
MOSSBAUER STUDY OF IRON COMPOUNDS IN HYPERACCUMULATOR PLANT *ALYSSUM MURALE* STUDIMI MOSSBAUER I KOMPONIMEVE TË HEKURIT NË BIMËN HIPERAKUMULATORE *ALYSSUM MURALE*

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PËRMBLEDHJE

Hekuri është një nga elementët themelore për bimët. Ne kemi studiuar përqendrimit dhe gjendjet kimike të hekurit në bimën hiperakumulatore *Alyssum Murale* duke përdorur tëkniken FRX dhe spektroskopinë Mossbauer të ⁵⁷Fe. Eksperimentët u kryen me bimë (rrënjë dhe gjethe) që u gjendën në zona minerare të pjesës lindore të Shqipërisë. Nga matjet FRX, vumë re se *Alyssum Murale* akumulon Ni dhe përqendrimi i tij në gjethe është 7 deri 9 herë më i madh se sa në rrënjë. Spektrat Mossbauer të mostrave të dheut u përshtatën me komponentë paramagnetike të Fe³⁺ dhe Fe²⁺ dhe një komponent magnetik, ndërsa spekat Mossbauer të bimëve (rrënjëve dhe gjetheve) u përshtatën vetëm me komponentë paramagnetikë. Analiza e spektrave Mossbauer të rrënjëve dhe gjetheve tregoi praninë e komponimeve të ndryshme të Fe³⁺, që janë identifikuar si ferrihidriti, lepidokrositi dhe Fe-nicotianamina. Në disa mostra ne dalluam një komponim të Fe²⁺ në sasi të vogël, me hekurin në vendndodhje oktaedrale.

Fjalë kyçe: bimë hiperakumulatore, komponime hekuri, spektroskopia Mossbauer, FRX

SUMMARY

The iron is one of the essential elements for plants. We have studied the elemental concentrations and chemical statës of iron in hyperaccumulator plant *Alyssum Murale* using XRF tëchnique and Mossbauer spectroscopy of ⁵⁷Fe. Experiments were carried out with plants (roots and leaves) grown in different metalliferrous sitës in east part of Albania. From XRF measurements, *Alyssum Murale* accumulatës Ni in their leaves, where the Ni concentration in leaves was 7 to 9 times more than in roots. Mossbauer spectra of soil samples could be fittëd to Fe³⁺ and Fe²⁺ paramagnetic components and one magnetic component, while the Mossbauer spectra of plants (roots and leaves) could be fittëd only to paramagnetic components. Mossbauer spectra analysis of roots and leaves indicatëd the presence of different Fe³⁺ compounds, which are identified as ferrihydritë, lepidocrocitë and Fe-nicotianamine. In some samples we differentiatëd a small Fe²⁺ compound with iron in octahedral sitë.

Key words: hyperaccumulator plant, iron compound, Mossbauer spectroscopy, XRF

INTRODUCTION

The iron (Fe) is essential for plant growth and development [3]. In aerobic conditions, soil Fe is usually found as oxihydroxide polymers, which have very low solubility, limiting the Fe supply for plant uptake [5, 8]. A small number of intëresting

plant species can grow in soils containing high levels of heavy metals, and can also accumulatë these metals to high concentrations. These metal hyperaccumulator species have the genetic potëntial to remove toxic metals from contaminatëd soil. The Nickel hyperaccumulator, *Alyssum Murale*, a herbaceous perennial

(*Brassicaceae* family) native to Mediterranean serpentine soils, has been developed as commercial crop for phytoremediation / phytomining [13].

