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Original Research

Studies of Plants Useful in the Re-Cultivation of Heavy Metals-Contaminated Wasteland – a New Hyperaccumulator of Barium?

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Abstract

The strategy of re-cultivation of wasteland contaminated with heavy metals based on phytoremediation was proposed. The ability of plants that colonized the investigated area to uptake pollutants from soils and their translocation to aboveground organs was examined. The metals mobility and bioavailability was assessed based on a single extraction with water and solutions of hydrogen peroxide and EDTA. A new hyperaccumulator of barium – great mullein (*Verbascum densiflora*) – useful in the phytoextraction process, was identified.

Keywords: barium, phytoremediation, ICP-MS, bioaccumulation, great mullein (*Verbascum densiflora*)

Introduction

In many countries burning coal remains a major source of energy. It should be remembered that there are a number of adverse environmental effects of coal mining and burning, especially in power stations. It is always related to coal combustion products (CCPs): fly ash, bottom ash, and flue gas desulphurization (FGD) materials. Fly ash and bottom ash are primarily composed of valuable industrial minerals such as alumina, silica, lime, and iron oxide. FGD is primarily composed of gypsum, the mineral used to make wallboard. CCPs can be used as a replacement for natural materials in construction, providing a quality product while reducing costs. Fly ash makes up the largest component of CCPs, with more than 70 million tons produced each year. Fly ash composed of very fine spherical particles is an excellent additive to concrete. Bottom ash is resistant to pressure and drains well, so it is often used for road base, embankment, and structural fill applications. Unfortunately reports measuring the health risks posed by disposal practices at coal ash dumps confirm their negative impact on the environment, especially the release of some toxic elements and degradation of groundwater quality. Moreover, as the areas of wasteland are usually very valuable and in many cases could be used for building, special interests and efforts are paid to restoration such sites [1].

Today, intensive investigation is directed at the usefulness of phytoremediation in recovery of some contaminated areas. The method of phytoremediation is based on the application of plants in the removal of contaminants from the soil. The plants, which are often identified as 'bioaccumulators', have the ability to take up soil contaminants and deposit them in their roots, as well as in the aboveground organs. Subsequently, they can be harvested in order to eliminate contaminants from the restored area permanently.

It is necessary to point out that bioaccumulating plant species are normally characterized by high concentration factors, i.e. concentrations of the toxic substances are higher in their tissues than in the soil. Bioconcentration factors of some plants can even reach 1000x. There are plant

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species capable of intensive uptake of soil contaminants and, at the same time, are characterized by a significant production of biomass [2-4].

Costs of area restoration using phytoremediation are normally much lower than in the case of any other *in situ*, and in particular *ex situ*, methods. In fact, the method of phytoremediation is often used in the USA and in Western Europe. In Poland there is on-going research aiming to assess the possibility of using different plant species in biorestoration of polluted soils, as well as in the recovery of precious elements from waste deposits (biomining).

The main task of the undertaken studies was to examine the usefulness of plants growing in the vicinity of an industrial plant near Warsaw for accumulation of wasteland contaminants and their translocation to aboveground organs. For this purpose a list of plants growing on loads of ash waste, as well as in zones remaining after the removal of the ash, was identified and, after the determination of heavy metal contents in particular plant organs and soil, the assessment of their suitability to biorestoration of that contaminated area was evaluated. Moreover, the bioavailability of heavy metals present in ash obtained after coal burning and stored in the wasteland was assessed. For this purpose a single extraction with water, hydrogen peroxide and EDTA was performed [5]. For determination of total element contents in digested soil and plant samples and in the extracts, we used mass spectrometry with inductively coupled plasma (ICP-MS) [6-10].

Experimental

Reagents

The following reagents were used: hydroxylamine hydrochloride (puriss p.a.) (Fluka, UK), nitric acid, perchloric acid, and fluoric acid (Ultranal) (Cheman). Standard solutions were prepared by diluting Spectroscan solutions (1000 $mg \cdot L^{-1}$) of the appropriate element. Ultrapure water obtained from Milli-Q-Water System (Millipore, USA) was used throughout the work.

