	0.03^{a}	7.74	±	0.04^{a}	7.86	±	0.02^{a}
	Fat (%)	8.94	±	0.03^{a}	5.05	±	0.05^{a}	4.95	±	0.11^{a}
	Protein (%)	6.55	±	0.07^{a}	9.62	±	0.03^{a}	9.49	±	0.08^{a}
	Ash (%)	10.9	±	0.3^{a}	9.05	±	0.14^{a}	9.03	±	0.21^{a}
	pH	6.27	±	0.02^{a}	5.81	±	0.01^{a}	5.72	±	0.11^{a}
2	Moisture (%)	7.66	±	0.02^{bc}	7.77	±	0.05^{a}	7.73	±	0.02^{bc}

	Fat (%)	8.81	±	0.22^{ab}	4.98	±	0.09^{a}	4.86	±	0.06^{a}
	Protein (%)	6.52	±	0.03^{a}	9.56	±	0.05^{a}	9.55	±	0.05^{a}
	Ash (%)	10.8	±	0.2^{a}	9.09	±	0.09^{a}	9.05	±	0.13^{a}
	pH	6.23	±	0.04^{a}	5.72	±	0.03^{b}	5.68	±	0.02^{ab}
4	Moisture (%)	7.62	±	0.01^{c}	7.75	±	0.03^{a}	7.69	±	0.04^{c}
	Fat (%)	8.80	±	0.03^{ab}	5.08	±	0.03^{a}	4.90	±	0.10^{a}
	Protein (%)	6.51	±	0.10^{a}	9.51	±	0.12^{a}	9.49	±	0.05^{a}
	Ash (%)	10.6	±	0.4^{a}	9.03	±	0.55^{a}	9.09	±	0.44^{a}
	pH	6.17	±	0.02^{b}	5.63	±	0.02^{c}	5.61	±	0.06^{ab}
6	Moisture (%)	7.69	±	0.04^{b}	7.73	±	0.06^{a}	7.75	±	0.03^{b}
	Fat (%)	8.71	±	0.06^{b}	4.99	±	0.11^{a}	4.83	±	0.16^{a}
	Protein (%)	6.51	±	0.04^{a}	9.56	±	0.05^{a}	9.46	±	0.11^{a}
	Ash (%)	10.8	±	0.2^{a}	9.07	±	0.03^{a}	9.04	±	0.15^{a}
	pH	6.16	±	0.01^{b}	5.61	±	0.02^{c}	5.57	±	0.02^{b}

Table 4.9: Physico-chemical and nutritional properties of spices (Red chili, Turmeric, Cumin and Coriander, Garlic, Ginger and Black pepper powder) during storage.

Dose (kGy)	Parameters	Coriander						Garlic		
		0 month		**SD**	**6 months**		**SD**	**0 month**		**SD**
0	Moisture (%)	5.75	±	0.03^{a}	5.85	±	0.03^{a}	7.21	±	0.02^{a}
	Fat (%)	1.27	±	0.12^{a}	1.19	±	0.03^{a}	4.21	±	0.03^{c}
	Protein (%)	3.71	±	0.15^{a}	3.63	±	0.07^{a}	3.0	±	0.07^{b}
	Ash (%)	10.1	±	0.1^{a}	9.93	±	0.38^{a}	7.03	±	0.12^{a}
	pH	6.47	±	0.03^{a}	6.44	±	0.04^{a}	5.86	±	0.05^{a}
2	Moisture (%)	5.64	±	0.03^{c}	5.64	±	0.03^{c}	7.25	±	0.03^{a}
	Fat (%)	1.13	±	0.06^{a}	1.20	±	0.01^{a}	4.27	±	0.02^{b}
	Protein (%)	3.85	±	0.05^{a}	3.64	±	0.14^{a}	3.10	±	0.02^{a}
	Ash (%)	10.1	±	0.1^{a}	9.97	±	0.15^{a}	6.97	±	0.16^{a}
	pH	6.42	±	0.03^{ab}	6.40	±	0.02^{ab}	5.83	±	0.02^{ab}
4	Moisture (%)	5.70	±	0.02^{b}	5.64	±	0.03^{c}	7.25	±	0.03^{a}
	Fat (%)	1.20	±	0.05^{a}	1.09	±	0.01^{b}	4.32	±	0.03^{a}
	Protein (%)	3.84	±	0.04^{a}	3.64	±	0.10^{a}	3.0	±	0.05^{b}
	Ash (%)	10.2	±	0.2^{a}	9.80	±	0.20^{a}	6.94	±	0.33^{a}
	pH	6.33	±	0.11^{bc}	6.36	±	0.02^{b}	5.81	±	0.01^{ab}
6	Moisture (%)	5.68	±	0.01^{bc}	5.71	±	0.02^{b}	7.21	±	0.02^{a}
	Fat (%)	1.12	±	0.09^{a}	1.07	±	0.05^{b}	4.25	±	0.02^{bc}
	Protein (%)	3.82	±	0.06^{a}	3.68	±	0.04^{a}	3.10	±	0.02^{a}
	Ash (%)	10.2	±	0.06^{a}	9.67	±	0.29^{a}	6.99	±	0.17^{a}
	pH	6.26	±	0.04^{c}	6.27	±	0.06^{c}	5.78	±	0.02^{b}