This study was realized with a representative of these hyperaccumulator plants, *Alyssum Murale*. This plant is taken out from four different metalliferous sites in east part of Albania (Shkumbin Valley – Ohrid lake). The study is focused in a specific region, which is composed from serpentine soils with elevated levels of metals, Mn, Ni, Cr, Co, Fe. This region is very rich with iron-nickel minerals and Fe contents vary from 7% to 11% [1]. Mössbauer spectroscopy of Fe-57 was used to characterize the chemical forms in which Fe was present in plant tissues (roots and leaves) and in the soils, to understand the mechanisms of transport soil – root – leaf of iron and other metals. Elemental concentrations in these plants (roots and leaves) were assessed using X-Ray Fluorescence with Energetic Dispersion (EDXRF).

MATERIALS AND METHODS

Samples were taken from four different sites noted K1 (Pojske), K2 (Prrrenjas), K3 (Xhyre) and K4 (Librazhd), which belong to Ohrid Lake(K1) and Shkumbin Valley (K2, K3 and K4). These samples included both the soil samples and the plant samples (roots and leaves).

Mössbauer spectroscopy: Samples (soils, roots, and leaves) were rinsed in distilled water, dried at room temperature, ground and pressed into tablets with surface 3 cm². For the soil samples were taken 150 mg material for each, for the root samples 700 mg material and for the leaf samples around 1000 mg.

The dried samples were sealed in a sample holder. The Mössbauer spectra were obtained using a conventional acceleration spectrometer in transmission geometry. A 50-mCi source of ⁵⁷Co in Rh matrix, kept at room temperature, was used. The velocity scale was calibrated with a Fe metal foil at room temperature. Spectra were acquired in the velocity range ± 10 mm/s. The hyperfine parameters (isomer shift relative to Fe metal – IS, quadrupole splitting – QS and hyperfine magnetic field - HMF) were obtained by a least-square procedure assuming Lorentzian line shapes constrained to equal half widths.

Energy Dispersion X-Ray Fluorescence - EDXRF: The concentrations of Fe, Ni, Cr, Mn, Zn, Cu, Pb, K, Ca, Ti, Br, Rb and Sr were evaluated in soils, roots and leaves

by EDXRF technique. This system works with Mo anode X-ray tube and Si(Li) detector with resolution of 160 eV at Mn K α .

RESULTS AND DISCUSSIONS

The plants growing on serpentine soils, which in the Ohrid Lake - Shkumbin Valley region are ultramafic soils, accumulated metallic elements, resulting in elevated metal concentration in plant tissue, relative to plants in normal soils [2].

Information regarding metal localization and elemental association in accumulator plants is crucial to understanding the mechanism of hyperaccumulation and tolerance. In this study we are focused in Fe behaviour in *Alyssum Murale* as an associated element of Nickel.

Site	Concentration ratio between leaves and roots			
	Fe	Ni	Cr	Mn
K1	0.780	7.067	1.304	1.811
K2	5.080	7.071	7.074	4.823
K3	0.498	8.750	0.190	1.905
K4	0.320	9.712	0.264	0.536

Table 1. Concentration ratio of different elements between leaves and roots

Analysis of EDXRF. From XRF measurement *Alyssum Murale* accumulated Ni in their leaves, where the Ni concentration in leaves was very high, compare to soils and roots. In the Table 1 are shown the concentration ratio of different elements between leaves and roots.

The Ni concentration in leaves, for the four sites, was 7 – 9 times more than in roots, while the Fe concentration in leaves was lower than in roots, with the exception of K2 site, where Fe concentration in leaves was 5 times more than in roots. *Alyssum Murale* has two year vegetative cycle, and may be need more careful investigation to evaluate the period of high accumulation and Ni – Fe correlation.

Mössbauer spectroscopy: All Mössbauer spectra generated from the analysed samples of soils

could be fitted to paramagnetic subspectra (two Fe^{3+} components and one Fe^{2+} component) and one magnetic spectrum. A typical Fe-57 Mossbauer spectrum, obtained from one of the soil samples (K2 soil), is shown in Figure 1.