Instrumentation

Total concentrations of studied elements in the digested samples were measured by spectrometer (Perkin Elmer SCIEX, Canada) with Meihard-type nebulizer and Scott-type spray chamber. Advanced Microwave Digestion System Ethos 1 (Milestone, Italy) was used for sample digestion.

Sampling and Sample Preparation

At the edge of the investigated area we collected a few dominant plant species: rapeseed (*Brassica napus*), grass (*Lolium perenne*) giant goldenrod (*Solidago gigantea*), great mullein (*Verbascum densiflora*), and tansy (*Tanacetum vulgare*). Rapeseed, giant goldenrod, tansy, and grass were collected from 1 m^2 areas. It gave above 50 plants per species. In the case of great mullein 5 plants were collected.

Harvested plants were carefully washed several times with Milli-Q water. Special attention was paid to *Verbascum densiflora* because their leaves were densely covered with grayish hairs. Then the plants were divided into leaves, roots, and flowers. Samples were dried at 60ºC overnight, milled in an agate mill, and sieved through a 0.125 mm nylon sieve.

Soil samples were collected using a multiple sampling procedure according to the Polish Standard [11]. Soils, collected to a depth of about 20-40 cm, were bulked into bags for transport to the laboratory. After removing foreign bodies such as twigs or stones, soil was air-dried at ambient temperature for two weeks. The dried material was sieved through a 1 mm sieve. The resulting material was homogenized by shaking. The measured pH (H_2O) of soil equalled 8.5.

Extraction Procedures

The extraction procedure was performed to assess the mobility and bioavailability of the contaminant from soils and ashes deposited in the wasteland area located in the vicinity of an industrial plant near Warsaw. One g of dried soil or ash samples were extracted with 50 mL of the extractant – water, 3% hydroxyperoxide or 0.05 $mol \cdot L^{-1}$ EDTA – in a 120 mL polyethylene container. Extracts were centrifuged at 2,000 rpm for 30 min and filtered through a 0.45 μm cellulose acetate filter into polyethylene containers. Extracts after filtration (with exception of EDTA) were acidified with 50 μl of concentrated HNO_3 (to pH about 2) and stored at 4ºC.

Sample Digestion

Plants – approximately 0.25 g of dried plant material and 3 mL of concentrated HNO_3 – were placed in PTFE vessels and digested in a microwave system. A three-stage program with a maximum temperature of 200ºC and maximum microwave power of 1000 W was used (5 min 20-90ºC, 10 min 90-170ºC, 50 min 170-200ºC). Digested samples were transferred to 25 mL volumetric flasks and diluted to volume with Milli-Q water. Digestion of all samples was triplicated.

Soil – approximately 0.20 g of dried material and a mixture of concentrated acids (2 mL of HNO_3 and 1 ml $HClO_4$) – were placed in PTFE vessels and digested in a microwave digestion system. A three-stage program with a maximum temperature of 200ºC and maximum microwave power of 1000 W, as in case of plants, was used. Then, after short cooling, the vessels were opened and 0.5 ml HF was added and the same three-stage program was applied. Digested samples were transferred into 50 mL volumetric flasks and diluted to volume with Milli-Q water. Digestion of all samples was triplicated.

Before barium determination, 0.1 g of dried samples of soil were weighed with 140 mg of hydroxylamine hydrochloride into quartz vessels and filled up to 50 mL with 2 $mol \cdot L^{-1}$ nitric acid. Then the vessels were covered with quartz watch glasses and heated to boiling for about 20 min on an electric heater to complete dissolution.

After cooling, the solutions were refilled to 50 ml, filtered, and stored a max 8 hours before determination.

Digested samples were diluted if required before measurements.

Total Metal Determination

ICP-MS measurements were performed under the following conditions: sweep 5, replicates 5, dwell time 100 ms, ICP RF power 1100 W, lens voltage 8 V, plasma gas flow 15 L·min^{-1}, auxiliary gas flow 1.2 L·min^{-1}, nebulizer gas flow 0.9 L·min^{-1}, measured isotopes: ^{52}Cr, ^{63}Cu, ^{58}Ni, ^{138}Ba, ^{208}Pb, ^{59}Co, ^{64}Zn. Iridium was applied as an internal standard.

