

Trace element analysis in food and beverages by neutron activation analysis: A review of fundamentals, performance, and practical utility

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Abstract

As a high-priority issue for sustainable global development, food security requires attention to both the quantity and quality of food. Trace elements pose a complex challenge for food security in this context; they can be beneficial as essential micronutrients or harmful as toxic contaminants that compromise food safety and public health. Consequently, advanced analytical tools, particularly elemental analysis, are employed to verify food quality, authenticity, and traceability. Since its first application in 1936 neutron activation analysis has proven to be a powerful analytical technique for determining the elemental composition of a wide variety of samples. The method's advantages include its multi-element character, non-destructive nature, high sensitivity and precision, ultra-low detection limits, and simple sample preparation. These advantages have led to its use in critical fields such as food safety, agriculture, and environmental monitoring. The paper provides an introduction to the biological role and toxicity of trace elements, discusses the fundamental principles, strengths, and limitations of neutron activation analysis, and presents an overview of NAA applications in food analysis, along with a summary of key findings.

Keywords: neutron activation analysis, trace elements, food, environment

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1. Introduction

Food security is a high-priority issue for sustainable global development and population health. Over the years, human populations have faced numerous adverse effects that have led to significant impairments in health. These effects are often associated with high levels of environmental pollution caused by chemical elements released through various anthropogenic activities (agriculture, industry, mining, vehicles) or deficiency of micronutrients critically important for life [1, 2].

Therefore, chemical elements play a dual role being essential nutrients and potential toxicants, raising significant environmental concerns [3, 4]. Based on their nutritional significance,

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trace elements can be conditionally divided three groups: essential, probably essential, and toxic (non-essential) elements [5, 6].

Essential elements, such as Fe, Cu, Zn, Mn, Mo, I, Ni, and Se [7, 8], are required in very small amounts, but play a crucial role for human health. They contribute to physical and intellectual development, oxygen transport, haematopoietic function, immunologic function, and biological metabolism [9]. Specific metal ions perform selective functions, ranging from antioxidant effects (Se, Zn, and Cu) to hormone production (I for thyroid hormones) [10]. For example, Fe, the most abundant trace element in the human body, participates in diverse metabolic processes, including oxygen transport (via hemoglobin), DNA synthesis, electron transport chains, and cellular respiration [11]. It is a key component of various metalloproteins and enzymes [12, 13]. Zinc, the second most abundant trace element, is vital for immune function and overall health. It regulates critical biological processes, including cell differentiation, proliferation, and apoptosis, thereby influencing growth and development. Bioinformatics studies estimate that nearly 3000 human proteins bind Zn, underscoring its widespread biological significance [14, 15]. Additionally, Zn plays a role in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids [16].

Copper acts as a cofactor for numerous cuproenzymes, facilitating energy production, Fe metabolism, neuropeptide activation, connective tissue formation, and neurotransmitter synthesis. Beyond Fe homeostasis, Cu is indispensable for antioxidant defence, neuropeptide processing, and immune regulation [17, 18]. Molybdenum is essential for almost all biological systems, however, in humans only four enzymes require Mo, where it functions as an enzymatic cofactor at their active sites [16, 19].

Deficiency or excess of essential trace elements can have serious consequences for living organisms. Iron deficiency, one of the most prevalent nutritional deficiencies worldwide, affects more than 25% of the global population and is the leading cause of anemia [9]. Zinc contributes to numerous metabolic and chronic diseases, including: diabetes, cancer, neurodegenerative diseases, and intestinal diseases [15]. Conversely, excessive accumulation of essential elements can also be detrimental, leading to organ damage and other health complications.

Probably essential elements, such as Li, B, Al, Si, Ti, V, Cr, Rb, Sr, and Ba, are those whose role as micronutrients or in metabolic activities within the human body remains unclear [5, 8]. Manganese is an essential element for bone mineralization, protein and energy metabolism, antioxidant defence against free radicals, and glycosaminoglycan synthesis. It acts as a cofactor for multiple enzymes, modulating both catalytic and regulatory activities [20, 21]. Additionally, it plays a role in glucose and lipid metabolism, protein synthesis acceleration, and the metabolism of vitamins C and B [16]. Although the biological function of Ni remains incompletely understood, it is found in the body in relatively high concentrations within nucleic acids, particularly RNA, and is thought to play a role in protein structure or function [16].

