PHYTOREMEDIATION OF SOIL AND GROUNDWATER CONTAMINATED WITH PESTICIDES: POTENTIAL USE OF POPLAR CLONES.

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Abstract
Clones of poplar (*Populus*) species and hybrids were cultivated in soil contaminated by a pesticide mixture originating from a pesticide tomb (Niedzwiady, Poland), in a field and a pot experiment. Growth responses of the *Populus* shoots and mycorrhizal colonization of the root systems were studied. Four poplar clones characterized by a high tolerance to the pesticide mixture were selected from 38 clones tested. Concentrations of certain pesticides in groundwater and an evaporation tank in the vicinity of the storage site (Niedzwiady, Poland) were monitored.

Introduction
Pesticide pollution of soil and groundwater may come from several sources, such as the leaking of surface applied pesticides (1), leaking after total weed control, from waste disposals (2) and from bunkers or other unsafe storage sites of unwanted obsolete pesticides (3, 4). The last example can be particularly dangerous to the environment and human health. In Poland approximately 60 000 tons of obsolete pesticides are stored in bunkers (4).
Phytoremediation, which uses plants and their associated microorganisms (mycorrhizal fungi and bacteria) to decontaminate polluted soil and groundwater, is a relatively new and promising technology. The key to efficient bioremediation lies in the selection of suitable plants and microorganisms.
Poplars - deep-rooted, water-loving trees stabilize soil and, by a high water uptake and transpiration, slow down the pesticide expansion in soil towards the groundwater (5). Poplar trees have been shown to have a relatively high potential in phytoremediation of soil and water contaminated with toxic compounds (5, 6, 7, 8, 9).
Mycorrhizal symbiosis is known to improve the uptake of water by plant root systems and ameliorate the effects of various environmental stresses (10). Mycorrhizal fungi and mycorrhiza helper bacteria (MHB) can stimulate the degradation of toxic compounds. Poplar roots may be associated with both ectomycorrhizal and arbuscular mycorrhizal fungi, but mechanisms of the dual colonisation are not well understood (12).
The aims of this study were to evaluate: 1) concentrations of selected pesticides in groundwater at a former storage site of unwanted obsolete pesticides in Poland, 2) the effect of soil contaminated by pesticides on the growth of 38 poplar clones, 3) the influence of pesticides on mycorrhizal colonization of poplar roots.

Materials and Methods
Field experiment The experiment was established at a site where unwanted, obsolete pesticides had been stored for more than 20 years (Niedzwiady, Central Poland) (Fig. 1). Detailed information about this site was presented elsewhere (4, 11, 13, 14). The pollutants were a mixture of pesticides, in part unidentified (13, 14). The main compounds identified in the soil and groundwater at the study site were: MCPA, DNOK, 2,4-D, α-HCH, γ-HCH (Lindane), atrazine, chlorpropham (4, 13, 14, 15).
In April 2000 one-year-old unrooted stem cuttings, each of 1.0 m in length, from 38 clones of *Populus* spp. (114 cuttings in total) were planted directly in the pesticide-contaminated soil at the upper surface of the pesticide tomb, at a depth of 1 m, spacing 0,5 x 0,5 m. The plant material was originated from the collection of *Populus* clones located at a nursery of woody plants in Kórnik, Poland. In October 2002 the poplar shoots were cut and growth parameters were measured. Concentrations of selected

pollutants were measured systematically in groundwater at the study site according the methods presented elsewhere (11, 14).

Pot experiment One-year-old unrooted poplar stem cuttings, each of 25 cm in length, from 13 clones of *Populus* spp. -were potted in April 2002 in soil transferred from *Populus* collection located in the Kórnik nursery (control) and in a mixture of control soil and the pesticide-contaminated soil originating from the pesticide tomb in Niedzwiady (3:1, v/v). In October the poplar plants were harvested and the growth parameters and mycorrhizal colonization evaluated.

Results

All 38 poplar clones tested in the field experiment were able to grow in the pesticide-contaminated soil, although they differed widely in growth parameters (Table 1). This result indicated a high tolerance of selected poplar clones (*Populus* No 1, No 35, No 19, No 38, No 24, No 33, No 15, No 2) to the pesticides present in the soil. The pot experiment showed an increased growth of some of the poplar clones tested (*Populus* No 38, No 1, No 35, No 2) in the presence of the pesticide-contaminated soil as compared to the poplar plants grown in the unpolluted soil (Table 2). It seems to be possible that the poplars can use some of the contaminants for growth purposes. However an experimental confirmation of such processes is needed. Stimulation of the growth parameters was followed by a good survival of the poplar plants.

Over a 3,5 year period (1999-2002), a decline in concentration of selected pesticides in the groundwater was documented (Fig. 2).