Results and Discussion

During the first part of the research the characterization of the investigated area was completed. In soil samples total concentrations of Ba, Ni, Co, Cr, Pb, and Cu were determined. Based of the obtained results (Fig. 1), soils showed relatively high concentrations of barium – above 300 mg·kg^{-1}. This was of a great interest, as the wasteland area was dedicated to building development, and the admissible norms recommended by Polish law [12] related to barium content were exceeded. Most Polish soils contain no more than 50 μg·g^{-1} of barium, but in submontane regions its content may increase up to 100 μg·g^{-1} [13].

General information about barium toxicity is limited. It can be found that significantly higher mortality rates, particularly among individuals 65 years of age and older, for cardiovascular disease and heart disease (arteriosclerosis) could be observed in communities with elevated barium in drinking water levels (0.06-0.3 mgBa·kg^{-1}·day^{-1}), as compared to communities with low barium levels (0.006 mgBa·kg^{-1}·day^{-1}) [14].

Concentrations of chromium, cooper and nickel in the soil samples were also relatively high: 140, 110, and 80 mg·kg^{-1}, respectively, but did not exceed the limits, while contents of lead and cobalt were relatively low: 70 and 20 mg·kg^{-1}, respectively.

As the total concentration of heavy metals in soil is not sufficient for environmental risk assessment, in the next step fractionation analysis was performed. The determination of extractable trace metal contents in soil using single extraction procedure is currently performed to access the bioavailable metal fraction and related phytotoxic effects [15-17].

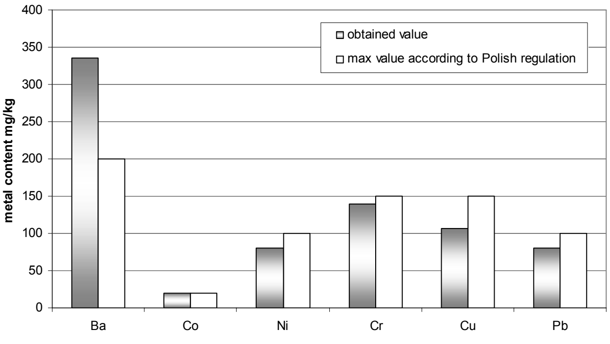

Fig. 1. The comparison of metal concentrations in studied soils with their acceptable amounts recommended by a decree of the Ministry of Environment.

It was discovered that the extractable amount of element by water or 3% solution of hydrogen peroxide did not exceeded 1% for all studied elements. While the bioavailability assessed after extraction by EDTA was much higher, especially in the case of Ba – with mean value about 35%. A relatively high bioavailability of this element gives a great opportunity for restoration of this area by phytoremediation methods. Phytoextraction, when plants take up contaminants from the soil and translocate them to aboveground organs, seems to be useful in this case. So the next task of the studies was to assess the suitability of plants to colonize this area for the phytoremediation purpose.

Five plant species, that predominantly colonized that area, were collected in the in the wasteland near the industrial plant in Warsaw. Total concentrations of studied elements were determined in particular plant organs (Table 1). To assess if plants can effectively take up the contaminants from ground and translocate them to aboveground organs, bioconcentration factors (BCF), defined as the ratio of concentration of element in plant organ to concentration of element in soil [18], were calculated (Table 1). It was found that the lower accumulation and the lower bioconcentration factors were observed for cobalt, while relatively high uptake was observed for copper. In the case of grass the BCF of Cu for aboveground organs was 0.95, slightly lower than characteristics for hyperaccumulators. Based on the literature data plant hyperaccumulators can be defined as plants possessing the ability to store elements in aboveground parts 10-500 times more than usual, with an enrichment coefficient (metal content in dry matter/metal content in soil) >1 [19, 20]. For some elements, hyperaccumulator thresholds are defined: 100 mg·kg^{-1} dry mass for Cd, Co, and Cr, 1000 mg·kg^{-1} dry mass for Cu, Ni, Pb, and Se, and 10,000 mg·kg^{-1} dry mass for Zn and Mn [21-23].

