10.2478/botlit-2014-0020

BOTANICA LITHUANICA
ISSN 1392-1665

2014, 20(2): 131–141

RESPONSE OF TEST-ORGANISMS TO DIFFERENT NA AND CU SALTS

Danutė Marčiulionienė[1], Danguolė Montvydienė[1*], Nijolė Kazlauskienė[1], Benedikta Lukšienė[2], Dalia Jasinevičienė[2], Stasys Tautkus[3]

[1]Nature Research Centre, Akademijos Str. 2, LT-08412 Vilnius, Lithuania
[2]Centre for Physical Sciences and Technology, Savanoriu av. 231, LT-02300 Vilnius, Lithuania
[3]Vilnius University, Department of Chemistry, Naugarduko Str. 24, LT-03225 Vilnius, Lithuania
[*]Corresponding author. E-mail: danguole.montvydiene@botanika.lt

Abstract

Marčiulionienė D., Montvydienė D., Kazlauskienė N., Lukšienė B., Jasinevičienė D., Tautkus S., 2014: Response of test-organisms to different Na and Cu salts [Testinių organizmų atsakas į skirtingų Na ir Cu druskų poveikį]. – Bot. Lith., 20(2): 131–141.

The scope of this research involves the evaluation of biological impact of different Na and Cu salts (nitrates, sulphates and chlorides) on test-organisms. The toxic impact of Na and Cu salts on seed germination and root growth of *Lepidium sativum* (garden-cress) as well as mortality, growth and physiological parameters of *Oncorhynchus mykiss* (rainbow trout) in early development stages (embryos and larvae) were determined. Among Na salts, nitrate was the most toxic to both test-organisms. Among tested Cu salts, sulphate caused the strongest toxic impact on *L. sativum* and nitrate – on embryos and larvae of *O. mykiss*. The accumulation of all tested anions and cations from the solutions of tested salts was higher in roots than in shoots of *L. sativum*. The highest transfer of Na^+ and Cu^{2+} from roots to shoots was determined for plants cultivated in sulphate salt. The transfer of SO_4^{2-} was the highest among tested anions of sodium salts and the transfer of NO_3^- was higher among tested anions of copper salts. The rather high correlation was found between root length and amount of Na^+, NO_3^- and SO_4^{2-} ions in plant roots and rather low correlation coefficient was calculated between root length and the amount of Na^+ and Cl^- ions in roots.

Keywords: accumulation, correlation, Na and Cu salts, test-organisms, toxicity, transfer.

INTRODUCTION

Release of chemical substances from inappropriately fertilized agricultural areas, energetic or industrial facilities, municipal waste water treatment plants or landfills into the environment can disturb health and stability of aquatic or terrestrial ecosystem (Skorbilowicz, 2009; Bonanno, 2011; Emenike et al., 2012). This pollution is especially dangerous as it is permanent and in many cases chemical substances are rather persistent (Manios et al., 2003). For example, the landfill's leachate composition is considered in four categories: (1) dissolved organic matter, (2) inorganic macrocomponents (Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+, Fe^{2+}, Mn^{2+}, Cl^-, SO_4^{2-}, HCO_3^-), (3) heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}) and (4) xenobiotic organic compounds (aromatic hydrocarbons, phenols, chlorinated aliphatics, pesticides and plastizers) (Kjeldsen et al., 2002). Heavy metal (HM) contamination due to its non-degradability is one of the most serious environmental problems limiting plant productivity and threatening human health.

Metal toxicity to various organisms has been widely studied (Kinraid, 1999; Montvydiene & Marciulioniene, 2004; Chiodi Boudet et al., 2011; Tao et al., 2012; Tang et al., 2013; Ben Salem et al., 2014; Harguinteguy et al., 2014). It is generally accepted that metal toxicity to organisms is positively correlated to the concentration of metals in organism tissues; higher metal concentrations in the tissues usually induce stronger damage in organism (Xiong & Wang, 2005). A lot of studies related to lethal, sublethal, acute and chronic toxic effect of various heavy metals and their mixtures on higher plants and animals as well as peculiarities of bioaccumulation of heavy metals in organisms have been carried out (Marciulioniene et al., 2002; Yruela, 2005; Vosyliene et al., 2005; Andersen et al., 2013; Ovečka & Takáč, 2014). Metals are found in various chemical compounds in the environment; consequently, their bioavailability and biological activity differ. Some facts suggest that the different salts of the same metal can cause different response of organism (Wang, 1992; Zaman et al., 2002). Information about the influence of anions on biological effects of metal salts, the impact of anions on accumulation and distribution of metals in the organisms as well as on accumulation and distribution of anions in organisms is scarce. The anions (nitrates, sulphates, chlorides) of Na and Cu salts, which were chosen in our study, are necessary for normal metabolism, growth and development of organisms (Marschner, 1995; Newman, 1998; Rahman et al., 2001; Yruela, 2005). Many studies have been conducted on Na salts (salinity), but most have focused on Na chloride accumulation in organisms and toxicity (Silva et al., 2003; Parida & Das, 2005). Considerably, less attention has been given to other salinities such as those caused by Na sulphate or nitrate (Mer et al., 2000; Zaman et al., 2002). Accumulation and toxicity to Cu on plants under natural and laboratory conditions have been studied rather extensively (Foy et al., 1978; Fernandes & Henriques, 1991; Dietz et al., 2001; Demirevska-Kepova et al., 2004; Muller et al., 2001; Montvydiene & Marciulioniene, 2004; Kopittke & Menzies, 2006; Lamb et al., 2012). However, the main attention has been focused on the impact of Cu sulphate on organisms (Mocquot et al., 1996; Lombardi & Sebastiani, 2005). The impact of other Cu salts on organisms has been investigated to a less extent (Kopittke & Menzies, 2006; Burzyński & Żurek, 2007).

The data on toxicity testing indicate that different organisms respond differently to various heavy metals or their mixtures, therefore, the use of test-organisms of different phylogenetic level is essential for a reliable assessment of the effects of heavy metals on the environment (Montvydiene & Marciulioniene, 2004; Šimonová et al., 2007; Chapman et al., 2013).

