POSSIBILITIES OF PLANT PRODUCTION FOR BIOREFINERY ON A SOIL CONTAMINATED WITH HEAVY METALS

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Abstract
About 17 % of the territory of Hungary is not cultivated and a significant part of this area is not suitable for agricultural use because of industrial pollution. Green biorefinery technologies allow the reutilization of hydrocarbon or heavy metal contaminated areas, since agricultural plants and herbs, which can be sources of valuable products can be produced on polluted sites.
The applicability of biorefinery was tested on a heavy metal polluted soil. The contamination originated from previous mining activity. Complete by-product utilization from the biomass is aimed to obtain cosmetic ingredients, pharmaceutical agents and precursors from plants cultivated on polluted areas. Consequently, we established a technology that ensures the biomass cycle and the utilization of solar energy bound in plants.
After the survey of the experimental areas (contaminants; microbial florae and endemic vegetation) 88 plant species and varieties were cultivated and tested for potential utilizable components. The level of possible contaminants in the plants was also monitored and the amounts of different plant materials were determined (carbohydrates, protein, organic acids, cellulose). Crude plant extracts were tested as potential sources of biologically effective components (for e.g. antimicrobial molecules) or as raw materials for lactic acid fermentation. Our results show that biorefinery is a real possibility for the utilization of polluted areas for industrial purposes. Numerous plants could be cultivated on contaminated fields without increased levels of contaminants in their tissues, thus they can be sources of industrially valuable compounds.

Keywords: heavy metal pollution, phytoremediation, plant biomass biorefinery

INTRODUCTION

Biorefinery is a complex technology where biomass can be converted to useful materials (e.g. fuels, solvents, plastics, cosmetic and pharmaceutical materials and food for humans) and/or energy carriers in an integrated manner and thereby it can maximize the economic value of the biomass used while reducing the waste streams produced (Ohara, 2003; Kaparaju et al., 2009). Use of agricultural lands to produce non-food plants causes social resistance currently; however, there are some so-called brown field areas (suffering from industrial contamination), where agricultural output is not feasible due to the high level of contaminants, such as heavy metal ions. Some plants are able to grow in heavy metal polluted environment (Murányi and Ködöböcz, 2008; Máthéné and Anton, 2004) and do not accumulate heavy metals in toxic levels for humans. Murillo et al. (1999) presented that sorghum and sunflower can easily absorb and translocate heavy metals to plant foliage. However, phytotoxicity limits the accumulation in plants at safe levels for humans and animals. On the other hand, various plants, such as lavender contain lots of different effective compounds, which can be utilized for different industrial purposes, like isolation of antimicrobial agents (Konstantia et al., 1998; Sato et al., 2007). Moreover, Valcho et al.

(2008) demonstrated that three essential oil species *Marrubium vulgare*, *Melissa officinalis* and *Origanum heracleoticum* can be grown as alternative high-value crops on metal polluted agricultural soils around smelters and provide metal free marketable products.

In 2007 a consortium was generated by the Elgoscar-2000 Ltd., by the Institute for Biotechnology and the Institute of Logistics and Production Engineering of Bay Zoltán Foundation for Applied Research, by the Karcag Research Institute of CASE Debrecen University and by the Biocentrum Ltd for the purpose of execution of a scientific research program supported by the Hungarian National Office for Research and Technology on the field of integration of phytoremediation of different polluted fields and of plant biomass biorefinery. The main goal of the project was to elaborate a new complex technology which can guarantee the proper treatment and the profitable utilization of polluted fields. For this purpose biomass production ability and potential availability for industrial utilization of numerous plant species and varieties were tested in a field experiment and in a pot trial on a soil extremely polluted by heavy metals.

Some results of the pot experiment are demonstrated in this paper to illustrate our research work and our screening technologies.

MATERIALS AND METHODS

With the purpose of fulfillment of our scientific program regarding to utilization of fields polluted by heavy metals a pot experiment (Fig. 1) was carried out at Gyöngyösoroszi in 2008. Great amounts of heavy metal containing slop were arisen at the Mátra Metal Mines during the ore enrichment processes. Some of this slop was filled into two types of pots. One type was of 50 dm^3 for herbaceous plants and the other type was of 300 dm^3 for arboreal plants. Biomass production capacity, heavy metal tolerance and potential availability for industrial utilization of 88 different plant species and varieties were tested in this experiment. The total number of the plots was 428.

