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Determination of elemental concentration profiles in tender wheatgrass (*Triticum aestivum* L.) using instrumental neutron activation analysis

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Abstract

Samples of shoots and roots of tender wheatgrass/wheat plants collected over a period of 20 days were analysed by instrumental neutron activation analysis. The wheatgrass (wheat: *Triticum aestivum* L.) samples analysed were grown in three different conditions namely (i) tap water, (ii) nutrient compounds with tap water, and (iii) soil and tap water. A total of 15 elements were determined in these samples. In addition, a commercially available wheatgrass tablet was analysed. Accuracy of the method was evaluated by analysing two biological reference materials, SRM 1573a (Tomato leaves) from NIST and ICHTJ-CTA-vtl-2 (Tobacco leaves) from INCT. The paper discusses the elemental concentration levels, their trends and concentration ratios of elements in shoot-to-root grown in these three conditions of growth. It was observed that the elements such as K, Na, Ca and Mg increased linearly in the shoots with the growth period whereas the concentrations of the elements namely Zn, Mn and Fe remained constant in shoots after 8th day of plant growth for all three conditions of growth. However, it was observed that the shoot to root concentration ratio in all the conditions increased linearly for K, Na, Ca, Mg and Cl and decreased for Zn, Fe, Mn, and Al with growth period.

Keywords: Wheatgrass; Growth conditions; Elemental concentration profiles; Neutron activation analysis

1. Introduction

It is known that food and food products provide carbohydrates, proteins and lipids, dietary fibres and essential elements required for the human beings. Many elements, in trace amounts, play an important role in metabolic processes and are essential for general well being of humans. Some of the important essential elements are Ca, K, Na, Mg, Fe, Mn, P, Zn, Cr, Cu, F, Se, Mo, and I (Aidid, 1988; Balaji et al., 2000a; Kanias & Philionos, 1979; Samudralwar & Garg, 1996). Though micronutrients such as Cr, Mn, Fe, Cu and Zn constitute only a small fraction of our diet, they play an important role in metabolic processes. Excess or deficiency of micronutrients might disturb normal biochemical functions of the body. Often it is required to supplement food materials with nutritional rich items e.g. fruits like citrus, banana and grapes, sprouts and herbs like wheatgrass. They contain antioxidants in addition to the compounds of nutrient elements (Niwa, Tominaga, & Yoshida, 1998). Tender wheatgrass and its juice are consumed for healthy growth of human body. It is also taken in the form of tablets available commercially as 'green food'. Although antioxidant activity of wheatgrass is well believed, the exact reasons are not well established. A very few publications are available

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in literature on nutritive and antioxidant properties of wheat sprout extracts where it is reported that these extracts inhibit the DNA oxidative damage and are effective in suppressing the superoxide radical that can further lead to various diseases (Falcioni, Calzuola, Marsili, & Gianfranceschi, 2002). It has also shown antimutagenicity property (Peryt, Szymczy, & Lesca, 1992). Wheatgrass was also reported to be helpful in curing certain diseases such as thalassemia (Marwaha, Bansal, Kaur, & Trehan, 2004) and distal ulcerative colitis (Ben-Arye et al., 2002). In order to understand the beneficiary role of wheatgrass to humans, a systematic study has been undertaken by determining mineral content as well as possible antioxidant activity, the later part is being studied separately. Wheatgrass was grown in three different conditions using (i) tap water, (ii) nutrient compounds with tap water, and (iii) soil and tap water. To study the effect of different growing conditions on elemental profiles and further possible differences in the antioxidant potential of wheatgrass (being studied separately) we used different growing conditions. Although, wheatgrass is consumed after a week's growth, in order to examine the variation of elemental concentrations, sampling was done on 5th, 6th, 7th, 8th, 10th, 15th and 20th days. Elemental concentrations were determined in the shoots and roots of wheat seedlings or wheatgrass as a function of growth period by instrumental neutron activation analysis (INAA).

INAA is one of the most popular and widely used analytical techniques for simultaneous multielement determination of major, minor and trace levels in diverse matrices. The technique involves irradiation of the samples in a neutron flux position of a nuclear reactor, leading to the formation of radionuclide whose radioactivity is measured by high-resolution gamma ray spectrometry. This non-destructive technique has many advantageous characteristics like high analytical sensitivity, good detection limit (ppm to ppb) and negligible matrix effect. In our laboratory the NAA methodologies have been routinely used to analyse various biological samples like medicinal leaves, cereals including wheat, pulses and, foodstuffs, and reference materials for multielement analysis (Acharya, Nair, Reddy, & Manohar, 2002; Balaji et al., 2000a, 2000b; Rajurkar & Pardeshi, 1997).

In the present study, wheatgrass growth was monitored over a period of 20 days. Elemental concentrations determined in wheat seeds, shoots (grass) and roots (root + remains of seeds) collected periodically during 5–20 days of wheatgrass growth are reported in this paper. The elemental concentration levels, their trends and ratios of elements in shoot-to-root are discussed. Additionally, a set of commercially available wheatgrass tablets, used as a dietary supplement, were analysed. Two biological reference materials SRM 1573a from NIST and CRM CTA-vtl-2 from INCT were analysed to evaluate the accuracy of the method.

