A METHOD FOR SILICON ANALYSIS IN CITRUS AND HORTICULTURE LEAF TISSUE

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Abstract

Silicon (Si) is classified as a beneficial element in higher plants. Although there is an abundance of literature on Si extraction and analysis in graminaceous crops such as rice and sugarcane, there is limited information on the validation of these methods for most dicotyledonous horticultural crops. These methods include gravimetric extraction, which requires a larger sample size the smaller the Si content in the test plant. An autoclave inductive digestion is considered effective by some and criticized by others due to the use of a strong oxidant and its sensitivity to the level of Si in the plant material. Microwave digestion occurs in a closed system which avoids contamination is quick and results in a high recovery rate due to its high extraction temperature. The aim of this experiment was to validate Si measurement following microwave digestion using inductively coupled plasma optical emission spectrometry (ICP-OES) and molybdenum blue colorimetry. The recovery of Si additions as SiO_2 was 82%-96% by ICP-OES and 85-94% by colorimetry. The recoveries did not differ between measurement methods ($P>0.05$), but the ICP-OES were more consistent. The limit of detection for Si determination by means of ICP-OES and that for the spectrophotometry method were 71 µg L^{-1} and 330 µg $L^{-}1$, respectively.

Keywords: microwave digestion; ICP-OES; spectrophotometer; detection limit

1. Introduction

Silicon (Si) is abundant in soil, it is only found in small quantities because of the low solubility of the complex it forms with Al (aluminosilicates) (Epstein 1999; Haynes 2014). Repeated cropping can further reduce the level of bioavailable Si (Epstein 1999; Savant *et al.*1999; Datnoff *et al.* 2001; Ma and Takahashi 2002). Silicon is absorbed by plants as monosilicic acid ($Si(OH)_4$), which can be found in the interstitial water of soils at concentrations $\leq$ 28 mg L^{-1} (Epstein 1999). Plants treated with Si fertilizers take up more Si and other nutrients (Raven 2003; Ma 2004). Nonetheless, Si is not considered essential for higher plants, although it benefits in the growth and development of some plants and alleviates biotic and abiotic stress (Miyake and Takahashi 1978; Adatia and Besford 1986; Liang *et al.* 2005; Romero-Aranda *et al.* 2006; Li *et al.* 2007; Liu *et al.* 2014; Li *et al.* 2015; Wang *et al.* 2015; Habibi 2015, 2016; Tubana *et al.* 2016; Rao and Susmitha 2017).

Many methods have been developed to extract and measure plant Si. For example, the gravimetric method is adequately sensitive for high-Si accumulators such as rice (*Oryza sativa L.*) and sugarcane (*Saccharum Officinarum* L.). However, gravimetry is not suitable for most horticultural crops which have a lower Si content King *et al.* (1955). Autoclave induced digestion (AID) uses a mixture of hydrochloric and hydrofluoric acids (HCl-HF) and microwave-assisted digestion using nitric acid (HNO_3) plus hydrogen peroxide followed by sodium hydroxide (NaOH) (Van der Vorm 1987; Elliott and Snyder 1991; Haysom and Ostatek-Boczynski 2006). The first published method for Si analysis was for rice *(Oryza sativa L.*) and entailed oxidative removal of the organic components then solubilisation of silica by fusion with NaOH in a nickel crucible, and colorimetry as the molybdate complex (Fox *et al.* 1969).

Autoclave induced digestion (AID) for Si extraction with NaOH and H_2O_2 has the advantages of low cost and rapid extraction (Elliott and Snyder 1991). It works well with rice but generates variable and typically low results, relative to extractants containing HF, for sugarcane, maize stalk (*Zea mays*), and leaves of peach (*Prunus persica*) and bluegrass (*Proa Pratensis*) (Taber *et al.* 2002; Ostatek-Boczynski and Haysom 2003; Kraska 2009; Kraska and Breitenbeck 2010).

However, HF can lead to the formation silicon tetrafluoride (SiF_4) and the routine handling of HF requires personal protection and HF-resistant equipment (Taber *et al.* 2002).

In contrast, microwave assisted digestion using HNO_3 and H_2O_2 followed by NaOH provides a safe reliable extraction method for Si from rice, sugarcane and pasture grasses and a strong linear relationship with Si extracted by dry ashing (Ostatek-Boczynski and Haysom 2003; Haysom and Ostatek-Boczynski 2006).

