

## **EFFECT OF LEAD EXCESS IN SOIL ON THE ACCUMULATION OF P, K AND Na IN INFECTED BY *FUSARIUM OXYSPORUM* F. SP. *RADICIS-LYCOPERSICI* IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) PLANTS**

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### **Abstract**

S. MANEVA, S., T. VATCHEV and E. MITEVA, 2013. Effect of lead excess in soil on the accumulation of P, K and Na in infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato (*Lycopersicon esculentum* Mill.) plants. *Bulg. J. Agric. Sci.*, 19: 972-980

Pot experiments were conducted in greenhouse of IPAZR, “N. Poushkarov”, Sofia, to study the effects of excess lead (Pb) in soils on the uptake of P, K and Na in tomato plants (*Lycopersicon esculentum* Mill.), cv. Ideal, grown in *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) infected soils. Lead at the rate of 0, 80 and 160 mg kg<sup>-1</sup> soil were added in soil in solution form as lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>]. The results showed that only the highest of applied doses of lead in soil leads to increase of P, K and Na in both the roots and the stems. Significant changes in the concentrations of the studied elements in plants caused FORL infection, as the increase was greatest for Na (P<0.001, 58% over the in the control stems). The combination of both stress factors had significant effect on P and Na uptake. It doubled the content of K in roots (60889 mg.kg<sup>-1</sup>) compare to control, which is out of the tolerance limit for tomato plants. The total amount of P, K and Na in plants increased with the increasing of DS of FORL infection. In case of Na this increase was due to increase of the Na content in roots while at P increased concentration in the stem. Positive correlations were found between soil Pb content and concentrations of P, K and Na in both stem and roots except the P in stems. FORL infection inverted this trend except for P in both part of the tomato plants.

*Key words:* lead, soil contamination, soil born pathogen, *Fusarium oxysporum* f. sp. *Lycopersici*, macronutrients, accumulation

*Abbreviations:* FORL - *Fusarium oxysporum* f. sp. *lycopersici*, DS – disease severity

### **Introduction**

Higher plants require the supply of certain inorganic elements to satisfy their nutritional requirements for growth and metabolic processes (Rice, 2007). These elements, especially heavy metals, are recognized as essential plant nutrients when they exist in soil in normal concentrations (Daroub and Syner, 2007). However, where they are found in higher concentrations in agricultural areas they become soil pollutants and they can have unexpected effects on plant and human health. Many authors draw attention to the fact that heavy metal pollution of soil is a significant environmental problem and has its negative impact on agricultural production and

associated human health (Cieslinski and Mercik, 1993; Lasat, 2002; Kiikkilä, 2003; Kobza, 2005; On-line 1, 2005; Romeiro et al., 2006; Jing et al., 2007).

Lead (Pb) is not recognized as essential nutrient but the higher plants have the ability to uptake and accumulate it. Among the existing pollutants, Pb is a major contaminant of the soil due to intensive use of fertilizers and pesticides, industrial activity and mining (Gratao et al., 2005; Romeiro et al., 2006). It may reach potentially toxic levels in soil close to busy streets and highways (Rosen, 2002). In Bulgaria, a number of large-scale examinations on the soil pollution have been carried out (Petrov et al., 1986). It was found out that in some regions the soil Pb content was 350 mg.kg<sup>-1</sup>. This great-

ly exceeds the soil limits of Pb published in Bulgarian Government Newspaper (Miteva et al., 1996). Dinev (2011) published data from monitoring of soil in which they are found lead contamination in the range of 172-280.4 and 180.8-191.4 mg.kg<sup>-1</sup> at a depth of 0-20 cm and 20-40 cm respectively.

There were two main aspects in published investigations of the uptake of heavy metals and their effect on agro-eco-systems. Some of them referred to hyperactive accumulation abilities of some plants, which could be useful for soil phytoremediation (Barlow et al., 2000, Lombi, 2001; Kos et al., 2003; Romeiro et al., 2006). The other authors pay attention on the plant nutrition and food quality with the relation to the human and plant health. Sharma and Dubey (2005) investigate the influence of Pb on mineral nutrition, photosynthesis and water balance of plants. Effect of excess Pb on plant nutrition was studied in strawberry plants (Cieslinski and Mercik, 1993), lettuce (Michalska and Hakan, 2001), *Ricinus communis* (Romeiro et al., 2002), sugar beet (Labri et al., 2002), and bean plants (Piechalak, 2008).

