B.2. Soil

Primary selection of poplars suitable for areas polluted with pesticides

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Summary

Objective

The feasibility of using plants for limiting the spread of pesticides released from inappropriate storage facilities was investigated. This was carried out during the present case study by the selection of plant species best suited for tolerance and uptake of pesticides from water and soil near the Niedźwiady village (50 km south of Poznań, Poland), where a leaking tomb containing xenobiotics is situated. The leakage is posing risks of heavy contamination of groundwater and consequently, to the surface water used in the area for drinking and for agricultural purposes. The extent of the problem, affecting a huge number of sites (more than 350 in Poland only) with different contamination issues, calls for the development of fast, economic and reliable methods of tolerant plant selection.

Materials and methods

The screening of plant species suitable for phytoremediation was approached through studies at four different levels: plant tissue culture, small-scale experiments, *in-situ* pilot-scale remediation, and the planning of full-scale procedures.

Results

The various poplar clones and cultivars that were tested have shown different degrees of tolerance to the xenobiotic solution obtained from the original contaminated site. Some clones have shown consistent responses both *in-situ* and in controlled environment experimental settings. Of the plants presenting high tolerance, the most promising cultivar was *P.*'DORSKAMP' (*P. x canadensis*).

Conclusions

The preliminary results obtained in this study suggest possibilities for the setting up of reliable methods of selecting woody plants suitable for phytoremediation purposes.

Keywords

Phytoremediation, pesticides, xenobiotics, poplar, *Populus*.

Introduction

Hazards posed by unwanted obsolete pesticides stored in places, which are unsafe for the surrounding environment is a widespread problem that concerns many countries, particularly in Central and Eastern Europe. In Poland, the total amount of obsolete pesticides stored in bunkers or similar facilities is estimated as about 60, 000 tonnes, located in more than 350 places (Stobiecki *et al.*1998). Only a few states have already disposed some limited amounts of stocks of obsolete pesticides and an additional problem of contaminated soils, especially around former production and storage sites, also still remains unsolved. The scale of the hazard (the number of contaminated places and the quantity of polluting substances involved), requires the implementation of solutions that are both a low-cost and environmentally friendly.

Pesticides and their metabolites are able to migrate in the groundwater over large distances. Vegetation can significantly help in reducing the impact of pesticides on the environment, by interacting with, direct up taking and metabolisation the available pollutants such as triazine (Nair and Schnoor 1993), or atrazine (Burken and Schnoor, 1997). Furthermore, the establishment of deep-rooted vegetation (like poplars) stabilises soil and water uptake and transpiration, slows down the downward pesticide spreading in the groundwater. Plants also exudate enzymes and increase the amount of organic carbon in the soil, thereby stimulating microbial activity and enhancing mineralisation in the rhizosphere that is attributable to mycorrhizal fungi and microbial consortia (Schnoor *et al*, 1995). The simple establishment of plants tolerant to the contaminants in the soil should be considered as only one of the steps required for soil reclamation. This is because the re-establishment of a soil, which is biologically impoverished by pollution calls for a complete recolonisation including vegetation and microorganisms such as mycorrhizas and bacteria. However, identification of tolerant plants is a basic requirement for phytoremediation and for revegetation. The approach to apply phytoremediation for the solution of pesticide soil pollution problems can benefit from studies on a different scale ranging from tissue culture through small-scale experiments to *in-situ* remediation. The present study represents a case study of contamination caused by a mixture of pesticides. The feasibility of the use of plants to prevent the spread of contaminants stemming from leaking storage facilities was the basis for an Italian - Polish bilateral project for evaluating methods for the selection of plant species suitable for the uptake of pesticides from water and soil. A tomb containing about 60 tonnes of organic xenobiotics situated near Niedzwiady village was chosen as project location. Here, leakage of pesticides into the surrounding soil has caused the destruction of a part of a 60 years old Scotch pine (*Pinus silvestris* L.) forest and is currently posing risks of heavy contamination of groundwater and consequently to the aquifer (drinking water intakes, household wells) and surface waters (fish ponds, streams) used for agricultural and drinking water purposes in the area. The set up of a consistent method for *ex-situ* plant screening could be of use to select the more suitable genotypes for the presented purpose. However, the set up of a fast, economic and reliable *ex-situ* selection method could provide more basic information and opportunities for facing similar issues, thus approaching many different situations with the help of basic preliminary plant tolerance studies.

