Effect of Pb-stress on selected physicochemical surface properties of barley (*Hordeum vulgare* L.) roots**

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A b s t r a c t. The objective of this research was to study the roots spring barley of the Ukrainian Peyas variety. The plants grew in a nutrient solution at pH 7, with a 16/8 h (day/night) diurnal light cycle and at temperatures of 20/16°C (day/night). At the beginning of the tillering stage, $Pb(NO_3)_2$ was introduced in to the nutrient medium in the amount of 320 mg per 1 dm^3 of the solution and the pH of the containers with Pb^{+2} was lowered to 4.5. The control setup consisted of containers free of Pb^{+2} ions at pH 7 and 4.5. Plants were grown for 10 days. Next, all the plants were collected and the surface charge of the roots was determined by the titration method. The titration was performed using the Titrino 702 MS titrator (Mertohem Ion analysis – Metrohem Ltd., CH-9101 Herisau, Switzerland) apparatus and 0.1 M solution of NaOH in 1 M NaCl as the titrate. Under the influence of Pb^{+2} ions, the total variable charge (Q) and cation exchange capacity (CEC) of roots increased. However, the total acidity of the root surface did not exhibit changes. Also, no significant differences were observed between roots growing without Pb^{+2} ions at pH 7 and pH 4.5. In addition to titration, water vapour isotherms were measured for the roots of the plants. From the isotherms, the apparent surface area was calculated using BET theory. Under Pb^{+2} stress the apparent surface area of the roots decreased.

K e y w o r d s: apparent surface area, CEC, lead, roots, surface charge, dissociation constants

INTRODUCTION

From the physicochemical point of view, quantities such as cation exchange capacity (CEC) and the value of the surface area (S) determine to a great extent sorption properties and transport of water and mineral elements (also toxic elements) through roots of plants. Up to now the measurement of potentiometric titration curves used in the studies of the surface charge of biological materials was predominantly interpreted in terms of either cation exchange capacity (CEC) or change of CEC under the influence of environmental pH variation (Morvan *et al*., 1979; Ganev and Arsowa, 1989). On the basis of potentiometric titration curves, it is also possible to determine the degree of heterogeneity of variable surface charge. The surface charge is described in terms of the dissociation reaction of surface functional groups. These groups are characterized by a distribution function of a surface dissociation constant, which defines the relative amount of groups with different acidic strength *ie* the groups generated with different charges (De Wit *et al*., 1990). The surface area of roots can be estimated from water vapour adsorption isotherm *ie* from measurements of a function relating the amount of adsorbed vapour to its equilibrium pressure at a constant temperature. The estimation of the surface area is done by finding the number of adsorbate molecules that cover the root adsorbing surface as a monolayer and multiplying this number by the area occupied by a single molecule.

Lead in plants is a completely unnecessary element. Low mobility of lead in soils (in the soil solution) causes it to be a heavy-metal contaminant in plants. In 1990 Dalenberg and van Driel (quoted by Kabata-Pendias and Pendias, 1999) confirmed that 73-95% of lead contamination of plants occurred due to atmospheric precipitation. The main amount of lead, the source of which is the soil solution, is detained on the roots. Lead is absorbed by roots and its amount is directly proportional to its concentration of the

soil solution. Stress conditions are signals for a plant to initiate in its cells different biochemical processes *eg* changes of cytoplasmic pH, changes of the electrostatic potential of cell membranes, flow of ions. As a consequence, metabolic and transport processes are affected, which lead to the lost of cell homeostasis and even to plant death. Anatomical and biochemical changes under stress are reflected in several physicochemical characteristics of the root. In particular, Józefaciuk and Szatanik-Kloc (2004), Szatanik-Kloc and Józefaciuk (1997), Szatanik-Kloc *et al.* (2001, 2007) and showed that the relative number of surface functional groups can vary under the influence of low pH and aluminium, as well as cadmium ions.

The aim of the present study was to determine the effect of pH and lead ions on surface charge properties of barley roots including CEC, the dependence of surface charge on pH, the average value and distribution of dissociation constants of charge generating surface functional groups and the apparent surface area.

