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1. Introduction

1.1 Constructed wetlands – ecological engineering technologies

Odum (1971) described ecological engineering as half science and half engineering: techniques of designing and operating the economy with nature. Ecological engineering or ecotechnology are defined by Guterstam (1996) as the design of human society with its natural environment for the benefit of both. One of the technologies included in ecological engineering is using constructed wetlands for wastewater treatment.

Constructed wetlands (vegetation filters or treatment wetlands) are artificial complexes of water, matrix, vegetation and the associated invertebrate and microbial communities designed to simulate the ability of natural wetlands to remove pollutants from water (Brix, 1987; Kangas, 2004). They provide an inexpensive and reliable method for treating a variety of waste waters such as sewage, landfill leachate, mine leachate, agricultural run-off, and are comparatively simple to construct, operate and maintain (Randerson, 2006, Randerson et al., 2007).

Based on hydraulic regime CWs may be divided into two main groups: surface flow systems (SF), and subsurface flow systems (SSF) (Kadlec et al., 2000). The latter may employ horizontal flow (HF), vertical flow (VF) or tidal flow (TF) hydraulic regimes and these may be combined in hybrid systems to optimize pollutant removal (Randerson, 2006). Both aerobic and anaerobic processes are involved, but degradation of carbonaceous matter to CO$_2$ and nitrification requires availability of oxygen. That may be achieved most efficiently in compact VF systems; as the surface is flooded, air is forced into the bed, while effluent percolates downwards through the matrix. HF beds typically achieve lower oxygen transfer rates but, with largely anaerobic conditions, they are effective in removing nitrogen to atmosphere via denitrification. SF wetlands most resemble natural wetlands, as the water level is typically above the soil surface (Randerson, 2006). With HF, water flows laterally below the surface, through a gravel bed. Oxygen is consumed by microbial activity, and oxygenation of the bed is limited by surface diffusion and transport via aerenchyma tissue to the root zone, so that anaerobic conditions predominate. Hence nitrification is limited by oxygen, and denitrification is limited by the supply of nitrate and usable carbon compounds. In the VF system, pulses of water flow downwards through layers of increasing particle size. Air is drawn into the bed between each pulse of water. Removal of BOD and
nitrification is very efficient, for a given surface area, but nitrate and phosphate are typically high in the outflow (Cooper, 1999). In subsurface systems the root zone is limited mostly to an upper layer of 30-40 cm, below which the influence of plants is reduced. Shallow HSSF beds (0.2–0.5 m in depth and with a water table close to the top substratum) removed organic matter and nitrogen at very high rates and higher than deeper beds (Garcia et al., 2004, Albuquerque et al., 2009). Those beds are normally more oxidized, possibly due to the shallow water and enhanced oxygen diffusion at the air–water interface.

1.2 Importance of plants in constructed wetlands

Plants commonly used in constructed wetlands include: cattail (*Typha latifolia* L.), reed (*Phragmites australis* (Cav.) Trin ex Steudel), rush (*Juncus effusus* L.), yellow flag (*Iris pseudacorus* L.), and manna grass (*Glyceria maxima* (Hartm.) Holmb.). As well as these typical natural wetland plant species, willow (*Salix sp.*) may be used in constructed wetlands with high efficiency. Willow vegetation filters have been shown to be effective in cleaning polluted drainage water from agricultural land, and as a final stage in wastewater treatment (Aronsson, 2000; Aronsson & Perttu, 2001; Elowson, 1999; Kowalik & Randerson, 1994; Perttu & Kowalik, 1997). Willows have been used successfully in the treatment of agricultural runoff and leachate from landfill sites (Bialowiec et al., 2007; Duggan, 2005; Randerson, 2006) and are especially successful at removing high levels of ammonia and nitrogen from solution. A review of the potential for the use of willow filter beds and short rotation willow coppice (SRC) to treat landfill leachate by Duggan (2005) concluded that a number of studies showed success of willow filter beds in treating leachate and that the leachate treatment improved with the number of the willows.

