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APPLICATIONS OF MÖSSBAUER SPECTROSCOPY IN PLANT PHYSIOLOGY

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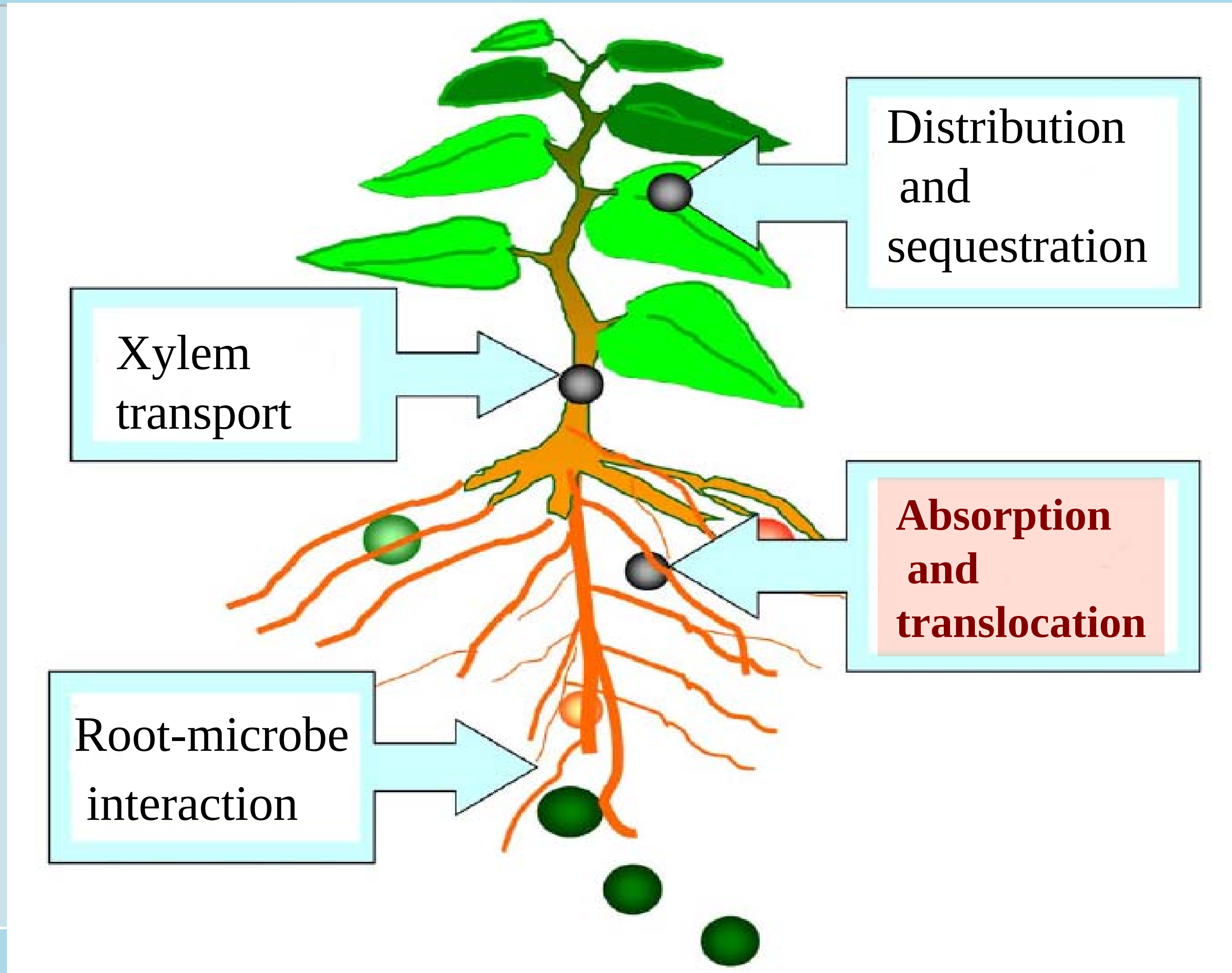
Introduction

- **Iron** is considered as a **plant micronutrient**
- **Iron** is considered the **key metal** in **energy transformations** needed for synthesis and other life processes of the cells.
- Nowadays, the **uptake, transport and the storage of** metal ions (including **iron**) in plants attract a considerable scientific interest, since the **plants can accumulate** trace elements especially **heavy metals**, thus plants are intermediate reservoir for the trace elements originated from the lithosphere, hydrosphere and atmosphere. **This is very important for the phytoremediation biotechnology**.

Major processes proposed to be involved in heavy metal hyperaccumulation by plants



Iron uptake strategies of plants

- **Strategy group I. Dicots and monocots not included in the grass family** (model: cucumber)

  plants reduce Fe(III) complexes at the root surface and absorb the Fe^{2+} ions produced via root associated reduction

- **Srategy group II Grass family** (model: wheat)

  plants excrete specific, low molecular weight, organic polydentate ligands, known as phytosiderophores, which solubilize Fe^{3+} ions and make them available for absorption in the form of Fe^{3+}

Previous Mössbauer studies

Previous Mössbauer investigations, performed with plants grown either at a high iron content of isotopically enriched nutrient solutions [1–3] or in an extremely acidic soil environment with a very high iron content [4], **indicated only Fe(III) species in the roots** of duckweed, stocks, pea [1,2], and a perennial grass [4].

