Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 3, July – September 2014, p. 1149 - 1157

UPTAKE AND INTRACELLULAR TRAFFICKING OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONs) IN PLANTS

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The uptake and movement of iron nanoparticles in elodea plants have been detected by the ESR method. This method is applied for the first time for the monitoring of the translocation, movement and uptake iron nanoparticles in plant organs. Exposition of stem, root or leaves of elodea in superparamagnetic iron nanoparticles (SPIONs) with concentration 12.9 mg/mL and less 10 times showed that iron nanoparticles may enter into the root tissue or cells and move to the stem and leaves. The localization and movement of iron nanoparticles depends on the exposure period and concentration.

(Received July 18, 2014; Accepted September 17, 2014)

Keywords: Iron nanoparticles, ESR method, Broad ESR signal, Elodea plant

1. Introduction

Studies of the transport and accumulation of nanoparticles (NPs) in plants can highlight the environmental consequences of nanotechnology. At present there are relatively a few investigations which have examined the interaction mechanisms of NPs with plants toward phytotoxcity and bioaccumulation . Despite the fact that plants could tolerate the presence of nanoparticles inside their issues, an important question to be addressed is what happens with such nanoparticles if they move into the simplistic or apoplastic transport . Could nanoparticles accumulated in a roots, shoots, stem or leaves of plants and pass through the xylem system of roots, intercellular face of plant tissues or enter the cell? At this point, there are no studies about the real mechanism by which nanoparticles can penetrate into the plant cells. However, there is a recent work dealing with internalization of gold nanoparticles using tobacco protoplasts (Onelly et al.,2008). The study of NPs uptake in plants is important for the understanding interaction mechanism of NPs with intracellular components and processes which cause to toxicity. Up still it is unknown how NPs migrate by plant organs and enter into cells . Understanding the principles of how NPs can transmigrate into cells could enable greater control over cellular uptake and would improve prediction of possible toxic effects. Nanoparticles may enter plant roots through osmotic pressure, capillary forces, pores across the cell wall, which has a thickness ranging from 5 to 20 nm, determines its sieving properties (Fleischer et al., 1999; Fujino and Itoh, 1998; Madigan et al., 2003; Zemke-White et al., 2000) and intercellular plasmadesmata (50–60nm at midpoint) (Smith, 1978) or via the highly regulated symplastic route.

A recent study showed significant uptake of nano-sized copper (nCu) by *Phaseolus radiates (Mung bean)* and *Triticum aestivum* (wheat), with reported bioaccumulation factors of 8 and 32 L/kg, respectively (Lee et al., 2008) Transmission electron microscopy analysis showed that nCu was absorbed and agglomerated into the cytoplasm of the root cells and the extent of absorption depended on the concentration of the nCu deposits on the roots' surface. Another study found individual nZnO particles in endodermal and vascular cells of ryegrass exposed to 1,000 mg/L of nZnO (Lin and Xing , 2008) ; the translocation factor (defined as Zn content ratio of shoot to root) was 0.01 to 0.02. Significant uptake, translocation, and accumulation of nFe3O4 in the roots and leaves of *Cucurbit maxima (pumpkin) h*as also been reported without any effect on

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growth and development of the test species (Zhu et al., 2008) . Therefore, some uptake of nanoparticles by plants is very possible. However, little is known about the maximum nanoparticle size amenable for plant uptake, and how uptake kinetics and toxicity are affected by plant type and rhizospheric chemistry. Recent research highlights the importance of transition metals that adsorb to nanoparticles and promote oxidative stress (Wilson et al., 2002) , whereas natural organic matter in soil or pore water can sorbs, coat, or stabilize nanoparticle suspensions and affect their mobility, bioavailability, reactivity, and toxicity (Handy et al., 2008). The transport and accumulation of nanoparticles in plant tissues and cells suggests a plausible mechanism for nanoparticle uptake: a dynamic competition between nanotransport driven by water and nanoparticle convections and the physical hindrances of plant tissues and nanoparticle aggregation.

