EFFECTS OF HEAVY METALS ON THE WATER BALANCE OF CUCUMBER DETECTED BY MRI MEASUREMENT

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Abstract

The purpose of our study was to extend the potential applications of MRI (applied in human diagnostics) to plant-water relations, and to verify the previous knowledge about it. Effects of heavy metals on the water balance of the plants can be examined by MR measurements.

Three-week-old seedlings of cucumber (Cucumis sativus) were polluted with Pb-nitrate, Zn-sulfate, Cd-nitrate and Hg-chloride solutions at 10^{-5} M concentration. The incubation time was 1 week. The plants were grown in nutrient solution in growing chambers. The effects of heavy metals on the water balance were measured by classical method and MRI technique. The MRI measurements were carried out at the Diagnostics and Oncoradiological Institute of the University of Kaposvár (Hungary), using a Siemens Avanto MRI equipment.

The MR measurements are made on the spin's system. The procedure is based on the interaction of the external magnetic field, electromagnetic waves and the hydrogen nucleus' in the substance. Namely the quantity and distribution of the protons are measured by MR. If we ask a question, where can we find relatively a lot of protons, our answer is in the water. So the MR doesn't measure the anatomic structure, but the quantity of protons. The anatomic structures are determined by the distribution and quantity of protons.

As classical method stomatal resistance was measured by AP4 porometer in conductance mode. Water content was determined by drying until weight consistence. Water content percentage is determined by the dry and fresh weight.

Significant differences can be detected between the different heavy metal treatments by MRI measurements, but the classical methods did not prove these deviations. In consequence the MRI measurements can provide more detail information about water content and transport. In addition MRI measurement is a non-destructive method, opposite to the classical techniques.

MRI measurement can increase our knowledge on the cycling and pathways of heavy metals in the plants.

Key words: MRI, heavy metals, water balance

Összefoglalás

A vizsgálatunk célja a humándiagnosztikában alkalmazott MRI alkalmazási lehetőségének növény-víz kapcsolatra történő kiterjeszté-

se, valamint a korábbi ismeretek igazolása volt. A nehézfémek növényi vízháztartásra gyakorolt hatása vizsgálható-e MRI mérés felhasználásával.

Három hetes uborka palántákat szennyeztünk ólom nitrát-, cink-szulfát-, kadmium nitrát- és higany klorid oldattal, melyek koncentrációja 10^{-5} M volt. A szennyezést egy héten keresztül végeztük. A növényeket tápoldaton, klímakamrában neveltük. A nehézfémek növényi vízháztartásra gyakorolt hatását klasszikus és MRI mérésekkel is vizsgáltuk. Az MRI méréseket a Kaposvári Egyetem Diagnosztikai és Onkoradiológiai Intézetében végeztük, egy Siemens Avanto típusú MR felhasználásával.

Az MR mérés elve nem más, mint a spinek rendszerén végzett mérések. A folyamat a külső mágneses tér, az elektromágneses hullámok, és az anyag hidrogén atomjainak kölcsönhatása révén valósul meg. Vagyis az MR a protonok mennyiségét és eloszlását méri. Ha feltesszük a kérdést, hogy hol található relatíve sok proton, akkor erre a kérdésre a válasz az, hogy ahol sok hidrogén van tehát elsősorban a vízben. Vagyis az MR nem az adott anatómiai struktúrát méri, hanem az adott anatómiai struktúrában levő víz mennyiségét és eloszlását.

A klasszikus mérések közül a sztóma ellenállást vizsgáltuk, AP4 porométer felhasználásával konduktancia üzemmódban. A növények víztartalom %-át a friss és a száraz tömegből határoztuk meg.

Szignifikáns különbséget tudtunk kimutatni az MRI mérés során a különböző nehézfém kezelések között, azonban a klasszikus mérésekkel ezt nem tudtuk kimutatni. Az MR mérés a víztartalom és vízszállítás folyamatáról egy sokkal részletesebb elemzést tesz lehetővé. Mellette nem destruktív mérési eljárás, ellentétben a klasszikus elemzésekkel.

Az MR mérések növelhetik ismeretanyagunkat a nehézfémek körforgásáról és áramlásáról a növényekben.