Fig. 1 **View of the pot experiment**

Some chemical properties of the used slop are shown in the Table 1. These analytical data proved the significant Zn-, Cu-, Cd-, and Pb-pollution of the slop. Ground limestone of 5 m% was mixed with the slop to mitigate the solubility of the heavy metal compounds. The used slop did not contain any phosphorus, hence artificial P-fertilization (analogous with 200 kg P_2O_5 ha^{-1} dose) was applied in each plot.

Table 1

Some chemical properties of the used slop polluted by heavy metals

pH(H_2O)	pH(KCl)	y1	Humic substances (%)	AL-soluble				
				P_2O5	K_2O	Ca	Mg	Na
				($mgkg^{-1}$)				
6,63	6,37	10	1,24	<2	112	21470	1178	43
KCl+EDTA soluble								
Zn	Cu	Fe	Mn	Cr	Co	Ni	Cd	Pb
($mgkg^{-1}$)								
648	230	258	270	4	2,4	2	6,3	356
Total element content								
Zn	Cu	Fe	Mn	Cr	Co	Ni	Cd	Pb
($mgkg^{-1}$)								
1715	675	42666	964	33	10	7	7	473

The pot experiment was two-factorial which provided possibilities to examine the tolerance of different plants (88 species) to heavy metal contaminations and the effects of different TERRASOL compost doses (analogous with 30 tha^{-1} and 50 tha^{-1} doses) on the biomass production of the tested plant species.

At the beginning of blooming or yield ripening plant samples were taken from the plant standings of plots to establish the biomass production and to make different chemical analysis.

Establishment of common plant ingredients

0.2 g plant sample was homogenized in 0.1 N filtered H_2SO_4 of 5 ml and centrifuged for 20 min (13500 rpm). The supernatant was diluted by H_2SO_4 to 20-fold and analyzed by GynkoTek isocratic HPLC arrangement, on CAR-H column and at 30 ºC, and the mobile phase was 0.01 N filtered H_2SO_4. Sugars were detected by refractive index detector and the organic acids were detected by UV detector. Data were integrated by the Chromeleon program. Cellulose content was determined from 1g plant sample by the nitric acid–ethanol mixture methodology (Wang Z H, 1995). During the protein assay 0.2 g plant sample was homogenized in 0.1 N NaOH of 6 ml and incubated in water-bath at 60 °C for 2 hours and centrifuged for 10 minutes at 13000 rpm. The supernatant was diluted by distilled water to 20-fold. 250 µl taken from the prepared sample was analyzed by the Lowry method (Lowry et al., 1951). The concentration of the reduced Folin's reagent is measured by absorbance with a Unicam Heλios α UV-VIS spectrophotometer at 750 nm.

Heavy metal analysis

Levels of heavy metal contaminants of the plant and soil samples were determined by Bálint Analitika Ltd. (Hungary) using HP 4500 plus ICP-MS.

Lactic acid fermentation

Shoots of the plants were physically crushed and fractioned by an Angel juice extractor. The liquid phases were tested as potential medium for lactic acid fermentation. *Lactobacillus delbruckii* spp. *lactis was* applied to convert the sugar content of plant extracts to lactic acid. The liquid phases were sterilized at 115 °C for 30 minutes then centrifuged by 5000 rpm for 20 minutes. The inoculum was grown in modified DSM-186 medium and incubated at 37 °C for 72 hours. Inoculum of 20 µl was added to 1 ml supernatant of the liquid phase and incubated at 37 °C for 48 hours. The initial glucose content and the amount of produced lactic acid were measured by HPLC.

RESULTS

Heavy metal pollution tolerance of the tested plant species

On the basis of the experimental data we established that the amount of biomass was increased by the compost application in the case of a part of the tested plant species and varieties. Data of Table 2 show some examples of the positive effect of the applied TERRASOL compost. We must mention that the extremely high compost dose of 50 tha^{-1} decreased the amount of the green biomass in the case of several plant species.

Under the circumstances of the experiment buckwheat, grain sorhum, energy poplars, energy willows, hemp, purple coneflower, white mustard and sufflower could reach relatively high amount of biomass yield.

