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CHARACTERIZATION OF IRON IN DIFFERENT REGIONS OF SPINACIA OLERACEA PLANT USING X- RAY ABSORPTION

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***Abstract-* Chemical environment and the amount of iron in different regions of Spinacia oleracea plant were investigated using X-ray absorption. The main edge energy position of X-ray Absorption Near Edge Spectrum (XANES) is similar in mature leaves, young leaves, stems, and roots. The amount of iron present depends on the region of the plant. Mature leaves contain the most amount of iron. The amount of iron in stems is comparable to that of young leaves but less than mature leaves. Mature leaves and stems contain small amounts of iron next to oxygen atoms.**

Keywords – Spinacia Oleracea, XANES, Iron, Pre-Edge,DIstribution

I. INTRODUCTION

Iron is an essential nutrient not only for humans, but also for all types of plants. Plants use iron for chlorophyll formation, RNA metabolism, and transpiration process regulation[1]. The presence of iron increases the thickness of a leaf and hence the flow of nutrients[2]. Therefore, iron is an essential element for the growth of plants. Iron is one of the most abundant metals in soil and occurs in a wide range of chemical forms[3]. Since plants can absorb only certain species, all of the iron in the soil is not available for plants. For example, plants can absorb ferrous ions, but not ferric ions[4]. If the pH in the soil is higher than 7, iron ions can be tied to the soil and become unavailable to plants[4]. Therefore, the amount of iron absorption by plants is not solely dependent on the amount of iron present in the soil, but also on the chemical nature of the iron and the pH of the soil. Typical garden soil contains different functional groups such as carboxyls and amines, which interact with ferrous and ferric ions[5]. These interactions may influence the changes in oxidation state and solubility of iron species and consequently the availability of iron.

The human body requires about 2 mg of iron per day. In general, this required dose comes from the food we consume. However, the type of iron present is not identical in all varieties of foods. The absorption of iron into the body depends on the type of iron and other nutrients in the food. For example, iron found in meat products, also known as heme-iron, is absorbed into the body at 15-35 % of total iron present in the meat and not influenced by other nutrients. On the other hand, the absorption percentage of non-heme iron is 2-15% and is influenced by the presence of other nutrients. People who are vegans receive all of their nutrients from plant based food products or supplements. But not all of them can afford to pay for supplements. The plant Spinacia oleracea, commonly known as spinach, is a well known iron source. Iron distribution in different regions of the plant has not yet been investigated. The aim of this work is to investigate the type iron in spinach plant and its distribution in different regions.

II. EXPERIMENTAL TECHNIQUES

A common method used to analyze samples for a specific element is wet chemical analysis. However, this chemical method may alter the nature of the element during analysis. For example, wet chemical methods may be able to determine the total amount of iron in a specific sample, but it is not possible to obtain information such as bonding or type of near neighbor atoms of iron species present in the sample. X-ray absorption Spectroscopy (XAS) using synchrotron x-ray radiation is a valuable alternate to wet chemical analysis. Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES), both of which use different regions of X-ray absorption spectrum, are very powerful tools to study the local structure and bonding of metal compounds[6,7]. In XAS experiments, absorption of x-rays by a typical sample is measured as a function of X-ray energy. In general, absorption is measured in the energy range of about 150eV below the edge (for example 7112 of Fe K edge) to about 800 eV above the edge. XANES is the study of the feature immediately before and after the edge (about 50 eV above the edge) of the absorption spectrum. The main edge position of the XANES is the primary indicator of oxidation state of iron species[8]. The height (normalized to the sample mass) of the main edge from the background level is proportional to the amount of iron presence in the sample[8]. Analysis of EXAFS provides information such as bond length, bond angle, number and type of near neighbor atoms. Both XANES and EXAFS data

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can be collected during single scan of absorption coefficient as a function of X-ray energy.

III.SAMPLE PREPARATION

several different pots with soil samples were prepared with regular garden soil. In each pot a spinach plant was grown starting from seeds. Each plant was given water as necessary. No other chemicals or fertilizers were used. Six weeks after the germination of seeds, tissue samples from each plant were collected from different regions of the plants together with soil samples. Plant samples include: roots, stems, mature leaves and very young leaves. Samples were dried at 200^0C and grinded to a fine powder. Equal masses of powder samples were then placed between two kapton tapes such that each sample has the same thickness and surface area.

IV.DATA COLLECTION

An energy-tunable intense X-ray source is needed to perform X-ray absorption fine structure experiments, such as XANES and EXAFS. The intensity of conventional X-ray tubes is not adequate to observe fine structure signals from low concentration samples. Typically EXAFS and XANES experiments require a synchrotron X-ray source. The x-ray absorption data were collection experiments were performed at the National Synchrotron Light Source at Brookhaven National Laboratory, Upton New York. All data were collected in fluorescence mode using Lytle detector to measure fluorescence intensity and iron chamber filled with Nitrogen to measure the intensity of incoming X-ray beam. Samples were mounted at 45^0 to the incoming beam and Lytle detector at 90^0 to the incoming beam. Samples were mounted such that the same area of each sample was exposed to the X-ray beam. The absorption of X-rays were measured as a function of the incoming X-ray energy from 200 eV below the iron K-edge (7112 eV) to about 800 eV above the absorption edge.