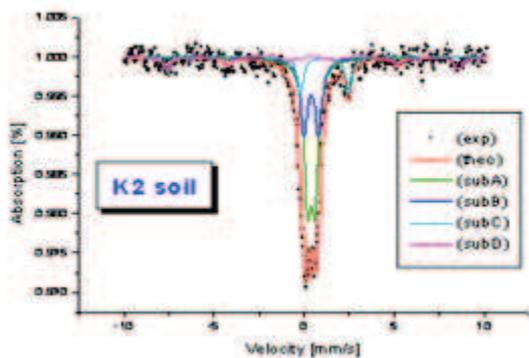


Figure 1. Fe-57 Mössbauer spectrum obtained from K2 group soil sample at RT.

The soil spectra were fitted to four subspectra, represented by the curves A, B, C and D. The subspectra A, B and C represent iron in paramagnetic states and from the hyperfine parameters of Fe^{2+} ($IS = (1.07\text{--}1.11)$ mm/s and $QS = (2.61\text{--}2.67)$ mm/s) and hyperfine parameters of Fe^{3+} ($IS = (0.32\text{--}0.41)$ mm/s and $QS = (0.30\text{--}1.21)$ mm/s). We identified ferrous iron in octahedral sites (pyroxene/olivine) and ferric iron in tetrahedral sites (Fe^{3+}_{IV}) and in octahedral sites (Fe^{3+}_{VI}) [4, 11].

From the hyperfine parameters of the magnetic component D ($IS = (0.32\text{--}0.44)$ mm/s, $QS = (0.005\text{--}0.022)$ mm/s and $HMF = (48.5\text{--}50.2)$ Tesla), we identified iron in magnetite/magnetite (iron oxide minerals).

The Mössbauer spectra of plant samples (roots and leaves) could be fitted to paramagnetic subspectra: two Fe^{3+} compounds and one Fe^{2+} compound (small amount). A typical spectrum, obtained from a root sample (K2 root) is shown in Figure 2 and leaf sample (K2 leaf) in Figure 3.

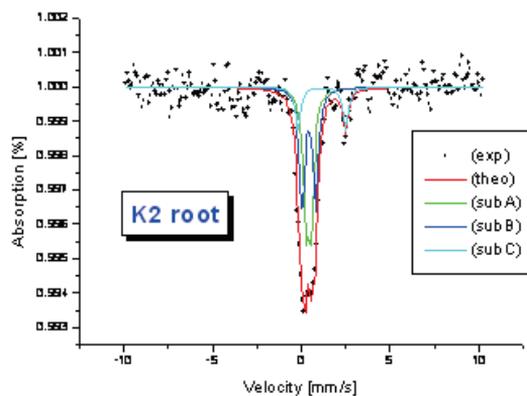


Figure 2. Fe-57 Mössbauer spectrum obtained from root sample of K2 group at RT.

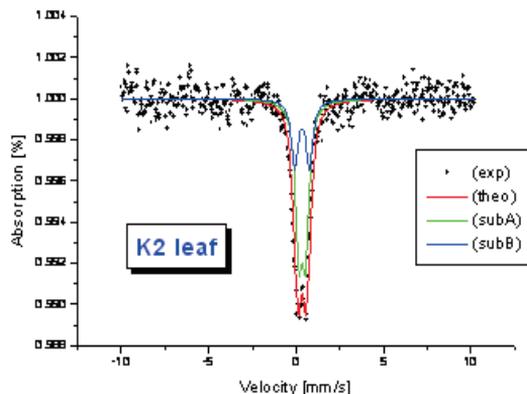


Figure 3. Fe-57 Mössbauer spectrum obtained from leaf sample of K2 group at RT.

In the Fig. 2 and Fig. 3 the subspectra A and B represent Fe^{3+} compounds and subspectrum C represents Fe^{2+} compound.

The data obtained at room temperature for plant samples (roots and leaves), we classified in four groups, which are shown in Table 2. It was possible to detect the same compounds in samples of roots and leaves from the same group but different compounds from different groups.