On the basis of the experimental data originated from the pot trial we established that the following plant species and varieties could tolerate the unfavorable chemical, hydrological and microclimatic conditions of the heavy metal polluted trial site: each sweet sorghum hybrid, castor-oil plant, chicling vetch, amaranth, lozenge, coriander, millet, facelia, bluebottle, oenothera, evening star, red poppy, Arundo donax, onion, French marigold, bean, dill, white clover.

From the tested plant species and varieties the following ones could not tolerate the unfavorable ecological conditions of the experiment: maize, sunflower, pumpkin, lupin, perennial flax, Canary grass, pine, spruce, oak, wattle, rosemary, milfoil, anise, basil, green pea, poppy, celery and parsley.

The sensitivity of these plant species to the heavy metal contamination was manifested in limited germination. Anthocyanine pigmentations were observable on the young seedlings and their growth was slow. As a consequence of these processed mentioned above, the sensitive indicator plants died in June and July when additional climatic stress (high air temperature) was formed out.

Table 2

Effect of compost doses on the green biomass (gm^{-1}) of some plant species grown in heavy metal polluted soil (pot experiment at Gyöngyösoroszi, 2008)

Plant species/varieties	Compost treatments		
	control	30 tha^{-1} compost	50 tha^{-1} compost
grain sorghum (Alföldi 1)	0	640	320
sweet sorghum (Cellu)	0	694	1 032
grain sorghum (Albita)	0	434	408
grain sorghum (GK Emese)	0	416	378
sweet sorghum (Monori édes)	0	502	706
sweet sorghum (Róna 1)	0	592	584
sudan grass (Gardavan)	0	370	482
grain sorghum (Zádor)	0	360	306
castor-oil plant	0	586	672
sweet sorghum (Sucrosorgo)	0	196	566
hemp	0	1016	848
evening star	0	286	312
lozenge	22	106	156
white clover	46	62	334
garlic	118	200	376
French marigold	0	650	664
onion	0	346	502
kitchen sage	38	384	560
Arundo donax	220	633	426

We observed that the actual biomass production of tolerant plant species covered on a part of their genetic potential only. By our opinion, the biomass production of these plant species can be increased by the improvement of the agroecological conditions (water regime, organic matter content, nutrient supply, etc.) of production sites polluted by heavy metals.

Analysis of heavy metal contents of the produced biomass

The heavy metal concentrations of the plants grown on contaminated soil were monitored. After the preparation of plant samples by juicer, 98-100 % of the heavy metals could be detected in the liquid fraction. We found that heavy metals were concentrated in roots (Table 3) consequently. The investigated plants did not transport them to the shoots. The compost treatments had no obvious influences on the heavy metal uptake and distribution.

Table 3

Heavy metal contents of different parts of the selected plant species and varieties

		Cd		Cu		Ni		Pb		Zn	
		30t/ha	50t/ha	30t/ha	50t/ha	30t/ha	50t/ha	30t/ha	50t/ha	30t/ha	50t/ha
	shoot	0.57	0.19	1.59	0.54	2.09	0.77	0.65	1.49	18.10	10.40
sorghum, Albita	root	7.71	1.69	43.20	11.40	8.44	1.51	204.00	193.00	486.00	31.90
	shoot	1.46	2.16	1.37	3.42	4.41	0.79	1.40	6.28	30.40	32.60
sorghum, Cellu	root	5.62	2.38	26.10	14.50	19.40	2.04	147.00	205.00	94.40	39.10
	shoot	0.31	0.31	0.53	2.06	1.31	1.51	0.70	12.80	27.00	21.10
sorghum,GK Emese	root	4.78	2.59	17.20	13.60	4.64	2.67	92.20	211.00	85.00	112.00
	shoot	1.86	0.17	2.67	2.51	2.15	1.93	2.87	1.04	99.80	30.60
sorghum, Monori édes	root	6.56	2.59	12.00	49.10	2.72	7.13	384.00	226.00	96.80	110.00
	shoot	0.44	0.18	0.57	1.05	2.34	1.93	0.97	1.33	23.50	11.40
sorghum, Róna 1	root	3.23	2.02	9.03	20.00	2.06	2.87	8.77	124.00	186.00	35.90
	shoot	0.69	0.11	1.23	0.55	0.46	2.47	1.90	1.00	30.50	12.30
sorghum, Zádor	root	7.22	1.29	31.60	8.19	2.54	33.70	315.00	32.30	216.00	87.00
balm	shoot	0.97	0.13	74.70	17.20	6.45	1.14	95.20	14.30	136.00	36.10
lavender	shoot	0.23	0.16	37.30	12.40	4.23	1.16	27.90	27.70	78.30	35.50

