Volume: 1, Issue: 2, (December, 2019), pp 26-50
www.rjt.ac.lk/agri/sljae

SRI LANKAN JOURNAL OF AGRICULTURE AND ECOSYSTEMS ISSN: 2673-1401

SLJAE

ORIGINAL ARTICLE

Sweet Potato Cultivar with Characteristic Acid-Green Foliage as an Alternative Leafy Vegetable to Water Spinach Grown in Polluted Habitats

R. D. G. A. Ranasinghe[1], A. P. D. T. Ranathunga[1], L. T. Ranaweera[1], S. W. Meepegamage[1], W. W. M. U. K. Wijesundara[1], S. M. N. K. Thilakarathne[1], C. K. Weebadde[2], and S. D. S. S. Sooriyapathirana[1,3]

[1]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya (20400), Sri Lanka.

[2]Department of Plant, Soil and Microbial Sciences, College of Agriculture and Natural Resources, Michigan State University, East Lansing, MI 48824, USA.

[3]Postgraduate Institute of Science, University of Peradeniya, Peradeniya (20400), Sri Lanka.

Correspondence:
[1,3]sunethuop@gmail.com,
ORCID: 0000-0002-5592-1742

Abstract

Water spinach (WS) is a leafy vegetable that accumulates toxic elements when grown in polluted habitats. Unfortunately, WS sold in the Sri Lankan local market, mainly comes from polluted sites. The fast-growing cultivar, acid-green sweet potato (AGSP), is an underutilized leafy vegetable in Sri Lanka. In this study, the applicability of AGSP shoot-tops to replace WS was assessed. WS samples were collected from an urban wastewater channel (WS-UWC), open market (WS-C), and AGSP leaves from a farming site to conduct elemental analysis using Inductive Coupled Plasma Mass Spectrometric (ICP-MS) method. The growth of WS and AGSP under standard growing conditions on soil beds were also compared. Greenhouse-grown WS and AGSP shoot-tops were prepared as dishes under three recipes and subjected to sensory evaluation. The phytochemical contents of WS and AGSP were also compared. Finally, DNA fingerprinting assay with *trnH-psbA* locus was performed to check the presence of WS in culinary preparations made with AGSP. The WS-C and WS-UWC contained significantly higher amounts of toxic heavy metals compared to AGSP. The toxic element Pb was detected only in WS-UWC. When considering growth, AGSP shoot-tops were grown faster and higher yield was given than WS. Consumers prefer WS over basic stir-fried AGSP with minimum flavours. However, consumers accepted AGSP when flavors and chicken cubes were added to the recipe, as if it was WS. The nutritional value of AGSP was higher than WS. The *trnH-psbA* could be used to detect the presence of WS/AGSP as an adulterant/ingredient in culinary dishes. Thus, AGSP can be proposed as a safer and healthier alternative to WS that is prone to bioaccumulation.

Keywords: *Bioaccumulation, heavy metals, sweet potato, Kankong, leafy vegetables.*

Date of Submission: 13-08-2019 Date of Acceptance: 24-11-2019

1. Introduction

Leafy vegetables are a significant component in culinary preparations worldwide. Water spinach (WS) (*Ipomoea aquatica* Forssk.) is a favourite leafy vegetable routinely used as an herb in various cuisines prepared in high profile hotels, as well as in daily meals of all social classes in many countries around the world (Marcussen et al. 2008). WS is very popular in traditional and hotel-based cuisines in Sri Lanka. Also, a high demand exists for fresh and attractive immature stems and leaves (i.e., shoot-tops) of WS. This species generally requires semi-aquatic/aquatic habitats for successful growth and often grows exceptionally well in water-logged areas, channels of urban drainage systems and polluted lakes which continuously contaminate with toxic elements and many other pollutants. This contamination scenario makes the consumption of the WS growing in the polluted habitats unsafe for humans. The WS sold in the Sri Lankan vegetable markets mainly originates from unhygienic habitats than from consumer safe commercial WS fields (Grubben, 2004; Kananke et al. 2014). Moreover, WS has a remarkable ability to accumulate toxic metals such as As, Cd, Pb and Hg in cells (Göthberg et al. 2002). The toxic elements can give rise to a wide range of chronic and acute diseases including cancers, neurological dysfunctions, organ malfunctions and skeletal fragilities (Sears and Genuis 2012). Furthermore, WS frequently exposes to thermo tolerant coliforms, intestinal helminth eggs and protozoan parasites making it further unsafe for human and livestock consumption (Anh et al. 2007). However, many people routinely consume WS samples from open market (WS-C), despite the potentially hazardous effects associated. Therefore, the exploration and recommendation of a suitable and safe alternative leafy vegetable to replace commercially available WS is a timely requirement.

Sweet potato (*Ipomoea batatas* L. Lam.), the sixth largest crop grown in the world, shows promising signs to be an economically beneficial and a healthy leafy vegetable (Islam 2014; Johnson and Pace 2010). The root tubers are the economically important part of sweet potato (Dhaliwal 2017) whereas the shoots are an underexploited leafy vegetable with insignificant economic importance in the current vegetable market (Woolfe 1992). Generally, sweet potato shoots are agricultural wastes used as animal feed and mulching material for crop fields (Li et al. 2017). People in the rural areas of Sri Lanka consume sweet potato shoot-tops as a leafy vegetable, and it is also popular in Africa, Taiwan, Japan and China (Li et al. 2017). If compared with many other leafy vegetables, the shoot-tops of sweet potato have a high potential for rapid growth enabling farmers to harvest them many times per year (Li et al. 2017). Moreover, many studies have reported that the nutritional properties of sweet potato shoot-tops are notably higher than most of the other major commercially available leafy vegetables such as broccoli, lettuce, spinach and cabbage (Sun et al. 2014; Mulokozi et al. 2007). Compared to other leafy vegetables, the sweet potato contains higher amounts of carbohydrates, vitamins, iron,

polyphenols, dietary fibres and many other nutritionally rich bio-active compounds, which promote health and confer protection from many diseases (Islam 2014; Johnson and Pace 2010). Among the recently identified medicinal benefits of sweet potato; its leaves contain the ability to inhibit mutagenicity, diabetes, and incidence of cancer (Nagai et al. 2011; Chary et al. 2008). Also, unlike WS which grows well in aquatic/semi-aquatic environments in urban areas of Sri Lanka, sweet potato thrives well only in upland, well-drained, sandy and loamy soils, irrespective of the climatic and seasonal changes (Islam 2014). Therefore, consuming sweet potato shoot-tops over WS would be a healthy alternative because sweet potato shoot-tops are not originating from polluted aquatic/semi-aquatic sites.

The Department of Agriculture recommends nine sweet potato cultivars for growers in Sri Lanka (DOA 2017). The cultivar composed of attractive acid-green coloured shoots and leaves [because of the leaf colour and no proper vernacular name is available this variety has been named as acid-green sweet potato (AGSP)]. This is the fastest growing cultivar out of the recommended varieties and all the other sweet potato landraces / selections available within the country. The cultivar AGSP produces harvestable shoots within five weeks of planting and also possesses a significant value as an ornamental plant. AGSP is a well-known sweet potato cultivar for its attractive foliage and frequently grown as an ornamental plant in the premises of government and private sector buildings in Sri Lanka. Even though, people from rural communities consume shoot-tops and leaves of sweet potato; no previous studies assessed the feasibility of using sweet potato in place of WS which is highly prone to bioaccumulation of toxic heavy metals. Moreover, data are not available on the consumer acceptance of sweet potato shoot-tops in comparison to WS. Therefore, the objectives of the present study were to compare the differences in growth, morphological features, elemental contents, organoleptic preferences, and phytochemical values of WS and AGSP. The study also aimed at providing a DNA based test to discriminate WS from AGSP when prepared as dishes.

2. Materials and Methods

Water spinach growth in a polluted habitat in comparison to cultivated samples

A total of 5 kg each from WS shoot-top samples were collected from a wastewater channel in Municipal Council area of Kandy, Sri Lanka harbouring a lush growth of WS (7.274344 °N, 80.612304 °E), an Experimental WS plot (7.272003 °N, 80.601231 °E), and a greenhouse in Kandy (7.258949 °N, 80.598030 °E) (Plate 1). The stem diameters and internodal lengths of WS samples collected were measured (30 shoot-top samples from each source).

Assessment of the growth rates

AGSP and WS cuttings were grown in soil beds (1 m × 1 m) to compare their growth rates based on time taken to fill the bed from foliage

Plate 1: The diverse habitats of water spinach in Sri Lanka. (A) - (D) Polluted habitats photographed from an urban wastewater channel in Kandy, Sri Lanka. A: Road-side waste water channel; B: A close-up photo showing lush growth of WS growing on passed-oil effluent; C: A close-up photo showing WS growing under a culvert containing waste water; D: WS growing in the proximity of train-tracks; E: Commercial WS plot in Sri Lanka; F: Experimental WS plot in Kandy.

cover under standard crop recommendations (DOA 2017) [14]. Twenty-five stem cuttings were planted per bed according to Completely Randomized Design with three replicates.

