INFLUENCE OF ANIONIC SURFACTANTS ON Zn^{2+} AND Sr^{2+} UPTAKE BY IVY (*Hedera helix* L.) LEAVES

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Abstract: Surfactants are frequently used as adjuvants for improving the efficiency of foliar applied fertilizers, pesticides and other biologically active substances. In our paper we used detached leaves of ivy (*Hedera helix* L.) for the study of the influence of anionic surfactants sodium dodecylsulfate (SDS) and sodium dicyclohexyl sulfosuccinate (DCSS) on zinc and strontium uptake by leaf surface and transport by radiotracer technique with $^{65}ZnCl_2$ and $^{85}SrCl_2$. Accumulated amounts of Zn^{2+} and Sr^{2+} ions by the surface of detached intact ivy leaves were 5.0 and 1.1 μg/g, respectively. Ivy leaves pretreated for 24 h in 1 mM SDS or DCSS solutions accumulated approx. twice more Zn and five time more Sr than non treated leaves. Pretreatment with surfactants increased mobility of zinc and strontium in leaf tissues. Separate experiments showed that both SDS and DCSS were sorbed onto the leaf tissue reaching equilibrium within several hours of immersing leaf blades to surfactant solutions. The process can be described in terms of partition equilibria $P = [C]_{leaf}/[C]_{solution}$ with log P = 1.396 within surfactant concentration studied $C_o \leq 100$ μmol/L. The mechanism of action of surfactants on metal ion uptake is discussed.

Key words: ivy, *H. helix* L., foliar uptake, surfactants, zinc, $^{65}ZnCl_2$, strontium, $^{85}SrCl_2$

1. Introduction

The cuticle is the main interface between plants and their environment. It covers the epidermis of all aerial primary parts of plant organs as a continuous extracellular matrix. This hydrophobic natural composite consists mainly of the biopolymers, cutin, and cuticular lipids called waxes. Water-repellent cuticle or waxes on a plant surface is the major barrier to the spreading, retention and penetration of solutes (BARGEL *et al*., 2006). Surfactants are frequently used as surface-acting adjuvants that improve the absorbing, emulsifying, dispersing, spreading, sticking, wetting or penetrating properties of foliar applied fertilizers. On the other hands, surfactants alone are able to accumulate in plants and to change physico-chemical properties of the leaf surface.

It has been proposed that there is a requirement for surfactants to be absorbed into plant leaves at rates similar to those for the active ingredient for the best uptake results (STOCK *et al*., 1993). However, there have been only a few studies of surfactant uptake (ZABKIEWICZ *et al*., 1995) compared to the multitude of studies quantifying active ingredient uptake, and all of these are reported on a percentage basis. The finding that active ingredient mass uptake can be related to initial dose applied (FORSTER *et al*., 2004; NIELSEN *et al*., 2005) raises the question of whether the surfactant component of a typical spray formulation will behave in a similar fashion.

Our paper is a step towards addressing the question of the influence of anionic surfactants sodium dodecylsulfate (SDS) and sodium dicyclohexyl sulfosuccinate (DCSS) on the uptake of Zn and Sr as bivalent metals by the leaf surface of ivy (*Hedera helix* L.).

Ivy (*H. helix*) is a common, easily available species, which possesses a number of advantages. The ultra-structure of leaves is well described (CANET *et al*., 1996; GILLY *et al*., 1997). Ivy leaf cuticle was used as a model to investigate cuticle permeability (CHAMEL, 1986). Fine structure and permeability of ivy leaf cuticles in relation to foliar development and after selective chemical treatments and relationship between structure and permeability are well described (GILLY *et al*., 1997).

In our previous papers are described some properties and behavior of sulfosuccinate esters in biological systems (VRBANOVÁ *et al*. 1997; CSERHÁTI *et al*. 1997) and leaf uptake and distribution of Zn ions by ivy (MAREŠOVÁ *et al*., 2009).

2. Materials and methods

2.1 Chemicals

Standardized $^{65}ZnCl_2$ solution (0.877 MBq/cm^3, 50 mg/dm^3 $ZnCl_2$ in 3 g/dm^3 HCl) and $^{85}SrCl_2$ (2.665 MBq/cm^3, 20 mg/dm^3 $ZnCl_2$ in 3 g/dm^3 HCl) were obtained from The Czech Institute for Metrology, Prague. Sodium dodecylsulfate (SDS) was obtained from Sigma, sodium dicyclohexyl sulfosuccinate (DCSS) from Cytec Corp., U.S.A. Solutions were prepared in deionized water, conductivity 0.05 µS/cm, pH was adjusted with NaOH.