The aim of this study was to estimate and compare the effect of Na and Cu salts (nitrates, sulphates, chlorides) on a rainbow trout (*Oncorhynchus mykiss* L.) in their early stages of development as well as seed germination and root growth of garden-cress (*Lepidium sativum* L.), to investigate the accumulation of cations (Na^+ and Cu^{2+}) and anions (NO_3^-, SO_4^{2-}, and Cl^-) in the roots and shoots of *L. sativum* from the solutions of different Na and Cu salts.

MATERIALS AND METHODS

Assay of *Oncorhynchus mykiss.* *O. mykiss* (rainbow trout) was tested in early ontogenesis (embryos and larvae) for mortality throughout the test and physiological indices: heart rate (HR, counts·min^{-1}), gill ventilation frequency (GVF, counts·min^{-1}) after 5–20 days of exposure, growth (average body mass at the end of the test, mg; integrated parameter – relative body mass increase, %). Long-term (30 days) toxicity tests with the rainbow trout (embryos and larvae were obtained from the Žeimena hatchery) were conducted under semi-static conditions and two replications were done. Artesian water of high quality was used as the control. The average hardness of water was approximately 284 mg·l^{-1} as $CaCO_3$, alkalinity 244 mg·l^{-1} as HCO_3^-, average pH ~ 8.0, temperature 9.5–10 ± 0.2ºC and the oxygen concentration ranged from 8 to 10 mg·l^{-1} (Kazlauskienė & Stasiunaitė, 1999; Kazlauskienė et al., 2002). Significance of all responses was verified by Student's t-test at $p \leq 0.05$, using the GraphPAD (InStat, USA).

Assay of *Lepidium sativum.* Bio-assay of *L. sativum* (garden-cress) was carried out following the method modified by Magone (1989). Briefly, 9 ml of distilled water (as control) or testing sample solution was pipetted onto three layers of filter paper fitted into a 9-cm glass Petri dish. Twenty-five healthy looking *L. sativum* seeds of similar size were distributed evenly on filter paper. The Petri dishes were placed in the darkness at 25 ± 1°C for 48 hours. Afterwards, the seed germination, root length of seedlings and fresh and dry biomass of roots and shoots were determined. The samples were dried at room temperature (t = 19–21°C). Germination power of seeds and length of *L. sativum* roots in distilled water were 92 ± 4% and 30.6 ± 1.5 mm, respectively. The experimental set of each testing scheme involved 10 control dishes and 10 replicates for each tested salt concentration. The range limits of pH values of experimental solutions were from 5.17 to 6.01. The EC50 values (i.e. toxicant concentrations that induce 50% growth inhibition of *L. sativum* as percentage of control, in 2-day experiments) were estimated by linear regression analysis of root length and logarithm of cation concentration in $mg \cdot l^{-1}$. The data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, at $p < 0.05$, using the Statgraphics plus Version 2.1. (Statistical Graphics Corp., USA).

Chemical reagents and measurement of cations and anions concentration in plant. Solutions of Na and Cu salts were prepared using reagents of analytical grade: Na_2SO_4, $NaNO_3$, NaCl, $CuSO_4 \cdot 5H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $CuCl_2$ (Merck (Darmstadt, Germany) and deionized water (pH 6.24 ± 0.04). Salt concentrations expressed in the amount of cations were used in the study for the evaluation of the toxic effect of Na and Cu salts on the test-organisms.

The measurement of Na and Cu concentrations in tested samples of *L. sativum* roots and shoots was performed using atomic absorption spectrometer (Hitachi 150–70, Japan). Anions SO_4^{2-}, Cl^- and NO_3^- were determined using ion chromatography – Dionex 2010i with conductivity detector, the column used for anion analyses was Ion Pac AS4A-SC. The eluent for anion analysis was 1.8 mM sodium carbonate + 1.7 mM sodium bicarbonate and the regenerator was 100 mM H_2SO_4.

The transfer (TF) of tested ions from roots to shoots was calculated using formula

$$TF = \frac{(Ion)shoot}{(Ion)root} \times 100\%$$

(Ion (root) – amount of accumulated ion (mg) in roots, dry weight (d. w.) (g); Ion (shoot) – amount of accumulated ion (mg) in shoot, d. w. (g)).

The amounts of cations and anions were presented for the dry weight (d. w.) mass. Uncertainties were evaluated bearing into account bias weighing, solution preparation and concentration determination. In all cases uncertainties did not exceed 10%.

RESULTS AND DISCUSSION

Effects of metal salts on *Oncorhynchus mykiss*

It is known that early stages of development are especially sensitive period in fish life, as during a rather short time (from four days to two months) their organisms undergo many critical periods (embryos, larvae, fry) till formation of self-sufficient individual (Kazlauskienė et al., 2002). Investigations showed that the mortality of *O. mykiss* embryos in examined sodium and copper sulphates and chlorides fluctuated from 5 to 11.5% (Fig. 1). It means that these salts did not have significant effect on the mortality of *O. mykiss* embryos in the tested concentrations, because allowable mortality of embryos in control water is 15% (Kazlauskienė et al., 2002). However, the treatment with 44.0 and 440 $mg \cdot l^{-1}$ Na^+ concentration in Na NO_3 resulted in, respectively, 32.5% and 38% mortality of embryos (Fig. 1).

Embryo immunity against effects of toxic substances is related to spawn embryonic membranes, which are a protective barrier between the embryo and the environment. However, this barrier is pervious to certain toxic substances. It is conditioned by the selective spawn membrane perviousness, wherefore spawn exhibit greater immunity against toxicants compared to larvae. Toxic substances have a harmful effect on gill, through which they enter the organism (Kazlauskienė et al., 2002).

$Cu(NO_3)_2$ has the greatest effect on larvae mortality, where 0.06 $mg \cdot l^{-1}$ Cu^{2+} concentration in this salt caused 28.1% larvae mortality. The 0.08 and 0.1 Cu^{2+} concentrations in copper sulphate and chloride accounted for 18.2% and 13.2% larvae mortality, respectively (Fig. 2). Consequently, *O. mykiss* larvae are significantly more sensitive to the effect of different sodium and copper salts compared to embryos.

Long-term investigations showed that sodium and copper salts not only reduce the survival of *O. mykiss* embryos and larvae, but also interfere with the functioning of the principle vital systems, because such salts are slowing down gill ventilation frequency (GVF) and heart rate (HR) of embryos and larvae. It was found that only NaCl did not have any effect on the HR of embryos (Fig. 3). The HR of rainbow trout embryos was more affected by $NaNO_3$ than by Na_2SO_4 (Fig. 3). However, larvae HR were negatively affected by all examined salts. NaCl did not have any effect on the embryo HR; however, the effect of this salt on HR of larvae was significant in comparison with control. The GVF of rainbow trout larvae was not affected only by Na_2SO_4, whereas all other sodium and copper salts reduced this index significantly (Fig. 3).