Table 4.9: Physico-chemical and nutritional properties of spices (Red chili, Turmeric, Cumin and Coriander, Garlic, Ginger and Black pepper powder) during storage.

Dose (kGy)	Parameters	Garlic		Ginger		
		6 months	**SD**	**0 month**	**SD**	**6 months**	**SD**

0	Moisture (%)	7.44	±	0.03[a]	6.85	±	0.03[a]	6.90	±	0.02[a]
	Fat (%)	4.18	±	0.03[b]	2.21	±	0.05[a]	2.20	±	0.02[a]
	Protein (%)	2.94	±	0.11[b]	2.79	±	0.06[b]	2.70	±	0.11[b]
	Ash (%)	6.99	±	0.20[a]	9.34	±	0.66[a]	9.04	±	0.90[a]
	pH	5.83	±	0.04[a]	5.93	±	0.04[a]	5.85	±	0.04[a]
2	Moisture (%)	7.24	±	0.03[b]	6.84	±	0.02[a]	6.82	±	0.02[b]
	Fat (%)	4.18	±	0.03[a]	2.26	±	0.03[a]	2.18	±	0.03[a]
	Protein (%)	2.92	±	0.14[a]	2.89	±	0.06[a]	2.87	±	0.03[a]
	Ash (%)	6.89	±	0.34[a]	9.37	±	0.32[a]	9.13	±	0.15[a]
	pH	5.80	±	0.03[ab]	5.90	±	0.02[a]	5.89	±	0.01[a]
4	Moisture (%)	7.26	±	0.02[b]	6.84	±	0.01[a]	6.78	±	0.01[c]
	Fat (%)	4.22	±	0.02[b]	2.24	±	0.04[a]	2.19	±	0.03[a]
	Protein (%)	2.88	±	0.17[b]	2.87	±	0.03[ab]	2.81	±	0.10[ab]
	Ash (%)	6.92	±	0.10[a]	9.28	±	0.38[a]	9.11	±	0.48[a]
	pH	5.73	±	0.02[ab]	5.82	±	0.01[b]	5.79	±	0.03[b]
6	Moisture (%)	7.23	±	0.02[b]	6.89	±	0.04[a]	6.83	±	0.03[bc]
	Fat (%)	4.20	±	0.03[b]	2.23	±	0.02[a]	2.17	±	0.01[a]
	Protein (%)	2.87	±	0.20[a]	2.85	±	0.03[ab]	2.88	±	0.06[a]
	Ash (%)	6.93	±	0.28[a]	9.25	±	0.27[a]	9.06	±	0.06[a]
	pH	5.70	±	0.02[b]	5.81	±	0.01[b]	5.76	±	0.01[b]

Table 4.9: Physico-chemical and nutritional properties of spices (Red chili, Turmeric, Cumin and Coriander, Garlic, Ginger and Black pepper powder) during storage.