Detection of standard colourimetric parameters of the foliage

Red, Green, and Blue (RGB) values of WS and AGSP foliage colours were detected using an online RGB calculator (Art Paints.Com 2017). Then the hex codes for the colours were determined by feeding their RGB values to the online tool available at Rapid Tables (2017). A total of 25 random leaves were separately collected from each replicated bed.

Elemental analysis using ICP-MS method

Samples for elemental analysis: An urban wastewater channel in Municipal Council area of Kandy where observed with lush growth of WS was selected for the present study. Immature shoot-top samples collected from this place (abbreviated WS-UWC hereafter) and the commercially available WS (WS-C) purchased from roadside vegetable stores were used for elemental analysis. Simultaneously, AGSP shoots were also collected from a farming site (AGSP-FS) in Kandy District (7.259984 °N, 80.596419 °E), for the elemental analysis. A total of three replicates were used for each of WS-UWC, WS-C and AGSP. Immediately after collection, all the samples were oven-dried at 80 ºC for three days and ground into fine particles using a mortar and pestle. The ground samples then sieved using a 0.5 mm sieve and sealed in plastic bags.

ICP-MS procedure: The trace metals were quantified in all collected samples using Inductive Coupled Plasma Mass Spectrometric (ICP-MS) Method. Approximately, 0.2 g of powdered shoot-top sample was mixed with 10 ml of conc. HNO_3 and 1 ml of H_2O_2. The digestion continued for 30 mins at 180 °C in a Mars-6 Microwave Digester (CEM; Matthews, NC). Then the elemental composition was analyzed in digested samples along with a standard control sample through ICP-MS using Thermo ICapQ (Thermo-Fisher Scientific Inc., Bremen, Germany) analyser (Diyabalanage et al. 2016; Diyabalanage et al. 2017). Finally, the elemental concentrations were calculated according to equation 1 (Van de Wiel 2003).

$$W\ (mgkg^{-1}) = (C_1 - C_0)D_f V/m \quad \text{.....eq. 1}$$

Where;

W: Weight of the element in the test sample ($mgkg^{-1}$)

C_1: Concentration of the element in the test sample given by ICP-MS

C_0: Concentration of the element in the standard control sample given by ICP-MS

D_f: Dilution factor

V: Volume of the sample (test or standard control)

m: Mass of the powdered sample

Estimation of the probable intake of elements in human consumption:

The weight (W $mgkg^{-1}$) values of the 36 elements of WS and AGSP samples obtained using ICP-MS were converted using the equation 2 to determine the probable intake amounts, if humans consumed these samples. The serving size of the leafy vegetable

in an average daily human diet is about 80 g on a fresh weight basis. According to literature, the approximate water content in WS is 85 % thus the dry weight of the 80 g fresh weight is 12 g (Umar et al. 2015).

Probable intake of an element (mg per day or serving) = (W in mg / kg) × Dry wt. of the serving in kg)…eq.2

Culinary preparations and sensory evaluation

WS seeds were purchased from an authorized seed supplier in Sri Lanka and grew those with AGSP stem cuttings in the greenhouse according to standard cropping practices (DOA 2017). The standard soil based potting mixture and the commercially available fertilizer (NPK) were used to grow the plants. A total of ten pots were raised for WS and AGSP separately. The tender shoot-tops of AGSP and WS were harvested from the pots and prepared three dishes according to three recipes namely basic, special and special with chicken. Table 1 contains details of the ingredients added. To prepare the basic dish; cut pieces (approximately 5 cm each) of WS and AGSP were separately stir fried in heated pans with oil and onion. Then salt and green chilies were added once the shoot-tops became darker in colour (Table 1). In preparation of the special dish, same procedure was followed as in basic dish except mixing additional ingredients as indicated in Table 1. Finally, the special dish with chicken which is more popularly known as "Chinese devilled dish" in Sri Lanka was prepared according to the recipe of the special dish with extra ingredients listed in Table 1. The tender shoot-tops used for all the culinary preparations were harvested on the same day and subjected all three prepared dishes to the taste panel evaluation immediately after cooking.

Table 1: Ingredients added to culinary preparations of AGSP and WS for sensory evaluation.

Ingredients for 100 g of tender shoots	Culinary preparation		
	Basic dish	Special dish	Special dish with chicken
Coconut oil	3 ml	3 ml	3 ml
Salt	5 g	5 g	15 g
Chopped green chili	5 g	5 g	5 g
Chopped onion	10 g	15 g	25 g
Chopped garlic	-	5 g	5 g
Red chili-flakes	-	5 g	5 g
Tomato slices	-	30 g	60 g
Tomato sauce	-	10 ml	100 ml
Soya Sauce	-	3 ml	5 ml
Black pepper powder	-	-	5 g
Curry pepper slices	-	-	40 g
Vinegar	-	-	5 ml
Sugar	-	-	10 g
Chicken cubes deep fried in coconut-oil	-	-	100 g

A modified sensory evaluation procedure described in Busari et al. (2016) was used in this study. For the sensory evaluation, 30 panellists were invited to rank the desired levels for colour, aroma, texture and overall taste according to a three-tier ranking system (1: least preferred, 2: moderately preferred, 3: highest preferred). In contrast, the relative bitterness level was ranked with a separate three-tier system (1: least bitterness, 2: moderate bitterness and 3: highest bitterness). In between the tasting of two dishes, drinking water was provided to the panellists for rinsing to avoid the carry-over effect on taste buds from the previously tasted sample.

Phytochemical assessment

The phytochemical tests were conducted in triplicate. Immature leaves of WS and AGSP from the greenhouse were collected randomly from the plants and washed them thoroughly using distilled water and air-dried the leaf samples for 24 hrs. Next, 10 g from the air-dried sample were crushed and dissolved in approximately 200 ml of distilled water. Then the mixture was sonicated for 30 mins and filtered through 8 µm Whatman filter paper (Cat NO 1002 125). Finally, 50 ml of the resultant filtrate was stored at 4 °C for the phytochemical analyses to detect the presence of anthocyanins, flavonoids, phlobatannins, reducing sugars, saponins, tannins, and terpenoids.

The presence of anthocyanin was tested by adding 1 ml of 2 M NaOH to 2 ml of leaf extract. This step results in a bluish green colour in the mixture indicating the presence of anthocyanin (Harborne 1973). To test the amountof flavonoids in the samples, 1 ml of leaf extract was mixed with 5 ml of diluted NH_3. The formation of an intense yellow colour indicates the presence of flavonoids. Then concentrated H_2SO_4 was added to the yellow coloured mixture until the colour disappeared. The amount of H_2SO_4 added until the yellow colour disappearance is proportional to the amount of flavonoids present in the sample (Hossain et al. 2013). The test for phlobatannin was conducted by adding 1 ml of leaf extract to 2 % HCl solution followed by boiling. The development of red precipitate confirms the presence of phlobatannin (Auwal et al. 2014). The presence of reducing sugars were confirmed by adding a few drops of Benedict's solution to 1 ml of leaf extract followed by boiling the mixture for a few minutes in a water bath. A brick-red precipitate indicates the presence of reducing sugars (Benedict 1909). The presence of saponins, were checked with the formation of a persistent froth in the leaf extract after being vigorously shaken (Singh et al. 2012). The tannin test was conducted by adding a few drops of freshly prepared 10 % $FeCl_3$ to 1 ml of leaf extract (Hossain et al. 2013). Finally, the Salkowski test was performed to confirm the presence of terpenoids by mixing 1 ml of leaf extract with 2 ml of chloroform and adding 3 ml of conc. H_2SO_4 along the wall of the tube. The development of a reddish-brown colour at the interface of the solvents confirms the presence of terpenoids (Benedict 1909).

DNA fingerprinting with trnH-psbA

DNA was extracted from fresh and cooked

samples of AGSP and WS using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1999). An adulterated dish was prepared with a mixture of AGSP and WS shoot-tops (all other components were similar to the special dish in Table 1). DNA was extracted from the shoot-top material taken from the adulterated dish prepared. Subsequently, the isolated DNA samples were amplified through PCR using *trnH-psbA*, a standard plant DNA barcoding marker (trnHf: CGC GCA TGG TGG ATT CAC AAT CC and psbA'3f: GTT ATG CAT GAA CGT AAT GCT C). PCR mix of 15 µl volume, consisting with 2× GoTaq® Green Master Mix, 0.3 pmol of forward and reverse primers and 50 ng of template DNA were prepared. The PCR program consisted of initial denaturation at 95 °C for 5 minutes, 35 cycles with denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 1 minutes and synthesis at 72 °C for 90 seconds, final extension at 72 °C for 4 minutes (Bolson et al. 2015). Two percent agarose gel stained with ethidium bromide was used to size separation and visualize the PCR products. The confirmed PCR products from each species were purified and subjected them to 3× sanger sequencing using ABI Genetic Analyzer (Version 4405186).