In a CW, the plant root-soil interface plays a significant role in the removal of pollutants. Oxygen released from roots creates aerobic conditions in the otherwise anaerobic rhizosphere, which induces growth of both heterotrophic and autotrophic aerobic bacteria (nitrifiers) and the aerobic breakdown of organic material (Brix, 1997). Reddy et al. (1989) reported that approximately 90% of the oxygen available in the rhizosphere was transported there by aquatic vegetation. The other 10% arrives by soil surface diffusion (Lloyd et al., 1998).

Plant uptake also plays an important role in the enhancement of N removal, especially in treatment wetlands containing fast-growing plants such as willows (Randerson, 2006). Release of oxygen into the rhizosphere and uptake of nutrients are not the only ways that the presence of plants can facilitate the removal of pollutants.

Most macrophytes release carbon compounds into the rhizosphere (e.g. polysaccharides, sugars, amino acids, organic acids and fatty acids) that may be removed through aerobic oxidation, fermentation and denitrification pathways (Pinton et al., 2007), especially the ones with low molecular weight. Other compounds may be converted into recalcitrant organics (e.g. humins). Organic rhizodeposition includes lysate, root border cells and root exudates, released passively (diffusates) or actively (secretions). The main mechanisms of root exudation are diffusion, ion-channels, vesicle transport and exocytosis. In addition these exudates, which make up 5-25% of photosynthetically fixed carbon, assist the degradation of toxic organic chemicals (Brix, 1987).

Aerenchyma also plays a role in the removal of nitrogen (N) from wetlands through releasing N\(_2\) and N\(_2\)O produced by anaerobic denitrification of NO\(_3\)\(^-\) into the atmosphere (Reddy et al., 1989). Plants are also largely responsible for the release of CO\(_2\) and CH\(_4\) from
the soil, with up to 90% of the net efflux of methane from peat lands being attributed to gas transport by plants (Beckmann and Lloyd, 2001). As well as the influence of plant species, the release of oxygen into the rhizosphere is related to some extent to photosynthesis, light intensity, stomatal aperture, and temperature (Stein and Hook, 2005). The amount of oxygen in the rhizosphere therefore fluctuates over diurnal periods and varies between seasons (Williams et al., 2010). There is higher oxygen release in periods of illumination and even in periods of relatively low light intensity the amount of oxygen released into the rhizosphere can meet the respiratory oxygen demand of the roots and micro-organisms in the rhizosphere (Sheppard and Lloyd, 2002). In view of the differences between plant species in the release of oxygen into the rhizosphere, assessing the oxidizing/reducing capability of the plant system may improve understanding of the effect of this flux on the remediation of LL in treatment wetlands. The majority of microbial processing that occurs in wetlands is attributed to biofilms made up of communities of algae, bacteria, protozoa and invertebrates. It has been shown that up to 90% of organic and inorganic N can be removed from LL by biofilms (Welander et al., 1998).

Other studies have shown the importance of evapotranspiration during hot periods in natural wetlands and also in constructed wetlands. Evapotranspiration may be defined as water evaporation from soil or and open water surface, supported by transpiration of plants (Allen et al., 1998). Tchobanoglous (1987) informs that water plants are more productive than terrestrial ones, because of higher resistance to environmental changes and high photosynthetic efficiency. Annual transpiration of water plants (hydrophytes) is in the range of 1000-1400 mm/year (Siuta, 1996). Williams et al. (1987) compiled a water balance for Jack Valley wetland (155 ha) in USA. Water loss due to evaporation was about 1524 mm/year. Reeds, cattails and, near banks, willows and poplars were the most common colonizing plants. Raisin (1999) assessed the annual evapotranspiration to be 1100 mm/year at the Reids Wetland in NE Victoria, Australia, where Phragmites australis, Juncus usitatus L., and Carex fascicularis Sol. Ex Boot dominated. Also, Wieënner et al. (1999) observed the loss of water at wetlands planted with reed for industrial sewage treatment. The measured annual evapotranspiration was about 1500 mm/year. Białowiec at al. (2006), reported the total lack of effluent from a hydrophyte system planted with reed designed for wastewater treatment.

