ISSN 1821-1046
UDK 630

INSTITUTE OF FORESTRY BELGRADE | **INSTITUT ZA ŠUMARSTVO BEOGRAD**

SUSTAINABLE FORESTRY ODRŽIVO ŠUMARSTVO

COLLECTION
TOM 63-64

ZBORNIK RADOVA
TOM 63-64

BELGRADE BEOGRAD
2011.

ISSN 1821-1046

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INSTITUTE OF FORESTRY BELGRADE

PROCEEDINGS

Publisher
Institute of Forestry
Belgrade, Serbia

For Publisher
Ljubinko Rakonjac, Ph.D.

Chief Editor
Snežana Rajković, Ph.D.

Technical Editor and Layout
Tatjana Ćirković-Mitrović, M.Sc.

Secretary
Tatjana Ćirković-Mitrović, M.Sc.

Printed in
150 copies

Printed by
Klik tim DOO
Beograd

INSTITUT ZA ŠUMARSTVO BEOGRAD

ZBORNIK RADOVA

Izdavač
Institut za šumarstvo
Beograd, Srbija

Za izdavača
Dr Ljubinko Rakonjac

Glavni i odgovorni urednik
Dr Snežana Rajković

Tehnički urednik i prelom teksta
Mr Tatjana Ćirković-Mitrović

Sekretar Zbornika
Mr Tatjana Ćirković-Mitrović

Tiraž
150 primeraka

Štampa
Klik tim DOO
Beograd

Cover Page: Author of the Photos Tatjana Ćirković-Mitrović, M.Sc.
Naslovna strana: Autor fotografije mr Tatjana Ćirković-Mitrović

CIP – Каталогизација у публикацији
Народна библиотека Србије, Београд

630

SUSTAINABLE Forestry : collection = Održivo šumarstvo = zbornik radova / chief editor = glavni i odgovorni urednik Snežana Rajković. - 2008, T. 57/58- . - Belgrade (Kneza Višeslava 3) : Institute of forestry, 2008- (Beograd : Klik tim). - 24 cm

Godišnje. - Je nastavak: Zbornik radova - Institut za šumarstvo = ISSN 0354-1894
ISSN 1821-1046 = Sustainable Forestry
COBISS.SR-ID 157148172

SADRŽAJ CONTENTS
TOM 63-64

INSTITUTE OF FORESTRY • BELGRADE
INSTITUT ZA ŠUMARSTVO • BEOGRAD

SUSTAINABLE FORESTRY **ODRŽIVO ŠUMARSTVO**
COLLECTION 63-64, 2011 ZBORNIK RADOVA 63-64, 2011

UDK 502.51:504.5]+502.174:582.548.25 Canna indica=111
Original scientific paper

INDIAN SHOOT (*CANNA INDICA* L.) IN PHYTOREMEDIATION OF WATER CONTAMINATED WITH HEAVY METALS

Nevena CULE[1], Ljubinko JOVANOVIC[2], Dragana DRAZIC[1], Milorad VESELINOVIC[1], Suzana MITROVIC[1], Marija NESIC[3]

Abstract: *This paper presents the results of experiments with the plant indian shoot (Canna indica L.), which were conducted in order to obtain the exact indicators of the plant potential for the removal of heavy metals (lead) from the aquatic environment and biomass production. Heavy metals such as Cd, Hg, Pb, As, Tl and U, which can be detected in industrial and other wastewaters have no biological value for living organisms, but are extremely toxic even in relatively low concentrations. Alternative methods that use plants to remove pollutants from contaminated water, soil and air, can be named as phytoremediation. This term refers to the diverse complex of technologies based on the use of natural or genetically created plants for the purpose of removal of pollutants from the environment or their transformation into nontoxic forms. Indian shoot is just one of the plants that have been used recently in constructed aquatic ecosystems. This plant has experimentally been proved to be very tolerant to the absence of nutrients, and able to produce large amounts of biomass. In the water it develops a very thick strong fibrous root system with a large area for the adoption of heavy metals. It is highly tolerant to the presence of lead and is able to absorb and store it in the root and rhizome due to low translocation to aboveground parts.*

Key words: *Canna indica* L., phytoremediation, heavy metals, aquatic environment, biomass