Sample	Fe ³⁺ I			Fe ³⁺ II			Fe ³⁺ III			Fe ³⁺ IV		
	IS	QS	Cont.	IS	QS	Cont.	IS	QS	Cont.	IS	QS	Cont.
K1-R				0.36	0.55	83.3	0.41	1.09	16.7			
K1-L				0.34	0.57	89.5	0.34	1.21	10.5			
K2-R	0.36	0.78	41.6							0.34	0.297	43.1
K2-L	0.32	0.86	28.1							0.33	0.38	65.9

Table 2. Hyperfine parameters for roots (R) and leaves (L) of K1 and K2 groups. Isomer shift (IS) and quadruple splitting (QS) values are given in mm/s. Contents of each component are given as percentages of total spectrum area.

The component I with hyperfine parameters (IS values 0.36 mm/s or 0.32 mm/s and QS values from 0.78 mm/s to 0.86 mm/s), corresponds to ferrihydrite ($\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$). Mössbauer parameters for ferrihydrite have been reported in studies with different plants [6, 17, 15, 9, 12]. Ferrihydrite is the inorganic compound usually associated with Fe accumulation in ferritin, which are large proteins with a central cavity that can store up to 4500 Fe atoms, which can be released when necessary [16]. Therefore, these proteins are believed to play a critical role in the cellular regulation of Fe storage and homeostasis [18].

In roots, ferrihydrite is also possibly found in the Fe plaque. The Fe plaque has been shown to be formed by 63% ferrihydrite ($\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$), 32% goethite ($\alpha\text{-FeOOH}$) and 5% siderite (FeCO_3) in the wetland plant *Phalaris arundinacea* [7].

The Component II (IS values 0.36 mm/s and 0.34 mm/s and QS values 0.55 mm/s and 0.57 mm/s respectively) has hyperfine parameters compatible with lepidocrocite ($\gamma\text{-FeOOH}$) which is very common. We detected its presence only in root and leaf samples of K1 group with respective content 83.3% and 89.5%.

Based on the hyperfine parameters of Fe^{3+} compounds we could not to identify the component III. In the experiments carried out by Kilcoyne et al. [9] in roots and leaves of rice plants, it is reported the presence of two major compounds in rice plants; ferrihydrite and lepidocrocite. Component III has a minor content in roots and leaves of K1 group (respectively about 16.7% and 10.5%), however, we

considered that further investigations are necessary.

Component IV (IS = 0.34 mm/s and 0.33 mm/s and QS = 0.297 mm/s and 0.38 mm/s) was present in root and leaf samples. Based on similarity between NA (nicotianamine) and MA (mugineic acid) [10], that have six ligands for Fe complexation and both chelate Fe in a similar way, and on the study of von Wiren et al. [14], we evaluated that the component IV belong to Fe complexed to NA (nicotianamine).

CONCLUSION

Alyssum Murale is a wild plant grown in metalliferous area in the east part of Albania. From Mössbauer measurements carried out in roots and leaves, it is concluded that predominant compounds of iron are ferric (Fe^{3+}) compounds. In some samples, we distinguished a small percentage of ferrous (Fe^{2+}) compound in octahedral site. Mössbauer study was also realized in soil samples where the above-mentioned plants were grown. Hyperfine parameters of soil samples Mössbauer spectra showed the presence of a magnetic component, which belong to iron oxides such as magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$), and a nonmagnetic component. The analysis of the plant spectra (roots and leaves) indicated that the magnetic component did not exist and the central paramagnetic part of spectrum based on the values of hyperfine parameters (isomer shift and quadrupole splitting) pertained to iron compounds of Fe^{3+} : i) ferrihydrite (an inorganic

compound) $\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$ which is decreased (from roots to leaves) from 42 % to 28 %; ii) lepidocrocite ($\gamma\text{-FeOOH}$) common which is decreased (from roots to leaves) from 17 % to 10 %; iii) Fe-Nicotianamine, the most important chelator of free Fe in plant cells, which is increased (from roots to leaves) from 43 % to 66 %.

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