Toxic (non-essential) elements, such as As, Cd, Sb, Hg, and Pb, can adversely affect biological functions in humans. These elements are known to induce multiple organ damage, even at low exposure levels, that is why they receive significant attention from policymakers and researchers [5]. The World Health Organization (WHO) has included As, Cd, Hg, and Pb in the list of the top 10 chemicals of major public health concern [22]. Anthropogenic activities, such as mining, metal processing, coal burning, petroleum combustion, chemical production, wood preservation, thermal power stations, high-voltage infrastructure, plastics, textiles, microelectronics and paper processing plants along with the natural processes as weathering and volcanic eruptions, significantly contribute to environmental metal pollution [23]. The presence of toxic elements in food, water and environment compartment can lead to numerous

disorders, including, nervous, respiratory, and cardiovascular, endothelial dysfunction, cancer, stomach and blood vessel disorders, liver, kidney, and brain damage, hormonal imbalances, miscarriages, skin lesions, and even death [23, 24].

From the foregoing discussion, it is evident that there is a critical need for the detection of trace elements in food, crops, beverages, and environmental compartments (air, water, and soil). To address these challenges, analytical techniques capable of simultaneous multiple elements analysis from a single sample with high accuracy and precision are required. Such techniques should also be sensitive enough to detect elements present in trace quantities [25]. The field of trace element analysis has advanced rapidly in response to the growing demand for accurate measurements at ultra-low concentrations in diverse matrices [26].

Currently, various analytical techniques are available for both single-element and multi-element analysis of food products. Routine determination of trace metals is typically performed using inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), X-ray fluorescence (XRF), etc., ideal for commercial and routine food laboratories. However, most of these techniques are destructive, require complex sample preparation, and are prone to spectral and non-spectral interferences, which can affect measurement accuracy [25, 27, 28]. These limitations can be overcome by neutron activation analysis (NAA), a powerful non-destructive technique capable of analysing elements across a wide range of concentrations in various matrices, with low detection limits (at $\mu\text{g/kg}$ to mg/kg level) for up to fifty elements, and negligible matrix influence. NAA offers high applicability, practicability, specificity, trueness, and precision [29], and can serve as a primary reference method to validate the accuracy of other techniques. The present review discusses the principle of NAA, its advantages and limitations, as well as its application for trace element analysis in food and beverage samples.

2. Neutron activation analysis: Principle and application

2.1. Neutron activation analysis — main steps, advantages and limitations

Neutron Activation Analysis (NAA) is a sensitive, multi-elemental, and non-destructive analytical technique widely used for both qualitative and quantitative analysis of trace elements across diverse fields, including environmental sciences, food safety, geological and geochemical sciences, material sciences, nanotoxicology, archaeological studies, forensic, etc. [30].

The method was first introduced in 1935 by George de Hevesy and Hilde Levi, who demonstrated that neutron irradiation could induce radioactivity in elements, enabling their identification and quantification. In their groundbreaking 1936 publication, they successfully determined the Dy content in yttrium preparations [31]. The subsequent development of nuclear reactors in the 1940s and sodium-iodide scintillation detectors in the early 1950s significantly expanded NAA's applications for trace element analysis across multiple disciplines. Further advancements, including the invention of high-resolution solid-state Ge(Li) detectors in the 1960s and the integration of computerized automation during the 1970s and 1980s, enabled NAA to be applied to large-scale research studies involving vast numbers of samples [32]. NAA is divided in many categories, among which Instrumental Neutron Activation Analysis (INAA) can be highlighted, which consists of irradiation, measurement, and spectral evaluation [33, 34].

Samples collection and preparation for INAA follow the procedures similar to other analytical techniques, while samples analysis consists of three main steps: activation and decay, measurement, and data processing.