2. Materials and Methods

Higher water plants *Elodea Canadensis* as biological targets for nanoengineered materials. In this study higher water plant *Elodea Canadensis* have been exposed as biological targets to nanoengineered materials. This plant have well known electrophysiological characteristics and can easily be cultured and handled in the laboratory conditions. The plants of Elodea Canadensis were selected for studies due to the following reasons: they represent one of key species of aquatic macrophytes with an extremely broad range; this species was used in pervious studies of accumulation and phytotoxicity of chemical pollutants, which provide material for comparative analysis; this tpecies has outstanding economical and ecological importance as one of aggressive introduced species with a strong potential for invasion into new aquatic bodies (Johnson et al., 2011). For these reasons, this species has been the focus of many experiments and research in the hopes of establishing a greater knowledge of growth habits, the true threat it causes, and possible prevention methods. The plants *Elodea canadensis* were purchased from Zoo magazine of Lausanne. Elodea plants was cultured at room temperature (18–23 °C), in a glass aquarium filled with tap water and under illumination with cool white light (from fluorescent lamps). Root, leaves and shoots of *Elodea Canadensis*, and cells of leaves were selected as biologically relevant targets in this nanophytotoxicity study. Subsequently, leafs, shoots or roots was placed in the experimental medium consisting of tap water and nanoparticles. Depending on a selected nanomaterial, the exposure will last from several hours to several days.

Fig. 1. Elodea Canadensis. The leaves of Elodea form a characteristic two-layer structure. The epidermal cells of the upper layer are large and visible by ordinary microscope. The chloroplasts and their motion can clearly be seen.

Superparamagnetic iron oxide nanoparticles – SPIONs . Maghemite, γ-Fe2O3, is a red-brown, ferromagnetic mineral isostructural with magnetite, but with cation deficient site. It occurs in soils as a weathering product of magnetite or as the product of heating of other Fe oxides, usuallay in the presence of organic matter (Cornell RM, Schwertmann U., 2003) .*Gamma*-Fe_2O_3 was synthesized by Prof. Alke FINK, University Fribourg, Fe content: 12.9 mg/mL; pH~ 3, at 10^{-3} M HNO_3; delivered in July 2012.

ESR studies. Spectra of plant objects were registered with the aid of an X range ESR spectrometer ESR Bruker ESP300E (Bruker, Germany) at room temperature (293 K) observing conditions indicated in captions to the corresponding figures. The uptake and internalization of superparamagnetic SPIONs (g-Fe2O3) was followed by electron spin resonance (ESR). In particular, ESR revealed a very fast uptake and a significant extent of biomagnification of waterborne SPIONs by leaves of plants.

3. Results

For the study of transport, accumulation and migration of nanoparticles in plants we used iron nanoparticles (SPIONs) . The application of iron nanoparticles for study of nanoparticles transport in plants is more interesting than two peculiarities. Firstly, the iron NPs has supermagnetic properties and by ESR signal we can identify the location, accumulation and translocation of iron NPs in keeping the native state of objects. Secondly, the iron NPs allow a very specific localization of particles to release their load, which is of great interest in the study of nanoparticulate delivery for plants. A few works have been reported regarding the uptake, translocation and specific localization of magnetic nanoparticles (less than 50 nm) in pumpkin plants (Gonzalez-Melendi et al.,2008; Zhu et al, 2008; Corredor, et al.,2009) . Magnetization signals of various strengths were observed from different portions (ranging from roots to leaves) of the treated plants which clearly indicates the successful translocation of nanoparticles in the entire plant system irrespective of the area of application.

ESR signal of ferro-fluid SPIONs. Before experiments we identified the ESR signal of original ferro-fluid SPIONs. For this purpose the quartz glass capillary with inside diameter 0.7 mm was filled by original ferro-fluid with concentration 12.9 mg/mL, which pH was 3. In figure 2 shown the ESR signal of original SPIONs which was identified in Bruker ESP300E. From figure 2 it is clear that the ESR signal of ferro-fluid is vastly strong signal and we can observe it in a wide range of magnetic field (2000 - 4500 G).

