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MINERALIZATION OF ALANINE ENANTIOMERS IN SOIL TREATED WITH HEAVY METALS AND NUTRIENTS

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Abstract

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This work deals with the determination of the effect of heavy metals and nutrients applied to the soil on alanine enatiomers mineralization with the main focus on evaluating the effect on L/D alanine respiration rate ratio. This study was initiated because previous research works revealed a change in L/D amino acid respiration under acid- or heavy metal-stress in soil. Generally, D-amino acids artificially supplied to soil are less utilized by microorganisms compared with their L-enantiomers. Stress of soil microorganisms cause decreased discrimination of D-amino acids utilization. Also, previous research showed that an application of fertilizers or combinations of fertilizers may affect the mineralization rate of L-amino acids differently, compared with their D-enantiomers. The results of this study show, that the effect of both heavy metals and nutrients on the L/D ratio was not clear, increasing or decreasing this ratio. Further research is necessary to broaden this study.

soil alanine, enantiomers, respiration, heavy metals, nutrients

D-amino acid enantiomers atificially supplied to soil are known to be less utilized by soil microorganisms compared with their L-enantiomers (Martens and Frankenberger, 1993; Hopkins and Ferguson, 1994; Hopkins *et al.*, 1994, 1997; O'Dowd *et al.*, 1997, 1999; O'Dowd and Hopkins, 1998; Landi *et al.*, 2000). This difference may be altered under stress conditions. A decrease in L/D glutamine or glutamic acid respiration due to acid-stress accompanied by increasing qCO_2 (the metabolic quotient, which represents the ratio between the respiration and the microbial biomass) was reported by Hopkins *et al.* (1997). The exact mechanism of this decrease is not yet known. Landi *et al.* (2000) reported a significant decrease in the L/D glutamic acid respiration ratio in forest soil incubated in the presence of highly available cadmium content. Various factors which are directly or indirectly involved in soil respiration may be affected by presence of heavy metals including substrate availability, enzyme inhibition, death of microorganisms, change in physiology and structure of microbial community (Brookes, 1995; Dahlin *et al.*, 1997; Landi *et al.*, 2000; Renella *et al.*, 2003, 2004; Dai *et al.*, 2004; Khan *et al.*, 2010) when the exact mechanism of heavy metals action on amino acid respiration is not known. The effect on amino acids enantiomers mineralization by nutrients applied to the soil has not been studied thoroughly (Hopkins *et al.*, 1997). Generally, fertilizer application may affect soil properties including microbial biomass (Zhong *et al.*, 2010).

The change of L/D amino acid respiration can be used as a method to indicate stress in different soils. Intensive research is necessary to verify the effect of stress factors on L/D amino acid respiration ratio and its usefulness as an indicator to control soils in agriculture etc. An application of fertilizers may affect mineralization rate of L-amino acids differently, compared with their D-enantiomers (Hopkins *et al.*, 1997). More research is necessary to understand this process and, perhaps in future, consider further use of these results in e.g. control of soil fertility.

In this study whe have attempted to determine the effect of selected heavy metals on C-mineralization of alanine enantiomers. This work represents preliminary study where the main aim was to determine if application of different heavy metals or nutrients change L/D alanine respiration ratio. Alanine belongs to amino acids which are characterized by low differences between mineralization of its enantiomers (Hopkins *et al.* 1997).