Additionally, to characterize transport efficiency of elements from roots to aboveground organs translocation factor (TF), defined as the ratio of metal concentration in shoots to roots, is used [18]. Metal concentration in roots and aboveground organs of tansy, giant goldenrod, and grass was similar for each investigated element, and calculated values of TFs were nearly 1.

The highest difference of metal concentration in plant organs was observed in the case of rapeseed. For Co, Ni, and Cr, the highest concentrations were present in roots and translocation of these elements to aboveground organs was the least. But Pb concentration was the highest in stems, while Ba content was nearly the same independent of plant organs. Despite the relatively low bioconcentration factor and transfer factors of metals observed for rapeseed, this species might be considered for phytoremediation of soils in an area designated for restoration because of its high density – naturally growing in this area, ca. 100 plants/m^2. The most valuable and useful finding of the undertaken studies was the ability of great mullein to take up, accumulate, and translocate into leaves, barium, cobalt, nickel, and lead.

Table 1. Total concentration of metals in particular organs of studied plants, expressed as mean values±SD [mg·kg^{-1}] (n≥3), and bioconcentration factors defined as a ratio of metal content in plant organ to metal content in soil (in brackets).

Me	Tansy *Tanacetum vulgare*		Giant goldenrod *Solidago gigantea*		Grass *Lolium perenne*		Rapeseed *Brassica napus*			Great mullein *Verbascum densiflora*		
	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots	Stem	Leaves	Roots	Stem	Leaves
Ba	23.1±1.5 (0.07)	25.2±1.1 (0.07)	28.5±1.2 (0.08)	18.9±0.9 (0.06)	16.2±0.9 (0.05)	19.4±0.8 (0.06)	21.6±0.9 (0.06)	19.5±1.1 (0.06)	17.1±0.8 (0.05)	63.9±2.1 (0.19)	35.0±1.8 (0.10)	343.6±7.4 **(1.02)**
Co	0.80±0.05 (0.04)	0.73±0.04 (0.04)	0.37±0.02 (0.02)	0.25±0.01 (0.01)	0.47±0.02 (0.02)	0.36±0.02 (0.02)	0.61±0.02 (0.03)	0.19±0.01 (0.009)	0.31±0.01 (0.02)	1.60±0.08 (0.08)	1.80±0.06 (0.09)	12.9±0.4 (0.65)
Ni	4.15±0.19 (0.05)	4.82±0.18 (0.06)	5.58±0.09 (0.06)	3.20±0.08 (0.04)	7.12±0.21 (0.08)	6.22±0.12 (0.07)	12.4±0.3 (0.14)	2.41±0.08 (0.03)	2.91±0.09 (0.03)	12.8±0.5 (0.15)	10.7±0.5 (0.12)	35.8±1.1 (0.41)
Cr	7.49±0.41 (0.05)	8.47±0.31 (0.06)	11.69±0.35 (0.08)	5.67±0.11 (0.04)	13.35±0.29 (0.09)	6.52±0.10 (0.05)	13.5±0.4 (0.10)	4.0±0.1 (0.03)	3.2±0.1 (0.02)	n.d.	n.d.	n.d.
Cu	26.62±0.91 (0.25)	18.91±0.82 (0.18)	22.30±1.05 (0.21)	13.28±0.40 (0.12)	32.23±1.80 (0.30)	101.26±1.15 (0.95)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.21±0.09 (0.03)	3.9±0.1 (0.06)	1.12±0.05 (0.02)	4.2±0.1 (0.06)	2.62±0.07 (0.04)	29.5±0.9 (0.42)

n.d. not determined

In the case of all these elements their concentrations in leaves were several times (5 to 10) higher than in roots and stems. Bioconcentration factors between 0.41 and 1.02 indicated this plant species as very suitable for the phytoextraction process. Great mullein is a widely distributed species found all over Europe and in temperate Asia as far as the Himalayas and in the eastern United States where it is exceedingly abundant as a naturalized weed. It grows on hedge-banks, roadsides, and on waste ground, more especially on gravel, sand, or chalk. It seems to be excellent for the recovery of wasteland areas in the vicinity of industrial plants before such reuse of such areas for building development.

