# Synchrotron X-Ray Based Investigation of As and Fe Bonding Environment in Collard Green Tissue Samples at Different Growth Stages

Sunil Dehipawala, Aregama Sirisumana, P. Schneider, G. Tremberger Jr, D. Lieberman, Todd Holden T. Cheung

***Abstract*****—**The arsenic and iron environments in different growth stages have been studied with EXAFS and XANES using Brookhaven Synchrotron Light Source. Collard Greens plants were grown and tissue samples were harvested. The project studied the EXAFS and XANES of tissue samples using As and Fe K-edges. The Fe absorption and the Fourier transform bond length information were used as a control comparison. The Fourier transform of the XAFS data revealed the coexistence of As (III) and As (V) in the As bonding environment inside the studied plant tissue samples, although the soil only had As (III). The data suggests that Collard Greens has a novel pathway to handle arsenic absorption in soil.

***Keywords*****—**EXAFS, Fourier Transform, metalloproteins, XANES

## I. Introduction

ARSENIC is a naturally occurring substance in the Earth's crust. Average arsenic concentration of the Earth's crust is approximately 1.5 ppm. But the amount present varies depending on the geographic location and type of soil and rock. In some areas it can go up to 35,000 ppm [1]. Arsenic and its compounds are known toxics. According to Environmental Protection Agency (EPA) and the World Health Organization (WHO) arsenic is a well known carcinogen [2]. But toxicity depends on the type of compound. For example, arsenic in organic compounds is less toxic but arsenic in inorganic compounds is more toxic. Arsine (ASH3), arsenite, and arsenate in particular are highly toxic compounds. Humans unknowingly consume arsenic through drinking water and the plants they consume. In addition to naturally occurring arsenic, the arsenic concentration of soil increases due to human activity. Arsenic compounds are in our environment and used in the industry sector in the manufacturing of semi-conductors, glass production, papers, metal adhesives, ceramics, wood preservatives, and explosives [3]. Inorganic arsenic compounds were used as pesticides in the US before the 1950's. Naturally occurring arsenic and arsenic from waste products from industries are becoming a growing concern to human health as use of material containing arsenic and expansion of human settlements to former agricultural and industrial lands widens. Small doses of arsenic in the body are considered harmless. Once arsenic enters the body it circulates within the blood stream and exits the body through urine. But high doses of arsenic can be very toxic. It can cause cancer, bladder, liver, and kidney problems. There are numerous studies concerning the amount of arsenic found in food crops. But most of them lack the identity or chemical nature of arsenic compounds in plants. X-ray absorption spectroscopy (XAFS), which includes X-ray Absorption Near Edge structure (XANES) and Extended X-ray absorption fine structure (EXAFS), is a very useful tool for the examination of local environments of specific elements. For example, absorption edge energy is sensitive to the oxidation state of absorbing atom. Arsenic occurs with several stable oxidation states such as -3, -1, 0, 3, and 5. XANES can be used to identify the oxidation state of arsenic compounds present in soil and plants. EXAFS is a valuable tool to determine parameters such as near neighbor bond length, number, and type of near neighbor atoms. Both of these techniques do not require long range order. Therefore it is ideal to study amorphous material such as plant leaves.

Recent X-ray absorption arsenic research activities as reported in the literature include the Monte Carlo simulation study on XANES As(III) data fitting [4], the X-ray accelerated photo-oxidation of As(III) to As(V) study under XANES condition [5], and the As sorption by Fe-Mn oxide sorbent study using XANES and EXAFS data [6]. The delivery of the anti-cancer drug arsenic trioxide via hollow silica nanoparticles was also reported [7]. On the other hand the health issue in vegetable consumption has always been a popular research topic. For example, a study showed that steamed green/leafy vegetables including collard greens kale, mustard greens, broccoli, green bell pepper, and cabbage would lower the cholesterol level, the risk of cardiovascular disease and cancer [8]. The phenolic compounds, potential antioxidants against cancer, in leafy and baby-leaf vegetables have been reported as well [9], [10]. Therefore the arsenic absorption in a leaf vegetable will be an important issue. This report will focus on the arsenic absorption in collard greens, a member of the *Brassica oleracea* Acephala Group, as revealed by synchrotron based X-ray absorption data. Iron and arsenic uptake correlation in water fern has been reported and that a low iron leafy vegetable would be expected to have less arsenic [11]. A 100 gram serving of raw collard contains 0.21 mg Zn and 0.47 mg Fe per 100 gram, which is a good sample system to test the X-ray absorption method in terms of arsenic

