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Growth and ion uptake in maize plants exposed to Pb, Cd and Ni depend on NO_3^-/NH_4^+ ratio

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ABSTRACT: Maize (*Zea mays* L.) seedlings (hybride ZP 330) were grown in hydropone culture on a standard nutrient solution containing 7.5 mM N. Nitrogen was applied in two forms: 1) as NO_3^- and 2) as NO_3^-/NH_4^+ (2:1). After two days in the culture, the plants were exposed to heavy metals and the two treatments were further divided into four groups: a) control; b) 0.5 mM Pb; c) 0.5 mM Cd; d) 0.5 mM Ni. After three days of exposure, the following parameters were determined in roots: heavy metal concentration, root length, root weight, the concentrations of proline, soluble proteins, K, Ca and Mg. The results show that almost all the determined parameters depend on nitrogen form. Lead and Cd accumulation were lower under NO_3^- treatment, while Ni accumulation was lower under NO_3^-/NH_4^+ treatment. The decrease of the root length depended on heavy metal concentration in roots, while root weight decrease depended only on N form. Soluble protein and K concentrations changed in dependence of both N form and heavy metal type. Proline concentration increased significantly in the presence of each metal under NO_3^- treatment, while under NO_3^-/NH_4^+ treatment the change was negligible except in the plants exposed to Ni. Cadmium and Ni induced a significant decrease of Ca and Mg concentrations, which was more pronounced under NO_3^-/NH_4^+ treatment in both cases. Lead induced a decrease of Mg concentration under NO_3^- treatment, accompanied with Ca concentration increase, and a decrease of Ca concentration accompanied with Mg concentration increase under NO_3^-/NH_4^+ treatment. Such results showed, apart from different maize responses to different metals, a wide potential for the modification of heavy metal impact by ionic form of nitrogen nutrition.

Key words: cadmium, growth, ion uptake, lead, nickel, root, *Zea mays*

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INTRODUCTION

Constantly increasing heavy metal pollution in both populated and unpopulated areas becomes an unavoidable external factor for artificial and native plant communities. Numerous investigations have been carried out in order to elucidate the mechanisms of heavy metal toxicity and of plant defense reactions. Those investigations show that heavy metal impact on plants is complex, detectable at various cellular and molecular levels and that it is difficult to distinguish between the disorders of primary and secondary origin (Seregin & Ivanov 2001). Similarly, plant responses to heavy metal toxicity appear at various metabolic levels. Some of them are indicators of metabolic disturbances and some are part of constitutional or induced defense responses. These responses have some common characteristics, but are also specific for the plant species and the individual metal (Hall 2002).

Lead, cadmium and nickel are the most widespread non-nutrient heavy metals. Some of the most common effects of these metals on plants are growth inhibition, ion uptake and transport disturbances, enzyme activation or inhibition, stimulation of antioxidative response, photosynthesis inhibition (Fargasova 2001; Seregin & Ivanov 2001; Geebelen *et al.* 2002; Kim *et al.* 2002; Pandey & Sharma 2002; Gajewska & Sklodowska 2006).

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Nitrogen, one of the major essential nutrients, is assimilated by plants in two ionic forms: as NO_3^- or NH_4^+. Most plants are capable to utilize both forms. Different N forms affect differently the uptake of other ions, as well as the metabolisms of organic and amino acids (Chaillou *et al*. 1994; Jeong & Lee 1996). There were few attempts to strenghten plant defense mechanisms against abiotic stress by modifying N ion forms in the nutritient solution. Irshad *et al.* (2008) found that mixed nitrogen source (urea + nitrate) exerted the best effects in alleviating saline stress in maize, in comparison with individually applied urea or nitrate. Sotiropoulos *et al*. (2003) found that kiwifruit (*Actinidia deliciosa*) plants grown on NO_3^- medium accumulated less B than those grown on NH_4^+ or mixed medium. Horst et al. (1999) found NO_3^- - treated *Vigna unguiculata* (L.) Walp. plants to be more tolerant to Mn than those treated with NH_4^+. Lou *et al*. (2005) found that NH_4^+ nutrition increased the uptake of heavy metals in maize compared to NO_3^- nutrition. Sady & Kowalska (2004) and Zaccheo *et al.* (2006) found increased Cd accumulation in *Spinacia oleracea* L. and *Helianthus annuus* L. plants treated with NH_4^+ in comparison with NO_3^- - treated plants.