Soil microbial communities are worthy of study, as they are diverse, abundant and show a wide range of tolerance to environmental stress and interact directly with soil contaminants (Trett et al., 2000). Many of the soil microorganisms are strongly sensitive to contamination (Ellis et al., 2002). The soil born fungi *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) causes tomato crown and root rot. The species is among the most economically important pathogens in Bulgaria and is characterized by a high frequency of occurrence, infecting up to 100% of the tomatoes grown in greenhouses and field (Vatchev and Ilieva, 1999; Vatchev et al., 2003).

There are a very limited number of publications on the relationship of heavy metals and pathogens in aspect of their effect on the host-plants. It was studied the correlation between Cd and *Phytophthora infestans* in two potato varieties (Stroinski and Floriszak-Wieczorek, 1990, 1993). Weicht and MacDonald (1992) studied the impact of *Phytophthora* root rot of sodium (Na) uptake in Safflower. Karagiannidis et al. (2002) studied the effect of the arbuscular micorrhizal fungus *Glomus mosseae*, the soil-born *Verticillium dahliae*, and their interaction on nutrient uptake in tomato. Bogoeva et al. (2007) reported the results concerning the effect of combined stress of soil copper (Cu) pollution and *Fusarium culmorum* infection on heavy metal content in wheat. The effect of Pb, Cu or combination of them, as well as arsenic (As) soil excess on the plant growth, peroxidase activity (POA), plastid pigments and nutrition elements uptake in tomato plants infected by cucumber mosaic virus (CMV) or tomato mosaic virus (ToMV) has been studied (Miteva et al., 2001a, b, Miteva et al., 2005). Maneva et al. (2010) analyze the impact of excess copper on development of bacterial spots caused by

*X. vesicatoria* in tomato and the combined effect of both biotic and abiotic stress factors on plant growth. Escobasa et al. (2010) pay attention on the potential role of the phytopathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* in the translocation of metals and metalloids from soil to plants.

The aim of this work was to investigate the combined effect of excess lead in the soil and FORL infection on the accumulation of P, K and Na in tomato plants.

## Material and Methods

### Plant growth conditions

**Soil:** Pot trials were carried out over four years in the greenhouse of the Plant Protection Institute, Kostinbrod. Alluvial-meadow soil was used with pH 6.8, humus content 3.8% and an initial lead content of 1.5 mg.kg<sup>-1</sup>. The soil was sterilized at 160°C for an hour and sifted out through 2 mm sieve. Sand was added in a rate of soil /sand 3:1 w / w. The mixture was poured into pots (five per variant) with a volume of 2 L each.

**Lead pollution:** Lead was introduced in form of Pb(NO<sub>3</sub>)<sub>2</sub> water solution at rate 0, 80 and 160 mg.kg<sup>-1</sup> soil. Soils were stabilized during 20 days keeping 70-75% PPW moisture by sterilized water and mixed regularly.

**Pathogen:** The soil of half of the pots was artificially inoculated with the soil born pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), using poor culture of the grown for 2-wk at 26 °C on sterilized by autoclaving (121 °C for one hour) oat kernel. 200 kernels were applied per pot and mixed with the soil. Tomato cv. Ideal seedlings were grown in pots with inoculated soils to allow the establishment of the pathogen. Inoculated and not inoculated (healthy) soils were placed in greenhouse on a concrete surface in a completely randomized experimental scheme were then incubated at greenhouse conditions for 30 days. Plants were uprooted after 30 days.

**Plants:** After soils were ready, the test tomato plants, cv. Ideal, previously grown in pure of both, Pb and FORL infection soil, were planted in respective variant's pots. They were grown in a greenhouse at 18-20°C, photoperiod of 16/8 hours day/night, air humidity of 75-80%, and keeping soil moisture 70-75% PPW watering by distilled water regularly from the pot's pads to avoid washing of lead from soil.