Most of the poplar clones grown in pot cultures in the soil transferred from the *Populus* collection developed both ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM). However wide differences in proportion of ECM and AM colonization between clones were observed (Fig. 3). Roots of the poplar clones grown at the pesticide-contaminated site in Niedzwiady were colonized only by arbuscular mycorrhizal fungi.

Conclusion

Clones of *Populus* characterized by a high survival and growth potential when grown in a soil heavily contaminated with pesticides and with a high ability to develop arbuscular mycorrhiza, such as poplar clones 1, 2, 35, 38 tested in this study, seem to have an advantage in pesticide polluted soils and could be used for afforestation of pesticide polluted sites.

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Table 1. Biomass and high growth of one-year-old shoots of poplar clones grown in pesticide-contaminated soil in the field experiment (Niedzwiady, Poland) (n = 3).

No	Poplar clones	Biomass [d.w.g]	± SE	Height [cm]	± SE
1	2	3		4	
1	*Populus* No 1	**24,8**	±3,2	**85,7**	± 5,2
2	*Populus* No 35	**23,9**	±9,3	**112,3**	± 25.4
3	*Populus* No 19	**22,9**	±5,2	**105,0**	± 15.3
4	*Populus* No 38	**20,2**	±5,0	**131,3**	± 13.6
5	*Populus* No 24	**19,6**	±3,9	**111,0**	± 6.4
6	*Populus* No 33	**18,0**	±3,1	**87,7**	± 8,8
7	*Populus* No 15	**17,7**	±2,1	**99,3**	± 4,7
8	*Populus* No 2	**17,3**	±1,9	**99,7**	± 7,8
9	*Populus* No 6	**17,1**	±2,3	**100,7**	± 7,9
10	*Populus* No 4	**17,1**	±3,3	**73,3**	± 3,2
11	*Populus* No 34	**16,0**	±5,0	**80,7**	± 21,9
12	*Populus* No 5	**15,6**	±2,6	**91,3**	± 10,7
13	*Populus* No 31	**15,2**	±3,0	**52,0**	± 16,8
14	*Populus* No 28	**14,7**	±2,5	**90,0**	± 0,6
15	*Populus* No 27	**14,6**	±1,4	**81,0**	± 11,5
16	*Populus* No 25	**14,0**	±3,3	**74,0**	± 15,7
17	*Populus* No 17	**14,0**	±0,6	**57,0**	± 4,7
18	*Populus* No 9	**13,9**	±6,4	**70,0**	± 29,8
19	*Populus* No 37	**11,9**	±4,8	**66,0**	± 15,7
20	*Populus* No 18	**11,8**	±3,7	**54,0**	± 10,0
21	*Populus* No 22	**11,6**	±2,8	**74,0**	± 3,1
22	*Populus* No 23	**11,1**	±2,0	**49,7**	± 13,3
23	*Populus* No 29	**10,6**	±2,6	**93,3**	± 6,8
24	*Populus* No 3	**10,4**	±0,4	**70,0**	± 13,1
25	*Populus* No 30	**10,1**	±1,2	**93,7**	± 5,9
26	*Populus* No 14	**10,0**	±2,0	**48,0**	± 37,6
27	*Populus* No 20	**9,9**	±0,8	**68,3**	± 3,7
28	*Populus* No 11	**9,8**	±2,6	**70,3**	± 14,9
29	*Populus* No 16	**9,3**	±0,2	**53,7**	± 23,8
30	*Populus* No 36	**8,9**	±2,4	**49,0**	± 7,5
31	*Populus* No 39	**8,4**	±4,7	**45,0**	± 16,3
32	*Populus* No 32	**8,2**	±7,3	**38,0**	± 25,5
33	*Populus* No 10	**8,1**	±0,2	**43,7**	± 15,8
34	*Populus* No 26	**7,5**	±1,0	**59,0**	± 4,2
35	*Populus* No 12	**6,9**	±3,1	**55,0**	± 16,5
36	*Populus* No 7	**4,7**	±2,2	**21,5**	± 9,4
37	*Populus* No 13	**4,6**	±1,1	**14,3**	± 9,0
38	*Populus* No 8	**3,7**	±1,5	**23,0**	± 13,1

± SE - standard error;

d.w. - dry weight

Table 2. Survival and high growth of one-year-old shoots of poplar clones grown in pesticide-contaminated soil and in an unpolluted soil (control) in the pot experiment in a greenhouse (n = 4).

