Certificate of measurement

ISO 9001 Quality Management certified by BSI under certificate number FS27613

Strawberry Leaves

Certified Reference Material LGC7162

Certified Values

Constituent	Number of Laboratories	Certified Value[1] g/100 g	Uncertainty[2] g/100 g	Weight of sample[3] g
Calcium	25	1.53	0.07	0.5
Magnesium	25	0.377	0.017	0.5
Nitrogen	12	2.01	0.06	0.5
Phosphorus	16	0.260	0.023	0.5
Potassium	21	1.96	0.10	0.5
Sulfur	11	0.174	0.016	0.5

Constituent	Number of Laboratories	Certified Value[1] mg/kg	Uncertainty[2] mg/kg	Weight of sample[3] g
Arsenic	9	0.28	0.07	2
Barium	11	107	10	0.5
Cadmium	12	0.17	0.04	2
Cobalt	10	0.47	0.11	2
Chromium	15	2.15	0.34	2
Iron[4]	1	818	48	0.5
Lead[4]	1	1.8	0.4	2
Manganese	23	171	10	0.5
Mercury	8	0.027	0.006	0.5
Molybdenum	8	0.32	0.08	2
Nickel	12	2.6	0.7	2
Strontium	9	64	6	0.5
Zinc	24	24	5	2

Notes:

1. Results are expressed on a dry weight basis.
2. The uncertainty quoted is an expanded uncertainty interval calculated using a coverage factor of 2, providing a level of confidence of approximately 95 %.
3. Weight of sample taken for the homogeneity assessment.
4. Lead and Iron were determined by Isotope Dilution Mass Spectrometry (IDMS)

Date of Issue: October 2001
Format revised March 2009

Signed: ____________________
Gill Holcombe (Mrs)
for the Government Chemist

Queens Road, Teddington, Middlesex, TW11 0LY, UK
Tel: +44 (0)20 8943 7000 • Fax: +44 (0)20 8943 2767 • www.lgcgroup.com

Indicative Values

The following figures have not been certified, but are provided for information and should be regarded as indicative values.

Constituent	Indicative Value[1] g/100 g
Total Aluminium	0.1
Extractable Aluminium	0.06

Constituent	Indicative Value[1] mg/kg
Copper[2]	10
Lithium	0.7
Total Sodium	210
Extractable Sodium	65
Selenium	0.04
Vanadium	1.8

Notes:

1. Results are expressed on a dry weight basis.
2. The material is not homogenous for this element.

Procedures Used in the Production of this Material

The raw material was collected from a private strawberry farm in the Czech Republic. The leaves were cut and then jet milled to pass a 250 μm nylon sieve. The resulting powder was homogenised for 72 hours before bottling in 20 g portions in 60 mL bottles. (The bottles had been pre-cleaned by leaching in dilute HCl and rinsed). Finally the bottled material was radiation sterilised by ^{60}Co at a dose of 25 kGy.

The material was tested for homogeneity by analysing randomly selected samples for each analyte using an appropriate technique. The material was judged to be homogeneous for all elements, apart from copper, as the variation between the samples tested was not significantly greater than the method variation. The analysis of the material for copper resulted in the occasional high value which was thought to be due to chemical treatment of the plants that had not been removed during the washing of the leaves.

This material has been certified by means of two inter-laboratory exercises. These involved the analysis of samples of the material by the participants using methods of their own choice. The certified values (excluding Fe and Pb) are based on the mean of laboratory means from both studies combined, following elimination of outlying results.

The uncertainties were calculated by combining the 95 % confidence intervals obtained from the inter-laboratory exercises with the uncertainties calculated from the homogeneity study.

The certified values for iron and lead were determined by the application of a primary method (isotope dilution mass spectrometry) in a single laboratory (LGC). For information only, the iron content derived from the inter-laboratory study was 709 ± 58 mg/kg and the lead content derived from the inter-laboratory study was 1.8 ± 0.5 mg/kg.