[1] B. A. Goodman, P.C. DeKock, J. Plant Nutr. 5 (1982) 345-353.
[2] B. A. Goodman, P.C. DeKock, J. D. Rush, J. Plant Nutr. 5 (1982) 355-362.
[3] S. H. Kilcoyne, P. M. Bentley, P. Thongbai, D. C. Gordon, B. A. Goodman, Nucl. Instrum. Meth. Phys. Res. B. 160 (2000) 157-166.
[4] N. Rodríguez, N. Menéndez, J. Tornero, R. Amils, V. de la Fuente, New Phytologist, 165 (2005) 781-789.

AIM

Our main aim was to **apply ^{57}Fe Mössbauer spectroscopy to evidence the validity of the iron uptake mechanism strategy I** in the case of cucumber, to **identify the iron species** resulted, and to study **the effect of Cd** on the iron uptake.

EXPERIMENTAL

Series 1. **Iron-sufficient roots**

grown in a **modified Hoagland** nutrient solution

1.25 mM KNO_3, 1.25 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.25 mM KH_2PO_4, 11.56 μM H_3BO_3, 4.6 μM $MnCl_2 \cdot 4H_2O$, 0.19 μM $ZnSO_4 \cdot 7H_2O$, 0.12 μM $Na_2MoO_4 \cdot H_2O$, 0.08 μM $CuSO_4 \cdot 5H_2O$.

Iron was supplied as Fe(III) citrate, 10 μM, containing 90% enriched ^{57}Fe.

After 3 weeks of growth the plants were **harvest**ed and the **roots were immediately frozen to 78 K.**

Series 2. **Iron-deficient roots**

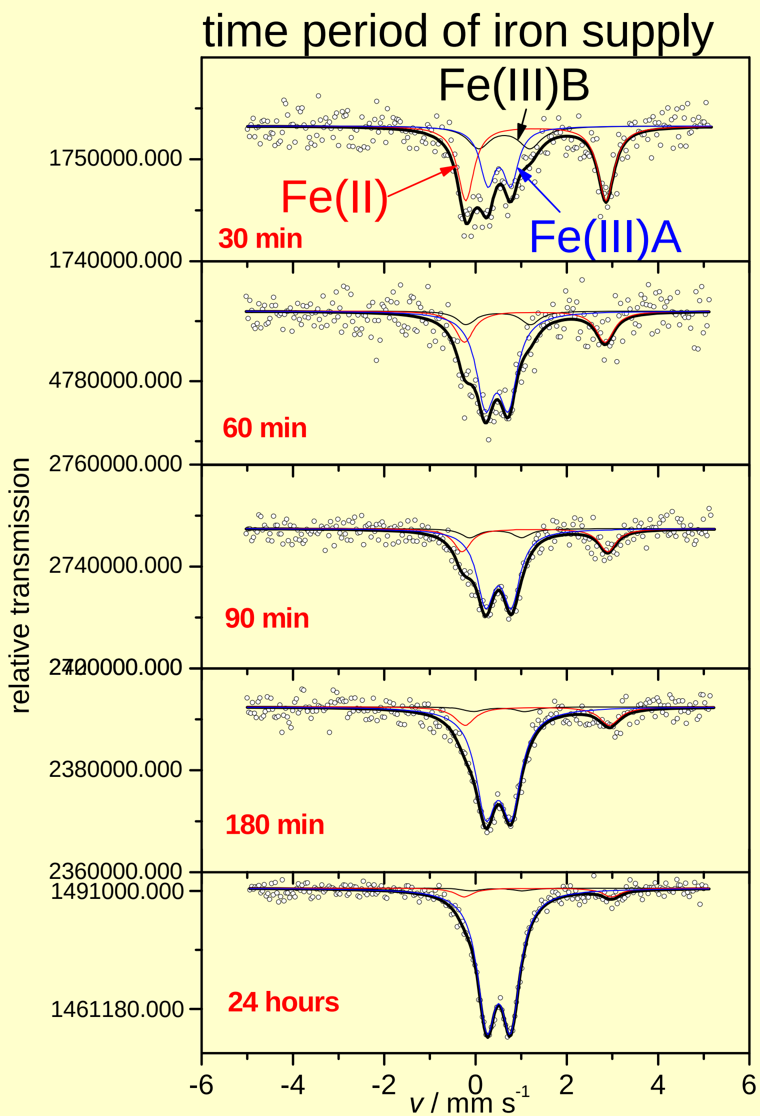
grown in **iron-deficient** nutrient solution

those plants were **supplied with iron only before harvesting** for 0.5 to 24 hours.

Series 3. **Cd-treatment**

Plants were grown **both** in **iron sufficient and iron deficient** media. In all cases, the nutrient solutions contained **Cd contaminants in different concentrations** from 10^{-7} M up to 10^{-4} M.

