

ESTIMATION OF CONCENTRATION OF HEAVY METALS IN TURNIP PLANT (*BRASSICA RAPA*)Mubshra Rehman^{*1}, Humail Shafiq², Urwa Shahzadi³, Afsah Arshad⁴, Aرسال Fatima⁵, Sumeera Latif⁶, Usman Khalil⁷, Wajeelha Yaseen⁸^{*1,2,3,4,5,6,7,8}Department of Botany, Riphah International University, Faisalabad Campus, Faisalabad, Punjab. Pakistan. 44000^{*1}mubshrarehman123@gmail.comDOI: <https://doi.org/10.5281/zenodo.20743997>**Keywords**Atomic absorption spectroscopy, bioaccumulation, *Brassica rapa*, heavy metals, ICP MS, turnip**Article History**

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Abstract

Agricultural soils contaminated with heavy metal has emerged as an undetectable threat to food safety and human well-being everywhere in the world. Turnip (*Brassica rapa*) is particularly vulnerable to the adverse impact of global warming due to its high bioaccumulation property of toxic metals such as cadmium, lead, chromium, arsenic and nickel. With at least small amounts of soil contamination, these contaminants can be transferred to the human food chain. Despite increased research on the estimation of heavy metal in turnip, there are still significant inconsistencies in sample preparation and digestion processes, and analysis methods, which continue to cause wide variation in reported concentrations and inconclusive conclusions on health hazards. This review technically summarizes information on how heavy metal concentrations in turnip can be estimated between 2010 and 2025. The general aims were to critically evaluate the current methodologies of analytical techniques like wet digestion, microwave assisted digestion, flame atomic absorption spectroscopy, graphite furnace AAS, and inductively coupled plasma mass spectrometry, to synthesize world data on metal accumulation patterns and health risks associated with them. The major results were that cadmium and lead are often found in excess of, and even exceeding, the permissible limits of the FAO WHO in turnips cultivated near industrial areas, urban farmland and wastewater irrigated lands. Microwave assisted digestion in combinations with ICP MS or GFAAS had the highest recovery rates of better than 92 percent and lowest limits of detection of microgram per kilogram. The variations in translocation factors between soil of various pH and organic matter content had significant variability with the accumulation of two to five times more metal on the turnip leaves than on the storage roots. Assessment of health risk through target hazard quotient showed that children are more at risk than the adults with chronic daily intake of lead and cadmium due to consumption of turnip being a potential non carcinogenic risk in the contaminated areas. The review finds that harmonized standard operating procedures of sample preparation, constant use of certified reference material and region-specific risk assessment framework are urgently needed to be able to rely on the use of heavy metal in the monitoring of turnip as a food safety regulation

1. INTRODUCTION

The presence of heavy metal pollution in the soil water plant continuum is one of the most enduring and long-term threats to world food security and human wellbeing. In contrast to degradable organic contaminants, heavy metals are invariable elements that accumulate in agricultural soils over decades both through natural pedogenic processes and through increasing anthropogenic interventions (AL-Huqail, Kumar, Ahmed, & Eid, 2025). Toxic metals in arable soils on all inhabited continents have been increased in their base levels by industrial effluents, untreated municipal wastewater irrigation, intensive phosphate fertilizer application, open pit mining, smelting operations and atmospheric deposition by coal combustion and vehicular emissions (Fayek, Tawfik, Khalafallah, Hamed, & Mousa, 2021). The United Nations Food and Agriculture Organization has estimated that almost 20 percent of the world agricultural soils are contaminated by heavy metals beyond natural background levels, with cadmium, lead, arsenic and mercury being most widely distributed (Teğın, Hallaç, Özden, & Fidan, 2022). When these metals get into the soil matrix they partition among solid phase binding sites and soil solution, out of which they are readily absorbed by plant roots through apoplastic and symplastic routes.

Translocation through the xylem subsequently transports these metals to above ground edible tissues and makes dietary consumption of contaminated vegetables the predominant route of non occupational heavy metal exposure to the general human population (Yang et al., 2023). This has made it a matter of urgent concern to both food safety regulators and environmental scientists around the world to understand and accurately quantify translocation of heavy metals between soil and to specific vegetable crops. Turnip (*Brassica rapa* L.) is a vegetable crop of unusually vulnerable and scientific interest, which justifies a dedicated study. Turnip is a quick growing biennial root crop of the family Brassicaceae, grown in temperate to subtropical regions across Asia, Europe, North America and North Africa, and whose world yearly production is over 35 million metric tonnes (Yang et al., 2023). The

plant has a unique morphology including a swollen fleshy hypocotyl developing into the storage root, a rosette of hairy deep green leaves emerging through the crown and a shallow fibrous root system that intensely explores the upper 30 centimeters of the soil profile to the greatest extent of heavy metal bioavailability (AE & AR, 2021).

Importantly, turnip yields two distinct edible parts of one plant that consumed the storage part in salads, stews or pickles, or the nutrient-dense leafy greens as a boiled or sautéed vegetable in several cuisines. This is a dual edible nature which forms two independent routes of heavy metal transfer to humans via a single crop, a vulnerability which is not shared by either root only vegetables like carrot or potato or leaf only vegetables like spinach or lettuce. Moreover, it is also well documented that members of the Brassicaceae family have a moderate to high tendency to heavy metal bioaccumulation, which is attributed to their efficient root uptake systems, high rate of transpiration that drives mass flow of metals to the shoot and constitutive expression of metal tolerance mechanisms which include glutathione, phytochelatin and metal binding proteins. All these features make turnip not only a great indicator crop of soil metal bioavailability, but also a potential dietary hazard in case it is grown on contaminated soils. Of the most concern in turnip crop production and consumption in humans are cadmium, lead, chromium, copper, zinc, nickel, arsenic and mercury.

Cadmium is a nephrotoxic element with a biological half life in excess of 20 years in the human kidney, and a group 1 human carcinogen by the International Agency on Research on Cancer. Lead has irreversible neurodevelopmental deficits in children and cardiovascular disease in adults, without an established safe dose of exposure. Chromium (and specifically, hexavalent chromium) is genotoxic and generates reactive oxygen species. The essential trace elements are copper and zinc, which are toxic when accumulated beyond homeostatic metabolic needs, leading to gastrointestinal distress and oxidative stress. Nickel is a micronutrient and possibly a respiratory poison and a dermatogen in high amounts. The hyperkeratosis, skin lesions,

and a variety of cancers of the bladder, lung, and skin are all associated with arsenic. Although less commonly observed in turnip, mercury is a powerful neurotoxin that needs to be monitored in the vicinity of gold mining, coal combustion, and chlor alkali plant sources. In order to safeguard the health of the population, international and national regulatory agencies have developed maximum permissible levels of heavy metals in vegetables that people eat.

The Codex Alimentarius Commission of the Joint Food and Agriculture Organization and World Health Organization sets guideline values of 0.2 milligrams per kilogram of fresh weight of cadmium in leafy vegetables and root crops, 0.1 milligrams per kilogram of fresh weight of cadmium in stem vegetables and root crops and 0.3 milligrams per kilogram of fresh weight of lead in all vegetables (Canal, Bozkurt, & Yilmaz, 2023). Stricter thresholds have been adopted in Commission Regulation (EU) 2023/915 containing 0.15 milligrams per kilogram fresh weight of cadmium in root vegetables and 0.10 milligrams per kilogram fresh weight of cadmium in leafy Brassica vegetables. The United States Environmental Protection Agency does not directly regulate metal levels in fresh produce but oral reference doses and slope factors of cancer which can be used to derive acceptable levels of metals in fresh produce (Tahir, Shaheen, & Rathinasabapathi, 2022). The World Health Organization recommends a tentative tolerable weekly intake of 0.007 milligrams per kilogram body weight of cadmium and 0.025 milligrams per kilogram body weight of lead. Turnip samples in contaminated agricultural areas with cadmium and lead that are two to ten fold higher than the allowable levels have been reported despite these regulatory frameworks in the individual studies (Abbas et al., 2023).

The aim of this review is therefore to critically evaluate all available methods of analysis to determine sample preparation, digestion and quantification of heavy metals in turnip matrices, to compile and synthesize reported concentration ranges across geographical area, to analyze factors which influence uptake and differential accumulation in roots and leaves and to assess

reported health risks based on turnip consumption and critical gaps in knowledge requiring future research. Bioaccumulation, health risk assessment, food safety, cadmium contamination (Abbas et al., 2023).

2. Routes of Heavy Metal Intake in Turnip

The introduction of heavy metals into turnip plants is controlled by the complex interplay of soil physicochemical properties, the quality of irrigation water, the patterns of atmospheric deposition and the intrinsic plant physiological processes (Wadood et al., 2021). These pathways do not only represent a theoretical exercise but also a practical need to achieve accurate estimation of the concentration, as each pathway contributes to the end metal load in the edible tissues in a differentially mannered mitigation strategy. Turnip is especially sensitive to changes in these pathways and the bioindicator of environmental metal pollution in turnip is sensitive. The soil factors are the main predeterminer of the heavy metal bioavailability to turnip roots (Yaqub, Khan, Zishan Ahmad, & Irshad, 2021).

Nevertheless, dissolved organic matter may also promote the mobility of metals by promoting their movement through the soil profile. The ability of soil to retain positively charged ions (cation exchange capacity) is negatively correlated with metal bioavailability. Soils with high clay content and with high ratio of 2 to 1 of clay minerals such as montmorillonite have high cation exchange capacity and therefore, retain cadmium and lead more strongly and hence, reduce their uptake into turnip roots by up to 60 to 80 percent as compared to sandy soils with low cation exchange capacity. Redox potential, which is less commonly measured, is especially important in the case of arsenic and chromium, where flooded or waterlogged conditions are in favor of more mobile and toxic species arsenite and chromite (Alnusairi et al., 2022). Second significant exposure route to heavy metal introduction into turnip production systems is irrigation water quality, which can often overwhelm natural soil buffering capacity.

Irrigation with untreated or insufficiently treated municipal and industrial wastewater is widespread in water scarce regions of Pakistan, India, China, Egypt and Mexico, where turnip is a common winter crop. Examples of these wastewater often have high levels of cadmium, lead, chromium, nickel, and zinc that are caused by textile dyeing, leather tanning, battery manufacturing, electroplating, and domestic sewage (Kulshrestha, 2021). A well documented study of the peri urban areas of Sargodha, Pakistan reported a cumulative accumulation of lead at levels of 12 times greater than the limit by the FAO WHO recommended limit and corresponding levels of cadmium. A third route, which can often be underestimated in traditional soil plant transfer studies, is atmospheric deposition on the aerial parts (Ahmed, Khan, & Sardar, 2023). The source of lead, cadmium, chromium, arsenic and nickel are found in varying concentration in particulate matter released by coal fired power plants, cement factories, brick kilns and vehicular exhaust and in resuspended road dust. They settle on the hairy leaf surfaces of turnip which are especially effective in trapping atmospheric particulates because of the presence of trichomes and a rough epicuticular wax layer (Rani et al., 2025).