Data analysis

The elemental contents were measured from three replicates from AGSP and WS. The GLM procedure was used with the main effect 'leafy vegetable' in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to analyze the elemental data generated by ICP-MS. The means were separated using Tukey option in GLM. The FREQ procedure was employed in SAS for non-parametric association analysis using 'leafy vegetable' and each of the sensory parameters as factors to assess the ranked data generated by the taste panel. The strengths of associations were interpreted using the raw percentage of associations and Chi-square values. Results of the qualitative phytochemical tests were descriptively analyzed. The DNA bands on agarose gel were used to check the presence of WS in a dish with AGSP where WS is present as an adulterant and confirmed the band sizes detected in agarose gels using DNA sequencing results.

3. Results

Habitats of WS growth

WS is commonly available in polluted aquatic habitats of urban areas and the shoot-tops (young shoots with tender leaves) get harvested and end up in the markets. The industrial effluents significantly contaminated the wastewater channels in urban areas which greatly support the prolific growth of WS. It was observed that a lush growth of WS in a concrete-walled wastewater channel located next to the train-tracks in Kandy city, Sri Lanka (Plates. 1A, 1B, 1C, and 1D). The water accumulated in these channels appears polluted and blackish coloured. Plate 1E displays a commercial WS farming field. It is irrigated from a channel stream polluted with wastewater coming from an urban area (not included in the photograph). Plate 1F shows a well-maintained experimental WS field without any contamination from an urban wastewater

system. The best foliage and shoot growth were observed in the polluted channel compared to other habitats observed for the study (Plates 1A-D and 2A-C). The greenhouse-grown WS showed the least shoot-top growth with significantly lowest mean stem diameter of 0.15 cm (Plate 2A). The WS-C showed significantly average mean stem diameter of 0.35 cm (Plate 2B). The WS-UWC collected from the urban wastewater channel showed the highest growth and the significantly highest mean stem diameter of 0.60 cm (Plate 2C) ($p<0.05$). The internodal lengths were not significantly different and but highly varied among the three WS types assessed ($p>0.05$).

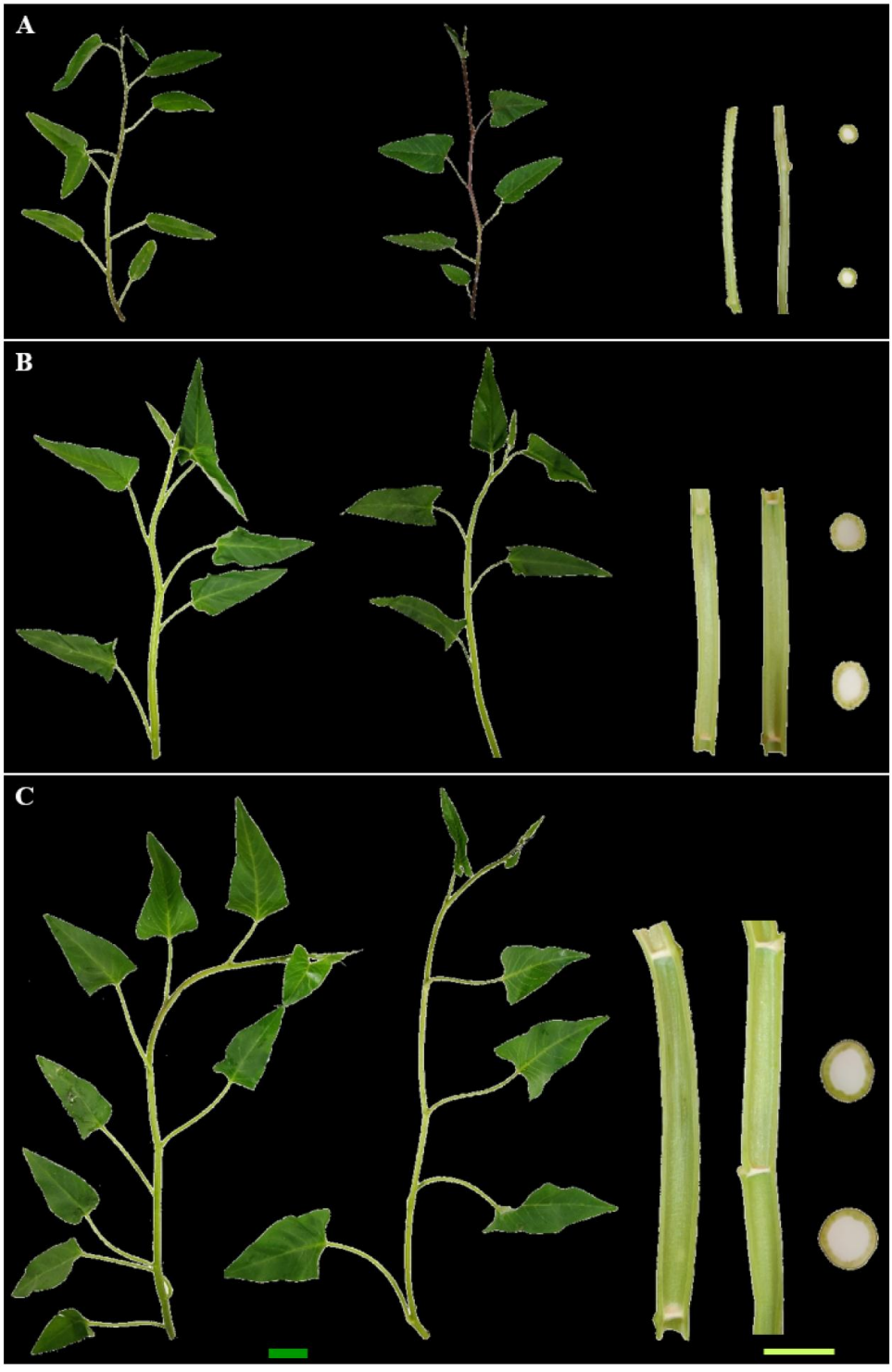

Plate 2: The comparison of the shoot-tops of WS collected. A: Greenhouse grown; B: Commercially available (WS-C); C: Collected from an urban waste water channel in Kandy, Sri Lanka (WS-UWC). The images exhibit shoot-tops, longitudinal and cross sections. The scale bars represent 1 cm (separately for shoot-tops and sections).

Growth rates of AGSP and WS

We compared the growth rates of AGSP and WS by growing them on 1 m^2 soil beds with standard potting mixtures and crop recommendations (DOA 2017). The growth rate of AGSP was higher than that of WS. Because of the larger foliage size in AGSP, it reached to the 100 % bed-fill (covering the bed without exposing the soil surface underneath) within five weeks of establishment (Plates 3A *vs.* 3C and 3E), whereas WS did not complete bed-fill even after ten weeks (Plates 3B *vs*. 3D and 3F). The mean yield of shoot-tops per bed for AGSP and WS were 2910 g (after five weeks) and 820 g (after ten weeks) respectively.