MATERIAL AND METHODS

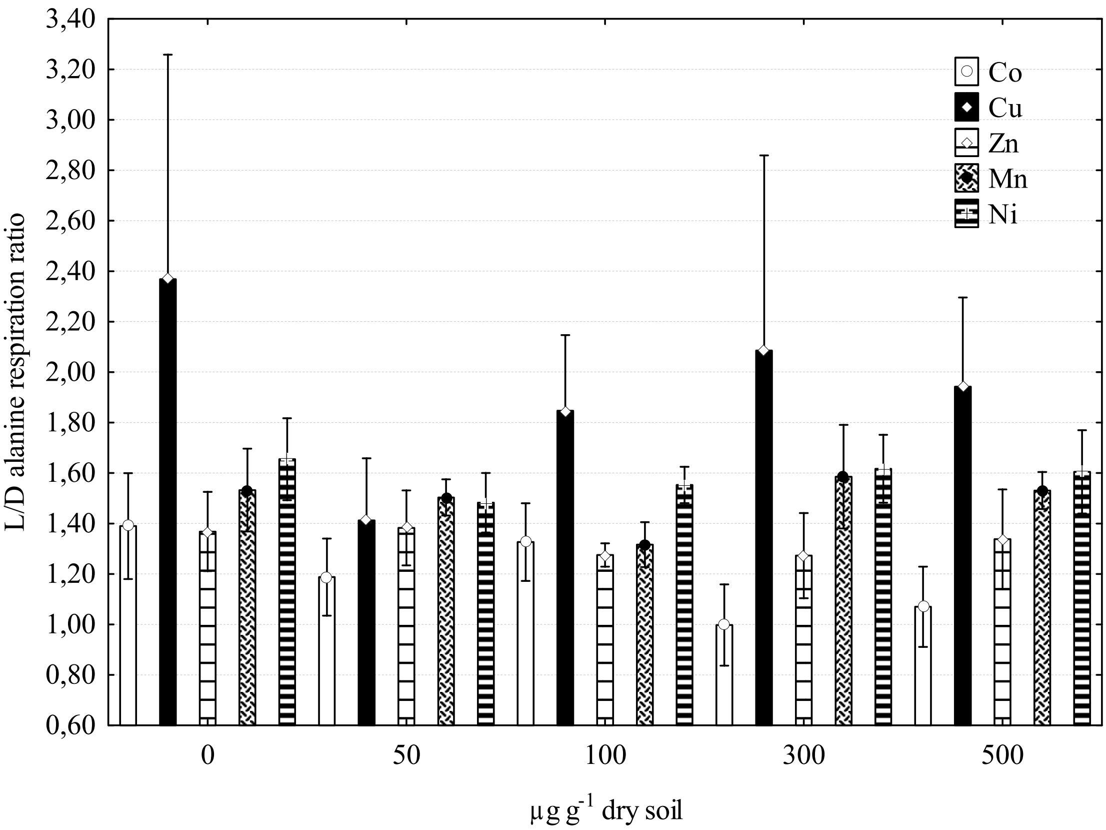
A mixed soil sample (= 10 random sub-samples) of up to10 cm depth for experiments was taken from cultivation of *Miscanthus* x *Giganteus* in Botanic Gardens and Arboretum of Mendel University in Brno, the Czech Republic (N 49°12'54.240", E 16°36'41.989", 235 meters a.s.l). This plant was established there more than >15 years ago on a plot of 291 m^2. After air-drying to reduce moisture to 20% (w/w) and sieving through 2 mm sieve, the soil contained 2 mg L- or D- amino acid enantiomers per g dry soil, heavy metal solution (Co, Cu, Zn, Mn, Ni in form of chlorides or sulphates) or nutrient solution (Ca, K, and NH_4^+-N in form of chlorides, and NO_3^--N in form of $NaNO_3$) to obtain 60% (w/w) moisture. Carbon dioxide which developed in the course of 24 hours was measured at 22 °C by its absorption into 1 ml 1 M NaOH. Heavy metals or nutrients were applied in concentrations 0–500 µg . g^{-1} dry soil, 2 mg amino acid enantiomers per g dry soil was reported to give maximum respiration rate (Hopkins and Fergusson, 1994; O'Dowd *et al.*, 1997, 1999). Each of the analyses were performed five times and soil properties are mentioned in Table I.