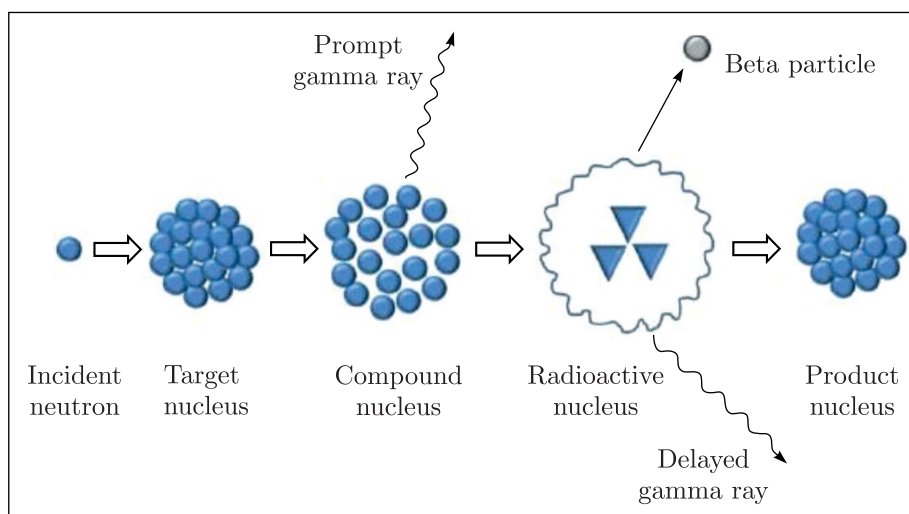


Figure 1. Diagram illustrating the process of neutron capture by a target nucleus followed by the emission of gamma rays [37].

Activation and decay. Thermal and epithermal neutrons are the primary projectiles used in INAA to induce nuclear reactions. Thermal neutrons are those in thermal equilibrium with the moderator atoms. The velocity of these neutrons is 0.025 eV. Thermal neutrons are highly efficient at inducing capture reactions in a wide variety of target nuclei. Epithermal neutrons are partially moderated neutrons with energies ranging from 0.5 eV to about 0.5 MeV. Epithermal NAA provides higher detection for about 10–15 specific trace elements (for example, As, Sb, U) by preventing the activation of common interfering macro-elements, extending the scope of INAA [35].

Neutron activation occurs when a nucleus interacts with neutrons, resulting in neutron capture by the target nucleus. This creates a highly energetic compound nucleus that immediately releases excess energy (usually 10^{-14} s) through emission of photons and/or particles. The newly formed nucleus may be unstable. If unstable after activation, it starts decaying to a stable state by the emission of radiation through one or more of the following processes: α decay, β^- decay, electron capture, β^+ decay, or internal transition decay. In most cases γ and X-radiation will be emitted too [25, 34, 36]. The most common nuclear reaction used in NAA is neutron capture (n, γ), whose sequence of events is illustrated in Figure 1.

Decay is the process by which an excited nucleus stabilizes through the emission of ionizing radiation, ultimately reaching a stable ground state. This nuclear transformation exhibits the following characteristics: the decay of any individual nucleus is a random process; the probability of decay depends solely on the observation period. It is not possible to predict when a given nucleus will decay, but the decay characteristics can be described by the physical laws of radioactive decay, which are comparable to first order chemical kinetics [34, 36]. Even different neutron sources exist, including nuclear reactors, particle accelerators, neutron generators, and radioisotope neutron emitters, nuclear research reactors are considered the most suitable source [36].

Measurement. In NAA, gamma radiation is nearly exclusively measured due to its higher penetrating power and the selectivity that can be obtained from distinct energies of the photons enabling precise identification (unlike beta radiation, which exhibits continuous energy distribution) [34, 38].