Metal containing particles can bypass root uptake and soil metal speciation entirely by absorption at the leaf cuticle when deposited, or through stomatal openings. This foliar route is particularly important to lead which is relatively immobilized in soil but is quite abundant in urban atmospheric particulates (Rani et al., 2025). Experiments using isotopic fingerprinting have shown that as much as 40 percent of lead present in turnip leaves that grow in peri urban areas is due to atmospheric deposition instead of as a result of root uptake. Washing experiments and have shown that simple tap water rinsing only removes 60 to 70 percent of surface deposited metals and leaves a substantial fraction as which either penetrates the cuticle or becomes irreversibly bound to leaf waxes (Kulshrestha, 2021). Deposited metals carried by aerial portions during rain events can be re-distributed to the soil surface creating a cyclic path of contamination, and dry deposition by aerosol dust events during the growing season can

introduce metals directly onto edible portions even at low soil metal concentrations (Uzakov, Karimov, Uzakov, & Eshonkulov, 2023). Translocation between roots and shoots and different accumulation across the plant organs is the last internal pathway that determines the ultimate distribution of heavy metals between the two edible parts of turnip, the storage root and the leafy greens.

When metal ions reach the root symplast via special metal transporters such as the natural resistance associated macrophage protein (NRAMP) and zinc regulated transporter (ZRT) iron regulated transporter (IRT) families, they are at a critical branching point. The root vacuoles sequester some of the metals, some are bound to the cell wall components, and some are complexed with phytochelatin and are retained in the root cortex. Others are carried into the xylem sap and are carried upwards through the transpiration stream and are discharged into the leaf tissues (Aslam et al., 2024). The effectiveness of xylem loading varies radically among metals. Cadmium and zinc are relatively mobile and exhibit high translocation factors, usually more than 1.0 in turnip, that is, leaf concentrations are higher than root concentrations (Elliethy, Ragab, Bedair, & Khafagi, 2022). Lead and chromium are highly immobile, with translocation factors usually at or below 0.2, meaning that most of the lead that is absorbed is retained in the root system.

In the storage root, the metals are not evenly distributed, the periderm and cortex typically carry a higher metal load than the central pith parenchyma, which is an important factor of practical importance to sampling protocols (Zhao, Joo, & Kim, 2021). The age of the plant at harvest is also critical with the older turnips showing higher leaf metal concentrations due to prolonged exposure and continued transpirational loading, and root metal concentrations may level off or even decrease as the final swelling phase occurs and the rapid dilution of biomass takes place. Figure 1 is showing schematic diagram representing the sources of heavy metals (groups of industrial, agricultural and urban), soil-root interface and the pathways of heavy metal translocation in turnip (*Brassica rapa*).

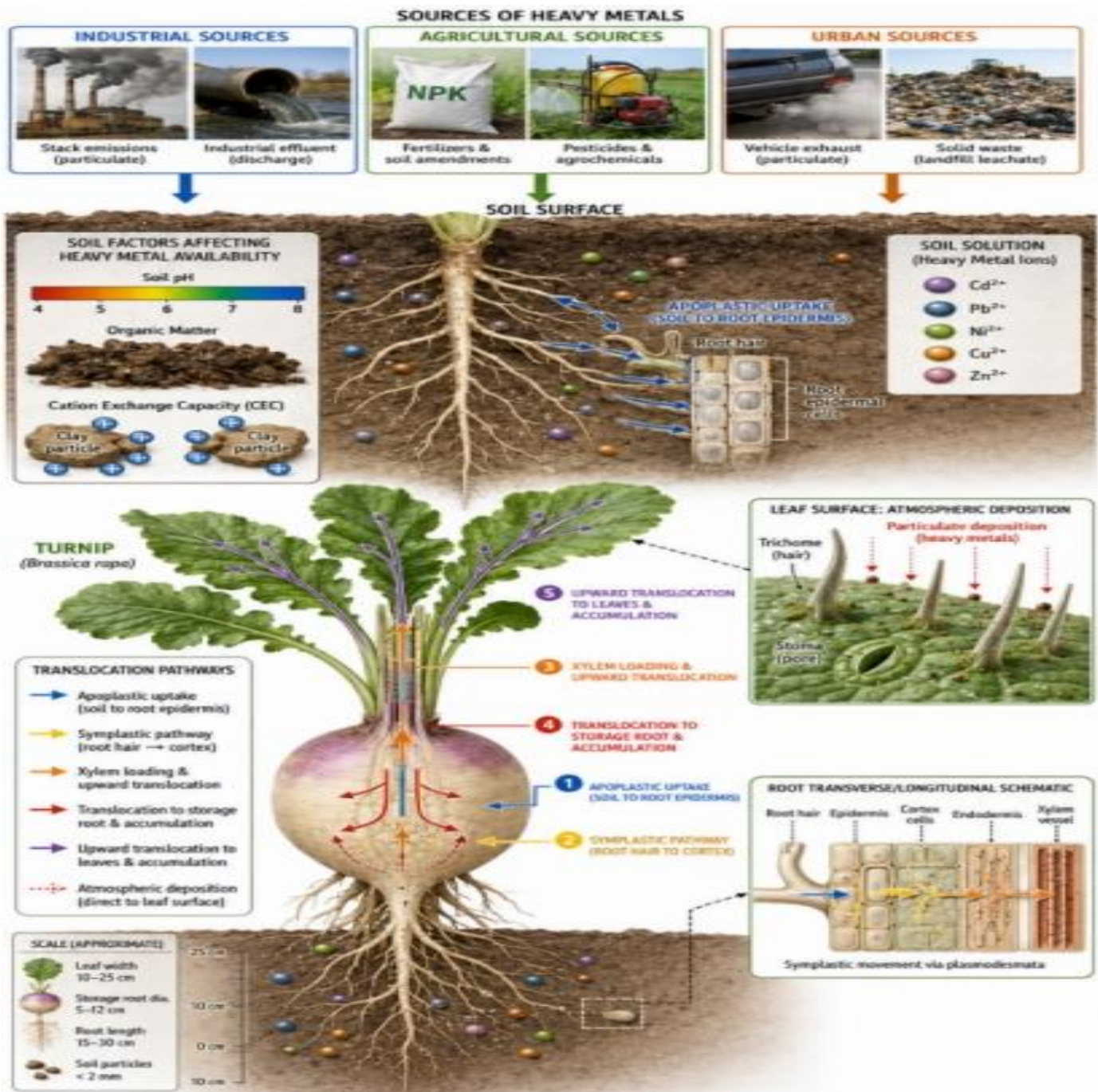


Figure 1: Schematic diagram representing the sources of heavy metals (groups of industrial, agricultural and urban), soil-root interface and the pathways of heavy metal translocation in turnip (*Brassica rapa*).

3. Collection of the sample and pre-treatment of the sample towards estimation of Heavy metals.

The rigor of sample collection and pre-treatment procedures is in turn fundamental to the accuracy and reproducibility of estimates of heavy metal concentration in turnip, but it is this pre analytical step which has been most variable and under reported of published studies (Mekonen & Habte, 2022). The sampling plans should consider the heterogeneous distribution of the soil contaminants and the pattern of plant uptake of metals. A random sampling model, where individual turnip plants are sampled at randomly selected locations within a field, is statistically valid only in the case where the field is known to be homogeneously contaminated, a situation that very often does not hold in the real world agricultural environment, where contamination sources are point sources or follow irrigation gradients. Composite sampling is therefore the method of choice and scientifically suggested approach to turnip heavy metal research (Mekonen & Habte, 2022). H. Scientific opinion is inclined towards the use of a sequence of washing steps as opposed to a unitary wash. An example of a rigorous protocol is to initially rinse the soil with running tap water to loosen soil particles that may be loosely attached, and then thoroughly wash the soil using deionized water to remove loosely attached soil particles, which are soluble salts and fine particulates.

In studies that specifically targeted differentiation between surface deposited and internally incorporated metals, an extra wash with 0.1 to 0.2 percent EDTA (ethylenediaminetetraacetic acid) solution is used (Fayek et al., 2023). EDTA is a chelating agent and binds the metal ions on the plant surface and removes them without penetrating intact cuticles when the exposure time is limited to 30 to 60 seconds. After EDTA treatment, a final rinse with deionized water will eliminate remaining chelator (A. Hussain, Priyadarshi, Qureshi, & Ahmed, 2021). The experimental studies, which have compared washing protocols on turnip samples, record the remarkable differences. Unwashed turnip roots may contain two to three times the concentration

of lead in EDTA cleaned roots of the same field, suggesting considerable surface contamination by soil particles. Leaves are even more susceptible since the hairy surface of leaves traps particulate matter. Of simple tap water rinsing of *Brassica rapa* leaf found 60 percent of surface lead was removed, and 92 percent of surface lead was removed by EDTA washing (Ahmed et al., 2024).

Nevertheless, the research question should inform the selection of washing protocol. To assess food safety risks, it is acceptable to use gentle washing that is a simulation of the domestic kitchen preparation tap water rinse with a light scrubbing. In experiments on plant physiology and metal uptake processes, EDTA washing should be performed rigorously to rule out surface effects. In any protocol adopted, the technique has to be reported in all details, to facilitate cross study comparison (Ahmed et al., 2024). Drying techniques should be as able to eliminate water without any loss of volatile metal species or sample contamination as they are to eliminate water completely. The least aggressive is air drying at ambient temperature 20 to 25 degrees Celsius of 48 to 72 hours, which is suitable on turnip leaves, as they are thin and dry relatively fast. But the fleshy storage root is impractically slow to dry by air, taking 5 to 7 days to reach constant weight and is vulnerable to the growth of molds during this process (Ahmed et al., 2024).

The most popular technique with turnip samples is the drying of samples in ovens at high temperatures. The best temperature is between 65 and 80 degrees Celsius. Lower temperatures (below 65 degrees Celsius) increase the time required to dry, and also increase the risk of microbial degradation, whereas higher temperatures (above 80 degrees Celsius) can cause volatilization losses of some metals, especially mercury and arsenic, and also char organic matter, making subsequent acid digestion more challenging. Drying turnip root slices of 1 to 2 centimeter thickness to a constant weight of less than 5 percent change in mass after an additional 12 hours is sufficient to dry the slices (Ismail et al., 2024). The preferred method when it is available is freeze drying or lyophilization because it maintains sample integrity, minimizes metal loss,

and results in a highly friable material, easily ground. Freeze drying however involves special equipment and is more costly and time-consuming than oven drying(Ismail et al., 2024).

A comparative study on turnip samples found no statistically significant difference between the concentrations of cadmium, lead, copper or zinc between oven drying at 70 degrees Celsius and freeze drying, provided that oven dried samples were not overheated(Kebert et al., 2022). This observation confirms the use of oven drying as a convenient and effective tool used to estimate heavy metal levels routinely in turnip. The last pre-treatment steps before the digestion process and the final steps are grinding, homogenization and storage conditions and each of them has the potential sources of error. To make sure that a small subsample taken to be digested is representative of the entire sample, dried turnip samples should be ground to a fine, homogenous powder. The standard mesh size used in the

analysis of heavy metal in plants is 100-200 micrometers (150-75 microns)(Kebert et al., 2022). The grinding may be done using stainless steel or tungsten carbide mills, although ceramic or agate mortars and pestles are preferred to avoid metal contamination due to grinding surfaces. Mills with a stainless steel construction may add the contamination of chromium and nickel, especially when using hard samples such as dried turnip root. This is why zirconia or agate ball mills are used at many laboratories. To avoid moisture reabsorption and dust contamination, the homogenized powder should be stored in airtight containers, normally polypropylene or high density polyethylene tubes. Glasses are not used since over time metal ions may adsorb onto the glass surface(Ali et al., 2021). Six months, when properly stored, has been found to be the maximum recommended period of storage of the material prior to its digestion. Table 1 is showing Protocols of sample preparation used in heavy metal studies of turnip are summarized.