2. Materials and methods

2.1. Sample collection

The seeds of wheat (*Triticum aestivum* L. C.V. Pbn-51) were procured from Marathwada Agricultural University, Parbhani, Maharashtra State of India. These seeds were washed with tap water followed by distilled water. The wheat plants were grown in (i) tap water, (ii) tap water with nutrients, and (iii) soil (black cotton soil) and tap water. These three conditions were named as conditions 1, 2 and 3, respectively. In conditions 1 and 2, the plants were grown in perforated plastic base open cylinders ($h = 5$ cm and dia = 20 cm) called 'wheat-sprout makers'. A total of 100 seeds were placed in each sprout maker. Two-hundred millilitre of water was added every day. Throughout the studies seeds, nutrient solution and soil were taken from the same stock and water was from the same source. Same quantity of water with nutrients was added to sprout maker for condition 2. Composition of nutrient solution was chosen as per the Hoagland solution (Hoagland & Arnon, 1950) and consists of 2 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 25 μM H_3BO_3, 2 μM $MnCl_2$, 2 μM ZnSO4, 0.5 μM $CuSO_4$ and 0.5 μM Na_2MoO_4. In condition 3, the seeds were transferred to the trays of dimension 15×35 cm^2, containing 7 kg of soil. The trays with soil were provided with sufficient tap water regularly and were placed in a room where normal airflow and sunlight were available. Samples were collected on 5th, 6th, 7th, 8th, 10th, 15th and 20th days and samples are numbered as 1–7. Accordingly seven trays were used for growth of plants under each condition. Fifty numbers of wheatgrass seedlings of similar height were plucked from each tray. These plants were washed thoroughly with tap water followed by distilled water. The shoots were separated, and the shoots and roots with the remains of seeds were dried in hot air oven at 80 °C till a constant weight was reached. These were stored and sub-samples of these were used for irradiation.

The commercially available wheatgrass tablets, used in our studies, were purchased from a local shop at Mumbai. The growth condition of the corresponding wheatgrass is unknown. The average weight of the tablets was 0.5 g and the recommended (by the producer) intake was three tablets per day. These tablets constitute 98% of wheatgrass, 1.5% silica and 0.5% vegetable stearates, as per the specifications.

2.2. Sample irradiation

Accurately weighed samples in the range of 50–250 mg were packed in polyethylene pouches. Reference materials SRM 1573a (Tomato leaves) and CTA-vtl-2 (Virginia tobacco leaves) were also packed in a similar

way to that of the samples. Elemental standards were prepared using stoichiometric compounds/metal foils. Samples, standards and reference materials were neutron irradiated together either at E8 position of APSARA reactor or at Pneumatic Carrier Facility (PCF) of DHRUVA reactor, Bhabha Atomic Research Centre, Trombay, Mumbai, India. Both short (1 min, at PCF) and long (7 h, at APSARA reactor) irradiations were carried out depending on the half-lives of the nuclides of interest. The thermal neutron fluxes at E8, APSARA and PCF, DHRUVA reactors are in the order of 5×10^{11} and 5×10^{13} cm^{-2} s^{-1} respectively.

2.3. Radioactivity measurements and concentration calculation

After appropriate cooling, the samples were counted using a 40% relative efficiency HPGe detector coupled to a PC-based multichannel analyser (8k MCA) in a fixed sample-to-detector geometry. The detector system had a resolution (FWHM) of 1.9 keV at 1332 keV of ^{60}Co. The irradiated samples were counted for suitable periods in order to obtain good counting statistics. The peak areas under the characteristic gamma rays were determined by a peak fit software PHAST developed at BARC (Mukopadhyay, 2001). The peak areas were used for the concentration calculation by the relative method of INAA. Using mass of the element in standard ($m_{x,\mathrm{std}}$) and count rates (counts per second, cps) of standard ($\mathrm{cps}_{x,\mathrm{std}}$) and sample ($\mathrm{cps}_{x,\mathrm{samp}}$), the mass of the element present in the sample ($m_{x,\mathrm{samp}}$) was determined for the same counting time by the following equation:

$$m_{x,\mathrm{samp}} = m_{x,\mathrm{std}} \times \frac{\mathrm{cps}_{x,\mathrm{samp}}}{\mathrm{cps}_{x,\mathrm{std}}} \times \frac{D_{\mathrm{std}}}{D_{\mathrm{samp}}},$$

where D is the decay factor ($\exp(-\lambda t_{\mathrm{d}})$), λ is the decay constant, and t_{d} is the decay time. The $m_{x,\mathrm{samp}}$ (μg) was converted to concentration (μg/g) by dividing with sample mass (g).

3. Results and discussion

Table 1 gives the various conditions used for growing the wheatgrass in the laboratory. During the entire experimental period lengths and weights of shoots and roots of wheat plants were recorded. On the 8th day, the lengths of shoots in conditions 1, 2 and 3 were 8–9, 11–14 and 13–15 cm whereas corresponding length on 20th day were 14–18, 20–23 and 20–25 cm, respectively. For accounting the total mineral content of the seeds, the seed remains are retained with the roots and sampled for the analysis. This becomes important for condition 1 where there is no external nutrient input other than through tap water. The elemental concentrations determined in shoots and roots for the three conditions are given in Tables 2–7. These concentrations were arrived from duplicate experiments. The quoted uncertainties in Tables 2–7 are arrived from propagated counting statistics of duplicate experiments. The concentration values from wheatgrass tablets and whole-wheat seeds are given in Table 8. The elemental concentration values of the CTA-vtl-2 (Tobacco leaves) and NIST SRM 1573a (Tomato leaves) are given in Table 9. The quoted uncertainties in Tables 8 and 9 are the standard deviations (±1 s) arrived from four independent experiments. The comparison of determined and recommended values indicates trueness of the results. In many cases, the % deviations of determined concentrations were found to be within ±10% of the certified values.

Table 1
Various conditions employed for growing wheatgrass in the laboratory

Condition	Treatment
Condition 1	Tap water
Condition 2	Nutrient solution
Condition 3	Soil + tap water

Although 15 elements were determined, the trends and comparisons for elements K, Mn, Zn, Ca, Mg, Na and Fe are discussed below. It was observed that in conditions 1 and 2, the concentration of K, Na, Ca, Mg, Mn and Cl increased linearly with plant growth period whereas Zn and Fe increased up to 8th day and then almost remained constant. The concentration of Al decreased with the plant growth period. In the case of condition 3, concentration of K, Na, Ca, Mg and Mn increased linearly with the plant growth. The concentration of Zn and Fe increased up to 10th day and then remained constant whereas concentration of Co and Cl did not show any trend. Figs. 1–3 show the concentration ratios for elements K, Na, Zn, Fe, Mn, Ca, Mg, Al and Cl in shoot and root as a function of growth period for growth conditions 1–3, respectively. It was observed that the ratio increased linearly for K, Mg, Ca, Na and Cl with growth period indicating that these elements can easily be transported to shoots. Whereas the said ratios decreased for the elements Zn, Fe, Mn and Al.