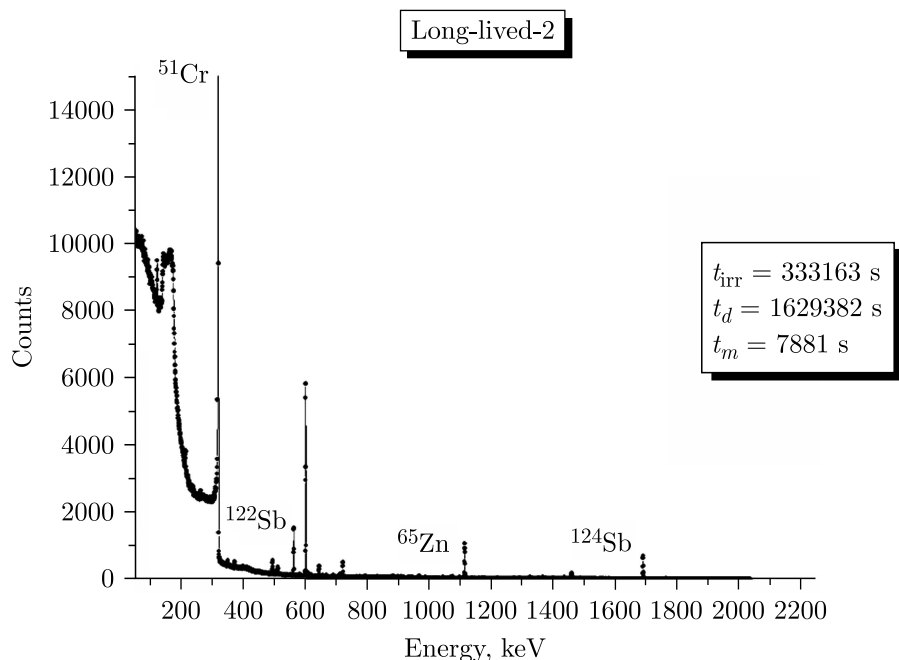


Figure 2. An example of the gamma spectrum obtained at the REGATA facility of the IBR-2 reactor (JINR, Dubna).

Gamma rays interact with matter through ionization processes, generating detectable electrical signals. Contemporary instrumentation used to measure gamma rays from radioactive samples generally consists of a semiconductor detector, signal processing electronics, and a computer-based multi-channel analyzer [33, 39]. For most NAA applications, detection is performed using a high-purity intrinsic germanium detector, which consists of a crystal mounted in a vacuum cooled cryostat [34].

Data processing. Nowadays, various algorithms and software are used for spectra processing. These tools enable the determination of the shape and energy of each gamma-ray peak in the spectrum, as well as the calculation of the peak area. Subsequent corrections, such as accounting for decay times, geometry, flux variations, interferences, and fission product effects, along with comparison to a standard, lead to quantitative analysis [36]. The elemental content in analyzed samples can be determined using either relative or absolute methods. In the relative method, samples are irradiated and measured under the same conditions as a reference material with a known elemental composition. The absolute method, on the other hand, involves calculating the element content by directly measuring the induced absolute activity (A_t) and using known nuclear characteristics of the nuclide as well as the irradiation conditions [36]. For further details on the principles and analytical characteristics of NAA, refer to the following review papers [25, 34, 36, 37].

If to consider as example the REGATA facility of the IBR-2 reactor (Fig. 2) to determine the elements producing short-lived radionuclides — Al, Mg, Si, Cl, Ca, Ti, V, and Mn in food samples — they are irradiated for 3 min at a thermal neutron flux of $1.1 \cdot 10^{12} \text{ cm}^{-2} \cdot \text{s}^{-1}$ followed by immediate measurement for 15 min. Elements with long-lived isotopes — Na, K, Sc, Cr, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Zr, Sb, Cs, Ba, La, Ce, Sm, Eu, Tb, Yb, Ta, Hf, W, Th, and U — are determined after 4-day irradiations at a neutron flux of $1.8 \cdot 10^{11} \text{ cm}^{-2} \cdot \text{s}^{-1}$. Gamma spectra of induced activity are acquired after 4 and 20 days using Canberra HPGe detectors with an efficiency of 70–100% and resolution of 1.8–2.0 keV at a 1332 keV ^{60}Co total absorption peak. The

analysis of the spectra is performed using the Genie2000 software (version 4.0) by Canberra, while elements mass fractions are calculated using the software developed at FLNP. To evaluate quantitatively the mass fractions of elements in a sample reference material with known elements, the mass fractions are irradiated under the same conditions as samples. Since a single reference material may not cover the entire set of elements to be determined, the standard practice is to irradiate several reference materials simultaneously. If a required element is missing in one reference material, its value is taken from reference material where it is present. Another option is to average values across multiple standards for each element. A reference material assembled in this way is called a group standard. The main nuclear-physical characteristics of radionuclides, by which the mass fraction of elements is determined are presented in Table 1.