No	Poplar clones	Survival [%]		Height [cm] ± SE		Test.*t* (5:6)
		Contaminated soil	Control	Contaminated soil	Control	
1	2	3	4	5	6	7
1	*Populus* No 38	100	100	**143.1** ± 3.5	**92,4** ± 5.2	0,0002
2	*Populus* No 1	100	75	**111.2** ± 5.6	**80,1** ± 5.4	0,0110
3	*Populus* No 35	100	75	**134.3** ± 9.2	**78,5** ± 2.5	0,0068
4	*Populus* No 2	100	75	**111.3** ± 12.4	**77,5** ± 2.6	0,0698
5	*Populus* No 12	75	75	**89.1** ± 3.2	**71,8** ± 1.2	0,0070
6	*Populus* No 4	75	50	**98.8** ± 4.2	**69,2** ± 2.2	0,0098
7	*Populus* No 13	25	75	112,5 */	**66,6** ± 3.4	--
8	*Populus* No 18	75	100	**92.0** ± 1.8	**64,9** ± 7.2	0,0294
9	*Populus* No 22	25	75	72,1 */	**63,9** ± 4.2	--
10	*Populus* No 36	100	50	**88.9** ± 1.2	**60,4** ± 2.4	0,0182
11	*Populus* No 8	50	50	**62.0** ± 10.5	**45,4** ± 2.3	0,2625
12	*Populus* No 25	0	50	N.S.	[66,0] ± 7.9	--
13	*Populus* No 28	0	50	N.S.	[39,0] ± 24.7	--
	MEAN	63,5	65,4	**92,3**	**70,1**	

± SE - standard error;

*/ - one plant (tree)

N.S. - not survive

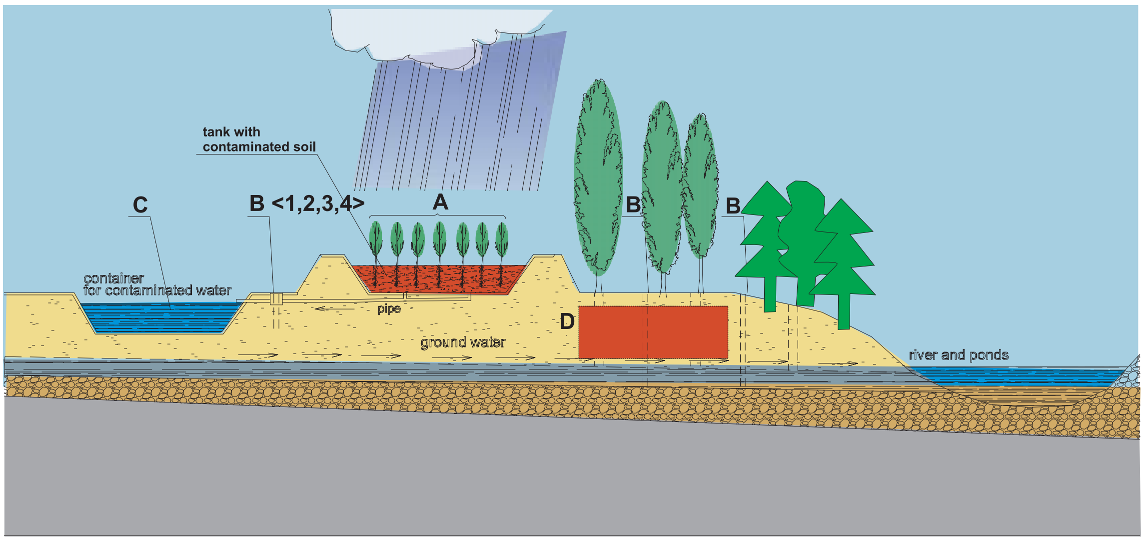

Fig.1. Niedzwiady (Poland): Layout of tomb containing obsolete pesticides. A - deep rooted poplar tree plot in excavated contaminated soil from former old tomb with obsolete pesticides; B - set of monitoring wells (piezometers); C - evaporation tank with contaminated water; D - location of old tomb; (adapted and modified from: L. Pilc and S. Stobiecki 1999)

