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NICKEL PHYTOREMEDIATORY POTENTIAL AND MECHANISMS OF ITS DETOXIFICATION IN PLANTS USING FOR DECONTAMINATION OF SOILS

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Plants have developed various mechanisms that allow them to tolerate soils that are polluted with heavy metals (Ni, Cd, Pb et al). One of their main protective mechanisms is excessive HM chelation by different organic compounds. The aim of this work was the analysis of a possibility to apply polyamines (putrescine and et al) as possible ligands for Ni detoxification and for Ni increasing content in plant biomass applying for phytoremediation of contaminated soils. Again, it was established by us that Ni can induce endogenous accumulation of free polyamines in rape plant (*Brassica napus)* by 4.5 times as compared with control.

Key words: heavy metals, soil, polyamines, chelates, phytoremediation.

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ФІТОРЕМЕДІАЦІЙНИЙ ПОТЕНЦІАЛ НІКЕЛЮ ТА МЕХАНІЗМИ ЙОГО ДЕТОКСИКАЦІЇ У РОСЛИНАХ, ЯКІ ВИКОРИСТОВУЮТЬСЯ ДЛЯ ОЧИЩЕННЯ ҐРУНТІВ

Забруднення ґрунтів важкими металами (ВМ) представляє велику небезпеку як для культивованих рослин, так і для людини із-за їх високої токсичності. Проблема розробки технології очистки забруднених ґрунтів від ВМ за допомогою рослин-зверхакумуляторів ВМ (Ni, Cd, Pb та ін.), здатних виносити їх з ґрунту з біомасою набуває все більшого значення. Пошук таких рослин ґрунтується на їх здатності акумулювати ВМ, розвиваючи різноманітні механізми знешкодження їх в клітинах. Одним з головних захисних механізмів являється виникнення координаційних зв’язків різних органічних речовин з ВМ, так зване хелатування. В цій роботі проведено аналіз застосування знешкодження нікелю поліамінів (путресцину та інших), які здатні створювати з ним хелати та тим самим знижувати його токсичність й підвищувати акумуляцію Ni в надземній масі. Цей прийом дозволить такі рослини як *Brassica napus* L., *Amaranthus cruentus* L., *Calamagrostis epigeios* L. застосовувати для очистки ґрунтів від Ni за допомогою фіторемедіації.

Ключові слова: важкі метали, ґрунт, поліаміни, хелати, фіторемедіація.

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ФИТОРЕМЕДИАЦИОННЫЙ ПОТЕНЦИАЛ НИКЕЛЯ И МЕХАНИЗМЫ ЕГО ДЕТОКСИФИКАЦИИ В РАСТЕНИЯХ, ИСПОЛЬЗУЕМЫХ ДЛЯ ОЧИСТКИ ПОЧВ

Загрязнение почв тяжелыми металлами (ТМ) представляет большую опасность как для культивируемых растений, так и человека из-за их высокой токсичности. Проблема разработки технологии очистки загрязненных почв от ТМ с помощью растений-сверхаккумуляторов ТМ (Ni, Cd, Pb и др.), способных выносить их из почвы с биомассой приобретает все большее значение. Поиск таких растений основывается на их способности аккумулировать ТМ, развивая различные механизмы обезвреживания их в клетках. Одним из главных защитных механизмов является образование координационных связей различных органических веществ с ТМ, так называемое хелатирование. В настоящей работе проведен анализ применения для обезвреживания никеля полиаминов (путресцина и других), которые способны образовывать с ним хелаты и тем самым снижать его токсичность и повышать аккумуляцию Ni в надземной массе. Этот прием позволит такие растения как *Brassica napus* L., *Amaranthus cruentus* L., *Calamagrostis epigeios* L. применять для очистки почв от Ni с помощью технологии фиторемедиации.

Ключевые слова: тяжелые металлы, почва, полиамины, хелаты, фиторемедиация.