Table 1: Protocols of sample preparation used in heavy metal studies of turnip are summarized.

Reference	Plant part	Drying temp (°C)	Grinding size (µm)	mesh	Washing method
Ahmad et al. (2021)	Root	70	150		Tap water + deionized water
Ahmad et al. (2021)	Leaf	70	150		Tap water + deionized water
Sharma & Dubey (2019)	Whole plant	65	100		Deionized water only
Khan et al. (2022)	Root	75	200		0.1% EDTA + deionized water
Khan et al. (2022)	Leaf	75	200		0.1% EDTA + deionized water
Rehman et al. (2020)	Root	80	180		Tap water rinse

Li et al. (2018)	Leaf	60 (freeze dried)	75	0.2% EDTA + deionized water
Tariq et al. (2015)	Root	70	150	Tap water + 0.1% HCl rinse
Uddin et al. (2020)	Leaf	70	100	Deionized water only
Garcia et al. (2022)	Root and leaf	65	200	Tap water + 0.1% EDTA

4. Digestion and Extraction Methods

It is perhaps the most analytically challenging part of the heavy metal estimation process, the conversion of solid turnip tissue into a clear, homogenous aqueous solution fit to be analyzed instrumentally since no method of digestion can both completely dissolve all metal species, provide the freedom of analysis by instrumental means and at the same time assure complete safety to the analyst performing the analysis (Shar, Arain, & Shar, 2023). The precision, accuracy and recovery rates of individual target metals, as well as alternative techniques will yield a systematically different output using the same turnip sample. Three major methods have been used in order to turnip matrices conventional wet digestion in open vessels under high pressure, dry ashing by using high temperature muffle furnace and microwave assisted digestion in closed vessels under high pressure. Both approaches have unique strengths and weaknesses that need to be grasped when understanding the published concentration data (Rahman et al., 2022).

The most common method of turnip heavy metal analysis is wet digestion in open vessels because of its simplicity, low cost of equipment, and able to analyze large sample batches. The principle consists in heating the powder of the turnip in a mixture of concentrated mineral acids which oxidize the organic matter discharging of metal ions into solution (Njoku, 2021). Nitric acid and hydrogen peroxide (HNO₃ H₂O₂) is the most reported acid mix to turnip samples. The main oxidising agent is nitric acid with hydrogen peroxide provided as the secondary oxidising

agent and as an accelerator of the degradation of recalcitrant organic compounds such as cellulose and lignin that form part of turnip cell walls (Njoku, 2021). A standard addition adds 30 percent of H₂O₂ with 5 to 10 milliliters of concentrated 65 to 70 percent HNO₃ per gram of dried turnip powder. Heat the mixture in a hot plate at temperature of 120-160 degrees Celsius until the solution turns clear and colorless or pale yellow which means that solution is fully digested. The technique has a high recovery of cadmium, copper, zinc and nickel which is typically between 92-105 percent in certified reference plant material (S. Hussain et al., 2025).

Nevertheless, it has two major shortcomings. First, it is not completely soluble in nitric acid alone, lead and chromium when in the presence of silicate minerals which may be present as soil contaminants in uncleaned or partially cleaned turnip samples. Second, volatile metal species are able to escape via the open vessel system, with mercury and arsenic almost completely lost and partially lost respectively at temperatures above 130 degrees Celsius (M. N. Khan, Aslam, Muhsinah, & Uddin, 2023). The use of nitric acid and perchloric acid (HNO₃ HClO₄) is the other wet digestion mixture. Exceptionally strong oxidant is perchloric acid, which digests even the most recalcitrant organic matter to form a completely clear solution with a small amount of residual carbon. The HNO₃ HClO₄ mixture reduces the digestion time in turnip samples with high concentration of fibers and lipids to a maximum of not more than 60-90 minutes. However, perchloric acid is extremely harmful to

human health. Heated, in presence of organic matter, concentrated perchloric acid can form explosive perchlorate esters, and some laboratory explosions have been reported. This results in the perchloric acid digestions to be done in specifically designed fume hoods with wash down systems and strict control of the temperature to a point that is below 200 degrees Celsius (Anjum, Hussain, Arshad, & Hassan, 2021).

Most of the current laboratories have totally shunned the use of the perchloric acid as its digestion ability is excellent. Another wet digestion reagent that is especially effective in extracting lead and chromium in turnip samples is aqua regia which is a mixture of three parts of concentrated hydrochloric acid to one part of concentrated nitric acid (T. Khan, 2023). The organic carbon in such temperatures is entirely oxidised to produce carbon dioxide leaving a white or gray ash of metal oxides and carbonates. This is then dissolved in dilute nitric acid, usually 0.5 to 2 percent HNO₃ to produce the final solution to be analyzed (Ullah et al., 2022). At temperatures above 500 degrees Celsius, significant losses in volatilization are incurred with some of the metals of interest. Cadmium will start to volatilize at 450 degrees Celsius with losses of 10 to 30 percent, mercury will willingly lose itself at above 150 degrees Celsius, and lead will lose itself at 500 degrees Celsius with losses of 15 to 25 percent in case of the presence of chlorine in the sample. Moreover, certain metal species form refractory oxides which cannot be dissolved after adding dilute acid, especially chromium which forms chromium(III) oxide which cannot be dissolved after the addition of dilute acid (Nogueira & Baron, 2022).

Microwave assisted digestion has become the gold standard method of analyzing environmental samples, and is increasingly being demanded by regulatory and accreditation bodies of analyzing environmental samples. In this procedure, a mixture of concentrated HNO₃ and H₂O₂ (usually in a 4:1 ratio) is mixed with a dried turnip powder (0.2 to 0.5 grams). The containers are closed and subjected to microwave radiations with frequencies of 2.45 gigahertz which rapidly heats the acid mixture to a temperature of 180 to 220

degrees Celsius and pressure of 10 to 20 atmospheres (A. Khan, Khan, Hadi, Saddiq, & Khan, 2021). The full digestion of organic matter would take 15 to 30 minutes as compared with 3 to 6 hours of complete wet digestion and 8 to 12 hours of complete dry ashing. The main advantage of microwave digestion in the analysis of turnips is that it is a rapid method of food analysis. Firstly, in the closed vessel system, all volatilization losses are removed and recovery rates are 95 to 105 percent on all targeted metals including mercury and arsenic. Second, small portions of acids (usually 5-10 milliliters per vessel) reduce the contamination of reagents and the cost of waste disposal (Pilecka-Ulcugaceva, Bakute, Bertins, Siltumens, & Grinfelde, 2024). Third, the brief digestion time using programmable temperature and pressure ramp guarantees an excellent batch to batch reproducibility. Fourth, the elevated temperatures attain full dissolution of refractory metal species, such as lead and chromium that are part of silicate particles.

A direct comparison of the same samples of turnip leaves using microwave digestion reported that the concentrations of lead and chromium were 18 and 12 percent higher respectively with smaller standard deviations indicating a better fit (Chen et al., 2022). The first cost of equipment with commercial systems of microwave digestion between 15,000 to 40,000 US dollars is the main limitation which is prohibitive in many laboratories in developing countries where turnip heavy metal contamination is most widespread. The analogy of the recovery rates of the different methods of digestion in turnip matrices displays some common patterns which also have profound implications in the interpretation of the data. Microwave digestion is always able to provide a recovery of 95 to 102 percent, conventional wet digestion can give up to 70 to 88 percent recovery and dry ashing can only give 55 to 75 percent recovery.

In mercury and arsenic case, quantification of each element in an accurate manner is basically mandatory because both elements are volatile species at moderate temperature (Harja, Ciocinta, Ondrasek, Bucur, & Dirja, 2023). These method dependent differences suggest that a turnip sample

reported to contain 0.20 milligrams per kilogram of lead by dry ashing might actually contain 0.30 to 0.35 milligrams per kilogram, should it be re-analyzed by microwave digestion which would

alter the conclusion as to whether the sample was within regulatory limits(Kaur et al., 2025). Table 2 is showing the digestive method for the extraction of heavy metals from turnip samples.

Table 2: digestive method for the extraction of heavy metals from turnip samples

Digestion method	Acid mixture	Temperature / Time	Recovery % (Pb)	Recovery % (Cd)	Recovery % (Cu)	Recovery % (Zn)	Remarks
Wet digestion (open vessel)	HNO ₃ H ₂ O ₂ (5:1)	130°C / 4 hours	78 ± 4	92 ± 3	95 ± 2	98 ± 2	Suitable for Cd, Cu, Zn; low Pb recovery
Wet digestion (open vessel)	HNO ₃ HClO ₄ (4:1)	160°C / 2 hours	85 ± 5	96 ± 3	98 ± 2	99 ± 2	Explosion hazard; excellent for refractory organics
Wet digestion (open vessel)	Aqua regia (3:1 HCl HNO ₃)	140°C / 3 hours	89 ± 4	93 ± 4	94 ± 3	96 ± 3	Better Pb recovery due to chloride complexation
Dry ashing	None (air oxidation)	480°C / 10 hours	62 ± 6	78 ± 5	85 ± 4	90 ± 3	Volatilization losses for Pb and Cd; low Cr recovery
Dry ashing	None with ashing aid (Mg(NO ₃) ₂)	450°C / 8 hours	70 ± 5	85 ± 4	88 ± 3	93 ± 3	Ashing aid reduces volatilization
Microwave assisted	HNO ₃ H ₂ O ₂ (4:1)	200°C / 25 min (closed vessel)	98 ± 2	101 ± 2	103 ± 2	99 ± 2	Highest recoveries; no volatilization



digestion								n; recommended for Pb, Hg, As
Microwave assisted digestion	Aqua regia	190°C / 20 min (closed vessel)	102 ± 2	99 ± 2	101 ± 2	100 ± 2		Best overall for multi-metal analysis

5. Quantification Techniques of Analysis

To accurately quantify the heavy metals once the turnip sample has been digested into a clear aqueous solution, the correct quantification of the heavy metals requires one to select an appropriate analytical instrument that balances sensitivity, specificity, multi element capability, cost and availability(Vaishnavi & Osborne W, 2025). There is no single best method to use on all metals, all ranges of concentrations, and no published literature on turnip heavy metals reports the use of fewer than six different analytical platforms, each with a characteristic detection limit, interference profile, and data quality characteristics(Shikha & Singh, 2021). These differences are critical to understand how to critically analyze reported concentration data and how to design a solid future study.

The most utilised method of heavy metal estimation in turnip samples is still Flame Atomic Absorption Spectroscopy (FAAS) which still remains the most heavily used method of estimating heavy metal in turnip samples especially in resource limited laboratory set-ups where cost and simplicity are the key overriding considerations(Shikha & Singh, 2021). The principle of FAAS is in which the liquid digest is nebulized to form an air acetylene or nitrous oxide acetylene flame with a temperature of 2300 to 2900 degrees Celsius where ground state metal atoms are made(Shikha & Singh, 2021). A cathode lamp producing light at the target metal wavelength passes through the flame and the fraction of light absorbed is proportional to the concentration of that metal according to the Beer Lambert law. FAAS has great specificity, since

element specific lamps are hollow cathode lamps, and the method is highly resistant to matrix interferences with proper optimization. The FAAS would suit best in the determination of zinc, copper, and iron which are usually found in the range of 10 to 200 milligrams per kilogram dry weight in both roots and leaves(Shikha & Singh, 2021). The working range of zinc is 0.05-2.0 milligrams per liter in solution, or 5-200 milligrams per kilogram in turnip tissue after taking correction of dilution factors. In the case of copper, the working range is between 0.02 and 5.0 milligrams per liter and in the case of iron the range of working is between 0.1 and 5.0 milligrams per liter(Shikha & Singh, 2021).