Evapotranspiration as a process might be used in constructed wetlands for decreasing the volume of treated wastewater or landfill leachate (Dobson & Moffat, 1995). Such soil-plant systems are planted with macrophytes like willows, poplars and reeds, as these are the plants that are recommended by the US EPA (2000) for landfill leachate evapotranspiration. Systems where evapotranspiration dominates might be called evapotranspirative soil-plant systems (Białowiec et al., 2007) or zero-discharge systems (Białowiec & Randerson, 2010). In this chapter the functioning of a zero discharge system located in Poland is described: an evapotranspirative system (ES) with reed at the landfill in Zakurzewo near Grudziądz. Additionally, the possibilities of employing transpiration measurements as a tool in phytotoxicity of landfill leachate assessment are presented.

2. Evapotranspirative system for landfill leachate treatment

2.1 Landfill leachate

One of the most important problems associated with landfill sites is the leachate, that may cause significant environmental impacts. Landfill leachate (LL) is formed when rain water
filters through a landfill becoming contaminated with pollutants (Kjeldsen et al., 2002). The composition of LL varies from site to site and includes various organic and inorganic compounds that may be either dissolved or suspended, but is generally characterised by high levels of total nitrogen (N), ammonia (\(\text{NH}_4^+\) or \(\text{NH}_3\), depending on pH), chemical oxygen demand (COD), sodium (Na), chloride (Cl) and a low ratio of BOD/COD (Jones et al., 2006). A high concentration of ammonia in the range from 50 to 5000 mg/dm\(^3\) (Yildiz and Unlu, 2003) is the main problem causing toxicity of LL, but also oxygen depletion and changes in the fauna and flora of surface water bodies (Christensen et al., 1992, Kjeldsen et al., 2002). It was shown that low phosphorus concentration, usually not exceeding 60 mg/dm\(^3\), can act as a limiting factor in applying LL as fertilizer (Hasselgren, 1998).

The most commonly used method of treatment is to transfer landfill leachate directly to a wastewater treatment plant. In this case, in practice the major treatment process is dilution. Another way of leachate treatment is its recirculation on to the original waste heap. Unfortunately, when this technique is poorly operated, it causes water overloading of the landfill system. There are several technologies of landfill leachate treatment on-site. However, because of high variation in the production and composition of leachate, treatment using just one process is not efficient. Therefore the combined systems of physical, biological and chemical technologies are needed, increasing the cost of treatment.

2.2 Using plants for landfill leachate treatment
Despite differing views on leachate treatment, many experts agree that on-site treatment is desirable, since it is easier to operate, and more economical in terms of cost and energy (Bulc, 2006). Owing to low investment and maintenance costs, there is a growing interest in applying plants in LL disposal (Ashbee & Fletcher, 1993). On-site treatment using constructed wetlands (CW) is one of the low cost methods of LL treatment which has been widely practiced in several countries for many years (Kadlec et al., 2000, Schnoor et al., 1995, Wallace and Knight, 2006, Vymazal & Kropfelova, 2008). CW with reed (Wojciechowska et al. 2009, Wojciechowska & Obarska-Pempkowiak, 2008) as well as with willow (Bialowiec et al., 2007, Randerson, 2006) have been shown to be effective in treating leachate from landfill sites and are especially successful at removing high levels of nitrogen from solution.

