PLANT PRODUCTION POSSIBILITIES ON A HEAVY METAL CONTAMINATED SOIL WITH THE PURPOSE OF BIOREFINERY

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Keywords

biorefinery, heavy metal pollution, phytoremediation,

Summary

Significant part of not cultivated area of Hungary is not suitable for agricultural utilization because of industrial pollution. Technologies of biorefinery make reutilization of contaminated areas possible. Biomass of plants produced on polluted soils can be raw material of valuable products. Applicability of biorefinery was tested on a heavy metal polluted soil, where the contamination originated from previous mining activity. Complete biomass utilization was aimed to obtain cosmetic ingredients, pharmaceutical agents, and precursors. During our research work 88 plant species and varieties were produced and tested for potential utilizable components. Levels of possible contaminants in these plants were monitored, and amounts of carbohydrates, protein, organic acid and cellulose were determined as well. Different plant extracts were tested as potential sources of biologically effective components or as raw materials for lactic acid fermentation. Our results show that biorefinery is a real possibility for utilization of polluted areas. Numerous plants could be cultivated on contaminated areas without increased levels of contaminants in their tissues, thus they can be sources of valuable compounds.

INTRODUCTION

Biorefinery is a complex technology where biomass can be converted to useful materials (e.g. fuels, solvents, plastics, cosmetic and pharmaceutical materials) or energy carriers in an integrated manner, thereby it can maximize the economic value of the biomass used while reducing the waste streams produced [8, 3]. Utilization of arable 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 use is not possible due to high levels of contaminants, such as heavy metal ions. Some plants are able to grow in heavy metal polluted environment [6, 5] and do not accumulate heavy metal ions in toxic levels for humans. Sorghum and sunflower can easily absorb and translocate heavy metals to plant foliage [7]. However, accumulation of heavy metal ions in plants is limited by their phytotoxicity at levels safe for humans and animals. Various plants, such as lavender contain lots of effective compounds, which can be utilized for different industrial purposes, like isolation of antimicrobial agents [2]. Moreover, plants of high sugar or starch content, such as sorghum or millet, provide valuable raw materials for ethanol or lactic acid production [10]. Lactic acid might be utilizable to produce biodegradable plastic [1].

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 CAAES RISF of 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.

MATERIAL AND METHODS

With the purpose of fulfillment of our scientific program regarding to utilization of heavy metal polluted fields a pot experiment (Fig. 1) was carried out at Gyöngyösoroszi in 2008.

Figure 1. View of the pot experiment

Great amounts of heavy metal containing slop were arisen at the Mátra Metal Mines during 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.

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.

The two-factorial pot experiment provided possibilities to examine the tolerance of the tested 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 these plant species.

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

Table 1

Some chemical properties of the used slop polluted by heavy metals

pH(H_2O)	**pH(KCl)**	**y1**	**Humic matter content (%)**	**AL-soluble**				
				P_2O_5	**K_2O**	**Ca**	**Mg**	**Na**
				($mg\ kg^{-1}$)				
6,63	6,37	10	1,24	<2	112	21470	1178	43
KCl+EDTA soluble								
Zn	**Cu**	**Fe**	**Mn**	**Cr**	**Co**	**Ni**	**Cd**	**Pb**
($mg\ kg^{-1}$)								
648	230	258	270	4	2,4	2	6,3	356
Total element content								
Zn	**Cu**	**Fe**	**Mn**	**Cr**	**Co**	**Ni**	**Cd**	**Pb**
($mg\ kg^{-1}$)								
1715	675	42666	964	33	10	7	7	473

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 [9]. 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 [4]. 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

Amounts 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 phase was sterilized at 115 °C for 30 minutes then centrifuged at 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.

Biogas production

After crushing and fractioning the solid phase of grain sorghum were tested for biogas production. The reactor volume was 1000 ml. 800 ml anaerob sludge and 20g dried solid phases were applied. Reactors were incubated at 37°C. The produced biogas were trapped and monitored for the total amount of gases in ml for 30 days. The methane content of the biogas was investigated at the end of the exponential phase (13th day). The gas analysis was carried out by Gas Chromatograph with 5975 MS Detector (Front Inlet: 250 °C; split rate: 1:50; GS-GASPRO (60m x 0,320mm) column; at 40°C for 6 min., heating to 130 °C by 30 °C/min; at 130° C for 2,0 min.; methode: C1-C2GAS.M.; detecting in AUTO tune mode; carrier gas: He (Linde).