For Aluminium and Sodium, indicative values are given for both total and extractable concentrations. The total concentration uses data from those laboratories employing an HF dissolution as part of the extraction procedure, or who employed a technique which did not require a digestion step. The extractable concentration was calculated from the results of laboratories (excluding outliers) that did not use HF. For the rest of the elements measured, the use of HF did not affect the concentration of element recovered.

The nature of the material is such that deterioration is not anticipated over its lifetime. The certified values will be monitored, and customers notified of any changes in values.

Intended Use

This material is intended for use in development, validation or quality control of analytical methods for the determination of elements in vegetation. The material may also be applicable to other matrices where suitable reference materials are not available.

Instructions for Use

Before opening, the contents should be mixed thoroughly by repeated inversion of the bottle. The moisture content should be determined on a separate test portion by drying for a minimum of 4 days over fresh P_2O_5.

Storage Conditions and Shelf Life

The unopened bottle should be stored at 20 ± 5 °C. Provided the sample is stored under the recommended conditions, its certification will remain valid for 12 months from the date of shipment.

Guidance on the Interpretation of Results

When comparing an analytical result obtained on the certified reference material to the appropriate certified value, users should not necessarily expect the result always to fall within the uncertainty limits of the certified value quoted on page 1. The uncertainty of the analytical result (U_{result}) must also be taken into account and if the uncertainty of the certified value is U_{RM}, then, as a simple test, a result should normally lie within the following limits of the certified value:

$$\pm \sqrt{U^2_{RM} + U^2_{result}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Both U_{RM} and U_{result} are expanded uncertainties providing a level of confidence of approximately 95 %. If a result lies outside these limits a problem with the measurement procedure and/or the uncertainty estimate of the result is indicated, which should be investigated.

If an estimate of the uncertainty of the result is not available, an approach recommended[1] by ISO Guide 33 is to obtain a value for the expected precision of the method (σ). The precision has two components, the within-laboratory standard deviation (s_w) and the between-laboratory standard deviation (σLm). The precision (σ) is given by:

$$\sigma = \sqrt{\sigma^2_{Lm} + \frac{s^2_w}{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

The within-laboratory standard deviation (s_w) is that observed when n replicate measurements are carried out under repeatability conditions (i.e. same analyst, same equipment, same reagents, and same calibration, over the shortest practical time period). The between-laboratory standard deviation (σLm) cannot be estimated by a single laboratory, but in many cases it is sufficient[1] to substitute the standard deviation observed in a single laboratory under intermediate precision conditions. The latter may be taken to refer to the situation where a series of measurements (10 or more) have been carried out over an extended period of time (1 month or more), preferably involving different analysts, equipment, reagents, calibrations, etc.

Once a value for σ has been obtained, a simple test for an analytical result is that it should normally lie within the following limits of the certified value:

$$\pm \sqrt{U^2_{RM} + (2\sigma)^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

If a result lies outside these limits a problem with the measurement procedure and/or the estimate of σ is indicated, which should be investigated.

It will be realised that the value 2σ is a substitute estimate of the expanded measurement uncertainty, U_{result}. In either case, a larger value for 2σ or U_{result} will make it more likely that an analytical result will lie within the limits calculated according to equations 1 or 3. The analyst must decide whether the adopted value for 2σ or U_{result} is consistent with their measurement, results being fit for the particular purpose for which they are intended.