Table 1. The main nuclear-physical characteristics of radionuclides, by which the mass fraction of elements is determined.

Element	Isotope	Half life	Main decay lines, keV
Na	^{24}Na	15 h	2753.99
Mg	^{27}Mg	9.5 min	843.8
Al	^{28}Al	2.2 min	1778.99
Cl	^{38}Cl	37.2 min	2167.68
Si	^{29}Al	6.6 min	1273.3
K	^{42}K	12.4 h	1524.58
Ca	^{49}Ca	8.7 min	3084.54
Sc	^{46}Sc	83.8 days	889.25
Ti	^{51}Ti	5.8 min	320.1
V	^{52}V	3.7 min	1434.1
Cr	^{51}Cr	27.7 days	320.1
Mn	^{56}Mn	2.6 h	1810.77
Fe	^{59}Fe	44.5 days	1099.25
Co	^{60}Co	5.3 yr	1332.5
Ni	^{58}Co	70.9 days	810.8
Zn	^{65}Zn	244.1 days	1115.5
As	^{76}As	1.1 days	559.1
Br	^{82}Br	35.3 h	554.3
Rb	^{86}Rb	18.7 days	1076.7
Sr	^{85}Sr	64.8 days	514
Sb	^{124}Sb	60.2 days	1691.0
Cs	^{134}Cs	2.1 yr	795.9
Ba	^{131}Ba	11.5 days	496.3
La	^{140}La	1.7 days	1596.5
Ce	^{141}Ce	32.5 days	145.4
Sm	^{153}Sm	46.7 h	103.2
Eu	^{152}Eu	13.5 yr	1408.0
Tb	^{160}Tb	72.3 days	879.4
Yb	^{169}Yb	32 days	277.2
Hf	^{181}Hf	42.4 days	482
Ta	^{182}Ta	115 days	1221.4
W	^{187}W	24 h	479.5
Th	^{233}Pa	27 days	312.2
U	^{239}Np	2.4 days	277.6

Table 2. INAA comparison with other analytical techniques.

Method	Detectable elements	Sensitivity
INAA	Na to U	mg/kg to ng/kg
Inductively coupled plasma mass spectrometry/ Inductively coupled plasma optical emission spectroscopy	Li to U	mg/kg to ng/kg
Atomic absorption spectrometry	Mainly metallic elements, up to 70 elements	mg/kg
X-ray fluorescence	Be to U	mg/kg to $\mu\text{g/kg}$
Scanning electron microscopy — energy dispersive X-ray spectroscopy	All except H, He, and Li	mg/kg

Like any analytical technique, NAA has both advantages and limitations [29, 30, 40–42]. The comparison of the technique with other analytical methods is presented in Table 2.

The main advantages of NAA include: no influence from the chemical state or physical form of the element being analysed, multi-element capability, high sensitivity, high accuracy and precision, non-destructive nature, simple sample preparation procedure, and self-validation [34, 41]. At the same time, it is important to mention that INAA is not the optimal technique for the determination of B, Li, S, Si, Cu, Cd, and Pb. In such cases, alternative analytical methods are more suitable.

Because of the necessity to access nuclear research reactor, the decrease in their number followed by stringent safety regulations, relatively high analysis costs, and sometimes long delay between sample collection and final results, the use of NAA remained restricted to a limited number of laboratories [43]. One of the challenges associated with the NAA method is the significant time required for operators to complete all steps of the analysis [33]. Recently, in many laboratories each individual stage of analysis is subjected to automation to some extent. This automation includes hardware and software development and improvement [29, 30, 32, 40–42]. However, only a few laboratories currently employ such automated systems, including the REGATA facility at the IBR-2 reactor [42]. Even some systems are commercially available, their high cost makes them inaccessible to most facilities [33].