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Translation: Dragana Ilić

KANA (*CANNA INDICA* L.) U FITOREMEDIJACIJI VODA ZAGAĐENIH TEŠKIM METALIMA

Izvod: *U radu su predstavljeni rezultat eksperimenata sa biljkom kana (Canna indica L.), koji su postavljeni u cilju dobijanja egzaktnih pokazatelja o potencijalu ove biljke za uklanjanje teških metala (olova) iz akvatičnih sredina i produkciji biomase. Teški metali, kao što su Cd, Hg, Pb, As, Tl i U i koji mogu da se detektuju u industrijskim i drugi otpadnim vodama nemaju biološku vrednost za žive organizme, već su izuzetno toksični i u relativno malim koncentracijama. Alternativne metode, koje koriste biljke za uklanjanje polutanata iz kontaminiranih voda, zemljišta i vazduha bi jednim imenom mogle da se nazovu fitoremedijacija. Ovaj pojam se odnosi na raznovrsan kompleks tehnologija, koje se baziraju na upotrebi biljaka, prirodnih ili genetski stvorenih, radi uklanjanje polutanata iz životne sredine ili radi njihovog pretvaranja u netoksične oblike. Kana je samo jedna od biljaka, koja se u poslednje vreme sve više koristi u konstruisanim akvatičnim ekosistemima. Ona se u eksperimentima pokazala kao biljka vrlo tolerantna na odsustvo hranljivih materija i biljka koja može da stvara veliku količinu biomase. U vodi razvija izuzetno gust, jak i žiličast korenov sistem sa velikom površinom za usvajanje teških metala.Vrlo je tolerantna na prisustvo olova, koje lako usvaja i koncentriše u korenu i rizomu, jer je translokacija u nadzemne delove slaba.*

Ključne reči: *Canna indica* L., fitoremedijacija, teški metali, akvatična sredina, biomasa

1. INTRODUCTION

The soil and water contaminated with heavy metals present a major environmental problem, which has an extremely negative impact on the environment and people, and is still in need of an efficient and cost-effective technological solution. The basic idea that plants can be used for environmental remediation is certainly very old and there is no information regarding its first-time use to remove various pollutants from contaminated media. However, a series of scientific discoveries, combined with interdisciplinary and multidisciplinary research supported the development of this idea into a promising environmental protection technology called phytoremediation. Phytoremediation is defined as the use of plants for the removal or immobilization of contaminants from the environment (Cunningham and Ow, 1996).

Phytoremediation of metals is a cost-effective green technology based on the use of specially selected plants that can accumulate and remove heavy metals, including radionuclides from soil and water. This technology takes advantage of the fact that a living plant can be compared to a solar-powered pump that can extract certain elements from the environment and concentrate them in its tissues. This method is becoming possible thanks to the successful basic and applied research.

Metals that can be removed through various processes of phytoremediation include lead, cadmium, chromium, nickel, arsenic and various radionuclides. Removed plants, rich in accumulated pollutants, can easily and safely be processed by drying, burning or composting. Some of the metals can be re-extracted from the ashes, which further reduces the generation of hazardous waste and accelerates the creation of profit.

A particularly significant method in the phytoremediation of water is rhizofiltration. This method is based on the use of plant roots for the absorption and adsorption of pollutants, mainly metals, from water. A particular type of *ex situ* rhizofiltration facility is a constructed aquatic ecosystem.

Wastewater treatment using these alternative systems is a process that is far cheaper than conventional wastewater treatment. With minimal maintenance and minor consumption of labor as well as no expenditure of electrical energy, these systems provide second category water - technical water, which can be used as drinking water after a minimal additional treatment. Through further development, this technology could become the future leading technology in wastewater treatment and the recovery of water bodies.

To date, over 400 plant species that can hyperaccumulate metals have been identified. Families with the largest number of such representatives are: *Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae* and *Euphorbiaceae* (Prasad and Freitas, 2003).

Most of these plants can successfully be used in the local climatic conditions of Serbia. The most significant species which stand out are: reed (*Phragmites communis* Trin.) bulrush (*Schoenoplectus lacustris* (L.) Palla), broadleaf cattail (*Typha latifolia* L.), yellow flag (*Iris pseudoacorus* L.), soft rush (*Juncus effuses* L.), duckweed (*Lemna minor* L.), water mint (*Mentha aquatica* L.) and water plantain (*Alisma plantago - aquatica* L.).

According to literature data, wastewater treatment also involves the use of other plants, including: *Butomus umbellatus* L., *Carex hirta* L., *Menyanthes trifoliata* L., *Bidens tripartita* L., *Carex rostrata* Stokes, *Myosotis scorpioides* L., *Caltha palustris* L., *Deschampsia cespitosa* (L.) P.Beauv., *Nasturtium officinale* L., *Canna indica* L., *Eupatorium cannabinum* L., *Phalaris arundinacea* L., *Carex vulpinoidea* Michx., *Euphorbia palustris* L., *Persicaria hydropiper* (L.) Delabre, *Carex vesucaria* L., *Filipendula ulmaria* (L.) Maxim., *Polygonum bistorta* L., *Carex pseudocyperus* L., *Gladiolus palustris* Gaudin., *Rumex hydrolapathum* Huds., *Carex pendula* Huds., *Gratiola officinalis* L., *Sagittaria sagittifolia* L., *Carex acutiformis* Ehrh., *Humulus lupulus* L., *Scirpus palustris* L., *Carex elata* All., *Lychnis flos-cuculi* L., *Solanum dulcamara* L., *Carex gracilis* R.Br., *Lysimachia nummularia* L., *Symphytum officinale* L., *Carex disticha* Huds., *Lysimachia vulgaris* L., *Valeriana officinalis* L., *Carex riparia* Curtis, *Lythrum salicaria* L., *Veronica beccabunga* L., and others (Gawronski and Gawronska, 2007, Matagi *et al.*, 1998, Kamal *et al*., 2004, Prasad and Freitas, 2003, Kumar *et al*., 1995).