2.2 Plant material

Ivy branches (*H. helix* L.) were picked during spring months from freely grown garden vegetation. The upper part of branches were cut from a wild ivy plant, washed repeatedly in deionized water and used for experiments. Leaves about 0.2 – 0.3 g of fresh weight and leaf area 11.5 - 12.5 cm^2 were used in experiments.

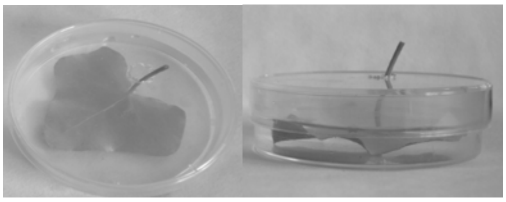

Fig. 1. Macro photo of ivy leaves in experiments. Leaf blades immersed by both sides in nutrient media in Petri dishes. Characteristic signs: short petioles; shallow sinus; well developed veins; terminal, lateral and basal lobes.

2.3 Bioaccumulation experiments

Pretreatment of leaves by surfactants was made by immersing of detached leaf blades into SDS or DCSS solution in deionized water. For metal uptake experiments leaf blades were immersed in 10 ml 25% HM medium supplemented with 5 µmol/L $^{65}ZnCl_2$ or $^{85}SrCl_2$ in dishes covered with plastic lids (Fig. 1.) in cultivation room at 22±2°C illuminated with artificial light (2 000 lx) in 12h/12h light/dark cycle. The following molar concentrations of salts were present in full-strength HM (mM):

$MgSO_4.7H_2O$ – 1.5; KNO_3 – 4.0; $CaCl_2$ – 4.0; $NaH_2PO_4.2H_2O$ – 1.87; $Na_2HPO_4.12H_2O$ – 0.13; $FeSO_4.7H_2O$ – 0.06; $NaNO_3$ – 4.0; NH_4Cl – 3.17; NH_4NO_3 – 2.0; H_3BO_3 – 0.14; $Na_2MoO_4.2H_2O$ – 0.0025; $MnSO_4.5H_2O$ – 0.21; $ZnSO_4.7H_2O$ – 0.023; $CuSO_4.5H_2O$ – 0.033.

2.4 Radiometric analysis

A gamma spectrometric assembly using the well type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, The Netherlands) and data processing software Scintivision 32 (ORTEC, USA) were used for ^{65}Zn and ^{85}Sr determination in leaf biomass and solutions. Counting time 600 s allowed obtaining data with measurement error <2 %, which do not reflect other source of errors.

2.5 Surfactant analysis

Anionic surfactants were determined by the MBAS method (ARAND *et. al*., 1992). Shortly, anionic detergents react with methylene blue to form a blue colored complex that is extracted into chloroform and blue coloration is measured at 651 nm.

3. Results and discussions

3.1 Uptake of surfactants by ivy (H. helix L.) leaves

Leaf surface of *H. helix* is able to accumulate anionic surfactants SDS and DCSS from water solutions (Fig. 2.). Concentration equilibrium C_{solid}/C_{water} is reached at 20 °C within several hours. Such slow processes are typical for partition equilibria between solution and solid materials at which the rate limiting step is the diffusion process in existing membrane systems.

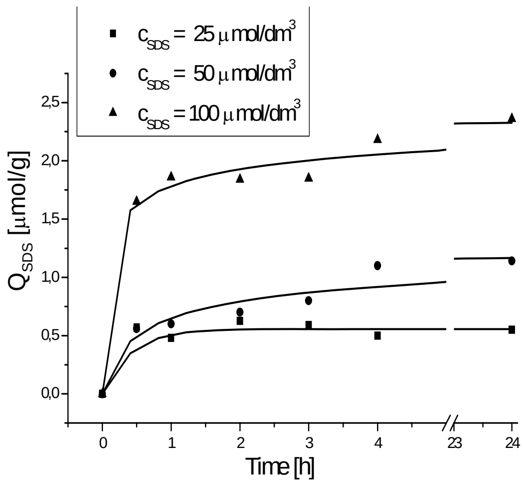

Fig. 2. Kinetics of SDS uptake (μmol/dm³, w.w.) by detached ivy leaves (*H. helix* L.) at 20 °C. The initial SDS concentrations: 25.0 (■-■-■), 50.0 (●-●-●) and 100.0 (▲-▲-▲) μmol/dm³. Leaf biomass: 40.3 ± 0.15 g/dm³ w.w. (±SD), leaf blades area 1 654 ± 58.0 cm²/dm³ (±SD).