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detectable limit as compared to kale, the other member of the *Brassica oleracea* Acephala Group, having 1.47 mg Fe and 0.56 mg Zn per 100 gram [12].

## II. Materials and Methods

The EXAFS and XANES data were collected at beamline X10C of National Synchrotron Light Source at Brookhaven national Laboratory. Rhodium coated cylindrical mirror is used to focus the beam on the sample. A double crystal Si(220) mono-chromator was used for energy selection. Ion chambers were used to measure beam intensity before the hutch, $I_0$ (before sample), and transmission intensity. A 7-element Si drift detector was used to measure fluorescence intensity. Piezoelectric driver using A/C feedback system locks the beam. Beam size on the sample was 10 mm x 2 mm. Beamline and data collection was controlled by microvax II computer running on VMS. The collard greens seed samples were purchased from consumer sources, and grown with water from New York City Water Supply and soil purchased from consumer sources. The calibration samples (As and Fe oxides) were purchased from Sigma Aldrich. The data analysis was done with EXAFSPAK and WIN-XAS packages.

## III. Results and Discussion

A typical Arsenic accumulated amount data curve is shown in Fig. 1. Fig. 2 is the iron accumulated amount data curve.

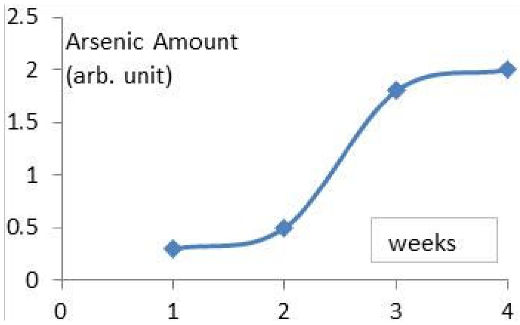

Fig. 1 Arsenic accumulated amount data curve

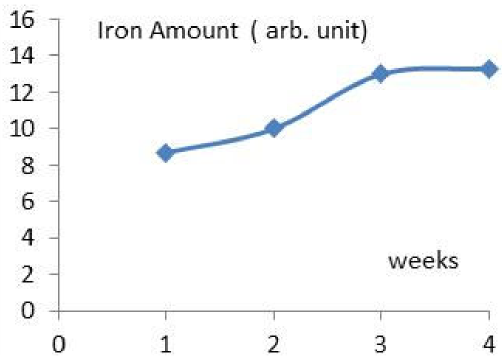

Fig. 2 Iron accumulated amount data curve

The X-ray Plant As XANES data is shown in Fig. 3.

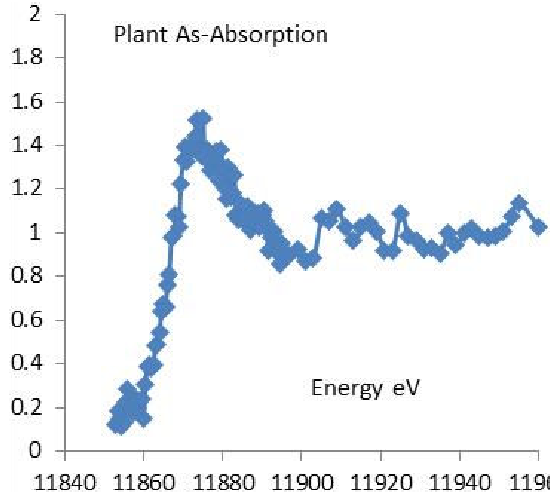

Fig. 3 The XANES Plant As data. The sample was harvested after one week of growth. The x-axis energy scale is in e