There are only a few reports concerning barium accumulation by plants. Relative to the amount of Ba found in soils, less is bioconcentrated by plants. Nevertheless, there are plants such as legumes, forage plants, Brazil nuts, and mushrooms that can accumulate Ba. Bioconcentration factors from 2 to 20 have been reported for tomatoes and soybean [24]. It also has been stated that *Indigofera cordifolia* can colonize the soils around barite mining area in India and accumulate Ba at 3.5 mg·g^{-1} dry weight [25] when *Juncellus serotinus* growing on uranium mill tailings repository accumulate Ba in the roots (179 μg·g^{-1} dry weight) [26].

Conclusions

Summarizing the undertaken studies and considering the fact that only partial removing of contaminants from the soil is necessary before the start of building development - phytoremediation can be successfully applied in the mentioned area. The required decline in amount of contaminants, according to Polish legislation, will probably be achieved after a few seasons of cultivation of a well-selected plant species. As the most important problem on this area is related to barium, the species *Verbascum densiflora*, showing the ability for barium, accumulation, could be useful for phytoextraction. This subject is worth studing, especially that Sagiroglu et.al [20] looking for hyperaccumulators among plants that are common and native throughout Turkey, found another plant from the same (Scrophulariaceae) family, i.e. *Verbascum cheiranthifolium Boiss*. This plant can accumulate some metals like Mo, Cu, Pb, Zn, Ag, and As, as high as 3-4 times more than their content in soil.

Future studies of Ba bioaccumulation by *Verbascum densiflora* should include relations with soil pH. Soil pH plays a crucial role in heavy metal bioavailability [27, 28]. In general, acidic soils present a low metal retention capacity. Comparing acid with basic soils, greater quantities of metals were extracted from the former.