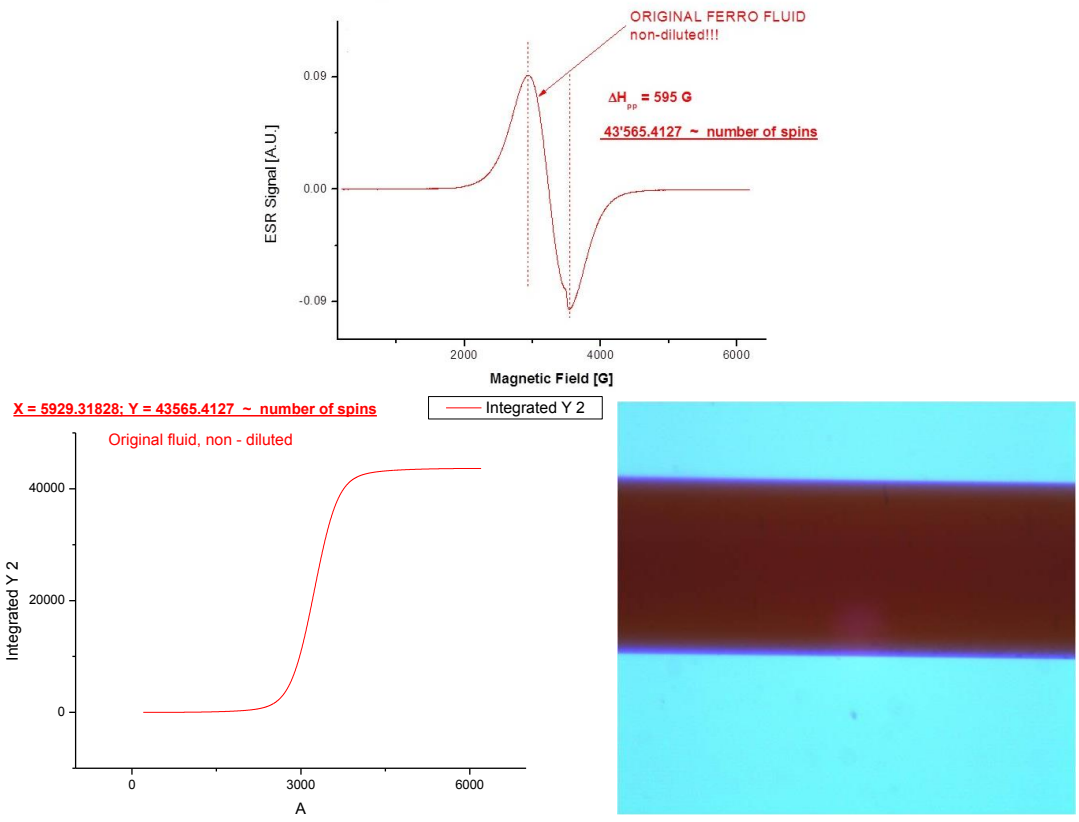

Fig 2. The ESR signal of original ferrofluid - SPIONs with concentration 12.9 mg/mL