Potassium is one of the most important macronutrients essential for the plant growth. An examination of Tables 2 and 3 shows that when plants are grown in condition 1, potassium concentration in shoots increased linearly, the highest being 5648 ± 114 mg/kg on 20th day while in roots it was found to decrease proportionally. In condition 2, K concentration remained constant after 8th day of plant growth around 4500 ± 150 mg/kg. In the case of condition 3, potassium concentration in the shoots increased from 4891 ± 91 to 18916 ± 210 mg/kg from 5th to 20th day. The ratios of shoot to root concentration of K in conditions 1–3 are 0.77, 0.91 and 0.37, respectively, and are found to be higher compared to other elements studied except for Ca, Mg

Table 2
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat shoots grown in the tap water during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	1537 ± 42	2198 ± 87	3042 ± 125	3722 ± 119	4257 ± 97	4896 ± 142	5648 ± 114
Mn	0.81 ± 0.04	1.3 ± 0.1	1.9 ± 0.1	2.8 ± 0.1	3.9 ± 0.2	4.5 ± 0.9	4.5 ± 0.4
Zn	0.51 ± 0.03	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.5 ± 0.4	1.6 ± 0.1	1.7 ± 0.4
Fe	2.3 ± 0.5	4.8 ± 0.9	5.9 ± 0.9	7.1 ± 0.6	8.5 ± 0.9	9.2 ± 1.0	9.2 ± 1.1
Na	82.3 ± 5.2	132 ± 8	199 ± 6	265 ± 10	352.2 ± 15.2	392 ± 22	410.5 ± 56.5
Ca	710 ± 38	1006 ± 51	1376 ± 79	1841 ± 102	2560 ± 71	4137 ± 147	5740 ± 192
Mg	541 ± 28	781 ± 46	952 ± 82	1239 ± 72	1867 ± 103	2579 ± 191	3080 ± 282
Al	21.5 ± 1.3	25.8 ± 2.6	11.5 ± 0.9	6.5 ± 0.8	4.3 ± 0.07	3.12 ± 0.05	0.9 ± 0.06
Cl	841 ± 48	1072 ± 68	1261 ± 77	1881 ± 140	2110 ± 98	2977 ± 210	3041 ± 225
La	0.62 ± 0.02	0.64 ± 0.02	0.8 ± 0.1	0.88 ± 0.04	1.0 ± 0.1	3.6 ± 0.6	8.9 ± 0.8
Rb	5.5 ± 0.4	2.5 ± 0.3	3.3 ± 0.4	16.8 ± 1.0	30.8 ± 1.2	79.5 ± 3.6	116 ± 9.5
Br	30.9 ± 2.6	43.4 ± 3.4	57.9 ± 4.1	66.7 ± 5.2	125 ± 7	149 ± 9	175 ± 6

Table 3
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat roots grown in the tap water during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	5149 ± 106	3921 ± 41	3726 ± 52	3413 ± 22	2964 ± 52	2536 ± 65	1921 ± 32
Mn	1.6 ± 0.1	3.9 ± 0.5	7.3 ± 1.1	9.1 ± 0.9	12.6 ± 0.2	21.7 ± 1.2	28.3 ± 1.8
Zn	1.23 ± 0.05	1.9 ± 0.1	2.5 ± 0.1	3.7 ± 0.4	4.7 ± 0.3	8.9 ± 0.9	15.2 ± 1.8
Fe	88.6 ± 3.8	85.8 ± 5.6	109.4 ± 4.8	140.7 ± 9.6	241.2 ± 12.4	292.1 ± 25.4	299.9 ± 10.7
Na	107.4 ± 5.1	144.6 ± 7.2	203.5 ± 15.2	239.8 ± 10.8	247 ± 11.6	263.5 ± 23.1	302.1 ± 18.8
Co[a]	3.5 ± 0.6	2.3 ± 0.4	4.6 ± 0.8	5.4 ± 0.1	4.3 ± 0.6	5.4 ± 0.9	4.3 ± 0.9
Ca	3852 ± 160	3281 ± 192	3032 ± 87	2597 ± 171	2465 ± 91	2398 ± 137	2271 ± 88
Mg	1049 ± 99	1256 ± 101	1489 ± 87	1720 ± 57	2132 ± 141	2078 ± 130	1856 ± 104
Al	60.5 ± 2.3	51.7 ± 3.7	77.1 ± 4.5	71.9 ± 8.5	59.9 ± 4.8	79.6 ± 7.9	81.5 ± 8.8
Cl	1891 ± 131	1587 ± 148	1027 ± 87	1001 ± 77	840.7 ± 51.8	570 ± 42.7	590 ± 69.8
La	6.3 ± 0.6	8.2 ± 0.4	9.8 ± 0.8	12.6 ± 1.2	15.3 ± 0.7	30.3 ± 1.4	42.2 ± 2.1
Rb	5.8 ± 0.4	6.1 ± 0.8	8.5 ± 0.6	5.7 ± 0.9	4.6 ± 0.4	3.4 ± 0.6	3.7 ± 0.1
Br	66.2 ± 2.1	98.2 ± 6.3	105.1 ± 8.1	113.9 ± 9.7	134 ± 10	66.2 ± 5.1	69.9 ± 6.3

[a] μg/kg.