Cucumber

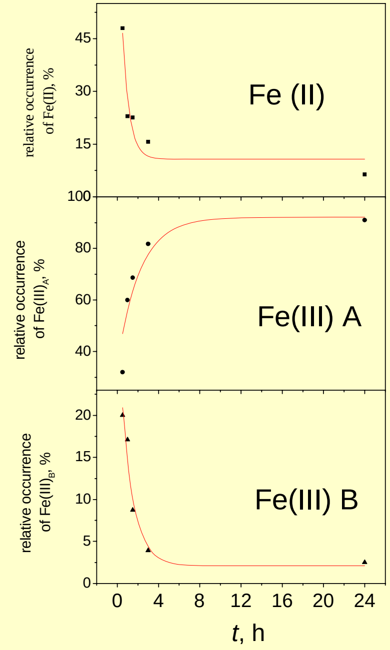


Time period of iron supply

Iron-deficient root

78K	Fe(III) A	Fe(III) B	Fe(II)
Doublet	DA	DB	D
δ (mms^{-1})	0.5	0.5	1.3
Δ(mms^{-1})	0.5	1.2	3.2

Fe(II) species was found

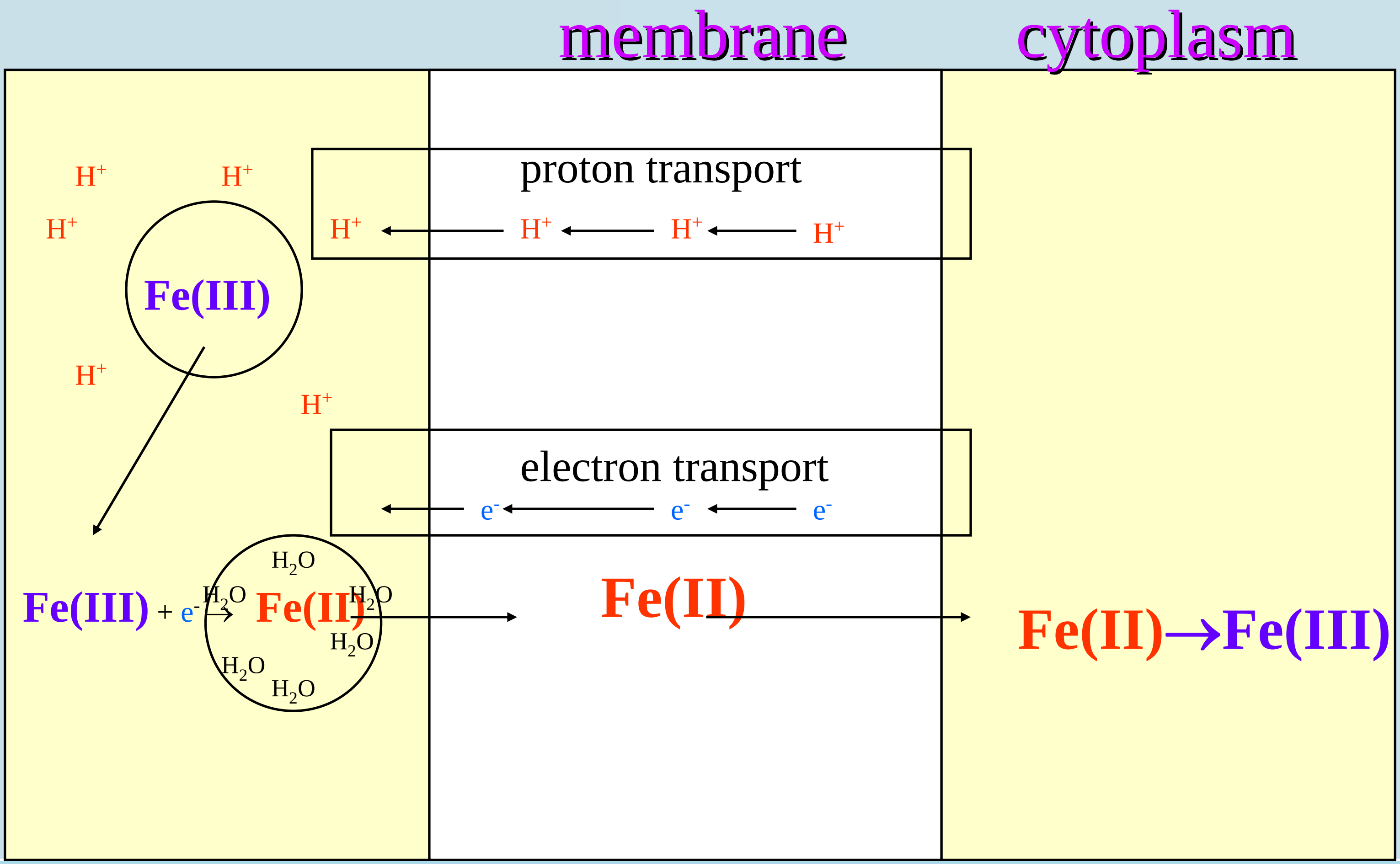


time period of iron supply

The **component D is attributed to ferrous hexaaqua complex** based on its characteristic Mössbauer parameters.

We have succeeded in showing by Mössbauer spectroscopy **the presence of divalent iron in the plant root** when the nutrient solution contained only Fe(III). **This finding gives a direct evidence** for the **existence of Fe^{2+} ions produced via root-associated reduction according** to the mechanism proposed for iron uptake for plants belonging to **strategy group I**.