After comparison of the effects of different salts of the same metal, it was found that nitrates showed the highest toxicity to the GVF of rainbow trout larvae. It is known that changes in respiratory and heart activity in embryos and larvae caused by toxicants influence the growth of embryos and larvae, disturb the duration of incubation, and impede their development (McKim, 1985). Insufficiently developed blood circulation in a yolk sack disturbs the use of yolk and negatively affects respiratory mechanisms; therefore, the size of larvae decreases, their growth rates slow down, biomass decreases, and a possibility of becoming a victim or dying increases (McKim, 1985).

It was found that the effect of tested salts depended on the development stage of *O. mykiss* (larvae were more sensitive than embryos), the sensitiveness of indices used in the experiment and on metal salts themselves (nitrates were more toxic than sulphates and chlorides).

Effects of metal salts on *Lepidium sativum*

Investigations of the effect of Na and Cu salts on *L. sativum* showed that in most cases seed germination in the solution of these salts differed insignifi-

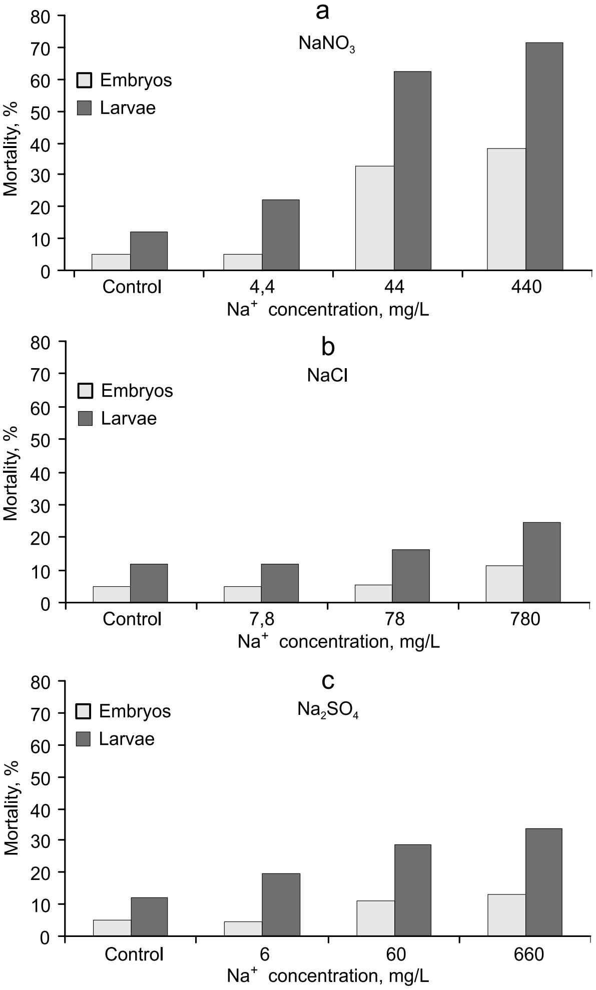

Fig. 1. Effect of sodium salts on the mortality of *O. mykiss* embryos and larvae (n = 200)

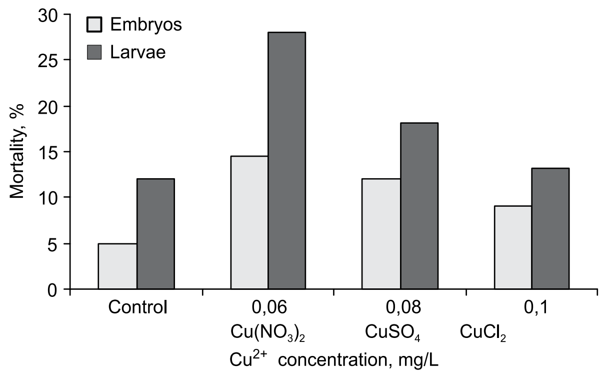

Fig. 2. Effect of copper salts on the mortality of *O. mykiss* embryos and larvae (n = 200)

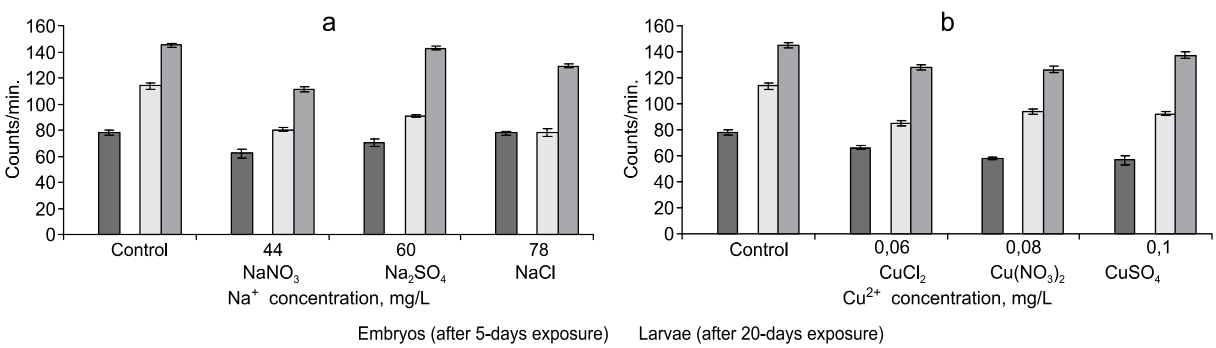

Fig. 3. Effects of Na and Cu salts on cardio respiratory indices of *O. mykiss* embryos and larvae: heart rate (HR) and gill ventilation frequency (GVF)

cantly from the control ($p < 0.05$), except in the highest concentrations of Na nitrate (1500 and 3000 mg·l^{-1} Na^+ ions in $NaNO_3$ solution), which decreased the seed germination by 35% and 58%, respectively, in comparison with control. Lutts et al. (1996) found significant reduction of rice seed germination at high NaCl concentration and explained it by the osmotic and toxic effects of NaCl.