The use of plants in landfill leachate evapotranspiration has been widely tested. Two willow species: \textit{Salix alba} and \textit{Salix nigra} were used for landfill leachate disposal by Alker et al. (2003). Evapotranspiration values ranging from 8 to 9 mm/d and 5 mm/d obtained from willow stands, were determined by Persson & Lindroth (1994), and Elowson (1999) respectively. In \textit{Salix viminalis} L. and \textit{Salix aquatica} Sm. stands, located at municipal and industrial landfill sites, evapotranspiration was significantly higher than the annual precipitation rate in Finland (Ettala, 1992). Transpiration by willow stands may be high enough to affect significantly the ground water table level (Dulohery et al., 2000) and exceed annual precipitation (Persson, 1995). Research on young willow sprouts revealed that shortly after planting, evapotranspiration was effective at reducing landfill leachate volume. Agopsowicz (1994) determined that evapotranspiration of 3-month willow sprouts was 1.6-1.8 times higher than an average precipitation rate in Poland, which is about 600 mm/y. Bialowiec et al. (2003) confirmed that transpiration of 3-month old sprouts of \textit{Salix amygdalina} L. resulted in evapotranspiration ranging from 80 to 90 % of the supplied volume.

The decrease in leachate volume due to evapotranspiration from soil-plant systems provides a great advantage in landfill leachate disposal. Evapotranspiration technology might therefore be used in soil-plant systems for landfill leachate treatment.
In Polish climatic conditions (mean precipitation about 550 mm/year), it is possible to achieve a negative water balance for the landfill. One of the most important advantages of this technology is the low cost of investment and operation in comparison to other ways of leachate treatment.

Currently, plant species resistant to pollutants in leachate and characterized by high transpiration and adaptation to a wide range of pollutant concentrations are sought. So far, the use of different species of willows for leachate evapotranspiration has been tested mostly in Sweden (Hasselgren, 1992, Dimitriou & Aronsson, 2003, 2005, Dimitriou et al., 2006), Finland (Ettala, 1992), Great Britain (Alker et al., 2003) and also in Poland (Agopsowicz, 1994, Białowiec et al, 2003, Białowiec 2005, Bialowiec et al. 2007). However, numerous reports concerning transpiration ability, gross biomass growth and resistance to salinity of halophytes, point to the suitability of common reed—Phragmites australis in soil-plant systems for landfill leachate evapotranspiration.

Reed can by characterized by high transpiration ability, gross biomass growth, resistance to high concentrations of pollutants in soil solution, resistance to waterlogged soil and anaerobic conditions in soil. There is also a simple method of breeding, planting and cultivation. Reed was found to be the most suitable plant for landfill leachate evapotranspiration, because of very high evapotranspiration about 1800 mm/year (5 mm/d), biomass yield about 12 Mg d.m./ha/y and high resistance to pollutant loads supplied with leachate up to 6.7 MgN/ha and 1.5 MgCl-/ha (Białowiec, 2005).

On the basis of recent results, soil-plant systems with reed—Phragmites australis have been implemented at the landfill in Zakurzewo near Grudziądz, Poland. This chapter presents the design and two years operational experiences with this system.

2.3 Landfill leachate treatment in evapotranspirative system – case study
2.3.1 Landfill site
The municipal landfill is located at Zakurzewo, about 15 km north of Grudziądz. Total area of the site is 13.5 ha, and over 2.5 ha are reserved for piles of waste material (Agopsowicz et al. 2006). Zakurzewo landfill was built in 1997 and is operated by SWECO company based on waste disposal in “energetic piles”. This was the second landfill in Poland involving waste disposal using this technology for energy production. Design guidelines were based on the results from the pilot installation in Sweden (Hagby) (Trzosińska & Podkański, 2000).

Economic analysis of the investment assumes biogas generation and recovery, and selling various recyclable materials (plastic, metal, glass, textiles), as well as the likely sale of part of the waste as a fuel (Trzosińska & Podkański, 2000).