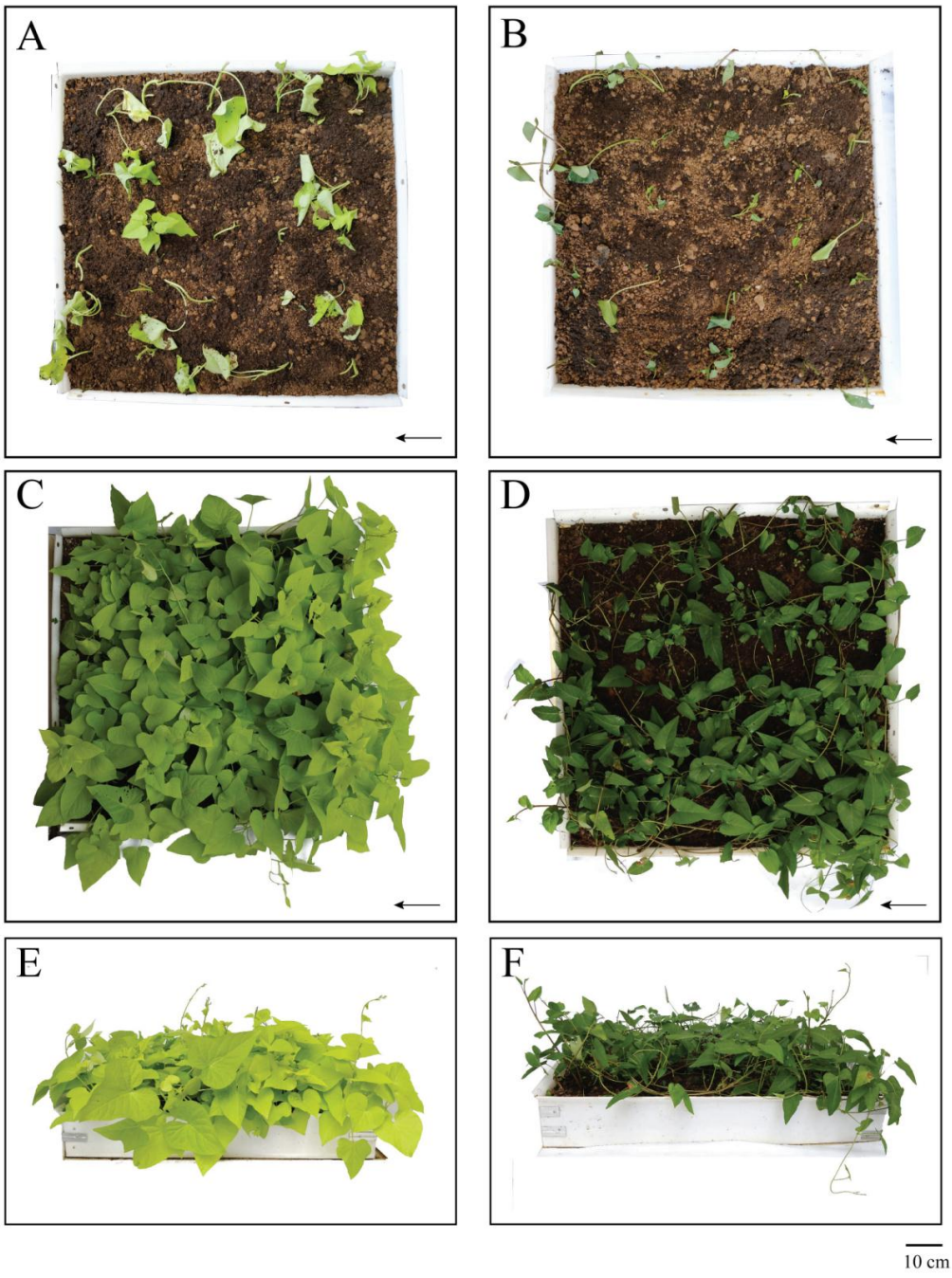

Plate 3: Growth of AGSP and WS on soil beds. A, C and E: AGSP; B, D and F: WS; A and B: On the day of establishment; C and E: After 5 weeks; D and F: After 10 weeks; C and D: top views; E and F: Lateral views from the East. Arrows in A, B, C and D indicate the direction of sunlight.

Standard colourimetric parameters of AGSP and WS leaves

The foliage of AGSP exhibited an acid-green colour with the mean RGB values of 176, 191 and 26 respectively. The hex code for the acid green colour of AGSP is #B0BF1A. The acid green colour was exhibiting a luminescence in its colour as well. The foliage of WS was dark green with mean RGB values of 60, 150 and 60 respectively. The colour hex code for WS foliage is #3c963c. Plates 4A and 4B display the colours of AGSP and WS. Overall AGSP was more ornamentally attractive than WS.

When comparing morphology of the shoot-tops of AGSP and WS where it was observed that the AGSP shoot-tops contained more leaves in the tender shoots compared to WS tender shoots (Plates 4Ai and 4Biv). The AGSP leaves are cordate in shape whereas WS leaves were spear in shape (Plates 4Aii, 4Aiii, 4Bv, and 4Bvi).

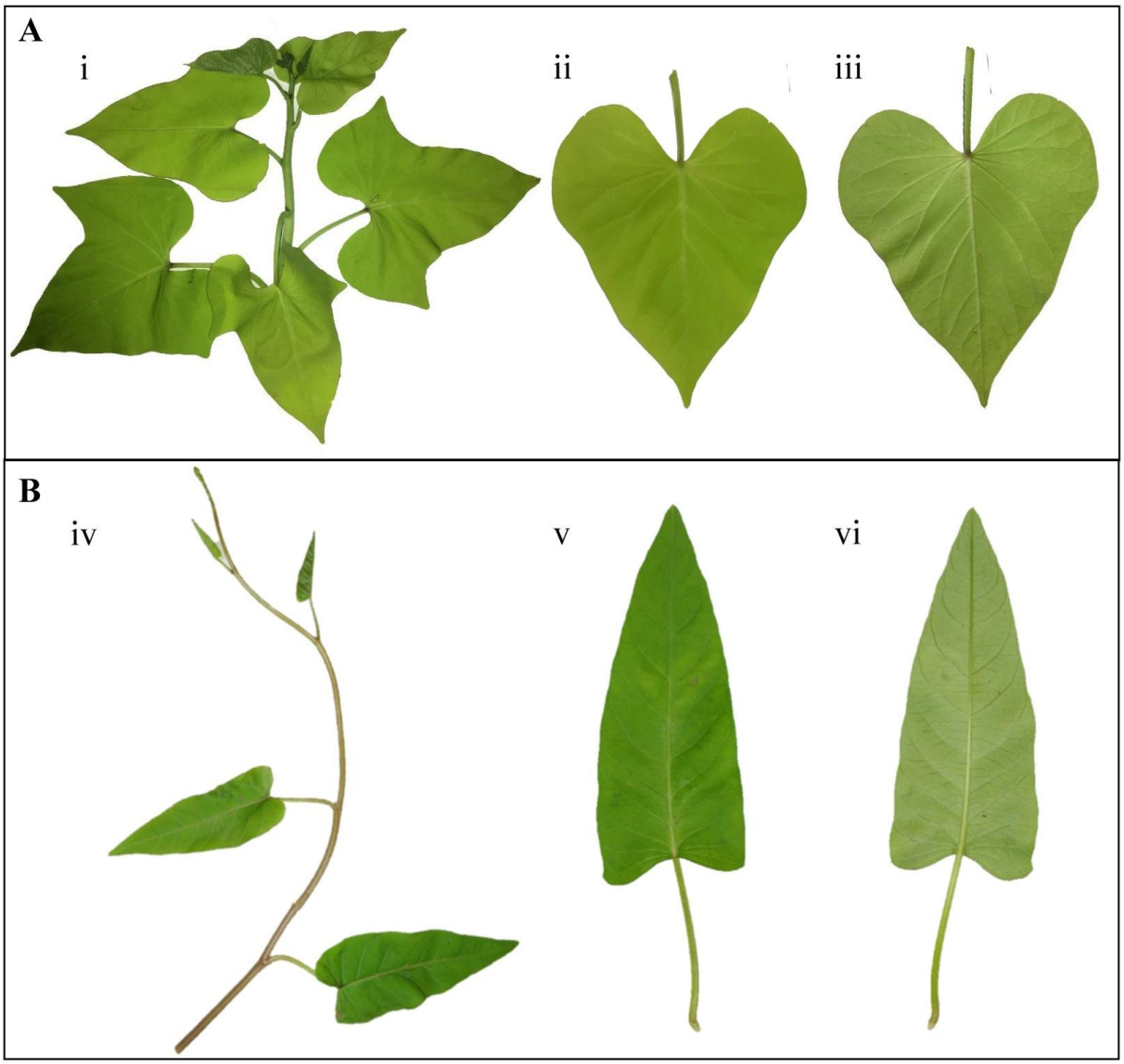

Plate 4: Edible shoot-tops and leaves of AGSP and WS. Edible shoot-tops and leaves of AGSP and WS. ai: immature edible shoot-top with leaves of AGSP; aii: Adaxial surface of AGSP leaf; aiii: Abaxial surface of AGSP leaf; biv: Immature edible shoot-top with leaves of WS; bv: Adaxial surface of WS leaf; bvi: Abaxial surface of WS leaf. AGSP leaves are simple, alternately arranged and, cordate-shaped. The leaf tip is acuminate, the base is cordate, and the margin is entire. WS leaves are simple, alternately arranged, and spear-shaped. The leaf tip is acute, the base is truncated-cordate, and the margin is entire (Norton-Brown Herbarium 2017). The leaf sizes are not according to a common scale.

ICP-MS elemental analysis

The concentrations (weight basis as mg/kg) of the 36 elements in shoot-top samples of AGSP, WS-UWC, and WS-C were detected using ICP-MS method (Table 2). Out of 36 elements tested, 16 elements gave significantly higher concentrations in WS-UWC. The concentrations of Na and Ca were significantly higher in WS-C. The concentrations of Sr and Cs were significantly higher in AGSP. The heavy metals Cr, In, Hg, Tl and all three isotopes of Pb were found to be significantly higher in WS-UWC. The As concentration was significantly higher in WS-C and WS-UWC (0.5434 and 0.2298 mg/kg respectively) compared to significantly lower As concentrations in AGSP (0.0103 mg/kg) (Table 2, $p<0.05$). The Pb isotopes were absent in AGSP or WS-C whereas the significantly higher levels of Pb isotopes were present in WS-UWC. The consumption of 80 g of fresh weight WS-UWC as a serving in the diet surpass or equal to the Pb permissible level indicated for a human adult (GED 2013) (Table 2), in contrast, the other elements would not reach the maximum permissible levels after consumption of a similar amount of WS-UWC or WS-C in a day. However, frequent consumption of WS-UWC or WS-C would exceed the maximum permissible levels of chronic and bioaccumulatory effects of heavy metals (Table 2).

Table 2: The elemental composition in shoot-top samples of AGSP and WS which was detected using ICP-MS and maximum permissible levels of elements allowed in food.