At present, biosphere pollution with heavy metals (HM), nickel in particular, becomes increasingly actual problem. HM are accumulated in air, soil and water rather rapidly and removed from it extremely slowly. Half-life of Pb, Cd and Cu in soil are more than thousand years. HM are one of the most dangerous pollutants for human and for most crops (fig. 1).

on most crops

- **Inhibition of photosynthesis**
- **Disturbance of mineral nutrition**
- **Suppress of growth**
- **Disturbance of water and hormonal status**

on human

- **Cancerogenic and mutagenicity action**
- **Disturbance of metabolism and nutrition**
- **Cytotoxic effect**
- **Action on nervous system**

Fig. 1. **Harmful effect of heavy metals**

However, many species of terrestrial plants could inhabit soil ecotops enriched in HM and accumulate toxic metals in their aboveground organs in high concentration without any signs of damage (Raskin, Ensley 2000). Most of them are Ni hyperaccumulators (from 1.0 to 30 g Ni/kg shoot dry wt). Potencial use of wild-type plants for extracting contaminant metals from polluted soils, as it is called, phytoremediation, has received much attention in recent years (fig.2). Countries in which technologies of phytoremediation are realized: USA, Bulgaria, Spain, Great Britain, New Zealand, China et al.

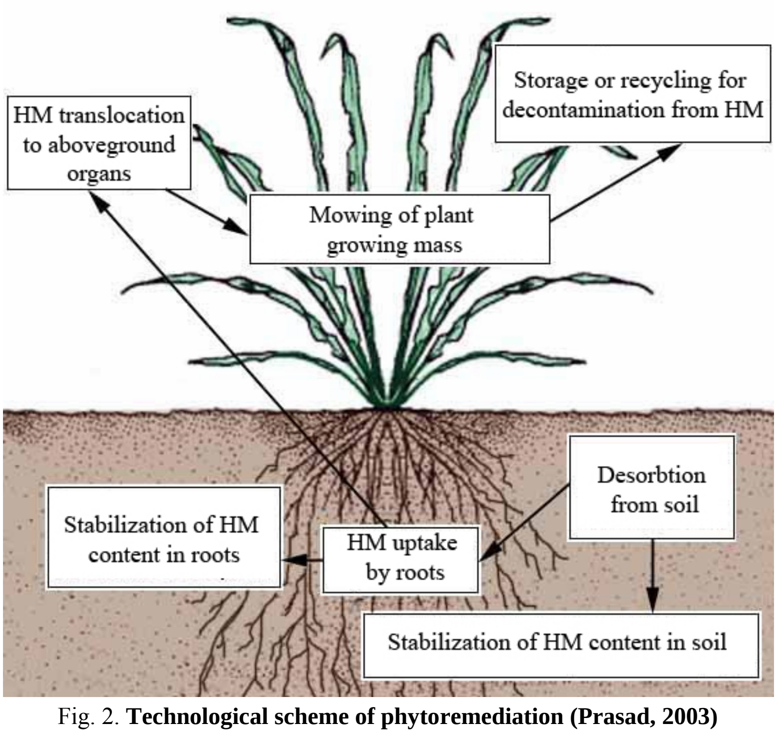


Fig. 2. **Technological scheme of phytoremediation (Prasad, 2003)**

However, the main obstacle for application of natural HM-hyperaccumulators for phytoremediation is their relatively small biomass and low growth rate. Therefore, for efficient phytoremediation it is necessary to select plant species, which are not only capable of HM accumulation but also produce large biomass and have developed various mechanisms of adaptation to HM.

Screening of Ni-accumulating species could be also performed among ruderal plants inhabiting waste buring soils in megapolis. As an example, perennial grass *Calamogrostis epigeios* (L) Roth is capable of Ni accumulation in the aboveground (more than seven hundred mg/kg dry wt) (Madzhugina, Shevyakova, Kuznetsov, 2008) (fig.3).