Morphological changes in the roots were observed only in solutions of some concentrations of Cu nitrate and chloride. Roots of *L. sativum* were undeveloped, with brown tips, without root hairs in two highest concentrations of Cu ion in Cu nitrate solution, as well as the brown tips of the roots were observed in two highest Cu ion concentrations in Cu chloride solution. However, solution of the tested Na salts did not cause any morphological changes in the plant roots. It was found that some concentrations of the tested Na salts and some concentrations of Cu nitrate and sulphate stimulated ($p < 0.05$) the growth of *L. sativum* roots (Fig. 4, 5).

The toxicity of Na and Cu salts to *L. sativum* was assessed according to the 50% effective concentration (EC50) of Na^+ and Cu^{2+} ions (mg·l^{-1}), which inhibits root growth by 50% in 2-day experiment (Table 1).

Nitrate was the most toxic to root growth of *L. sativum* among the tested Na salts; while sulphate was the most toxic to this plant among tested Cu salts. Chloride of both tested salts was less toxic to root growth of *L. sativum*. Obtained data showed that the values of EC50 of Na salts were 100 times lower than that of the same Cu salts (Table 1). According to the literature sources (Ouzounidou, 1995; Montvydiene & Marciulioniene, 2004; Lombardi & Sebastiani, 2005; Lamb et al., 2012) the value of the effective concentration (EC) of Na and Cu varied in very wide limits and depended on plant species.

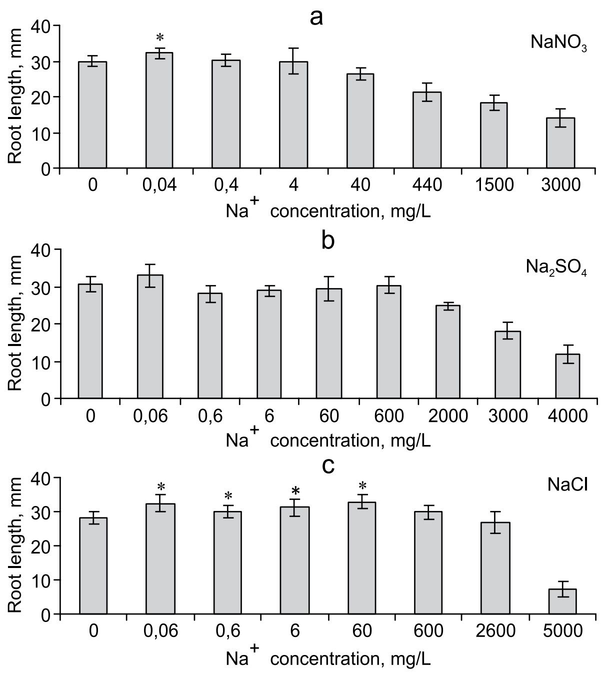

Fig. 4. Impact of Na salts on *Lepidium sativum* root length

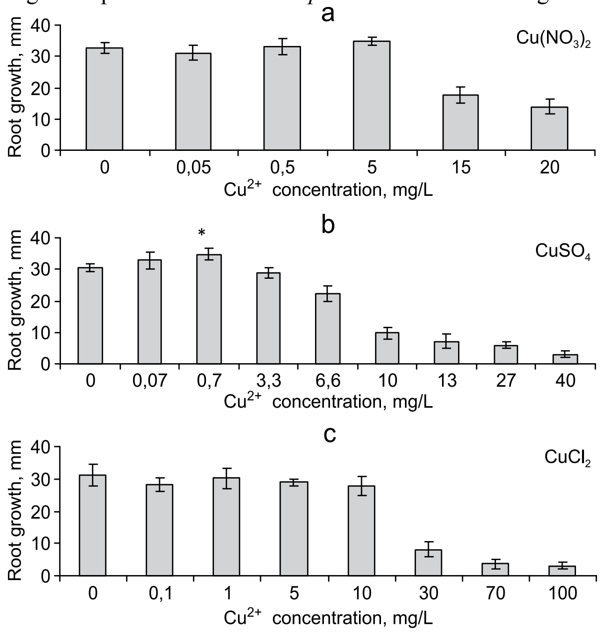

Fig. 5. Impact of Cu salts on *Lepidium sativum* root length

Accumulation and transfer of cations and anions in *Lepidium sativum*

The EC50 values of Na^+ and Cu^{2+} ions in tested salts were markedly higher than the environmental concentrations of these metals. Na concentration in surface water and bottom sediments varied in rather high limits depending on season and pollution sources around water basins. For example, average values of Na in quite polluted lake water varied from 420 mg·l^{-1} in winter to 640 mg·l^{-1} in summer, as well as the average values of Na in bottom sediments varied from 2000 mg·kg^{-1}, d. w. in winter to 4000 mg·kg^{-1}, d. w. in summer (Arain et al., 2008). Rather similar average values of Na amounts in river water and bottom sediments were determined by Shomar et al. (2005). It is considered that practically 15% of total Na in bottom sediments is bioavailable (Arain et al., 2008). Cu concentration in non-contaminated soil and bot-

Table 1. Toxic impact of Na and Cu salts on root growth of *Lepidium sativum* (2-day EC50, mg·l^{-1} Na^+ or Cu^{2+} ion concentrations in tested salt solution)

Tested solution	EC50, mg·l^{-1} of Na^+ ion	Tested solution	EC50, mg·l^{-1} of Cu^{2+} ion
$NaNO_3$	2030 ± 480	$CuSO_4·5H_2O$	14.5 ± 0.8
Na_2SO_4	3590 ± 680	$Cu(NO_3)_2·3H_2O$	17.1 ± 0.9
NaCl	5050 ± 1750	$CuCl_2$	27.0 ± 2.4

tom sediments and surface water is relatively low (20 ppm, 30 ppm and 2 ppb, respectively) (Moore & Ramamurthy, 1987). Besides that, Cu easily makes complexes with organic ligands, consequently, their presence in the medium sharply reduces copper bioavailability (Fernandes & Henriques, 1991). However, it is currently well accepted that both total and soluble Cu concentrations are poor predictors of Cu toxicity (Hassler et al., 2004). Consequently, it is very important to know how Na and Cu ions and anions of their salt are accumulated from medium solution to plant, how they distribute in plant vegetative parts and how it can influence plant growth and development.