Therefore, the development of NAA is closely intertwined with advancements in nuclear physics, developments of powerful and safe sources of neutrons, and precise radiation detection methods, high speed electronics, and increased processing ability [25]. Today, NAA remains a gold standard for trace element determination owing to its non-destructive nature and high accuracy. Further examples of NAA application for trace elements determination in food and beverages will be discussed.

2.2. Neutron activation analysis — in food and beverage analysis

NAA began to be used in food and beverage analysis in the 1960s. According to the PubMed database, a search using the keywords “neutron activation analysis”, “trace elements”, and “food” from 1960 to 2016 yielded 962 papers. Adding the keyword “beverage” identified 39 additional publications. From 2000 onward, between 8 and 17 papers have been published annually. It should be noted that a similar search for ICP-MS techniques revealed approximately 2800 published papers for the period 1992–2026, confirming its broader applicability for food elemental analysis, largely due to its wide availability. At the same time, it is important to

highlight that NAA can produce more accurate data for halogens (Cl, Br, I) compared with other analytical methods [44]. The method has also been shown to be a more powerful technique with high detection accuracy and precision for rare earth element determination [45]. In some cases, it also provides more accurate data for As, Sb, Se, and Hg.

Speaking about food analysis, different variants of the techniques started to be used in 1960s. The first paper dealing with the application of INAA for food analysis was published in 1962 by Krug and Gruverman [46]. A key reference from the period 1975–1987 is presented by Cunningham and Stroube [47], who also performed multielement analyses of 240 food composites from the Food and Drug Administration’s Total Diet Study. Chatt and co-authors in 1988 [48] applied five NAA methods — cyclic, epithermal, conventional, radiochemical, and preconcentration — to determine numerous elements in food samples, including Sc, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Br, Rb, Mo, Sn, Sb, Au, Hg, Th, and U. The main part of the elements was determined using INAA, while other cyclic and preconcentration NAA were used for determination of specific elements (Se, Th and U). In the same period NAA was widely applied for tea samples analysis in order to determine the level of toxic and essential elements [49–51].

Throughout the 1990s, INAA remained a widely employed technique among several research groups for the determination of the elemental composition of food products [2, 52–55]. During this decade, a total of 223 peer-reviewed papers on this specific application were published. In the following decade, spanning 2000 to 2010, the number of publications on this topic declined noticeably to 140. This decrease can most likely be attributed to the growing availability and routine use of alternative analytical techniques, such as AAS, ICP-OES, ICP-MS, XRF, etc., which offered faster sample analysis and lower operational costs. Concurrently, the decommissioning of research reactors across various European countries and in Russia further constrained access to the neutron irradiation facilities essential for INAA, thereby limiting its practical applicability for many laboratories. The number of publications for the period 2010–2025 remained almost the same as in the previous period (181 papers).

Some examples of INAA application for food and beverage analysis in the period 2000–2025 are presented in Table 3 (only trace elements are listed).

Table 3. Some examples of INAA application for different types of food and beverages analysis.

Type of product/beverage	Determined elements	Facility	Reference
Dietary supplements and agricultural by-products	Fe, Co, Zn, Rb, Cs	IEA-R1 nuclear research reactor, Brazil	[56]
Dairy food (flesh and meat products, dairy products, bread, vegetables, legumes, roots, fruits, and mushrooms)	Sc, Cr, Mn, Fe, Co, Zn, Se, Rb, Sr, Sb, Cs, Hg	VVR-C-type research reactor, Russia	[57]
Fruits	Sc, V, Cr, Mn, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Mo, Sb, Ba, Cs, La, Sm, Ta, Th, U	REGATA IBR-2, JINR, Dubna	[58, 59]
Fruits	K, Sc, Cr, Fe, Co, Zn, Br, Rb, Cs, La	IEA-R1 nuclear research reactor, Brazil	[60]