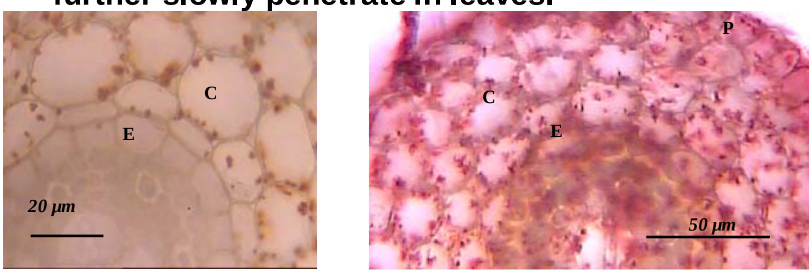
This plant is a typical flora representative in the central climatic zone characterized by a high tolerance to soil pollution. On the polygons for Megapolis Waste *Calamogrostis epigeios* plants produced the monopopulation of high productivity of green mass.

Fig. 3. **Perennial grass *Calamogrostis epigeios* L (wood small reed-grass)**

The main task of our investigations was to characterize plants as *C. epigeios* tolerance to HM on basis of cytological and physiological parameters: hystochemical analysis of Cd, Pb (ditizon) and Ni (dimethylglyoxime) distribution in tissues after seed germination, potencial of seed germination in the presence of HM and so on.

Like other plant species reed-grass (*C. epigeios)* accumulated various metals (Pb and Cd) and especially large amounts in roots (fig. 4).

By means of specific for each metal hystochemical test (Ivanov, Seregin, 2003) was indicated that Cd and Pb were accumulated in the main of cortex and endoderms. The hypothesis provides an explanation for slowly penetration of these metals in conductive vessels and may be further slowly penetrate in leaves.



In presence of 200 µM $Pb(NO_3)_2$ In presence of 200 µM $CdCl_2$

Fig. 4. **Cd and Pb distribution in tissues of *C. epigeios***

However, distribution of Ni in root tissues of this plant indicates that in distinct of Cd and Pb, root endodermis is not a barrier limiting Ni transport into stele and Ni rapidly penetrated in aboveground organs of reed-grass (fig.5).

(C – cortex; P – pericycle; MX – metaxylem; CV – conductive vessel; E – endodermis; Rh - rhizodermis)

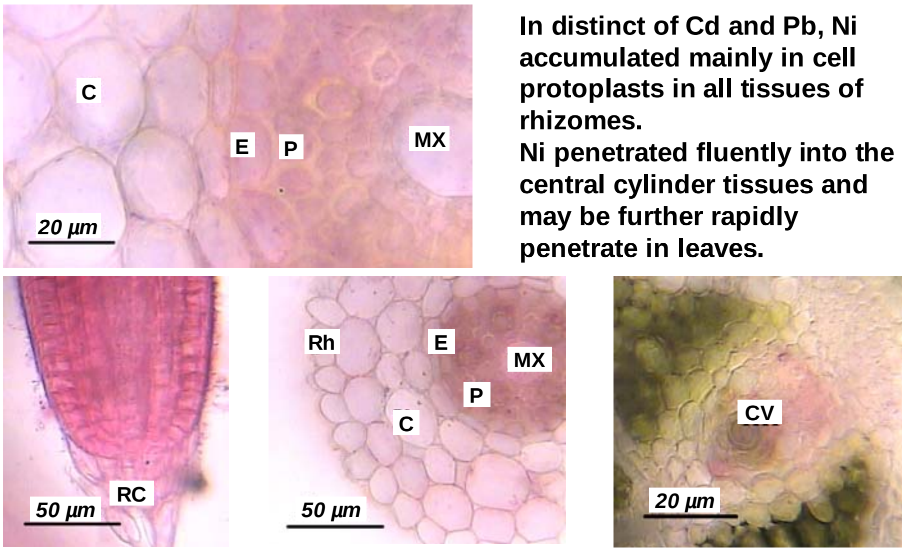


Fig. 5. **Ni distribution in tissues of *C. epigeios***

The assessment of HM action on seed germination of this plant demonstrated their relatively more higher tolerance to Zn and Ni. Thus, seed germinability was not affected by 1 mM $ZnSO_4$, or Ni $(NO_3)_2$ whereas 1 mM $CuSO_4$ and 1 mM Pb $(NO_3)_2$ were more toxic (fig. 6).