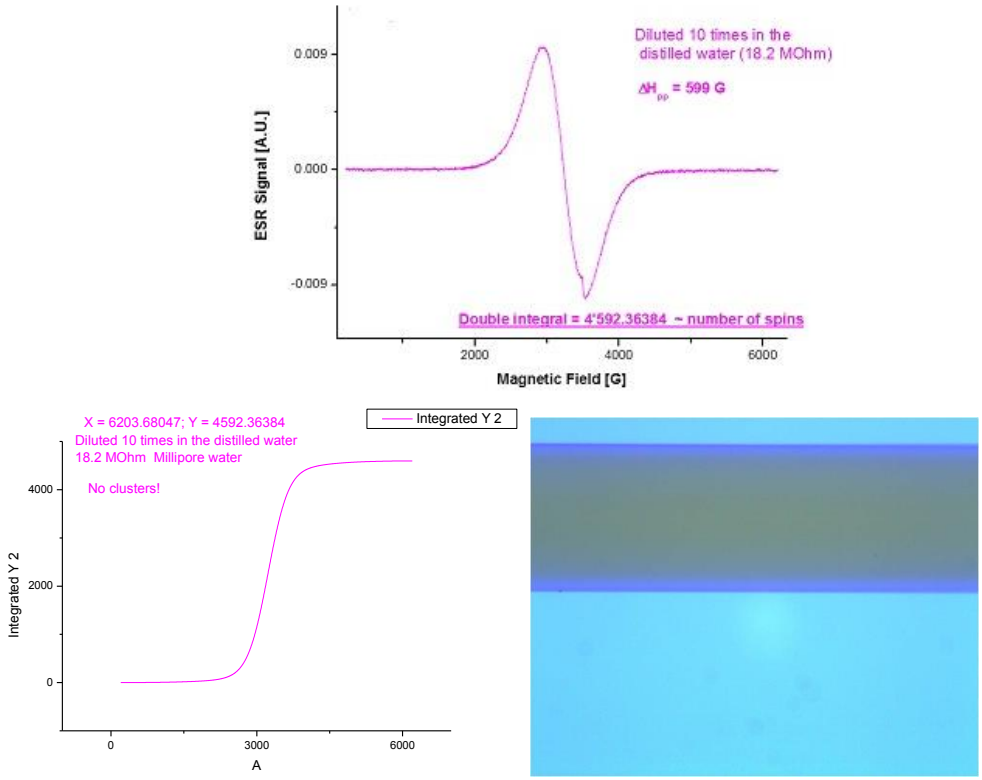

Fig 3. ESR signal of diluted 10 times of original SPIONs liquid

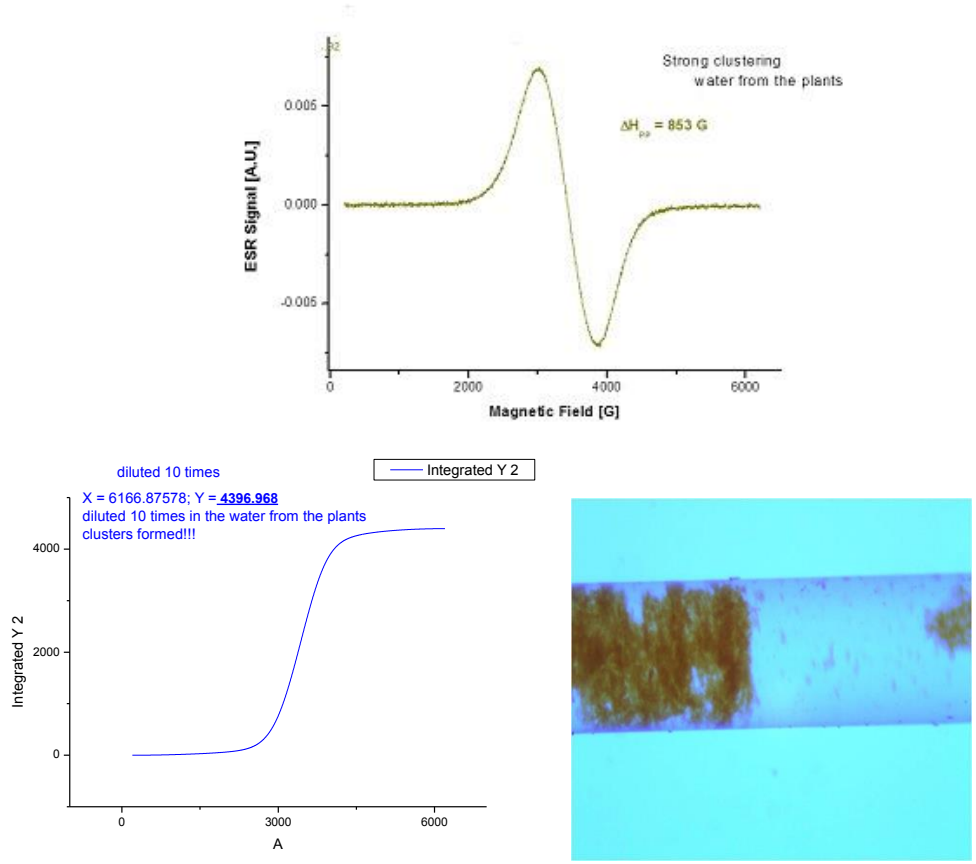

Fig 4. ESR signal of strong clustering water which containing SPIONs after exposure of elodea plant