Silicon measurements made using the inductively coupled plasma optical emission spectrometry (ICP-OES) are sensitive, and can be accurate and precise (Lichte *et al.* 1980; Hou *et al.* 2006). Consequently, for plant extracts, comparison of Si measured by using ICP-OES and by older colorimetric methods should be a suitable means of validating a method of measuring total plant Si (Elliott and Snyder 1991; Kraska 2009). Indeed, for leaves of apple (*Malus domestica*), peach, maize and bluegrass a correlation of 0.99 has been reported between the two methods (Taber e*t al*. 2002) with the sensitivity of colorimetry being 6% below that of ICP-OES (Taber *et al.* 2002; Kraska and Breitenbeck 2010).

The sensitivity is important as it determines the lower quantification limit of a method (Hou *et al.* 2006; Sivakumar *et al.* 2006).

The aim of this study was to validate an accurate and rapid procedure for Si extraction and quantification in horticultural crops such as tomato, lettuce, and citrus by using a microwave digestion followed by ICP-OES analysis and spectrophotometer analysis. We also measured surface bound Sion leaves.

2. Materials and Methods

2.1. Pot trial

The pot trial was conducted in a climatically controlled glasshouse at the Hatfield Experimental Farm of the University of Pretoria (S25^{o} 44' E28°15'). Valencia ("Delta"), Clementine ("Nules"), lettuce (*Lactuca sativa* L.), and tomato ((*Solanum lycopersicum* L.) leaves were grown in pots with different levels of Si supply.

The Si content in citrus trees was determined in young and old leaves Valencia and Clementine varieties to compare the pattern of uptake found in previous experiments (Matichenkov *et al.* 2001; Ma and Takahashi 2002).

2.2. Removal of surface bound Si

Three liquids were tested to remove surface bound Si from the leaves Valencia, Clementine, lettuce, and tomato leaves, namely deionised water, 10% acetone and 10% ethanol (Rossini Oliva and Raitio 2003). The leaves were left for five min then wiped dried before placing them in the oven dried at 60^{o}C for 48 h. The experiment was done in triplicate.

2.3. Silicon extraction

Polypropylene and polyfluorocarbon containers were used during the preparation and digestion of the samples. All containers had been with 10% NaOH solution e to minimize Si

contamination (Taber *et al.* 2002). The microwave-assisted digestion method was adapted from Haysom and Ostatek-Boczynski (2006). Three mL of 65% HNO_3 (EMSURE, reagent grade, Merck) was added to 500 mg of dry, ground leaf samples in the 55 mL polyfluorocarbon reaction vessels tubes were capped and left to stand for five min before two mL of 30% H_2O_2 (reagent grade, Merck) was then added, and thoroughly mixed, recapped while left to stand overnight. The tubes were placed in the ceramic tube holders of a microwave system (Mars 5 CEM Corporation, North Carolina, United States of America) and ramped to 180^{o}C over 15 min, at which it was maintained for 15 min. The tubes were left to cool, and then 20 mL of 10% NaOH solution was added, the tubes were recapped and microwaved as before. The digests were allowed to cool then the contents of the tubes were neutralized with HNO_3 (2 M) in a plastic beaker, using phenolphthalein as the indicator, and then diluted to 250 mL with Type I deionised (DI) water (0.18 µS cm^{-1}) in a volumetric flask to reduce the concentration of sodium nitrate matrix. The Si concentrations were determined using both colorimetric and ICP-OES analysis.

2.4. Silicon measurement

2.4.1. ICP-OES analysis

An ICP-OES procedure was used to measure the Si concentration. The plasma power was 1000 W with a plasma flow rate of 15 L min^{-1} and an integration time of 1s. Calibrating standards for the ICP-OES instrument (Agilent 720 series, Victoria, Australia) were prepared from a 1000 mg L^{-1} standard (Fluka, Switzerland). The ICP was fitted with an alkaline resistant torch, Sturrman Master spray-chamber and a V Groove nebulizer assembly in axial view.

The delay time for washing between samples and signal measurement was set to 3min.

To reduce signal interference, background corrections were done 0.01 nm to the right side of peak and the matrices of the calibrating standards and extracts were matched. The upper end

of the working range was set beyond the highest expected concentration in the samples, i.e. 0, 0.5, 1, 2, 5, 10 and 15 mg Si L^{-1}. The calibration was linear (r = 0.999). The measurement of a blank solution after measuring 1 mg L^{-1} calibration standard indicated the lack of memory effect.

