Environment Protection Engineering

Vol. 35 2009 No. 2

MAŁGORZATA KACPRZAK*, KRZYSZTOF FIJAŁKOWSKI*

MYCORRHIZA AND SEWAGE SLUDGE EFFECT ON BIOMASS OF SUNFLOWER AND WILLOW DURING PHYTOREMEDIATION OF DEGRADED TERRAINS WITHIN ZINC FOUNDRY ZONE

The research estimated the effect of sewage sludge fertilization of soil (seriously contaminated with heavy metals) and simultaneous mycorrhization of sunflower and willow roots on the biomass obtained yields.

The experiment was carried out under controlled conditions in a large-size phytotron chamber. The process was conducted for 3 months at a temperature ranging from 25 °C (day) to 17 °C (night), photoperiod of 16/8, and humidity from 60 to 90%, using sewage sludge from pulp industry and mycorrhizal fungi occurring naturally (endo- and ectomycorrhiza). The plants were grown on the soil from the terrain of zinc mill (Miasteczko Śląskie). It was found that the addition of 30% (w/w) of sewage sludge caused approximately a six-fold increase of willow biomass production (mainly above ground) and the use of mycrorrhizal fungi only (two strains) caused a one- and three-fold increase of willow biomass. The mycrorrhizal roots of sunflower allowed almost 0.5-fold increase in its biomass. The biomass of this plant increased five-fold and even thirteen-fold as a result of adding sewage sludge at the respective rates of 20% (w/w) and 30% (w/w). Simultaneous root mycorrhization and sewage sludge added as above caused additional increase in sunflower crops by 18 and 38%, respectively

1. INTRODUCTION

Phytoremediation is one of the most promising methods of the reclamation of strongly degraded terrains. Growing plants are able not only to accumulate contaminations, which offers the possibility of their biorecovery (e.g. of metals), but also create environmental conditions for the reconstruction of ecosystems, mainly by the maintenance of the biological activity and physical structure of soils [1]. The success of the process depends on the appropriate selection of plant species, soil fertilization and irrigation and the use of biological agents (bacteria or fungi) [2]–[4].

Willow and sunflower play an important role not only as hyperaccumulators [5], [6], but also as the source of renevable bioenergy. However, the plants grown for biomass as an

* Institute of Environmental Engineering, Częstochowa University of Technology, ul. Brzeźnicka 60a, 42-200 Częstochowa, Poland. E-mail: mkacprzak@is.pcz.czest.pl

alternative energy source decrease arable area. Hence, some researchers propose that such plants should be grown on degraded soils. Willows are resistant to adverse environmental conditions and may play a double role of bioproducts and reclamation agents.

The aim of this work was to estimate the effect of sewage sludge fertilization of soil (seriously contaminated with heavy metals) and simultaneous mycorrhization of sunflower and willow roots on the biomass of the yields obtained.

2. MATERIALS AND METHODS

2.1. SOIL AND SEWAGE SAMPLES

The soil samples were taken from at least five different places north (200–300 m) of the zinc plant. The samples were transported in a cooler to laboratory where they were thoroughly mixed, passed through a 2 mm sieve and used for the experiment. Sewage sludge was derived from the wastewater treatment plant of pulp industry and its samples being taken from lagoons, mixed and transported to laboratory in plastic containers.

2.2. PLANTS AND MYCORRHIZA ISOLATES

The willow cuttings of 1054 genotype came from the producer of bush and trees "lasprywatny AB". The sunflower seeds were commercially available. Mycorrhizal isolates came from mycorrhizal vaccines producer "Mykoflor", where mycorrhizal fungi of willow (ECTO) were isolated from natural willow root environment in Karkonosze Mountains. The mycorrhizal fungi of sunflower (ENDO) belong to *Glomus* spp.