Materials and methods

The situation in Niedźwiady is outlined in Figure 1. A tomb, made of concrete, containing the unwanted pesticides (a) was established on a small hill some decades ago. Flow of contaminants has polluted the surrounding environment; pesticides are migrating through the soil and groundwater (arrows); excavation and isolation of highly contaminated soil (A) has been performed, but without the establishment of obstacles, pollutants already spread, over a large volume of soil, will still migrate towards creeks and surface water (c).

The problem calls for:

- highly tolerant plants (both: woody and perennial) to be established in the heavily contaminated soil in/near the tomb (Fig.1.A, a);
- trees with a deep root system able to limit downward pesticide migration into groundwater and possibly to reach the water table;
- fast growing (with deep rooting system and pesticides tolerant) trees can be used to establish barriers to pollutants before they are delivered to the surface water (C).

The capacity of plants, particularly of trees characterised by a deep root system, to tolerate and remove the xenobiotics is studied on different levels.

Part of the selection work is conducted in Poland and included *in-situ* experiments with plants growing in heavily contaminated soil inside the original forest (a); with plants growing on soil removed from the nearest surrounding of the bunker (A); screening of potted plants irrigated with the xenobiotic solution. Groundwater was obtained from a piezometer (B) in Niedźwiady. The Institute of Dendrology in Kórnik conducted researches. More than 36 poplar clones and cultivars are actually in the process of evaluation. The composition of the solution (Stobiecki *et al.* 1999 - unpublished data), used in the pot experiment is presented in Table 1.

Complementary experiments are also being conducted at ISTEA-CNR in Italy using the same xenobiotic solution obtained from Niedźwiady. These experiments aim at developing fast screening methods for the selection in both in a controlled environment and *in-vitro*, and to compare results with those obtained in Poland. Fifteen poplar genotypes have been chosen on the basis of their observed development *in-situ* or on known desirable plant traits (e.g. a fast growing root system) system). These included *P. x canadensis* (=*P. x euramericana*) (*P.*'I-214', *P.*'BOCCALARI', *P.*'ROBUSTA', *P.*'DORSKAMP', *P.*'GELRICA', *P.*'MARILANDICA', *P.*'SAN MARTINO'); *P. alba* (*P.*'VILLAFRANCA', *P.*'PB TICINO'); P. *x interamericana* (*P.*'BOELARE', *P.*'ERIDANO'); *P. nigra* (*P.*'JEAN POUTRET', *P.*'PN TICINO'); *P. deltoides* (P.'LUX'), and P. *x canescens* (*P.* 'PG TICINO'). Experiments are conducted on potted plants, on excised shoots, or on *in-vitro* cultures.

Table 1. Pesticide concentrations in groundwater [µg/l]; Niedźwiady - control well, piezometer (B); 31.05.1999

		MCL [µg/l]			MCL [µg/l]
Atrazine	1.3	2.0	alpha-HCH	10.0	
Chlorfenson	239.0		beta-HCH	524.0	2.0
Chlorpropham	183.0		MCPA	1.8	2.0
2,4-D	31.0	30.0	Mekoprop	16.0	
op'-DDT	11.0	2.0	Metoxychlor	18.7	20.0
pp'-DDT	1.3	2.0	Metylparathion	0.9	
Dichlorfos	1.5		Simazine	4.1	2.0
DNOC	247.0		Tiometon	15.0	
Fenitrothion	161.0				

MCL = Maximum Concentration Limit in drinking water [in: µg/l], according to WHO, 1993

Field experiments

For the field experiment (*in-situ*), one-year-old unrooted cuttings of 1 m length (from a stool-bed) of 36 various good growing clones and cultivars were used. The experiment was established directly inside the tank (A) with contaminated soil (Stobiecki *et al.*1998). Plant development and appearance of injuries (leaf and shoots chlorosis, necrosis and deformation) were monitored. Plant growth was measured as the number of new shoots developed and the total length of the shoots in each plant.

Potted plant tests

For the potted plant tests, equivalent experiments were conducted in both Kórnik and Bologna. Woody cuttings were rooted in quartz sand in a greenhouse. After rooting, only one shoot per cutting was allowed to develop. Control plants were irrigated only with tap water, and the other plants were treated with increasing doses up to 100 ml of 100% solution of xenobiotics derived from bunker leakage (NIED). Appearances of injury symptoms and plant growth were monitored over a six-week period in Bologna and a twelve-week period in Kórnik.

Excised shoots

For the experiment with excised shoots, actively growing shoots were taken from mother plants and placed in 10 ml tubes containing NIED or water. Evidence of damage and shoot water uptake was monitored over a three-week period.