Element	Weight of element present in the Test sample (mg/kg)			Estimated weight of element present in the Test sample if one serving (~80 g) was ingested, Unit: mg/serving			Standard maximum permissible level (mg/day) for an adult human	
	AGSP	WS-C[#]	WS-UWC[$]	AGSP	WS-C	WS-UWC	Value	Reference
Li^{7}	2.740×10^{-2c}£	3.644×10^{-1b}	5.374×10^{-1a}	3.000×10^{-4}	4.400×10^{-3}	6.400×10^{-3}	7.800×10^{-1}	Guideline for Elemental Impurities (2013)
Be^{9}	2.230×10^{-2c}	2.350×10^{-2b}	2.820×10^{-2a}	0.000	3.000×10^{-4}	3.000×10^{-4}	1.200×10^{-2}	Toxicological profile for Beryllium (2002)
Na^{23}	4.968×10^{1c}	5.287×10^{3a}	2.259×10^{3b}	5.962×10^{-1}	6.344×10^{1}	2.710×10^{1}	3.500×10^{3}	Van Amburgh (2018)
Mg^{24}	1.265×10^{3a}	1.456×10^{3a}	9.726×10^{2b}	1.518×10^{1}	1.747×10^{1}	1.167×10^{1}	4.000×10^{2}	
Al^{27}	2.363×10^{1c}	2.236×10^{2b}	9.693×10^{2a}	2.835×10^{-1}	2.683	1.163×10^{1}	6.000×10^{1}	Uluozlu et al. (2009)
K^{39}	2.198×10^{4a}	2.798×10^{4a}	2.306×10^{4a}	2.638×10^{2}	3.358×10^{2}	2.767×10^{2}	3.900×10^{3}	Van Amburgh (2018)
Ca^{44}	4.767×10^{3b}	9.748×10^{3a}	5.332×10^{3b}	5.721×10^{1}	1.170×10^{2}	6.398×10^{1}	1.200×10^{3}	
Ti^{48}	1.179×10^{1c}	4.888×10^{1b}	8.977×10^{1a}	1.415×10^{-1}	5.865×10^{-1}	1.077	2.000	Weir et al. (2012)
V^{51}	9.000×10^{-2c}	1.011^{b}	3.238^{a}	1.100×10^{-3}	1.210×10^{-2}	3.890×10^{-2}	1.200×10^{-1}	Guideline for Elemental Impurities (2013)
Cr^{52}	0.000^{b}	7.021×10^{-1b}	2.147^{a}	0.000	8.400×10^{-3}	2.580×10^{-2}	1.100×10^{1}	
Mn^{55}	3.191×10^{1b}	9.000×10^{1a}	9.276×10^{1a}	3.829×10^{-1}	1.080	1.113	5.000	Environmental Fact Sheet (2006)
Fe^{56}	1.058×10^{2c}	3.822×10^{2b}	1.306×10^{3a}	1.270	4.587	1.568×10^{1}	5.000×10^{1}	WHO (2003)
Co^{59}	7.130×10^{-2c}	7.140×10^{-1b}	8.659×10^{-1a}	9.000×10^{-4}	8.600×10^{-3}	1.040×10^{-2}	5.000×10^{-2}	Guideline for Elemental Impurities (2013)
Ni^{60}	1.114^{a}	7.682×10^{-1a}	1.431^{a}	1.340×10^{-2}	9.200×10^{-3}	1.720×10^{-2}	6.000×10^{-1}	
Cu^{63}	9.608^{a}	9.030^{a}	6.482^{b}	1.153×10^{-1}	1.084×10^{-1}	7.780×10^{-2}	1.300	
Zn^{66}	8.484^{c}	8.464×10^{1b}	2.745×10^{1a}	1.018×10^{-1}	1.016	3.294×10^{-1}	2.000×10^{1}	WHO (2003)
Ga^{71}	3.380×10^{-2c}	2.419×10^{-1b}	6.981×10^{-1a}	4.000×10^{-4}	2.900×10^{-3}	8.400×10^{-3}	4.000×10^{-4}	Bibak et al. (1998)
As^{75}	1.030×10^{-2b}	5.434×10^{-1a}	2.298×10^{-1a}	1.000×10^{-4}	6.500×10^{-3}	2.800×10^{-3}	1.500×10^{-2}	Guideline for Elemental Impurities (2013)
Se^{78}	4.266×10^{-1a}	7.970×10^{-2c}	2.007×10^{-1b}	5.100×10^{-3}	1.000×10^{-3}	2.400×10^{-3}	1.700×10^{-1}	

Table 2 (Cont.)

Element	Weight of element present in the Test sample (mg/kg)			Estimated weight of element present in the Test sample if one serving (~80 g) was ingested, Unit: mg/serving			Standard maximum permissible level (mg/day) for an adult human	
	AGSP	WS-C[#]	WS-UWC[$]	AGSP	WS-C	WS-UWC	Value	Reference
Rb^{85}	4.329×10^{1a}	4.055×10^{1a}	3.449×10^{1a}	5.195×10^{-1}	4.866×10^{-1}	4.139×10^{-1}	-	-
Sr^{88}	2.186×10^{1a}	1.263×10^{1b}	7.978^{c}	2.623×10^{-1}	1.515×10^{-1}	9.570×10^{-2}	1.2000	Committee on Toxicology (2006)
Ag^{107}	1.710×10^{-2b}	1.320×10^{-2b}	7.930×10^{-2b}	2.000×10^{-4}	2.000×10^{-4}	1.000×10^{-3}	1.700×10^{-1}	Guideline for Elemental Impurities (2013)
Cd^{111}	1.080×10^{-2a}	1.910×10^{-2a}	5.730×10^{-2a}	1.000×10^{-4}	2.000×10^{-4}	7.000×10^{-4}	5.000×10^{-3}	
In^{115}	3.000×10^{-4b}	6.000×10^{-4b}	2.400×10^{-3a}	0.000	0.000	0.000	1.900×10^{-2}	Committee on Toxicology (2006)
Cs^{133}	1.130×10^{-1a}	3.190×10^{-2c}	5.330×10^{-2b}	1.400×10^{-3}	4.000×10^{-4}	6.000×10^{-4}	1.000×10^{-2}	ATSDR (2004)
Ba^{137}	1.639×10^{1a}	1.709×10^{1a}	1.963×10^{1a}	1.967×10^{-1}	2.051×10^{-1}	2.356×10^{-1}	1.300×10^{1}	Guideline for Elemental Impurities (2013)
Hg^{202}	3.280×10^{-2b}	3.370×10^{-2b}	5.460×10^{-2a}	4.000×10^{-4}	4.000×10^{-4}	7.000×10^{-4}	4.000×10^{-2}	
Tl^{205}	1.000×10^{-3c}	4.610×10^{-2b}	2.460×10^{-2a}	0.000	6.000×10^{-4}	3.000×10^{-4}	8.000×10^{-3}	
Pb^{206*}	0.000^{b}	0.000^{b}	4.220×10^{-1a}	0.000	0.000	$\mathbf{5.100\times10^{-3}}$	5.000×10^{-3}	
Pb^{207*}	0.000^{b}	0.000^{b}	3.897×10^{-1a}	0.000	0.000	$\mathbf{4.700\times10^{-3}}$	5.000×10^{-3}	
Pb^{208*}	0.000^{b}	0.000^{b}	3.993×10^{-1a}	0.000	0.000	$\mathbf{4.800\times10^{-3}}$	5.000×10^{-3}	
Bi^{209}	1.200×10^{-3a}	1.190×10^{-2a}	1.740×10^{-2a}	0.000	1.000×10^{-4}	2.000×10^{-4}	2.000×10^{-4}	Committee on Toxicology (2006)
U^{238}	9.000×10^{-4a}	1.550×10^{-2a}	6.830×10^{-2a}	0.000	2.000×10^{-4}	8.000×10^{-4}	1.500×10^{-3}	Uluozlu et al. (2009)

[#]WS shoot-top sample collected from commercially available stocks.
[$]WS shoot-top sample collected from an urban wastewater channel in Kandy, Sri Lanka.
[*]Different isotopes were tested.
[£]Means denoted by same letters within rows are not significantly different at P<0.05.
Pb206, Pb207 and Pb208 isotopes are naturally available in the percentages of 24.1, 22.1 and 52.4 respectively.
The cells which have the significantly higher numbers shaded in grey tone for better visualization.
- Not available

Sensory evaluation

The basic preparation of AGSP (Plate 5A) was dull green compared to the basic preparation of WS which was dark green (Plate 5D) whereas the dull green colour of AGSP reduced significantly in the special preparation of AGSP (Plate 5B). However, special preparation of WS (Plate 5E) got an attractive dark green colour compared to the special preparation of AGSP. Even though the AGSP special preparation with fried chicken cubes got the dull green colour of AGSP shoot-tops, that colour masked due to the addition of other ingredients (Plate 5C). However, because of the dark green colour of the shoot-tops of WS, the special preparation with chicken exhibited a more appealing overall colouration with a dark green touch (Plate 5F).