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References

1. International Ash Utilization Symposia and the Word of Coal Ash Conference WOCA 1999-2007. University of Kentucky Center for Applied Energy Research. www.whocares.caer.uky.edu/wasp/AshSymposium.AshLibraryAgenda.asp
2. BAROCSI A., CSISTALAN Z., KOCSANYI L., DUSHENKOV S., KUPERBERG J.M., KUCHARSKI R., RICHTER P.J. Optimizing phytoremediation of heavy metals – contaminated soil by exploiting plants stress adaptation, Int. J. Phytoremediation **5**, (1), 13, **2003**.
3. HERRERO E.M., LOPEZ-GONZALLVES A., RUIZ M.A., LUCAS-GARCIA J.A., BARBAS C. Uptake and distribution of zinc, cadmium lead and copper in *Brassica napus* var. *oleifera* and *Heliantus annus* grown in contaminated soils, Int. J. Phytoremediation **5**, (2), 153, **2003**.
4. KOWALSKA J., ASZTEMBORSKA M., BYSTRZEJEWSKA-PIOTROWSKA G. Platinum uptake by mustard (*Sinapsis alba* L.) and maize (*Zea mays*) plants, Nukleonika **49**, 31, **2004**.
5. ALVAREZ J.M., LOPEZ-VALDIVIA L.M., NOVILLO J., OBRADOR A., RICO M.I. Comparison of EDTA and sequential extraction tests for phytoavailability prediction of manganese and zinc in agricultural alkaline soils, Geoderma **132**, 450, **2006**.
6. PUCHELT H., SETIOBUDI I.J. New dissolution technique for rarely soluble sulfate minerals for AAS and ICP investigations, Fresen. J. Anal. Chem. **335**, 692, **1989**.
7. MONTEIRO M.I.C., CURTIUS A.J. Determination of barium by electrothermal atomic absorption spectrometry, J. Anal. Atom. Spectrom. **10**, 329, **1995**.
8. MIERZWA J., SUN Y.C., CHUNG Y.T., YANG M.H. Comparative determination of Ba, Cu, Fe, Pb and Zn in tea leaves by slurry sampling electrothermal atomic absorption and liquid sampling inductively coupled plasma atomic emission spectrometry, Talanta **47**, 1263, **1998**.
9. ARSLAN Z., ERTAS N., TYSON J.F., UDEN P.C., DENOYER E.R. Determination of traces elements in marine plankton by inductively coupled plasma mass spectrometry (ICP MS). Fresen. J. Anal. Chem. **366**, 273, **2000**.
10. BOEVSKI I., DASKALOVA N., HAVEZOV I. Determination of barium, chromium, cadmium, manganese, lead and zinc in atmospheric particulate matter by inductively coupled plasma atomic emission spectrometry (ICP AES), Spectrochim. Acta Part B, **55**, 1643, **2000**.
11. BN-78/9180-02. Analysis of soil samples.
12. Decree of the Ministry of Environment 9.09. **2002** (J. L. No. 165, Item 1359).
13. KABATA-PENDIAS A., PENDIAS H. Biogeochemistry of trace elements. Warsaw: PWN, **1999**.
14. CICAD 33 2001. Barium and Barium Compounds.
15. QUEVAUVILLER P., RAURET G., URE A., RUBIO R., LOPEZ-SANCHEZ J.F., FIEDLER H., MUNTAU H. Preparation of candidate Certified Reference Material for quality control of EDTA- and Acetic Acid-Extractable trace metal determination in sewage sludge-amended soil and Terra Rossa Soil, Mikrochim. Acta, **120**, 289, **1995**.
16. ARAIN M.B., KAZI T.G., JAMALI M.K., JALBANI N., AFRIDI H.I., SHAH A. Total dissolved and bioavailable elements in water and sediment samples and their accumulation in *Oreochromis mossambicu*s of polluted Manchar Lake, Chemosphere, **70,1845**, **2008**.
17. URE A.M., QUEVAUVILLER P., MUNTAU H., GRIEPINK B. Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European Communities, Int. J. Environ. An. Ch. **51**, 135, **1993**.
18. YOON J., CAO X., ZHOU Q., MA L.Q. Accumulation of Pb, Cu, and Zn in native plants growing on contaminated Florida site, Sci. Total Environ., **368**, 456, **2006**.
19. HAJIBOLAND R., MANAFI M.H. Flora of heavy metal-rich soils in NW Iran and some potential hyper-accumulator and accumulator species, Acta Bot. Croat. **66**, (2), 177, **2007**.
20. SAGIROGLU A., SASMAZ A., SEN Ö. Hyperaccumulator Plants of the Keban Mining District and Their Possible Impact on the Environment, Pol. J. Environ. Stud. **15**, 317, **2006**.
21. BOYD R.S. The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions, Plant Soil **293**, 153, **2007**.
22. SURESH B., RAVISHANKAR G. A. Phytoremediation – A Novel and Promising Approach for Environmental Clean-up, Critical Reviews in Biotechnology **24**, (2-3), 97, **2004**.
23. VERBRUGGEN N., HERMANS CH., SCHAT H. Molecular mechanisms of metal hyperaccumulation in plants, New Phytol. **181**, 759, **2009**.
24. NOGUEIRA T. A. R., MELO W. JFONSECA ., I. M., MARQUES M. O., HE Z. Barium uptake by maize plants as affected by sewage sludge in a long-term field study, J. Hazard. Mater. **181**, 1148, **2010**.
25. RAGHU V. Accumulation of elements in plants and soil in and around Mangampeta and Vemula barite mining areas, Cuddapah District Andhra Pradesh, India, Environ. Geol., **40**, 1265, **2001**.
26. LI G., HU N., DING D., ZHENG J., LIU Y., WANG Y., NIE X. Screening of Plant Species for Phytoremediation of Uranium, Thorium, Barium, Nickel, Strontium and Lead Contaminated Soils from a Uranium Mill Tailings Repository in South China, Bull Environ Contam Toxicol **86**, 646, **2011**.
27. SORIANO-DISLA J. M., GÓMEZ I., NAVARRO-PEDREÑO J., LAG-BROTONS A. Evaluation of single chemical extractants for the prediction of heavy metal uptake by barley in soils amended with polluted sewage sludge, Plant Soil **327**, 303, **2010**.
28. WATMOUGH SH. A. Element mobility and partitioning along a soil acidity gradient in central Ontario forests, Canada, Environ Geochem Health, **30**, 431, **2008**.