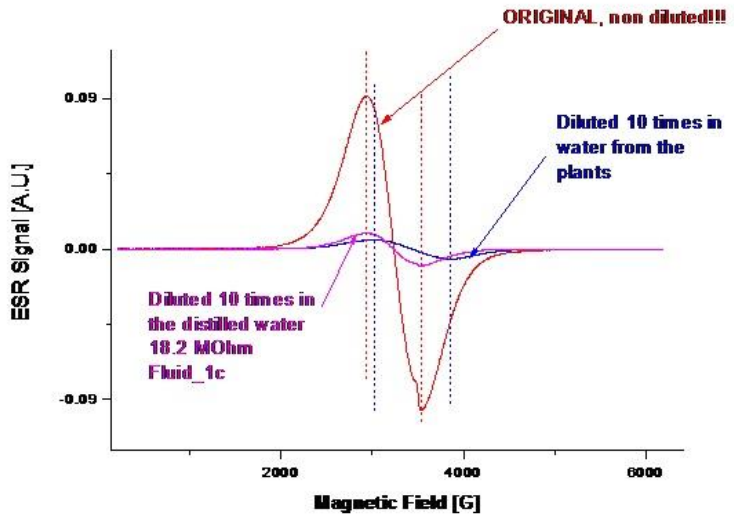

Fig 5. The compare of ESR siqnals ferro-fluid original (red), diluted 10 times in water from the plants (blue) and diluted the distilled water 18.2 MOhm (rose).

Then we have diluted 10 times of original ferrofluid with distilled water from Millipore 18.2 MOhm and identified the ESR signal of this liquid. The ESR signal of this diluted SPIONs remains ~ 600 G, as for the original ferro-fluid. The ESR signal of diluted SPIONs was shown in fig.3. It is known that when nanoferrofluid remains the long-term, when interacts with organic polymeric materials clusters is forming. It is interesting what may happen with ESR signal of this fluid. We observed that during keeping the elodea leaves in the diluted ferrofluid the clusters is forming. In fiq.4 is shown the ESR signal of this ferrofluid. As shown in figure 4 the form of ESR signal of this ferrofluid remains same. There may be some characteristically changes, but the form of signal is as original ferrofluid. In the fig.5 shown all three ESR signal of SPIONs and was compared their parameters.

This case allow us to say that iron nanoparticles after entering into the plant roots, shoots or leaves the clusters may forming and even if the clusters forms the ESR signal of ferrofluid will same as the ESR signal of the origin ferrofluid. Therefore the strong ESR signal of SPIONs allow us to monitor the uptake, transport and accumulation of iron nanoparticles in different organs of plants. These experiments with SPIONs may explain the transport mechanisms of nanoparticles in roots, shoots, leaves and even of cells of plants.

The absorption of iron nanoparticles in elodea leaves. The use of ferrofluids in plant requires colloidal stability at physiological pH, which is close to the isoelectric point of the magnetic iron oxide nanoparticles.The pH of original ferrofluid – SPIONs was 3 – 3.5 and this value does not suitable for plants. Therefore we have changed the pH of SPIONs up to pH6.5. Then we have diluted it 4 time and used as experimental liquid**.** The some broken off leaves of elodea from stems has remained 5 days in diluted ferrofluid with pH6.5. Then we identified ESR signal of two types leaves from ferrofluid. First leaves was directly taken from fluid and was dried. The second types of leaves were strongly washed and dried. The ESR signal of directly taken from fluid leaves was very strongly, but the ESR signal of washed leaves was weak in comparison the directly taken from fluid leaves. The results of this experiment were shown in fig.5. Figure 4 shows that iron nanoparticles strongly absorb on surface of leaves, but a small signal from of washed leaves allow us to say that iron nanoparticles may entre into the cell.