Q_{SDS} and Q_{DCSS} (µmol/g, d.w.) values are proportional with the initial concentration of surfactants in solution within the concentration range studied $C_0 \leq$ 100 µmol/L (Fig. 3.). The process of SDS and DCSS accumulation by *H. helix* leaf tissues can be described in terms of partition equilibria with partition coefficient log *P* = 1.349 for both substances.

Both SDS and DCSS contain C_{12} hydrocarbon part and one ionisable anionic group, what explain similar behaviour in the contact with the leaf structures.

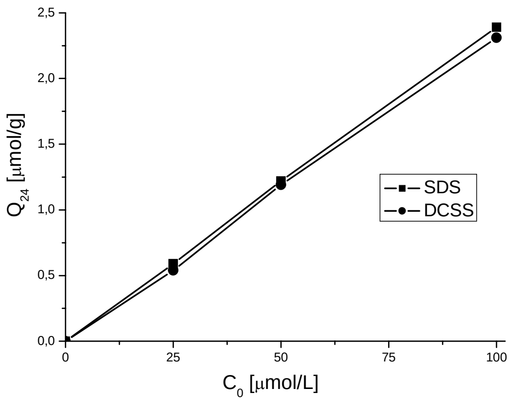

Fig. 3. Uptake of SDS (■) and DCSS (●) by the surface of detached ivy leaves (*H. helix* L.), expressed as Q_{24} (µmol/g) in dependence on the initial concentration C_0 in solution. Log *P* = 1.394

3.2 Influence of pretreatment of leaves by surfactants on Sr and Zn uptake and distribution

Ivy leaves treated with SDS or DCSS solution accumulated higher amounts of Zn^{2+} and Sr^{2+} ions, comparing with non-treated control leaves. Enhancement ratio ER for Zn and Sr is shown in Tab. 1. Non-treated ivy leaves accumulated 4.55 times more zinc than strontium and SDS-treated leaves 3.2 times more zinc than strontium. Similar behavior can be expected also in the case of other bivalent metals. The effect of surfactants on metal sorption of inorganic sorbents was studied by AHN *et al.* (2009). SDS and DCSS -impregnated activated carbon sorbed Cd^{2+} up to 0.198 mmol/g, which was more than one order of magnitude better than Cd^{2+} sorption by activated carbon without surfactants.

Treating of ivy leaves with SDS or DCSS solution caused the increase of zinc and strontium mobility in plant tissues. As can be seen from data presented in Fig. 4 both metals are transported with higher efficiency from immersed part of leaf blades to petiols and to other parts of plants. Strontium and zinc foliar uptake and transfer in tomato plants (*Lycopersicum esculentum* L.) was studied by BRAMBILLA *et al.* (2002). Leaf to fruit transfer coefficient for ^{65}Zn was one order magnitude higher than for ^{85}Sr.

Tab.1. Zinc and strontium uptake (µg/g, d.w.) by non treated and pretreated leaf surface of ivy (*H. helix* L.) after 24 h in 1.0 mmol.dm^{-3} SDS or DCSS. Zn and Sr uptake from 25% HM spiked with $^{65}ZnCl_2$ (5 µmol.dm^{-3}) or $^{85}SrCl_2$ (5 µmol.dm^{-3}).

Metal	Uptake [µg/g]		ER*	Uptake [µg/g]		ER*
	Non-treated	SDS-pretreated		Non-treated	DCSS-pretreated	
Zn^{2+}	5.0	17.0	3.4	3.9	11.1	2.8
Sr^{2+}	1.1	5.4	4.9	-	-	-

* Enhancement ratio ER is the ratio of the metal concentration in leaves treated with surfactants to the metal concentration in non-treated leaves.