In addition to the above plants, various edible plants, agricultural and vegetable crops and ornamental and woody plants are used in phytoremediation. Various biofilters are also in use (Gawronski and Gawronska, 2007).

Indian shoot (*Canna indica* L.) is one of the plants, which has several important characteristics suitable for phytoremediation.

1.1 Indian shoot (Canna indica L.)

Canna species constitute important floral material of all urban areas. They are most often planted over large areas, such as for example, squares, areas along roads, parks and parterres of representative green spaces, thereby increasing their aesthetic effect. In addition to their exceptional decorativeness, they are important for phytoremediation because they successfully remove heavy metals and other pollutants from soil and water. Cannas are known for their leaves, on whose large surface areas various pollutants from the air (dioxins, polycyclic aromatic hydrocarbons and polychlorinated biphenyls) are deposited and thus removed from the atmosphere (Gawronski and Gawronska, 2007). The plants of this family are particularly interesting because of the high biomass that they develop in different soils and especially in the aquatic environment.

Indian shoot (*Canna indica* L.) is a perennial tropical and subtropical plant growing to a height of 0.5 m to 2.5 m, with an underground stem (rhizome) (Maas-van de Kamer and Maas, 2008). The natural range of this plant are South America and India, but it is widespread in almost all cities of the world, where it is used as a decorative floral species in different categories of green areas and their various compositions.

Table 1. *Systematics of the Canna species (Canna indica L.)*

Regnum	*Plantae*
Clade	*Angiospermae*
Clade	*Monocotyledoneae*
Clade	*Commelinidae*
Ordo	*Zingiberales*
Familia	*Cannaceae*
Genus	*Canna sp.*
Species	*Canna indica* L. - indian shoot

It has a very wide application. It is used in medicine, and its starchy rhizome is used in nutrition. Paper is made from the fibers of its leaves and stems. The seed is used in jewelry making, and it used to be used instead of rifle bullets. Purple color is obtained from the seeds. Young seeds and young shoots can be used in nutrition (Maas-van de Kamer and Maas, 2008). The fibers obtained from stems are used as a substitute for jute. This plant has a large biomass production and in some countries is used for thermal energy production i.e. as biofuel (State Master, 2010).

In recent years, indian shoot is increasingly grown in constructed aquatic ecosystems, in order to improve the quality of lakes and rivers and remove various pollutants from wastewater (Zhang *et al*., 2008). An example of such usage are floating islands made of indian shoot, which are ever more widely used for the refining of eutrophic water, mainly because of their low cost and easy construction.

Fig. 1. *Floating islands with indian shoot (Canna indica L.)*

Such floating islands can remove nitrogen from polluted water to a large extent, especially if the medium is supplemented with denitrifying bacteria and if aeration is introduced into the system (Sun *et al.*, 2009). Five days after the onset of the experiment the total removal of nitrogen (N) in the combined islands is 72.1%, oxidation of ammonia nitrogen (NH4 +-N) 100%, oxidation of nitrate nitrogen (NO3 - N) 75.8%, oxidation of nitrite nitrogen (NO2 - N) 95.9% and chemical oxygen consumption is reduced by 94.6% (*Sun et al.*, 2009).

In their experiment Bose *et al.* (2008) proved that *Canna indica* L. absorbs different heavy metals (Cr, Fe, Cd, Cu, Ni, Zn, Mn and Pb) well, when grown on contaminated soils supplemented with different amounts of industrial sludge. The order of absorbed metals in indian shoots, on the 90^{th} day from the experiment setup, was Fe> Cr> Mn> Zn> Ni> Cu> Cd> Pb, whereas the translocation was almost two times higher in roots than in shoots. With the increasing percentage of sludge in the soil, the concentration of the metal in different parts of the plant grows. The length of roots and shoots also depends on the changes in soil sludge. The growth is good in the soil supplemented with 10% of sludge, whereas at 20% and 30% concentrations of sludge, a slow decline in root and stem growth can be observed, although without visible toxic signs. The decline in root growth suggests that most of the metals are accumulated in the underground parts of these plants.