Mechanism of iron uptake via strategy I through the membrane in the cucumber root



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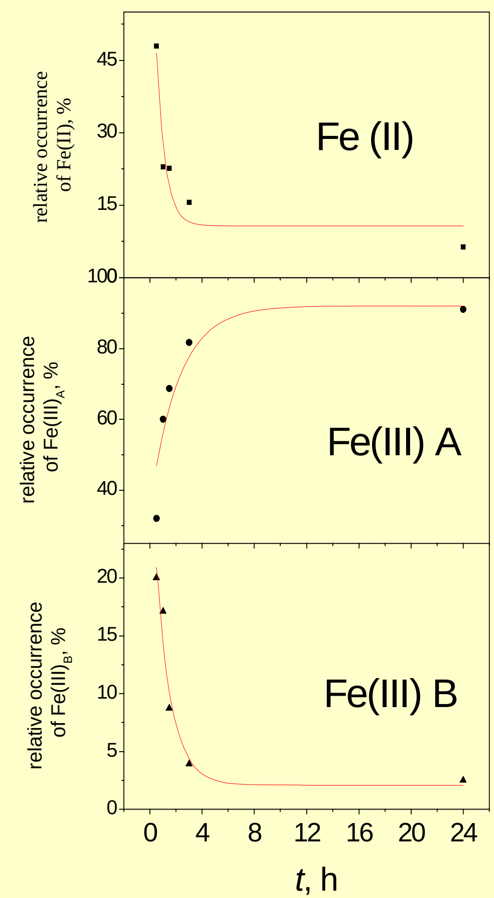
Fe(III)→Fe(II) | membrane | Fe(II)→$Fe(III)_A$

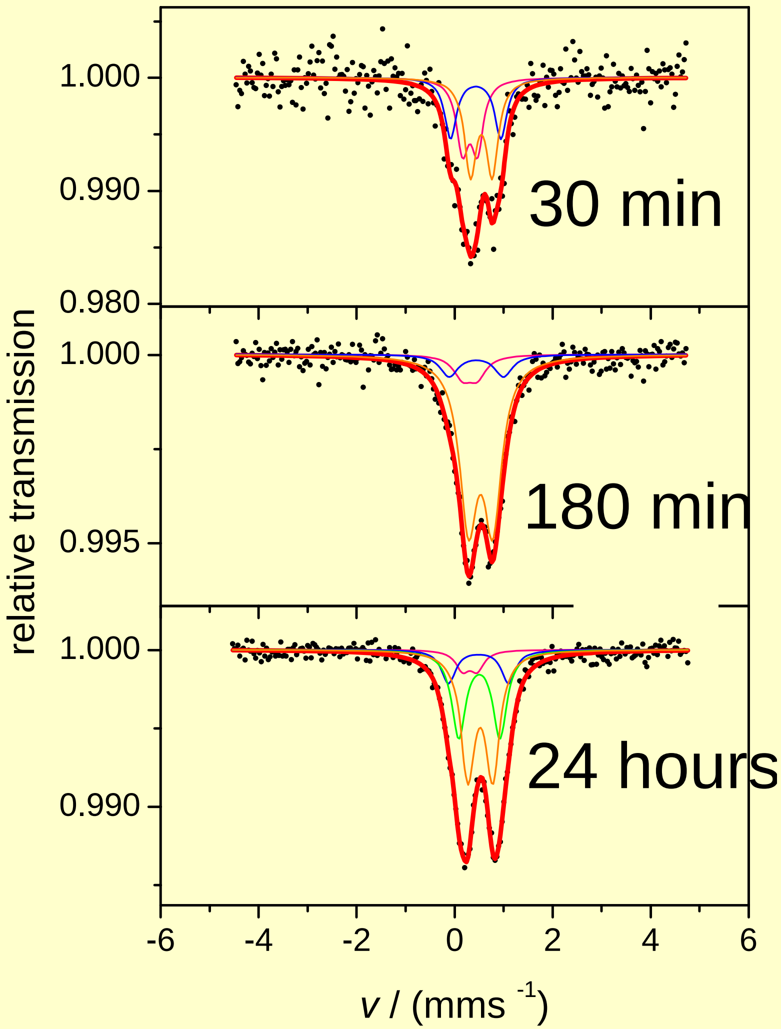
78K	Fe(III)A	Fe(III) B	Fe(II)
Doublet	DA	DB	D
$\delta\ (mms^{-1})$	0.5	0.5	1.3
$\Delta(mms^{-1})$	0.5	1.2	3.2

The changes in the relative content of the components shown in Figure can indicate the **transformation of Fe(II) hexaaqua complex into the $Fe(III)_A$ species**. This agrees with the accepted viewpoint that the translocation and storage of iron inside the root cells take place in the form of Fe(III) compounds. The **reaction rate** of this **Fe(II)-to-Fe(III) transformation** is **much higher than that of** of the Fe(III) reduction outside the membrane, **when sufficient amounts of iron are taken up by the root**, possible due to significantly lower Fe(II) reduction rate in these plants. Consequently, the **Fe(II) species cannot be detected** on the background of the Fe(III) components using Mössbauer spectroscopy in the case of iron-sufficient roots.