2.3. EXPERIMENT DESIGN

The experiment was conducted in plastic containers and soil microcosms (microenvironments) were designed according to table 1. The plants were grown under control conditions in a large-size phytotron chamber (Biogenet) for 3 months at a temperature from 25 °C (day) to 17 °C (night), 16/8 photoperiod, and humidity from 60 to 90%.

Table 1

Combinations in indivudual microcosms (microenvironments)

Control	Sunflower	Willow
Soil (5 kg)	soil + mycorrhiza	soil + 30% sewage sludge
	soil (3.5 kg) + 20% sewage sludge	soil + mycorrhiza 1 (ECTO)
	soil + mycorrhiza + 20% sewage sludge	soil + mycorrhiza 1 + 30% sewage sludge
	soil + 30% sewage sludge	soil + mycorrhiza 2 (ENDO)
	soil + mycorrhiza + 30% sewage sludge	soil + mycorrhiza 2 + 30% sewage sludge

2.4. PHYSICAL-CHEMICAL ANALYSES

The following physical-chemical properties were analysed: dry matter by gravimetric method [7], pH in H_2O by potentiometric method [8]; pH in 1 mol KCl and sorption capacity according to [9]. Total nitrogen was determined by Kjeldahl method [10], and available phosphorus (phosphates) – by spectrophotometric method [11]. Humic acids were extracted from air-dried soil with 0.1 M NaOH and 0.1 M $Na_4P_2O_7$. After 24 h, they were precipitated by acidification to pH 1 with HCl and separated by oven-drying at 60 ºC. Heavy metals were extracted from the air-dried samples after their digestion in concentrated HNO_3 at 80 ºC for 120 minutes and quantified by the inductively coupled plasma atomic emission spectrometry (ICP AES, Optima 2000DV model). Total carbon was analysed using a combustion analyzer (Analytik Jena).

3. RESULTS AND DISCUSSION

Top-soil surface from the terrain of the zinc foundry contained heavy metals in very high concentration, which in most cases exceeded their average content in soil (table 2). The addition of sewage sludge improved the most important soil parameters, such as total carbon, nitrogen, phosphorus, humic acids or sorption capacity (table 3).

Table 2

Average concentrations of heavy metals in soil within zinc foundry Miasteczko Śląskie in years 2004–2007 (mg/kg dry matter)

Metal	Minimum	Maximum	Average concentration in Polish soils[1]
Zn	428.0±48	1220±43	35–80
Pb	530.0±52	1123.0±81	13–25
Cd	4.0±0.2	10.9±0.9	0.05–0.3

[1] According to [12].

Table 3

Parameters of soil and sewage sludge-amended soil taken for experiment

Parameters	Soil	Soil + 20% sewage sludge	Soil + 30% sewage sludge
N_{tot} (mg/g)	0.20	1.19	1.52
TC (mg/g)	13.76	20.88	28.69
C:N	71:1	18:1	18:1
P_2O_5 (mg/100 g)	2.88	23.87	28.86
Humic acid (mg/g)	10.74	10.89	11.21
pH in H_2O	5.24	6.14	6.53
pH in KCl	5.03	5.88	6.37
CEC (cmol(+)/kg)	2.15	4.10	8.50
Sorption capacity (cmol(+)/kg)	5.25	6.30	10.90

It was found that the addition of 30% (w/w) of sewage sludge caused approximately a 6-fold increase of willow biomass (mainly above ground) and the use of only mycorrhizal fungi (both strains) caused one- and three-fold increase of willow biomass. However, in the containers with the plants whose roots were colonized by fungi and which are grown on sewage sludge, a decrease of biomass production compared with the combinations without mycorrhizal fungi was noted (table 4, figure 1). In the case of sunflower, root mycorrhization increased the plant biomass about 0.5 time and the sewage sludge added at the rates of 20% (w/w), and 30% (w/w) caused a five-fold and even a thirteen-fold increase, respectively. Simultaneous root mycorrhization and sewage sludge addition increased the biomass by 18 and 38% (table 5, figure 2).