Firstly, four waste piles were constructed according to design guidelines. A rectangular basin (70 x 38 m), was prepared with a height of 3 m and a vertical section as an inverted trapezium, enclosed by a 5 m high embankment. The basin was sealed by a 50 cm layer of clay. The predicted capacity of each pile was 20 000 Mg of waste (in practice the mass of collected waste in the piles ranged from 19 154 Mg to 23 269 Mg, comprising 96-116 % of predictable capacity). Currently the piles are covered by an isolation layer, and are excluded from biogas recovery. Two bigger waste piles (90 x 90 x 10 m), working alternately from 2002, are now almost filled. The predictable capacity of these piles was 84 000 Mg of waste (in practice the collected waste mass ranged from 62 869 Mg to 89 333 Mg, comprising 75-106 % of predictable capacity). During the period of landfilling, degassing wells with a diameter of 1 m were installed, consisting of broken stone inside which was a 100 mm
diameter perforated pipe made of PE. In five pipes there are heads joined to a gas collector. According to the landfill operator, in the years 2002 and 2003, 6 700 Mg of sewage sludge with moisture about 80 % was deposited in one of the bigger piles.

Leachate is collected by a system of drainage pipes, and flows by gravity to a collection well, and then pumped to the main collector. From there, leachate passes to a concrete tank with a volume of 76 m$^3$ that is joined to a newly built tank with a working volume of about 2 500 m$^3$. SWECO technology recommends heating the waste to 40 °C before pumping under pressure into the waste piles, using elastic hoses inserted near the surface of the waste layer. This allows irrigation of the waste independently of the degree of filling of the pile. Currently this system is not working (leachate is not heated and not forced into the piles). Hence about 700 m$^3$ of leachate per month were taken for treatment to the wastewater treatment plant, at a total cost of about 29 000 $/year.

### 2.3.2 Evapotranspirative system construction and leachate characteristics

On the top of the one of the big waste piles, a soil-plant system with area of 2 500 m$^2$ was built. The base of the soil-plant system consists of two liners: 0.5 m of compacted clay soil, overlain with stabilized sewage sludge (dosage 250 Mg/ha). The soil-plant system is surrounded by a 0.5 m high clay bank with a gradient of 1:3 (H:L) (figure 1). Existing gas wells inside the soil-plant systems have been isolated by additional clay banks to a height of 0.5 m to reduce water percolation into the gas wells (Fig. 1) (Białowiec et al., 2007).

![Fig. 1. Soil-plant system construction.](image)

Seedlings of reed (rhizomes) were placed into the sewage sludge liner and irrigation of the soil-plant system started from that time (1 July 2006). Firstly, clean rain water, gathered in a chamber, was supplied. After the reed had established (5 weeks), irrigation of leachate started, using the existing leachate recirculation system. Reed was flooded by both incoming rain water and leachate in accordance with the design. From the beginning of the project the amount of irrigated clean water, leachate, and also precipitation were measured.
2.3.3 Field measurements
The efficiency of leachate treatment is evaluated in respect of water balance, based on the amount of leachate pumped into the soil-plant system, precipitation measurements, and meteorological data of evaporation measured with a Peach Evaporometer.
Treated landfill leachate was characterized by low concentration of organic compounds (COD - 997.0 mg\textsubscript{O}_2/dm\textsuperscript{3}) which consisted of about 20 % dissolved solids. The nitrogen concentration was very high (ammonium nitrogen 576 mg/dm\textsuperscript{3}). It caused a slightly alkaline reaction about 8.5 pH.
During the second year, in the peak of the vegetative season, the degree of leaf stomata opening (transpiration), was measured on 100 reed plants, using a Porometer AP-4-UM-3 Delta-T Devices [mmolH\textsubscript{2}O.m\textsuperscript{2}.s\textsuperscript{-1}]. Measurements were made every day during July. These results were used to assess the behavior of reeds in the toxic conditions of landfill leachate supply. During recent laboratory studies, the parameter - degree of leaf stomata opening (transpiration) had been used to determine the resistance of reeds to pollutants contained in leachate. The lowest effective concentration causing a toxic effect (LOEC) was estimated for leachate from Zakurzewo, to be at a level of 9 % share of leachate in water supplied to plants (Białowiec & Kasinski, 2008). Direct measurements of the degree of leaf stomata opening were compared to the value obtained for LOEC.