MATERIALS AND METHODS

Plant growth

The research was carried out using roots of spring barley of the Peyas Ukrainian variety. The plants were grown in a nutrient solution at pH 7, prepared according to Marshner and Romheld (1983), in 16/8 h day/night light regime. The temperature was kept at 20°C during days and at 16°C during nights. All the experiments were performed with three replications. At the beginning of the tillering stage, $Pb(NO_3)_2$ was added in the amount of 320 mg per 1 dm^3 of the nutrient medium. Then in one part of the experimental setup the pH of the solution was lowered to 4.5, while in the other it was kept equal to 7. Two control sets without Pb^{+2} ions were also investigated. In the first set, the pH was 7 during the entire experiment, whereas in the second set the pH was lowered to 4.5 at the beginning of the tillering stage. The plants were grown under the stress conditions for 10 days. Control plants were also grown at pH 7 and 4.5 for 10 days. During the experiment, the pH of the solutions was measured every 24 h. After 10 days, all roots were harvested, rinsed 3 times in 0.01 mol dm^{-3} HCl , 5 times in distilled water, air dried, and collected for further analyses. The aim of HCl washing was to replace surface exchangeable ions by protons. This procedure was necessary to standardize conditions for further experimentation.

Adsorption isotherms

Water vapour adsorption isotherms were measured in triplicate for all root samples using a vacuum chamber method. During the measurements, temperature was kept constant at 298 K. The air dry root samples *ca.* 0.3 g, which were in weighing vessels, were placed in a vacuum chamber over sulfuric acid solutions with stepwise decreasing concentrations. Decreasing concentrations of sulfuric acid resulted in an increase of the relative water vapour pressure, $x=p/p_0$, where p is the pressure and p_0 is the saturated vapour pressure at the temperature of the measurements. The amount of water sorbed by the samples at a given relative pressure, $a(p/p_0)$, was determined by weighing the samples after 48 h of equilibration. At the beginning the adsorption measurements, the adsorption equilibration time was 96 h. The dry mass of the roots was determined after drying the samples overnight at a temperature of 378 K. The differences among adsorption data evaluated for the replications did not exceed 5%.

Measurement of titration curves

The titration curves for the roots were determined using the auto-titrator Titrino 702 MS (Mertohem Ion Analysis-Metrohem Ltd., CH-9101 Herisau, Switzerland). The back titration was carried out according to the procedure of Nederlof *et al.*, (1993) with a few modifications that were described by Szatanik-Kloc *et al.* (2001) and by Józefaciuk and Szatanik-Kloc (2004). Samples of 0.0500 ± 0.0001 g (dry mass) of the barley roots were equilibrated overnight with 20 g of 1 mol dm^{-3} NaCl solution. Prior to the titration, pH of every sample was adjusted to 2.95 with 1 mol dm^{-3} HCl after 5 mins of mixing. The suspension was titrated using 0.1 mol dm^{-3} NaOH in 1mol dm^{-3} $NaCl_2$ solution added at the rate of 0.01ml min^{-1}. The amount of the titer consumed between pH 3 and 10 was recorded in steps of 0.1 pH unit. As mentioned before, to find the amount of base consumed by the roots itself, the base consumption by the supernatant should be known. The titration measurements were performed in three-replicationes with the deviation not exceeding 5%.

Theory

Interpretation of apparent surface area

Surface area of the roots was determined from the water vapour adsorption isotherms. The linear form of an BET equation (Ościk, 1982):

$$y/a = 1/(a_m C) + x(C-1)/(a_m C), \qquad (1)$$

was fitted to the experimental adsorption data. In the above, $y = x/(1-x)$, $x = p/p_0$ where p the pressure and p_0 (Pa) is saturated vapour pressure at a measuring temperature T (K), (in kg 1 kg^{-1} of the dry roots) is the statistical monolayer capacity and C is the constant that is related to the adsorption energy (Ościk, 1982). Within the range of the relative pressures $0.2 < x < 0.35$ the fit was very good and the squared correlation coefficient was higher than 0.98. Having calculated values of a_m from the slope and the intercept of the straight line of Eq. (1), the surface area of the roots, S (m^2 kg^{-1}), was calculated according to:

$$S = N\omega a_m / M_r, \qquad (2)$$

where: N (mol^{-1}) is the Avogadro number, M_r (kg) is molecular weight of water, and ω (m^2) is the area occupied by a single water molecule equal to 1.08×10^{-19} m^2.