References

1. ISO Guide 33:2000. Uses of Certified Reference Materials.

Participants in the Interlaboratory Exercise

The number of participants results used in the calculation of the certified values is given in the table on page 1. The following organisations took part in the interlaboratory exercise:

Agri-Food and Veterinary Authority (Singapore)

Al Hoty Stanger Ltd Co (Kingdom of Saudi Arabia)

Analytica Ltd (Czech Republic)

ARC - Analytical Laboratory (Republic of South Africa)

Associated Laboratory Services (UK)

Australian Government Analytical Laboratories (Australia)

Austrian Research Centres – (Austria)

Avecia Ltd. (UK)

Butterworth Laboratories Ltd. (UK)

CCFRA Technology Ltd (UK)

Central Laboratories (UK)

Comision Chilena de Energia Nuclear (Chile)

ENEA (Italy)

The Environment Agency – Llanelli Laboratory (UK)

Forest Research – Environmental Research Branch (UK)

Government Laboratory (Hong Kong)

LGC (Teddington) Ltd (UK)

National Institute of Health (Portugal)

Nestec (Switzerland)

NRM Ltd. (UK)

Public Analysts Laboratory (Ireland)

Services Laboratory – Agro and Food Technology Division (Sri Lanka)

Sheffield Hallam University (UK)

South African Bureau of Standards (Republic of South Africa)

Thailand Institute of Scientific and Technological Research (Thailand)

Universidad Nacional Autonoma de Mexico –Instituto de Geologica (Mexico)

University of Hertfordshire (UK)

University of Paisley (UK)

University of Sheffield (UK)

WRC-NSF Ltd (UK)

Unit Number:	Date of Shipment:

Legal Notice

(revised March 2009)

The values quoted in this certificate are the best estimate of the true values within the stated uncertainties and based on the techniques described herein. No warranty or representation, express or implied, is made that the use of the product or any information, material, apparatus, method or process which is the subject of or referred to in this certificate does not infringe any third party rights. Further, save to the extent: (a) prohibited by law; or (b) caused by a party's negligence; no party shall be liable for the use made of the product, any information, material, apparatus, method or process which is the subject of or referred to in this certificate. In no event shall the liability of any party exceed whichever is the lower of: (i) the value of the product; or (ii) £500,000; and any liability for loss of profit, loss of business or revenue, loss of anticipated savings, depletion of goodwill, any third-party claims or any indirect or consequential loss or damage in connection herewith is expressly excluded.

Production of this reference material was supported under contract with the Department of Trade and Industry as part of the National Measurement System Valid Analytical Measurement Programme.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Calcium (mg/d)	Chromium (μg/d)	Copper (μg/d)	Fluoride (mg/d)	Iodine (μg/d)	Iron (mg/d)	Magnesium (mg/d)	Manganese (mg/d)	Molybdenum (μg/d)	Phosphorus (mg/d)	Selenium (μg/d)	Zinc (mg/d)	Potassium (g/d)	Sodium (g/d)	Chloride (g/d)
Infants															
0 to 6 mo	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6 to 12 mo	260*	5.5*	220*	0.5*	130*	**11**	75*	0.6*	3*	275*	20*	**3**	0.7*	0.37*	0.57*
Children															
1–3 y	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 y	**1,000**	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 y	**1,300**	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 y	**1,300**	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1,250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 y	**1,000**	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 y	**1,000**	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 y	**1,000**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
> 70 y	**1,200**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 y	**1,300**	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 y	**1,300**	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1,250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 y	**1,000**	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 y	**1,000**	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 y	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
> 70 y	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*
Pregnancy															
14–18 y	**1,300**	29*	**1,000**	3*	**220**	**27**	**400**	2.0*	**50**	**1,250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 y	**1,300**	44*	**1,300**	3*	**290**	**10**	**360**	2.6*	**50**	**1,250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin* B_6, *Folate, Vitamin* B_{12}, *Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and* Sulfate (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Vitamins