An interval correction was done after every ten consecutive samples run by running a known Si standard concentration to monitor the stability of the ICP-OES and to detect any drift in the readings. When this occurred, the ICP-OES was re-calibrated. After twenty consecutive samples were run, a known Si standard and a blank were introduced into the sequence to reduce contamination and to make automatic correction of any drift in the readings. The blank sample was run through the instrument at the beginning of each element concentration series and between series. Between series, the blank sample helped check for the element residue to confirm that it had been completely washed out.

2.4.2 Colorimetry

Fifty mL of the aliquot extracted was placed in a polycarbonate test tube. The Si concentration was measured using the method of Elliott and Snyder (1991). Briefly, 35mL of 20% acetic acid and 10 mL of ammonium molybdate (54 g L^{-1}, pH 7.0) were added, mixed and the solution left to stand for five minutes. Then 5 mL of tartaric acid (20%) was added, followed by 1 mL of a reducing solution containing: sodium sulfite, sodium bisulfite and 1-amino-2-naphthol-4-sulfonic acid. The solutions were mixed and stood for 30 min before absorbance was measured using a cell with a 10 mm path at 650 nm using a UV/Vis spectrophotometer (Beckman Coulter DU 530) calibrated at 650 nm. Finally, the absorbance was compared to a standard calibration curve of known Si concentrations combined with both soluble Si and the reagents as described previously.

Sensitivity of the method

According to Froes *et al.* (2009) the detection limit of ICP-OES is estimated as the limit of quantification (LOD) using the equation:

$$LOD = (3 \times RSD_{Blank} \times BEC) /100 \qquad \text{Eq 1,}$$

where BEC is the background equivalent concentration and RSD_{Blank} is the relative standard deviation of the blank (n = 10).

The BEC equation is expressed as,

$$BEC=C_{Element}/SBR \qquad \text{Eq 2,}$$

Where $C_{Element}$ is the concentration and SBR is the signal to background ratio.

Accuracy

The recovery efficiency of Si was determined for the microwave assisted digestion. Known amounts of Si (0, 2000 and 4000 mg kg^{-1}), based on the sample Si content, were added to three replicates of leaf samples for the different crops. An interval correction was done after every ten consecutive samples run by using a known Si standard concentration to monitor the stability of the ICP-OES and to detect any drift in the readings. When this occurred the ICP-OES was re-calibrated. In addition, after twenty consecutive samples run, a known Si standard and a blank were introduced in the sequence to reduce contamination and make automatic correction of any drift in the readings.

The samples and standards were measured in triplicate and the relative standard deviation (RSD) was < 3%.

Limit of detection

The limit of detection (LOD) was calculated based on the formula 3.3 δ/S, where δ was the standard deviation of a blank replicate determined under the same conditions, and S was the sensitivity, taken from the slope of the calibration graph (Al-Ghannam and Al-Olyan 2008).

Accuracy

The recovery efficiency of Si was determined using microwave assisted digestion followed by Si quantification using the spectrophotometer. Known amounts of Si (0, 1000 and 2000 mg kg^{-1}), based on the sample Si content range determined in the Si treated leaves, were added to three replicates of leaf samples for the different crops.

2.6. Statistical analysis

The collected data was subjected to analysis of variance by using Statistical Analysis System software (SAS) version 9.4 (Cary, NC, USA) to determine treatment mean effects. Differences between treatments were determined using Fisher's Least Significant Difference (LSD) at 5% level of significance. The paired t test was conducted to find out if there was any significant difference between these two methods.

The method was validated according to the international guidelines ISO/IEC year (International Organization for Standardization year).

3. Results

3.1. Foliar bound Si removal

The most effective method of removing Si bound to the leaf surfaces was investigated using three solvents and a control. The Si content of the unwashed control and ethanol-washed lettuce and tomato leaves were significantly higher than leaves washed with deionised water and acetone for all leaf tissues (Table 1). Within solvent treatment, Si content of the Valencia and Clementine leaves were not significantly different from each other (Table 1).