I: *Selected properties of soil used for experiments*

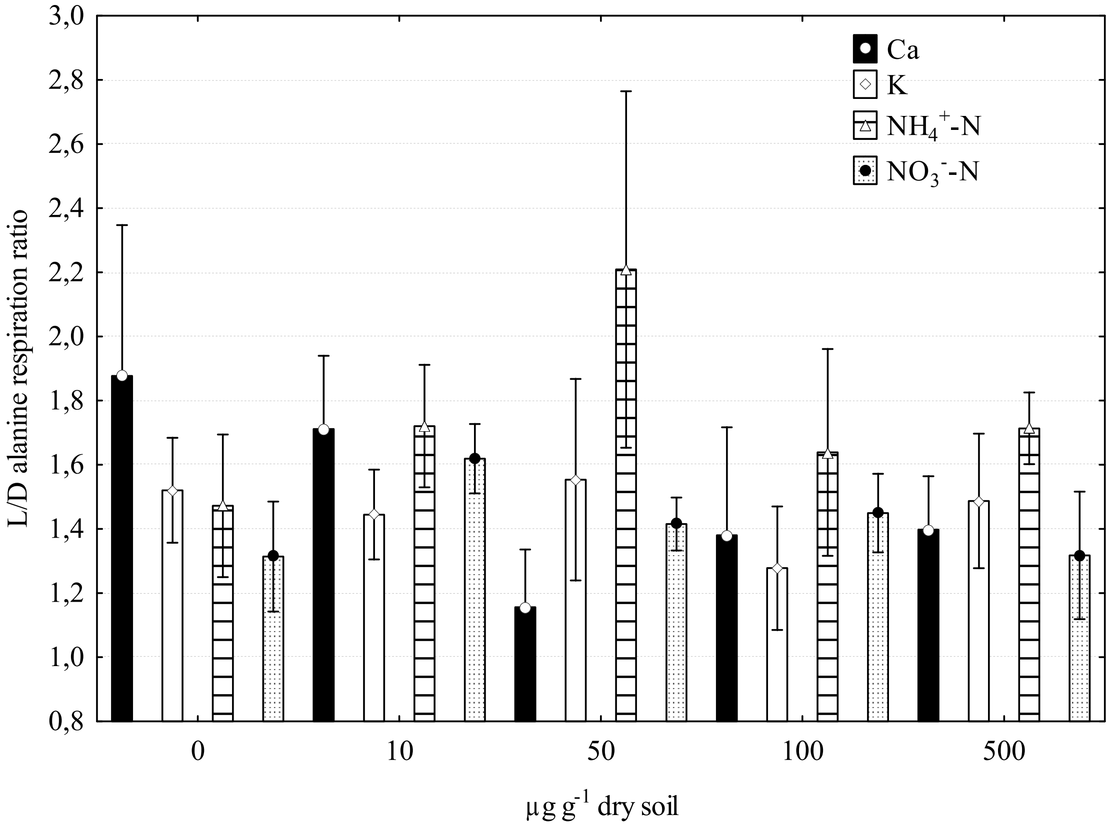
Soil properties	Value
Clay (< 0.002 mm) (%)	28.2
Silt (0.05–0.002 mm) (%)	45.8
Sand (2–0.05 mm) (%)	26.0
pH/H_2O	7.99
pH 0.01 M $CaCl_2$	7.62
C_{ox} (%)	2.98
N_t (%)	0.24
C/N	12.42
Cation exchange capacity effective (mmol chem eq . kg^{-1})	263

RESULTS AND DISCUSSION

C-mineralization rate after application of alanine enatiomers without application of heavy metals or nutrients ranged from 9.60 to 26.5 µg C-CO_2 . g^{-1}. h^{-1}. The L/D alanine respiration in presence of different heavy metals showed no clear trend (see Fig. 1).



1: *L/D ratio of alanine respiration in soil supplied by different heavy metals concentrations (mean ± SE, n = 4–5)*



2: *L/D ratio of alanine respiration in soil supplied by different nutrients concentrations (mean ± SE, n = 5)*

In many cases was L/D ratio of alanine respiration in presence of heavy metals higher compared with the control. Landi *et al.* (2000) reported a significant decrease in the L/D glutamic acid respiration ratio in forest soil incubated in the presence of highly available cadmium content. Highly available cadmium content decreased amino acid enantiomer respiration rate of soil up to the 4th day from the treatment, while a decrease in the L/D glutamic acid respiration ratio was reported during the first 14 days of incubation of soil, when respiration was attributed to the native microbial biomass being measured after 6 hours. Measurement after 24 hours (the start of the microbial growth phase) showed no clear trend. This is consistent with our results when measurements were also performed after 24 hours. Contrary to the work of Landi *et al.* (2000), the change of pH after application of heavy metals to soil was not measured in our work, and thus change in soil microbial community due to this reason can not be predicted. Other processes, including substrate availability, enzyme inhibition, death of microorganisms, change in physiology and structure of microbial community due to occurence of heavy metals and shifts to utilization of different substrates, could be involved (Brookes, 1995; Dahlin *et al.*, 1997; Landi *et al.*, 2000; Renella *et al.*, 2003, 2004; Dai *et al.*, 2004; Khan *et al.*, 2010).

Application of nutrients in different concentrations did not show a clear effect on L/D alanine respiration (Fig. 2). An application of fertilizers and their combinations may effect mineralization rate of L-amino acids differently, compared with their D-enantiomers. For example, an application of farm yard manure increased respiration of L-amino acids or D-alanine and decreased respiration of some D-amino acids; farm yard manure in combination with mineral fertilizers (NPK) or only mineral fertilization using NPK reduced or did not effect respiration of L-amino acids whereas mineralization of D-amino acids was mostly stimulated (Hopkins *et al.*, 1997). Fertilizer application may effect soil properties including microbial biomass (Zhong *et al.*, 2010). As discussed in work of Hopkins *et al.* (1997) farm yard manure may contain a substantial amount of faecal material rich in bacterial D-amino acids and its application may function as a soil conditioner with D-amino acid enantiomers enhancing D-amino acid metabolism (Hopkins and Ferguson, 1994). Further research of this topic is necessary to better understand these processes.