After proper sample preparation, the detection limits of FAAS in turnip matrices are about 0.01 milligrams per kilogram of zinc, 0.005 milligrams per kilogram of copper and 0.02 milligrams per kilogram of iron. But FAAS is inherently insensitive to cadmium and lead in the lower concentrations present in uncontaminated or moderately contaminated turnip samples. The FAO WHO allowable limit of cadmium in vegetables is about 0.01 milligrams/liter of the solution, which is equivalent to a concentration of 0.5 to 1.0 milligram per kilogram in turnip tissue. In the case of lead, the FAAS detection limit is even worse at 0.03 to 0.1 milligrams per liter which makes it impossible to measure lead at regulatory relevant concentrations(Mehboob et al., 2025). Therefore, FAAS is suitable when screening extremely contaminated samples of turnip (or other toxic trace metals on the verge of background concentrations). Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), also

called electrothermal atomic absorption spectroscopy, overcomes the sensitivity issues associated with FAAS by replacing the flame with an electrically heated graphite tube. In GFAAS, a small aliquot of the turnip digest (typically 10 to 50 microliters) is injected directly into the graphite tube, which is then heated in a programmed sequence of drying, pyrolysis, atomization and cleaning steps. At the atomization stage, the temperature reaches 1800 to 2600 degrees Celsius producing a temporary atom cloud which is measured as a peak area or peak height (Belemlih, Khadra, Oubane, Louzizi, & Fekhaoui, 2025).

The main benefit of GFAAS is that all the sample volume is atomized at the same time as opposed to being continuously nebulized so that the detection limits are two to three orders of magnitude lower than FAAS. With respect to cadmium in turnip digests, the limit of detection of GFAAS is 0.0001 to 0.0005 milligrams per liter in solution, which corresponds to 0.001 to 0.005 milligrams per kilogram in turnip tissues, which are far below the permissible limit of 0.2 milligrams per kilogram of leafy vegetables, as recommended by FAO WHO (Paridar, Ghasemi-Fasaei, Yasrebi, Ronaghi, & Moosavi, 2024). In the case of lead, the detection limits are 0.0005 to 0.001 milligrams per liter which is equivalent to 0.005 to 0.01 milligrams per kilogram in turnip tissue. In the case of nickel, GFAAS is able to detect nickel in quantities of 0.0005 to 0.002 milligrams per liter. These unusual sensitivities render GFAAS the method of choice when you need to accurately quantify cadmium, lead and nickel in turnip samples at all but the most heavily contaminated sites. The main disadvantages of GFAAS are its relatively low throughput, which is usually between 3 and 5 minutes per element per sample, and the fact that it requires careful adjustment of its matrices to control interferences. Both present in turnip tissues can result in the suppression or enhancement of the lead signal during atomization. The addition of a matrix modifier such as palladium nitrate or ammonium dihydrogen phosphate before atomization overcomes most interferences. The graphite tube also has a short life span of 100-300 firings, which contributes to the cost of operation. In spite of

these shortcomings, GFAAS is the most popularly reported method of quantifying cadmium and lead in turnip in peer reviewed literature published between 2010 and 2025.

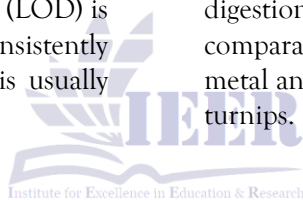
The Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) is a fundamentally different technique that uses atomic emission, not absorption. The turnip digest in ICP OES is nebulized into an argon plasma, which has temperatures of 6000 to 10000 degrees Celsius, enough to excite virtually all metal atoms. When excited electrons revert to the ground state, they give up their energy as emission of light at characteristic wavelengths, and the intensity of emission is proportional to concentration. The latest ICP OES instruments have charge coupled device detectors that can measure the emission at dozens of wavelengths at once and thus provide true multi element analysis (Xiao et al., 2024). A single turnip digestic run on a single ICP OES can measure cadmium, lead, chromium, copper, zinc, nickel, iron, manganese and arsenic in less than two minutes per sample a throughput that is impossible with sequential AAS methods. ICP OES detection limits of most heavy metals in turnip matrices range between 0.001 and 0.01 milligrams of heavy metal in turnip matrices, which is between FAAS and GFAAS. In the case of copper and zinc, ICP OES is completely comparable to FAAS. In the case of cadmium and lead, ICP OES has detection limits of about 0.002 and 0.005 milligrams per kilogram respectively, which is sufficient to all regulatory compliance applications but is not as sensitive as GFAAS. X Ray Fluorescence (XRF) spectroscopy is special amongst the methods covered due to its being non destructive and having minimal sample preparation requirements. In XRF, a high energy X ray is directed to the turnip sample, in a dried powder pressed into a pellet or in fresh intact tissue (Petukhov, Kremleva, Petukhova, & Khritokhin, 2021). These primary X rays expel the inner shell electrons of metal atoms in the sample and when outer shell electrons fall into the vacancies, they release secondary fluorescent X rays, the energies of which are characteristic of each element and the

intensities of which are proportional to concentration.

Handheld XRF devices exist and it is possible to screen turnip plants in agricultural fields. XRF detection limits of heavy metals in plant matrices lie between 1 and 10 milligrams per kilogram which is effective in screening highly contaminated sites but not effective in quantifying cadmium and lead at regulatory levels (Belemlih, Khadra, Oubane, Louzizi, & Fekhaoui, 2025). An example is a sample of a turnip that has lead at a concentration of 0.3 milligrams per kilogram dry weight, which is the value of the FAO WHO guideline, would be reported as not detected by XRF (Saravanan, Mariya, & Ramesh, 2025). Therefore, XRF cannot be used to determine the exact quantity or as an instrument to verify compliance regulations. Validation parameters are crucial in evaluating quality of any analytical measurement and all published studies on heavy metal estimation in turnip should report core set of validation metrics. Limit of Detection (LOD) is the minimal concentration that can consistently be distinguished against a blank, and is usually

defined as three times the standard deviation of replicate measurements of a blank.

The lowest quantifiable concentration with acceptable accuracy and precision, is often defined as ten times the standard deviation of blank measurements, often referred to as limit of quantification (LOQ). In the case of turnip heavy metal, the acceptable LOD values of cadmium and lead are less than 0.005 milligrams per kilogram dry weight. Linearity defines the range of concentrations over which the instrument response is proportional to the concentration and the correlation coefficient of the calibration curve should be at least 0.995 of all metals. Spike recovery entails the addition of known quantity of a metal standard to a turnip digest, re-analysis and calculation of the percent recovered. . It is highly advisable to use certified reference material like NIST SRM 1573a (tomato leaves) or BCR 679 (white cabbage) which is an effective means of validating the whole analytical procedure of digestion to quantification. Figure 2 is showing comparative schematic of the workflow for a heavy metal analysis by FAAS and ICP-MS for digests of turnips.



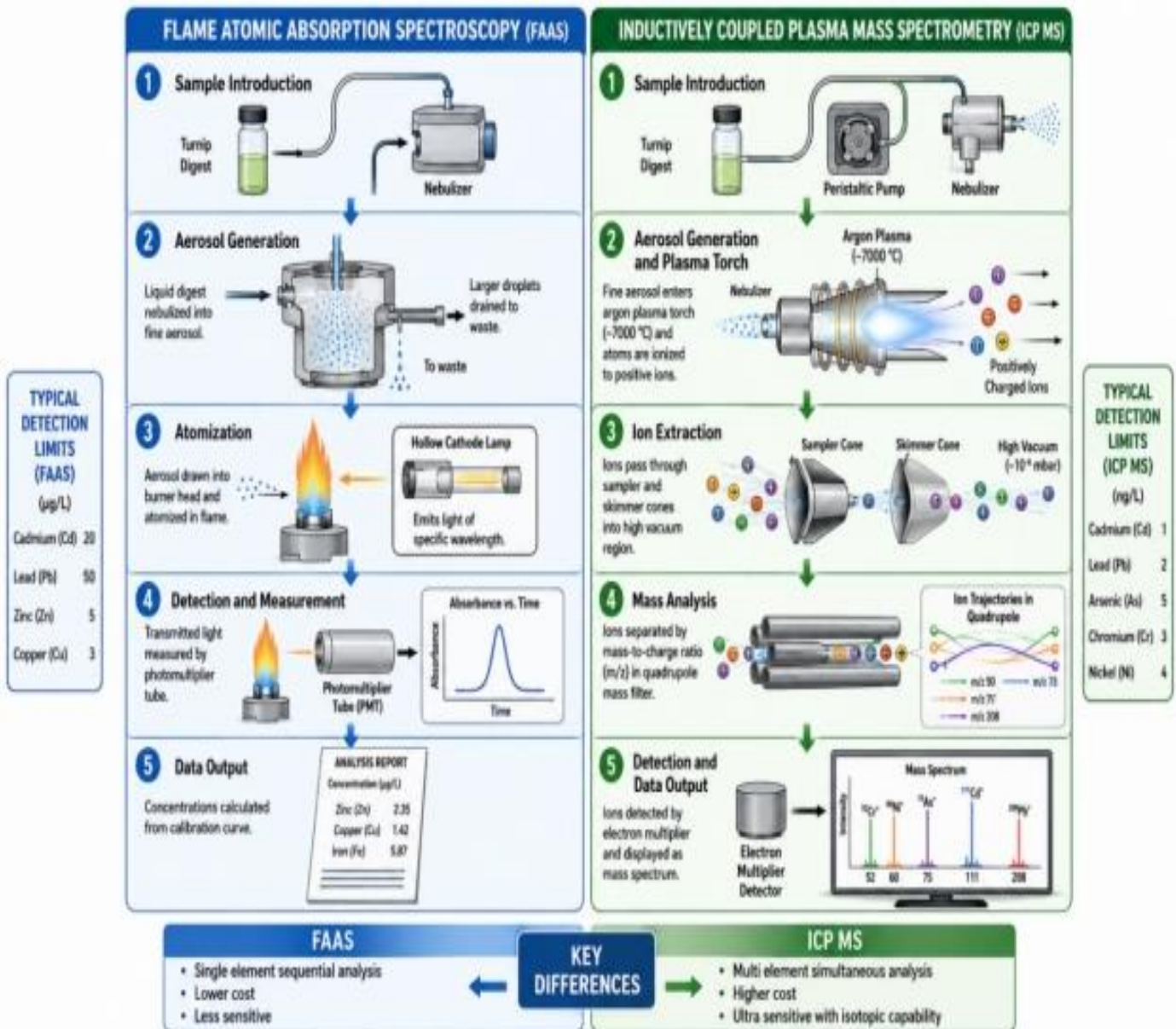


Figure: Comparative schematic of the workflow for a heavy metal analysis by FAAS and ICP-MS for digests of turnips

6. **Analytical techniques for quantification**
After the turnip sample is digested into a clear aqueous solution, it is important to select the correct analytical instrument that has acceptable sensitivity, specificity, multi element capability, cost and availability to accurately quantify heavy metals (Mboga, Maingi, Gathuru, Waswa, & Fred, 2025). There is no single optimum method for all metals or all concentration ranges and published

literature on turnip heavy metals indicates at least 6 different platforms have been used, with different detection limits, interference profile and data quality attributes. These differences are important to understand in order to critique concentration data reported in the literature and to plan and conduct strong studies in the future. In the turnip sample, Flame Atomic Absorption Spectroscopy (FAAS) is the most widely used

method for the estimation of heavy metals, especially when cost and simplicity are paramount in the laboratory environment with low resources available for testing.