Table 4
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat shoots grown in the nutrient solution during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	2876 ± 21	3126 ± 37	3742 ± 25	4325 ± 49	4577 ± 96	4506 ± 102	4648 ± 114
Mn	1.83 ± 0.04	3.49 ± 0.07	3.91 ± 0.08	4.86 ± 0.15	5.72 ± 0.22	6.53 ± 0.37	7.49 ± 0.48
Zn	0.63 ± 0.03	0.89 ± 0.06	0.82 ± 0.09	1.36 ± 0.06	1.94 ± 0.19	1.57 ± 0.08	1.92 ± 0.13
Fe	4.91 ± 0.53	6.52 ± 0.94	9.57 ± 0.87	10.12 ± 0.65	10.52 ± 0.94	10.69 ± 1.03	10.72 ± 1.13
Na	124 ± 5	212 ± 8	289 ± 6	395 ± 11	422 ± 15	562 ± 22	620 ± 56
Co[a]	3.92 ± 0.89	4.25 ± 0.56	4.76 ± 0.44	4.98 ± 0.23	4.88 ± 0.34	5.03 ± 0.89	4.93 ± 0.96
Ca	1571 ± 125	2157 ± 97	2228 ± 110	2561 ± 147	3148 ± 205	4152 ± 288	5183 ± 298
Mg	982 ± 71	1260 ± 125	1920 ± 109	2547 ± 210	2967 ± 174	3548 ± 259	4251 ± 288
Al	18.4 ± 1.9	27.1 ± 1.4	21.7 ± 2.1	15.7 ± 0.9	14.7 ± 0.5	11.5 ± 0.7	12.6 ± 1.1
Cl	1121 ± 158	1479 ± 124	1738 ± 163	2268 ± 141	2570 ± 201	2608 ± 288	2672 ± 185
La	0.63 ± 0.01	0.81 ± 0.03	0.93 ± 0.06	1.11 ± 0.05	1.3 ± 0.1	4.5 ± 0.1	11.8 ± 0.1
Rb	10.9 ± 1.2	18.3 ± 1.9	25.1 ± 2.0	41.8 ± 5.1	81.7 ± 4.1	135 ± 5	168 ± 7
Br	29.7 ± 1.5	44.6 ± 2.9	61.5 ± 5.4	106.8 ± 9.4	145 ± 11	187 ± 15	215 ± 16

[a] μg/kg.

and Cl, which indicates that K can easily be transported to the shoot irrespective of the growth conditions. In all the three conditions, potassium in the wheatgrass after 8th day onwards was enriched compared to that in the seed. It is possible that K^+ supply could be from nutrients and soil in conditions 2 and 3, respectively. In the case of condition 1, the source of potassium is seed and the growing medium (water).

Manganese is an essential element for plant growth, though it is required in small quantity. It is also an

Table 5
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat roots grown in the nutrient solution during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	9853 ± 128	9112 ± 246	8327 ± 339	7789 ± 189	8026 ± 486	8157 ± 695	8556 ± 244
Mn	12.4 ± 0.9	18.6 ± 0.9	25.8 ± 1.4	32.9 ± 2.8	48.4 ± 3.2	89.5 ± 6.0	109.6 ± 9.7
Zn	3.2 ± 0.05	10.4 ± 0.8	15.3 ± 1.3	22.5 ± 1.1	40.3 ± 3.0	81.8 ± 5.8	94.3 ± 7.1
Fe	61.9 ± 3.2	118.8 ± 9.1	248.7 ± 11.2	321.4 ± 20.6	491.3 ± 33.6	720.2 ± 41.3	791.1 ± 20.6
Na	1188 ± 26	1925 ± 55	2749 ± 189	3591 ± 320	4461 ± 99	5886 ± 156	7008 ± 285
Co[a]	37.5 ± 3.6	31.3 ± 1.4	44.6 ± 4.9	55.4 ± 2.1	42.3 ± 3.6	57.4 ± 4.9	47.3 ± 1.0
Ca	3520 ± 251	3789 ± 189	4052 ± 298	4635 ± 356	4711 ± 312	4963 ± 396	4848 ± 291
Mg	1328 ± 56	1950 ± 107	2689 ± 241	2841 ± 127	3256 ± 235	3496 ± 286	3783 ± 261
Al	65.3 ± 1.2	112.8 ± 5.9	126.5 ± 7.5	156.9 ± 10.3	189.3 ± 11.8	152.3 ± 8.9	178.9 ± 10.4
Cl	1568 ± 89	1782 ± 104	1956 ± 141	1894 ± 111	1788 ± 109	1811 ± 51	2009 ± 105
La	7.5 ± 0.5	11.9 ± 1.2	25.6 ± 2.1	37.9 ± 2.9	58.6 ± 3.1	89.4 ± 4.1	99.6 ± 5.2
Rb	6.4 ± 0.4	11.5 ± 1.0	18.9 ± 0.8	44.6 ± 2.5	74.8 ± 5.4	101 ± 8	95.4 ± 4.1
Br	41.3 ± 3.1	64.8 ± 5.4	77.5 ± 6.1	91.4 ± 7.1	104.3 ± 5.2	115 ± 11	104.6 ± 9.8

[a] μg/kg.