In-vitro experiments

For the *in-vitro* experiments, tissue cultures were established for six chosen genotypes. In vitro tests for tolerance have been conducted with the NIED mixture and with some of the single compounds determined to be present in the mix (2,4-D, MCPA, DNOC) (Predieri and Gatti, 1999) on *P.*'PG TICINO' and *P.*'PB TICINO'. Microcuttings had the basal part immersed in the solutions prior to transfer to standard micropropagation media.

Results

Field experiments

During the first growing season the tested poplar clones already exhibited high variability in growth responses. Some of the clones showed a satisfactory growth level, producing up to 15 new shoots including *P.*'DONK', *P.*'DORSKAMP', *P.*'HOOGVORST', and *P.* 'FLEVO'. Regarding total shoot length, the most vigorous clones were:

P.'HOOGVORST' (>600 cm); *P.*'DONK' (560 cm); *P.*'VILLAFRANCA' (525 cm); *P.*'DORSKAMP' (520 cm). In contrast very poor growth were observed in *P. deltoides* 270/81 (43 cm); *P. deltoides* S 6-36 (46 cm); *P.* 'ANDROSCOGGIN' (96 cm). Additionally some shoots of those poplars showed a degree of necrosis. Some of the clones included in this study exhibited injuries and deformation of leaves.

Potted plants

The only consistent result of experiments conducted in Bologna and Kórnik was the growth reduction observed on plants treated with 100 ml of 100% xenobiotics solution but not at lower doses (with 100 ml of 10%). The various genotypes included in this series of experiments exhibited different degrees of tolerance. In the experiment conducted in Kórnik (Figure 2), only one cultivar (*P.*'NE 42') exhibited a significant growth rate reduction when exposed to both 10 % and 100 % doses of the solution. Among the genotypes tested at both locations *P.*'DORSKAMP' exhibited the best growth rate in the presence of xenobiotics. In the experiment conducted in Bologna the most sensitive genotype was *P.*'BOCCALARI'. Six genotypes: *P.*'DORSKAMP', *P.*'GELRICA', *P.*'VILLAFRANCA', *P.*'PB TICINO', *P.*'PG TICINO' and *P.*'ROBUSTA' showed no evidence of growth reduction (Figure 3).

Excised shoots

This test was developed to allow the direct transport of the solution into shoot tissues without a previous selective uptake step that would have normally taken place through the roots. Only some of the genotypes that exhibited high tolerance in the potted plants experiment had this confirmed (*P.*'DORSKAMP', *P.*'PG TICINO', *P.*'ROBUSTA', *P.*'VILLAFRANCA') while others evidenced growth reduction as compared to control shoots grown in water alone (*P.*'GELRICA' 18%, P.'PB TICINO' 29%).

In-vitro cultures

Plant tissue culture's response to NIED was very rapid. These cultures proved very sensitive to pollutants and appeared to be potentially very useful for fast and early screening allowing the use of low amounts of toxic compounds, an important issue for the operator's safety. The observed responses need to be investigated further to establish a solid correlation with field performance.

Discussion and conclusions

Field experiments showed significant differences among the tested clones in growth rate and overall health when grown in the contaminated soil. These variations, observed inside the genus *Populus*, suggest that it is possible to select plant genotypes best suited for reclamation of a given polluted area. A more tolerant genotype can be more successful in its interaction with microorganisms toward the restoration of the environment. The results of the experiments conducted with potted plants allowed the identification of two clones very sensitive to the xenobiotics solution (*P.*'NE 42' and *P.*'BOCCALARI'). These genotypes can be useful as bioindicators of the solution toxicity and allow studies on specific toxic effects.

Some of the other genotypes tested, in particular *P.*'DORSKAMP', exhibited a consistent tolerance level to the pesticide solution, both in the field and in experiments conducted with potted plants as well as with excised shoots. Further studies will indicate if this cultivar has a higher tolerance to the mixture of pesticides present in Niedzwiady, and thus becoming useful in the creation of barriers to pesticide migration in soil and water. The results obtained in these tests will be validated with other field experiments conducted in Niedzwiady.

Tissue cultures results are very preliminary, but the possibility of using tissue culture for early screening and collection of germplasm suitable for phytoremediation purposes indicated the need for a deeper investigation of *in-vitro/in-vivo* correlations. The reliability of selection at tissue culture level could also result in the use of mutation-induced genetic variation (e.g. somaclonal variation), a technique that has already been shown to induce increased tolerance to pesticides in poplars (Michler and Haissing, 1988). All the methods tested can be especially useful for the selection of genotypes with high bio-remediation capacity, but only the correlation of these results with the appropriate field experiments will indicate the ultimate usefulness of the proposed selection methods.