In their study, Cheng *et al.* (2002) showed the effects of Cd^{+} on the growth of the plant *Canna indica* L., its chlorophyll content, photochemical efficiency and photosynthetic intensity. It is shown that this species can tolerate concentrations from 0.4 to 0.8 mg L^{-1} Cd^{2+}, which indicates that it can be used in the phytoremediation of heavy metals.

The field of research of indian shoot, as a plant for the production of biofuels, is still relatively new, but many studies have shown that it has great potential, due to its extremely high starch production and equally successful cultivation in tropical and temperate climates (State Master, 2010).

For the purpose of the project "Research opportunities for the production of biomass for the energy from short rotation plantations within the electrical power system of Serbia" (TR 18201A) several experiments were set up by the Institute of Forestry in Belgrade in order to determine the ability of indian shoot to remove heavy metals from wastewater and determine the amount of biomass produced by this plant in contaminated water.

2. MATERIAL AND METHOD

Plant material was obtained from the rhizome of the species *Canna indica* L. - indian shoot. In early April rhizomes were planted in peat in order to get well-cultivated seedlings, which can be transferred to aqueous solutions.

An experiment in the open was set up, within the research area of the Institute of Forestry in Belgrade, to monitor the growth of indian shoot in the aquatic environment. In late July each plant was transferred to a 10 l volume bucket, which was half-filled with tap water. The experiment lasted until mid-September, and the water was changed several times. The measuring of the parameters of growth to determine biomass production was carried out at the beginning and the end of the experiment.

The efficiency of indian shoot in the removal of heavy metals was tested in an experiment under laboratory conditions. In early May, seedlings of indian shoot were transferred to buckets with 5 l of modified 50% Hoagland solution. In late May, this nutrient solution was changed and supplemented with three different concentrations of lead (10μM, 50μM and 150μM). Two parallel experiments were set up, in which lead was supplemented to one group of plants in the form of $Pb(NO_3)_2$, and to the other in the form of $Pb(CH_3COO)_2 \times 3H_2O$. The experiments lasted 20 days. The measurements of lead content in the solution and the plants were carried out every 5 days.

The plants were taken out from the solution and divided into roots, rhizomes, stems and leaves. The solution was sampled to determine the residual Pb. All plant parts were washed three times in distilled water. The fresh weights of stems, leaves, rhizomes and roots were measured, as well as the volumes of the rhizomes and roots. The leaves and shoots of the rhizomes were counted and leaf area was determined. Plant parts were then dried for 24 hours at 80^{o}C. Dry weights of the stems, leaves, rhizomes and roots were measured after drying.

Microwave digestion (CEM MDS 2000, Berghof, Germany, Mod. Speedwave MWS3 +) was used for sample preparation. About 250-300 mg of dried homogenized plant material was added to special Teflon vessels and the quantities of 5 ml of 69% HNO_3 and 2 ml of 30% H_2O_2 were used for destruction. After microwave digestion the samples were diluted in distilled H_2O (total volume 25 ml). The measuring of lead content was performed using an ICP-AES spectrometer (Spectro Genesis FEE, Germany).

3. RESULTS AND DISCUSSION

The two most important characteristics that a plant suitable for phytoremediation should have are the ability to quickly produce large amounts of biomass and the ability to absorb metal in large quantities in the shoots (Kumar *et al*., 1995, Cunningham and Ow, 1996, Blaylock *et al*., 1997). So, the combination of high metal accumulation and high biomass production provides the best results in the removal of metals.

Other desirable characteristics of plants are tolerance to poor environmental conditions, production of a dense root system, the ease of establishment and growing and resistance to pests and diseases. Dushenkov and Kapulnik (2000) describe the characteristics of an ideal plant for rhizofiltration. These plants have to be able to accumulate and tolerate significant amounts of targeted metals, but should also be easy to handle, have low maintenance costs and low production of secondary waste that requires disposal. It is desirable that these plants produce significant amounts of root biomass or have large root area.

The results of the experiment that examined the growth of indian shoot in the aquatic environment suggested that *Canna indica* L. possesses one of the most important characteristics of the plants suitable for phytoremediation and that is high biomass production in a short time. In mid-September, after a month and a half of growth without fertilization, the Canna plants produced a significant amount of biomass both of the aboveground and underground parts.