Heavy metal recovery was calculated for different sorghum varieties based on biomass-yields and the measured heavy metal contents. Similarly to heavy metal distribution, the varied compost treatment did not cause observable differences in heavy metal recovery (Fig. 2). However, we would like to highlight that approximately 0.5-1 kgha^{-1} zinc was recovered in the “Cellu” and “Monori édes” sweet sorghum varieties. This founding indicates the possibility of utilization of these varieties for phytoremediational purposes.

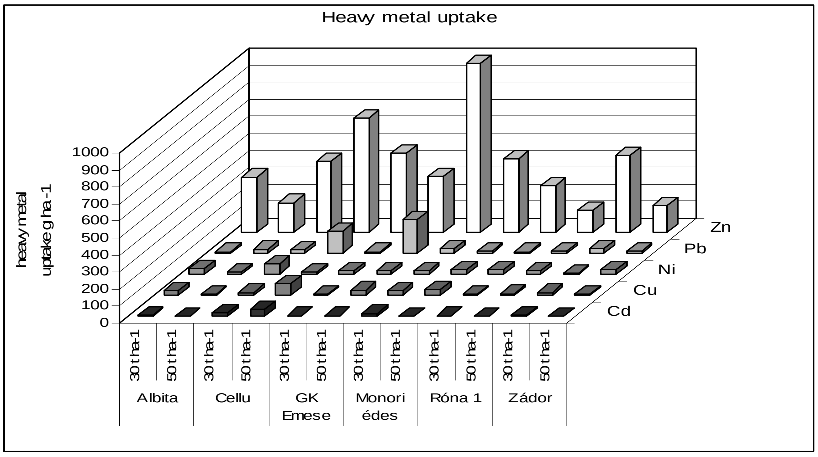


Fig. 2. **Heavy metal uptake of different sorghum varieties originating from soils treated by different compost doses (30 and 50 tha^{-1})**

Analysis of common plant ingredients

Amounts of common plant ingredients, for example cellulose, sugars, organic acids and proteins were measured in all tested plant species grown on an area extremely polluted by heavy metals and treated with different compost doses. According to the experimental data, the varied amount of compost had no effect on the amounts of the monitored plant ingredients. However, the biomass production was found to be lower on the experimental site than the usual biomass production on agricultural soils, the proportion of the compounds were not affected by the presence of heavy metals.

Lactic acid fermentation

The shoots of different Sorghum varieties were ground in an Angel juice extractor, and the liquid phases of the produced samples were inoculated with *Lactobacillus delbruckii spp. lactis* cells. In some cases, the proportion of lactic acid to glucose was higher than one; consequently, other carbon sources from the plant extract could be used for lactic acid fermentation by the bacterium. Compost treatment did not affect the effectiveness of lactic acid production. In some cases the yield of lactic acid was more than 50 g l^{-1} (Table 4.), which can be considered as a quite high concentration compared to the literature results obtained with starch of sorghum seeds (Zhan et.al, 2003.). The high lactic acid concentration is beneficial for the purification of lactic acid, which might be utilizable to produce polylactate, a biodegradable plastic(Feerzet Achmad et al. 2009)

Table 4

Lactic acid fermentation from the liquid phase of different sorghum varieties originated from pots treated with different compost doses (30 and 50 tha^{-1})

Lactic acid fermentation			
	Plants	glucose g l^{-1}	produced lactic acid g l^{-1}
Albita	30 t ha^{-1}	40.61	24.29
	50 t ha^{-1}	25.29	8.52
Cellu	30 t ha^{-1}	60.14	22.37
	50 t ha^{-1}	20.32	20.30
GK Emese	30 t ha^{-1}	98.54	54.28
	50 t ha^{-1}	66.79	52.25
Monori édes	30 t ha^{-1}	53.43	31.75
	50 t ha^{-1}	93.85	53.65
Róna 1	30 t ha^{-1}	48.81	47.09
	50 t ha^{-1}	182.37	62.53
Zádor	30 t ha^{-1}	86.82	60.71
	50 t ha^{-1}	107.23	63.85