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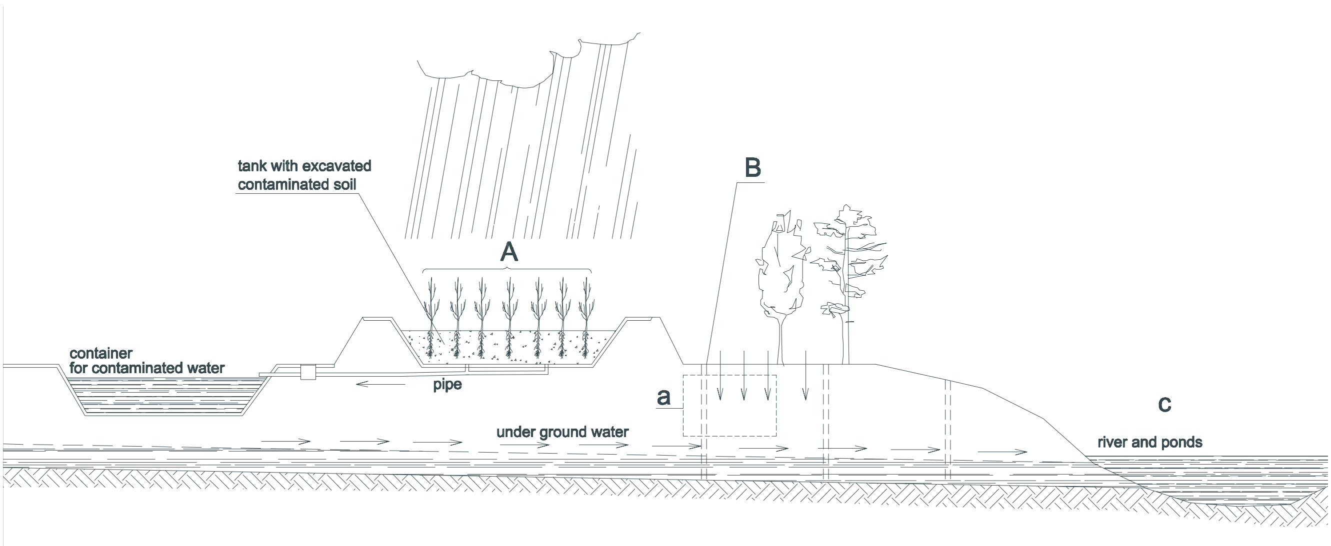

Figure 1. Layout of the Niedźwiady experimental field

A - Field experiment: deep-rooted poplar tree plot; plants are growing on contaminated soil excavated from the former tomb and surroundings;

a - Place were the tomb (bunker) containing pesticides was established some decade ago;

B - Control well (piezometer) - source of contaminated water (leakage) for greenhouse and laboratory experiments;

c - Creeks and superficial water;