Table 1. The effect of foliar bound Si removal on Si leaf content (mg kg^{-1})

	Washing Solvents			
Leaf Samples	**Acetone**	**Deionized water**	**Ethanol**	**Unwashed Control**
Valencia	1389[b]	1404[b]	2136[a]	2177[a]
Clementine	1380[b]	1415[b]	2093[a]	2104[a]
Lettuce	883[b]	873[b]	1251[a]	1258[a]
Tomato	2029[b]	2045.70[b]	2368[a]	2369[a]

*The means in rows followed by similar letters do not differ at ($P \leq 0.05$) according to Fisher's LSD test. LSD: 50.01, CV%: 25.6 and Sed: 7.2. Values are means of silicon content **(**mg kg^{-1}) in three replicates of leaves of the four species.

3.2. Validation of inductively coupled plasma optical emission spectroscopy

After microwave extraction, Si levels were analyzed using ICP-OES. Its recovery efficiency ranged between 82- 96% and the recovery rate increased as the amount of Si added to the leaf samples increased (Table 2). The highest recovery rates were observed for the leaves of Clementine and Valencia. The coefficient of variation was generally low, in the range of 0.4-3.5%.

Table 2. Silicon recovery (%) from mature plant tissue extracted with microwave technique using nitric acid/hydrogen peroxide and sodium hydroxide, followed by analysis in triplicate using ICP-OES

Plant tissue	Si added (mg kg^{-1})	Recovered Si (mg kg^{-1})	SD	CV (%)	Recovery (%)
Valencia	0	2358	41	1.7	-
	2000	3954	92	2.30	91
	4000	5956	43.7	0.73	94
Clementine	0	2269	42	1.30	-
	2000	3930	48	1.20	92
	4000	5998	30	0.50	96
Lettuce	0	967	20	3.50	-
	2000	2675	82	0.75	82
	4000	4702	51.51	1.09	95
Tomato	0	5201	14	1.40	-
	2000	6169	21	0.40	86
	4000	8422	48.18	0.57	91

*****Values in the four species are means of three replicates with CV% and SD. The recovery efficiency was calculated from Si recovered and applied Si.

The ICP-OES sensitivity was tested by measuring the lowest concentration of an analyte in a sample that is detectable and distinguishable from the noise level of the system. The limit of detection was 0.071 mg L^{-1} (Table 3)**.**

Table 3. Silicon limit of detection using ICP-OES analysis

Parameters for detection limit quantification	**ICP-OES detection limit**
BEC	0.075 mg L^{-1}
RSD_{Blank}	28.7%
LOD	0.071 mg L^{-1}

*Values are Si measured by determine Si concentration from the blank. BEC= background equivalent concentration and LOD= Limit of detection

3.3. Validation of colorimetric analysis

After a standard extraction, the Si levels in leaf tissues were analyzed using a spectrophotometer, which was evaluated for accuracy and precision. The recovery efficiencies ranged between 85-94%. Recovery % were lower at the higher Si level added except for lettuce (Table 4). The standard deviation was generally low with exception of the control treatments. The CV% was in the range of 0.35-12.01%. In addition, lower CV% were observed in all leaf tissues at the higher Si level added.

Table 4. Silicon recovery (%) from mature plant tissue extracted with nitric acid/hydrogen peroxide and sodium hydroxide analyzed in triplicate using a spectrophotometer

Plant tissue	Si added (mg kg^{-1})	Recovered Si (mg kg^{-1})	SD	CV (%)	Recovery (%)
Valencia	0	1957.75	19.80	1.01	-
	1000	2758.70	0.05	5.40	92
	2000	3455.90	0.003	0.35	85
Clementine	0	1433.39	41.10	2.87	-
	1000	2334.75	0.01	1.10	94
	2000	3221.40	0.01	1.08	92
Lettuce	0	811.17	5	0.62	-
	1000	1701.70	0.01	1.45	94
	2000	2658.7	0.013	1.34	94
Tomato	0	2429.23	291.87	12.01	-
	1000	3122.4	0.0544	5.82	93
	2000	3951.7	0.028	3.06	91

*Values in the four species are means of three replicates with CV% and SD. The recovery efficiency is calculated from Si recovered and applied Si.

The spectrophotometer detection limit (LOD) was computed from the sensitivity and standard deviation of the set measurements. The LOD was 0.3 mg L^{-1} (Table 5), which was five times higher than the detection limit of ICP-OES (Table 3).