CONCLUSIONS

The application of different heavy metals or nutrients in increasing concentrations to soil did not show any clear effect to change L/D alanine respiration. More research is necessary to test these treatments, using other type of amino acids and soils of different properties. This future research should be aimed to find out if the L/D amino acid respiration may be used as an indicator of soil quality.

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REFERENCES

BROOKES, P. C., 1995: The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and fertility of soils, 19, 4: 269–279. ISSN 0178-2762.

DAHLIN, S., WITTER, E., MÅRTENSSON, A., TURNER, A., BÅÅTH, E., 1997: Where's the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. Soil biology and biochemisty, 29, 9–10: 1405–1415. ISSN 0038-0717.

DAI, J., BECQUER, T., ROUILLER, J. H., REVERSAT, G., BERNHARD-REVERSAT, F., LAVELLE, P., 2004: Influence of heavy metals on C and N mineralization and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. Applied soil ecology, 25, 1: 99–109. ISSN 0929-1393.

HOPKINS, D. W., FERGUSON, K. E., 1994: Substrate induced respiration in soil amended with different amino acid isomers. Applied soil ecology, 1, 1: 75–81. ISSN 0929-1393.

HOPKINS, D. W., ISABELLA, B. L., SCOTT, S. E., 1994: Relationship between microbial biomass and substrate induced respiration in soils amended with D- and L-isomers of amino acids. Soil biology and biochemistry, 26, 12: 1623–1627. ISSN 0038-0717.

HOPKINS, D. W., O'DOWD, R. W., SHIEL, R. S., 1997: Comparison of D- and L-amino acid metabolism in soils with differing microbial biomass and activity. Soil biology and biochemistry, 29, 1: 23–29. ISSN 0038-0717.

KHAN, S., HESHAM, A. E. L., QIAO, M., REHMAN, S., HE, J. Z., 2010: Effects of Cd and Pb on soil microbial communitry structure and activities. Environmental science and pollution research international, 17, 2: 288–296. ISSN 0944-1344.

LANDI, L., RENELLA, G., MORENO, J. L., FALCHINI, L., NANNIPIERI, P., 2000: Influence of cadmium on the metabolic quotient, L-: D-glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. Biology and fertility of soils, 32, 1: 8–16. ISSN 0178-2762.

MARTENS, D. A., FRANKENBERGER JR., W. T., 1993: Metabolism of tryptophan in soil. Soil biology and biochemistry, 25, 12: 1679–1687. ISSN 0038-0717.

O'DOWD, R. W., PARSONS, R., HOPKINS, D. W., 1997: Soil respiration induced by the D- and L-isomers of a range of amino acids. Soil biology and biochemistry, 29, 12: 1665–1671. ISSN 0038-0717.

O'DOWD, R. W., HOPKINS, D. W., 1998: Mineralization of carbon from D- and L-amino acids and D-glucose in two contrasting soils. Soil biology and biochemistry, 30, 14: 2009–2016. ISSN 0038-0717.

O'DOWD, R. W., BARRACLOUGH, D., HOPKINS, D. W., 1999: Nitrogen and carbon mineralization in soil amended with D- and L- leucine. Soil biology and biochemistry 31, 11: 1573–1578. ISSN 0038-0717.

RENELLA, G., ORTIGOZA, A. L. R., LANDI, L., NANNIPIERI, P., 2003: Additive effects of copper and zinc on cadmium toxicity on phosphatase activities and ATP content of soil as estimated by the ecological dose (ED_{50}). Soil biology and biochemistry, 35, 9: 1203–1210. ISSN 0038-0717.

RENELLA, G., LANDI, L., NANNIPIERI, P., 2004: Degradation of low molecular weight organic acids complexed with heavy metals in soil. Geoderma, 122, 2–4: 311–315. ISSN 0016-7061.

ZHONG, W., GU, T., WANG, W., ZHANG, B., LIN, X., HUANG, Q., SHEN, W., 2010: The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant and soil, 326, 1–2: 511–522. ISSN 0032-079X.

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