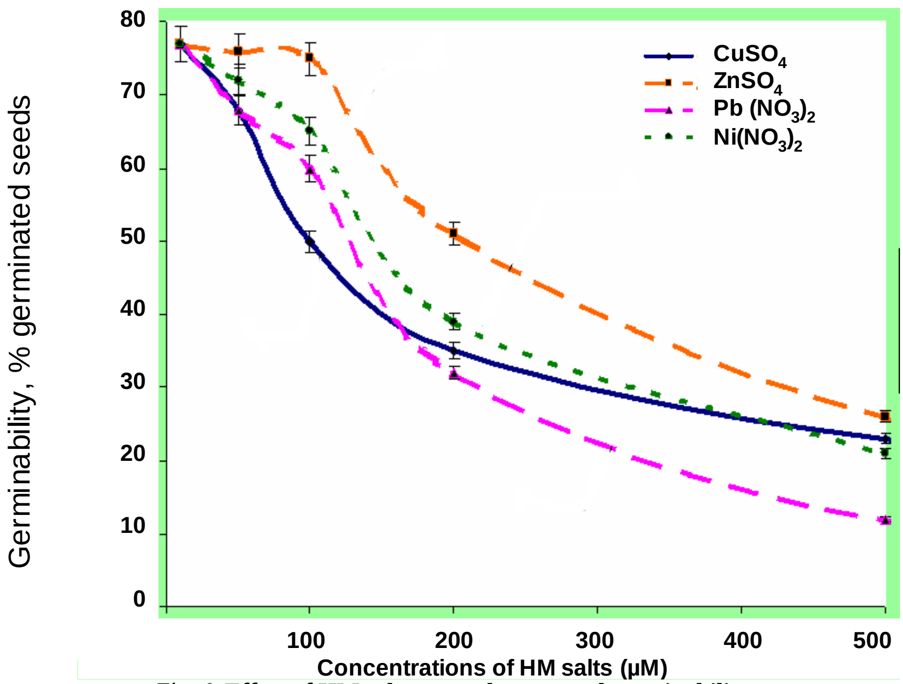


Fig. 6. **Effect of HM salts on reed-grass seed germinability**

On the important characteristics of this plant as a possible phytoremediant could be its capability to accumulate HM in green mass. To assess a possibility for this plant usage for recultivation of polluted soils, it was important to evaluate plant tolerance to mowing and to determine the level of metals removal with mowed plant biomass in plant grown in soil culture in the presence of HM (fig. 7).