The association analysis between the sensory parameters (desired levels of colour, aroma, texture, relative bitterness level and overall taste) for the two species (AGSP and WS) prepared under basic, special and special with chicken preparations (Table 3) revealed the potential for using AGSP as a substitute to WS. In all basic, special and special with chicken preparations, the association between colour preference and the two species were significant at $p<0.05$. In all three preparations, consumers highly preferred the colour of WS compared to the colour of AGSP. However, the strength of the significant association between colour and species decreased in the special preparation with (χ^2 = ~27) or without chicken (χ^2 = ~23), implying that the addition of specific flavor enhancers would reduce the dis-likeness expressed by consumers on the colour of AGSP

(Table 3). The association between the desired aroma and species was significant only in basic preparation in which 27 % of the tasters ranked the aroma of WS as the best whereas 10 % tasters ranked AGSP as the best ($p< 0.05$). The association between the desired level of aroma and species for special ($\chi^2 = \sim1$) and special with chicken preparation ($\chi^2 = \sim4$) was not significantly different ($p>0.05$) (Table 3). The association between the desired level of texture and species for basic and special preparations was significant while the majority of the tasters ranked WS as the species having the better texture. However, after the addition of chicken to the special preparation, the association between the desired texture and species became insignificant ($p>0.05$; $\chi^2 = \sim3$) (Table 3). The relative bitterness of the prepared dish and the species was significant in basic preparation in which 33 % of the respondents said AGSP had the highest felt bitterness. However, a significant association between bitterness and species became insignificant in special with chicken preparations ($\chi^2 = \sim3$; Table 3). The association of the desired overall taste, the most important sensory parameter, between both species in special preparation was significant ($p<0.05$), and the majority of tasters preferred WS preparation. However, the strength of the association got weakened after addition of specific flavor enhancers to the special preparation as revealed by the reduced chi-square values. Interestingly, the association between the desired overall taste of the two species was not significantly different for the special preparation with chicken ($\chi^2 = \sim4$; $p>0.05$), indicating that healthier and more nutritious AGSP is a suitable substitute in place of bioaccumulation prone WS in mixed dishes (Table 3).

Plate 5: Culinary preparations subjected to taste panel and subsequent association analyses between sensory parameters and two species. A: AGSP basic preparation (dish); B: AGSP special preparation; C: AGSP special preparation with chicken.; D: WS basic preparation; E: WS special preparation; F: WS special preparation with chicken. The scale bar given in blue colour represents 1 cm.

Table 3: Association analysis between the sensory parameters and two species (AGSP and WS).

Culinary preparation[$]	Species	Desired Colour				Desired Aroma				Desired Texture				Relative bitterness level				Desired Overall taste			
		% respondents			Level of significance	% respondents			Level of significance	% respondents			Level of significance	% respondents			Level of significance	% respondents			Level of significance
		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
Basic preparation: only routine flavor enhancers added	AGSP	73	10	17	χ^2= 34.649*	53	37	10	χ^2= 13.301*	63	30	7	χ^2= 22.019*	27	40	33	χ^2= 13.280*	77	20	3	χ^2= 33.944*
	WS	3	77	20		10	63	27		7	86	7		57	43	0		3	70	27	
Special preparation: routine and specific flavor enhancers added	AGSP	55	45	0	χ^2= 27.333*	15	55	30	χ^2= 0.533	25	70	5	χ^2= 9.733*	40	40	20	χ^2= 1.207	40	35	25	χ^2= 9.077*
	WS	5	15	80		10	50	40		5	50	45		55	35	10		5	30	65	
Special preparation with chicken: with routine and specific flavor enhancers and deep oil fried chicken cubes added	AGSP	37	47	16	χ^2= 23.105*	10	43	47	χ^2= 3.540	14	43	43	χ^2= 2.373	56	27	17	χ^2= 2.085	10	40	50	χ^2= 3.514
	WS	3	20	77		0	40	60		3	40	57		74	13	13		0	37	63	

Grey shaded cells represent the significant associations (* represent $p<0.05$)

[$]As in Table 1

Phytochemical content

Fig. 1 displays the relative presence of phytochemicals in AGSP and WS. AGSP has all the tested phytochemicals in a higher amount than in WS. According to the qualitative ranking, AGSP contained 33 % more anthocyanin and flavonoids, 50 % more phlobatannins, reducing sugars and terpenoids, 66.7 % more saponins and 40 % more tannins compared to WS.

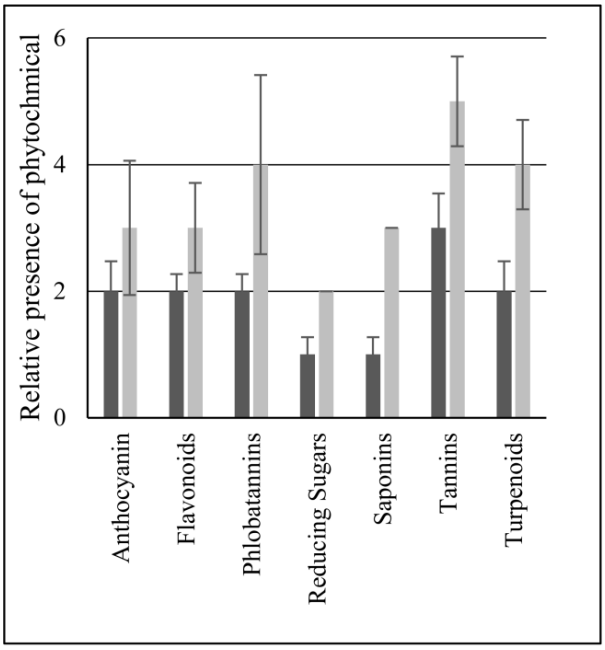

Figure 1: Comparison of the relative presence of phytochemicals in AGSP (gray) and WS (black). Note that AGSP has higher contents for all the phytochemicals qualitatively assessed.

DNA based detection of WS adulteration in dishes containing AGSP

The genomic DNA extracted from uncooked and cooked AGSP and WS displayed distinct bands in agarose gel, whereas raw AGSP and WS displayed the polymorphic bands. A band having the length of 350 bp was present in AGSP whereas 550 bp band in WS. Consistently both uncooked (fresh) and cooked samples had similar sized bands. In cooked mixture containing both AGSP and WS to mimic the adulteration scenario, both 350 bp, and 550 bp bands were detected indicating the presence of shoot-tops from both AGSP and WS. The positive control of the experiment, the rice DNA with a band having the length of 650 bp was present (Plate 6). Sanger sequencing of the *trnH-psbA* PCR products of AGSP and WS confirmed these lengths and SNP and INDEL differences (Fig. 2). The sequence length of the AGSP sample and WS were 342 bp and 550 bp respectively (GenBank Submission ID: 2120848). A total of 11 INDELS and 86 SNPs were present among WS and AGSP for the *trnH-psbA* locus and, the nucleotide difference was 17.02 % (Fig. 2). A Basic Local Alignment Search Tool (BLAST) search confirmed that the detected barcodes of the two species were *I. aquatica* and *I. batatas* validating our sequence analysis.

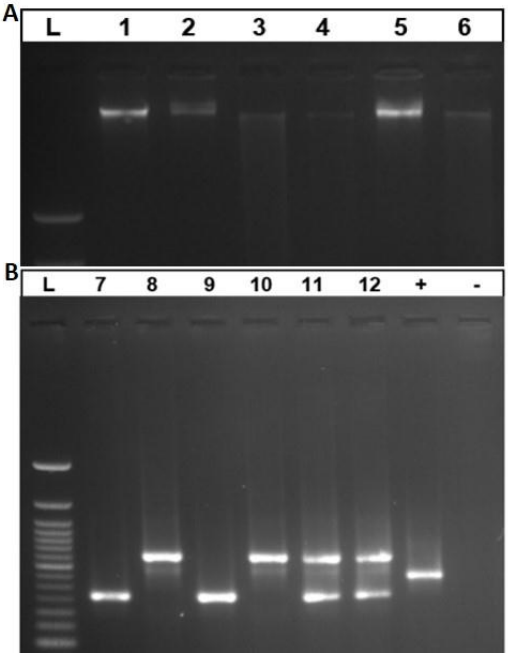

Plate 6: DNA fingerprinting with *trnH-psbA* locus to detect the presence/adulteration of WS in culinary preparations made with AGSP. A-1-6: gDNA; B-7-12: *trnH-psbA* PCR products; L: 50 bp ladder. 1 and 7: Uncooked (fresh) AGSP; 2 and 8: Uncooked (fresh) WS; 3 and 9: Cooked AGSP; 4 and 10: Cooked WS; 5 and 11: Uncooked AGSP and WS mixture; 6 and 12: Cooked AGSP and WS mixture; +: Positive control (rice); -: negative control.

Figure 2: The DNA sequence alignment for *trnH-psbA* locus in WS and AGSP. The INDELs, SNPs and shared bases are colour coded for easy visualization. The numbers of bases in each section of the alignment are in the right. The band sizes of the Plate 6 are matching with the sequence lengths shown here.

4. Discussion

Leafy vegetables are essential in our diets for the nutritional benefits. Out of many leafy vegetables available in the world, WS is undoubtedly one of the most preferred species frequently consumed by human beings despite their social classes in many parts of the world, especially in Asia. Descriptive data for the nutritional values of WS are available amply in the literature (Islam 2014; Edie and Ho 1969). Many reported studies and the present study confirmed that WS is highly prone to bioaccumulation of heavy metals (Table 2) (Göthberg et al. 2004; Kumar et al. 2008; Wang et al. 2008; Rai and Sinha 2001; Igwe and Abia 2006; Cobb et al. 2000). WS exhibits a remarkable growth pattern in polluted sites (Plate 1). In developing countries like Sri Lanka, polluted habitats are common harvesting sites of WS and vendors sell them in the open market (Pers. Comm.). After washing and packing into bunches, consumers or consumer protection agencies would not be able to detect the origin of growth when displayed in the market. In practice, due to limitations in traceability especially in informal open markets including roadside vendors, it is challenging to avoid the entry of such leafy vegetables to the market. Therefore, we believe that the identification and deployment of an alternative leafy vegetable to replace WS is essential. Similarly, public awareness programmes can be conducted to aware the consumers to buy WS from reliable sources.