Whereas toxic impact of the tested metal salts (nitrates, sulphates, chlorides) on *L. sativum* differed, we can state that the difference in toxicity can be attributed to the tested anions (NO_3^-, SO_4^{2-}, Cl^-). Amount of Na^+ and Cu^{2+} as well as of the tested anions in plants was not measured in all variants of growing solution; it was done only in variants, which were interesting for us regarding the caused toxicity effects. In addition, concentration of Cu^{2+}, NO_3^- and Cl^- ions in some tested variants was lower than detectable limit of measurement.

The amounts of Na^+ ions and tested anions in roots and shoots of *L. sativum* are presented in Table 2.

Accumulation of Na^+ ion in roots and shoots of control plants fluctuated from 4.2 to 6.3 mg/g and from 0.74 to 1.6 mg/g, d. w., respectively, and transfer coefficient varied from 16 to 25%. The similar content of sodium ions in control plants was reported by Queirós et al. (2011), however, the content of Cl^- in their study was higher than those in ours. In our case the amounts of NO_3^- and Cl^- in control plant roots and shoots were lower than detectable limit of measurement. Therefore, only the amount of SO_4^{2-} was measured in control plant, and it reached 16.1 mg/g, d. w. in roots and 3.8 mg/g d. w. in shoots, as well as the transfer coefficient of sulphate ion was 23.6% (Table 2). The values of the amount of Na^+ ions were rather similar in roots from all tested solutions (Table 2). However, the amount of Na^+ ions in shoots was lower in plants growing in NaCl solution in comparison with plants growing in other tested Na salts. The tested anions (NO_3^-, SO_4^{2-} and Cl^-) of Na salts in roots accumulated in larger amount than in shoots. It corresponded with the data reported in literature (Queiros et al., 2011; Yildiztugay et al., 2011). Additionally, accumulation of Cl^- ions in roots of *L. sativum* was higher than that of SO_4^{2-} and NO_3^- (Table 2). Obtained data showed strong correlation between *L. sativum* root length and content of Na^+ ions in shoots of plants growing in Na nitrate solution ($r = 0.99$, $p = 0.001$). Correlation was also found between root length and content of NO_3^- in roots and shoots of plants growing in Na nitrate solution ($r = 0.93$, $p = 0.022$ and $r = 0.99$, $p = 0.0007$, respectively). The other correlations between sodium ions and anions content and root length were not statistically significant. The transfer of Na^+ from roots to shoots increased up to a certain Na^+ ions concentration, which differed from all tested salts, then transfer of Na^+ started to decline (Table 2). The highest transfer of this ion from roots to shoots in plants growing in Na nitrate and sulphate solutions was estimated at the concentration following the concentration in which the highest accumulation was measured (Table 2). It is known that regulation of NO_3^- uptake involves the induction of high-capacity, high-affinity uptake system and negative feedback regulation of NO_3^- uptake by increasing internal NO_3^- concentration (White, 2011). The negative feedback regulation may be caused not only by high NO_3^- concentration in vacuoles, but also by elevated concentrations of reduced N in the form of amino acids glutamine and asparagines or NH_4^+ (White, 2011). Very important role in the plant cell is played by nitrate reductase, which reduces nitrate to nitrite, but according to Reddy & Menary (1990) the activity of this enzyme is inhibited by high endogenous NO_3^- concentrations. It is now assumed that the dominant signal of plant tissues S status that regulates sulphate uptake and assimilation is the accumulation of reduced S compounds such as cysteine or reduced glutathione and sulphate storage in vacuole is less important for negative feedback regulation of sulphate uptake (White, 2011).

Table 2. The amount (mg·g^{-1}, d. w.) of accumulated Na^+, SO_4^{2-}, NO_3^- and Cl^- in roots and shoots of *Lepidium sativum* and transfer (%) of these ions from roots to shoots

Tested solution	Concentration, mg·l^{-1} of Na^+ ion	Accumulation of Na^+, mg·g^{-1} d. w.		Accumulation of anion, mg·g^{-1} d. w.		Transfer of ions from roots to shoots, %	
		Roots	Shoots	Roots	Shoots	Na^+	Anion
Na_2SO_4	0	4.2	0.74	16.1	3.8	17.6	23.6
	600	4.7	0.6	27.9	11.6	12.7	41.6
	2000	44.4	4.1	43.3	12.2	9.2	28.2
	3000	11.1	7.1	31.7	23.5	60.7	74.1
	4000	22.9	2.1	34.9	13.5	9.1	38.7
$NaNO_3$	0	6.3	1.4	–	–	*	*
	40	9.6	2.8	12.9	2.1	29.2	16.3
	440	11.5	5.8	14.3	6.3	50.4	44.1
	1500	36.4	8.4	28.5	7.2	22.9	25.3
	3000	24.1	9.6	30.2	10.3	39.7	34.1
NaCl	0	5.8	1.6	–	–	*	*
	60	0.02	0.005	12.6	3.6	25	28.6
	600	0.13	0.03	50.5	12.7	23.1	25.1
	2600	0.17	0.04	80.9	15.6	23.5	19.3
	5000	0.19	0.02	69.4	10.4	10.5	14.5

(−) – lower than detectible limit of measurement; * − not possible to estimate due to lack of data

Table 3. The amount (mg·g^{-1}, d. w.) of accumulated Cu^{2+}, SO_4^{2-}, NO_3^- and Cl^- in roots and shoots of *Lepidium sativum* and transfer (%) of these ions from roots to shoots

Tested solution	Cu^{2+} ion, mg·l^{-1}	Accumulation of Cu^{2+}, mg·g^{-1} d. w.		Accumulation of anion, mg·g^{-1} d. w.		Transfer of ions from roots to shoots, %	
		Roots	Shoots	Roots	Shoots	Cu^{2+}	Anion
$CuSO_4·5H_2O$	0	0.08	0.01	–	–	12.5	*
	0.7	0.012	0.001	–	–	8.1	*
	3.3	0.34	0.003	–	–	7.2	*
	6.6	0.052	0.002	1.41	0.06	9.3	4.3
$Cu(NO_3)·3H_2O$	0	0.11	0.01	–	–	10.0	*
	5	0.073	0.002	14.6	0.58	2.8	4.1
	15	0.04	0.002	7.3	1.02	5.5	10.6
	20	0.73	0.004	9.1	0.81	5.4	7.1
$CuCl_2$	0	0.07	0.009	–	–	12.8	*
	1	0.013	–	0.001	–	7.7	*
	5	0.013	–	0.0012	–	9.2	*
	10	0.07	0.095	0.004	0.002	5.1	2.1