Table 6
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat shoots grown in the soil during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	4891 ± 91	5331 ± 85	6148 ± 112	7520 ± 138	9127 ± 107	13,821 ± 164	18,916 ± 210
Mn	3.6 ± 0.2	4.1 ± 0.6	5.6 ± 0.5	7.8 ± 0.7	10.9 ± 0.9	18.7 ± 1.0	27.6 ± 1.3
Zn	3.9 ± 0.1	5.6 ± 0.6	5.9 ± 0.9	6.2 ± 1.0	6.8 ± 0.2	6.7 ± 0.8	7.1 ± 0.4
Fe	12.3 ± 0.4	29.8 ± 2.6	46.3 ± 1.9	51.3 ± 5.9	68.5 ± 3.1	71.6 ± 4.4	75.8 ± 6.3
Na	120 ± 9	251 ± 13	312 ± 17	377 ± 22	571 ± 15	709 ± 38	957 ± 21
Co[a]	10.8 ± 1.4	13.4 ± 0.9	7.9 ± 0.6	18.7 ± 1.8	15.6 ± 1.6	35.1 ± 2.1	25.1 ± 2.0
Ca	1092 ± 18	1130 ± 13	1242 ± 56	1421 ± 94	1912 ± 126	2257 ± 178	2735 ± 54
Al	14.6 ± 0.9	13.2 ± 0.7	10.8 ± 0.1	10.6 ± 0.8	8.3 ± 0.5	5.4 ± 0.6	4.9 ± 0.1
Mg	3885 ± 59	3342 ± 128	2838 ± 97	1665 ± 102	1421 ± 104	1520 ± 119	1776 ± 129
Cl	5654 ± 176	3878 ± 190	2856 ± 168	2541 ± 198	4211 ± 356	5681 ± 193	3851 ± 255
La	1.72 ± 0.11	1.83 ± 0.05	2.23 ± 0.07	3.24 ± 0.08	4.27 ± 0.23	10.1 ± 6.2	38.9 ± 3.1
Rb	7.1 ± 0.3	11.8 ± 0.8	14.1 ± 1.5	60.3 ± 2.7	95.2 ± 4.3	101 ± 6.2	104 ± 4
Br	19.6 ± 0.7	20.5 ± 0.5	25.9 ± 0.6	24 ± 0.7	37.4 ± 0.6	41.6 ± 0.7	78.2 ± 1.7
Cr	0.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	2.6 ± 0.2	3.1 ± 0.1	7.1 ± 0.4	10.5 ± 0.6

[a] μg/kg.

Table 7
Elemental concentration (mg/kg unless μg/kg is specified) of the wheat roots in the soil during the seedling stage

Element	Growth period (d)						
	5	6	7	8	10	15	20
K	8547 ± 29	9352 ± 104	11,557 ± 225	11,948 ± 349	11,042 ± 296	10,982 ± 102	10,842 ± 514
Mn	14.2 ± 0.4	15.5 ± 0.7	21.6 ± 0.8	25.9 ± 1.1	28.7 ± 2.2	33.1 ± 3.4	40.1 ± 3.5
Zn	1.96 ± 0.08	2.31 ± 0.05	2.98 ± 0.07	4.11 ± 0.04	8.90 ± 0.08	17.12 ± 0.57	21.7 ± 1.3
Fe	49.2 ± 1.5	67.6 ± 3.5	74.3 ± 7.3	96.2 ± 4.4	95.8 ± 6.9	98.6 ± 7.0	99.9 ± 10.7
Na	1259 ± 98	1835 ± 57	2438 ± 86	3546 ± 105	3841 ± 108	3935 ± 116	4012 ± 245
Co[a]	35.5 ± 3.6	27.3 ± 1.4	48.6 ± 4.9	52.4 ± 2.1	40.3 ± 3.6	55.4 ± 4.9	42.3 ± 0.9
Ca	1665 ± 154	3885 ± 320	3529 ± 312	3290 ± 254	3429 ± 289	3342 ± 99	3864 ± 256
Mg	1120 ± 103	1728 ± 46	2509 ± 67	3878 ± 56	4162 ± 123	4219 ± 211	4278 ± 256
Al	41.8 ± 1.5	51.4 ± 1.2	55.2 ± 2.9	66.5 ± 3.1	69.6 ± 3.2	78.9 ± 4.5	91.6 ± 3.2
Cl	714 ± 53	1282 ± 107	1179 ± 52	1387 ± 77	1526 ± 112	1840 ± 70	2003 ± 109
La	51.2 ± 0.7	23.5 ± 0.2	7.7 ± 0.7	14.1 ± 0.8	20.3 ± 1.2	27.3 ± 0.2	31.3 ± 1.4
Rb	10.3 ± 0.3	10.7 ± 0.3	18.1 ± 0.6	14.4 ± 1.0	14.3 ± 0.6	12.3 ± 0.6	14.4 ± 0.8
Br	10.3 ± 0.9	17.4 ± 1.3	22.3 ± 1.5	29.0 ± 1.9	36.9 ± 2.0	27.0 ± 2.4	50.2 ± 4.1
Sm	0.08 ± 0.01	0.05 ± 0.01	1.04 ± 0.02	1.34 ± 0.04	2.12 ± 0.13	4.85 ± 0.31	4.7 ± 0.2
Cr	0.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	2.6 ± 0.2	3.1 ± 0.1	7.1 ± 0.4	10.5 ± 0.6

[a] μg/kg.

Table 8
Elemental concentrations (mg/kg) in commercially available wheatgrass tablet and whole-wheat seed (used for growing wheatgrass)

Element	Wheatgrass tablet	Quoted values	Whole-wheat seed
K	50,638 ± 511	48,400	3017 ± 30
Mn	85.0 ± 6.3	NA	37.1 ± 0.4
Br	26.6 ± 2.0	NA	88.3 ± 9.6
Fe	1895 ± 77	1744	143.4 ± 1.2
Zn	80.1 ± 3.0	NA	9.18 ± 0.18
Co	0.25 ± 0.05	NA	0.02 ± 0.0005
Na	771 ± 10	NA	14.7 ± 0.4
Cr	8.9 ± 1.2	NA	ND
Mg	5108 ± 212	4252	6019 ± 572
Ca	3911 ± 51	3299	2185 ± 406
Al	376.7 ± 9.3	NA	50.8 ± 4.6
Cl	4225 ± 177	NA	1107 ± 40

ND, not detected.
NA, not available.