Fig 2. *The beginning of the experiment in late July (left), the size of plants at the beginning of the experiment (middle) and the size of plants in mid-September (right).*

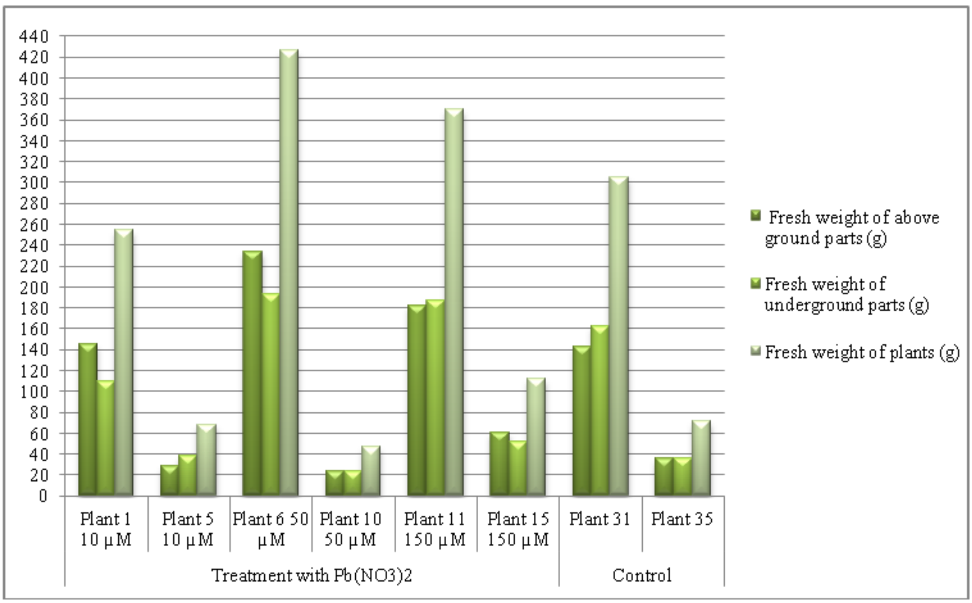
Besides that, these plants developed very dense strong fibrous roots with a large area for the sorption of metals. This further confirmed the fact that terrestrial plants are more suitable for rhizofiltration than aquatic plants. Aquatic macrophytes often have limited rhizofiltration potential, because of their insufficient effectiveness in the removal of metals due to their relatively small root and its slow growth (Dushenkov *et al.*, 1995). The same authors argue that the high water content in these plants complicates their drying, composting or burning.

In this experiment indian shoot revealed its tolerance to the absence of plant nutrients in the solution (plants were grown in pure water without fertilizers). The establishment and cultivation of the seedlings was very easy. The total absence of pests and diseases that could threaten the plant was observed.

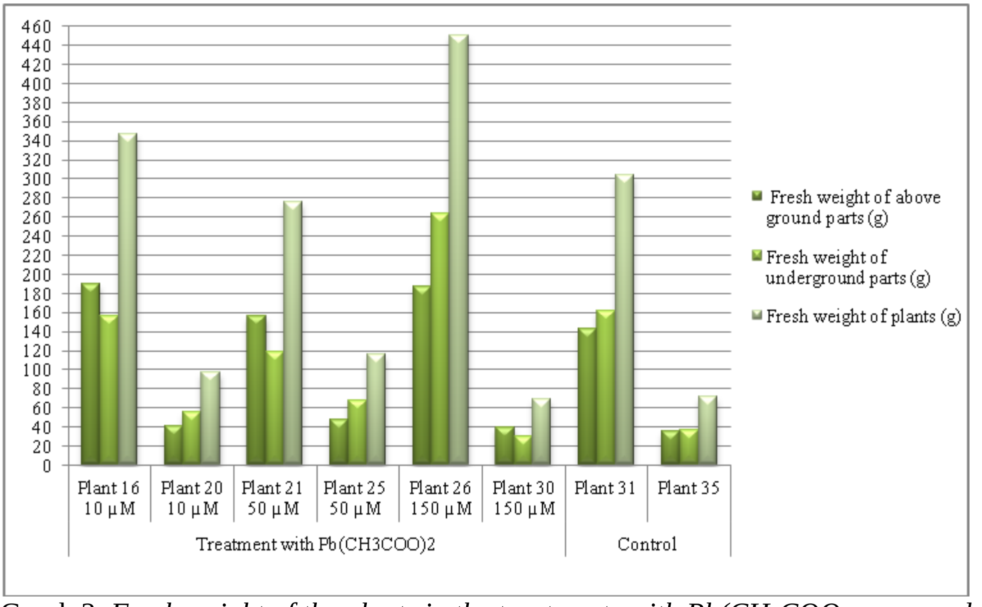
The second experiment indicated that *Canna indica* L. plants were extremely tolerant to the presence of heavy metals, in this case lead, in the medium in which they grew. Specifically, they revealed excellent growth even in the medium with the highest lead concentration (150μM $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ x $3H_2O$).

Fig. 3. *A part of Canna indica L. plants at the beginning of the experiment (left) and the appearance of the plants in the mid-experimental period (right).*

The graphs below show the results of the measurements of fresh and dry weights of the aboveground and underground parts of the *Canna indica* L. plants on the 9th day of the experiment.