Evolutionary, the closest leafy vegetable to WS is sweet potato, which belongs to the same genus and used as a potential alternative leafy vegetable to WS in rural areas. The sweet potato shoot-tops are a favorite leafy vegetable in ethnic ranges of the world. As identified in the present study and many reported studies, sweet potato shoots are nutritious (Fig. 1) (Sun et al. 2014; Mulokozi et al. 2007; Luis et al. 2014), fast-growing (Woolfe 1992) (Plate 3) and less prone to bioaccumulation (Table 2) (Mahlangeni et al. 2012; Lockley and Bardsley 2000). Out of the many sweet potato genotypes in Sri Lanka, AGSP exhibits the fastest foliage growth, grows as an ornamental cover crop and is edible. In addition, the tubers of AGSP are very similar in appearance and taste to the tubers of other yellow sweet potato varieties grown. The shoot-tops of AGSP can be harvested within five weeks of planting and used in any form of culinary preparation. AGSP can provide 3.6 times of shoot-top yield in five weeks compared to WS harvest that could be obtained only after ten weeks (Plate 3). The elemental analysis revealed that AGSP contains essential elements but does not accumulate toxic heavy metals implying that sweet potato is not prone to bioaccumulation (Table 2). Furthermore, the phytochemical analysis revealed that AGSP contains higher amounts of essential phytochemicals than WS (Fig. 1).

However, the association analysis for sensory parameters revealed that consumers are hesitant to accept AGSP as a leafy vegetable preparation over WS. The hesitation is probably due to being accustomed to the taste, aroma, texture, and colour of WS for long periods of use (Table 3). However, in the modern cuisines, WS is mainly used as an ingredient in devilled dishes with or without protein sources like

chicken meat. When we used AGSP in place of WS in special preparation with chicken, the association analysis revealed that the consumers prefer the AGSP's aroma, texture, and overall taste similar to the special dishes prepared with WS (Table 3). Therefore, for such special dishes, we strongly recommend the use of AGSP as an alternative to WS, as a way of minimizing the consumption of toxic metals when the origin of WS cannot be determined. Also, we can readily consume WS if their shoot-tops are coming from standard farms with no possibility of bioaccumulation. However, under practical situations in the open market, street suppliers, and vendors, it is evident that attractive and bountiful harvests of WS shoot-tops available for purchase are most likely those contaminated with high levels of toxic elements (Table 2).

The DNA based detection of food adulterations is a common practice in the modern world (Lockley and Bardsley 2000; Woolfe and Primrose 2004; Reid et al. 2006; Madesis et al. 2014; Dhanya and Sasikumar 2010). The *trnH-psbA* barcode is ideal to discriminate WS from AGSP in agarose-based gel platforms because of the observed length polymorphism due to 11 INDELs. Unlike many other plant DNA barcoding loci, the sequence length of the *trnH-psbA* spacer varies due to frequent INDELS from 152-1006 bp across plant families, (Pang et al. 2012) and detected barcodes for WS and AGSP fall into this range. The DNA barcoding locus *trnH-psbA* could be used in a PCR based diagnostic test to detect the presence of WS in commercial dishes if consumer protection agencies wish to detect the adulteration of dishes with WS. As the first of its kind, this study records the potential of using AGSP as an alternative leafy vegetable in place of bioaccumulation prone WS to safeguard the health of human beings.

The ICP-MS elemental analysis revealed that WS-C and WS-UWC contain significantly higher concentrations of heavy metals than AGSP (Table 2, $p<0.05$). The WS-UWC contain the significantly highest levels of Pb (all three isotopes), Hg, Tl, In and Al indicating the higher degree of the bioaccumulation of heavy metals in WS. Although, one serving of WS-UWC surpasses the maximum permissible levels of Pb; in cases of Ti, two servings and for V, three servings have to be consumed to exceed the maximum permissible levels per day. Similarly, if a person consumes three servings of WS-C, the maximum permissible level of As would be surpassed. However, the smaller quantities of heavy metals could get accumulated in body tissues and cause unfavorable consequences at a later stage in a chronic pattern. Thus, maximum permissible levels per day cannot be considered in deciding the safety levels of the heavy metals. It is also apparent that the urban wastewater channel, polluted with toxic heavy metals and they are readily being bioaccumulated by WS causing deleterious health effects on consumers if they unknowingly purchase and consume these leafy vegetables from the market on a regular basis. The WS-C also contained higher levels of As indicating that commercial samples might have come from As-contaminated fields. The present elemental analysis implied that people should avoid commercial WS samples and, in

unavoidable circumstances should eat in smaller servings and less frequencies. Our study also noted that Se, an essential micronutrient (Zeng 2009) was present in AGSP in significantly higher amounts highlighting its nutritional value. Despite all the harmful effects of WS to human health due to the high accumulation of heavy metals; WS is a potential aquatic plant to use in the phytoremediation (Göthberg et al. 2004; Chen et al. 2010).

5. Conclusions

The growth of WS is prolific in polluted aquatic habitats compared to commercial fields. The ICP-MS elemental analysis highlights the fact that WS-C samples in the Sri Lankan market would not be safe for frequent consumption. The WS-UWC and (WS-C) samples contain significantly higher amounts of toxic elements including heavy metals such as Hg and As. Furthermore, significantly higher quantities of Pb were detected in WS-UWC. The present analysis demonstrates that AGSP could be considered as a potential alternative leafy vegetable in place of bioaccumulation prone WS because AGSP contains higher amounts of phytochemicals and nutritionally essential elements and does not contain any of the heavy metals tested even at minimum permissible levels. However, when we prepared AGSP as a basic culinary dish with minimum flavors, consumer acceptance levels were low. When flavors and fried meat sources like chicken were added and prepared as favorite Sri Lankan devilled dish, consumer acceptance was as same as for WS, indicating that AGSP is an ideal substitute for WS for mixed dishes. Furthermore, a faster growth was detected in the tender shoots of AGSP than that of WS highlighting the feasibility of using AGSP as a cost-effective leafy vegetable in Sri Lankan cuisines. The DNA based detection using the *trnH-psbA* locus could be readily implemented to ensure consumer trust by detecting the adulterations of WS in AGSP containing dishes if consumers opted to eat AGSP in place of WS in the future.

6. Acknowledgments

Departments of Geology and Physics of the Faculty of Science, University of Peradeniya, Sri Lanka for generous support in elemental analysis.

Ms. S.I. Karunarathne, Ms. G.K.S. Ananda, Ms. S.K. Kannangara, Mr. H.S.M. Jayarathne, Ms. T.B. Salpadoru and Mr. P.G.R.G. Rathnayake for the valuable assistance provided during the study.

7. References

Agency for Toxic Substances and Disease Registry (ATSDR) (2004) Public Health Statement for Cesium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available from: https://www.atsdr.cdc.gov/phs/phs.asp?id=575&tid=107. Accessed on 1st June 2018.

Anh V T, Tram N T, Klank L T, Cam P D, Dalsgaard A (2007) Fecal and protozoan parasite contamination of water spinach (*Ipomoea aquatica*) cultivated in urban

wastewater in Phnom Penh, Cambodia. Trop Med Int Health 12: 73-81.

Art Paints.com (2017) Available at: http://www.art-paints.com. Accessed on 19th December 2017.

Auwal M S, Saka S, Mairiga I A, Sanda K A, Shuaibu A, Ibrahim A (2014) Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Vet Res Forum 5: 95-100.

Benedict S R (1909) A reagent for the detection of reducing sugars. J Biol Chem 15: 485-487.

Bibak A, Behrens A, Stürup S, Knudsen L, Gundersen V (1998) Concentrations of 63 major and trace elements in Danish agricultural crops measured by inductively coupled plasma mass spectrometry. 1. Onion (*Allium cepa* Hysam). J Agric Food Chem 46: 3139-3145.

Bolson M, de Camargo Smidt E, Brotto M L, Silva-Pereira V (2015) *ITS* and *trnH-psbA* as efficient DNA barcodes to identify threatened commercial woody angiosperms from Southern Brazilian Atlantic rainforests. PloS ONE 10: e0143049.

Busari K R, Oyeyinka S A, Akinoso R, Aworh O C (2016) Nutritional and sensory properties of wild lettuce (*Lactuca taraxacifolia*) leaves as affected by sun drying alone or in combination with blanching. Croatian Journal of Food Technology, Biotechnology and Nutrition 11: 28-35.

Chary N S, Kamala C T, Raj D S S (2008) Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Saf 69: 513-524.

Chen J C, Wang K S, Chen H, Lu C Y, Huang L C, Li H C, Peng T H, Chang S H (2010) Phytoremediation of Cr (III) by Ipomonea aquatica (water spinach) from water in the presence of EDTA and chloride: Effects of Cr speciation. Bioresour Technol 101: 3033–3039.