important element from biochemical activity point of view, since it is associated with an antioxidant enzyme superoxide dismutase (Mn-SOD) (Halliwell & Gutteridge, 1999). It can be seen that Mn concentration increased with plant growth period in both shoots and roots (Tables 2–7). In all the cases, Mn concentration in roots was found to be higher than that in the shoots. Manganese concentrations in shoots of the plants grown in conditions 1 and 2, are less than that in the wheat seeds indicating that manganese present in the seed is the source for shoots during the early period of growth. On the other hand, in the case of plants grown in soil culture, the Mn concentrations are higher than the corresponding values in seeds. The analysis of soil showed that the concentration of Mn in the soil was 219.9 ± 8.6 mg/kg. It is well known that oxygen atom in water co-ordinates with Mn(II). These aquo complexes are mobile in the soil solution facilitating the uptake by plant roots (Marschner, 1995). Thus the enhanced value of Mn concentration in condition 3 is due to the contribution from the seed as well as soil.

Zinc is directly required in the production of plant hormones and is also an activator of many plant enzyme (Cu–Zn SOD) systems. Antioxidant properties of Zn (Powell, 2000) and the physiological role of Zn (Bray & Bettger, 1990) are well known. It also prevents oxidation of lipids and proteins initiated by heavy metals particularly by Fe^{2+} (Zago, Verstraetan, & Oteiza, 2000). Zinc concentration in wheat shoots was found to be constant after 8th day of plant growth in experimental conditions 1 and 2 (Tables 2 and 4), whereas in condition 3, the highest concentration was observed on 20th day, about 21.7 ± 1.3 mg/kg (Table 6). The Zn concentration in root was found to be higher than in shoot under all growing conditions.

Iron is one of the most essential elements needed by plants as well as human beings. Though it is an essential element, excess intake can lead to iron toxicity and can damage lipids and proteins (Bothwell, Charlton, Cook, & Finch, 1979; Fraga & Oteiza, 2002). Iron concentration in the shoots was found to be almost constant, around 10 ± 2 mg/kg after 8th day of growth in experimental conditions of 1 and 2. In the case of condition 3, iron concentration in shoots was found to be much higher than that of previous conditions. As discussed above, the concentrations of Mn, Zn and Fe in the shoots are lower than the same in the seeds. It is possible that the plants could get enough quantities of these elements during their growth from the seeds. However, when the plants are grown in soil culture, it is clear that the corresponding value could be more, depending on their bioavailability in the soil/soil and water.

Table 9
Comparison of measured and literature values (mg/kg) of biological reference materials CTA-vtl-2 and NIST SRM 1573a

Element	CTA-vtl-2 (Tobacco leaves)		SRM 1573a (Tomato leaves)	
	Present work	Literature value (info)	Present work	Literature value (info)
Ca	35,892 ± 196	36,000 ± 1440	48,938 ± 487	50,500 ± 1010
Br	14.8 ± 0.15	14.3 ± 1.43	1123.8 ± 21	(1300)
Cl	8005 ± 96	7430 ± 297	5885 ± 118	(6600)
Co	0.315 ± 0.011	0.429 ± 0.026	0.47 ± 0.08	0.57 ± 0.023
Cr	1.65 ± 0.08	1.87 ± 0.15	1.89 ± 0.02	1.99 ± 0.06
Fe	1106 ± 48	1083 ± 32.5	330 ± 8.5	368 ± 7.36
K	9882 ± 112	10,300 ± 412	31,378 ± 891	27,000 ± 540
La	0.98 ± 0.06	1.01 ± 0.10	3.71 ± 0.06	(2.3)
Mg	5548 ± 116	5100 ± 255	13,520 ± 126	(12,000)
Mn	78.1 ± 2.3	79.7 ± 2.4	232 ± 6	246 ± 7.38
Rb	56.8 ± 6.5	48.6 ± 2.43	15.2 ± 0.9	14.89 ± 0.30
Sm	ND	0.157 ± 0.024	0.078 ± 0.005	(0.19)
V	6.8 ± 1.5	4 ± 0.44	0.69 ± 0.038	0.835 ± 0.008
Zn	40.6 ± 2.8	43.3 ± 2.16	32.1 ± 1.8	30.9 ± 0.62
Al	1211 ± 86	(1682)	611 ± 13	598 ± 12
Na	380 ± 18	(312)	163 ± 29	136 ± 4.1
Sc	0.291 ± 0.005	(0.268)	0.15 ± 0.05	(0.1)

info, information value; ND, not detected.

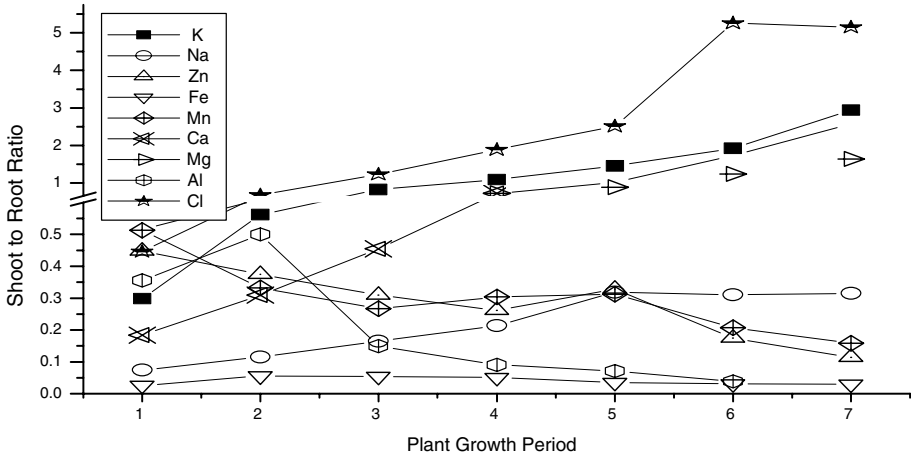

Fig. 1. Shoot to root concentration ratios of elements of wheatgrass grown in tap water. Plant growth stage 1, 2, 3, 4, 5, 6 and 7 corresponds to growing period of 5, 6, 7, 8, 10, 15 and 20 days.