Introduction

Environmental pollution by heavy metals is a global problem (*Xiangyang et al.* 2009). Heavy metal contamination also occurs in industrial zones, where the sources include heavy vehicular traffic, refuse dumps and sewage sludge (*Larcher 2003*). The trace metals are not essential elements for plants take up from soil and atmosphere and accumulate them in their edible parts in various concentrations. Increasing heavy metal uptake causes stress by modifying the water transport in plant.

An everyday problem in the classical measurements of the components of plant water balance is the way in which a component of the plant – as the "random taken" component of the soil-plant-atmosphere system – will respond to interventions by the study and the parameter to be measured is affected, and to what extent, by the measurement procedure itself. Another basic problem of measurements applied in studying classical plant-water relations is their destructive nature; the opportunity of repeating the measurement is entirely eliminated for a given test specimen. Of the deficiencies of traditional approaches, MRI is capable of eliminating the second potential error – it is a non-destructive method, thus a particular sample of live and functional plant can be tested even several times in succession.

Studies of plant physiological aspects often involve an inaccuracy of measuring the components of plant water balance (*Pearcy et al.* 1991). The duty of researchers is made fairly difficult by the sensitivity of living organisms responding immediately to any minor or major changes, external or internal ones. The sensitivity of a plant often manifests itself in the broad scattering of the parameter being measured; this may often be due to the measurement itself – covering the actual relations as well as the comparisons. MRI appears to be an extremely efficient means of eliminating that potential error. The procedure involves a measurement of spin systems in the live plant; it will not intervene harshly into the system under test. The spins only have weak interactions with macroscopic parameters of the biological system under test that will affect its behavior from biological and chemical aspects. Magnetic properties play a fairly irrelevant role in biochemical processes at levels of cells (*Berényi et al.* 1997). On the other hand, biochemical parameters will affect the behavior of spin systems in a readily measurable manner – i.e. conclusions may be drawn from measurements of spin systems on behavior of the biological system under test (*Berényi et al.* 1997, *Földes et al.* 2003).

A few decades ago the plant-water relations were studied primarily in their components, as fractional processes. They are the water absorption of the root, its transmission to the evaporating surface, and the transpiration through the leaf. This subject has a literature too broad to be listed (*Ketelapper* 1963, *Kanemasu* 1969, *Lange et al.* 1976, *Lange et al.* 1976, *Jarvis and Mansfield* 1981, *Monteith 1*973, 1976, *Monteith et al.* 1990, etc.) – occasionally discussing in detail the potential errors of measurement (*Johnson* 1981, *Norman et al.* 1981, *Meyer et al.* 1985, *McDermitt* 1990, etc.). It must have been the error of measurement that led to the need of studying the complex behavior of the soil – plant – atmosphere system. That need may not be referred to as having been focused recently (*Shawcroft et al.* 1974, *Norman* 1979, *Goudriaan and van Laar* 1974, *Bouman et al. 1996*) –although its real renaissance coincides with the energetic-based approaches, the spreading of simulation models. Spreading of the systems outlook has given rise to the need of viewing the living beings in conjunction with their environments, holding in mind all possible and negative consequences of removing them from the environment (*Brisson et al.* 2003, *Pronk et al.* 2007). Although the importance of systems' outlook is recognized, most of the analyses of plant – water relations focuses on two end points of the system even today – on water intake from the soil (e.g. *Jackson et al.* 2000, *Novak et al.* 2005) or delivery of water, transpiration (e.g. *Langensiepen et al.* 2009). The transport of water inside the plant the changes occurring in the stem are reviewed less frequently. This may be due to the fact that, since the exact determination of transport of water inside the plant – definition of water potential – it is a familiar fact that such a process requires no extra energy input by the plant; the process is maintained by the water potential difference between the soil and the atmosphere (*Sutcliffe* 1984). (*Jakusch et al.* 2010).

Our analysis has focused on the effects of heavy metals on the water balance of the plants can be examined by MR measurements.