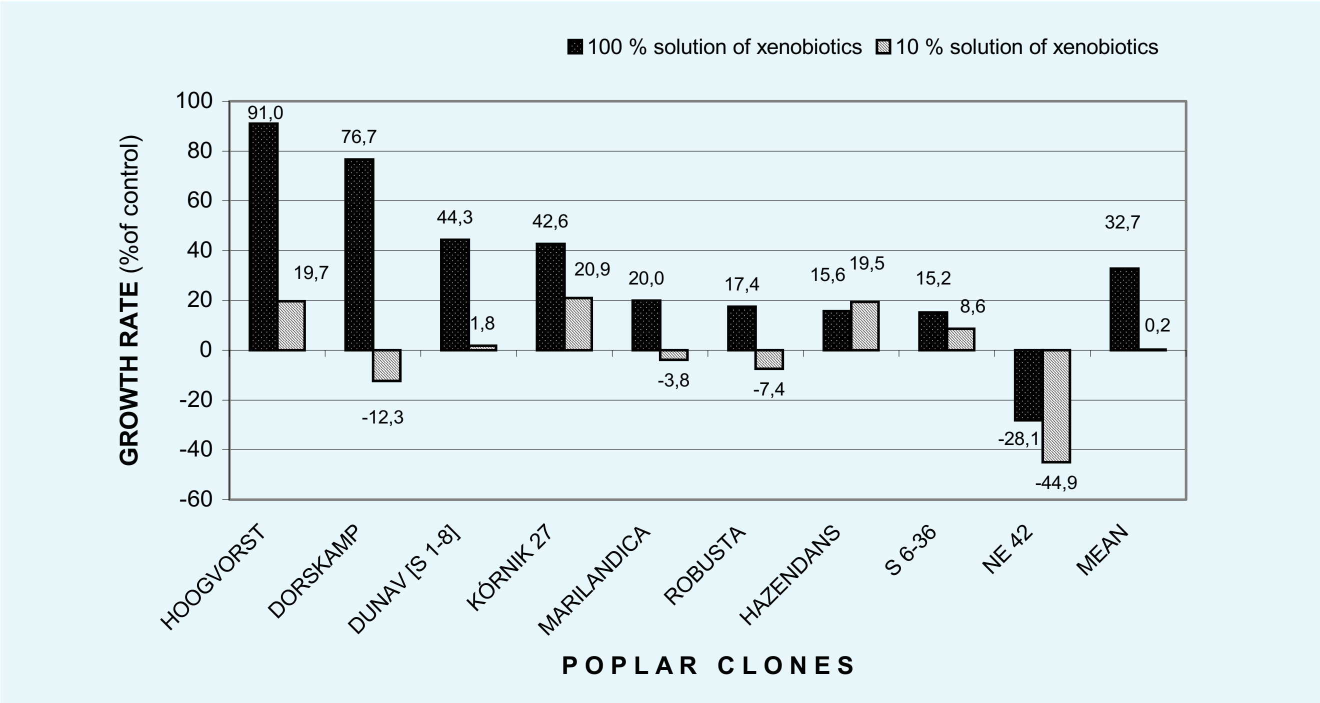

Figure 2. Relative growth rate of investigated poplar clones and cultivars after treatment with 100 ml of 10% and 100% of xenobiotics solution, in a greenhouse pot experiment, Kórnik (Poland). Mean of 4 replicates.

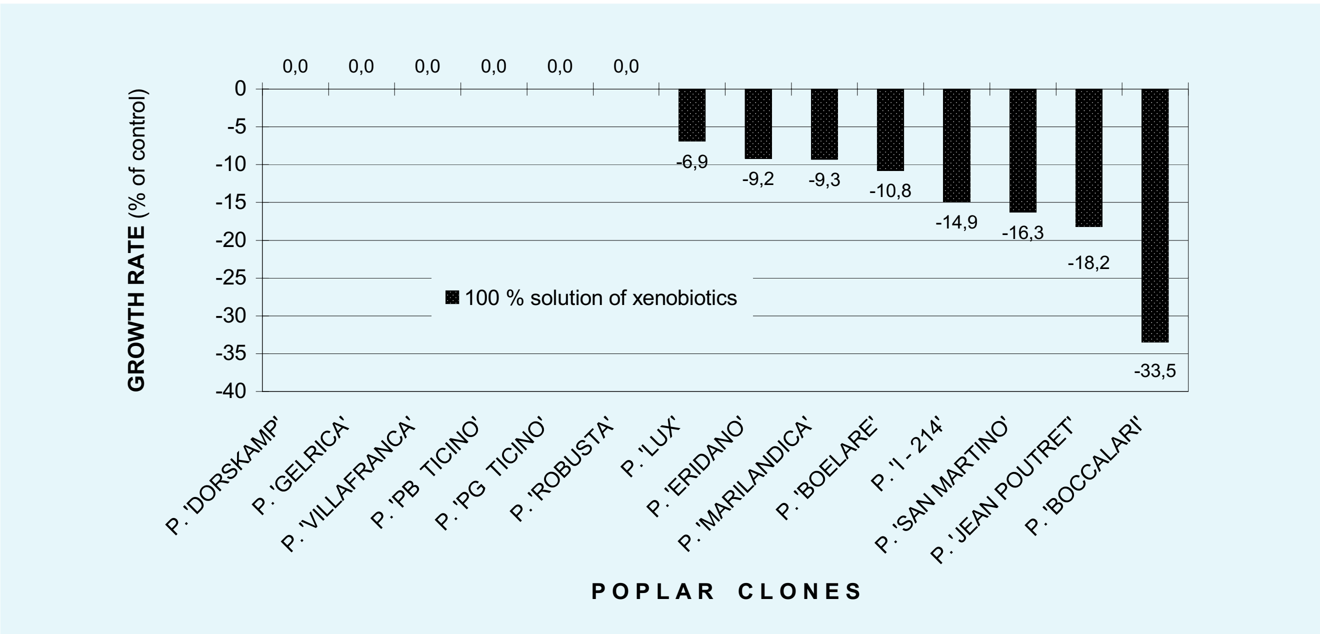

Figure 3. Relative growth rate of investigated poplar clones and cultivars after treatment with 100 ml of 100% of xenobiotics solution in Bologna (Italy).