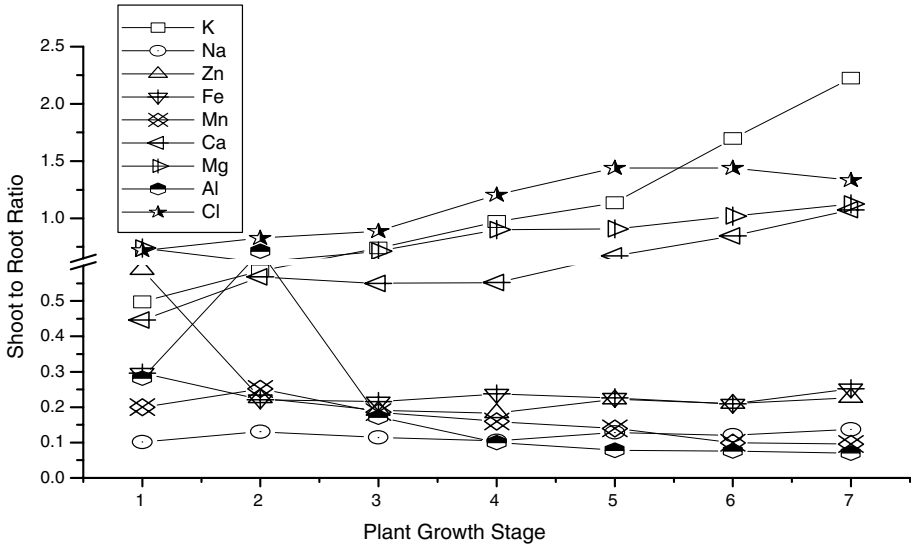

Fig. 2. Shoot to root concentration ratios of elements of wheatgrass grown in nutrient solution. Plant growth stage 1, 2, 3, 4, 5, 6 and 7 corresponds to growing period of 5, 6, 7, 8, 10, 15 and 20 days.

Role of sodium is still not clearly understood in plant physiology, but there are a few evidences suggesting that Na as a beneficial micronutrient. Though its concentration is only 14.7 ± 0.4 mg/kg in the seed, higher quantities are present in shoots and further higher quantities in roots. This may be due to the uptake from water, water and nutrients and soil during the plant growth. The other elements like Ca, Mg, Al, and Cl are essential minerals for the plant. Calcium concentration was found to increase almost linearly with the plant growth period (Tables 2–7). The roles of these elements are also well known in plant physiology (Marschner, 1995).

A comparison of recommended dietary allowance (RDA) by Indian Council of Medical Research (ICMR, 1985) of various essential elements was carried out with that present in the wheatgrass grown in our laboratory and commercially available wheatgrass tablet. On an average daily intake of fresh wheatgrass is 100 g and it is consumed either directly or in the form of its juice. The concentration values for each element are converted to the corresponding amount in 100 g of fresh wheatgrass (dry weight basis) for the three growth conditions and also in wheatgrass tablet (dosage = 1.5 g) and these results are given in Table 10 along with RDA values (ICMR, 1985). A comparison for each element suggests that the amounts are much less than the RDA values for all the cases. It indicates that wheatgrass is taken by humans for good health rather than food supplement. It is observed that the amounts of elements Ca, Mn and Mg in wheatgrass in all conditions are more than corresponding values in commercial tablet whereas the values of Fe and Zn are less than tablet except for Zn in condition 3. Preliminary antioxidant study of wheatgrass extracts showed similar trends indicating that fresh grass

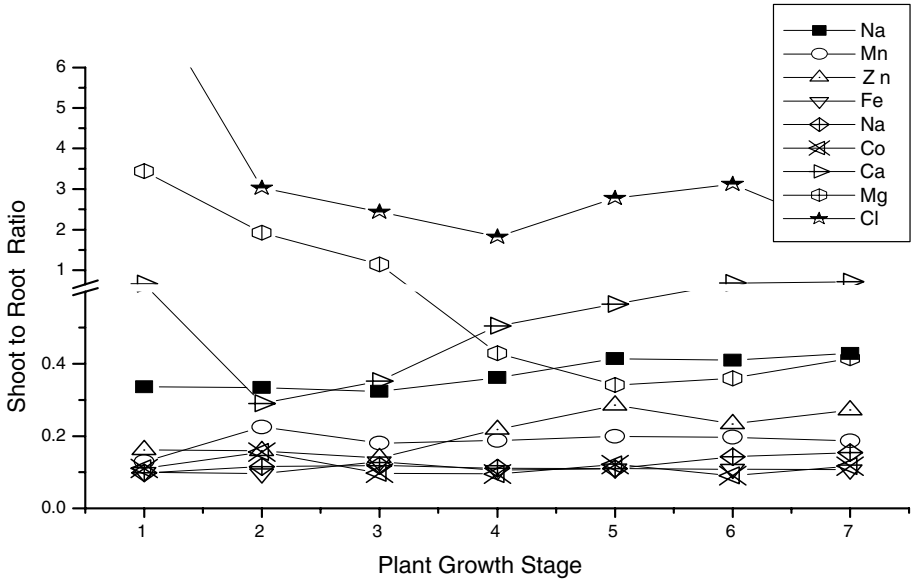

Fig. 3. Shoot to root concentration ratios of elements of wheatgrass grown in soil and tap water. Plant growth stage 1, 2, 3, 4, 5, 6 and 7 corresponds to growing period of 5, 6, 7, 8, 10, 15 and 20 days.