Materials and methods

Our test plant was cucumber (Cucumis sativus) (*Fig 1*), which was grown in nutrient solution in growing chamber. The seeds were germinated in culture dish, on filter paper moistened with distilled water for two days in darkness at 30 ^{o}C. The seedlings with 15-30 mm-long primary roots were transferred to $CaSO_4$ solution at $5*10^{-4}$ M concentration for 24 hours in darkness. The radicles of cucumbers were gained by the $CaSO_4$. After the $CaSO_4$ incubation the plants were put in the nutrient solution and the growing chamber. The lighting was given fluorescent and metal-halogen lamp. The light intensity was 100-140 µmol photon$*s^{-1}*m^{-2}$, and the period of the light intensity was 14/10 hour (light/dark). The temperature of the room was 22/26 ^{o}C. The cucumbers were grown at fourfold dilution Hoagland-solution (*Table 1*). Fe-citrate was the Fe source. The cucumbers were polluted with Pb-nitrate ($PbNO_3$), Zn-sulfate ($ZnSO_4$), Cd-nitrate ($CdNO_3$) and Hg-chloride ($HgCl_2$) solutions at 10^{-5} M concentration (*Fig 2*). The treatments held 7 days.

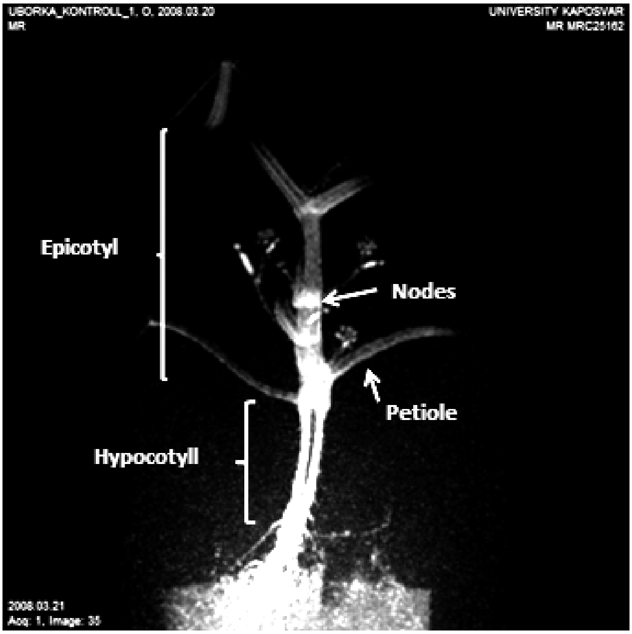

Fig. 1.

MRI record of cucumber

Compound	Concentration (mol/dm3)
KNO_3	$1.25*10^{-3}$
$Ca(NO_3)_2$	$1.25*10^{-3}$
$MgSO_4$	$0.5*10^{-3}$
KH_2PO_4	$0.25*10^{-3}$
H_3BO_3	$1.156*10^{-5}$
$MnCl_2*4H_2O$	$4.6*10^{-6}$
$ZnSO_4*7H_2O$	$1.9*10^{-7}$
$Na_2MoO_4*2H_2O$	$1.2*10^{-7}$
$CuSO_4*5H_2O$	$8*10^{-8}$
Fe-citrate	$1.0*10^{-5}/2.0*10^{-5}$

Table 1

Components of Hougland-solution

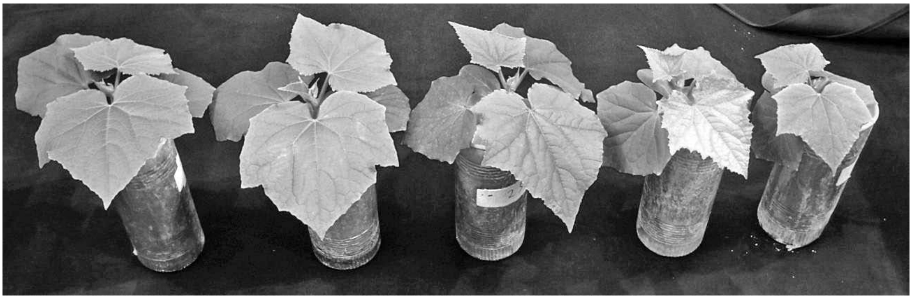

Fig. 2.
Polluted cucumber plants (from the left: control, lead-, zinc-, cadmium-, mercury treatment)

The MR measurements are made on the spin's system. The procedure is based on the interaction of the external magnetic field, electromagnetic waves and the hydrogen nucleus' in the substance. Namely the quantity and distribution of the protons are measured by MR. When we ask the question, where we can find relatively a lot of protons, our answer is: in the water. So the MR doesn't measure the anatomic structure, but the quantity of protons. The anatomic structures are determined by the distribution and quantity of protons.