Fruits	Cr, Mn, Fe, Co, Cu, Mo, Hg	VVR-SM, Uzbekistan	[61]
Fruits	Cl, V, Cr, Mn, Fe, Ni, Cu, Zn, As, I	TRIGA reactor, USA	[62]
Dried fruits	Na, Sc, Cr, Fe, Co, Zn, As, Se, Br, Rb, Sr, Mo, Sb, Cs, La, Ce, Eu, Hg	Pakistan Research Reactor 2, Pakistan	[63]
Vegetables and fruits	Cr	NIRR-I reactor, Nigeria	[64]
Vegetables	Mn, Fe, Zn, Br	TRIGA Mark II research reactor, Morocco	[65]
Mussels	Al, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Zn, Se, Rb, Sr, Sb, I, Cs, Th	REGATA IBR-2, JINR, Dubna	[66–68]
Cereals	Se and Hg	LVR-15, Czech Republic	[69]
Cereals	Se	RPI-ITN, Portugal	[70]
Cereals	Sc, Cr, Fe, Co, Zn, As, Se, Br, Rb, Sb, La, Sm, Eu, Yb, Lu, Hg, Th, U	Nigeria Research Reactor-1, Nigeria	[71]
Nuts	Mn, Zn, Se, Ba	IEA-R1, Brazil	[72]
Areca nuts	Na, Mg, Al, Si, Cl, K, Ca, Mn, Co, Ni, Cu, Zn, As, Sb	REGATA IBR-2, JINR, Dubna	[73]
Fish	Na, K, Ca, Sc, Cr, Fe, Co, Ni, Zn, As, Se, Br, Rb, Sr, Zr, Sb, Ba, La, Ce, Sm, Eu, Tb, Hf, Ta, W, Au, Hg, Th, U	TRIGA reactor, Romania	[74]
Cheese, eggs, fish, fowl and meats	S, Ti, Cu, Br, Rb, I	Slowpoke-2 reactor, Canada	[75]
Fish	Fe, Co, Zn, As, Hg	RA-3 reactor, Argentina	[76]
Sugar products	Na, Mg, Cl, K, Ca, Cr, Mn, Fe, Co, Zn	Pakistan Research Reactor 2, Pakistan	[77]
Moldavian wine	Al, Ca, Mn, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Sb, Cs, Ba, U	REGATA IBR-2, JINR, Dubna	[78]
Macedonian wine	Na, K, Ca, Sc, Cr, Fe, Co, Zn, As, Br, Rb, Sr, Ag, Sb, Ba, La, Ce, Sm, Yb, Th	250 kW TRIGA Mark II reactor, Slovenia	[79]
Indian tea	Na, K, Sc, Cr, Mn, Fe, Co, Zn, As, Br, Rb, Ba, La, Ce, Nd, Sm, Yb, Th	DHRUVA reactor, India	[80]

Black tea brands in Iran	Na, Mg, Al, K, Sc, Cr, Mn, Fe, Co, Zn, Br, Rb, Ba, La, Sm	Tehran Research Reactor, Iran	[81]
<i>Coffea arabica</i>	Na, K, Ca, Sc, Fe, Co, Zn, Br, Rb, Sr, Ba, La, Sm	EA-R1 nuclear research reactor, Brazil	[82]
Coffee species (Arabica and Robusta)	Sc, Cr, Fe, Co, Zn, Rb, Sr, Cs, La, Ce, Sm, Lu, Th	Algerian Es-Salam research reactor, Algeria	[83]

Conclusions

Although the application of NAA requires access to a nuclear reactor or neutron source, which limits its widespread use in routine food testing, it remains an effective technique for detecting trace elements in food samples. The integration of automated sample changers and artificial intelligence in γ -spectra analysis can significantly increase the number of analysed samples and enhance data accuracy. Furthermore, coupling NAA with other analytical techniques (e.g., inductively coupled plasma mass spectrometry, inductively coupled plasma optical emission spectroscopy, atomic absorption spectrometry) can provide a more comprehensive elemental profile of food samples. The values obtained from NAA analyses are critically important for national and international institutions involved in assessing food quality and establishing maximum permissible concentrations of trace elements, particularly toxic ones, in widely consumed products.

Author contributions

I. Z. and D. G: Conceptualization, Methodology, Literature search; Writing, Reviewing and Editing.

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Conflicts of interest

The authors declare no conflicts of interest.

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