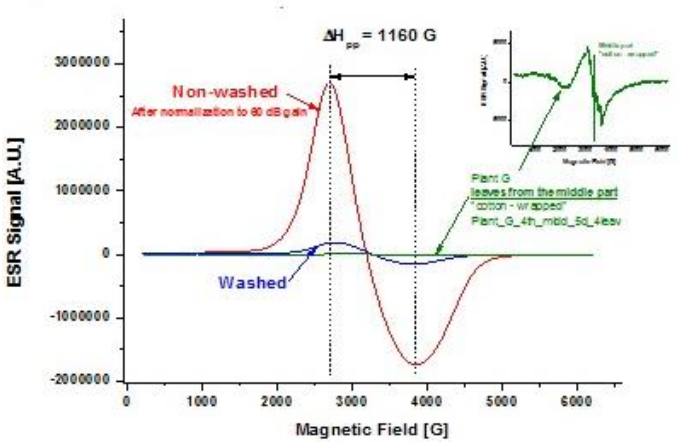

Fig 6 . The ESR signals of elodea leaves which were exposure 5 days in the citrated ferrofluid of SPİONs

In other experiment we have study the dependence of amplitude of ESR signal on the period of exposure in the ferrofluid. For this goal the leaves of elodea was kept in the diluted ferrofluid in different period. The exposure times was 1min, 30 min, 60 min and 1100 min. The mass of leaves was normalized for every variants. Leaves of elodea was strongly washed and dried after withdrawn from ferrofluid. The ESR signal of dried leaves was identified. The results of this experiment were shown in fig.7. The fig.7A shows that the amplitude of ESR signal increase with increasing exposure period. The dependence of amplitude of ESR signal was linear in the range of 1-60 minutes. But in the long-time interval the saturation phenomenon has been observed (fig.7B).

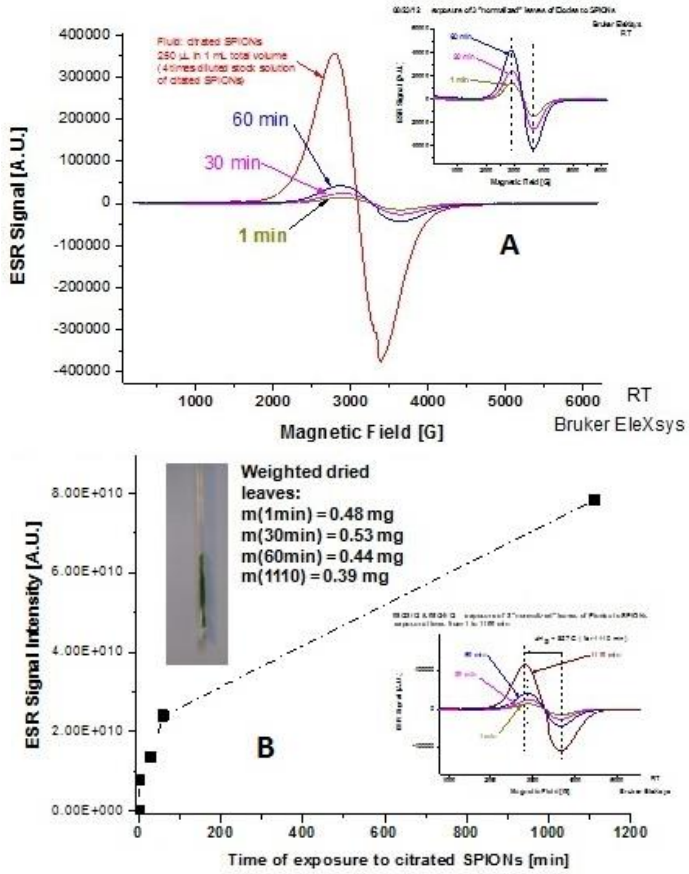

Fig. 7 The dependence of amplitude of ESR signal of elodea leaves which were exposure by different times in SPİONs solution