### Disease severity rating

The trail was terminated 90 days after the planting. The plant roots were washed free of soil and wiped dry between sheets of filter paper. Sections of the symptomatic roots, randomly taken from inoculated plants, were surface sterilized (1% NaOCl for 1 min) and placed on oat agar (OA) and po-

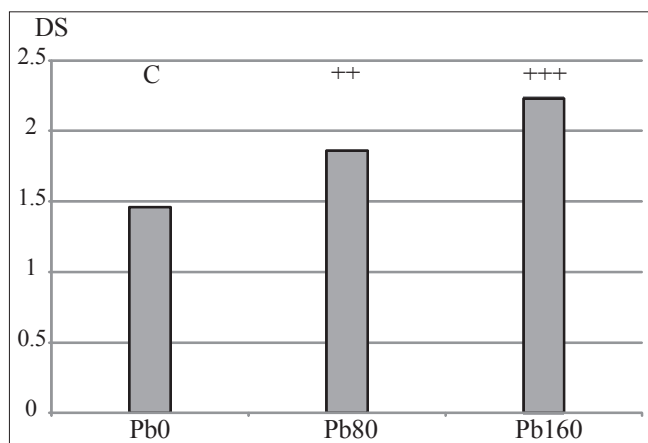


**Phosphorus (P):** Increasing of Pb content in soil decrease the stem content of P insignificantly within the experimental Pb amounts (Figure 2). FORL inoculation increases P uptake and obtained differences between the variants with same Pb content in soil are significant (Table 1). The combination of both stress factors strengthen this trend as it was most obvious at 80 mg.kg<sup>-1</sup> Pb in soil (P<0.01). In this variant, the P content in stem was 31% higher than the control one (Figure 2). The combination of infection and highest Pb dose suppress the P uptake in stems but the accumulation remained higher than in the healthy plants. Both factors, applied separately, increase the P content in the roots (Figure 2). Combination of both fac-

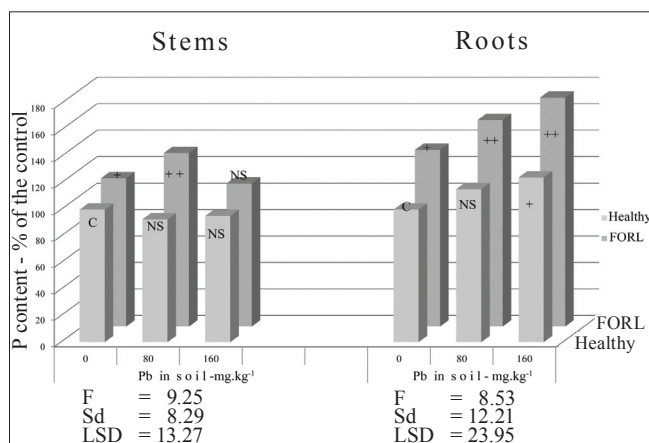
tors increased this effect significantly, and plants reached 73% root P content over the control (Table 3, Figure 2).

Increases of Pb concentrations in soil caused the increase of total P in the tomato plants. FORL infection enhances the effect, thereby increasing both DS and Pb content increased amount of P. As percent of total content P in roots was less than in stem but it is growing faster than in the stem (Figure 5A).

**Potassium (K):** Soil contamination has increased content of K in the stems, and this is statistically significant at 160 mg.kg<sup>-1</sup> Pb - 22.67% over that in control plants (P<0.05, Figure 3). The FORL infection has increased significantly stem uptake of K compared to the control (Table 3). The combina-



**Fig. 1. Development of FORL infection depending on the soil content of Pb (mg.kg<sup>-1</sup>) estimated on the base of 6-grade scale (0-5). C – control (soil with the initial content of Pb), ++ - P<0.01; +++ - P<0.001; F = 17.28, Sd = 0.53, LSD<sub>0.05</sub> = 1.039**



**Fig. 2. Accumulation of P in tomato plants sv. Ideal grown on Pb polluted soils and infected by FORL C – control, NS – not statistical significance, + - P<0.05, ++ - P<0.01, +++ - P<0.001**