2.3.4 Leachate treatment efficiency
In the period (152 days) from 1 July 2006 to 30 November 2006 (end of vegetation season), the following amount of water had been supplied to the soil-plant system: clean rain water 231.0 m\textsuperscript{3} (collected and stored during July in the unused waste deposition cell), precipitation 648.1 m\textsuperscript{3} and leachate 283.0 m\textsuperscript{3}. The share of landfill leachate in the total amount of water supplied to the soil-plant system was 24.4 % (Tab. 1). During that period the hydraulic loading rate (HLR) of the soil-plant system by leachate was low, about 0.7 mm/d. In next year the share of leachate increased to 38 %, higher HLR (about 2.4 mm/d), and a much higher efficiency of water loss (about 4200 m\textsuperscript{3} compared to about 1100 m\textsuperscript{3}), was obtained. Recent experience indicates that hydraulic loading rate of reed stands by leachate may be higher than the value required to balance transpiration loss (5 mm/d) (Białowiec & Wojnowska-Baryła, 2007). We decided to use a low HLR because we wanted to avoid the toxic influence of leachate on young, developing plants.

<table>
<thead>
<tr>
<th>Year of operation</th>
<th>The share of leachate in total water supplied into soil-plant system [%]</th>
<th>Leachate hydraulic loading rate [mm/d]</th>
<th>Evapotranspiration efficiency [m\textsuperscript{3}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 (initial 35 days)</td>
<td>0.0</td>
<td>0.00</td>
<td>270</td>
</tr>
<tr>
<td>2006 (total 152 days)</td>
<td>24.4</td>
<td>0.74</td>
<td>1100</td>
</tr>
<tr>
<td>2007 (270 days)</td>
<td>37.6</td>
<td>2.36</td>
<td>4241</td>
</tr>
<tr>
<td>Recommended values</td>
<td>9.0*</td>
<td>&lt;5.00**</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Parameters of soil-plant system with reed in Zakurzewo. * LOEC – (Białowiec, Ksiński, 2008), ** (Białowiec & Wojnowska-Baryła, 2007)
The calculation of water balance showed that evapotranspiration from the soil-plant system did not exceed the total hydraulic loading rate; the difference was 84 mm after the end of first year (Fig. 2), and about 60 mm at the end of second year of operation (Fig. 3). However, the retention volume of the soil-plant system (500 mm) was high enough to accommodate all incoming water within the system, and evapotranspiration rate was high enough to reduce level of water gathered in the system during the second year (Fig. 3).

Measurements of the degree of leaf stomata opening indicated the mean value of transpiration rate to be 261.3 [mmolH₂O.m⁻².s⁻¹]. Comparison with laboratory results (Białowiec & Kasiński, 2008), indicated a strong capacity for adaptation by reed growing at the landfill. The electrical conductivity (EC) of the leachate collected in the soil-plant system at the landfill was 10.8 mS/cm. During laboratory tests a similar degree of reed leaf stomata opening, 250.3 [mmolH₂O.m⁻².s⁻¹] was measured for a solution with EC of only 1.1 mS/cm (Fig. 4). This indicates that during one year of operation of the system, the plants’ resistance to higher salinity had increased about ten times. This is a promising aspect for implementation of this technology.

In addition the influence of the soil-plant system on biogas production was examined. Static measurements of CH₄ content in biogas gathered from the waste pile with the soil-plant system (biogas sample taken from a gas well) showed the CH₄ concentration to be 60 %. Dynamic measurements gave similar results (biogas sample taken from the pump station) during 0.5 h of pumping, indicating that the soil-plant system had no negative influence on fermentation processes inside the pile.
Fig. 3. Water balance elements of the soil-plant system supplied by landfill leachate during 270 days period of the year 2007.