Table 5. Silicon limit of detection using Spectrophotometer analysis

Parameters for detection limit quantification	**Spectrophotometer detection limit**
Sensitivity	0.0067
Standard deviation	0.06
LOD	0.3 mg L^{-1}

The regression analysis of the citrus leaf data indicated a linear relationship of 99% between colorimetric analysis and ICP-OES (Figure 2). These two analytical methods have a strong relationship implying as the Si levels measured by ICP-OES increased, the same pattern was observed with colorimetric analysis.

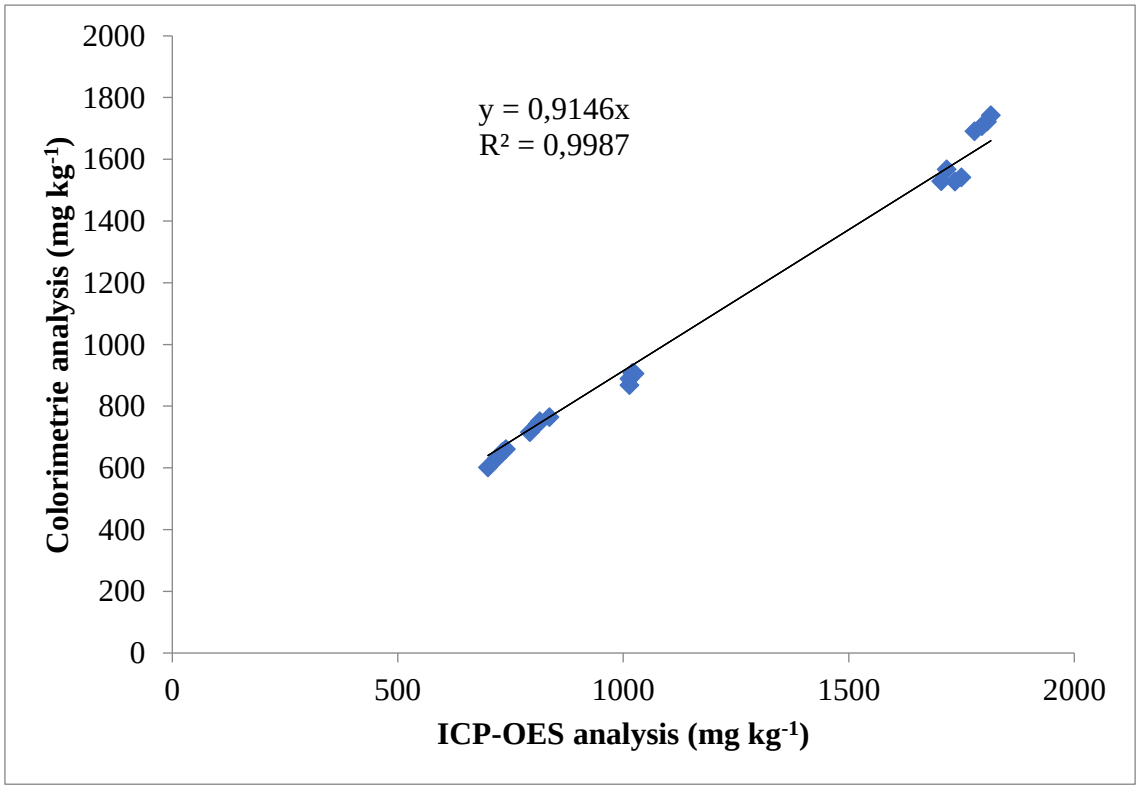

Figure 1. The correlation between results from the ICP-OES analysis and results from the colorimetric analysis of Si in citrus leaf tissues.

Silicon uptake was investigated in young and mature leaves in both citrus species (Table 6). A significant increase in Si content was observed in old leaves compared to young leaves in both species.

Table 6. Silicon content (mg kg^{-1}) in young and old Valencia and Clementine leaves

Varieties	Leaf maturity	Si (mg kg^{-1})
Valencia	Young	462[b]
Valencia	Old	1311[a]
Clementine	Young	439[b]
Clementine	Old	1260[a]

*****LSD: 15.48 means followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test. Values are means of three replicates.

4. Discussion

Leaf surface cleaning assists in the removal of dust particles, which is made up of elements such as Si, Al, Mg, K, Ca, S and Fe Shi *et al.* (2008). The removal of foliar bound Si was most effective using deionised water or acetone treatments for all leaf types. It proposed that the deionized water was able to dissolve surface-bound Si. In contrast, acetone is non-polar, and it is hypothesized that it dissolved the epidermal waxes binding superficial Si. Use of one of these treatments is needed before leaves are analysed for their Si content in uptake studies (Rossini Oliva and Raitio 2003).