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Vitamin A (μg/d)[a]	Vitamin C (mg/d)	Vitamin D (μg/d)	Vitamin E (mg/d)[b,c]	Vitamin K	Thiamin	Riboflavin	Niacin (mg/d)[c]	Vitamin B_6 (mg/d)	Folate (μg/d)[c]	Vitamin B_{12}	Pantothenic Acid	Biotin	Choline (g/d)	Carotenoids[d]
Infants															
0 to 6 mo	600	ND[e]	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6 to 12 mo	600	ND	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Children															
1–3 y	600	400	63	200	ND	ND	ND	10	30	300	ND	ND	ND	1.0	ND
4–8 y	900	650	75	300	ND	ND	ND	15	40	400	ND	ND	ND	1.0	ND
Males															
9–13 y	1,700	1,200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 y	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
51–70 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
> 70 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
Females															
9–13 y	1,700	1,200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 y	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
51–70 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
> 70 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
Pregnancy															
14–18 y	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
Lactation															
14–18 y	2,800	1,800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND	35	100	1,000	ND	ND	ND	3.5	ND

NOTE: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a] As preformed vitamin A only.

[b] As α-tocopherol; applies to any form of supplemental α-tocopherol.

[c] The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

[d] β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency.

[e] ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamine E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Arsenic[a]	Boron (mg/d)	Calcium (mg/d)	Chromium	Copper (μg/d)	Fluoride (mg/d)	Iodine (μg/d)	Iron (mg/d)	Magnesium (mg/d)[b]	Manganese (mg/d)	Molybdenum (μg/d)	Nickel (mg/d)	Phosphorus (g/d)	Selenium (μg/d)	Silicon[c]	Vanadium (mg/d)[d]	Zinc (mg/d)	Sodium (g/d)	Chloride (g/d)
Infants																			
0 to 6 mo	ND[e]	ND	1,000	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
6 to 12 mo	ND	ND	1,500	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
Children																			
1–3 y	ND	3	2,500	ND	1,000	1.3	200	40	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
4–8 y	ND	6	2,500	ND	3,000	2.2	300	40	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
Males																			
9–13 y	ND	11	3,000	ND	5,000	10	600	40	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 y	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 y	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
> 70 y	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
Females																			
9–13 y	ND	11	3,000	ND	5,000	10	600	40	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 y	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 y	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
> 70 y	ND	20	2,000	ND	10,000	10	1,100	45	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
Pregnancy																			
14–18 y	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	3.5	400	ND	ND	34	2.3	3.6
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6
61–50 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6
Lactation																			
14–18 y	ND	17	3,000	ND	8,000	10	900	45	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6

NOTE: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a]Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.

[b] The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.

[c]Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.

[d]Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals and this data could be used to set a UL for adults but not children and adolescents.

[e]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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			

S2 Table. Permissible limits (ppm) for heavy metals in food, water and soil according to international and local standards. International standards used are displayed in bold.

Matrices	Area of application	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)	Source
Food	**International**	**0.1-0.2**	**0.05-2**	**0.5-1**	**0.01-3**	**[1]**
	European Union	0.1-0.2	0.05-3	0.1-1	0.02-3	[2,3]
	USA	NA	NA	NA	NA	The U.S. Food and Drug Administration has not established regulatory limits for trace metals in finished food products other than bottled water.
	China	0.5	0.05	0.01	0.2	Retrieved from [4]
Drinking water	**International**	**0.01**	**0.003**	**0.001**	**0.01**	**[1]**
	European Union	0.01	0.005	0.01	0.001	[5]
	USA	0.01	0.005	0.002	0.015	[6]
	China	0.01	0.005	0.001	0.01	[7]
Irrigation water	**International**	**0.1**	**0.01**	**NA**	**5**	**[8]**
	USA	NA	0.005-0.01	NA	5	[9]
	China	0.05	NA	0.01	NA	Retrieved from [10]
Soil	**International**	**20**	**0.9-3**	**0.03-2**	**30-50**	[11,12]
	European Union	NA	NA	NA	NA	The European Union has not established limits for heavy metals in soils. There is

						however on-going policy to manage contamination, see [13] which states limit values in sludge for use in agriculture (Cd: 1-3 ppm; Hg:1-1.5 ppm; Pb:50-300 ppm)
	Finland	5-100	1-20	0.5-5	60-750	[14]. The Finnish standard values represent a good approximation of different national systems in Europe have been applied in an international context for agricultural soils as well.
	UK	32-640	10-230	1-3600	450-750	[15]
	USA	0.11	0.48	1	200	[16] [17] stated limit values in sludge for use in agriculture (As: 75 ppm; Cd: 85 ppm; Hg: 420 ppm; Pb: 840 ppm)
	China	20-40	0.3-0.6	0.3-1	80	[18]