The site of studies was the Diagnostics and Oncoradiological Institute of the University of Kaposvár (Hungary). The studies were carried out by using a Siemens MR apparatus Type Avanto (*Fig 3*) capable of generating a magnetic field of 1.5 T. The repetition and echo times applied in the measurements were 5.27 and 2.38 sec, respectively. The study made by us involved a pixel spacing of 0.78 mm and a slice thickness of 0.7 mm; according to this the resolution referred to a pixel slab/block of 0.43 mm^3 (*Jakusch et al.* 2010).

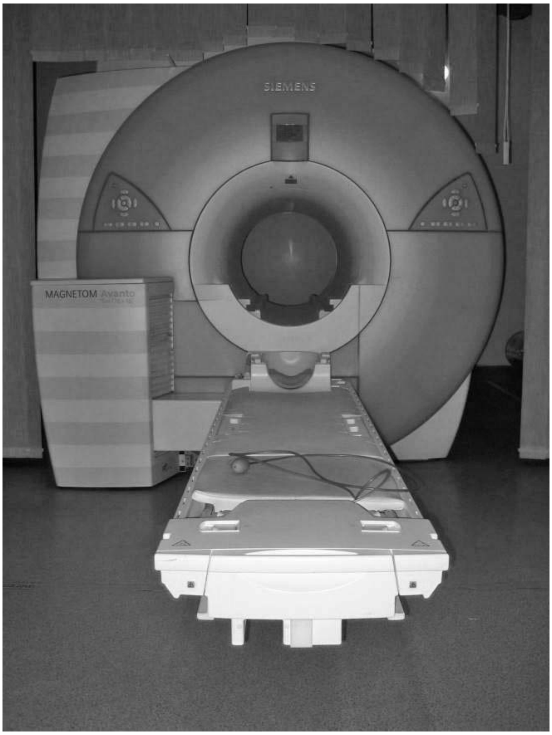

Fig. 3.
Siemens Avanto MRI

The stomatal resistance was measured by AP4 porometer in conductance mode in the growing chamber. The data were collected in mmol H_2O m^{-2} s^{-1} in conductance mode.

After all measurement, the water content measurement was followed. Water content was determined by drying until weight consistence. Water content percentage is determined by the dry and fresh weight.
Being a non-normal distribution involved, the distribution of data has been examined by the chi^2 test. The findings have been evaluated by the application of correlation analysis in studying the relation between the signal intensity and water content in the stem.

Results

Classical measurements

Several experiments confirm that the stomatal resistance, the water content of the root and the shoot are diminished by trace metals (*Hernandez et al.* 1997, Lozano-Rodriguez et al. 1997).

The stomatal resistance was detracted by all heavy metals however the Pb and Zn pollution did not prove significant differences. The strongest inhibition was effected by the Cd and Hg contamination. Significant differences can be detected between these heavy metal treatments and the control cucumber (*Fig 4*).

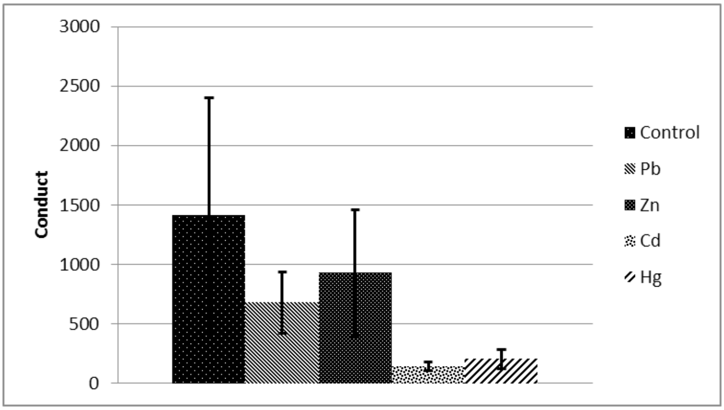

Fig. 4. Stomatal resistance

Heavy metals' effects on plants' development usually decrease their water content (*Seregin et al.* 2004). Some percent of water content deviation can cause remarkable changes in plants. The water content of the root, hypocotyl and epicotyl of the 6-week-old cucumber plants treated by lead and zinc did not differ significantly from the control (*Fig 5, 6, 7*).