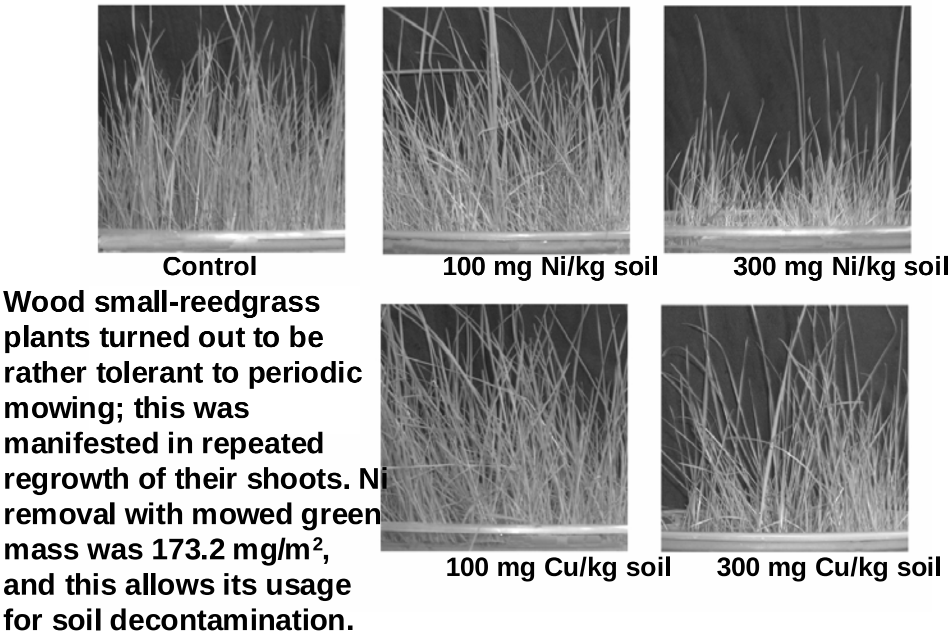


Fig. 7. **Shoot regrowth after mowing of reed-grass**

These data indicate that, at a short-term action of the high Ni concentration in nutrient medium, wood small reed-grass plants turned out to be rather tolerant to periodic mowing. Ni removal with mowed green mass was 173.2 mg/m^2 and this allows its usage for soil decontamination.

Potentials of *C. epigeos* plants for polygon recultivation for megapolis waste in Moscow oblast (Russia) are demonstrated in figue 8.

Fig. 8. **Green mass of plants *C. epigeios* periodic mowing on polygons of waste buring soils would result in soil cleaning of Ni for 5-10 years**

Thus, in this work, we performed a search of Ni accumulator among wild ruderal plants inhabiting polygons. However, to apply this plant species as phytoremediant of agricultural lands, it is necessary to produce on its basis new cultivars with a higher efficiency of HM removal and the increased rate of shoot productions.

The main task of further investigations was to characterize

To assess a possibility usage cultivated plants for recultivation of polluted soils, it was important to evaluate not only the level of metals removal with mowed plant biomass but also plant tolerance to HM. Therefore, in the next work we plans for new lines of approaches to phytoremediatory methods.

It is evident from the foregoing equation (Raskin, Ensley 2000) many species of terrestrial plants could inhabit soil ecotops enriched in heavy metals and accumulate these toxic metals in their aboveground organs in high concentration without any signs of damage. Most of them are wild Ni hyperaccumulators and belong mainly to the Brassicaceae family but have relatively small biomass and low growth rate. There are in more than 400 such plant species in the genus *Alyssum*.

For the goal of such plants selection among cultivated crops we have chosen rape plants (*Brassica napus*) (fig. 9). In this connection, we planned to establish upper limit of rape tolerance to Ni during plant vegetative growth and to reveal physiological targets of nickel toxicity. Rape (canola) is not HM hyperaccumulator. However, it can be suitable species for sol decontamination fron Ni due to its genetics (Brassicaceae family) and large biomass (Prasad 2003). Rape is annual oil plant of hybrid origin resulting from spontaneous cross-pollination between cabbage (Brassica oleracea) and field mustard (Brassica campestris), i.e. it is a closest turnip relative. Rape is cultivated in varios countries; it is characterized by a short growth period, great biomass production, and easy acclimation. Yield potential for spring cultivar: 2000 to 2800 $kg.ha^{-1}$, for winter canola – 4400 to 5600 $kg.ha^{-1}$ for central Washington (Hang et al. 2008). Rape is a promising target for agrobacterial transformation aimed the increase in its phytoremediatory potential. It is also widely applied as a biofue (Hang et al. 2008).

Fig. 9. **Rape plants (*Brassica napu*s L.)**

Ni accumulation by different leaves of adult rape plants shown in figue 10 A. At 125 μM $NiCl_2$ in medium cotyledonary leaves and young leaves accumulated 150 and 325 mg Ni/kg dry wt, respectively. At 250 μM $NiCl_2$ the content of Ni in young leaves almost doubled, whereas at 500 μM $NiCl_2$, it increased to 1000 mg/kg dry wt. However, in young leaves content of Fe (B) and Cu (C) – essential elements, were reduced. In result of it, chlorosis in leaves was manifested.

