Elemental analysis and health risk assessment of an edible plant grown along a National Road

Experimental

Materials and methods

The study was conducted along a National Road. The study area was selected due to high automobile activity from daily commuters and leisure travelers and since it is the main logistic route for trucks, which constitutes about 38% of the traffic. At peak times, traffic volumes exceed 2000 vehicles per hour. Plant samples of edible leafy green vegetable were collected from 10 sites that were ten kilometres apart due to the plant's dispersed growing patterns. These samples were collected from roadside soils approximately one meter away from the road. All samples were placed in polyethylene bags and stored in cooler bags for transportation.

Digestion was performed using the CEM Discover SP-80 microwave system with activent technology (CEM Corporation, USA). A mass of 2.00 g of certified reference material (CRM) and 0.5 g of dried and crushed plant leaves were accurately weighed into the microwave vessels to which 10 mL of nitric acid (70%) was added and allowed to pre-digest for 30 minutes before digestion in the microwave. After that, the digests were cooled (15 min) and gravity filtered through Whatman No. 1 filter papers into volumetric flasks (100 mL) and the volume was made up to the graduation mark with double distilled water. All samples were stored in a refrigerator at 4 ºC in polyethylene bottles until elemental analysis which was done within a week of digestion.

All samples were analysed by inductively coupled plasma – optical emission spectrometry (ICP-OES) (PerkinElmer, Optima 5300 Dual View, Billerica, Massachusetts, USA). Method validation was performed using the CRM, Strawberry Leaves (LGC7162) (LGC Limited, United Kingdom). CRMs were prepared and analysed like samples for method validation. Plant samples were analysed in triplicate whilst eight replicates of the CRM were analysed for method validation.

To eliminate matrix effects, standards and reagent blanks were prepared for calibration by addition of double distilled water to 70% HNO_3 using the same volume as the samples.

Working standards were prepared from stock standard solutions (1000 mg L^{-1}) and HNO_3 (70%) to match the matrix of digested samples. Calibration curves were obtained by preparing a blank and five standard solutions within the estimated ranges for each element. Wavelengths were chosen based on maximum analytical performance and minimum spectral interference. Spectral overlaps and inter-element interferences were eliminated by choosing the best of the three most sensitive lines. The Background Equivalent Concentration was checked daily by realigning the Hg lamp before analysis.

Quality assurance

Accuracy of the method was assessed by evaluating the closeness of the mean test result from replicate analyses for an analyte to the true or certified value of that analyte using the CRMs. The repeatability precision of the analytical method, which shows the closeness of individual measurements of an analyte to each other after being measured repeatedly, was evaluated by comparing the % RSD of the CRM test results to the appropriate limit of RSD, which should be within 10% of the true value. The equation for the calculation of %RSD is given below:

$$\%RSD = \frac{SD}{Mean} \times 100$$

Additionally, percentage recoveries were determined and were considered acceptable if between 90-110%.

Comparison of mean concentration to maximum permissible limits

The mean concentration of the elements (n=3) from each of the sites was determined and compared to threshold values or maximum permissible limits set in food or leafy vegetable. Some limits are provided below.

Element	FAO/WHO MPL (mg/kg)
Ni	67.9
Fe	425.5
Cu	73.3
Zn	99.4

Health risk assessment

The risk to human health due to consumption of the plant was evaluated by calculating the target hazard quotient (THQ) and carcinogenic risk (CR) for the analysed elements. The THQ was calculated as per the USEPA Region III Risk-Based Concentration Table (USEPA, 2011). THQ provides the non-carcinogenic risk of exposure from the ratio of a determined level of a potentially hazardous metal to its reference dose considered toxic (Song et al. 2009).

$$THQ = [X] \times IR / (B_w \times RfD_0)$$

[X] is the metal concentration in the plant (mg/kg dry weight), IR is the ingestion rate of plant per person (0.033 kg per day) (Sharma et al., 2016), B_W is the average body weight of an adult (70 kg), and RfD_0 is the oral reference dose of metals that adults can be exposed to (mg kg^{-1} per day). The RfD_0 values are as follows: As (0.0003), Cd (0.001), Co (0.043), Cr (0.003), Cu (0.04), Fe (0.7), Mn (0.14), Ni (0.02), Pb (0.004) and Zn (0.3) (USEPA, 2011). A THQ < 1 indicates a low risk of adverse effects due to exposure to that element, while a THQ > 1 suggests possible health risks due to exposure to that element.

The CR estimates the likelihood of an individual developing cancer from exposure to a potential carcinogen over a lifetime (Kortei et al. 2020).

$$CR = ([X] \times IR / B_w) \times CPS_0$$

CPS_o is the oral slope factor of the carcinogen in mg/kg BW per day, 1.5 for As, 0.0085 for Pb, and 6.3 for Cd. A value above 10^{-4} indicates a high probability of CR (Javed, 2016).

Contribution of elements to the diet

The concentrations of the essential elements in the plant were compared to recommended dietary allowances (RDAs) or adequate intakes (AIs) for most adults (ages between 14-70 years) (IOM, 2006). The average daily intake in mg day^{-1} was reported based on a serving size of 10 g per day (dry mass).

Element	Average concentration in 10 g (mg/day dry mass)	RDA/AI (mg/day)	UL	Contribution to diet (%)
Ca	140	1000-1300	2500	11-14
Mn	2.8			