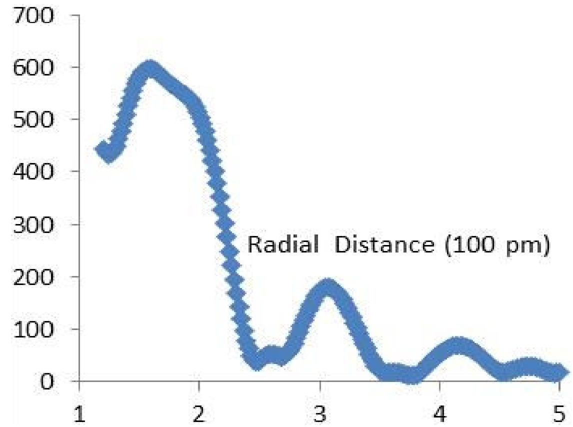

Fig. 4 The Fourier transform of Plant As k-cubed--weighted EXAFS (Y-axis). The x-axis is in Angstrom (100 pm)

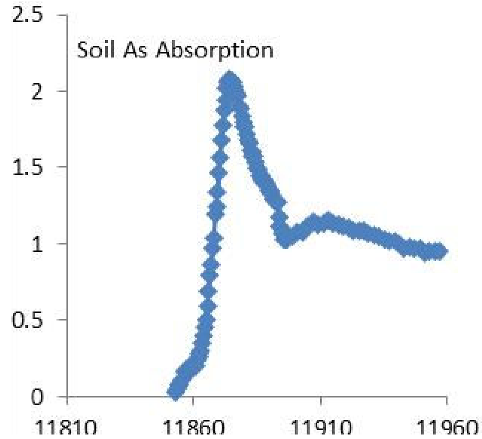

Fig. 5 The XANES Soil As data. The x-axis energy scale is in eV

The first peak in the Plant As Fourier transform k-cubed weighted EXAFS data in Fig. 4 was observed to be at 153 pm, which is shorter than that of the As bond length in the soil. The X-ray Soil As XANES data is shown in Fig. 5.

The X-ray Soil As XANES data is shown in Fig. 6.

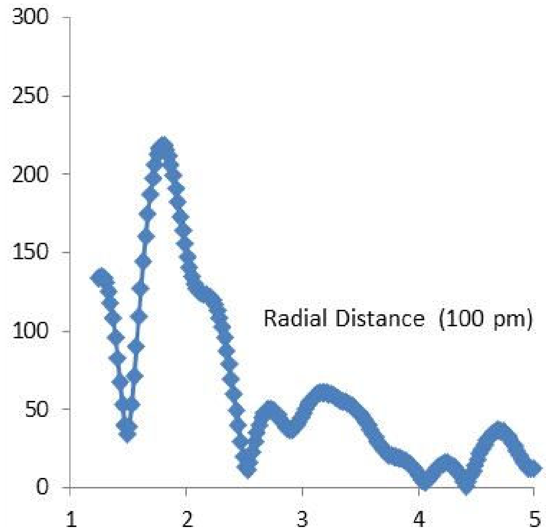


Fig. 6 The Fourier transform of Soil As k-cubed--weighted EXAFS (Y-axis). The x-axis is in Angstrom (100 pm)

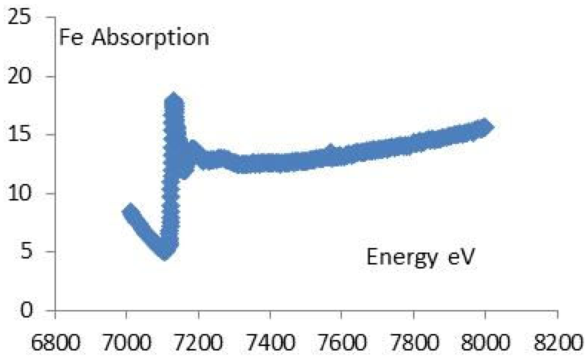


Fig. 7 The EXAFS Plant Fe data. The sample was harvested after one week of growth. The x-axis energy scale is in eV