V. RESULTS AND DISCUSSION

XANES of a soil sample is shown in Figure 1.

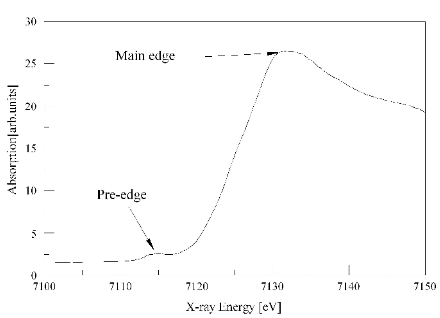

Figure 1: XANES of soil sample.

As shown in Figure 1, when x-ray energy is less than the K-edge energy of iron, no absorption occurs. But as x-ray energy reaches the edge energy of iron, a sharp increase of absorption occurs because of removal of core electrons. The main absorption edge energy position is sensitive to the charge state of iron and the height of the main edge peak above background level is dependent on the iron concentration. The small absorption peak appears before the main absorption edge, at energy 7112 eV, is known as pre-edge. This pre-edge feature occurs due to the 1s to 3d transition. This transition is forbidden by selection rules but becomes allowed due to mixing of the iron 3d orbital and oxygen 2p orbital. Therefore, only when iron atoms are surrounded by oxygen atoms does this pre edge appear. Also, intensity of pre edge feature depends on the symmetry of the iron compound. For example tetrahedrally coordinated iron compounds exhibit higher pre-edge intensity than octahedrally coordinated iron compounds. The appearance of the pre edge feature is indicative of the presence of iron-oxygen compounds. Therefore at least some of the iron present in soil is in some form of iron oxides.

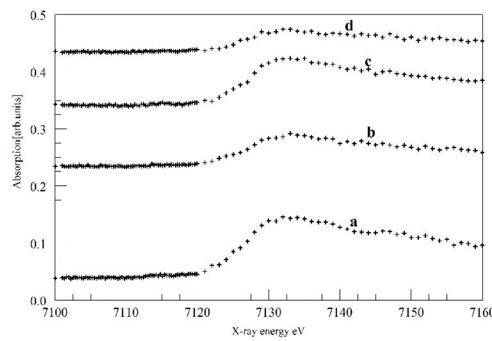

Figure 2: XANES of different regions of Spinach plant. a-mature leaves, b-young leaves, c-roots, d-stem.

Figure 2 shows the XANES of samples from different regions of spinach plants. Table 1 summarizes the main edge energy positions and absorption intensity of all samples together with standard iron supplement.

Sample	Description	Main edge position	Absorption Intensity
A	Mature leaves	7133	0.108
B	Young leaves	7133	0.058
C	Roots	7133	0.083
d	Stem	7133	0.048
Standard 1	Soil	7132	25
Standard 2	Centrum-Iron supplement	7123	2.4
Standard 3	Fe_2O_3	7123	180

As shown in the Figure 2 and Table 1, main absorption edge position is the same for all samples from the spinach plant. This indicates that the chemical environment of iron is similar in different regions of the plant. However, both Fe_2O_3 and iron supplement Centrum have main edge energy at 7123 eV which is 10 eV below that of the plant samples. The higher edge energy indicates that oxidation state of iron in plant samples is greater than 3+. The absorption edge energy of the soil sample is only 1 eV less than that of the plant samples. This indicates that chemical environment of iron in soil and plant samples are very close to each other. The absorption intensity shown in the Table 1 was obtained from subtracting background absorption before the edge from the main absorption height. This absorption intensity is proportional to the amount of iron present in the samples. According to Figure 2 and Table 1, mature leave samples contain about 50% more iron than the young leaves with the same mass. The roots of the plant contain more iron than young leaves but stems contain almost the same amount of iron as young leaves. In addition, XANES spectra of the mature leaves and the stems exhibit small absorption at the pre-edge region. This indicates that as leaves mature some of the iron in leaves become surrounded by oxygen because only iron-oxygen compounds contribute to the pre-edge. It is possible that as leaves mature some of the iron oxidizes.

VI. CONCLUSIONS

The chemical environment of most of the iron from different regions exhibit similar properties. Both mature leaves and stems contain some iron in iron-oxygen form. Mature leaves hold twice as much iron as young leaves of the same mass. The stems contain less iron compared to mature leaves. The oxidation state of iron in plants is higher than that of the iron supplement Centrum.

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