**Table 3**

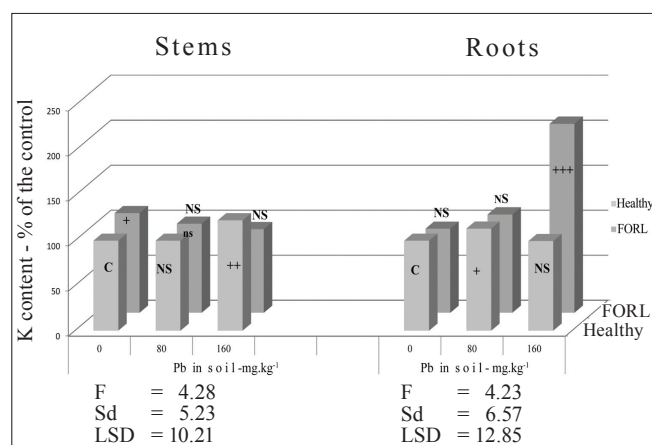
**Content of Macronutrients and Pb in tomato plants grown under combined stress factors- excess of Pb at soils and infected by FORL- statistical estimation of the obtained differences**

Variables Pb / FORL mg.kg <sup>-1</sup> / DS	Content of macronutrients – mg.kg <sup>-1</sup>					
	Stems			Root		
	P	K	Na	P	K	Na
0 -	2100 C <sub>1</sub>	28450 C <sub>1</sub>	1125 C <sub>1</sub>	1125 C <sub>1</sub>	18575 C <sub>1</sub>	3175 C <sub>1</sub>
0 +1.46	2350 +	31525 ++	1775 ++	1500 +	17350 +	4500 ++
80 -	1950 C <sub>2</sub>	28450 C <sub>2</sub>	1100 C <sub>2</sub>	1300 C <sub>2</sub>	21075 C <sub>2</sub>	3050 C <sub>2</sub>
80 +1.86	2750 ++	28175 NS	1625 ++	1754 +	20297 NS	4796+++
160 -	2000 C <sub>3</sub>	34900 C <sub>3</sub>	1275 C <sub>3</sub>	1400 C <sub>3</sub>	18500 C <sub>3</sub>	4250 C <sub>3</sub>
160 + 2.23	2275 +	26425 +++	1650 +	1945 ++	60889+++	4765 +
F	12.82	8.6	9.13	12.43	8.7	10.4
Sd	121.43	1000.5	132.31	178.00	614.8	208.2
LSD <sub>0.05</sub>	238.98	1961.0	259.34	349.00	1205.2	408.1

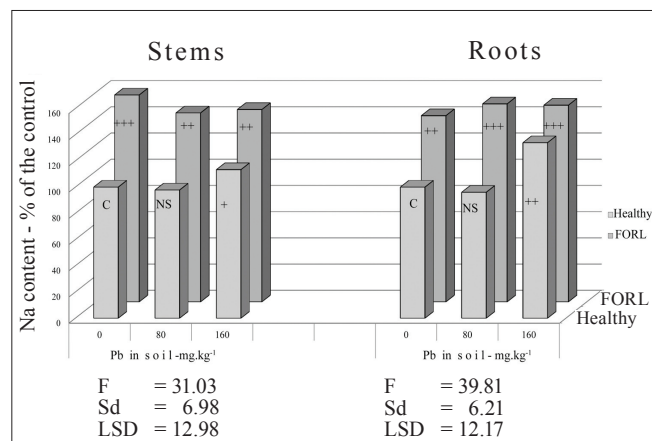
C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub> respective control, NS – no statistical significance (P>0.050), + - P<0.05, ++ - P< 0.001, +++ - P<0.001, DS – disease severity of FORL

tion of the both stress factors suppressed this effect (Figure 4). At the highest dose of Pb in soil, content of K in stems decreased by 29.79% compare to the variant with the same soil contamination ( $P < 0.01$ , Figure 3). Root K content is lower than the stem's one at the lower Pb values but at 160 mg.kg<sup>-1</sup> increasing of disease severity caused significant increase of root K over the both healthy and inoculated stem.