Dose (kGy)	Parameters	Black pepper					
		0 month		**SD**	**6 months**		**SD**
0	Moisture (%)	7.59	±	0.09[a]	7.67	±	0.03[a]
	Fat (%)	2.49	±	0.04[a]	2.40	±	0.03[a]
	Protein (%)	3.20	±	0.02[a]	3.16	±	0.05[a]
	Ash (%)	9.41	±	0.36[a]	9.38	±	0.33[a]
	pH	5.88	±	0.05[a]	5.81	±	0.01[a]
2	Moisture (%)	7.55	±	0.04[a]	7.57	±	0.03[b]
	Fat (%)	2.56	±	0.04[a]	2.43	±	0.07[a]
	Protein (%)	3.20	±	0.02[a]	3.14	±	0.04[a]
	Ash (%)	9.34	±	0.18[a]	9.39	±	0.10[a]
	pH	5.84	±	0.04[ab]	5.77	±	0.03[b]
4	Moisture (%)	7.58	±	0.02[a]	7.55	±	0.03[b]
	Fat (%)	2.52	±	0.04[a]	2.41	±	0.07[a]
	Protein (%)	3.17	±	0.05[a]	3.13	±	0.02[a]
	Ash (%)	9.14	±	0.31[a]	9.28	±	0.15[a]
	pH	5.81	±	0.02[ab]	5.71	±	0.01[c]
6	Moisture (%)	7.55	±	0.04[a]	7.62	±	0.02[a]
	Fat (%)	2.32	±	0.11[b]	2.25	±	0.05[b]
	Protein (%)	3.16	±	0.05[a]	3.13	±	0.03[a]
	Ash (%)	9.12	±	0.11[a]	9.20	±	0.06[a]
	pH	5.80	±	0.03[b]	5.65	±	0.01[d]

[a-d]Mean ±SD (n=3), mean values with same row with different lower case letter are significantly different at $p \leq 0.05$ among the gamma radiation dose.

Chapter 5

Discussion on Results and Relevance

5.1 Comparison of our Data with Literature

Our results were compared with available reported literature data from different countries (India, Algeria and Ethiopia) and given in Table 5.1. For coriander powder, Algerian samples are relatively rich in Sodium (1681 mg/kg), almost three times higher than Bangladeshi samples (600 mg/kg), India and Ethiopia sample (350, 244 mg/kg) are relatively low compared with Algeria samples, while Ethiopian samples are rich in the potassium (90400 mg/kg) and are comparable with Bangladeshi and Indian result (19500, 19100 mg/kg), but the concentration of K contents in Algerian samples are twice higher than the Bangladeshi and Indian sample (38045 mg/kg). In Algerian samples, contents of Ca (17324 mg/kg) were two times higher than those of Bangladeshi samples (8180 mg/kg). Zinc contents in Bangladeshi samples (66.1 mg/kg) that is twice higher than the Indian and Algerian sample (33.6, 37.22 mg/kg, respectively). The higher Fe contents were found in Ethiopian sample (3053 mg/kg), but a low concentration of Fe was found in Bangladeshi (535 mg/kg), Indian (102 mg/kg) and Algerian (239 mg/kg) samples. Chlorine content was similar in the Bangladeshi and Indian sample, but low content in Ethiopian sample. The concentration of Br in Ethiopian samples (13910 mg/kg) was found well above than Bangladeshi (5.07 mg/kg), Indian (2.37 mg/kg) and Algerian (65.78 mg/kg) samples. In Cumin powder, Na concentrations were found in the Indian and Algerian sample that were 4060 and 3149 mg/kg from literature, but in Bangladeshi samples Na concentrations were found in 2980 mg/kg. The K concentrations of Algerian