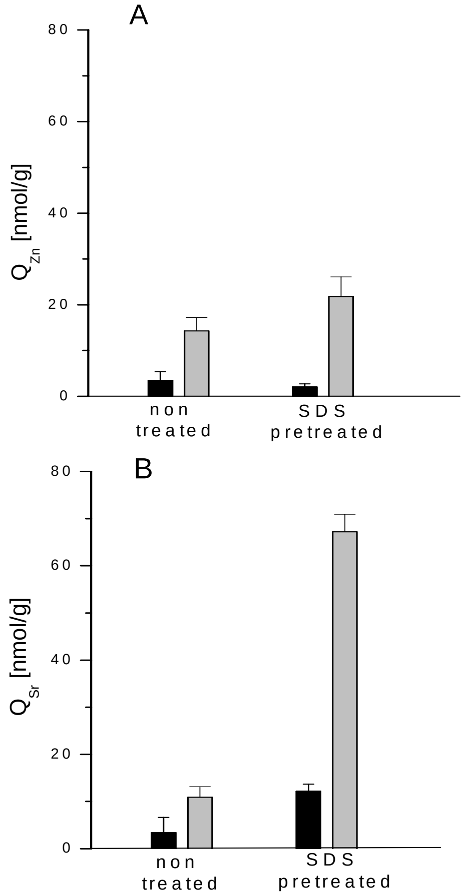

Fig. 4. Influence of SDS pretreatment on Zn **(A)** and Sr **(B)** uptake and distribution in ivy leaves (*H. helix* L.). Leaves were pretreated for 24h with 1.0 mmol/dm^3 SDS, then immersed in 5.0 µmol/dm^3 $ZnCl_2$ or $SrCl_2$ in 25 % HM, spiked with $^{65}ZnCl_2$ (114 kBq/dm^3) or $^{85}SrCl_2$ (311 kBq/dm^3) without SDS. Uptake *via* the surface of fully immersed leaves. Cultivation at illumination 12h/12h light/darkness (2 000 lx), pH 5.5 and 22±2°C. Data as the mean of two replicates. Error bars represent standard deviation (SD) of the mean. Wet weight of leaves: **A.** 0.29 ± 0.02 (±SD) g /10 ml, **B.** 0.24 ± 0.01 (±SD) g/10 ml. Leaf area [cm^2] – **A.** 10.82 ± 0.67 (±SD), **B.** 11.7 ± 0.36 (±SD). Data of leaf blades (■■) and non immersed leaf stalks (■■) expressed as Zn or Sr concentration (nmol/g), d.w.

To explain the effect of anionic surfactants on metal uptake by leaf surface and distribution in leaf structures will require a more detailed study. Stimulating effect can be caused by the following factors: increase of capacity of polar or water path for ion transport or the decrease of viscosity of cuticular and wax structures on the leaf surface, or metal cation - surfactant anion interactions improving metal mobility in the lipophilic leaf structures.

RIEDERER and SCHÖNHERR (1999) showed that treating the outer surfaces of isolated cuticles of Seville orange (*Citrus aurantium* L.) and pear (*Pyrus communis* L. cv. Bartlett) leaves with a number of nonionic (polyoxyethylene) surfactants increased their permeability to water by factors ranging from 4.1 to 14.7 and from 7.2 to 152.4, respectively. None of the surfactant treatments altered the amounts or composition of waxes in the cuticles used for transport measurements. Anionic surfactants can react with cations of bivalent ions.

TALENS-ALESSON (2007) found that SDS micelles are able to bind Zn^{2+} ions and ions of other metals. However in all sorption experiments we used SDS and DCSS solutions in concentrations $\leq$ 1 mmol/L, what is approximately 7 times lower concentration than critical concentration of micelle formation CMC = 6.9 mM (NAKAGARAJAN, 2003).

According to KIRKWOOD (1999) the physico-chemical properties of the cuticle may affect the rate and efficiency of cuticle permeation. The permeation of organic solutes is influenced by their solubility characteristics as indicated by octanol/water partition coefficients (log K_{ow}) and cuticle/water partition coefficient (log K_{cw}). Penetration of hydrophillic organic compounds (low log K_{ow}) may be enhanced by hydration of the cuticle**,** while transcuticular transport of non-polar organic solutes (high log K_{ow}) is increased by factors which reduce the wax viscosity.

4. Conclusions

Anionic surfactants sodium dodecylsulfate $C_{12}H_{25}OSO_3Na$ and sodium dicyclohexyl sulfosuccinate $C_{16}H_{25}O_4SO_3Na$ are sorbed by ivy leaves (*Hedera helix* L.) immersed in surfactant water solution. Pretreatment of leaves by surfactants increases their capacity to sorb Zn^{2+} and Sr^{2+} ions. Obtained data are discussed from the point of view of the effect of surfactants on the leaf structures and metal uptake.

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