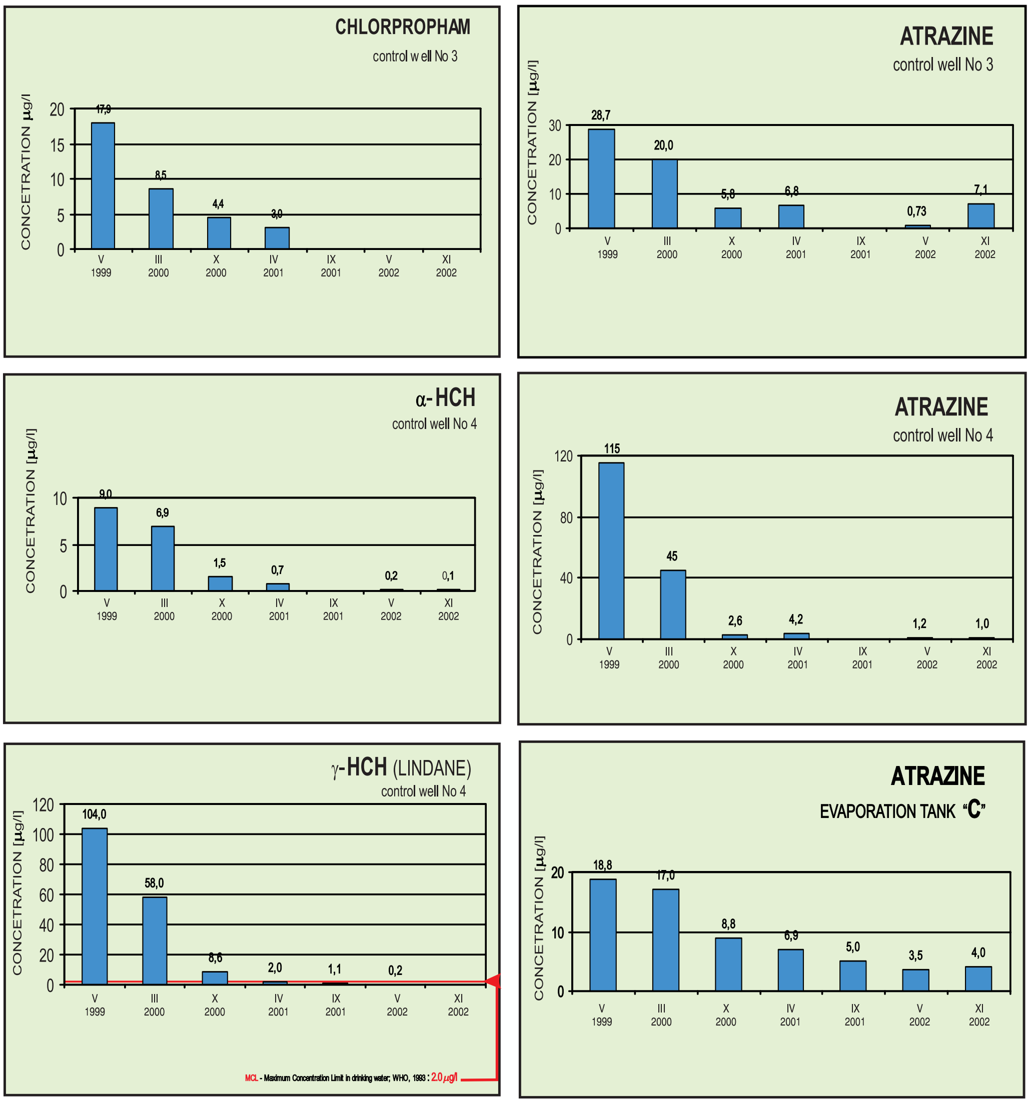

Fig. 2. Concentration of selected pesticides in control wells (for ground water) and evaporation tank C; (compare Fig. 1) between May 1999 and November 2002.

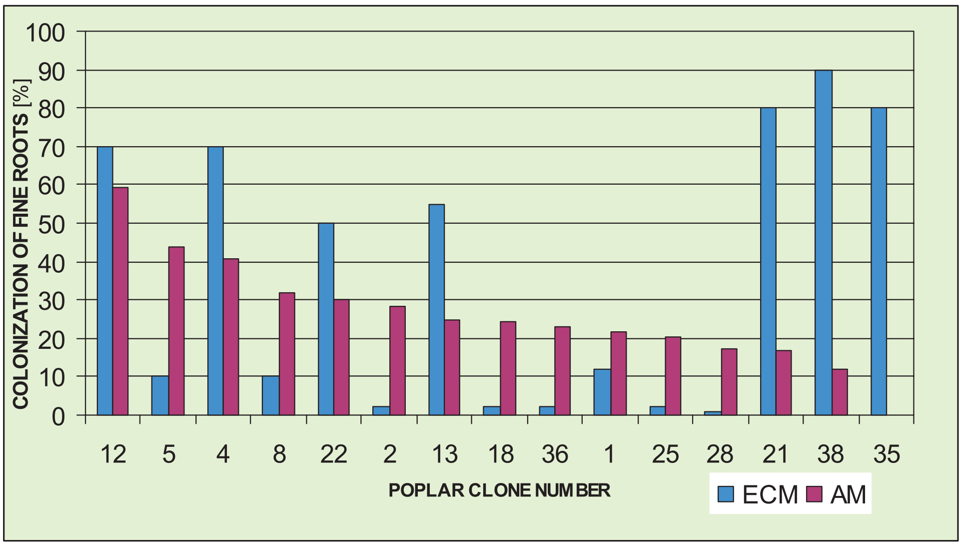

Fig. 3. Average ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) colonization of *Populus* fine roots potted in soil transferred from the *Populus* collection in Kórnik nursery.