Thus, contrasting NI-induced changes in the content of Fe in the leaves of different age could indicate that Ni prevents Fe and Cu uptake by rape plants. This clearly demonstrates the disruption of this plant nutrition with essential metals. Moreover, Ni accumulation in aboveground organs induced oxidative stress and increase the intensity of lipid peroxidation (content of malondialdehyde –indicator of membrane disturbance) (D).

These features do not allow this plants to extract sufficiently high amounts of Ni from contaminated soils. Various biotechnological strategies were developed to overcome these difficulties. One of such technology is application of various plant protective metabolites and low-molecular ligands for HM, enhancing their absorption and long-distance transport over the plant.

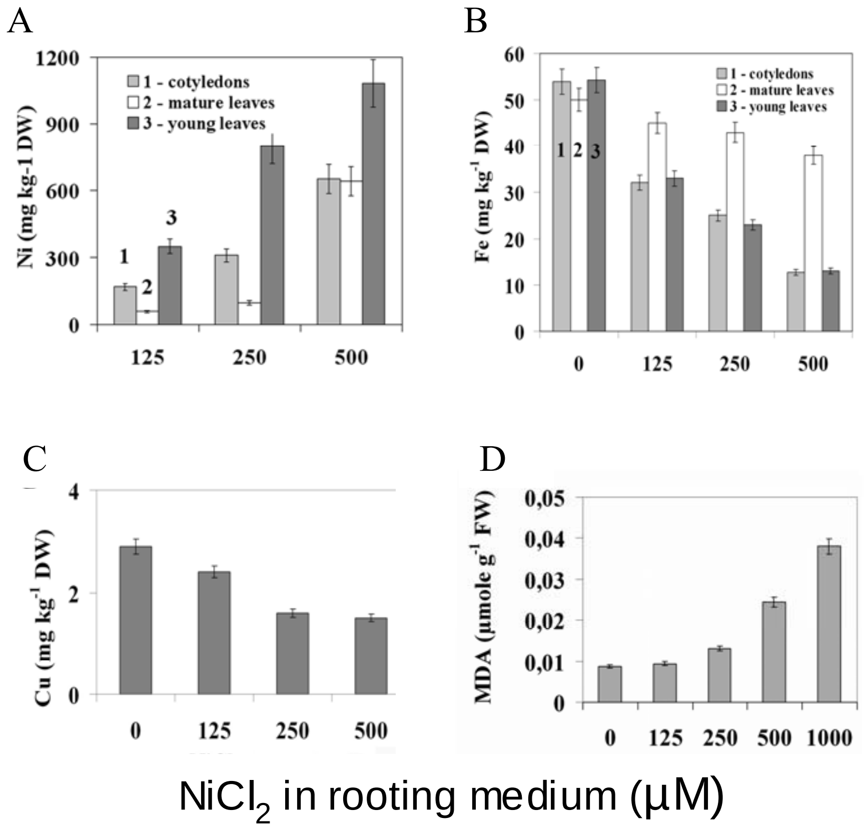
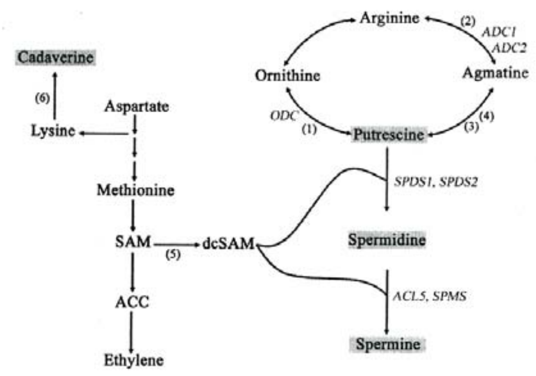


Fig. 10. **Effect of $NiCl_2$ on accumulation Ni, Fe, Cu and MDA**

Various natural low molecular chelators (citrate, oxalate, malate, histidine, and others) and high molecular chelators (phytochelatines, thioneines and so on) can form HM-chelate complex which detoxifies metal and accelerate its movement along root xylem and further to shoots (Shevyakova et al. 2010).