The total content of K did not vary significantly from the control and all variants except for the combination of the highest values of both dose of Pb in the soil and DS of FORL where the increases of the total K was due to the increase of K content in roots (Figure 5B).

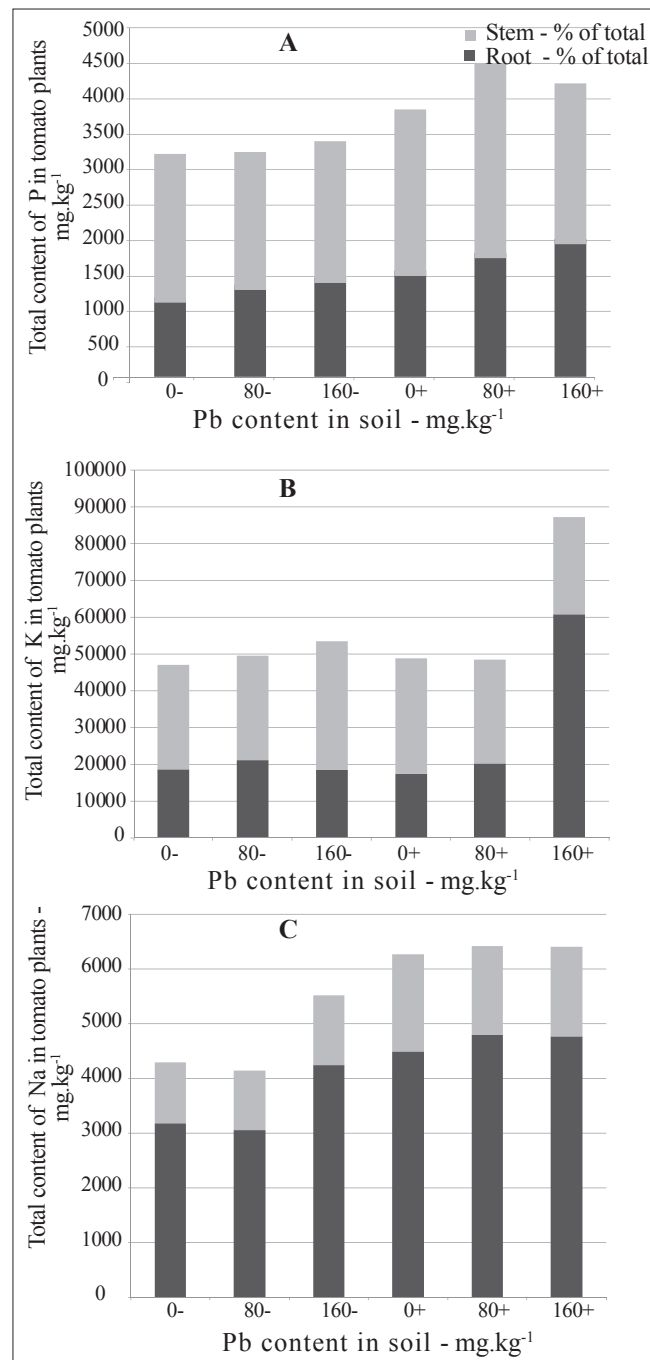


**Fig. 3. Accumulation of K in tomato plants sv. Ideal grown on Pb polluted soils and infected by FORL**  
 C – control, NS – not statistical significance,  
 + -  $P < 0.05$ , ++ -  $P < 0.01$ , +++ -  $P < 0.001$



**Fig. 4. Accumulation of Na in tomato plants sv. Ideal grown on Pb polluted soils and infected by FORL**  
 C – control, NS – not statistical significance,  
 + -  $P < 0.05$ , ++ -  $P < 0.01$ , +++ -  $P < 0.001$

**Sodium (Na):** The plants grown in polluted soils showed enhanced content of Na in the stems, and this was statistically significant at 160 mg.kg<sup>-1</sup> Pb content in soil – 13.33% over that in control plants ( $P < 0.05$ , Figure 4). Soil pathogen