(−) – lower than detectible limit of measurement; * − not possible to estimate due to lack of data

According to Berstein et al. (2010), unlike leaves, roots did not suffer oxidative damage in either growing or mature cells and demonstrated reduced antioxidant response under salinity stress. There are some mechanisms, which restricted transfer of excess Na^+ from roots to above-ground parts of plant, because the high concentrations of Na salts alter equilibrium of $Ca^{2+}$and Na^+ in the root environment, which affects membrane properties due to displacement of membrane-associated Ca^{2+} by Na^+, thus changing the membrane integrity and selectivity (Kinraid, 1999; Tester & Davenport, 2003; Silva et al., 2003; Lamb et al., 2012). The increase of Na^+ inside the cells could: change enzyme activity resulting in cell metabolic alterations; cause disturbance in K^+ uptake and partitioning in the cells and throughout the plant (Epstein, 1998). It is presumable that removal of Na^+ from the cytoplasm and compartmentation of Na^+ into vacuoles done by a salt-inducible Na^+/H^+ antiporter provides an efficient mechanism to avert the deleterious effects of Na^+ (and Cl^-) in the cytosol (Apse et al., 1999).

It is rather complicated to discuss the accumulation of anions from Cu salts solutions and their transfer from roots to shoots because the amount of anions in roots and shoots in half of the cases of the investigation was lower than the detectable limits of measurements (Table 3). Data showed that Cu^{2+} accumulated more in *L. sativum* roots than shoots and it coincided with the results reported in literature (Stolz & Greger, 2002; Lamb et al., 2012). The accumulation of Cu^{2+} ions in both roots and shoots from all tested Cu solutions was quite similar (Table 3). Therefore, for the deeper understanding of the Cu salts toxicity on plants more comprehensive investigations are needed.

The transfer of anions from roots to shoots was significantly lower in plants growing in Cu salts than in Na salts (Tables 2, 3). The transfer of Cu^{2+} ions from roots to shoots of *L. sativum* was also lower than that of Na^+ ions (Tables 2, 3). There are some mechanisms, which restrict transfer of excess Cu^{2+} from roots to above-ground parts of plants. One of these is storage of excess copper as copper-binding proteins in vacuole of root cells (Yruela, 2005). Excess Cu in medium inhibits respiration, negatively affects nitrogen and protein metabolism, decreases membrane integrity, causes a reduction of chlorophyll contents and inhibits some photosynthetic functions in leaves (Maksymiec, 1997; Demirevska-Kepova et al., 2004).

CONCLUSIONS

In the most cases, Na and Cu salts (chlorides, sulphates and nitrates) did not have any significant effect on the mortality of *O. mykiss* embryos. The strongest effect on larvae mortality was exerted by Na and Cu nitrates. Nitrates also caused the strongest effect on the heart rate (HR) of embryos and larvae. Na nitrate had the strongest effect on the gill ventilation frequency (GVF) of embryos. Effects of Cu nitrate and sulphate on GVF of larvae were similar and statistically higher than those of control and Cu chloride solution. It was found that toxicity of the investigated sodium and Cu salts to *O. mykiss* depended on the stage of fish development, different salts of the metal and sensitiveness of the indices used in the experiment.

The highest effects of Na nitrate among tested Na salts may be related to the relatively high transfer of Na^+ and NO_3^- from roots to shoots of *L. sativum*. The highest effects of Cu sulphate to *L. sativum* in comparison with other tested Cu salts may depend on the rather high transfer of SO_4^{2-} from roots to shoots, although Cu^{2+} accumulation in roots and shoots was quite similar in plants growing in all tested Cu salts. The rather high correlation was found between root length and amount of Na^+, NO_3^- and SO_4^{2-} ions in plant roots and rather low correlation coefficient was calculated between root length and the amount of Na^+ and Cl^- ions in roots.

The obtained results showed that all investigated Cu salts were more toxic (about 100 times) to *O. mykiss* in early stages of the development and root growth of *L. sativum*, compared to Na salts. Among Na salts, $NaNO_3$ caused the strongest toxic effect on both the *L. sativum* and *O. mykiss* embryos and larvae, as well as among Cu salts, $CuSO_4$ had the strongest toxic effect on the *L. sativum*, and – $Cu(NO_3)_2$ mostly affected *O. mykiss* embryos and larvae. It could be concluded that the toxicity of Na and Cu salts to organisms depends upon anions in salts.

All these investigations on the toxicity of Na and Cu salts to tested organisms, accumulation of cations and anions in the roots and shoots and their transfer from roots to above-ground parts of plant are the first steps to further examination of the toxicity of the mixtures of various metal salts and other chemical substances (including radionuclides), behaviour of the cations and anions in those mixtures and the importance of anions to the toxicity of metal salts. Fundamental investigations also are needed for the better understanding of the mechanisms of the behaviour of anions and cations in organisms of different phylogenetic and ontogenetic stages.

ACKNOWLEDGEMENTS

This work was funded by the Research Council of Lithuania No. MIP-038/2012.

REFERENCES

Andersen E., Opitz J., Thomas G., Stärk H.J., Dienemann H., Jenemann K., Dichinson B.C., Küpper H., 2013: Effects of Cd and Ni toxicity to *Ceratophyllum demersum* under environmental relevant conditions in soft and hard water including a German lake. – Aquatic Toxicology, 142–143: 387–922.

Apse M.P., Aharon G.S., Snedden W.A., Blumwald E., 1999: Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. – Science, 285: 1256–1258.

Arain M.B., Jamail T.G., Jalbani N., Afridi H.I., Shah A., 2008: Total dissolved and bioavailable elements in water and sediment samples and their accumulation in *Oreochromis mossambicus* of polluted Manchar Lake. – Chemosphere, 70: 1845–1856.

Ben Salem Z., Laffraya X., Ashooura A., Ayadib H., Aleyaa L., 2014: Metal accumulation and distribution in the organs of Reeds and Cattails in a constructed treatment wetland (Etueffont, France). – Ecological Engineering, 64: 1–17.

Bernstein N., Shoresh M., Xu Y., Huang B., 2010: Involvement of the plant antioxidative response in the differential growth sensitivity to salinity of leaves vs roots during cell development. – Free Radical Biology & Medicine, 49: 1161–1171.