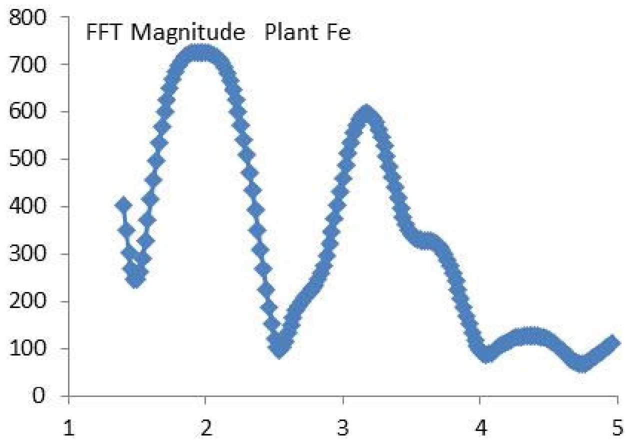


Fig. 8 The Fourier transform of Plant Fe k-cubed--weighted EXAFS (Y-axis). The x-axis is in Angstrom (100 pm)

The first peak in the Soil As Fourier transform k-cubed weighted EXAFS data in Fig. 6 was observed to be at 175 pm, which is consistent with the calibration standard of arsenic oxide. Therefore the interpretation that a plant would use its metabolism to change the absorbed As(III) to a mixture of As(III) and As (V) in its bonding environment would be a consistent inference. The As-O bond length could be less than that of Fe-O. It was reported that Fe-O and Fe-S have EXAFS bond lengths of 182 pm and 224 pm respectively in cytochrome 450 using the Advanced Photon Source at Argonne National Lab, USA [13]. They also listed the range of other reported Fe-O EXAFS bond lengths with a span from 164 to 193 pm. Therefore As-O bond length could be in the 150 pm range, and that the signal strength displayed in Fig. 3 would suggest that X-ray absorption is sensitive to pick up the As signal in one week old Collard Green. Other growth stage samples had stronger Arsenic X-ray absorption signal (as shown in Fig. 1) with similar FFT results.

In comparison the Plant Fe X-ray absorption and the FFT result are shown in Figs. 7 and 8 respectively.

The first peak in the Plant Fe Fourier transform k-cubed weighted EXAFS data in Fig. 6 was observed to be at 184 pm, which is consistent with the calibration standard of iron oxide.

The As and Fe absorption data shown in Figs. 1 and 2 are linearly correlated with the mass values. The absorption amount data resemble the standard S-shape growth curve and would suggest a regular uptake process from the studied As and Fe in the collard greens samples. The bond length information as extracted by FFT of the EXAFS and broad rise appears in the XANES suggests a mixture of As (III) and As (V) inside plant tissue samples although the soil samples carry the characteristics of As (III). Recent report suggests that biological gene mutation could enhance the ability to tolerate arsenic for people living in South America Argentinean Andes [14]. The AS3MT (arsenic [+3 oxidation state] methyltransferase) gene has mutated and the local people can tolerate arsenic up to 20 times above the accepted safety value. The human AS3MT gene product would produce an enzyme that would start the detoxification pathway and methylates arsenite to form methylarsonate. Then the methylarsonate reductase would reduce the methylarsonate to methylarsonite, which then be converted to the much less toxic compound dimethylarsinate [15]. A probable arsenite(III)-methyltransferase in *Halobacterium salinarum* was also cataloged [16]. According to our Uniprot databank search, collard green does not harbor an AS3MT equivalent pathway. It would be interesting to investigate if the As(III) oxidization to As(V) would have a similar pathway in the Brassica oleracea Species such that the vegetable could support a human-like AS3MT arsenic detoxification process. A recent review of arsenic stress in plants could be used to expand the list of vegetables having possible human-like AS3MT arsenic detoxification process [17]. The genetic study results on a chemoautotrophic As(III)-oxidizing bacterium in soil that uses As(III) oxidation as a detoxification mechanism could be used to provide additional pathway searching guidance [18]. Added source of guidance could be obtained from the recent results in the use of sodium meta-arsenite $NaAsO_2$ and arsenic trioxide ($As_2O_3$) in cancer treatment experiments [19], [20]. The duality role of arsenic as a toxin and as an anti-cancer agent could have a parallel analogy to soy where soy flour as whole food item could suppress cancer while soy isoflavone as a dietary supplement could promote cancer [21], [22]. The threat of arsenic and other toxic metals has been reported coming from seafood sources recently such that synchrotron X-ray based techniques are indispensible tools for future studies [23].