**Fig. 5. Distribution of content of P, K and Na (mg.kg<sup>-1</sup>) in root and stem of tomato plants as percent of the total amount**

has increased vastly the stem uptake of Na (Table 3). The increase of Na content in the stems was 44-58% over in the control (Figure 4). The obtained differences were with higher statistical level if the data are presented as a percent from the control (Figure 4) than as mg.kg<sup>-1</sup> (Table 1). The combination of both stress factors decreases this effect. Only the highest dose of Pb in soil caused significant increase of the Na in the tomato plant (Figure 4). FORL infection has increased this effect significantly (Table 3). The combination of both biotic and abiotic factors caused strengthening the Na accumulation in the roots. Accumulated by roots Na was 42-51% over the control (Figure 4)

The total content of Na has increased for the highest dose of Pb in soil as well as for the combination of the two stress factors. The percentage of the Na in the roots is considerably greater than in the stem. The variation in the applied factors of influence did not disturb the balance between stem and roots (Figure 5C).

Correlation between the factors of influence and the content of P, K and Na in the stem and root are presented in Table 4. The content of K and Na in stems and P and Na in root correlated positively with the content of Pb in the un-inoculated soils, as well as P in stems and K and Na in root showed negative correlation. FORL infection of the roots inverted this trend except for the P content in both root and stems. Inoculation of the roots lowered the level of significance of the obtained relationships except for P and K in roots and for K in stems.

## Discussion

Natural soils usually have less than 50 mg.kg<sup>-1</sup> of Pb (Dharmananda, On-line 2). According Bulgarian government standards for soil pollution in soils with 5.5<pH<7, the limit of Pb has to be within (50; 80) mg.kg<sup>-1</sup> (Bulgarian Government Newspaper, 2002, N39). In the results presented here soil has been given a dose of Pb equal to the upper value of the limit and a dose twice as high as the limit which gave us right to accept that the experiment covered a risk interval of

soil Pb content. Plants generally do not absorb or accumulate lead in quantities that are of concern (Blaylock et al, 1997; Rosen, 2002). However, Ang and Ng (2000) reported for 5.0 mg.kg<sup>-1</sup> dry weight Pb content in control plant stem. According Jones (2002) the amounts of 0, 5-30 or 10-300 mg.kg<sup>-1</sup> are deficient (normal), sufficient (toxic) or excessive respectively. The results obtained of this work showed that the excess of Pb in soil combined with FORL infection could increase the Pb concentration in plant stems over the 5 mg.kg<sup>-1</sup> and 244 times more in the roots compare to plants grown on healthy soils unpolluted with Pb .

In the literature, P and K are defined essential macronutrients for plants. Some authors define Na as a plant macronutrient (Ruiz et al., 1997) and, since then, other authors have included it in the list of micronutrients (Rice, 2007). In our work, we have scrutinized Na as well as P and K as primary plant nutrients. The macronutrients (P and K) are consumed in larger quantities and are present in plant tissue in quantities from 0.2% to 4.0% on a dry matter weight basis (2000 to 40 000 mg.kg<sup>-1</sup>) (Retrieved, 2010). Ruiz et al. (1997) reported the concentration of P, K and Na in control plant leaves 2 250, 20 180 and 2 400 mg.kg<sup>-1</sup> respectively. The total P concentration in agricultural crops generally varies from 1000 to 5000 mg.kg<sup>-1</sup> (on-line 4). Approximate content of K according Rice (2007) was 25 000 mg.kg<sup>-1</sup>. The concentrations of the examined macronutrients in the plants of this experiment grown in pure of Pb and healthy soils were in the same amounts.

According to Sharma and Dubey (2005), Pb alters the mineral nutrition. Michalska and Halan (2001) reported that there was not a significant effect of the lead on the concentrations of macro-elements. Results of the presented investigation showed that excess Pb in the soil influenced significantly the stem and root content of Na as well as the root content of P and decrease stems content of P. In all examined combinations of the both stress factors DS of FORL increased this effect in both stems and roots. For K only significant effect occurred at the combination of the highest values of both Pb content and FORL infection. According to Escobosa et al. (2010), the increase of uptake of soil elements in roots of plant