The movement of superparamagnetic iron oxide nanoparticles in elodea plants. In this experiment we studied the iron nanoparticles uptake, transport or movement through the root, stem and leaves. For this goal part of the end of the elodea stem with leaves were keeping in the original ferrofluid (12.9mg/mL) . The branch to the other side of elodea stem with leaves were keeping in water which was from aquarium. The exposure period was 24 hours. After exposure 24 hours was taken the examples from different part of elodea branch and identified the ESR signals. The results of this experiments were shown in fig.8. The A part of stem of elodea was directly in the ferrofluid. In first variant of experiment leaves and shoot were taken from A part and strongly washed. As seen from fig.8 in the leaves which are taken from A part of stem observed a normal ESR signal. The ESR signal also was identified in leaves which were taken from B part of stem. This part of stem was exposed in water. The amplitude of ESR signal is less then the signal from A part of stem, but the parametrs and form of the signal are same. Then we verified the signal in C part of stem. In this part of stem we did not observed the ESR signal.

In an other experiments we exposed the roots of elodea in the original ferrofluid. The exposure time was same as first variant of experiments. The stem of elodea with leaves were keeping in water. Firstly the examples was taken from part of root which is directly in the ferrofluid and were strongly washed. The ESR signal of this part of root was very strong and same as the signal of original ferrofluid. The second example were taken far from part of root which was in water. The signal roots of this part was weak , but the form and parametrs were same as first part of root. In the stem, leaves or in the roots of elodea which were keeping in the water we did not observed strong signal of ESR.The results of this experiment are shown in the fig.9