The aim of the present study was to find the extent of modification of maize response to Pb, Cd and Ni by changing N ion form in nutrient solution. The plants were treated only with nitrate and mixed N forms, because maize belongs to the plants incapable to grow completely deprived of NO_3^- ions. Selected parameters (root growth, soluble protein content, proline, K, Ca and Mg concentrations) were those known to be susceptible both to heavy metals and to N ion form.

MATERIAL AND METHODS

Maize (*Zea mays* L.) hybrid ZPSC 330 seeds used for the experiment were purchased from the Maize Research Institute „Zemun Polje", Zemun, Serbia. The seeds were germinated for three days in the dark, between moist filter papers at 25°C. They were subsequently transferred to hydropone culture under the following conditions: relative humidity 70%, day/night temperature 25°C/18°C and light intensity 150 μmol m^{-2} min^{-1}.

The composition of the culture medium matched a slightly modified Knop solution that contained 7.5 mM N. Nitrogen was applied in two forms: 1) NO_3^- and 2) NO_3^-/NH_4^+ (2:1). The compositions of the two culture solutions were as follows: 1) 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.5 mM KH_2PO_4, 0.8 mM $MgSO_4 \cdot 7\ H_2O$, 0.1 mM $FeCl_3$ (previously dissolved in equimolar EDTA solution), 0.012 mM H_3BO_3, 0.002 mM $MnSO_4 \cdot 4\ H_2O$, 0.0005 mM $ZnSO_4 \cdot 7\ H_2O$, 0.0002 mM $CuSO_4 \cdot 5\ H_2O$, 0.0001 mM $NH_4Mo_7O_{24}$; 2) 2.5 mM NH_4NO_3, 2.5 mM KNO_3, 2.5 mM $CaCl_2$, 1.5 mM KH_2PO_4, 0.8 mM $MgSO_4 \cdot 7\ H_2O$, 0.1 mM $FeCl_3$ (with EDTA), 0.012 mM H_3BO_3, 0.002 mM $MnSO_4 \cdot 4\ H_2O$, 0.0005 mM $ZnSO_4 \cdot 7\ H_2O$, 0.0002 mM $CuSO_4 \cdot 5\ H_2O$, 0.0001 mM $NH4Mo7O_{24}$. The pH value of the solutions was adjusted daily to 6.0. After two days in the culture, the plants were exposed to heavy metals and the two treatments were further divided into four groups: a) control; b) 0.5 mM Pb; c) 0.5 mM Cd; d) 0.5 mM Ni.

After three days of exposure, the following parameters were determined in roots: heavy metal content, root length, root weight, proline accumulation, soluble protein content, K, Ca and Mg concentrations.

The contents of heavy metals, K, Ca and Mg were determined in plant material mineralized by wet procedure, in the presence of HNO_3 and H_2O_2 at 130°C, by atomic absorption spectrometry. Soluble protein concentration was determined according to Lowry *et al*. (1956). Proline concentration was determined by the method of Bates *et al*. (1973). Proline was extracted by sulfosalycilic acid and measured spectrophotometrically after the reaction with ninhydrine dissolved in glacial acetic acid and H_3PO_4 and subsequent extraction with toluene.

Statistical analysis of the data was performed by one-way ANOVA using Origin 7.0.

RESULTS AND DISCUSSION

Accumulation of heavy metals in Pb-, Cd- and Ni-treated plants (Table 1) shows that, in the presence of NO_3^-/NH_4^+ mixture, Pb and Cd concentrations are higher by about 58 and 51 percent, respectively. Nickel concentration is about two times higher under NO_3^- treatment. In a similar experiment with sunflower plants exposed to Cd, Zaccheo *et al.* (2006) found about three times higher concentration of Cd in NH_4^+- than in NO_3^--treated plants. Heavy metal uptake stimulation by NH_4^+ is frequently explained by lowered rhizosphere pH value, which is favorable for heavy metal availability for plants (Sady & Kowalska 2004č Lou *et al*. 2005). Lower Ni uptake in the presence of NO_3^-/NH_4^+ observed in our experiment demonstrates some more specific mechanism of NH_4^+ interference with Ni transport, confirming specific features of the uptake of each heavy metal.