**Table 4**  
**Relationship between content of Pb in stem and roots on the content of P, K and Na in tomato plants**

	Impact of PB content and DS of FORL - Correlation coefficients					
	S t e m			R o o t		
	P	K	Na	P	K	Na
Pb(-DS)	-0.655	0.866 ++	0.798 +	0.888 +++	-0.026	0.815 ++
PB(+DS)	-0.147	-0.984 +++	0.277	0.997 +++	0.895 ++	-0.457
DS(+Pb)	-0.139	-0.985 +++	0.270	0.997 +++	0.891 ++	-0.450

+ - P<0.05; ++ - P<0.01; +++ - P<0.001; (Pb-) - healthy soil with Pb contamination;  
(Pb+) - infected soil with Pb

infected by *Fusarium oxysporum* f. sp. *Lycopersici* was due to fungus-assisted solubilization of soil humic substances contribute to element traffic from soil to roots in tomato plant.

P is one of 17 nutrients essential for plant growth and its functions cannot be performed by any other nutrient. Karagiannidis et al. (2002) reported that P uptake was higher in mycorrhizal treatment than in controls. In our case, stem P contents remained in the normal for healthy plants contents, but the excess of Pb in soil decrease them. On the base of obtained correlations between P content and both applied factors of influence, we are of the opinion that, at higher Pb concentrations in soil than those tested for, Pb in soil would reduce the P in the plant sufficiently to cause actual P deficiency. The results of our study also gave reason to claim that the FORL infection increases the accumulation of P in plants and this effect is more significant for the roots. However, the highest dose of Pb in soil depresses this effect in stems.

The K content in stems of experimental plants were found out to be higher than the amounts reported by Ruize et al. (1997) but remained within the values presented by Retrieved (2010). No difference in K uptake was observed between treatments. A significant increase of K content in roots has occurred at the combination of both stress factors in their highest observed values.

Tomato plants grown under the influence of both stress factors appeared to be most sensitive in the Na uptake. The main factor for this was the infection but the combination of the two stress factors, especially at high doses of Pb in soil also contribute to this. Despite the significant increase of Na content compare to the control, it remains within the levels of Na content reported in literature. We did not find much published information about the relationship between the content of Pb in soil and the Na content in plants as well as about the effect of soil born phytopathogen fungi. Botrini et al. (2000) reported that the high levels of K reduced the Na concentration in the roots and stems, but not in the leaves. According Zue (2001), excess Na in the cytoplasm can lead to enzyme inhibition, which in turn causes necrosis and chlorosis. We found out that concentrations of Na in plants negatively correlated with the concentrations of K in soil and plant stem ( $R_{Kst/Na.st} = -0.999$  +++ and  $R_{K.r/Na.st} = -0.556$  ns, stem and roots respectively) when plants a grown on health of FORL soil. The infection of FORL inverted correlation to positive one ( $R_{Kst/Na.st} = 0.877$  ++).

The obtained correlations between the concentrations of P, K and Na in stems and roots with the content of soil or DS of FORL infection showed that the uptake of the investigated plant nutrient depends significantly on both stress factors as well as the inverted the effect of soil Pb caused by the FORL.

It could be supposed that the influence of the soil Pb excess combined with the root rot on tomato plants caused by *Fusarium oxysporum* f. sp. *lycopersici*, on the nutrient elements P, K and Na uptake is a complicate proces. There is a need to improve our understanding of the mechanisms involved in the transfer and mobilization of P, K and Na by Pb soil content and FORL infection and to conduct research on the selection of microbial isolates from *Fusarium oxysporum* infected plants growing on Pb contaminated soils.

## Conclusion

The results of this investigation allow us to draw the following conclusions:

Only the highest of applied doses of Pb in soil caused the increase of P, K and Na in both the roots and the stems. Significant changes in the concentrations of the studied nutrients in plants caused FORL infection. Combined effect of the both stress factors depended on the nutrients.

The total amount of P, K and Na in plants increased with the increasing of DS of FORL infection. In case of Na this increase was due to increase of the Na content in roots while at P increased concentration in the stem.

Positive correlations were found between soil Pb content and concentrations of P, K and Na in both stem and roots except the P in stems. FORL infection inverted this trend except for P in both part of the tomato plants.

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