## IV. Conclusion

The project studied the EXAFS and XANES of tissue samples using As and Fe K-edges. Collard Greens tissue sample results suggest a novel pathway that As(III) from soil had been transformed to a mixture of As(III) and As(V) after absorption. Future X-ray absorption studies could include As absorption investigation in other leafy vegetables such as kale and the effect of As absorbing bacteria on plant growth.

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## References

[1] P. L. Smedley and D. G. Kinniburgh, Appl. Geochem. 17, 517-568 (2002).

[2] World Health Organization, Arsenic in Drinking Water. Fact Sheet No. 210, Revised May 2001. (http://www.who.int/inf-fs/en/fact210.html)

[3] P. J. Potts, M. H. Ramsey, J. Carlisle, Portable X-ray fluorescence in the characterization of Arsenic contamination associated with industrial buildings at a heritage Arsenic works near Redruth, Cornwall, UK. J. Environ. Monit, 4, 1017-1024, 2002.

[4] Canche-Tello J, Vargas MC, Hérnandez-Cobos J, Ortega-Blake I, Leclercq A, Solari PL, Den Auwer C, Mustre de Leon J. Interpretation of X-ray absorption spectra of As(III) in solution using Monte Carlo simulations. J Phys Chem A. 2014 Nov 20; 118(46):10967-73.

[5] Canche-Tello J, Vargas MC, Hérnandez-Cobos J, Ortega-Blake I, Leclercq A, Solari PL, Lezama-Pacheco J, Den Auwer C, Mustre de Leon X-ray Accelerated Photo-Oxidation of As(III) in Solution. J Phys Chem A. 2015 Mar 17. (Epub ahead of print) http://www.ncbi.nlm.nih.gov/pubmed/ 25730736

[6] Zhang G, Liu F, Liu H, Qu J, Liu R. Respective role of Fe and Mn oxide contents for arsenic sorption in iron and manganese binary oxide: an X-ray absorption spectroscopy investigation Environ Sci Technol. 2014 Sep 2; 48(17):10316-22.

[7] Zhao Z, Wang X, Zhang Z, Zhang H, Liu H, Zhu X, Li H, Chi X, Yin Z, Gao J. Real-Time Monitoring of Arsenic Trioxide Release and Delivery by Activatable T1 Imaging. ACS Nano. 2015 Feb 18. (Epub ahead of print) http://www.ncbi.nlm.nih.gov/pubmed/25688714

[8] Kahlon TS, Chiu MC, Chapman MH. Steam cooking significantly improves in vitro bile acid binding of collard greens, kale, mustard greens, broccoli, green bell pepper, and cabbage. Nutr Res. 2008 Jun; 28(6):351-7.