Root lengths after 3 days of exposure to heavy metals show that the process of root growth inhibition, which is the first and the most direct consequence of heavy metal action (Fargasova 2001; Seregin & Ivanov 2001; Hall 2002), depends primarily on heavy metal concentration in roots. It is more marked in the plants with higher metal accumulation (Table 1 and 2).

Heavy metal presence did not change root weights under NO_3^- treatment, in spite of the inhibition of root elongation, which means that the roots only became

Table 1. Accumulation of heavy metals in the roots of maize seedlings exposed to Pb, Cd and Ni, respectively, treated with different N ion forms. Each value ± S.D. is mean of four replicates (each consisting of 15 plants)

Treatment	**Heavy metal concentration (mg · kg^{-1})**		
	Pb	**Cd**	**Ni**
NO_3^-	0.77^{a}±0.12	1.54^{a}±0.18	1.69^{a}±0.20
NO_3^-/NH_4^+	1.22^{b}±0.10	2.33^{b}±0.40	0.87^{b}±0.20

Different small letters indicate statistically significant differences between the two N treatments (p ≤ 0.05).

Table 2. Root growth parameters of the maize seedlings exposed to Pb, Cd and Ni, respectively, treated with different N ion forms. Each value ± S.D. is mean of twenty (root length) or ten (root weight) plants

	Treatment	**Control**	**Pb**	**Cd**	**Ni**
Root length (cm)	NO_3^-	19.8^{Aa}±1.16	16.2^{Aa}±1.76	10.1^{Ba}±0.72	7.00^{Ba}±2.00
	NO_3^-/NH_4^+	15.4^{Ab}±1.48	10.3^{Bb}±2.64	8.5^{Ba}±1.00	9.60^{Ba}±3.12
Root weight per plant (g)	NO_3^-	0.30^{Aa}±0.05	0.29^{Aa}±0.04	0.29^{Aa}±0.04	0.29^{Aa}±0.04
	NO_3^-/NH_4^+	0.33^{Aa}±0.03	0.29^{Aa}±0.03	0.18^{Bb}±0.02	0.18^{Bb}±0.02

Different small letters indicate statisticaly significant differences between the two N treatments (p ≤ 0.05). Different capital letters indicate statisticaly significant differences within the same N treatment (p ≤ 0.05).

Table 3. Accumulation of soluble proteins and proline in the roots of maize seedlings exposed to Pb, Cd and Ni, respectively, treated with the two different N forms. Each value ± S.D. is mean of four replicates (each consisting of 3 plants)

	Treatment	**Control**	**Pb**	**Cd**	**Ni**
Soluble proteins mg · g^{-1} f.w.	NO_3^-	1.39^{Aa}±0.11	1.34^{Aa}±0.40	1.67^{Ba}±0.12	1.55^{ABa}±0.10
	NO_3^-/NH_4^+	1.64^{Aa}±0.18	1.42^{Aa}±0.12	0.93^{Bb}±0.12	0.92^{Bb}±0.07
Proline μmol · g^{-1} f.w.	NO_3^-	0.12^{Aa}±0.01	0.21^{Ba}±0.01	0.28^{Ba}±0.04	0.47^{Ca}±0.05
	NO_3^-/NH_4^+	0.40^{Ab}±0.05	0.48^{Ab}±0.05	0.48^{Ab}±0.04	0.63^{Bb}±0.03

Different small letters indicate statistically significant differences between the two N treatments (p ≤ 0.05). Different capital letters indicate statistically significant differences within the same N treatment (p ≤ 0.05).

more thick und stunted (Table 2). However, NO_3^-/NH_4^+ treatment appeared to enhance negative effects of Pb, Cd and Ni (the roots were lighter than control by about 12, 15 and 15 percent, respectively). The loss of weight seems to correlate more with N ion form, than with heavy metal concentration in roots.

The increase of soluble protein concentration under the influence of stress factors is a common finding and it indicates intensified metabolic activity within plant defense response. In our experiment (Table 3) soluble protein accumulation was stimulated in Cd- and slightly in Ni-treated plants in the presence of NO_3^-. In the presence of NO_3^-/NH_4^+ treatment, however, Cd and Ni decreased soluble protein concentration.