In the case of several adsorbents (such as ideal crystals), the surface area obtained from Eqs (1) and (2) can be treated as a real surface of an adsorbent (per unit of its mass), which takes part in adsorption process. However, for a great majority of real solids, the surface of an adsorbent should be considered rather as a relative parameter *ie* used to compare different adsorbents, because the adsorbate molecules can enter (and thus 'detect') only the surface irregularities of a size greater than their diameter (Ościk, 1982). Moreover, the BET equation was derived in accordance with the model of a localized adsorption on energetically homogeneous, flat surface. In the case of numerous, natural adsorbents, it is difficult to make a distinction between adsorption and absorption processes that take place simultaneously. This is why, in the case of organic sorbents, the surface determined from water vapour adsorption data is usually called the 'apparent surface area' (Chiou *et al.*, 1990).

Interpretation of titration data

The titration data were interpreted in terms of the site-heterogeneity theory (De Wit *et al.*, 1990; Józefaciuk and Shin, 1996; Nederlof *et al.*, 1993), which is briefly outlined below. The ratio of the amount (in moles) of base used for titration of suspension, N_{susp} minus the amount of the titre consumed by the supernatant, N_{sol}, is equivalent to an increase of the variable surface charge of the solid phase, ΔSO^- (pH):

$$\Delta SO^-(pH) = [\Delta Q_v(pH) = \Delta SO^-(pH)] - SO^-_{ini} = N_{susp} - N_{sol}, \quad (3)$$

where: SO^-(pH) holds for the charge at a given pH and SO^-_{ini} is the initial charge at the beginning of titration. Assuming that the variable charge originates from dissociation of surface acidic groups of kind i: $SOH_i = SO^-_i + H_s^+$ (H_s^+ denotes a proton in a plane of dissociation) and assuming the intrinsic dissociation constant, K_i, is:

$$K_i = [SO_i^-][H_s^+] / \{[SOH_i] - [SO_i^-]\}, \qquad (4)$$

where the brackets denote surface activities, the variable charge at given pH is:

$$\Delta Q_v(pH) = \Sigma[SO^-_i](pH) = \Sigma N_i \alpha_i (K_i\, pH_s), \qquad (5)$$

where: $\alpha_i(K_i, pH_s) = [SO_i^-]/[SOH_i]$ is the degree of ionization of groups of kind *i* and $N_i = [SOH_i]$ is their amount. For any pH during the titration one has:

$$\Delta Q_v(pH)/N_t = \alpha(pH) = \Sigma\, \alpha_i(K_i, pH_s)\, f(K_i),$$

$$N_i/N_t = \Sigma\, \alpha_i(K_i, pH_s) \qquad (6)$$

where: N_t is the total amount of groups and $f(K_i) = N_i/N_t$ is the fraction of i-th group. To solve Eq. (4) with respect to $f(K_i)$ values, one often assumes that the groups of weaker acidity start to dissociate when the stronger groups are fully neutralized (a continuous titration curve is replaced by a step function). This is called 'condensation approximation', CA (Nederlof *et al.*, 1993). The CA approaches a real situation when the variety of surface dissociating groups is large. Applying the CA, the $f(K_i)$ values are:

$$f(K_i) = 1/N_t\, \Delta Q_v(pH)/\Delta K_i \qquad K_i = [H_s^+], \qquad (7)$$

and on a logarithmic scale:

$$f(pK_i) = 1/N_t\, \Delta Q_v(pH)/\Delta K_i \qquad pK_i = pH_s, \qquad (8)$$

The distribution function of surface dissociation constants *ie* the dependence of $f(K_i)$ vs. K_i can be calculated knowing both surface proton activity (pH_s) and the total amount of surface groups, N_t. Because these values are difficult to establish, one usually replaces the surface proton activity by the solution activity and takes N_t as equal to N_{max} = maximal value of (N_{susp}-N_{sol}) measured within the experimental window. In this way, the intrinsic distribution functions of apparent surface dissociation constants, K_{app}, are determined:

$$f(pK_{app,i}) = 1/N_{max}\, \Delta Q_v(pH)/\Delta pK_{app} \quad pK_{app} = pH, \quad (9)$$

and the average $pK_{app\,(av.)}$ can be calculated as:

$$pH_{app(av.)} = \Sigma\, pK_{i,app}\, f_i(pK_{app}). \qquad (10)$$

Cation exchange capacity (CEC)