Table 10
A comparison of recommended dietary allowance (RDA) of various essential elements with that present in the wheatgrass (10th day sample) grown in our laboratory and commercially available wheatgrass tablet (dry weight basis)

S. No.	Element	Condition 1 (mg)	Condition 2 (mg)	Condition 3 (mg)	Commercial tablet (mg)[a]	RDA (mg/d)
1	Zn	0.054	0.061	0.211	0.12	40
2	Fe	0.311	0.333	2.13	2.84	40
3	Mn	0.143	0.181	0.34	0.13	11
4	Ca	93.7	99.8	59.5	5.87	1300
5	Mg	68.6	94.1	44.2	7.66	300

The values of the wheatgrass are calculated for 100 g fresh weight (dosage for fresh wheatgrass per day) of wheatgrass. For calculation of absolute amount of each element 100 g fresh weight has been converted to dry weight for all conditions.
[a] Indicated the total amount present in the prescribed dosage of 1.5 g per day.

is preferred over wheatgrass tablets. It also indicates that wheatgrass grown indoors using only tap water could be adequate to provide bare minimum health support. It is also advantageous to consume wheatgrass grown using only tap water since there is no extraneous addition of elements/compounds either during growth period or processing.

4. Conclusion

The INAA method has effectively used to arrive at the elemental concentration values in the tender wheatgrass (shoots and roots) during the growth in three different conditions. It was observed in shoots that in all conditions of growth the elemental concentrations of K, Ca, Mg, Mn and Na increased linearly. It was also observed, in general, that shoot to root concentration ratios increased linearly for K, Ca, Mg, Na and Cl with the growth period and the said ratio decreased for Zn, Fe, Mn and Al. The wheatgrass tablet, obtained from the market, was found to contain many elements in higher concentrations compared to cultivated wheatgrass.

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References

Acharya, R. N., Nair, A. G. C., Reddy, A. V. R., & Manohar, S. B. (2002). Validation of neutron activation analysis method using k_0-standardisation. *Applied Radiation and Isotope, 57*, 391–398.

Aidid, S. B. (1988). Determination of trace elements in leaves of tropical trees in Malaysia by neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry, 120*, 335–344.

Balaji, T., Acharya, R. N., Nair, A. G. C., Reddy, A. V. R., Rao, K. S., Naidu, G. R. K., et al. (2000a). Multielement analysis in cereals and pulses by k_0 instrumental neutron activation analysis. *Science of the Total Environmental, 253*, 75–79.

Balaji, T., Acharya, R. N., Nair, A. G. C., Reddy, A. V. R., Rao, K. S., Naidu, G. R. K., et al. (2000b). Simultaneous determination of essential elements in ayurvedic medicinal leaves by k_0 instrumental neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry, 243*, 783–788.

Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R., & Berry, E. (2002). Wheatgrass juice in the treatment of active distal ulcerative colities: a random double-blind placebo-controlled trial. *Scandinevian Journal of Gastroenterology, 37*, 444–449.

Bothwell, T. H., Charlton, R. W., Cook, J. D., & Finch, C. A. (1979). *Iron metabolism in man*. Oxford: Blackwell Scientific Publications.

Bray, T. M., & Bettger, W. J. (1990). The physiological role of zinc as an antioxidant. *Free Radicais in Biology and Medicine, 8*, 281–291.

Falcioni, G., Calzuola, L. M., Marsili, V., & Gianfranceschi, G. (2002). Antioxidant activity of wheat sprout extracts in vitro: inhibition of DNA oxidative damage. *Journal of Food Science, 67*, 2918–2922.

Fraga, C. G., & Oteiza, P. I. (2002). Iron toxicity and antioxidant nutrients. *Toxicology, 180*, 23–32.

Halliwell, B., & Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine* (3rd ed.). Oxford: Oxford University Press.

Hoagland, D. R., & Arnon, D. I. (1950). The water culture method for growing plants without soil. *California Agricultural Experimental Research Station Circular, 347*, 1–39.

ICMR. (1985). *Recommended dietary intakes for Indians* (p. 79). New Delhi: Indian Council of Medical Research

Kanias, G. D., & Philionos, S. M. (1979). Neutron activation analysis study of distribution of certain elements between plants and soil. *Journal of Radioanalytical and Nuclear Chemistry, 120*, 335–344.

Marwaha, R. K., Bansal, Deepak, Kaur, Siftinder, & Trehan, Amita (2004). Wheatgrass juice reduces the transfusion requirement in patients with Thalessemia major: a pilot study. *Indian Pediatrics, 41*, 716–720.

Marschner, H. (1995). *Mineral nutrition in higher plants* (5th ed.). London: Academic Press, p. 561.

Mukopadhyay, P. K. (2001). In *Proceedings of the symposium on intelligent nuclear instrumentation* (p. 307), Mumbai, India

Niwa, Y., Tominaga, K., & Yoshida, K. (1998). Successful treatment of several atopic dermatitis, complicated cataract and male infertility with natural antioxidants. *International Journal of Tissue Reaction, 20*, 63–69.

Peryt, B., Szymczy, K. T., & Lesca, P. (1992). Mechanism of antimutagenicity of wheat sprout extracts. *Mutation Research, 269*, 201–205.

Powell, S. R. (2000). Antioxidant properties of zinc. *Journal of Nutrition, 130*, 1447S–1454S.

Rajurkar, N. S., & Pardeshi, B. M. (1997). Analysis of some herbal plants from India used in control of diabetes Mellitus by NAA and AAS techniques. *Applied Radiation and Isotope, 48*, 1059–1062.

Samudralwar, D. L., & Garg, A. N. (1996). Minor and trace elemental determination in the Indian herbs and medicinal preparations. *Biological Trace Elemental Research, 54*, 113–121.

Zago, M. P., Verstraetan, S. V., & Oteiza, P. I. (2000). Zinc in the prevention of Fe^{2+} initiated lipid and protein oxidation. *Biological Research, 33*, 143–150.