Heavy metal – stimulated accumulation of proline, a factor counteracting oxidative stress, has been found by many authors (Seregin & Ivanov 2001; Rai *et al.* 2004; Gajewska & Sklodowska 2006). In our experiment, it may be clearly seen that higher proline stimulation is achieved in the presence of NO_3^- ions. Under NO_3^-/NH_4^+ treatment, it is apparent only in Ni-treated plants (Table 3).

In the most studies on the heavy metal impact on plants, the common feature is ion uptake inhibition. It has been found that heavy metals inhibit K, Ca and Mg uptake

Table 4. Accumulation of K, Ca and Mg in the roots of maize seedlings exposed to Pb, Cd and Ni, respectively, treated with the two different N ion forms. Each value ± S.D. is mean of four replicates (each consisting of 15 plants).

	Treatment	Control	Pb	Cd	Ni
K (mg · g^{-1} d.w.)	NO_3^-	20.79^{Aa}±3.14	37.49^{Ba}±3.70	15.11^{Ca}±2.00	19.04^{Ca}±3.00
	NO_3^-/NH_4^+	17.03^{Aa}±1.00	20.57^{Ab}±2.90	16.79^{Aa}±2.30	16.93^{Aa}±2.02
Ca (mg · g^{-1} d.w.)	NO_3^-	16.31^{Aa}±0.6	18.05^{Ba}±0.5	6.40^{Ca}±0.8	10.56^{Da}±0.6
	NO_3^-/NH_4^+	26.11^{Ab}±3.0	15.53^{Ba}±1.8	7.90^{Ca}±1.1	10.42^{BCa}±1.5
Mg (mg · g^{-1} d.w.)	NO_3^-	6.50^{Aa}±0.3	5.74^{Ba}±0.3	5.00^{Ba}±0.4	6.47^{ABa}±0.9
	NO_3^-/NH_4^+	5.29^{Ab}±0.5	7.20^{Bb}±0.4	2.62^{Cb}±0.3	4.41^{Ab}±0.6

Different small letters indicate statistically significant differences between the two N treatments ($p \leq 0.05$). Different capital letters indicate statistically significant differences within the same N treatment ($p \leq 0.05$).

(Seregin & Ivanov 2001; Sharma & Dubey 2005), but in some cases, stimulation has been reported (Päivöke 2002). In our experiment, there are changes of K, Ca and Mg accumulation in the presence of heavy metals (Table 4) that also show a significant influence of N ion form. The heavy metals affected most severely the concentration of Ca, which was decreased by each of the three metals under NO_3^-/NH_4^+ treatment, and by Cd and Ni under NO_3^- treatment. Magnesium concentration was decreased in Cd-treated plants under both N treatments and in Ni-treated plants under NO_3^-/NH_4^+ treatment. Potassium and magnesium accumulations were even stimulated by Pb. The stimulation of Mg accumulation under NO_3^-/NH_4^+ treatment is probably a process induced by simultaneous Ca loss, and is aimed towards the recovery of ion balance. Potassium accumulation stimulation by Pb in the presence of NO_3^- is possibly connected with Pb-stimulated uptake of NO_3^-, K^+ counter-ion, which had been reported earlier (Singh *et al.* 1997/98, Päivöke 2002). In the experiment with Cd-treated sunflower plants, Zaccheo *et al.* (2006) found NO_3^- and NH_4^+ effect on K, Ca and Mg uptake similar to ours except that Mg concentration was increased in the roots of NH_4^+-treated plants. However, the differences they found were statistically insignificant probably due to the fact that the applied Cd concentration was about 12 times lower than in our experiment.