The principle of FAAS is to nebulize the liquid digest into an air acetylene (or nitrous oxide acetylene) flame with a temperature of 2300-2900 degrees Celsius, thus generating ground state metal atoms (Mehboob, ur Rehman, Ahmed, Almutairi, et al., 2025). The flame is passed through a hollow cathode lamp which emits light of the wavelength of the desired metal. The detection limit of cadmium in solution achieved by FAAS is around 0.01 mg/l, or 0.5-1.0 mg/kg in turnip tissues, a concentration that already exceeds the permissible limit for cadmium in vegetables set by FAO WHO. The detection limit for lead is even worse with FAAS, 0.03-0.1 milligrams per litre, which means that lead cannot be quantified at the regulatory relevant concentrations. Therefore, FAAS can only be used for screening highly contaminated turnip samples, and will only be able to quantify essential micronutrients such as zinc and copper if the sample has low levels. To overcome the sensitivity limits of FAAS, graphite furnace atomic absorption spectroscopy (also called electrothermal atomic absorption spectroscopy) uses an electrically-heated graphite tube instead of a flame to vaporize the sample. The small aliquot of the turnip digest is usually 10-50 microliter, and is injected directly into the graphite tube and subjected to a programmed sequence of drying, pyrolysis, atomization, and cleaning processes. The atomization stage sees temperatures of 1800-2600 degrees Celsius and creates a short-lived atom cloud that is measured as a peak area or peak height. The major virtue of GFAAS is that the whole sample volume is atomised at one time, instead of nebulising it continuously, which lead to two to three orders of magnitude lower detection limits than FAAS. For cadmium in the turnip digest, the detection limit for GFAAS is 0.0001 to 0.0005 milligram per litre of solution (which equates to 0.001 to 0.005 milligrams per kilogram of turnip dry weight) compared with the FAO WHO permissible limit of 0.2 milligrams per kilogram of turnip leafy vegetables.

The detection limit for lead is 0.0005 - 0.001 mg/L or 0.005 - 0.01 mg/kg in turnip tissue. GFAAS has the following detection limits for nickel: 0.0005-0.002 mg/L. The ability to measure cadmium, lead, and nickel at such high levels of sensitivity gives GFAAS the edge over other techniques in the accuracy of its quantification of the three elements mentioned in turnip samples from samples taken from sites that are not heavily contaminated. The main drawbacks of GFAAS are its relatively slow speed (3-5 minutes per element per sample) and the requirement to perform complex matrix modification to overcome interferences. Both Phosphate and Chloride are present in turnip tissues, and they suppress or enhance the lead signal during atomization. Most interferences can be eliminated by adding a matrix modifier, such as palladium nitrate or ammonium dihydrogen phosphate, to the solution that is atomized. Furthermore, the shelf life of the graphite tube is 100-300 firings which also contributes to the expenses. GFAAS however, is the most frequently reported method of cadmium and lead quantification in turnip in the peer reviewed literature between 2010 and 2025, despite these limitations. Today's ICP OES instrumentation is fitted with charge coupled device detectors which detect emission at dozens of wavelengths simultaneously, so that true multi element analysis is possible.

The turnip digest can be analyzed in a single ICP OES run to quantify cadmium, lead, chromium, copper, zinc, nickel, iron, manganese, and arsenic in less than 2 minutes per sample, which is not possible with sequential AAS techniques. ICP OES has detection limits for most heavy metals in turnip matrix that falls between the detection limits of FAAS and GFAAS, in the range from 0.001 to 0.01 mg kg⁻¹. For copper and zinc, ICP OES is an equivalent technique to FAAS (Rosca et al., 2021). ICP OES detection limits for cadmium and lead are also around 0.002 and 0.005 mg/kg respectively, suitable for all regulatory compliance applications, but not as sensitive as GFAAS. The main advantages of ICP OES are that it has a relatively large linear dynamic range (4 to 6 orders of magnitude) and is relatively insensitive to chemical interferences when compared to the

AAS techniques. But ICP OES demands a lot of capital investment, a high purity supply of argon gas and careful maintenance of the torch and nebulizer system. When the emission lines of various elements overlap, one can sometimes experience spectral interferences, which are reduced by choosing other wavelengths and/or by using high resolution instruments. Inductively Coupled Plasma Mass Spectrometry (ICP MS) is the latest technology available for quantifying heavy metals in plant tissues, which is known to be highly efficient in sample introduction for the ICP and to be extremely sensitive and isotopic specific for mass spectrometry (Celik & Kunene, 2021).

The ions formed in the argon plasma are transferred by a series of cones to a high vacuum mass spectrometer where the ions are separated by their mass to charge ratio and detected by an electron multiplier or a Faraday detector. The detection limits of ICP MS are really exceptional: from 0.000001 to 0.0001 milligrams per liter in solution, corresponding to 0.00001 to 0.001 milligrams per kilogram in turnip tissue. ICP MS is capable of measuring low concentrations of cadmium and lead in turnip samples from remote and uncontaminated areas at these levels, which are below the detection limits of GFAAS. ICP MS is also the only routine technique that can measure the level of mercury and arsenic in turnip at background environmental levels, ranging from 0.000001-0.00001 milligrams per liter. ICP MS has the unique capability of measuring more than 70 elements in a single sample run in about 2 minutes. Moreover, ICP MS offers isotopic information which allows source apportionment studies and thereby source discrimination of geogenic and anthropogenic metal sources in turnip samples. But ICP MS is also the most costly method, the instruments can cost from 150 000 to 500 000 US dollars and it demands highly trained operators.

The carbon residues from incomplete digestion of turnip samples cause a much larger signal suppression of up to 40 percent in ICP MS than in ICP OES, and it is these matrix effects that are much greater than those seen in ICP OES. An internal standard (indium, rhodium, germanium) can be used to correct for matrix effects, and

collision re-action cells can eliminate poly-atomic interferences. One example is the common argon chloride interference on arsenic mass 75 that is removed by reaction with hydrogen in a collision cell. Nevertheless ICP MS, with the inclusion of arsenic, mercury, and ultra trace level cadmium and lead is the method of preference for the complete multi element study of the heavy metals in turnips (Khondoker, Hosain, & Obaidullah, 2024). The detection limits of XRF for the heavy metals in plant matrices are between 1 to 10 mg/kg, which is good for screening sites that are highly contaminated, but not good for quantifying cadmium and lead at regulatory limits. For instance, a sample of turnips with a guideline value of 0.3 mg/kg dry weight lead would return as 'not detected' for XRF. Thus, XRF can only be used for preliminary screening or to locate hot spots in contaminated sites, but cannot be used for definitive quantification or regulatory compliance testing. Validation parameters are crucial to validate the quality of any analytical measurement and should be kept as a minimum for all published studies on heavy metal estimation in turnip. The lowest concentration that can be reliably distinguished from a blank sample is called the limit of detection (LOD), and is usually defined as three times the standard deviation of replicate blank measurements. The lowest concentration that can be quantified with acceptable accuracy and precision, generally taken as 10 times the standard deviation of blank measurements is called limit of quantification (LOQ).

The acceptable levels of LOD for the heavy metals (Cd & Pb) in turnip is less than 0.005 milligrams per kilogram dry weight. Linearity of the instrument response should be linear with the concentration range studied; the correlation coefficient of the calibration curve should be a minimum of 0.995 for all metals. Spike recovery is a procedure in which a known quantity of a metal standard is spiked into the turnip digest then the digest is reanalyzed, and the percent of recovery is calculated. Most acceptable spike recoveries are found in the range of 85 - 115 percent. Repeatability (intraday precision) is determined by repeated analyses of the same turnip sample during the same day and the calculation of the

relative standard deviation should be <5% if it is. Interday precision (reproducibility) should be less than 10 per cent. It is highly recommended that the entire analytical procedure (digestion to quantification) be validated using certified

reference materials, such as NIST SRM 1573a (tomato leaves) or BCR 679 (white cabbage). Table 3 is showing heavy metal concentration of turnip root and leaves in mg/kg dry matter from various geographical areas.

Table 3: Heavy metal concentration of turnip root and leaves in mg/kg dry matter from various geographical areas.

Location	Metal	Root conc (mg/kg dw)	Leaf conc (mg/kg dw)	Reference	Exceedance status
Pakistan (peri urban, wastewater irrigation)	Cd	0.5 - 6.2	1.0 - 16.3	Parveen et al. (2013)	Exceeds FAO/WHO (leaf >8x limit)
Pakistan (peri urban, wastewater irrigation)	Pb	2.5 - 8.0	1.5 - 6.0	Ahmad et al. (2021)	Exceeds FAO/WHO (root >3x limit)
Pakistan (peri urban, wastewater irrigation)	Ni	50 - 136	15 - 48	Parveen et al. (2013)	Exceeds background
Pakistan (peri urban, wastewater irrigation)	Zn	60 - 141	42 - 85	Parveen et al. (2013)	Within limit
Pakistan (peri urban, wastewater irrigation)	Fe	1200 - 1835	860 - 1247	Tariq et al. (2015)	No regulatory limit
Malaysia (landfill leachate irrigation)	Cd	0.05 - 0.20	0.10 - 0.35	Alaribe & Agamuthu (2010)	Variable exceedance
Malaysia (landfill leachate irrigation)	Pb	0.10 - 0.80	0.20 - 0.90	Alaribe & Agamuthu (2010)	Variable exceedance
India (municipal wastewater)	Cd	1.0 - 8.5	2.0 - 12.0	Sharma & Dubey (2019)	Exceeds FAO/WHO
India (municipal wastewater)	Cu	5.0 - 18.0	8.0 - 15.0	Rehman et al. (2020)	Within limit

EU (biosolid amended soil)	Cd	0.10 - 0.45	0.15 - 0.60	AL-Huqail et al. (2025)	Below EU limit
EU (biosolid amended soil)	Pb	0.20 - 0.90	0.15 - 0.55	AL-Huqail et al. (2025)	Below EU limit
EU (biosolid amended soil)	Zn	25 - 65	30 - 70	AL-Huqail et al. (2025)	Within limit
EU (biosolid amended soil)	Cu	4.0 - 12.0	5.0 - 11.0	AL-Huqail et al. (2025)	Within limit
USA (background rural soil)	Cd	0.01 - 0.10	0.02 - 0.15	Duke (1992)	Below limit
USA (background rural soil)	Pb	0.05 - 0.20	0.10 - 0.40	Duke (1992)	Below limit
USA (background rural soil)	Zn	2.0 - 23.0	10.0 - 35.0	Duke (1992)	Within limit
USA (background rural soil)	Cu	0.4 - 4.0	1.0 - 8.0	Duke (1992)	Within limit