time period of iron supply

Time of iron supply

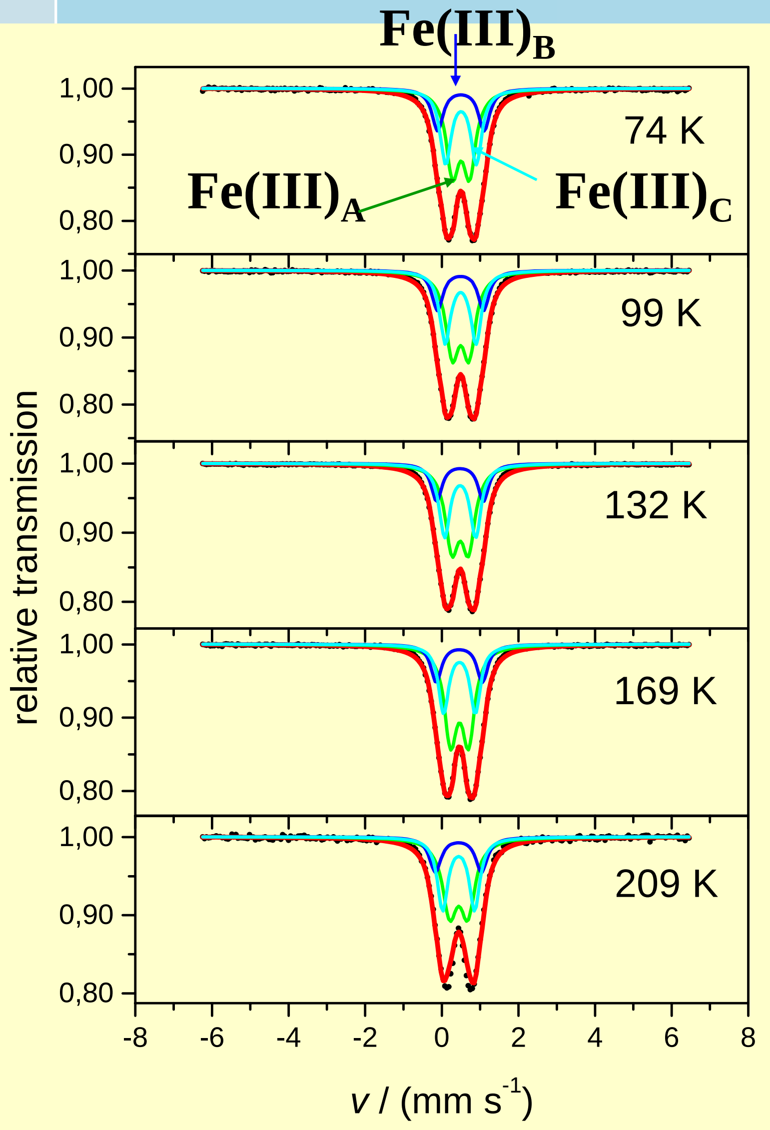


Iron-deficient root

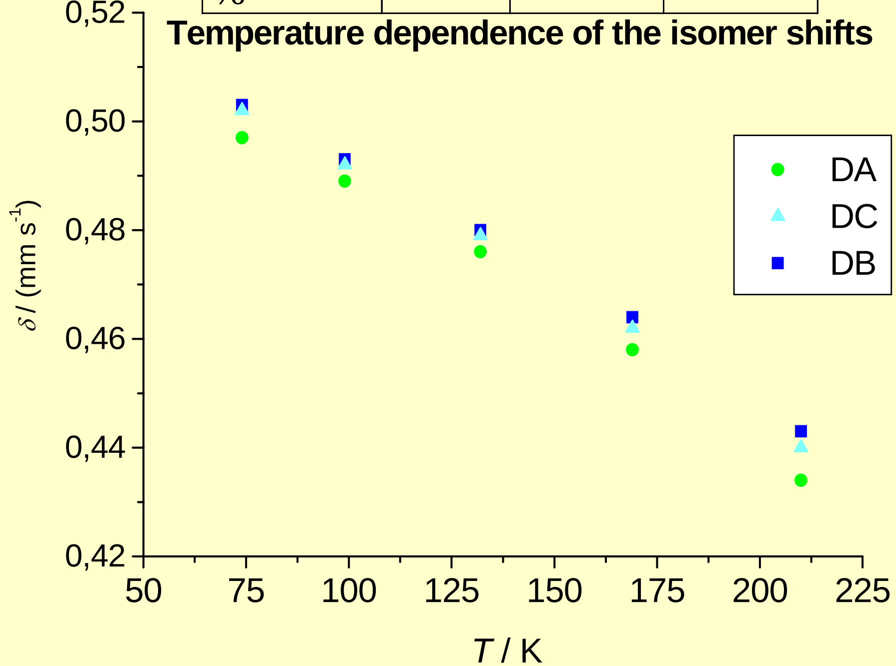
Wheat Strategy II

In the case of wheat which belongs to the strategy group II, grown under similar iron deficient conditions as cucumber **no ferrous iron could be found**. In the view of the results obtained for the cucumber this **proves that wheat does not belong to the strategy group I.**