Bonanno G., 2011: Trace element accumulation and distribution in the organs of *Phragmites australis* (common reed) and biomonitoring applications. – Ecotoxicology and Environmental Safety, 74(4): 1057–1064.

Burzyński M., Żurek A., 2007: Effects of copper and cadmium on photosynthesis in cucumber cotyledons. – Photosynthetica, 45(2): 239–244.

Chapman E.E., Dave G., Murimboh J.D., 2013: A review of metal (Pb and Zn) sensitive and pH tolerant bioassay organisms for risk screening of metal-contaminated acid soil. – Environmental Pollution, 179: 326–342.

Chiodi Boudet L., Escalante A., Haeften G. von, Moreno V., Gerpe M., 2011: Assessment of heavy metal accumulation in two aquatic macrophytes: a field study. – Journal of the Brazilian Society of Ecotoxicology, 6(1): 57–64.

Demirevska-Kepova K., Simova-Stoilova L., Stoyanova Z., Hölzer R., Feller U., 2004: Biochemical changes in barley plants after excessive supply of copper and manganese. – Environmental and Experimental Botany, 52: 253–266.

Dietz K.J., Tavakoli N., Kluge C., Mimura T., Sharma S.S., Harris G.C., Chardonnens A.N., Golldack D., 2001: Significance of the V-type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. – Journal of Experimental Botany, 52: 1969–1980.

Emenike C.U., Fauziah S.H., Agamuthu P., 2012: Characterization and toxicological evaluation of leachate from closed sanitary landfill. – Waste Management and Research, 30(9): 888–897.

Epstein E., 1998: How calcium enhances plant salts tolerance. – Science, 40: 1906–1907.

Fernandes J.C., Henriques F.S., 1991: Biochemical, physiological and structural effects of excess copper in plants. – Botanical Review, 57: 246–273.

Foy C.D., Chaney R.L., White M.C., 1978: The physiology of metal toxicity in plants. – Annual Review of Plant Physiology, 29: 511–566.

Harguinteguy C.A., Fernández Cirelli A., Pignata M.L., 2014: Heavy metal accumulation in leaves of aquatic plant *Stuckenia filiformis* and its relationship with sediment and water in the Suquía River (Argentina). – Microchemical Journal, 114: 111–118.

Hassler C.S., Slaveykova V.I., Wilkinson K.J., 2004: Some fundamental (and often overlooked) considerations underlying the free on activity and biotic ligand models. – Environmental Toxicology and Chemistry, 23: 283–291.

Kazlauskienė N., Stasiūnaitė P., 1999: The lethal and sublethal effect of heavy metal mixture on rainbow trout (*Oncorhynchus mykiss)* in its early stages of development. – Acta Zoologica Lituanica. Hydrobiologia, 9(2): 47–55.

Kazlauskienė N., Vosylienė M.Z., Svecevičius G., 2002: Sublethal effects of heavy metal model mixtures in fish: Consequences, after-effects and predictions. – In: Anke M., Müller R., Schäfer U., Stoepler M. (eds), Macro and Trace Elements, 21: 717–723. – Leipzig.

Kinraid T.B., 1999: Interactions among Ca, Na and K in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. – Journal of Experimental Botany, 50: 1495–1505.

Kjeldsen P., Barlaz M.A., Rooker A.P., Baun A., Ledin A., Christensen T.H., 2002: Present and long-term composition of MSW landfill leachate: a review. – Critical Reviews in Environmental Science and Technology, 32(4): 297–336.

Kopittke P.M., Menzies N.W., 2006: Effect of Cu toxicity on growth of Cowpea (*Vigna unguiculata*). – Plant and Soil, 279: 287–296.

Lamb D.T., Naidu R., Ming H., Megharaj M., 2012: Copper phytotoxicity in native and agronomical plant species. – Ecotoxicology and Environmental Safety, 85: 23–29.

Lombardi L., Sebastiani L., 2005: Copper toxicity in *Prunus cerasifera*: growth and antioxidant in *Prunus cerasifera*: Growth and antioxidant enzymes responses of in vitro grown plants. – Plant Science, 168: 797–802.

Lutts S., Kinet J., Bouharmont J., 1996: Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. – Plant Growth Regulation, 19: 207–218.

Magone I., 1989: Bioindication of phytotoxicity of transport emission. – In: Kachalova O.L. (ed.), Bioindication of toxicity of transport emissions in the impact of highway emissions on natural environment, 108–116. – Riga.

Maksymiec W., 1997: Effect of copper cellular processes in higher plants. – Photosynthetica, 34(3): 321–342.

Manios T., Stentiford E.I., Millner P.A., 2003: The effect of heavy metals accumulation on the chlorophyll concentration of *Typha latifolia* plants, growing in a substrate containing sewage sludge compost and watered with metaliferus water. – Ecological Engineering, 20: 65–74.

Marciulioniene D., Montvydiene D., Kazlauskiene N., Svecevicius G., 2002: Comparative analysis of the sensitivity of test-organisms of different phylogenetic level and life stages to heavy metals. – Environmental and Chemical Physics, 24(2): 73–78.

Marschner H., 1995: Mineral nutrition of higher plants. – London.

McKim J.M., 1985: Early life stage toxicity tests. – In: Rand G.M., Petrocelli R. (eds), Fundamentals of Aquatic Toxicology, 58–95. – New York.

Mer R.K., Pajith P.K., Pandya D.M., Pandey A.N., 2000: Effect of salts on germination of seeds and growth of young plants of *Hordeum valgare*, *Triticum aestivum*, and *Brassica juncea*. – Journal of Agronomy and Crop Science, 185(4): 209–217.

Mocquot B., Vangronsveld J., Clijsters H., Mench M., 1996: Copper toxicity in young maize (*Zea mays* L.) plants: effect on growth, mineral and chlorophyll contents, and enzymes activity. – Plant Soil, 182: 287–300.

Montvydiene D., Marciulioniene D., 2004: Assessment of toxic interactions of heavy metals in a multicomponents mixture using *Lepidium sativum* and *Spirodela polyrrhiza*. – Environmental Toxicology, 19(4): 351–358.

Moore J.W., Ramamurthy S., 1987: Heavy metals in natural waters. – Moscow.