To determine of CEC of the roots, the method described by Morvan *et al.* (1979) was applied. It consists of titration of plant roots, which were preliminary rinsed by 0.01 M HCl and next, several times by distilled water, with KOH or NaOH solution to the value of pH = 7. The amount of moles of consumed is taken as the value of CEC.

Interpretation of surface charge density (SCD)

Surface charge density (SCD, C m^2) of the roots was calculated from:

$$SCD = Q_v/S, \qquad (11)$$

where: Q_v (mmol g^{-1}) is the total surface charge and S (m^2 g^{-1}) is the apparent surface area of roots, evaluated from water vapour adsorption isotherms.

RESULTS AND DISCUSSION

The roots of the studied plants, cultivated without added lead ions at pH 7 and 4.5, had practically the same charge. Therefore, it can be deduced that an increase of proton

T a b l e 1. Variable charge of characteristics of the studied barley roots (averages. of 3 replicas. ±95% confidence)*

Properties	Control	pH 4.5	pH 7+Pb	pH 4.5+Pb
S (m^2 g^{-1})	146.6±3.5	145.8±3.1	122.5±2.08	121.1±2.06
CEC (mM g^{-1})	0.546±0.12	0.537±0.29	0.594±0.004	0.604±0.01
Qv (mM g^{-1})	0.871±0.01	0.863±0.01	0.912±0.006	0.91±0.003
pK$_{app\ (av.)}$	6.2±0.03	6.1±0.01	6.2±0.02	6.02±0.005
SCD (C m^2)	0.58±0.004	0.571±0.002	0.729±0.009	0.733±0.007

*No statistically significant differences were obtained for: control (pH 7), pH 4.5 and pH 7+Pb, pH 4.5+Pb.
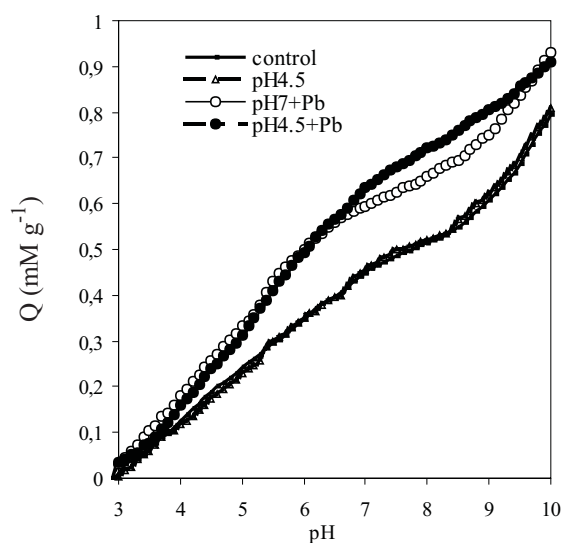
Fig. 1. Variable surface charge vs. pH for barley roots.
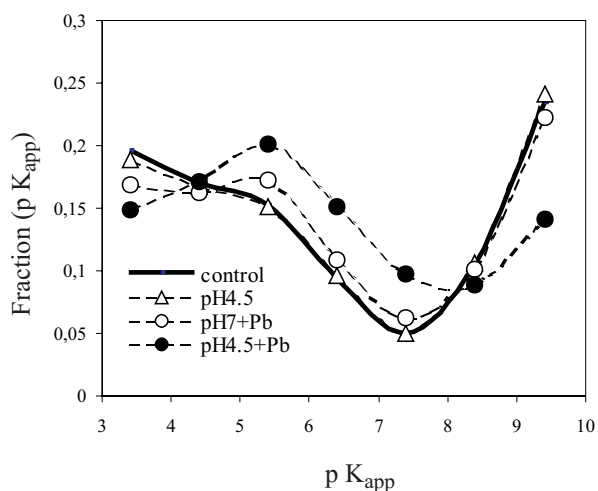
Fig. 2. Distribution function of apparent surface dissociation constants for the studied roots as related to acid and alkali treatment.