One of chelators, forming Ni—PAs, as showed by Koutensky et al (1995) and others, could be polyamines (PAs). In several studies, a Ni-induced accumulation of universal organic polycations polyamines was observed (Hauschild 1993; Bergmann et al. 2001). Stress-induced polyamines accumulation in plants is known to fulfill a multifunctional protective role against damaging action of various abiotic factors, including HM pollution (Kuznetsov and Shevyakova 2007; 2010).

As known in plants putrescine, spermidine and spermine are most abound polyamines (fig. 11). But the PA metabolic pathways are regulated by a limited number of key enzymes and genes. The main precursor of polyamines are aminoacids of ornitine cicle and S-adenosylmethionine (SAM).



(1) - ODC, ornitine dacarboxylase,

(2)- ADC, arginine decarboxylase,

(3)- agmatine iminohydrolase,

(4)- N-carbomoylputrescine amidohydrolase,

(5)- SAMDC, SAM decarboxylase,

(6)- SPDS, spermidine syntase,

(7)- SPMS, spermine syntase,

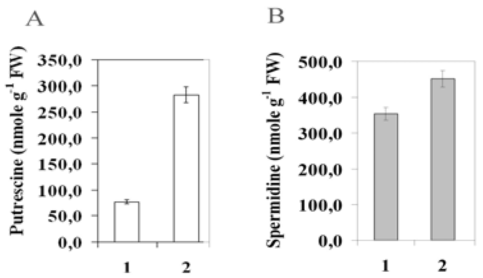
(8)- LDS, lysine decarboxylase, ACC, 1-aminocyclopropane-1-carboxylic acid; dcSAM, decarboxylated S-adenosyl-methionine.

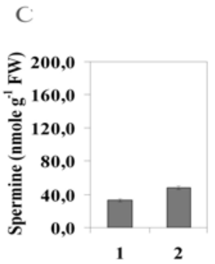
The genes involved are ADC1, ADC2, ODC, SPDS1, SPDS2, ACL5, SPMS

Fig. 11. **Pathway of biosynthesis of main plant polyamines (putrescine, Spermidine, Spermine and Cadaverine)**

On the basis of these data, we proposed that polyamines accumulation in plants might improve plant tolerance to Ni due to chelate formation.

The next aim of this work was to study a possibility of polyamines accumulation in rape plants (*Brassica napus* L.) under Ni pollution in soil. We showed that exogenous Ni stimulates biothynthes of endogenous polyamines of putrescine family in rape plants (Fig. 12).





1 – control, 2 – 250 μM $NiCl_2$,

A – Putrescine, B – Spermidine, C – Spermine.

Fig. 12. **Effect of $NiCl_2$ on endogenous content of free polyamines in young leaves of 5-day-old rape plants**

On the basis of these data, we propose rape plant can be as potential Ni accumulator for soil decontamination. However, it is necessary to increase plant capability of metal accumulation, aboveground organ biomass and plant tolerance to Ni toxicity. Therefore we attempted to improve the aforementioned parameters by increasing polyamines content in leaves (fig. 13). Earlier we have shown, that treatment of adult plants with putrescine increased biomass of roots and shoots, the content of chlorophyll in leaves. Taking it into account, we expected that exogenous putrescine would activate plant growth and improve tolerance to nickel-induced stress, and what is especially important, activated Ni accumulation in leaves. We assumed that exogenous putrescine penetrates from leaf surface via phloem into leaf parenchyma and then into xylem, where it could a complex with nickel causing Ni detoxification and increasing its transport into aboveground biomass. The obtained data demonstrated that treatment of exogenous putrescine increased the amount of Ni in leaves to the level attained by natural Ni-accumulators (~ 3000 mg/kg dry wt).