1. Codex Alimentarius. Codex general standard for contaminants and toxins in food and feed - CODEX STAN 193-1995. Joint FAO/WHO; 2015 p. 59. Available: 10.13140/RG.2.1.4910.2560

2. Commission Regulation (EU) 2015/1006. Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs (OJ L161). EU; 2015 Jun. Report No.: OJ L161. Available: http://data.europa.eu/eli/reg/2015/1006/oj/eng

3. Commission Regulation (EC) No 1881/2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L364). EU; 2006. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1881-20100701

4. Li J, Xie ZM, Xu JM, Sun YF. Risk assessment for safety of soils and vegetables around a lead/zinc mine. Environ Geochem Health. 2006;28: 37–44. doi:10.1007/s10653-005-9009-x

5. European Commission. Proposal for a Directive of the European Parliament and of the Council on the quality of water intended for human consumption (COM/2017/0753). EU; 2019.

6. US EPA. Drinking water regulations (Safe Drinking Water Act). 2017. Available: https://www.epa.gov/dwreginfo/drinking-water-regulations

7. Ministry of Health, China. Standards for drinking water quality (GB 5749-2006). 2006.

8. Ayers RS, Westcot DW. Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome; 1994. Available: http://www.fao.org/3/t0234e/T0234E06.htm#ch5.5

9. US EPA. Aircraft industry waste water recycling (EPA-600/2-78-130). 1978.

10. Zhao X, Wang H, Tang Z, Zhao T, Qin N, Li H, et al. Amendment of water quality standards in China: viewpoint on strategic considerations. Environ Sci Pollut Res. 2018;25: 3078–3092. doi:10.1007/s11356-016-7357-y

11. de Vries W, Schütze G, Lots S, Meili M, Römkens P, de Temmerman L, et al. Critical limits for cadmium, lead and mercury related to ecotoxicological effects on soil organisms, aquatic organisms, plants, animals and humans. In: Schütze G, Lorent U, Spranger T, editors. Proceedings of the expert meeting on critical limits for heavy metals and methods for their application, Berlin, 2–4 December 2002 Held under the UN/ECE Convention on Long- Range Transboundary Air Pollution UBA-Texte 47/03. Berlin: Umweltbundesamt; 2003. pp. 29–78.

12. WHO/FAO. Codex alimentarius commission. Food additives and contaminants. Food Standards Programme, ALINORM 10/12A. Joint FAO/WHO; 2001. Available: 10.13140/RG.2.1.4910.2560

13. Council Directive 86/278/EEC. Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (OJ L181). EU; 1986.

14. Ministry of the Environment, Finland. Government Decree on the assessment of soil contamination and remediation needs (214/2007). 2007.

15. UK Environment Agency. Soil guideline values for arsenic, cadmium, mercury and lead in soil (SC050021). 2009.

16. US EPA. Supplemental guidance for developing soil screening levels for superfund sites. Office of Solid Waste and Emergency Response, Washington, D.C. 2002. Available: http://www.epa.gov/superfund/health/conmedia/soil/index.htm

17. US EPA. Clean Water Act (Sec. 503, vol. 58, no. 32). 1993. Available: https://www.govinfo.gov/content/pkg/CFR-2019-title40-vol32/xml/CFR-2019-title40-vol32-part503.xml

18. Environmental Protection Ministry of China. Standards of soil environmental quality of agricultural land. Huangbanhang 69: Office of Environmental Protection Ministry of China, Beijing, China. 2015.