concentration in the nutrition had no influence on the charge of the root tissue. Only after addition of lead ions to the nutrient solution did the curves of the charge Q versus pH change (Fig. 1). The addition of lead ions to the nutrient medium caused an increase of the total surface charge and CEC (Table 1). Even more pronounced was an increase in the density of charge per unit of apparent surface area (Table 1). This was due to the fact that the presence of lead resulted in a decrease of the apparent surface area. The distribution function of surface dissociation constants (Fig. 2), which characterizes surface functional groups with different acidity *ie* with different generated charge, pointed to an increase in the role played by the functional groups of intermediate acidic sites *ie* with $4.5 < pK_{app} < 7$) and a decrease in the role played by weakly acidic sites ($pK_{app} > 7$), as well as strongly acidic sites ($pK_{app} < 4.5$). The average value of apparent dissociation constants ($pK_{app\ av.}$) for barley roots under the stress conditions of lead ions was practically the same for all the samples studied.

Toxic concentration of lead induces disturbance of photosynthesis, cell division and water status. Toxicity symptoms result in a dark-green color of leaves, and leaves fade and twist roots (Kabata-Pendias and Pendias, 1999). However, many plants develop different tolerance mechanisms to high lead concentration. These mechanisms are: a change of the cell membrane properties that due to removal of pectin increase their own sorptive properties (Wierzbicka, 1998) and fixation of lead in the cellular walls that limits lead inclusion into metabolism (Abratowska, 2006). In addition, lead may precipitate in the ortho and pyrophosphates forms or another crystalline forms on the cellular membranes (Seregin *et al.*, 2002). Lead can be also transported inside cells and stored in vacuoles. Any fixation of lead may cause structural changes (Seregin and Ivanov, 2001). These changes can cause changes of the apparent surface area of roots. Moreover, an increasing surface charge on roots due to the presence of lead ions can be connected with removal of pectin and/or precipitation of lead in crystalline forms on the cellular membranes.

The statistical analysis of the obtained results is shown in Table 2.

T a b l e 2. Statistical analysis of the obtained results (Student-t-Two tailed, Type 2 (homoscedastic) variances for averages of samples do not differ significantly from themselves (averages are the same for ±95% confidence interval))

Reaction	pH 7 (Control)	pH 4.5	pH 7+Pb	pH 4.5+Pb
S (m² g⁻¹)				
pH 7 (Control)	1*	1	0	0
pH4.5	1	1	0	0
pH7+Pb	0**	0	1	1
pH4.5+Pb	0	0	1	1
CEC				
pH 7 (Control)	1	1	0	0
pH4.5	1	1	0	0
pH7+Pb	0	0	1	1
pH4.5+Pb	1	0	1	1
pK_{app}				
pH 7 (Control)	1	1	1	1
pH4.5	1	1	1	1
pH7+Pb	1	1	1	1
pH4.5+Pb	1	1	1	1
SCD				
pH 7 (Control)	1	1	0	0
pH4.5	1	1	0	0
pH7+Pb	0	0	1	1
pH4.5+Pb	0	0	1	1

*1 – truth, **0 – false.

CONCLUSIONS

1. There are several different mechanisms leading to the tolerance of plants to heavy metals, which function on the symplastic as well the apoplastic level.

2. In the case of lead ions, the mechanisms must provide a possibility of cell protection against the toxic influence of the metal. Usually, different protection mechanisms act simultaneously with different intensities depending on the sensitivity of a plant. Therefore, it is difficult to explain reasons for the observed changes in the physicochemical properties of the spring barley roots in this experiment.

3. It was shown that lead ions caused an increase of CEC, as well as an increase in the total, variable surface charge of the roots.

4. A decrease of apparent surface area with a simultaneous increase in the total, variable surface charge caused an increase of the charge density on the surface.

5. No statistically significant differences were observed between roots growing without lead ions at pH 7 and 4.5 and no differences were obtained for pH 7+Pb and pH 4.5+Pb.

6. No significant differences were obtained for the total acidity of roots surface.

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