Muller S.L., Huggett D.B., Rodgers J.H. Jr., 2001: Effects of copper sulfate on *Typha latifolia* seed germination and early seedlings growth in aqueous and sediment exposure. – Archives of Environmental Contamination and Toxicology, 40: 192–197.

Newman M.C., 1998: Fundamentals of Ecotoxicology. – Chelsea, MI.

Ouzounidou G., 1995: Cu-ions mediated changes in growth, chlorophyll and other ions contents in a Cu-tolerant *Koeleria splendens*. – Biologia Plantarum, 37(1): 71–78.

Ovečka M., Takáč T., 2014: Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. – Biotechnology Advances, 32: 73–86.

Parida A.K., Das A.B., 2005: Salt tolerance and salinity effects on plants: a review. – Ecotoxicology and Environmental Safety, 60: 324–349.

Queirós F., Rodrigues J.A., Almeida J.M., Almeida D.P.F., Fidalgo F., 2011: Differential responses of the antioxidant defence system and ultrastructure in a salt-adapted potato cell line. – Plant Physiology and Biochemistry, 49: 1410–1419.

Rahman M., Matsumuro T., Miyake H., Takeoka Y., 2001: Effects of salinity stress on the seminal root tip ultrastructures of rice seedlings (*Oryza sativa* L.). – Plant Production Science, 4:103–111.

Reddy K.S., Menary R.C., 1990: Nitrate reductase and nitrate accumulation in relation to nitrate toxicity in *Boronia megastigma*. – Physiologia Plantarum, 20(3): 430–434.

Shomar B.H., Muller G., Yahya A., 2005: Seasonal variations of chemical composition of water and bottom sediments in the wetland of Wadi Gaza, Gaza Strip. – Wetlands Ecology and Management, 13: 419–431.

Silva J.V., Lacerda C.F., Costa P.H.A., Eneas Filho J., Gomes Filho E., Prisco J.T., 2003: Physiological responses of NaCl stressed cowpea plants grown in nutrient solution supplemented with

$CaCl_2$. – Brazilian Journal of Plant Physiology, 1: 99–105.

Skorbilowicz E., 2009: Aquatic plants as bioindicators of contamination of upper Narew River and some of its tributaries with heavy metals. – Environment Protection Engineering, 35(1): 65–77.

Stoltz E., Greger M., 2002: Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. – Environmental and Experimental Botany, 47(3): 271–280.

Šimonová E., Henselová M., Masarovičová E., Kohanová J., 2007: Comparison of tolerance of *Brassica juncea* and *Vigna radiate* to cadmium. – Biologia Plantarum, 51(3): 488–492.

Tang W., Zhao Y., Wang C., Shan B., Cui J., 2013: Heavy metal contamination of overlying waters and bed sediments of Haihe Basin in China. – Ecotoxicology and Environmental Safety, 98: 317– 323.

Tao Y., Yuan Z., Xiona H., Wei M., 2012: Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihu lake, China. – Ecotoxicology and Environmental Safety, 81: 55–64.

Tester M., Davenport R.A., 2003: Na^+ tolerance and Na^+ transport in higher plants. – Annals of Botany, 91: 503–527.

Vosyliene M.Z., Kazlauskiene N., Joksas K., 2005: Toxic effects of crude oil combined with oil cleaner simple green on yolk-sac larvae and adult rainbow trout *Oncorhynchus mykiss*. – Environmental Science and Pollution Research, 12(3): 136–139.

Wang W., 1992: Use of plants for the assessment of environmental contaminants. – Reviews of Environmental Contamination and Toxicology, 126: 88–127.

White P., 2011: Ion uptake mechanisms of individual cells and roots. Short distance transport. – In: Marschner P. (ed.), Marschner's mineral nutrition of higher plants: 7–44. – San Diego.

Xiong Z.T., Wang H., 2005: Copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis* Rupr.). – Toxicology, 20: 188–194.

Yildiztugay E., Sekmen A.H., Turkan I., Kucukoduk M., 2011: Elucidation of physiological and biochemical mechanisms of an endemic halophyte *Centaurea tuzgoluensis* under salt stress. – Plant Physiology and Biochemistry, 49: 816–824.

Yruela I., 2005: Copper in plants. – Brazilian Journal of Plant Physiology, 17(1): 145–156.

Zaman B., Ali A., Salim M., Hussain K., 2002: Growth of wheat as affected by sodium chloride and sodium sulphate salinity. – Pakistan Journal of Biological Science, 5(12): 1313–1315.

TESTINIŲ ORGANIZMŲ ATSAKAS Į SKIRTINGŲ NA IR CU DRUSKŲ POVEIKĮ

Danutė Marčiulionienė, Danguolė Montvydienė, Nijolė Kazlauskienė, Benedikta Lukšienė, Dalia Jasinevičienė, Stasys Tautkus

Santrauka

Darbo tikslas – įvertinti skirtingų Na ir Cu druskų (nitratų, sulfatų ir chloridų) poveikį testiniams organizmas. Buvo nustatytas Na ir Cu druskų poveikis sėjamosios pipirnės (*Lepidium sativum*) sėklų daigumui ir šaknelių augimui bei vaivorykštinio upėtakio (*Oncorhynchus mykiss*) ankstyvųjų vystymosi stadijų (embrionų ir lervų) mirtingumui, augimui bei fiziologiniams parametrams. Tarp Na druskų abiems tirtiems organizmams toksiškiausia buvo nitrato druska, o tarp tirtų Cu druskų stipriausią toksinį poveikį *L. sativum* sukėlė sulfato, o *O. mykiss* embrionams ir lervoms – nitrato druska. *L. sativum* šaknelėse kaupėsi didesni tirtų anijonų ir katijonų kiekiai nei stiebeliuose. Didžiausia Na^+ ir Cu^{2+} jonų pernaša iš šaknelių į antžeminę augalo dalį buvo nustatyta augaluose, augintuose sulfato druskose. Tačiau anijonų pernaša augaluose, augintuose Na ir Cu druskose skyrėsi. Augaluose, augintuose Na druskose, didžiausia pernaša pasižymėjo SO_4^{2-} jonai, o Cu druskose – NO_3^- jonai. Stipri koreliacija buvo nustatyta tarp *L. sativum* šaknelių ilgio ir Na^+, NO_3^- ir SO_4^{2-} jonų kiekio augalo šaknyse.