RESULTS AND DISCUSSION

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 case of a part of the tested plant species and varieties. Data of the Table 2 show the positive effect of the applied TERRASOL compost on the green biomass of some plant species. We must mention that the extremely high compost dose of 50 t ha^{-1} decreased the amount of the green biomass in case of several plant species. Under the circumstances of the experiment buckwheat, grain sorghum, energy poplars, energy willows, hemp, purple coneflower, white mustard and sunflower could reach relatively high amount of biomass.

Table 2

Effect of compost doses on the green biomass (g m^{-1}) of some plant species grown in a heavy metal polluted soil (Gyöngyösoroszi, 2008)

Plant species/varieties	Compost treatments		
	control	30 t ha^{-1}	50 tha^{-1}
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
sweet sorghum (Sucrosorgo)	0	196	566
hemp	0	1016	848
evening star	0	286	312
lozenge	22	106	156
French marigold	0	650	664
Arundo donax	220	633	426

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 trial site: 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 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 processes mentioned above, the sensitive indicator plants died in June and July when additional climatic stress (high air temperature) was formed out.

We observed that the actual biomass production of the 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 improvement of 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

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

Heavy metal recovery was calculated for different sorghum varieties based on their biomass yields and the measured heavy metal contents. Similarly to the heavy metal distribution, the applied compost treatments did not resulted in observable differences in the heavy metal uptake (Fig. 2). However, approximately 0.5-1 kg ha^{-1} zinc was recovered in the biomass of Cellu and Monori édes sweet sorghum varieties. This founding indicates the possibility of utilization of these varieties for phytoremediational purposes.

Table 3

Heavy metal contents of different parts of some plant species and varieties

Crop varieties	crop part	Cd		Cu		Pb		Zn	
		compost treatments (t ha^{-1})							
		30	50	30	50	30	50	30	50
Albita (sorghum)	shoot	0.57	0.19	1.59	0.54	0.65	1.49	18.10	10.40
	root	7.71	1.69	43.20	11.40	204.00	193.00	486.00	31.90
Cellu (sorghum)	shoot	1.46	2.16	1.37	3.42	1.40	6.28	30.40	32.60
	root	5.62	2.38	26.10	14.50	147.00	205.00	94.40	39.10
GK Emese (sorghum)	shoot	0.31	0.31	0.53	2.06	0.70	12.80	27.00	21.10
	root	4.78	2.59	17.20	13.60	92.20	211.00	85.00	112.00
Monori édes (sorghum)	shoot	1.86	0.17	2.67	2.51	2.87	1.04	99.80	30.60
	root	6.56	2.59	12.00	49.10	384.00	226.00	96.80	110.00
Róna 1 (sorghum)	shoot	0.44	0.18	0.57	1.05	0.97	1.33	23.50	11.40
	root	3.23	2.02	9.03	20.00	8.77	124.00	186.00	35.90
Zádor (sorghum)	shoot	0.69	0.11	1.23	0.55	1.90	1.00	30.50	12.30
	root	7.22	1.29	31.60	8.19	315.00	32.30	216.00	87.00
balm	shoot	0.97	0.13	74.70	17.20	95.20	14.30	136.00	36.10
lavender	shoot	0.23	0.16	37.30	12.40	27.90	27.70	78.30	35.50