Volatile compounds

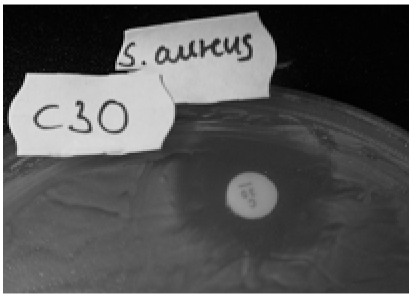
In this study, 20 different, well-known medicinal volatile oils (VOCs) were detected from the alcoholic extracts of the spice plants. Although, no VOCs were detect from lavender samples originating from the pots treated with 30 tha^{-1} compost dose, five different compounds were found in the samples taken from pots treated with 50 tha^{-1} compost dose. The difference in composting also affected the balm content of the plants (Table 5.). The heavy metal content of the alcoholic plant extracts were found to be low, thus, the further refining of the extracts for pharmaceuticals or cosmetic base materials seems to be possible.

Table 5
Volatile oils and heavy metal contents in alcoholic extract of balm and lavender grown on different compost treated soil.

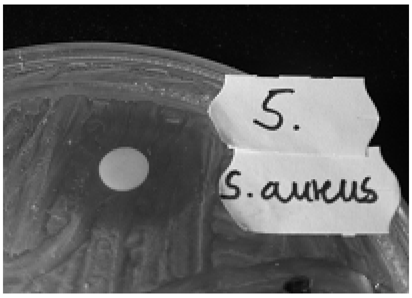
		Volatile oils in balm and lavender							Heavy metal content in the plant extracts				
		chamfor	p-cimene	borneol	eucaliptol	cisze-citral	γ-terpinene	carvacrol					
plants	treatment by compost	Rt=21,885	Rt=19,342	Rt=22,042	Rt=19,513	Rt=22,977	Rt=19,826	Rt=23,66	Zn	Cu	Pb	Cd	Ni
balm	30 t ha^{-1}		+		+	+		+	0,00	7,84	7,84	0,00	0,00
balm	50 t ha^{-1}		+		+		+		0,00	0,00	0,00	7,92	0,00
lavender	30 t ha^{-1}								0,00	1,98	1,98	0,00	0,00
lavender	50 t ha^{-1}	+	+	+	+		+		0,00	0,00	0,00	0,00	0,00

Antimicrobial effects

The sterile filtered liquid fraction of some sorghum varieties, like Albita and Zádor showed antiviral activity in the 8-fold dilution. The 16-fold dilutions were too diluted to exert their effect on VSV and the concentrated ones were toxic to Hep2 tissue.
The sterile filtered liquid fractions of plant samples were tested for containing potential biologically effective components, such as antimicrobial compounds. The antibacterial activity was tested against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The tested plants did not have any antibacterial effect against *P. aeruginosa* and *E. coli*, however, some sorghum varieties, like Monori édes and Cellu, had antibacterial activity against *S. aureus*. The inhibition zone had the same size as 30 µg ml^{-1} chloramphenicol (Fig. 3).



Inhibiton of
Staphylococcus aureus
by Chloramphenicol 30µg



Inhibiton of
Staphylococcus aureus
by extractum of sweet *Sorghum*

Fig. 3. **Antibacterial effect of sweet *Sorghum***

Some of the samples demonstrated antifungal activity against the *Paecilomyces variotii* only, however, this fungi was sensitive for all investigated plant extracts.

CONCLUSION

On the basis of the experimental data we established that some plant species can adapt to the unfavorable ecological conditions of heavy metal polluted soils. The amount of biomass of these plant species was increased by the compost application significantly.
According to the results, numerous plant species were able to synthesize some biologically active compounds, like antimicrobials, without the accumulation high level of heavy metal contaminants. These results suggest that plants cultivated on industrial, heavy metal contaminated areas can serve as valuable sources of base materials for biorefinery.
As a next step, the development of a complex purification method of the active compounds is necessary, considering the presence of heavy metal contamination.

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