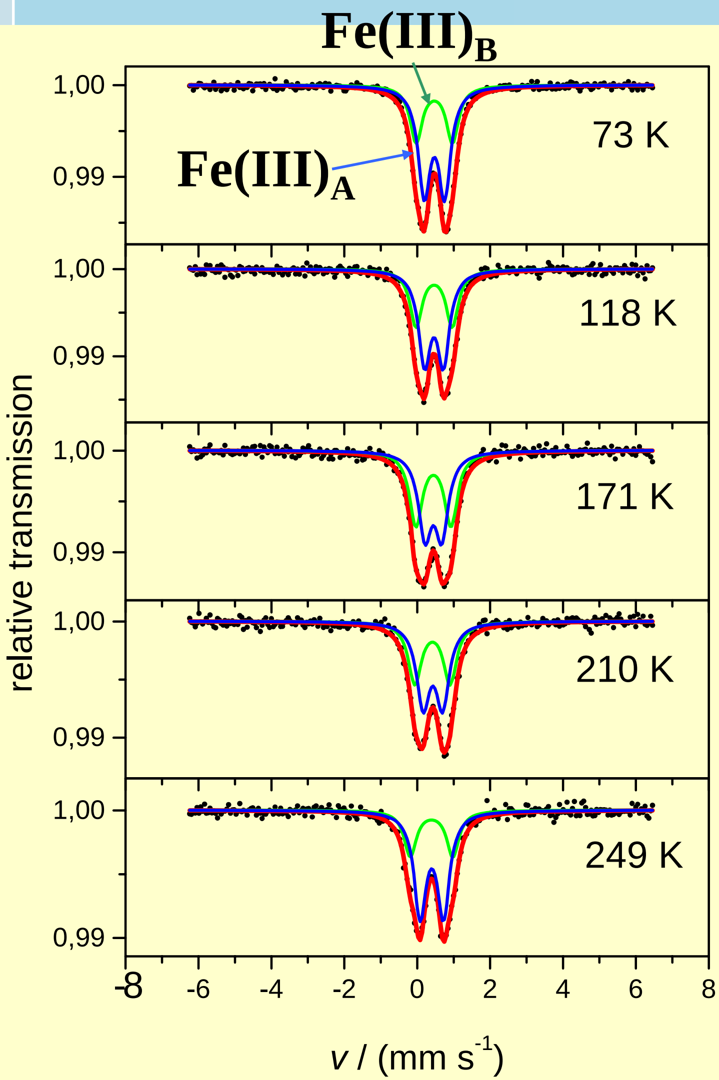
Mössbauer spectra of iron-sufficient cucumber root



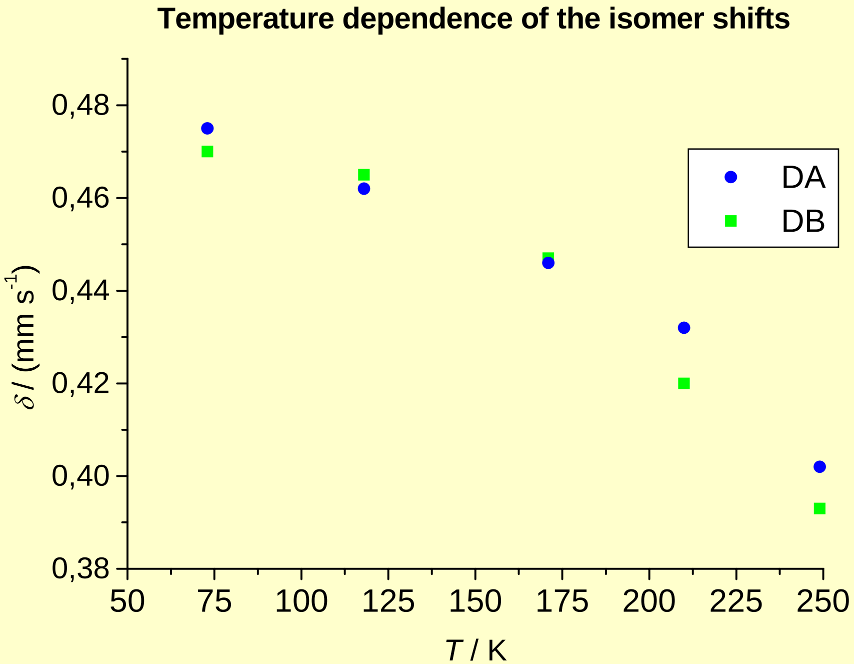
Doublet	DA	DB	DC
δ / (*mms*⁻¹)	**0.5**	**0.5**	**0.5**
Δ / (mms⁻¹)	**0.5**	**1.2**	**0.8**
A / %	46	21	33



Mössbauer spectra of **dithionite washed** cucumber root



Doublet	DA	DB	DC
δ / (mms^{-1})	**0.5**	**0.5**	**0.5**
Δ / (mms^{-1})	**0.5**	**1.2**	**0.8**