[9] Lin LZ, Harnly JM. Identification of the phenolic components of collard greens, kale, and Chinese broccoli. J Agric Food Chem. 2009 Aug 26;57(16):7401-8

[10] Santos J, Oliveira MB, Ibáñez E, Herrero M. Phenolic profile evolution of different ready-to-eat baby-leaf vegetables during storage. J Chromatogr A. 2014 Jan 31;1327:118-31

[11] M. Azizur Rahman, H. Hasegawa, K. Ueda, T. Maki, M. Mahfuzur Rahman. Influence of phosphate and iron ions in selective uptake of arsenic species by water fern (*Salvinia natans* L.). Chemical Engineering Journal Volume 145, Issue 2, 15 December 2008, Pages 179–184

[12] United States Department of Agriculture Agricultural Research Service National Nutrient Database for Standard Reference Release 27 http://ndb.nal.usda.gov/ndb/foods (Brassica oleracea)

[13] Newcomb M, Halgrimson JA, Horner JH, Wasinger EC, Chen LX, Sligar SG. (2008) X-ray absorption spectroscopic characterization of a cytochrome P450 compound II derivative. Proc Natl Acad Sci U S A. Jun 17; 105(24):8179-84.

[14] Schlebusch CM, Gattepaille LM, Engström K, Vahter M, Jakobsson M, Broberg K. Human Adaptation to Arsenic-Rich Environments.. Mol Biol Evol. 2015 Mar 3. pii: msv046. http://www.ncbi.nlm.nih.gov/pubmed/25739736

[15] http://www.uniprot.org/uniprot/Q9HBK9

[16] http://www.uniprot.org/uniprot/B0RA90

[17] Kumar S, Dubey RS, Tripathi RD, Chakrabarty D, Trivedi PK. Omics and biotechnology of arsenic stress and detoxification in plants: current updates and prospective. Environ Int. 2015 Jan; 74:221-30.. http://www.ncbi.nlm.nih.gov/pubmed/25454239

[18] Zhang J, Zhou W, Liu B, He J, Shen Q, Zhao FJ. Anaerobic arsenite oxidation by an autotrophic arsenite-oxidizing bacterium from an arsenic-contaminated paddy soil. Environ Sci Technol. 2015 Apr 2 http://www.ncbi.nlm.nih.gov/pubmed/25905768

[19] Tong D, Ortega J, Kim C, Huang J, Gu L, Li GM. Arsenic Inhibits DNA Mismatch Repair by Promoting EGFR Expression and PCNA Phosphorylation. J Biol Chem. 2015 Apr 23. http://www.ncbi.nlm.nih.gov/pubmed/25907674

[20] Li X & Sun WJ. The clinical activity of arsenic trioxide, ascorbic acid, ifosfamide and prednisone combination therapy in patients with relapsed and refractory multiple myeloma.. Onco Targets Ther. 2015 Apr 9; 8:775-81. http://www.ncbi.nlm.nih.gov/pubmed/25914547

[21] Yunxian Liu, Leena Hilakivi-Clarke, Yukun Zhang, Xiao Wang, Yuan-Xiang Pan, Jianhua Xuan, Stefanie C. Fleck, Daniel R. Doerge and William G. Helferich. Isoflavones in soy flour diet have different effects on whole-genome expression patterns than purified isoflavone mix in human MCF-7 breast tumors in ovariectomized athymic nude mice. Molecular Nutrition & Food Research, forth coming in press. http://onlinelibrary.wiley.com/doi/10.1002/mnfr.201500028/abstract.

[22] Juan E. Andrade, Young H. Ju, Chandra Baker, Daniel R. Doerge and William G. Helferich. Long-term exposure to dietary sources of genistein induces estrogen-independence in the human breast cancer (MCF-7) xenograft model. Molecular Nutrition & Food Research Volume 59, Issue 3, pages 413–423, March 2015. http://onlinelibrary.wiley.com/doi/10.1002/mnfr.201300780/abstract

[23] Salvo A, Cicero N, Vadalà R, Mottese AF, Bua D, Mallamace D, Giannetto C, Dugo G. Toxic and essential metals determination in commercial seafood: *Paracentrotus lividus* by ICP-MS. Nat Prod Res. 2015 Apr 28:1-8 http://www.ncbi.nlm.nih.gov/pubmed/25919907