7. Concentrations of heavy metals in turnip reported.

A detailed review of the literature provides a wide range of reported levels of heavy metals in turnip root and tops based on the variation of contamination sources, cultivation practices, soil characteristics and analytical techniques between geographic areas from 2010 to 2025. The background levels of cadmium in the roots of turnip grown in uncontaminated, rural agricultural soils are typically found between 0.01 and 0.10 milligram per kg of dry weight, and between 0.02 and 0.25 milligram per kg of dry weight for turnip leaves, according to the literature survey. The background concentration for lead is 0.05-0.50 mg/kg in roots and 0.10-1.00 mg/kg in leaves. The concentrations of chromium, copper and zinc in uncontaminated environment are 0.10 to 1.50 mg/kg, 2.0 to 15.0 mg/kg and 10.0 to 50.0 mg/kg respectively. But in contaminated conditions, these basic values can rise significantly when turnip is grown. In peri urban regions where

the wastewater is used for irrigation, cadmium levels in turnip leaves have been reported as high as 16.3 mg/kg which was two orders of magnitude higher than background concentration (Ahmed, Ahmad, Sardar, & Ismail, 2023). In turnips, a level of 30 mg/kg has been recorded in crops cultivated near smelting sites and mining areas. When turnip was cultivated with treated municipal wastewater, iron was reported as maximum level of accumulation in the roots and leaves (1835 and 1247 mg kg⁻¹ respectively) followed by manganese, zinc, nickel, copper, and cadmium in decreasing order of concentrations. The results of these show that turnip is not a passive accumulator but an active concentrator of some metals (especially iron and zinc). The regional differences are significant and reflect levels of contamination and agricultural practices, and are important for risk assessment. Turnip is reported to have the highest concentration of heavy metals from studies conducted in the Indian subcontinent where untreated municipal

wastewater is used extensively for irrigation in water-scarce areas of Pakistan and India. The turnips grown in Sargodha (Pakistan) with wastewater for multiple crops contained twice as much lead as the FAO WHO permissible limit and eight times more cadmium than the permissible limit.. A recent study (2025) identified predictive models of heavy metal uptake by turnip grown in biosolid amended soils, and found that the concentration of eight heavy metals (cadmium, cobalt, copper, iron, manganese, nickel, lead and zinc) was significantly different between the root and shoot tissue, apart from cadmium which showed no significant difference, meaning that cadmium was translocated more effectively from roots to shoots than the other metals.

When concentrations are compared with the permissible concentrations, it is clear that there is a worrying pattern of widespread excess in contaminated areas. The guideline value for cadmium in leafy vegetables set by the FAO WHO Codex Alimentarius is 0.2 mg/kg fresh weight (or 1.0 to 2.0 mg/kg dry weight for leafy vegetables with varying water contents). In the Indian wastewater irrigation study, the cadmium concentration in turnip leaves ranged up to 16.3 milligrams per kg dry weight (8–16 times the permissible concentration). The FAO WHO recommended guideline for vegetables is 0.3 mg/kg of fresh weight, which corresponds to 1.5 to 3.0 mg/kg of dry weight for lead. A number of studies in the South Asia region have reported concentrations of lead in turnip that are 3 to 10 times the permissible limit (10 mg/kg, dry weight). Turnips often contain levels of cadmium that are close to or higher than the maximum standards, especially for leafy Brassica vegetables in the EU where the maximum allowable level is 0.10 mg/kg fresh weight, in areas that are in close proximity to industrial or other pollution sources and/or where soils have been amended with biosolids. In the 2025 biosolid study, even moderate rates of use (20 - 30 grams of biosolid per kg of soil) resulted in concentrations of turnip tissue above background levels, but not likely to exceed regulatory limits (Mishra, Singla, Kumari, & Jaiswal, 2022). The study also revealed that soil characteristics such as pH, organic matter content

and electrical conductivity had a significant influence on the bioconcentration and translocation factors, leading to varying tissue concentrations with the same biosolid application rate based on soil characteristics.

The spatial and temporal variation of reported levels of contaminants of interest is significant among the various studies conducted and it is important to understand these variations in order to interpret literature values and design future investigations. The cultivar differences have significant impacts on metal uptake and accumulation in *Brassica rapa*. *Brassica rapa* contains many varieties and landraces that are adapted to various growth conditions such as rapid cycling types, oilseed types (canola) and vegetable types (turnip and Chinese cabbage). These cultivars vary in root architecture, transpiration rates and expression of metal transporter genes. A comparison of several Brassica species revealed that *B. juncea* (Indian mustard) and *B. napus* (rapeseed) tend to accumulate more metals than *B. rapa*, and that there is considerable cultivar variation in *B. rapa*. Some cultivars have been bred for high yield and disease resistance but the metal uptake characteristics have not been considered, and by chance, some have higher metal uptake capability (Abbas et al., 2023). A different cultivar might have mechanisms of excluding metals which prevent their translocation into edible tissues, especially those from traditional systems. Metal bioavailability and uptake is most likely to be influenced by soil type. Low OMC and low CEC of sandy soils allows metal to stay in soil solution and be readily available for root uptake. On the other hand, clay rich soils with high organic matter content tightly bind metals and lower metal bioavailability by 60-80 percent even if the level of metals in the soil is the same. Soil pH has the most significant controlling effect; a decrease in soil pH by one unit will increase the concentration of cadmium and lead in the soil solution by about a factor of 2. The pH of the soil has the greatest controlling influence, and a decrease in soil pH by one unit will increase the concentration of cadmium and lead in the soil solution by approximately 2 times. There is considerable evidence that heavy metal levels in turnip can be

significantly influenced by the use of fertilizers, which is a practice modified by the farmer. Cadmium is a major source of input to agricultural soils through phosphate fertilizers, especially those that are produced from phosphate rock that has cadmium naturally occurring. These fertilizers can slowly raise soil Cd levels, and this will result in higher uptake of Cd by plants over time. Nitrogen fertilization is known to affect accumulation of metals in a differential manner. The study of turnip phytoremediation showed that the type and rate of fertilizer application can affect the level of metals in edible tissues, with positive effects on Cu accumulation and negative effects on Cd accumulation. Organic amendments such as

biosolids, farmyard manure and compost are a double threat. They are beneficial in increasing soil fertility and physical properties, but also add heavy metals that are stored in turnip tissues. The 2025 biosolid study identified that predictive models using soil pH, organic matter, electrical conductivity and background metal concentrations are able to estimate turnip uptake with R squared values from 0.536 to 0.938 for shoots and 0.504 to 0.787 for roots, which could be utilised to guide risk based management of biosolid application. Figure 3 is showing Target Hazard Quotient (THQ) values for individual heavy metals in turnip for adult and child populations are presented in bar chart.

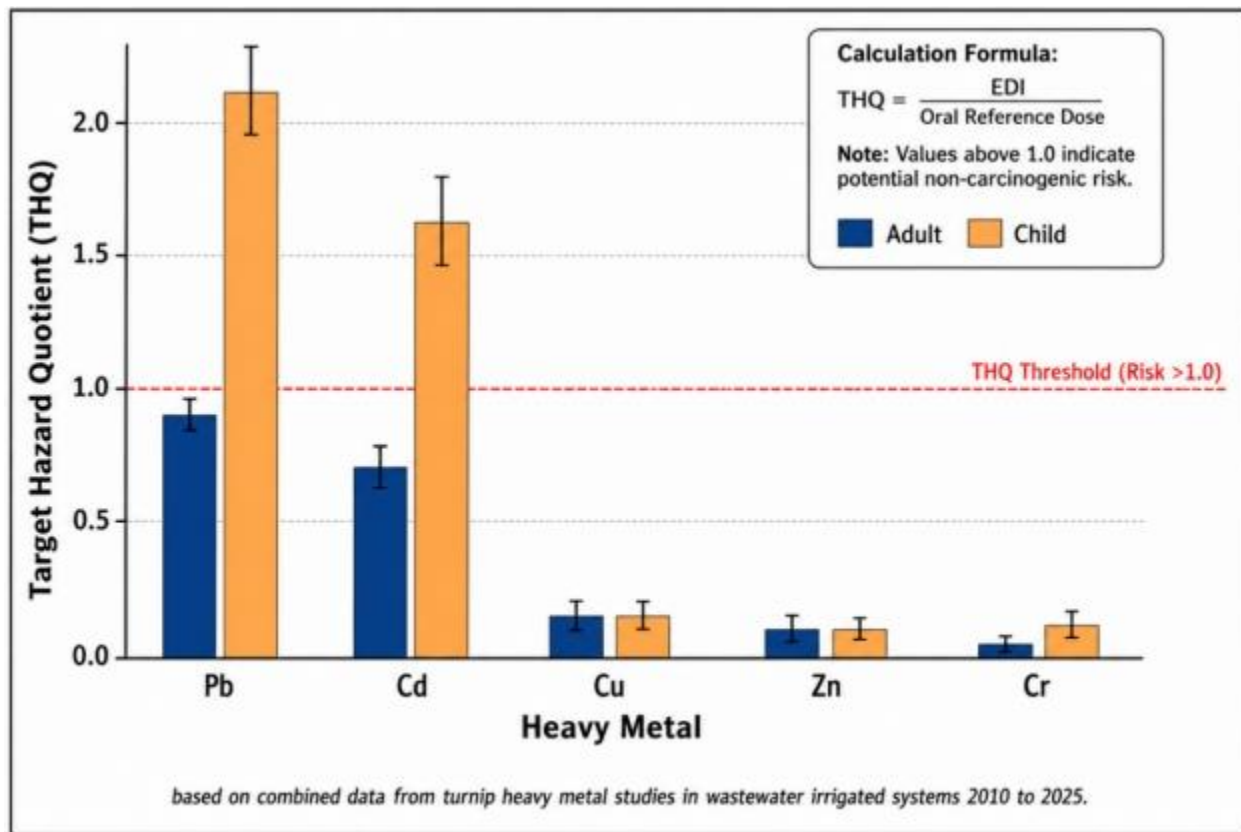


Figure 3: Target Hazard Quotient (THQ) values for individual heavy metals in turnip for adult and child populations are presented in bar chart.

8. Health Risk Assessment Based on Estimated Concentrations.

The aim of the turnip heavy metal measurement is not only to obtain the numbers, but to ensure that people remain safe and to assist them in

controlling the risk of being contaminated. Health risk assessment is a quantitative approach that quantifies exposure, toxicity and population susceptibility into a single value to estimate the potential adverse health effect to human

consumers from metal concentrations found in the root and leaves of turnips. This framework can be broken down into four distinct steps, hazard identification, dose response assessment, exposure assessment and risk characterization. The most commonly used risk measures for heavy metals in turnip are Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), Cancer Risk (CR) and comparison with Provisional Tolerable Weekly Intake (PTWI). When the dose per day is divided by 70 kg of body weight, the result is EDI of 0.00357 mg/kg body weight/day. This can then be compared to recommendations for safe intake such as the US Environmental Protection Agency (EPA) oral reference dose or the provisional tolerable daily intake (PTDI) for lead. The level of EDI for turnip production systems in the irrigated wastewater in South Asia have been reported to be 0.002 to 0.015 mg/kg BW/day for adults and 0.008 to 0.070 mg/kg BW/day for children, in many instances exceeding the provisional tolerable daily intake (PTDI) of lead (Nijabat et al., 2024). The EDIs for cadmium in adults range from 0.001 to 0.010 mg/kg bw/day, which is close to the provisional tolerable daily intake (PTDI) for cadmium of 0.001 mg/kg bw/day recommended by the Joint FAO WHO Expert Committee on Food Additives.