Table 4

Willow biomass (g dry mass ± standard deviations) obtained after experiment

Combination	Root (g)	Stem (g)	Leaf (g)	Aboveground (g)	Plant (g)
Control soil	2.82±1.16	3.17±1.12	6.27±1.57	9.98±2.69	12.80±3.85
Soil + 30% sewage sludge	39.65±0.59	17.65±2.18	18.27±1.21	35.93±3.40	75.85±4.00
Soil + mycorrhiza 1	9.85±4.94	6.04±1.60	9.73±0.88	15.78±0.87	25.63±5.10
Soil + mycorrhiza 1 + 30% sewage sludge	5.81±0.89	8.69±1.74	13.20±3.74	21.89±4.88	27.71±4.96
Soil + mycorrhiza 2	6.25±2.64	4.35±1.19	8.76±0.49	13.11±4.96	19.36±1.46
Soil + mycorrhiza 2 + 30% sewage sludge	5.34±1.52	11.07±2.36	13.32±1.32	24.40±0.87	29.74±5.10

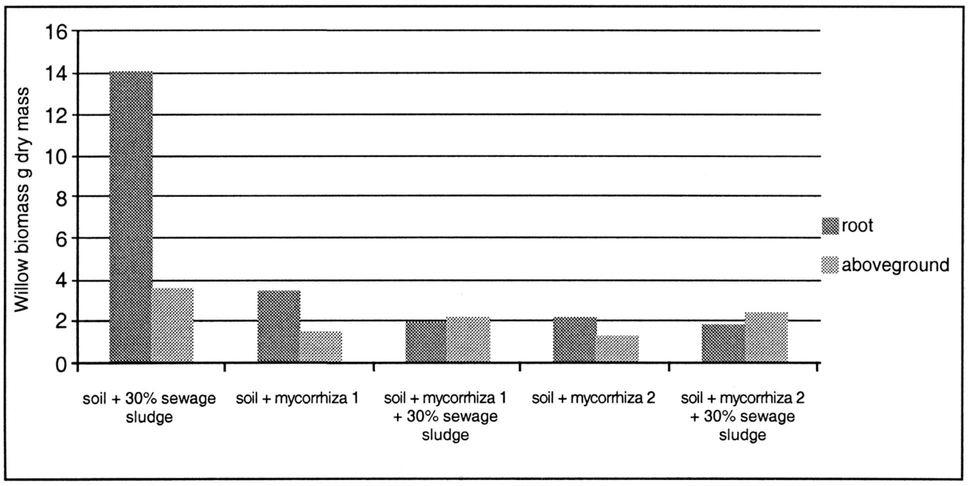

Fig. 1. Effect of soil improvement on willow biomass increase

Table 5

Sunflower biomass (g dry mass ± standard deviations) obtained after experiment

Combination	Root (g)	Stem (g)	Leaf (g)	Flower (g)	Aboveground (g)	Plant (g)
Control soil	0.80±0.49	1.35±0.70	1.46±0.50	*	2.81±0.40	3.61±0.81
Soil + mycorrhiza	0.94±0.11	1.39±0.50	2.42±0.63	*	3.81±1.13	4.75±1.25
Soil + 20% sewage sludge	2.09±0.65	3.17±1.33	7.95±1.29	1.38±0.45	12.50±1.46	14.59±2.11
Soil + mycorrhiza + 20% sewage sludge	2.16±0.63	4.34±1.20	8.98±1.37	2.30±1.87	15.62±4.45	17.78±5.08
Soil + 30% sewage sludge	7.48±0.74	11.92±0.62	20.41±0.46	5.19±0.75	37.52±1.44	45.00±1.57
Soil + mycorrhiza + 30% sewage sludge	16.20±6.90	14.07±3.22	22.05±0.43	9.60±2.36	45.72±2.12	61.92±4.79