samples (32797 mg/kg) were found two times higher than Indian samples (16500 mg/kg) but K concentration of Bangladeshi samples were found 22800 mg/kg. The Zn concentrations of Indian samples (56.3 mg/kg) were found two times higher than Algerian samples (25.8 mg/kg) but Bangladeshi samples were found 37.5 mg/kg. The Fe concentrations of Bangladeshi samples (258 mg/kg) were found three times higher than Indian samples (71.1 mg/kg) but Algerian samples were found 182 mg/kg that was two times higher than Indian samples. The Ca concentrations of Algerian samples were measured more than three times of Bangladeshi samples. For the results of black pepper, the content of Na (777 mg/kg), K (18800 mg/kg), Fe (1160 mg/kg), Cl (5240 mg/kg) and Br (25.8 mg/kg) in our black pepper powder are relatively rich, to be higher than the Na (300 mg/kg), K (14600 mg/kg), Fe (76.0 mg/kg), Cl (1240 mg/kg) and Br (8.38 mg/kg) concentrations in Indian data. Zinc concentrations in Indian samples were more than two times higher than those of Bangladeshi samples. In red chili samples, a significant level of Ca, Na, K, Zn, and Fe were found in Indian samples compare to Bangladeshi samples.

Table 5.1: Comparison of our results (mg/kg) with other available literature data.

Elements	Coriander Our data (Bangladesh)			Indian data [28]	Algeria data [65]	SD	Ethiopia data [65]		SD
As	0.061	±	0.021	-	0.673	0.083	-	±	-
Br	5.07	±	1.58	2.37	65.78	7.77	13910	±	83
Ca	8180	±	803	-	17324	3908	-	±	
Cl	4280	±	539	4230	-	-	839	±	40
Co	0.368	±	0.024	0.014	0.140	0.017	-	±	
Cr	2.19	±	1.08	0.33	4.367	0.139	-	±	
Fe	535	±	209	102	239	12	3053	±	284
K	19500	±	1081	19100	38045	1384	90400	±	2260
Mn	15.6	±	3.9	38.9	-	-	61	±	2
Na	600	±	100	350	1681	153	244	±	7
Sc	0.253	±	0.020	0.009	0.316	0.107	BDL	±	-
Zn	66.1	±	3.7	33.6	37.22	1.46	BDL	±	-

Table 5.1: Comparison of our results (mg/kg) with other available literature data.

Elements	Cumin							Black pepper		
	Our data		SD	Indian data [28]	Algeria data [65]		SD	Our data		SD
As	0.060	±	0.012	-	BDL	±	-	0.143	±	0.004
Br	29.8	±	8.41	32.9	76.8	±	9.1	25.8	±	0.8
Ca	9610	±	917	-	34087	±	7542	5570	±	330
Cl	7300	±	871	BDL	-	±	-	5240	±	197
Co	0.451	±	0.110	0.021	0.98	±	0.11	0.492	±	0.018
Cr	2.87	±	1.02	0.23	1.74	±	0.06	3.04	±	0.29
Fe	258	±	15	71.1	182	±	81	1160	±	132
K	22800	±	811	16500	32797	±	1199	18800	±	959
Mn	31.4	±	0.5	BDL	-	±	-	253	±	9
Na	2980	±	955	4060	3149	±	286	777	±	25
Sc	0.118	±	0.051	0.016	0.45	±	0.05	0.248	±	0.006
Zn	37.5	±	13.2	56.3	25.8	±	1.1	13.8	±	1.7

Table 5.1: Comparison of our results (mg/kg) with other available literature data.