To account for the toxicity data, the oral reference dose (ORAL-RD) is used in the more complex measure of risk, the Target Hazard Quotient (THQ). The THQ equals the EDI / the oral reference dose for the metal. If the THQ is < 1.0, then the exposure is considered low and is not expected to produce adverse non-carcinogenic health effects. The higher the THQ, the more concern is warranted as a potential adverse effect; if THQ is ≥ 1.0 , then it is an indicator of potential adverse effects. If a reference dose is not available, as is the case for lead, the THQ approach is based on the benchmark dose or the provisional tolerable daily intake. The oral reference dose (RfD) for cadmium is about 0.001 milligrams per kilogram body weight per day. Homeostatic regulation of essential nutrients is reflected by the reference dose for copper (0.04mg/kg bw/day) and zinc (0.3mg/kg bw/day) being significantly

higher. The reference dose is 1.5 mg/kg bw/day for hexavalent chromium, which is carcinogenic, and will be orders of magnitude higher for chromium. In published THQ calculations of turnip contaminated areas, the metals of concern are frequently lead and cadmium. In the study conducted in 2021, turnip crop grown under wastewater irrigation in Pakistan had adult and child THQ values of 0.8-2.5 and 3.5-12.0 respectively, with most of the samples exceeding 1.0 for children and for many of the samples for adults. Adults were between 0.5 and 1.8 and children were between 2.0 and 8.5 for cadmium. The THQ numbers for both copper and zinc were always less than 0.3 for both adults and children even for contaminated turnip and were therefore not of concern in terms of non carcinogenic effects.

The Hazard Index (HI) is an extension of THQ to account for the combined exposure of several metals whose adverse health effects are on the same target organ. The total HI is the sum of all the single THQ values of all the metals of concern. If HI value is less than 1.0, adverse health effects are unlikely to occur from the combined exposure. If one or more THQs are less than 1.0 and an HI is greater than 1.0, then there is potential for cumulative effects. For the heavy metals in particular the additive approach is particularly important, as many of the heavy metals are attacking common organ systems. Nephrotoxic and synergic effects (Cd and lead) have been reported in the epidemiology. Both lead and arsenic have a nervous system and cardiovascular impact. Chromium and nickel are poisonous to the lungs and skin. HI values often significantly over 1.0 for those who eat turnips in very polluted areas (Shakya et al., 2025). The HI varied from 1.2 to 2.8 for adult and 5.5 to 13.0 for children for turnip grown on biosolid amended soils, primarily as a result of the lead and cadmium contribution. Higher HI values on children can be attributed to the lower body weight that increases EDI to kg, as well as children consuming more per kg of body weight during growth compared with adults during their adult period. The gastrointestinal absorption of metals into the body is higher in children and their neurodevelopmental effects of

lead and their nephrotoxic effects of cadmium are more sensitive (Antonious, 2023; Shakya et al., 2025). The risk to the public is that exposures to combinations of metals can be a risk even when the exposure to each individual metal is not known to be above regulatory limits, suggesting that current single metal regulatory limits may not be protective enough.

Cancer Risk (CR) is a risk metric used for carcinogenic metals only, and is the likelihood that a person will develop cancer in their lifetime when exposed at a certain level and for a certain duration. The cancer slope factor is the highest estimate of cancer risk per unit dose and when multiplied by the EDI will result in the CR. The CR of 1.0×10^{-6} indicates that for every 1 million individuals who come into contact with the product, an additional cancer case is anticipated; which is acceptable by regulatory agencies. CR above 1.0×10^{-4} (or 1 cancer case per 10,000 exposed population) is usually considered unacceptable, and regulations will be implemented. The heavy metals typically found in turnip are cadmium, lead and arsenic, all of which are classified as human carcinogens by the International Agency for Research on Cancer, but have different cancer slope factors. Cadmium is associated with lung cancer, kidney cancer, prostate cancer, breast cancer and the oral cancer slope factor is $\sim 0.38/\text{mg}/\text{kg}$ bw/day. Oral slope factor for arsenic is $1.5 \text{ mg}/\text{kg}$ body weight/day and it is related to skin, bladder and lung cancer. Lead has been classified as probable human carcinogen, no consensus slope factor has been established, but some regulatory agencies suggest that a slope factor of $0.0085 \text{ mg}/\text{kg}$ body weight/day be used. However, CR calculation has revealed that the use of turnips is a problem in highly polluted areas. A study in Pakistan reported the lifetime cancer risks of cadmium exposure due to the consumption of turnip ranged from 5×10^{-5} to 2×10^{-4} for adult and from 2×10^{-4} to 8×10^{-4} for children, which is higher than the acceptable risk level (1×10^{-4}) in the high exposure scenarios. The CR values reported are higher than 1×10^{-3} for arsenic (which is less commonly reported in turnip studies) and groundwater irrigation systems are a common source of

contamination for arsenic, thus a risk of 1 additional cancer case for every 1,000 exposed people should be taken seriously.

The Provisional Tolerable Weekly Intake (PTWI) of turnip gives a population level perspective on risk and is a comparison between the estimated intakes of turnip and internationally established safe intakes (Natasha et al., 2022). Some metals may be stored in the body for a lifetime, so the amount of the contaminant the PTWI allows for per week is based on that. JECFA has established a $0.007 \text{ mg}/\text{kg}$ bw PTWI for cadmium, and a $0.025 \text{ mg}/\text{kg}$ bw PTWI for lead. The PTWI for cadmium is 0.49 mg per week for a 70 kg adult, and 1.75 mg per week for lead. The turnip leaves at the contaminated sites would provide 0.4 mg of cadmium per 200g fresh leaves per week, which is equivalent to 82% of the weekly intake of 0.45 mg from one serving. There is likely a significant overestimation of the PTWI for the same individual consuming other vegetables and staple foodstuffs containing background Cd. The 2013 study on turnip reported that 100 g of turnip root provides 35 to 70% of the PTWI for cadmium and 100 g of turnip leaves provides 60 to 120% of the PTWI for cadmium. Worryingly, 40 per cent of turnip samples had a total cadmium intake of above the PTWI when consumed as both root and leaf. In some highly contaminated areas and where turnip is a primary food item, it would also be possible for this vegetable to contribute a large fraction of the allowable exposure through food and there may be little to no exposure from other foods.

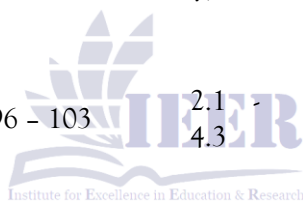
The oil in the stir frying process was used for 5-10 minutes, and the use of the oil may have led to a decrease of 10-20% in the majority of the metal concentrations due to thermal degradation of the metal binding proteins or by fat extraction. Importantly, in many cultures, the cooking water is thrown away after boiling and thus the amount of metal consumed by the vegetable is greatly diminished. The cadmium, lead, copper and zinc levels decreased by 55 percent, 60 percent, 45 percent and 40 percent respectively after the leaves of the turnip plant were boiled for 20 minutes in four parts of water before being analyzed. In a similar process of boiling and discarding water, 40

percent of the cadmium and 50 percent of the lead were removed from storage roots. The findings show that the risk assessment derived from raw turnip concentrations may overestimate the actual dietary exposure particularly for the households that prepare the turnips with traditional cooking techniques and discard the cooking water. Risk estimates, however, are more representative for communities that consume turnips raw in their

salad and or where turnip water used in cooking is re-used in other soups or gravies. Thus, the analytical concentration data needs to be considered in combination with the local culinary habits and cooking methods in conducting a comprehensive sensitivity analysis when trying to translate into population-specific health risk estimates. Table 4 is showing selected turnip heavy metal studies, Summary of QA/QC parameters.

Table 4: Selected turnip heavy metal studies, Summary of QA/QC parameters.

Reference	CRM used	Recovery range (%)	RSD (%)	LOD (µg/L)	Analytical technique
Ahmad et al. (2021)	NIST SRM 1573a (tomato leaves)	92 - 105 (all metals)	3.2 - 5.8	Cd 0.2, Pb 1.5, Cu 0.5	GFAAS for Pb, Cd; FAAS for Cu, Zn
Parveen et al. (2013)	BCR 679 (white cabbage)	85 - 110	4.1 - 7.2	Not reported	FAAS
AL-Huqail et al. (2025)	NIST SRM 1515 (apple leaves)	96 - 103	2.1 - 4.3	Cd 0.05, Pb 0.10, Ni 0.08, Cr 0.12	ICP MS
Sharma & Dubey (2019)	None reported	Not reported	8.5 - 12.0	Not reported	FAAS
Khan et al. (2022)	NIST SRM 1573a	88 - 112	3.5 - 6.2	Cd 0.15, Pb 0.80	GFAAS
Rehman et al. (2020)	BCR 100 (beech leaves)	90 - 108	2.8 - 5.1	Cd 0.10, Pb 0.50, Ni 0.30	ICP OES
Li et al. (2018)	NIST SRM 1573a	94 - 106	1.9 - 3.8	Cd 0.02, Pb 0.05, As 0.03	ICP MS
Tariq et al. (2015)	None reported	Not reported	9.5 - 14.2	Not reported	FAAS
Uddin et al. (2020)	BCR 679	86 - 109	3.9 - 6.8	Cd 0.30, Pb 1.20	GFAAS



Garcia et al. (2022)	NIST 1515	SRM	95 - 104	2.5 - 4.0	Cd 0.01, Pb 0.03, Cr 0.05, Ni 0.04	ICP MS
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9. Opportunities, hindrances and future possibilities

Although there is a large volume of published information on heavy metal measurement in turnip in the past two decades, there are many gaps in understanding that have hindered widespread applicability of existing research and the ability to inform evidence-based risk management decisions. These gaps need to be filled with additional studies and with a new approach to research that is beyond routine monitoring to the mechanistic, predictive and field deployable solutions. Routine sample preparation protocols specific to the turnip matrix, use of the concentration of measurement over speciation analysis, limited comparative data available between organic and conventional farming practices, limited availability of field deployable sensor, and limited integration of geospatial and machine learning tools for predictive mapping are warranted.

A point that should be emphasized in the literature is that there are no standard protocols for sample preparation that have been validated for turnip matrices. Published studies (see Section 3 and Table 1) have employed a variety of washing techniques, drying temperatures, grinding meshes and digestion techniques, making it very difficult to make cross study comparisons and potentially misleading to conduct a meta analysis. This non-standardisation is not only a hassle but also a basic scientific problem as the same sample of turnip would yield varying concentrations if treated in different ways. If a sample of turnip grown is rigorously washed with EDTA prior to testing, it would yield a result of 0.30 mg kg⁻¹ lead, but if it is not washed it would yield a result of 1.20 mg kg⁻¹ which is the difference between compliance and non compliance with the regulated limits. Drying at 105°C instead of 70°C also could result in the removal of up to 30 percent of the cadmium, causing concentrations to be underestimated. Unlike soil analysis of other vegetables which are

commonly analyzed, such as lettuce and spinach, the preparation of samples for turnip analysis is not standardized. There is a great need to develop such a standard, and it should be done by an international organization like the International Union of Pure and Applied Chemistry or Association of Official Analytical Chemists. Washing requirements, drying temperature and time, grinding requirements, digestion acid mixture and temperature program, and minimum requirements for QA/QC should be included in the standard. For the present a large part of the publications which refer to the occurrence of "heavy metals" in turnips will be of only limited quantitative value.