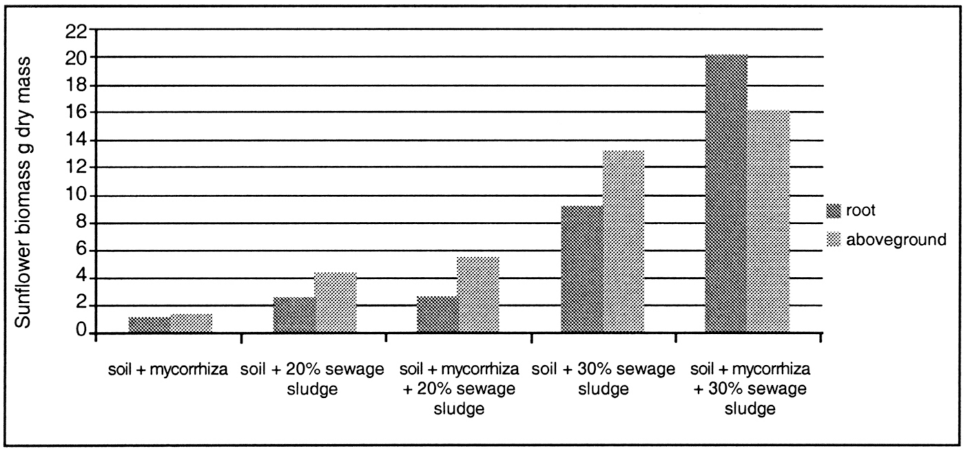

Fig. 2. Effect of soil improvement on of sunflower biomass increase

The current paper is a continuation of the studies described in [13], [14]. The addition of sewage sludge had a promising effect on the cultivation of Scots pine (mycorhized with *Hebeloma crustuluniforme*), birch and alder used for reclamation of degraded land. In the case of willow, an important role of humidity during the experiments was observed. At higher temperature (up to 30 °C) and relatively low humidity (<60%), the biomass of *Salix viminalis* grown on soil with 30% sewage addition after 6 months was even 27 times higher (the aboveground plant organs) and 7 times higher (underground plant organs) than that of control [14].

To sum up, mycorrhization and sewage sludge addition enhanced both plant biomass (willow and sunflower) and the plant tolerance to heavy metals. Sewage sludge provides soil with water and nutrients, and mycorrhiza improves the growth of plants by enhancing the uptake of water and nutrients (mainly P). Fast growing willow and sunlfower with micorrhizal fungi colonized roots can be extremely useful for phytoremediation and ecosystem reclamation under natural conditions

ACKNOWLEDGEMENTS

The study was supported by the Polish Ministry of Science and Higher Education (grant No. R1400603) and by the university internal grant BS/401/301/2008.The phytotron chamber was bought within the project: Equipment of Central Environmental Laboratory WKP_1/1.4.3/2/2005/61/180/365/ 2006/U.

REFERENCES

[1] PULFORD I.D., WATSON C., *Phytoremediation of heavy metal-contaminated land by trees. A review*, Environ. Int., 2003, 29, 529–540.

[2] KRÄMER U., *Phytoremediation: novel approaches to cleaning up polluted soils*, Current Option in Biotechnology, 2005, 16, 133–141.

[3] TURNAU K., JURKIEWICZ A., GRZYBOWSKA B., *Rola mikoryzy w bioremediacji terenów zanieczyszczonych*, Kosmos, 2002, 51, 2, 185–194.

[4] LYNCH J., MOFFAT A.J., *Bioremediation – prospects for the future application of innovative biological research*, Annals of Applied Biology, 2005, 146, 217–221.

[5] DICKINSON N.M., PULFORD I.D., *Cadmium phytoextraction using short rotation coppice Salix: the evidence trail*, Environ. Int., 2004, 31, 609–613.

[6] DAVIES Jr. F.D., PURYEAR J.D., NEWTON R.J., EGILLA1 J.N., SARAIVA GROSSI J.A., *Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (Helianthus annuus)*, J. Plant Physiol., 2001, 158, 777–786.