In case of the cadmium and mercury treatment significant changes could be detected. The water content of the cucumber plant treated by cadmium declined by 1% compared to the control, and mercury treatment caused more than 2% of decrease in it (*Fig 5, 6, 7*). Differences between the control and these two treatments are statistically proved.

Regarding the first and the second leaf of the plants significant modification cannot be found in any treatment (*Fig 8, 9*). In case of the third and fourth leaves the water content was equal to the control for lead and zinc treatment, but mercury and cadmium treatment both caused 1% of decline in it (*Fig 10, 11*).

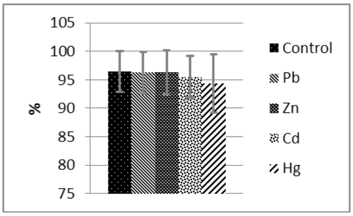

Fig 5 Water content of the root

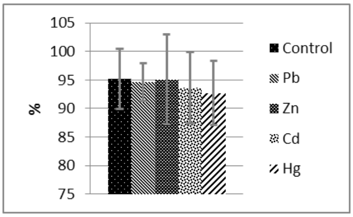

Fig 6 Water content of the hypocotyl

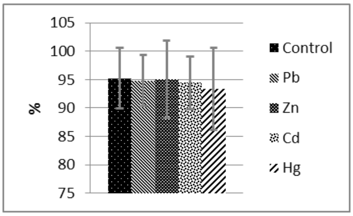

Fig 7 Water content of the epicotyl

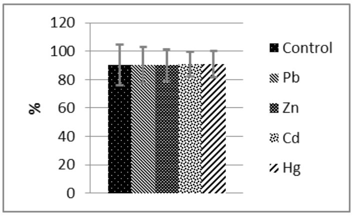

Fig 8 Water content of the first leaf

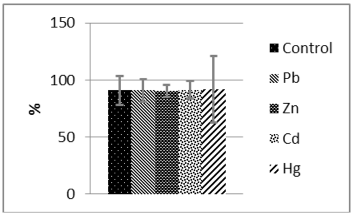

Fig 9 Water content of the second leaf

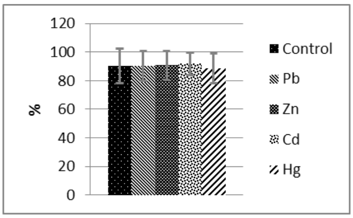

Fig 10 Water content of the third leaf

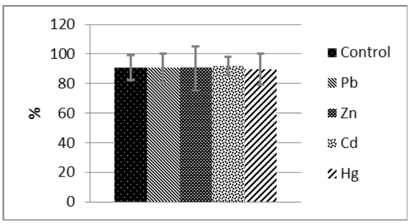

Fig 11 Water content of the fourth leaf

Findings by MRI measurements

During the research heavy metal treatments were applied when the test plants developed their third leaf, and in consequence heavy metals affected these leaves. According to the previous findings of *Sárvári et al.* (1999) heavy metals are transported to the youngest, actually developing part of the plant. Our measurements are shown on *Fig 12*.