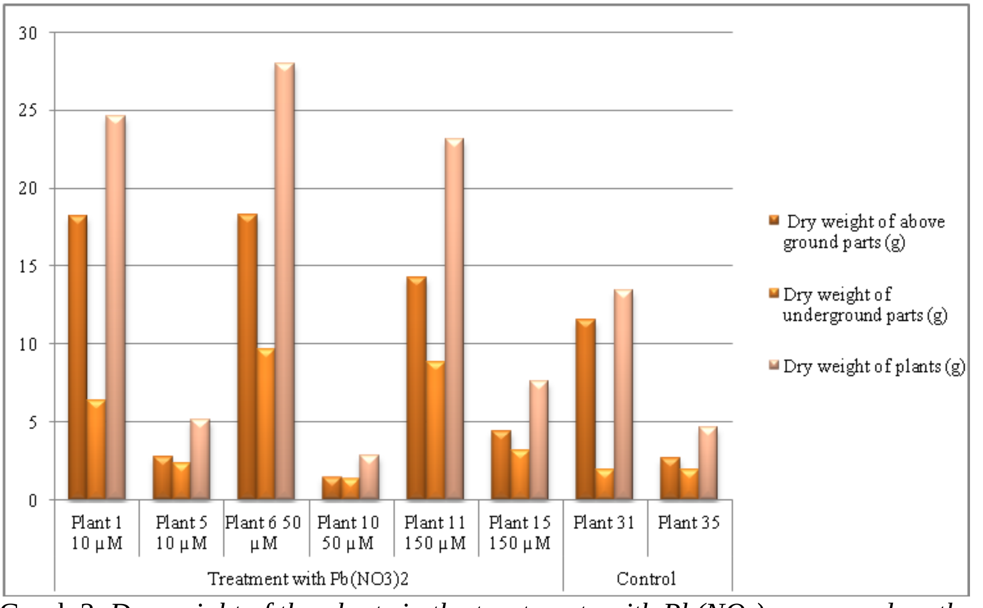
Graph 1. *Fresh weight of the plants in the treatments with $Pb(NO_3)_2$ measured on the 9th day of the experiment*



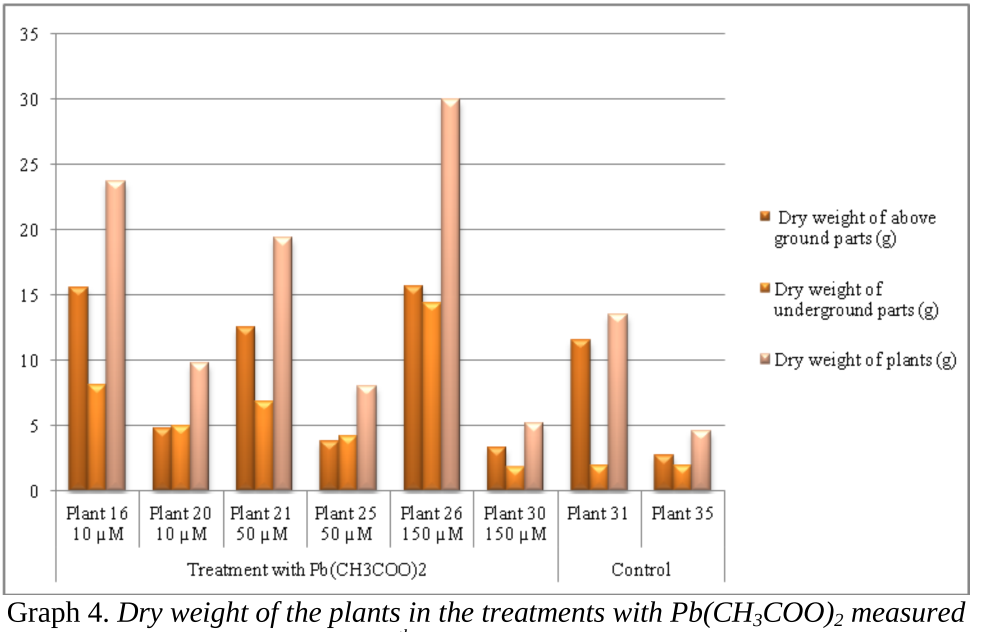
Graph 2. *Fresh weight of the plants in the treatments with Pb(CH_3COO_2 measured on the 9th day of the experiment.*

As can be seen from the results, fresh weight ratio of the aboveground and underground parts of the plants does not vary and it is on average 50:50. This is another indication that, in water, *Canna indica* L. plants tend to develop good root system and rhizome in which they storage substances.