In the presence of one or other N ion form, plant ion uptake, organic acid and amino acid metabolisms show differences (Vanbeusichem *et al.* 1988). Ammonium nutrition would be expected to be favorable under stress conditions, for some heavy metals do not inhibit the uptake of NH_4^+ as strictly as that of NO_3^-. Besides, the energy cost of NH_4^+ assimilation is lower than the assimilation of NO_3^- ion which needs to be first submitted to reduction, leaving more energy-rich compounds at the plant's disposal for the activation of defense processes. On the other hand, NH_4^+ uptake causes rhizosphere acidification, leading to increased heavy metal availability and uptake (Lou et al. 2005). In our experiment, although NO_3^-/NH_4^+ treatment appeared to be less favorable for plants, it did not show correlation with heavy metal concentration in roots. It is more probable that the presence of only NO_3^- stimulated the synthesis of oxalate and citrate, which had been found for *Vigna unguiculata* under Mn stress (Sotiropoulos *et al.* 2003), and for spinach under Cd stress (Sady & Kowalska 2004), enabling thus heavy metal inactivation either by exudation or vacuolar compartmentalization.

CONCLUSION

Maize (*Zea mays* L.) seedlings were grown in the presence of Pb, Cd or Ni under the treatment of two N ion forms: NO_3^- and NO_3^-/NH_4^+ (2:1). Heavy metal accumulation, root growth and the concentrations of soluble proteins, proline, K, Ca and Mg were monitored.

The results showed that the monitored parameters in maize were more susceptible to Cd and Ni than to Pb. Also, NO_3^-/NH_4^+ treatment made them significantly more susceptible to these metals. These results are in accordance with previous experiments demonstrating harmful effects of NH_4^+ on heavy metal tolerance. But, since there was no apparent connection between N form and heavy metal concentration in roots, we concluded that NO_3^- treatment probably contributed to organic ions (oxalate and citrate) accumulation and successful binding and inactivation of heavy metal ions.

The results indicate that, apart from the specificity in maize response degree to different metals, there exists a wide genetic potential for increasing heavy metal tolerance that can be induced by properly selected N ion balance.

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REZIME

Rast i usvajanje jona kod biljaka kukuruza izloženih Pb, Cd i Ni zavisi od odnosa NO_3^-/NH_4^+

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Klijanci kukuruza (*Zea mays* L.) (hibrid ZP 330) gajeni su u hidroponskoj kulturi na standardnom hranljivom rastvoru koji je sadržao 7.5 mM N. Azot je dodavan u dva oblika: 1) kao NO_3^- i 2) kao NO_3^-/NH_4^+ (2:1). Posle 2 dana gajenja, biljke su izložene dejstvu teških metala i svaki od dva tretmana je podeljen u četiri grupe: a) kontrola; b) 0.5 mM Pb; c) 0.5 mM Cd; d) 0.5 mM Ni. Posle 3 dana izlaganja dejstvu teških metala, odredjivani su sledeći parametri u korenovima klijanaca: sadržaj teških metala, dužina korena, težina korena, akumulacija prolina, sadržaj rastvorljivih proteina, koncentracije K, Ca i Mg. Rezultati pokazuju da je skoro svi odredjivani parametri zavise od oblika azota. Akumulacija Pb i Cd bila je niža u NO_3^- tretmanu, dok je akumulacija Ni bila niža u NO_3^-/NH_4^+ tretmanu. Smanjenje dužine korena pokazalo je zavisnost od akumulacije teških metala, dok je smanjenje težine korena zavisilo isključivo od oblika azota. Promena sadržaja rastvorljivih proteina i kalijuma zavisila je i od N oblika i od vrste teškog metala. Sadržaj prolina značajno se povećao u prisustvu svakog teškog metala u NO_3^- tretmanu, dok su ove promene u NO_3^-/NH_4^+ trertmanu bile zanemarljive, osim kod biljaka izloženih niklu. Kadmijum i nikal izazvali su značajno smanjenje sadržaja Ca i Mg koje je u oba slučaja bilo izraženije u NO_3^-/NH_4^+ tretmanu. Olovo je izazvalo smanjenje sadržaja Mg u NO_3^- tretmanu, praćeno povećanjem koncentracije Ca, i smanjenje koncentracije Ca praćeno povećanjem koncentracije Mg u NO_3^-/NH_4^+ tretmanu. Ovi rezultati pokazuju, osim različitih reakcija kukuruza na različite teške metale, širok potencijal modifikacije uticaja teških metala promenom jonske forme azota u ishrani biljaka.

Ključne reči: kadmijum, koren, nikal, olovo, rast, usvajanje jona, *Zea mays*