Method accuracy in analytical determination is conducted by evaluating the recovery efficiency (González and Herrador 2007). Silicon concentration correlated with recovery efficiency and CV%, hence the samples with the highest Si contents were extracted and analysed with the most precision (Table 2). The high recovery efficiency by ICP-OES analysis is more likely due

to the measurement of total Si (George *et al.* 2000; Haysom and Ostatek-Boczynski 2006). The low CV% values confirmed the consistency of the analysis it provided.

The sensitivity of a method is linked to the lowest level of detection by the instrument (Al-Ghannam and Al-Olyan 2008). The ICP-OES limit of detection was 71 µg L^{-1} (Table 3). The detection limit was 12 times lower than the lowest Si concentration measured in the samples, which gave an indication of high signal to noise ratio of ICP-OES, which reflects the high sensitivity and accuracy of this analytical method (Hou *et al.* 2006; González and Herrador, 2007). The recovery efficiency of Si using the spectrophotometer ranged between 85-94%, which was within the range of experimental error, and it also had a low variability (Table 4). In this study, the limit of detection for the spectrophotometer technique was 0.3 mg L^{-1} (Table 5), which suggested that ICP-OES is a more sensitive analytical method, possibly because it has fewer steps and less interferences Lichte *et al.* (1980).

The two analytic methods were compared by the correlation between results from the ICP-OES and colorimetric analysis. The correlation of Si in the leaf tissues was 99% and the regression equation shown in Figure 2 had a slope of 0.914. The strong linear relationship between ICP-OES analysis and colorimetric analysis technique corroborates with previous studies Taber *et al.* (2002). This suggested that ICP-OES can be used to determine Si content in the leaves of horticultural crops such as citrus. The Si content of leaves using the colorimetric technique was lower than those detected by ICP-OES, probably due to the instability of colour development. Another explanation could be that ICP-OES measures all forms of Si in solution, and the fact that Si standards were prepared in the same matrix as the samples, which resulted in the greater accuracy, as observed by Kraska and Breitenbeck (2010). In contrast, the development of the molybdenum blue colour measures Si ions only, therefore, it underestimates the total Si content (Taber *et al.* 2002; Kraska and Breitenbeck 2010).

In this experiment, the pattern of Si uptake in Valencia and Clementine was investigated to verify the reliability of the validated method. The Si content was significantly higher in the older leaves of both Valencia and Clementine than young leaves (Table 6). This agrees with previous results that reported Si content increases significantly with maturity in citrus, suggesting that Si is not easily redistributed within citrus plants (Wutscher 1989; Matichenkov *et al.* 2001; Ma and Takahashi 2002). This implies that Si accumulated in plants cannot be used during stress period because it polymerizes into silica gel, which makes it immobile in the plants (Epstein 1994; Ma and Yamaji 2006). The distribution pattern of Si within the citrus leaves can be explained by the transportation of Si along the transpiration stream as identified in cucumber and barley (Ma and Takahashi 2002).

Conclusions

Use of water or acetone removed much more of the foliar bound Si prior to extraction than ethanol. The inductively coupled plasma optical emission spectrometry (ICP-OES) analysis agreed with the conventional Spectrophotometer (colorimetric analysis) method. The ICP-OES analysis had a detection limit 10 times lower than the lowest Si concentration in the leaf tissues. It was more sensitive, precise, and faster than the colorimetric analysis. Microwave-assisted digestion extraction followed by ICP-OES analysis can be used as a sensitive, precise, and fast method for the analysis of horticultural crops for their Si content.

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References

ADATIA, M, and R. BESFORD. 1986. The effects of silicon on cucumber plants grown in recirculating nutrient solution. *Annals of Botany* 58: 343-351.

AL-GHANNAM, S, and A. AL-OLYAN. 2008. Spectrofluorometric determination of nicardipine, nifedipine and isradipine in pharmaceutical preparations and biological fluids. *Open Chemistry* 6: 222-228.

ALEXANDER, G.B., W. HESTON, and R.K. ILER. 1954. The solubility of amorphous silica in water. *Journal of Physical Chemistry* 58: 453-455.

BIRCHALL, J. 1995. The essentiality of silicon in biology. *Chemical Society Reviews* 24: 351-357.

DATNOFF, L.E., G.H. SNYDER, and G.H. KORNDÖRFER. 2001. Silicon in agriculture Elsevier.