The ratio of the dry weights of the aboveground and underground parts of the plant is slightly different. In small plants (plants 5, 10, 15 and 35) this ratio is retained, i.e. on average amounts to 55:45, whereas in large plants (plants 1, 6, 11 and 31), which have a larger rhizome, the ratio is different (on average 70:30), indicating that the underground parts of these plants contain plenty of water. This may be an aggravating circumstance in the further processing of biomass after the process of refining. On the other hand, this water content is far lower than that of the aquatic plants used for the same purpose, whose drying, composting or burning often consumes more energy. The most frequently-mentioned examples of these plants are *Eichhornia crassiper* (Mart.) Solms. (water hyacinth), *Hydrocotyle umbellata* L., and *Lemna minor L.* (duckweed), which have a high ability to absorb heavy metals, but a limited rhizofiltration potential due to their relatively small root, its slow growth and high water content (Dushenkov *et al.*, 1995)

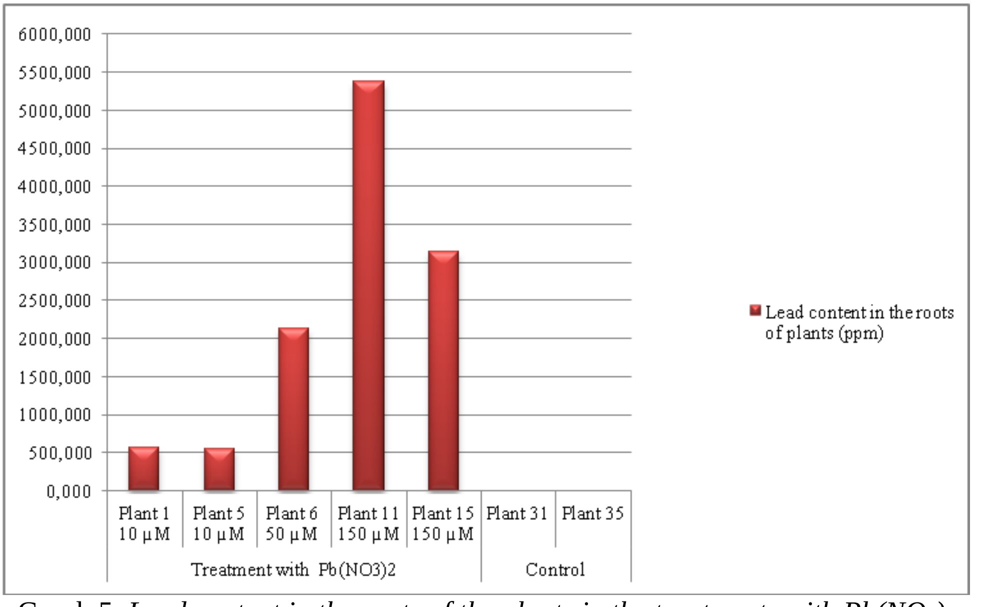


Graph 3. *Dry weight of the plants in the treatments with $Pb(NO_3)_2$ measured on the 9^{th} day of the experiment.*

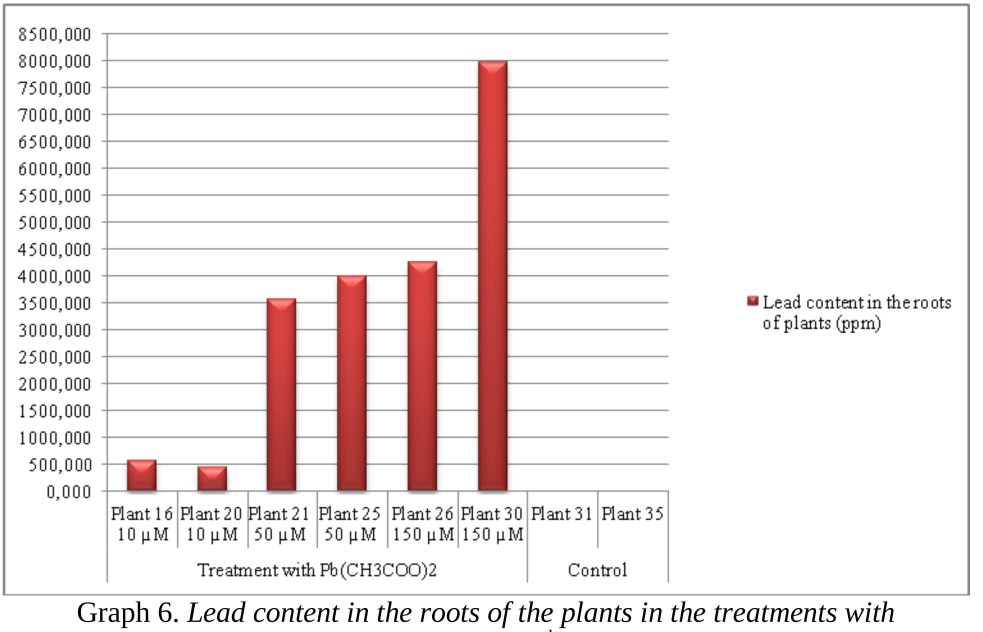


Graph 4. *Dry weight of the plants in the treatments with $Pb(CH_3COO)_2$ measured on the 9^{th} day of the experiment.*

According to the analysis of lead content in the plants and the solution it was observed that the *Canna indica* L. plants absorbed most of the lead from the solution until the fifth day of the experiment. The absorption was continued until the last day, and the highest lead content in plant tissue was recorded in the root.