Elements	Indian data [28]	Red Chili Our data (Bangladesh)		SD	Indian data [28]
As	-	0.071	±	0.020	-
Br	8.38	7.53	±	2.81	83.5
Ca	-	2440	±	416	12800
Cl	1240	3060	±	300	-
Co	0.033	0.296	±	0.030	0.465
Cr	BDL	1.82	±	0.63	5.54
Fe	76.0	164	±	17	287
K	14600	25300	±	2076	52000
Mn	73.3	15.4	±	2.1	BDL
Na	300	77.6	±	45.2	1230000
Sc	0.016	0.081	±	0.005	0.086
Zn	37.1	20.4	±	3.1	105

5.2 Prevalence of Foodborne Organisms in Un-irradiated and Irradiated Spices

Presence of foodborne microorganisms in spices of un-irradiated and irradiated with different doses are given in Table 5.2. In this investigation,selective media were used for identifying the selective type of organism presence or not in the samples. Likewise, *Bacillus spp.* was detected in all spices powder but *Salmonella spp.* was detected in only

red chili powder. *Listeria spp.* was found in red chili, turmeric and black pepper powder. This study also investigated, *Pseudomonas, E. coli* and *vibrio spp.* were not detected in spice samples. From previous study, *Bacillus spp.* [99], *Salmonella spp*. *Listeria spp.E. coli spp.* [100] and *Clostridium spp.* were found in spices in many researches. For these reasons, gamma irradiation is an effective method to reduce the level of contaminations in spices samples. It is also effective to reduce the growth of foodborne organisms in the samples [101]. In this study, the optimum doses (2 kGy=ginger; 4 kGy=cumin, coriander, garlic and black pepper; 6 kGy=red chili and turmeric) were identified for the spices samples. The similar observations were found in spices during 6 months storage period. From literature study, 2-3 kGy are extremely effective in reducing *Salmonella* spp. [102], 2.5 kGy is also effective in reducing *Listeria spp*. The previous studies reported a dose of 6 kGy reduced the total aerobic microbial contamination in red pepper effectively without changes major quality in foodstuffs. Another study also reported that 10 kGy was used for decontamination in dried red pepper and black pepper; overall dose of 10 kGy is accepted in many countries. From a previous study in Bangladesh reported that 10 kGy was used for total bacterial count below the detectable limit and coliforms were totally eliminated by 5 kGy in red chili, turmeric, coriander and cumin powder [44].

Table 5.2: Presence of foodborne microorganisms in control and irradiated samples for 6 months storage.

Spices	Gamma dose (kGy)	*Bacillus spp.*		*Salmonella spp.*		*Pseudomonas spp.*		*Listeria spp.*	
		0 m	**6 m**	**0 m**	**6 m**	**0 m**	**6 m**	**0 m**	**6 m**
Red chili	0	++	++	++	++	--	--	++	++
	2	++	++	++	++	--	--	++	++
	4	++	++	--	--	--	--	++	++
	6	--	--	--	--	--	--	--	--
Turmeric	0	++	++	--	--	--	--	++	++
	2	++	++	--	--	--	--	++	++
	4	++	++	--	--	--	--	++	++
	6	--	--	--	--	--	--	--	--

Cumin	0	++	++	--	--	--	--	--	--
	2	++	++	--	--	--	--	--	--
	4	--	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--	--
Coriander	0	++	++	--	--	--	--	--	--
	2	++	++	--	--	--	--	--	--
	4	--	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--	--
Garlic	0	++	++	--	--	--	---	--	--
	2	++	++	--	--	--	--	--	--
	4	--	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--	--
Ginger	0	++	++	--	--	--	--	--	--
	2	--	--	--	--	--	--	--	--
	4	--	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--	--
Black pepper	0	++	++	--	--	--	--	++	++
	2	++	++	--	--	--	--	++	++
	4	--	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--	--

Table 5.2: Presence of foodborne microorganisms in control and irradiated samples for 6 months storage.