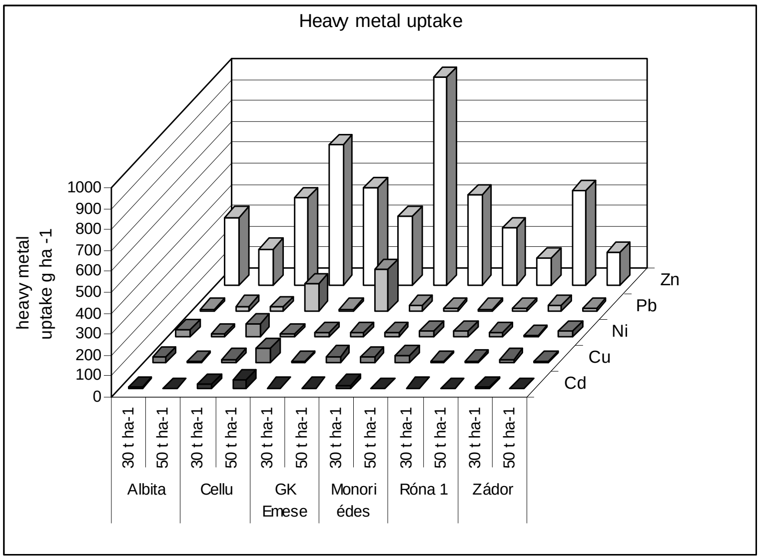


Figure 2. Effects of compost doses (30 and 50 t ha^{-1}) on the heavy metal uptake of different sorghum varieties

Analysis of common plant ingredients

Amounts of common plant ingredients, for example cellulose, sugars, organic acids and proteins were measured in the biomass of all tested plant species grown on a soil extremely polluted by heavy metals and treated with different compost doses. According to the experimental data, the applied compost doses had no effect on amounts of the monitored plant ingredients. While the biomass production was much lower in the experiment than the usual biomass production, the proportion of these compounds were not affected by the presence of heavy metal ions.

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 to be quite high concentration in comparison with the literature data [10]. The high lactic acid concentration is beneficial for the purification of lactic acid, which might be utilizable to produce polylactate [1].

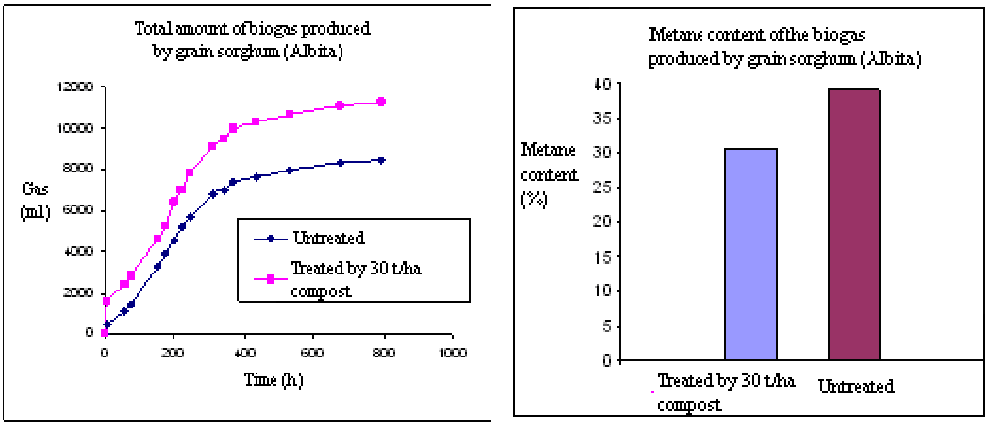
Biogas production

The solid phase of grain sorghum biomass was tested for energetic application. We established that the total amount of biogas formed was higher in case of compost treatment, but the methane content of the biogas measured at the end of the exponential phase of gas formation was higher in case of biomass samples originated from untreated pots (Fig. 3A and 3B).

__Table 4__

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



A

B

Figure 3. Biogas production of grain sorghum

A: Total amount of biogas B: Methane content of the biogas at the end of the exponential phase (untreated and treated by 30 t ha^{-1} compost)

We found that the methane content changed during the incubation, hence definite effect of the compost treatment on the quality of formed biogas could not be observable.

CONCLUSIONS

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, biomass of numerous plant species can be utilized as raw material of valuable products, like lactic acid, biogas and biologically active compounds (e.g. antimicrobials), without significant accumulation of heavy metal contaminants. These results suggest that plants cultivated on heavy metal contaminated areas can be valuable sources of base materials for biorefinery.

Development of a complex purification method of these active compounds is necessary, taking the presence of heavy metal contamination into consideration.

ACKNOWLEDGEMENTS

This work was supported by the grants NKFP_07_4-BIOFINOM.

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