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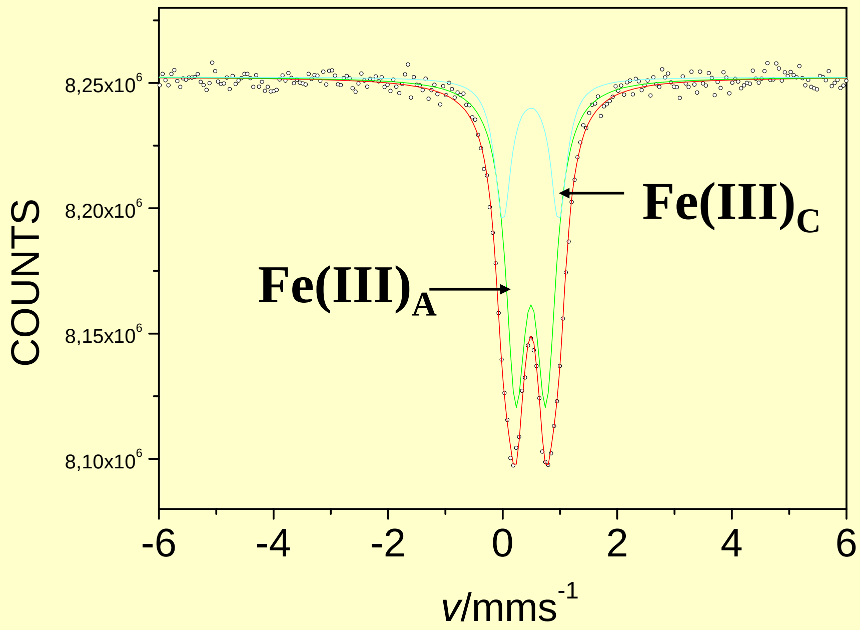
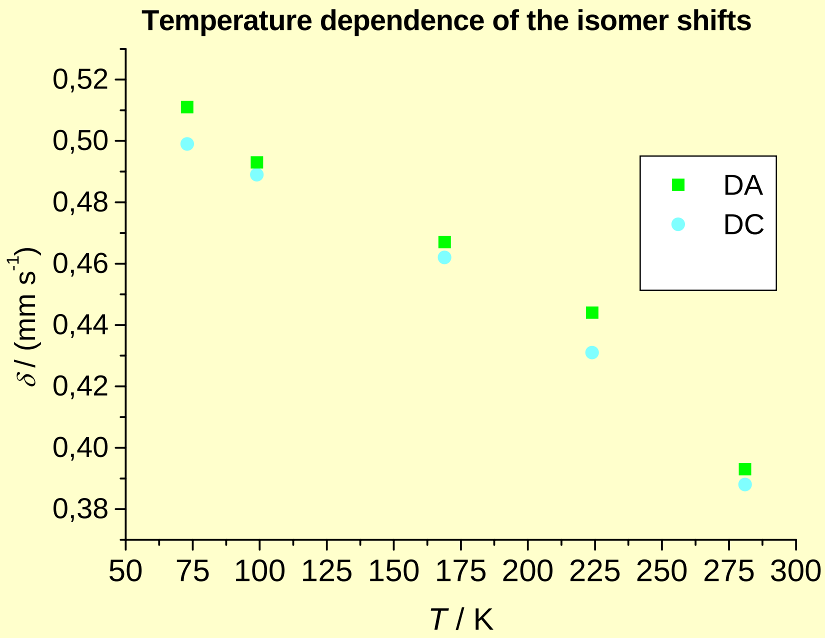
Mössbauer spectra of **EDTA washed** cucumber root

Mössbauer parameters of **DC** correspond to those of **ferritin**

Mössbauer parameters of **DB** correspond to those of **jarosite**

Doublet	DA	DB	DC
δ / (mms^{-1})	**0.5**	**0.5**	**0.5**
Δ / (mms^{-1})	**0.5**	**1.2**	**0.8**





Assignment of the spectral components

K. Kovács, E. Kuzmann, F. Fodor, A. Vértes, A. A. Kamnev, **Hyp. Int.** 165 (2005) 289-294
K. Kovács, E. Kuzmann, E. Tatár, A. Vértes, F. Fodor, **Planta** (2008) DOI 10.1007/s00425-008-0826x (available in electronic form)

According to the results of the different washing procedures for the separation of iron distributed between the apoplasm and the symplasm, we **assign**