Graph 5. *Lead content in the roots of the plants in the treatments with $Pb(NO_3)_2$ measured on the 9^{th} day of the experiment.*



Graph 6. *Lead content in the roots of the plants in the treatments with $Pb(CH_3COO)_2$ measured on the 9^{th} day of the experiment.*

As can be seen from the results shown in graphs 5 and 6, the plants were slightly more successful in the absorption of lead from the treatment with $Pb(CH_3COO)_2$. This can be explained by the fact that lead (II) acetate dissolves well in water. The maximum absorption of lead until the 9^{th} day of the experiment occurred in the roots of plant 11 from the treatment with 150µM $Pb(NO_3)_2$ and it amounted to 5369.384 ppm of lead and the root of plant 30 from the treatment with 150μM Pb $(CH_3COO)_2$, which amounted to 7973.518 ppm of lead. The concentration of lead was the lowest in the leaves, which indicates low mobility of this heavy metal through the plant.

Plants used for phytoremediation have to be tolerant to the metal or metals, which are being removed, as mentioned before. In addition to that, they also have to be efficient in the translocation of the metals absorbed by the roots to the aboveground parts of plants that are to be mowed (Blaylock and Huang, 2000). It should be noted that there are different opinions regarding the benefits of the translocation of absorbed metals from the roots to other parts of plants, especially when rhizofiltration is concerned. Many researchers believe that plants used for phytoremediation should accumulate metals only in their root system (Dushenkov *et al.*, 1995, Salt *et al.*, 1995; Flathman and Lanza, 1998). Dushenkov *et al.* (1995) explained that the translocation of metals to the aboveground shoots would decrease the efficiency of rhizofiltration by increasing the amounts of contaminated plant residues, which would have to be disposed of. In contrast, Zhu *et al.* (1999) suggest that the efficiency of the process can be increased if plants have an increased capacity for the absorption and translocation of metals in the plant. Despite these differences in opinions, it is obvious that proper selection of plants is the key in ensuring the success of rhizofiltration as a strategy for water refining.

If further experiments confirm that the translocation of lead from root to leaves in indian shoot is low, this will indicate the possibility of using the aboveground biomass of this plant for a variety of purposes, and not only for the production of biofuels.

4. CONCLUSION

Heavy metals, radionuclides and other inorganic pollutants are some of the prevailing forms of environmental pollutants and their remediation in soil, sediments and water is very hard. Unlike many organic pollutants most heavy metals cannot be eliminated from the environment by chemical and biological transformation. Therefore attention should be paid to the prevention of their entry into the environment and there is a need to work on new alternative methods for their removal.

The accumulation of metals and especially hyperaccumulation using plants have caused a lot of interest in recent years. Ebbs *et al.* (1997) argued that, in order to achieve successful phytoremediation, it is necessary to apply a combined strategy of rapid screening of the plant species with an ability to tolerate and accumulate heavy metals and agronomic practices that would increase the biomass of shoots and the availability of metals in the rhizosphere (Kamal *et al* ., 2004). Finding the right plants remains the main goal of many researchers involved in plant breeding and genetic engineering.

In the experiments, Canna (*Canna indica L.)* proved to be a very promising plant. Although it is a terrestrial plant, which has so far mainly been used as a decorative floral species in green areas and needs some kind of support in the water for refining, such as floating platforms, it generally removes larger quantities of pollutants than many aquatic plants capable of phytoremediation.

As shown this plant is very tolerant to the lack of nutrients. It can produce a large amount of biomass in a short time. It develops a very dense, strong fibrous root system in water, with a large surface area for the filtration and absorption of heavy metals. It is easy to produce and cultivate, and is resistant to pests and diseases. It is highly tolerant to the presence of lead, which is easily absorbed and concentrated in its root and rhizome, due to the low translocation to aboveground parts.

Rhizofiltration is a competitive technology in terms of cost, when used for the treatment of surface and ground waters, which contain low but significant concentrations of heavy metals such as Cr, Pb and Zn. The commercialization of this technology is supported by the economic and technical advantages such as its applicability to different metals, the possibility to treat large quantities of water, decreased need for a variety of toxic chemicals, reduced volume of secondary waste, the possibility of recycling and the likelihood of acceptance of this technology by the public and authorities.

ACKNOWLEDGEMENTS

This paper is a result of the research project "Research opportunities for the production of biomass for the energy from short rotation plantations within the electrical power system of Serbia" (TR 18201A) of the Institute of Forestry in Belgrade, financed by the Ministry of Science and Technological Development of the Republic of Serbia.

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