Spices	Dose (kGy)	*E. coli spp.*		*Vibrio spp.*	
		0 m	**6 m**	**0 m**	**6 m**
Red chili	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--
Turmeric	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--
Cumin	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--
Coriander	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--
Garlic	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--

Ginger	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--
Black pepper	0	--	--	--	--
	2	--	--	--	--
	4	--	--	--	--
	6	--	--	--	--

Each + or – symbols represent respectively, presence or Absence of the microorganisms in one of duplicate plates.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

In this study, the total concentrations of seventeen essential and toxic elements (Al, As, Br, Ca, Cd, Cl, Co, Cr, Fe, K, Mn, Na, Ni, Pb, Sc, V and Zn) in Bangladeshi common spices were determined by NAA and AAS. The concentrations of the chemical elements in the studied spices were compared with available literature data of the world. This study reveals that concentration of the elements As, Cd, Cr and Pb in some spices were higher than the WHO and FAO permissible levels. This study also reveals that calculated total dietary intake values for Cd, Cr, Mn and Pb were higher than the MTDI values, suggesting a considerable risk to the consumers. However, the cumulative risks of the studied elements through the intake of spices not exceeded THQ recommended value (<1.0), indicated that people would experience no potential risks if they being exposed to the elements from consumption of the studied spices. Gamma irradiation doses can be effective to reduce foodborne bacterial contaminations during storage, extended shelf-life and improvement of storage safety in spices. From this study, the optimum dose was identified as 6 kGy for red chili and turmeric; 4 kGy for cumin, coriander, garlic and black pepper; 2 kGy for ginger powder. This study also reveals that low irradiation gamma doses do not significantly change physico-chemical and sensory properties in spices.

6.2Recommendations for future works

- Determination of concentrations of some elements in the spices by epithermal neutron activation analysis can be done in future.
- Molecular interactions due to exposure of gamma ray will be analyzed by Fourier transform infrared spectroscopy (FT-IR).
- The level of fungal mycotoxin contaminations in spices will be done by HPLC.

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Appendix-A

Uncertainty Calculation:

The total uncertainty in the concentration calculation of Mn (847 keV energy) for sample is given below-

Various types of uncertainty parameter added in our experiment are the followings,

Sample comparator preparation, S= 0.55

Irradiation, T= 0.1

Counting statistics for Sample, U=$\frac{100}{\sqrt{Np}}$=$\frac{100}{\sqrt{132836}}$= 0.27

Where N_p is the peak area,

Counting statistics for comparator, V= U= 0.67

Geometry difference, W= 0.29

Losses (random coincidences), X= 0.5

Correction, Y= 0.3

Uncertainty combination, Z= $\sqrt{(S\text{^}2 + T\text{^}2 + U\text{^}2 + V\text{^}2 + W\text{^}2 + X\text{^}2 + Y\text{^}2)}$= 0.94

The concentration of Mn in sample-2.1, P= 658

Total uncertainty for the sample sample-2.1 = $\frac{P*Z}{100}$= 6.19

Appendix-B

Journal Publications

1. M. Rahman, M. A. Islam,Ruhul. A. Khan,"Characterization of chemical elements in common spices of Bangladesh for dietary intake and possible health risk assessment by INAA and AAS techniques," *Journal of Radioanalytical and Nuclear Chemistry*, vol. 318, pp. 1347-1357, 2018.
2. M. Rahman, M. A. Islam, Keshob C. Das, Md. Salimullah, M. Z. I. Mollah Ruhul. A. Khan, "Effect of gamma radiation on microbial load, physico-chemical and sensory characteristics of common spices for storage," *Journal of Food Science and Technology*. (Manuscript Number: JFST-D-19-02901; Submission date: 15 November, 2019).

ConferencesPublications

1. M. Rahman, M. A. Islam, M. S. Rahman, M. M. Zaved, Ruhul. A. Khan "Assessment of Essential and Toxic Elements in Common Spices of Bangladesh by Neutron Activation Analysis." Presented (Oral) at 2nd International Conference on Physics for Sustainable Development and Technology 2017. Organized by Department of Physics, Chittagong University of Engineering and Technology (CUET). Date: 10-11 December, 2017.
2. M. Rahman, M.A. Islam, M. Z. I Mollah, A. Z. M. Salahuddin, Ruhul. A. Khan (2018). "Effects of Gamma Radiation on Physico-Chemical Properties and Elemental Characterization of Common Spices of Bangladesh." Presented (Oral) International Conference on Physics-2018. Organized by Bangladesh Physical Society, Dhaka University. Date: 08-10 March, 2018.