[7] KARCZEWSKA A., KABAŁA C., *Metodyka analiz laboratoryjnych gleb i roślin*, Akademia Rolnicza we Wrocławiu, 2005.

[8] PN-ISO 10390: 1997. *Jakość gleb. Oznaczanie pH.*

[9] MOCEK A., DRZYMAŁA S., MASZNER P., *Geneza i klasyfikacja gleb*, Wyd. Akademii Rolniczej w Poznaniu, 2000.

[10] PN-ISO 11261:2002. *Jakość gleby. Oznaczanie azotu ogólnego. Zmodyfikowana metoda Kjeldahla*.

[11] PN-EN 1189: 2000. *Jakość wody – Oznaczanie fosforu – Metoda spektrofotometryczna z molibdenianem amonu.*

[12] KABATA-PENDIAS A., PENDIAS H., *Biogeochemia pierwiastków śladowych*, WN PWN, Warszawa, 1999.

[13] FIJAŁKOWSKI K., KACPRZAK M., BIEŃ J., *The evaluation of possibility to use seedlings of Helianthus annuus colonized with mycorrhizal fungi in reclamation of soils contaminated with heavy metals in addition of the sewage sludge*, Conference proceedings of Central European Conference ECOpole'06, Duszniki Zdrój, 19–21.10.2006, 186–190.

[14] FIJAŁKOWSKI K., KACPRZAK M., *Evaluation of possibility of municipal sewage sludge using to improve the physicochemical properties of polluted soil and plant-biomass production*, Polish Journal of Environmental Studies, 2007, Vol. 16, No. 2 A (III), 564–568.

WPŁYW MIKORYZ I OSADÓW ŚCIEKOWYCH NA WIELKOŚĆ BIOMASY SŁONECZNIKA I WIERZBY WICIOWEJ W PROCESIE FITOREMEDIACJI GRUNTÓW ZDEGRADOWANYCH W STREFIE HUTY CYNKU

Określono, jak nawożenie osadami ściekowymi gleb zanieczyszczonych metalami i równoczesna mikoryzacja korzeni słonecznika zwyczajnego i wierzby wiciowej wpływają na wielkość biomasy obu roślin.

Doświadczenie wazonowe przeprowadzono w warunkach sterowanych w wielkogabarytowej komorze fitotronowej. Trwało ono trzy miesiące w temperaturze 25 °C (dzień) i 17 °C (noc) z zaindukowanym fotoperiodem 16/8 i wilgotności od 60 do 90%. W badaniach wykorzystano osady ściekowe pochodzące z oczyszczalni przy zakładach płyt pilśniowych oraz grzyby mikoryzowe z izolatów naturalnie występujących symbioz (ekto- i endomikoryza). Rośliny uprawiano na zdegradowanej glebie pochodzącej ze strefy oddziaływania huty cynku i ołowiu w Miasteczku Śląskim. Stwierdzono, że 30% (w/w) dodatek osadów ściekowych spowodował średnio 6-krotny przyrost biomasy wierzby (głównie części nadziemnej), natomiast zaszczepienie jej korzeni dwoma szczepami grzybów mikoryzowych (podłoże bez dodatku osadów ściekowych) zwiększyło biomasę odpowiednio jedno- i trzykrotnie. Jednak mikoryza miała wpływ na ograniczenie przyrostu roślin. Mikoryza korzeni słonecznika zwiększyła przyrost biomasy o około 0.5 raza, dodatek osadów ściekowych zaś w stosunku 20% (w/w) ok. 5 razy, a 30% (w/w) ok. 13 razy. Zaszczepienie korzeni mikoryzą i dodatek do gleby osadów ściekowych w wyżej wymienionych proporcjach spowodowały jeszcze intensywniejszy przyrost biomasy – odpowiednio o 18% i 38%.