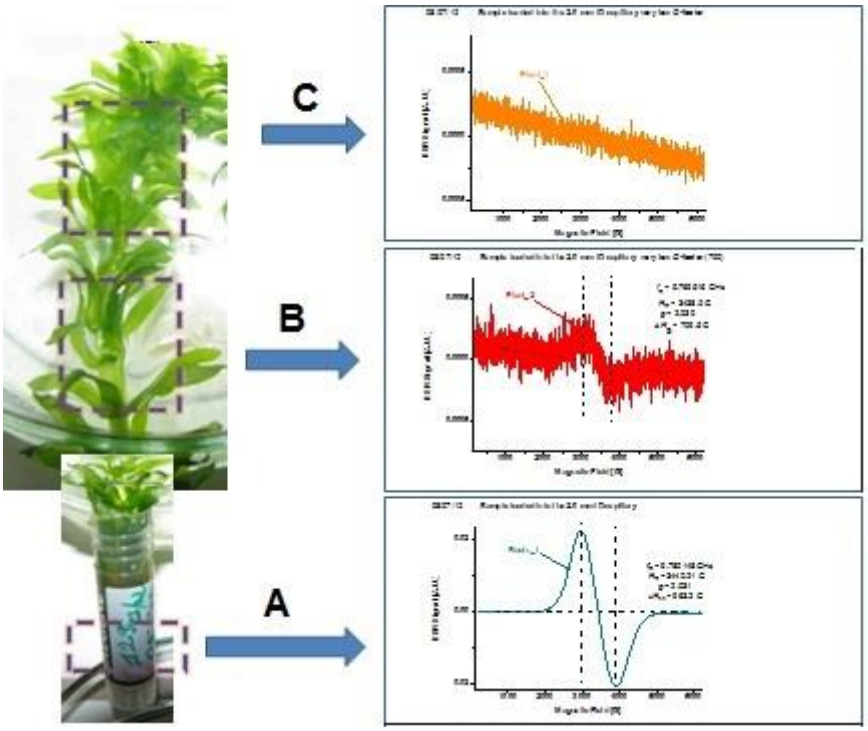

Fig. 8. The ESR signal of SPIONs from different part of elodea shoot

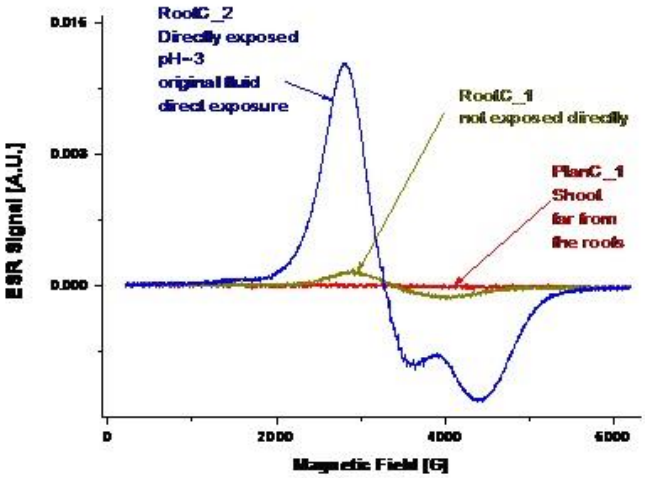

Fig.9 ESR siqnal of elodea root exposure in original SPİONs solution during 24 h

4. Discussion

Plants are one of the important link and are a potential pathway for the bioaccumulation of nanoparticles into the food chain. Except all of this water plants are a good ecological cleaners in aquatic ecosystems. Therefore the study of mechanism accumulation of nanoparticles in water plants is actual and urgent in such intensive development of nanotechnology. There are a few researches about uptake, accumulation, transport and localization of nanoparticles in organs and tissues of plants (Lin and Xing, 2008; Zhenyu Wang and et al.,2012; Zuny Cifuentes, et al.,2010). In the work of Zhu H. and his employees have been detected that plants can take up magnetite nanoparticles from soil, accumulating then in different parts, especially in leaves (Zhu et al., 2008). The originality of our investigation consists in that for the first time we applied ESR method for the study the uptake, transport and movement of iron nanoparticles in plants.

The ESR signal of ferrofluid is remains very strong even diluted it many times. Furthermore the sintering does not change it character and we observe same signal. In the experiments have been detected decreasing amplitude of ESR signals after strong wash of leaves. It may be means that iron nanoparticles accumulate just on the surface of roots or leaves. However we observed the ESR signal in examples which were washed many times. In other experiments, which have been detected saturation the value of amplitude during exposure leaves on long time. It means that part of iron nanoparticles even so inside of root or leaves. In the experiments where root or part of stem with leaves were keeping in ferrofluid directly we observed the ESR signal even in the stem or leaves which were not exposure in ferrofluid. It means that iron nanoparticles capable migrating to different regions of stem or leaves of elodea.

Results of our experiments allow us to conclude the following statements: 1) if the iron nanoparticles are inside of the tissue of plants (even in symplastic or apoplastic) and are interact with organic materials and sintered we can detect them by ESR signal; 2) iron nanoparticles capable of penetrating into roots of living elodea plant tissues; 3) iron nanoparticles capable of penetrating to stem and leaves of elodea; 4) iron nanoparticles capable migrating from root to the stem and from the stem to leaves; 5) the movements of iron nanoparticles over short distances to be favored; 6) long range movement of the iron nanoparticles through the plant organs was also detected, nanoparticles having been found in leaves a little after long term exposure of elodea in diluted solution of SPIONs.

To our knowledge, this is the first study showing the root − stem − leaves redistribution of iron nanoparticles within elodea plants. The current study provides direct evidence for the bioaccumulation and biotransformation of nanoparticles in plants which has significant implications on the potential risk of nanoparticles. These findings show that the use of ESR signal of iron nanoparticles or other paramagnetic resures suitable method for detecting uptake, accumulation and transport of nanoparticles toward resolve their toxicity.

Acknowledgements

We acknowledge the financial support of the Swiss National Science Foundation through the project: SCOPES JRP/IP: IZ73Z0_128068 /1. " Interactions of nanoparticles with membrane systems of cells of higher water plants – towards mechanisms of nano-phytotoxicity".

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