ELLIOTT, C. and G.H. SNYDER. 1991. Autoclave-induced digestion for the colorimetric determination of silicon in rice straw. *Journal of Agricultural Food & Chemistry* 39: 1118-1119.

EMICH, F., F.L. SCHNEIDER, and F. FEIGL. 1932. Microchemical laboratory manual.

EPSTEIN, E. 1994. The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences* 91: 11-17.

EPSTEIN, E. 1999. Silicon. *Annual Review of Plant Biology* 50: 641-664.

FOX, R.L., J.A. SILVA., D.L. PLUCKNETT, and D.Y. TERANISHI. 1969. Soluble and total silicon in sugar cane. *Plant Soil* 30: 81-92.

FRANTZ, J.M., J.C. LOCKE., L. DATNOFF., M. OMER., A. WIDRIG., D. STURTZ., L. HORST, and C.R. KRAUSE. 2008. Detection, distribution, and quantification of silicon in floricultural crops utilizing three distinct analytical methods. *Communication in Soil Science & Plant Analysis* 39: 2734-2751.

FROES, R.E., W.B. NETO., R.L. NAVEIRA., N.C. SILVA., C.C. NASCENTES, and J.B. DA SILVA. 2009. Exploratory analysis and inductively coupled plasma optical emission spectrometry (ICP-OES) applied in the determination of metals in soft drinks. *Microchemical Journal* 92: 68-72.

GEORGE, S., S. STEINBERG, and V. HODGE. 2000. The concentration, apparent molecular weight and chemical reactivity of silica from groundwater in Southern Nevada. *Chemosphere* 40: 57-63.

GONZÁLEZ, A.G, and M. Á. HERRADOR. 2007. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *Trends in Analytical Chemistry* 26: 227-238.

HABIBI, G. 2015. Exogenous silicon leads to increased antioxidant capacity in freezing-stressed pistachio leaves. *Acta Agriculturae Slovenica* 105: 43-52.

HABIBI, G. 2016. Effect of foliar-applied silicon on photochemistry, antioxidant capacity and growth in maize plants subjected to chilling stress. *Acta agriculturae Slovenica* 107: 33-43.

HAYNES, R.J. 2014. A contemporary overview of silicon availability in agricultural soils. *Journal of Plant Nutrition & Soil Science* 177: 831-844.

HAYSOM, M.B, and Z.A. OSTATEK-BOCZYNSKI. 2006. Rapid, wet oxidation procedure for the estimation of silicon in plant tissue. *Communication in Soil Science & Plant Analysis* 37: 2299-2306.

HOU, X., R.S. AMAIS., B.T. JONES, and G.L. DONATI. 2006. Inductively coupled plasma optical emission spectrometry. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*: 1-25.

KING, E., B. STACY., P. HOLT., D.M. YATES, and D. PICKLES. 1955. The colorimetric determination of silicon in the micro-analysis of biological material and mineral dusts. *Analyst* 80: 441-453.

KRASKA, J.E. 2009. Assessing the silicon status of rice (*Oryza sativa*). Louisiana State University, Louisiana, United States of America.

KRASKA, J.E, and G.A. BREITENBECK. 2010. Simple, robust method for quantifying silicon in plant tissue. *Communication in Soil Science & Plant Analysis* 41: 2075-2085.

LI, H., Y. ZHU., Y. HU., W. HAN, and H. GONG. 2015. Beneficial effects of silicon in alleviating salinity stress of tomato seedlings grown under sand culture. *Acta Physiologiae Plantarum* 37: 71.

LI, Q., C. MA, and Q. SHANG. 2007. Effects of silicon on photosynthesis and antioxidative enzymes of maize under drought stress. *Ying yong sheng tai xue bao= The journal of applied ecology* 18: 531-536.