Cobb G P, Sands K, Waters M, Wixson B G, Dorward-King E (2000) Accumulation of heavy metals by vegetables grown in mine wastes. Environ Toxicol Chem 19: 600-607.

Committee on Toxicology (2006) COT statement on the 2006 UK total diet study of metals and other elements. COT Secretariat, Food Standards Agency, London. Available at: https://cot.food.gov.uk/sites/default/files/cot/cotstatementtds200808.pdf. Accessed on 1st June 2018.

Department of Agriculture (DOA). Sri Lanka: Ministry of Agriculture, Government of Sri Lanka. Available at: www. Doa.gov.lk. Accessed on 17th July 2017.

Dhaliwal M S (2017) Tuber vegetable crops. In: Handbook of Vegetable Crops. 3rd edition. Kalyani Publishers.

Dhanya K, Sasikumar B (2010) Molecular marker-based adulteration detection in traded food and agricultural commodities of plant origin with special reference to spices. Curr Trends Biotechnol Pharm 4(1): 454-489.

Diyabalanage S, Fonseka S, Dasanayake D M S N B, Chandrajith R (2017) Environmental exposures of trace elements assessed using keratinized matrices from patients with chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka. J Trace Elem Med Biol 39: 62-70.

Diyabalanage S, Navarathna T, Abeysundara H T, Rajapakse S, Chandrajith R (2016) Trace elements in native and improved paddy rice from different climatic regions of Sri Lanka: implications for public health. Springer Plus5: 1864.

Doyle J J, Doyle J L (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Edie H H, Ho B W (1969) *Ipomoea aquatica* as a vegetable crop in Hong Kong. Econ Bot 23: 32-36.

Environmental Fact Sheet (2006) Manganese: Health information summary. New Hampshire Department of Environmental Services, 29 Hanzen Drive, Concord, New Hampshire. Available at: https://www.des.nh.gov/organization/commissioner/pip/factsheets/ard/documents/ard-ehp-15.pdf. Accessed on June 2018.

Göthberg A, Greger M, Bengtsson B E (2002) Accumulation of heavy metals in water spinach (*Ipomoea aquatica*) cultivated in the Bangkok Region, Thailand. Environ Toxicol Chem 21: 1934-1939.

Göthberg A, Greger M, Holm K, Bengtsson BE (2004) Influence of nutrient levels on uptake and effects of Mercury, Cadmium, and Lead in water spinach. J Environ Qual 33: 1247-1255.

Grubben G J H (2004) Vegetables (Vol. 2). Prota.

Guideline for Elemental Impurities (2013) Q3d Current Step 2b Version, International Conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Aviailable at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Step2b.pdf. Accessed on 1st June 2018.

Harborne J B (1973) Phytochemical Methods: A guide to modern techniques of plant analysis. London: Chapman and Hall Ltd: 279.

Hossain M A, Khulood A S R, Zawan H M, Afaf M W, Qasim A R (2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pac J Trop Biomed 3: 705-710.

Igwe J, Abia A A (2006) A bioseparation process for removing heavy metals from waste water using biosorbents. Afr J Biotechnol 5: 1167–1179.

Islam S (2014) Nutritional and medicinal qualities of sweet potato tops and leaves. Cooperative Extension Service, University of Arkansas.

Johnson M, Pace R D (2010) Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. Nutr Rev 68: 604-615.

Kananke T, Wansapala J, Gunaratne A (2014) Heavy metal contamination in green leafy vegetables collected from selected market sites of Piliyandala area, Colombo District, Sri Lanka. Am J Food Technol 2(5): 139-144.

Kumar J N, Soni H, Kumar R N, Bhatt I (2008) Macrophytes in phytoremediation of heavy metal contaminated water and sediments in Pariyej Community Reserve, Gujarat, India. Turk J Fish Aquat Sci 8: 193-200.

Li M, Jang G Y, Lee S H, Kim M Y, Hwang S G, Sin H M, Kim H S, Lee J, Jeong H S (2017) Comparison of functional components in various sweet potato leaves and stalks. Food Sci Biotechnol 26: 97-103.

Lockley A K, Bardsley R G (2000) DNA-based Methods for food authentication. Trends Food Sci Technol 11: 67-77.

Luis G, Rubio C, Gutiérrez Á J, González-Weller D, Revert C, Hardisson A (2014) Evaluation of metals in several varieties of sweet potatoes (*Ipomoea batatas* L.): comparative study. Environ Monit Assess 186: 433-440.

Madesis P, Ganopoulos I, Sakaridis I, Argiriou A, Tsaftaris A (2014) Advances of DNA-based methods for tracing the botanical origin of food products. Food Res Int 60: 163-172.

Mahlangeni N, Moodley R, Jonnalagadda S B (2012) Soil Nutrient Content on Elemental Uptake and Distribution in Sweet Potatoes. International Journal of Vegetable Science 18: 245-259.

Marcussen H, Joergensen K, Holm P E, Brocca D, Simmons R W, Dalsgaard A (2008) Element contents and food safety of water spinach (*Ipomoea aquatica* Forssk.) cultivated with wastewater in Hanoi Vietnam. Environ Monit Assess 139: 77-91.

Mulokozi G, Mugyabuso J, Modaha F (2007) Potential of cassava and sweet potato leaves to contribute to the vitamin A requirements. Proceedings of the 13th ISTRC symposium; Dar Salam, Tanzania: 755-762.

Nagai H, Okazaki Y, Chew S H, Misawa N, Yamashita Y, Akatsuka S, Ishihara T, Yamashita K, Yoshikawa Y, Yasui H, Jiang L (2011) Diameter and rigidity of multiwalled Carbon nanotubes are critical factors in Mesothelial Injury and Carcinogenesis. Proc Natl Acad Sci USA 108: E1330-E1338.

Norton-Brown Herbarium (2017) Plant identification guide. Available at: http://www.nbh.psla.umd.edu/guides/guides.html. Accessed on 30th January 2018.

Pang X, Liu C, Shi L, Liu R., Liang D, Li H, Cherny S S, Chen S (2012) Utility of the *trnH–psbA* intergenic spacer region and its combinations as plant DNA barcodes: a meta-analysis. PLoS ONE 7: p.e48833.

Rai U N, Sinha S (2001) Distribution of metals in aquatic edible plants: Trapa natans (Roxb.) Makino and *Ipomoea aquatica* Forsk. Environ Monit Assess 70: 241-252.

Rapid Tables (2017) Available at: https://www.rapidtables.com/web/colour/RGB_Colour.html. Accessed on 19th December 2017.

Reid L M, O'donnell C P, Downey G (2006) Recent Technological Advances for the Determination of Food Authenticity. Trends Food Sci Technol 17: 344-353.

SAS version 9.4. SAS Institute Inc., Cary, NC, USA.

Sears M E, Genuis S J (2012) Environmental determinants of chronic disease and medical approaches: recognition, avoidance, supportive therapy, and detoxification. J Environ Public Health: 356798.

Singh D, Singh B, Goel R K (2012) Role of saponins for the anticonvulsant effect of adventitious roots of Ficus religiosa. Pharm Biol 50: 816-822.

Sun H, Mu T, Xi L, Zhang M, Chen J (2014) Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. Food Chem 156: 380-389.

Toxicological profile for Beryllium (2002) Agency for Toxic Substances and Disease Registry [Internet]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available at: https://www.atsdr.cdc.gov/phs/phs.asp?id=339&tid=33; Accessed on 1st June 2018.

Uluozlu O D, Tuzen M, Mendil D, Soylak M (2009) Assessment of trace element contents of chicken products from turkey. J Hazard Mater 163: 982-987.

Umar K J, Hassan L G, Dangoggo S M, Ladan M J (2015) Nutritional composition of water spinach (*Ipomoea aquatica* Forsk.) leaves. Eur J Nutr 54: 11–16.

Van Amburgh J A (2018) What are the maximum oral doses of Potassium, Calcium Phosphorus, and Magnesium? Available at: https://www.medscape.com/viewarticle/771121. Accessed on 21st September 2018.

Van de Wiel H J (2003) Determination of elements by ICP-AES and ICP-MS. National Institute of Public Health and the Environment (RIVM). Bilthoven, The Netherlands:1-9.

Wang K S, Huang L C, Lee H S, Chen P Y, Chang S H (2008) Phytoextraction of Cadmium by *Ipomoea aquatica* (water spinach) in hydroponic solution: effects of Cadmium speciation. Chemosphere 72: 666-672.

Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N (2012) Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 46: 2242–2250.

Woolfe J A (1992) Sweet potato: an untapped food resource. Cambridge: Cambridge University Press.

Woolfe M, Primrose S (2004) Food forensics: using DNA technology to combat misdescription and fraud. Trends Biotechnol 22: 222-226.

World Health Organization (WHO) (2003) Zinc in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/zinc.pdf. 3rd April 2018. Available from: http://www.who.int/water_sanitation_health/dwq/chemicals/zinc.pdf.

Zeng H (2009) Selenium as an essential micronutrient: roles in cell cycle and apoptosis. Molecules 14: 1263-1278.