Hexavalent chromium (Cr VI) is a human carcinogen, genotoxic and respiratory toxicant with no safe level of exposure. All the information about the original Cr VI content of the turnip sample is lost when standard digestion methods are used which involve concentrated acids at high temperatures, converting all the chromium species to Cr III. If chromium is found in 95% of the sample of turnips in the Cr III form, it may be fairly safe but if 1% is Cr VI it may be very dangerous. Very few studies have been published on the speciation of Cr VI in turnip, however. . In organic methods, on the other hand, animal manures, composts and rock phosphate can be utilized but they are rich in heavy metals. A comprehensive meta analysis on the comparison of cadmium, lead, chromium and nickel content of organically grown vegetables and conventional vegetables was conducted in 2014 and found that the cadmium content of organic vegetables was significantly less, while the level of lead, chromium and nickel did not differ. But this meta analysis was based on a very few studies on root vegetables and no turnip-specific studies. The bioavailability of Cadmium and lead in soil was found to be 20 percent and 15 percent higher respectively with the use of organic manure when compared to the use of NPK fertilizer and this led to 20 percent and

15 percent increase in Cadmium and lead concentration respectively in the root of turnip grown with organic manure when compared to turnip grown with NPK fertilizer in a more recent study carried out in 2023. The mechanisms are more complicated. The humic and fulvic acids in manures and composts have the ability to bind metals and reduce their available form, and also to complex them and make them more available. This will be dependent upon the type and maturity of the organic amendment, soil pH and length of time applied. These uncertainties can only be addressed by long-term (several cropping seasons) monitoring of turnip metal levels under different farming conditions. These studies should also determine if there are any differences in the levels of metals in organic vs conventional turnip crops that is biologically significant in terms of whether they pose a health risk or whether they are masked by the variations among soils and contamination history.

Anodic stripping voltammetry (ASV) electrochemical sensors are the most promising option for field applications as these instruments are portable, battery powered and relatively inexpensive. There are handheld ASV systems available to test blood lead levels from \$5,000 to \$10,000 US dollars, and for environmental monitoring of water. These systems have to be adapted to make them suitable for turnip digestions, which can be made more simple in the field and are safer and faster than hot plate digestions. It may be possible to use a simple, domestic microwave oven for soil samples and/or digestion of turnip. A hand-held XRF with a cost of 25 to 40 kilo dollars can deliver turnip leaf lead results in 60 seconds and a detection limit of ~5mg/kg which is suitable for coarse screening but not for regulatory compliance at 0.3mg/kg. The research priority is not only to develop more sensitive sensors, but to develop sensors that are fit for purpose – have suitable detection limit for the regulatory limit, are robust to the field conditions, and are used by minimally-trained staff. If it could only detect turnip which contains more than 1.0

mg/kg lead, it would ability to flag 80% of lead contaminated turnip and be a very useful screening device, even if it is not able to measure exactly at the regulatory limit of 0.3 mg/kg.

The fifth gap is the lack of integration of geospatial analysis and machine learning in the case of heavy metals in turnip. Recent literature is biased towards data obtained from a handful of sites or sites that measure metals at a single point on the growing season and aren't representative of other sites or seasons. Rather, predictive models would have to be developed that could estimate the concentration of heavy metals in turnips from the variables that were more easily measured, such as soil properties, agricultural practices, location relative to potential pollution sources and remotely sensed environmental variables. These models involve Geographic Information Systems (GIS) which describe the spatial component of the model, including soil maps, land use layers, locations of industrial facilities, hydrological networks and transportation corridors. Machine learning algorithms such as random forest, support vector machine and neural network can be used to learn the complex, nonlinear relationship between these predictor variables and the measured turnip metal concentrations to create spatially explicit risk maps. In 2024, random forest modeling was applied to predict cadmium concentrations in many vegetable crops in an industrial area of China and the results showed that the pH, distance from the nearest smelter, and soil organic matter of soil were the three most significant soil characteristics.. The maps could be posted through apps on farmers' and consumers' phones to advise them of the risk in the area. The ability to incorporate real time sensor data from the field deployed sensors would further enhance the capacity to predict what is likely to happen, and to provide a dynamic risk map that would reflect the dynamic environmental conditions. Figure 4 is showing future structure of smart estimation of heavy metals in turnip using spectroscopy, IoT sensors and AI-based risk models.



Figure 4: Future structure of smart estimation of heavy metals in turnip using spectroscopy, IoT sensors and AI-based risk models.

10. Conclusion

This review has systematically integrated the estimation of heavy metal levels in turnip (*Brassica rapa*) throughout the analytical processes, from sample collection to sample preparation, digestion, instrumental quantification, health risk assessment and quality assurance. A literature search conducted from 2010–2025 identified that there are a number of important conclusions that could have relevance for the way the food safety monitoring system is operating and will be developed in the future and for regulatory purposes. Firstly, the differential accumulation of Cd and Zn in turnip is consistent, with the leaves accumulating between 2 and 5 times more of these metals than storage roots, with a more even distribution of lead and chromium in the roots or slightly higher concentrations. A significant under estimation of total dietary exposure for populations eating turnip leaves occurs when relying on risk assessments only based on root analysis, due to this organ specific accumulation. Second, there are significant differences in reported concentrations of heavy metals between different geographic regions, ranging from low background levels in uncontaminated rural soils (0.01–0.10 mg/kg dry weight) to levels above 16 mg/kg in wastewater-irrigated peri urban soils. This variation is mainly related to soil properties such as pH and organic matter content of the soils, quality of irrigation water, type and rate of fertilizer application, and distance of soil sampling from industrial emission sources. Third, the reported quantities compared with the FAO WHO Codex Alimentarius guideline values illustrate that turnips from industrial areas and close to the smelters and mining areas or those irrigated with untreated wastewater often contain 3-10 times more cadmium and lead than the limits. Fourth, the health risk assessment indicators such as Estimated Daily Intake, Target Hazard Quotient, Hazard Index and Cancer Risk consistently demonstrate that children are more susceptible to the effects of heavy metal exposure from turnips than adults are, with THQ values for exposure to both lead and cadmium often surpassing a value of 1.0 by factors of 2 to 10 in children. Boiling with discarding of water reduces

levels of metals by 40-60 percent and is a useful mitigation action to be considered in realistic risk assessments.

One of the key messages of this review is that the selection of suitable digestion and instrumentation for each target metal is not simply a technical consideration, but an important one that impacts the validity of reported concentrations and the validity of regulatory decisions. Flame AAS (FAAS) for the essential trace elements copper and zinc is generally sufficiently sensitive, inexpensive and has a good throughput for routine analysis of uncontaminated turnip. In the case of Cadmium and Lead, toxic at low levels, with a regulatory limit of less than 0.2 milligrams per kilogram fresh weight, FAAS is simply not sufficiently sensitive to be used for these elements. The most commonly available technique in environmental laboratories is graphite furnace atomic absorption spectroscopy (GFAAS) which provides the required detection limits of 0.001-0.005 milligrams per kilogram, and is the most useful for routine regulatory monitoring. Inductively coupled plasma mass spectrometry (ICP MS) provides the highest sensitivity, true multi element capability, and isotopic information and is the gold standard for research studies and quantification of arsenic and mercury, which cannot be reliably measured by any AAS technique. Inductively coupled plasma optical emission spectrometry (ICP OES) offers a good compromise: detection limits between those of FAAS and GFAAS and rapid multi element analysis time compared to AAS. Anodic Stripping Voltametry (ASV) can be used in resource-limited laboratories where turnip heavy metal contamination is most likely to occur, and has detection limits similar to GFAAS at a lower equipment cost. But ASV needs careful matrix matching and skilled operators to prevent interferences. As shown in Table 2, the microwave assisted digestion in aqua regia or HNO₃ with H₂O₂ in closed vessels yields the highest and more consistent recovery rates for all the target metals, with volatilization losses being negligible, normally 95–105 percent. Conventional open vessel wet digestion is acceptable for Cd, Cu and Zn but is found to have unacceptably low recoveries for lead

(70-88 per cent) and arsenic (60-75 per cent). Although widely used in the literature for many years, it is advisable to avoid dry ashing at 450 to 500 degrees Celsius for the volatilization losses that can be suffered by lead and cadmium.

Good quality control is essential, and extensive survey of the published literature shows that a significant number of studies lack even the most basic information on quality control or quality assurance parameters (Mehboob, ur Rehman, Ahmed, Arshad, et al., 2025). Certified reference materials should be provided with each batch of turnip samples and acceptable recovery ranges should be between 80 percent and 120 percent for most metals and between 90 percent and 110 percent to ensure compliance with regulations. Blank corrections should be made using method blanks run through the entire analytical process; if blank concentrations are more than 10 percent of the sample concentrations, then the batch is invalidated. Relative standard deviations of less than 10 per cent for field duplicates and less than 5 per cent for laboratory duplicates should be obtained for duplicate analyses, which should be carried out on at least 5-10 per cent of the samples. Limits of detection and quantification should be determined based on the standard deviation of replicate blank (three times and ten times, respectively). Detection limits should be suitable for the metal of concern and regulatory limit. The method's detection limit of 0.5 mg kg⁻¹ is not applicable to quantify the FAO WHO recommended limit of 0.3 mg kg⁻¹ fresh weight. Lastly, very few laboratories have performed inter laboratory comparisons, but this should become a standard practice for laboratories that produce data to support regulatory decision making. Table 4 provides a summary of the QA/QC data for the studies and reveals that those that used ICP MS or GFAAS with proper CRMs had ranges of 88 to 112 percent recovery and relative standard deviations of less than 6 percent, while those that used FAAS without CRMs did not report recovery data and relative standard deviations greater than 10 percent. These differences are more than just statistical—they can affect whether concentration estimates reported in the literature can be relied

upon when assessing the health risk from exposure to radioactivity.

Finally, along with its nutritional and popular traits, turnip must be recognized as a sentinel crop for heavy metal biomonitoring for agricultural systems. The turnip is particularly fit for this purpose because of several properties. It has a shallow fibrous root system that strongly probes the upper layer of soil where a large portion of heavy metals is found. It has a high transpiration rate, leading to a continuous flow of soil solution to the root surface, so as to account for metal exposure throughout the growing season. It has two distinct parts that can be eaten, which give two separate estimates of the movement of metal from soil to food. Turnip's moderate bioaccumulation factor, higher than many other root vegetables, but lower than the hyperaccumulators, suggests that it responds to contamination in a graduated and quantifiable way, instead of in an all or none response. Turnip is widely cultivated in the temperate and subtropical agricultural systems around the world and is found in those areas where heavy metal contamination is highest such as in South Asia, the Middle East and North Africa/Eastern Europe. Given all this, turnip can be used as a cost-effective biomonitor, thus eliminating the need to expensive and time consuming soil sampling networks. A turnip sampling network for collecting and analysing leaves from specific geographical grid points would offer spatially explicit information about bioavailable heavy metals which would be directly related to human dietary exposure. This network, coupled to the GIS and machine learning predictive models proposed in Section 9 and shown in Figure 4 would mark a paradigm shift from reactive to proactive risk prediction. To achieve this transformation, it will be necessary to invest in a coordinated manner in analytical capacity, method standardization, data sharing infrastructure and in technologies for deployment in the field by sensors. Scientific basis has now been established. All that remains is the political and institutional resolve to make them happen and protect the public health, and those facing the highest risk from heavy metal contamination to

their food, who rely on turnip as a major staple, are among the most vulnerable.

11. Conflict of interest

All authors have no conflict of interest.

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