LIANG, Y., J. WONG, and L. WEI. 2005. Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays L*.) grown in cadmium contaminated soil. *Chemosphere* 58: 475-483.
LICHTE, F., S. HOPPER, and T. OSBORN. 1980. Determination of silicon and aluminum in biological matrixes by inductively coupled plasma emission spectrometry. *Analytical Chemistry* 52: 120-124.
LIU, P., L. YIN., X. DENG., S. WANG., K. TANAKA, and S. ZHANG. 2014. Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in Sorghum bicolor L. *Journal of Experimental Botany* 65: 4747-4756.
MA, J.F. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science & Plant Nutrition* 50: 11-18.
MA, J.F, and E. TAKAHASHI. 2002. Soil, fertilizer, and plant silicon research in Japan. 1st ed., Amsterdam/ Netherlands: Elsevier.
MA, J.F, and N. YAMAJI. 2006. Silicon uptake and accumulation in higher plants. *Trends in Plant Science* 11: 392-397.
MARKERT, B. 1996. Instrumental element and multi-element analysis of plant samples: methods and applications Wiley.
MATICHENKOV, V., E. BOCHARNIKOVA, and D. CALVERT. 2001. Response of citrus to silicon soil amendments. *Proceedings of Florida State Horticultural Society* 114: 94-97.
MIYAKE, Y, and E. TAKAHASHI. 1978. Silicon deficiency of tomato plant. *Soil Science & Plant Nutrition* 24: 175-189.
NOVOZAMSKY, I., R. VAN ECK, and V. HOUBA. 1984. A rapid determination of silicon in plant material. *Communication in Soil Science & Plant Analysis* 15: 205-211.
OSTATEK-BOCZYNSKI, Z, and M. HAYSOM. 2003. The determination of silicon in sugarcane tissue using a microwave-assisted acid and alkali digestion procedure. *Tools for nutrient and pollution management (Occasional report No 17). Fertilizer and Line Research Centre, Massey University, Palmerston North*: 419-424.
RAO, G.B, and P. SUSMITHA. 2017. Silicon uptake, transportation and accumulation in Rice. *Journal of Pharmacognosy & Phytochemistry* 6: 290-293.
RAVEN, J.A. 2003. Cycling silicon–the role of accumulation in plants. *New Phytol* 158: 419-421.
ROBERT, B., G. BERTONI., D. SAYAG, and P. MASSON. 1996. Assessment of mineral nutrition of cork oak through foliar analysis. *Communication in Soil Science & Plant Analysis* 27: 2091-2109.
ROMERO-ARANDA, M.R., O. JURADO, and J. CUARTERO. 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *Journal of Plant Physiology* 163: 847-855.
ROSSINI OLIVA, S, and H. RAITIO. 2003. Review of cleanning techniques and their effects on the chemical composition of foliar samples. *Boreal Environment Research* 8: 263-272.
SAVANT, N.K., G.H. KORNDÖRFER., L.E. DATNOFF, and G.H. SNYDER. 1999. Silicon nutrition and sugarcane production: a review. *Journal of Plant Nutrition* 22: 1853-1903.
SHI, Z., D. ZHANG., M. HAYASHI., H. OGATA., H. JI, and W. FUJIIE. 2008. Influences of sulfate and nitrate on the hygroscopic behaviour of coarse dust particles. *Atmospheric Environment* 42: 822-827.
SIVAKUMAR, V., L. ERNYEI, and R.H. OBENAUF. 2006. Guide for determining ICP/ICP-MS method detection limits and instrument performance. *Atomic Spectroscopy* 21: 13.
SPOSITO, G. 2008. The chemistry of soils. 2nd ed. Oxford university press.
TABER, H.G., D. SHOGREN, and G. LU. 2002. Extraction of silicon from plant tissue with dilute HCl and HF and measurement by modified inductive coupled argon plasma procedures. *Communication in Soil Science & Plant Analysis* 33: 1661-1670.
TUBANA, B.S., T. BABU, and L.E. DATNOFF. 2016. A review of silicon in soils and plants and its role in US agriculture: history and future perspectives. *Soil Science* 181: 393-411.
VAN DER VORM, P. 1987. Dry ashing of plant material and dissolution of the ash in HF for the colorimetric determination of silicon. *Communication in Soil Science & Plant Analysis* 18: 1181-1189.
WANG, S., P. LIU., D. CHEN., L. YIN., H. LI, and X. DENG. 2015. Silicon enhanced salt tolerance by improving the root water uptake and decreasing the ion toxicity in cucumber. *Frontiers in Plant Science* 6: 759.
WUTSCHER, H. 1989. Growth and mineral nutrition of young orange trees grown with high levels of silicon. *HortScience* 24: 3.

ZHU, J., Y. LIANG., Y. DING, and Z. LI 2006. Effect of silicon on photosynthesis and its related physiological parameters in two winter wheat cultivars under cold stress. *Zhongguo nongye kexue* 39: 1780-1788.