the **DA component** to **Fe(III)-carboxylate compound**

the **DB component** to **jarosite**

the **DC component** to **ferritin**

Location of Fe(III) species in the cucumber root

nutrient solution

cell wall

membrane

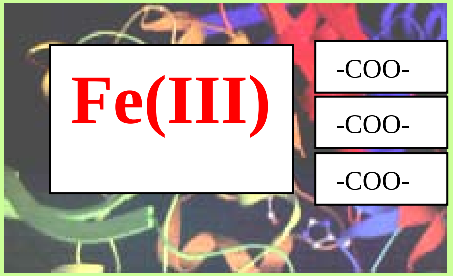
cytoplasm

Fe(III) citrate

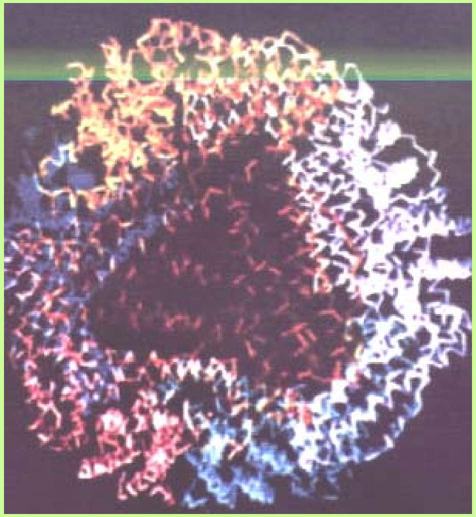
$MgSO_4$
$CuSO_4$
$ZnSO_4$

Jarosite

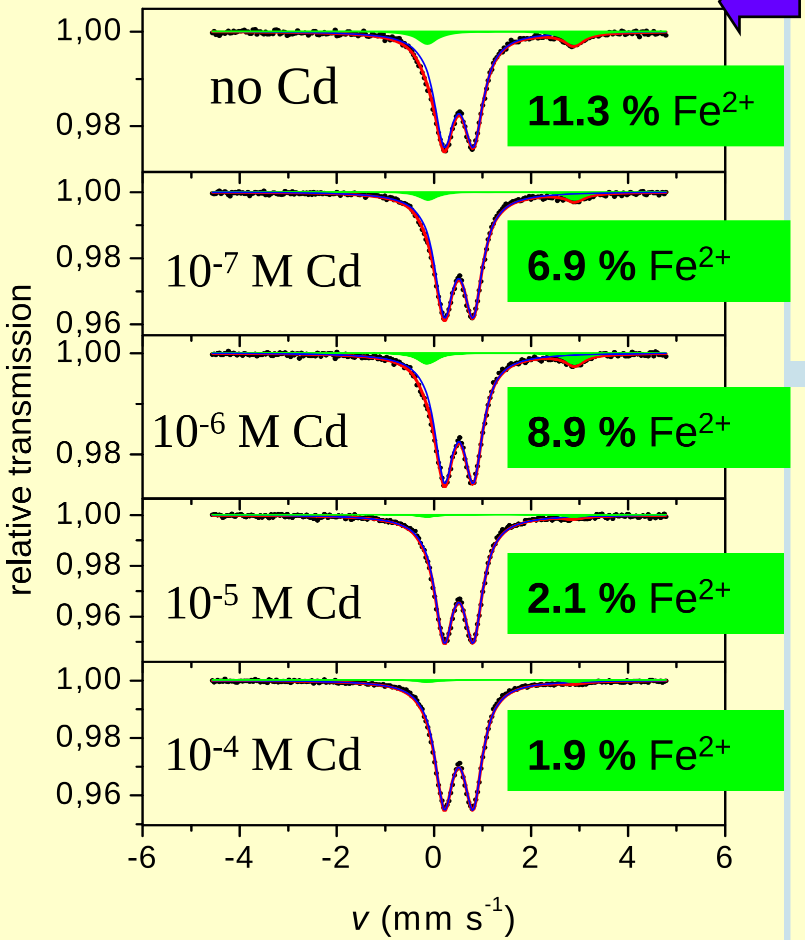
$KFe_3(OH)_6(SO_4)_2$



ferritin

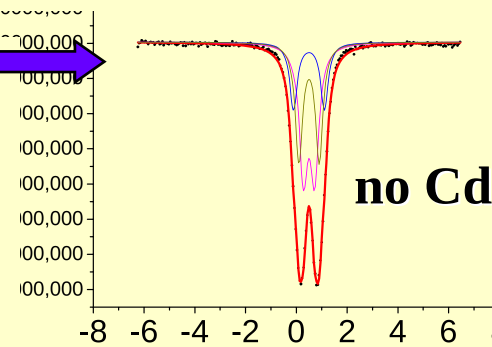


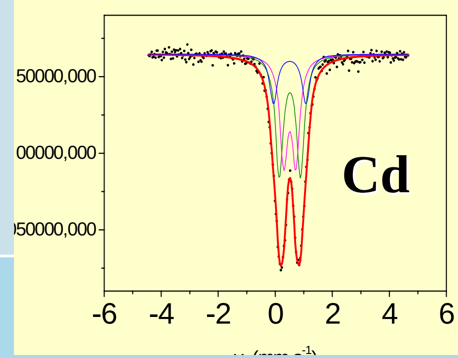
THE EFFECT OF Cd ON THE IRON UPTAKE AND STORAGE IN CUCUMBER



In the iron-deficient case the amount of the reduced iron (δ=1.3 mms^{-1} Δ=3.1 mms^{-1}, Fe^{II}-hexaqua complex) is decreasing upon Cd treatment Cd inhibits the ferric-chelate reductase enzyme activity. **Cd inhibits iron reduction** during iron uptake.

In the iron sufficient case the **relative amount of iron(III)-carboxylates decreases upon Cd contamination** supporting that **Cd accumulates** in the root in competition with the Fe^{3+} ions





SUMMARY

Iron is distributed among **3** characterisitic **main species in** (cucumber and wheat) **root** under nutrition condition sufficient for iron. They can be asssociated with **jarosite** related to the apoplasm and **ferritin** as well as **Fe(III)-carboxylate compound** located in the symplasm.

We have succeeded in showing by Mössbauer spectroscopy the **presence of divalent iron in the plant root** when the nutrient solution contained only Fe(III). **This gives a direct evidence** for the **existence of Fe^{2+} ions produced via root-associated reduction** according to the mechanism proposed **for iron uptake in plants** belonging to strategy group I. **In** the root of wheat belonging to **strategy group II**, **no Fe^{2+} were found at all.**

The presence of **Cd** in the the nutrient solution **inhibits iron reduction during iron uptake** and **accumulates in the root in competition with the Fe^{3+}** ions.

- THANK YOU VERY MUCH FOR YOUR ATTENTION