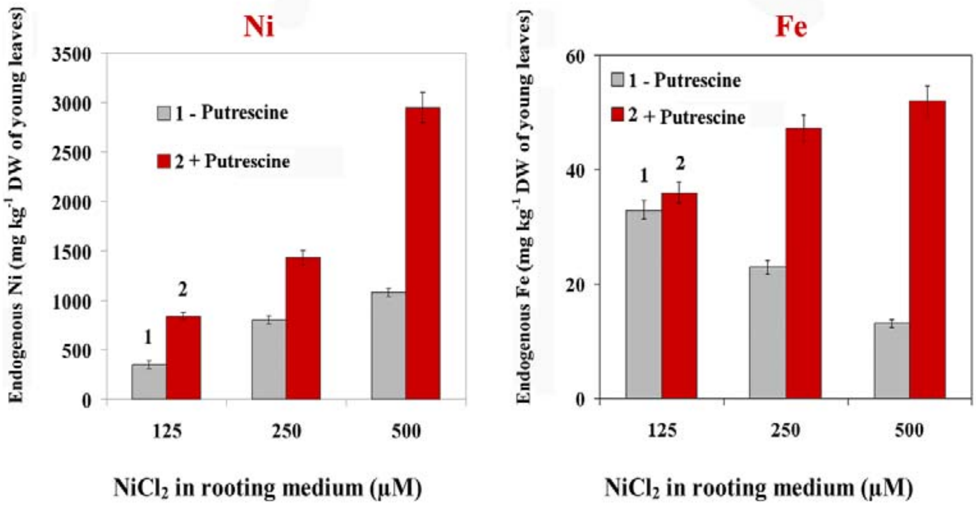


Fig. 13. **Effect of leaf treatment with exogenouse putrescine on Ni and Fe**

In an analogous way leaf spraying with exogenous putrescine of another cultivated plant (*Amaranthus cruenthus*) demonstrated the increase of Ni accumulation and its transport into aboveground biomass. It was shown in figure 14.

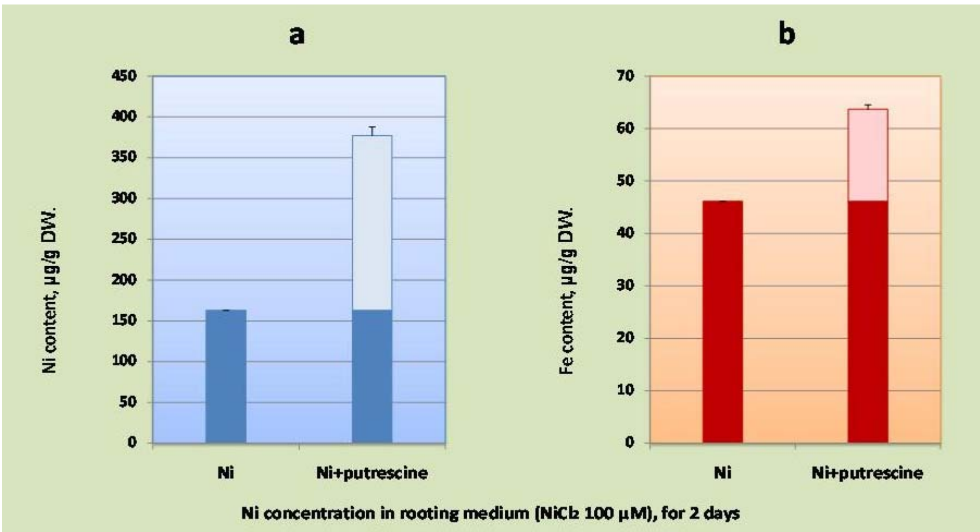


Fig. 14. **Effect of leaf treatment with exogenouse putrescine on Ni and Fe accumulation by young leaves of *Amaranthus cruentus* L. plants**

Such effects opens a possibility for creation of transgenic plants with enhanced production of endogenouse polyamines, which would be of interest for phytoremediation. This could be one of the efficient strategies for accelerated hyperaccumulator-mediated soil decontamination from HM.

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