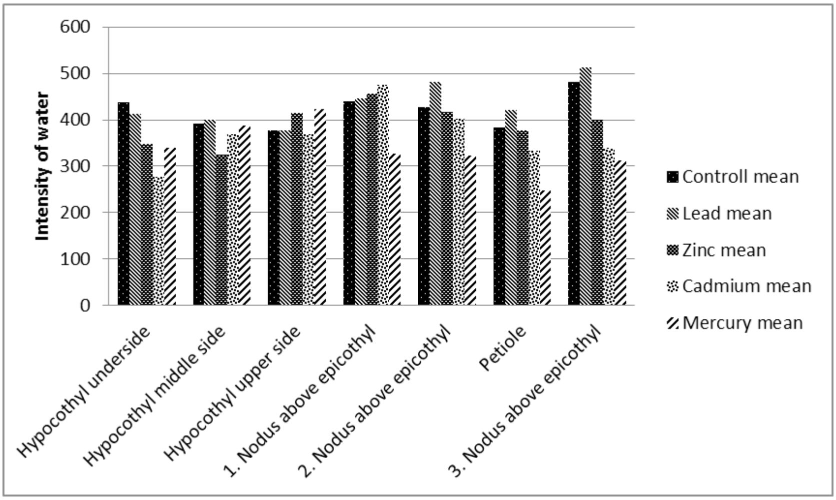

Fig. 12.
Intensity of water in the xylem of cucumber

Signal intensity detected by MRI was high in the hypocotyl and epicotyl of the control cucumber plant. Regarding the hole plant the distribution of water was balanced. Examining the hypocotyl slight increase can be detected towards the top of the shoot. This can be explaned with the decline of root pressure. The signal intensity increased by more than 60 units in the epicotyl above the first node compared to the upper part of the hypocotyl. In the epicotyl above the second node the signal intensity decrased. Above the third node increase of

50 unit can be observed. These modifications in signal intensity can be explained in more ways. According to the theory of cohesion the continious fluid column is moving towards the leaves in the elements of the xylem. In this case deviations in the signal intensity are caused by anatomic properties (*Jakusch et al.* 2010). An other solution can be that the decrease and increase of signal intesity in the epicotyl is caused by different speed of transpiration.

The intensity of the plant's shoot towards its tip also decreased under Pb treatment. The bottom side of the hypocotyl showed 25 units less intensity than the control. The reason is that the Pb accumulated and started blocking transpiration (*Fig 4*) at the location of the intake, the root. This affected the bottom of the hypocotyl as well. The increase in intensity at the center area of the hypocotyl, and the intensity noticed at the top of the hypocotyl was equal to the control cucumber, presumably because of the transpiration blocking. All of the intensities in the epicotyl over the first node were equal to the control plant's intensity, because of the Pb was blocking transpiration, however the water uptake was slightly modified. Significant differences were noticeable during the tests between the Pb treated and the control plant (Chi^2: 6972; p-value: 0.2389).

The intensity grew from the plant's (treated with Zn) hipocotyl towards its epicotyl over the first node. The Zn is essential to plant's growth, and it fastens the process until a certain level. However higher doses are toxic to plant and can easily be transported to the shoot. The intensity decreased in the epicotyl over the first node in comparison to the control plant. It was due to Zn infection. At the end of the treatment the leaves turned clorotic (light green with spots), and turned necrotic. This process was followed during the tests. Significant differences were noticeable during the tests between the Zn treated and the control plant (Chi^2: 6972; p-value: 0.2389).

The intensity of the Cd treated plant's hypocotyl's bottom side was 150 units lower than the control plant's. This was because the Cd blocks the root's growth, water uptake, and affects the hypocotyl's bottom side. However in the hypocotyl towards the tip of the shoot until reaching the epicotyl over the first node the intensity decreases. The Cd blocked transpiration, so the leaves were filled with water. In the epicotyl over the second node the intensity decreased along with the water content (*Fig 7-11*). Significant differences were noticeable during the tests between the Cd treated and the control plant (Chi^2: 6972; p-value: 0.2389).

The intensity of the Hg treated plant's hypocotyl's bottom side was 100 units lower than the control plant's, this maximum is due to the blocking of the root's aquaporins. In the hypocotyl towards the tip of the shoot the intensity increased, and in the upper part of the hypocotyl it even exceeds the control cucumber's results, because the Hg blocked not just the water uptake, but the transpiration as well. The intensity in the treated plant's epicotyl decreased without exception, in comparison to the control and all the other subjects. This was noticeable in the decreased water content too. Significant differences were noticeable during the tests between the Hg treated and the control plant (Chi^2: 6972; p-value: 0.2389).

Discussion

MRI may offer a novel procedure of studying the water flows of plants. The measurement accuracy of the system surpasses by far the errors of traditional plant and water studying procedures. This non-destructive technique can be carried out on live plants offering an unlimited number of repetitions.

Significant differences can be detected between the different heavy metal treatments by MRI measurements, but the classical methods did not prove these deviations. MRI measurement can increase our knowledge on the cycling and pathways of heavy metals in the plants.

MRI as a technique is bound to perform important roles in science and education as well. Its applications are expectable particularly among the research workers and teachers.

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