Fig. 4. Comparison of degree of leaf stomata opening (transpiration) in laboratory tests with measurements at the soil-plant system, Zakurzewo landfill, during July 2007.
3. Transpiration – a useful tool for landfill leachate phytotoxicity evaluation

3.1 Phytotoxicology in ecological engineering

Phyto-indicative methods for characterization of the environment have recently become popular. In such methods, observations of plant performance provide information about environmental conditions. Algae have been used as active components in phytotoxicological tests (Baun et al., 1998; Baun et al., 2002; Fargasowa et al., 1999; Halling-Sorensen, 2000; LeBlond & Duffy, 2001; Okamura et al., 2002; Rojickowa-Padrtowa & Marsalek, 1999; Sepic et al., 2003; Toricelli et al., 2002; Wundram et al., 1996). Multi-celled plants are also in use, mostly floating plants like *Lemna minor* L. but also rooted aquatic and terrestrial plants. Usually, such plants are used for toxicity assessment of pesticides, polycyclic aromatic hydrocarbons, and heavy metals as well as for landfill leachate toxicity determination. Similar studies have been conducted with willows: *Salix amygdalina* L., *Salix viminalis* L., *Salix purpurea* L. (Bialowiec & Agopsowicz, 2007), *Salix viminalis* (Dimitriou et al., 2006), and reed *Phragmites australis* (Cav.) Trin. ex Steud. (Bialowiec & Kasinski, 2008). Phytotoxicological tests may be important in environmental engineering in designing phytoremediation technologies for treatment of harmful substances (wastewater, landfill leachate) or for remediation of polluted soils. Such tests can determine the maximal dose of treated pollutant which causes no negative effect on the plants. In the case of phytoremediation using higher plants, with complex tissue and organ structures, there is a question of selecting the best parameter to indicate plant response to the toxicant. Different parameters have been chosen individually for each case of plant and phytotoxic compound. Fairchild et al. (1999) counted number of leaves and total biomass of *L. minor* L. Marwood et al. (2001) measured the concentration of chlorophyll a and b in cells of *Lemna gibba* L. Fairchild et al. (1998) measured biomass growth of *Ceratophyllum demersum* L., *Elodea canadensis* Michx., and *Myriophyllum heterophyllum* Michx. whereas growth inhibition of *Lolium perenne* L., and *Raphanus sativum* L. was employed by Prokop et al. (2003). Moreover, Dimitriou et al. (2006) examined the usefulness of leaf length and asymmetry measurements for determination of tolerance of five willow clones to landfill leachate. Dimitriou et al. (2006) used relative growth rate (RGR) based on measurements of shoot, leaf, root and total plant dry weights, leaf area and shoot length. Changes caused by toxicants are usually not visible in short term experiments, and size measurement with a ruler is not accurate enough to indicate small changes in the width, length, shape and symmetry of leaves. Selecting the most appropriate parameter from such a list as an indicator of phytotoxicity may lead to confusion. The parameter which may include all possible changes in leaf morphology is the fractal dimension of the leaf (Kopik & Bialowiec, 2007).

In ecological engineering, phytotoxicological tests are used in soil remediation, waste management and wastewater treatment in addition to chemical analysis (Wilson et al., 2002) and they may be used for landfill leachate toxicity assessment. Many studies indicate the utility of macrophytes in toxicological tests (Barbero et al., 2001; Kirk et al. 2002; Wang, 1987; Wang & Williams, 1988), and involve measuring basic parameters such as seed survival (germination), biomass growth, stem and root elongation. From a technological point of view, the most important parameters to determine the landfill leachate dose rate are transpiration and biomass growth, these being processes with the greatest impact on treatment efficiency. Successful implementation of a soil-plant (evapotranspirative) system with willow irrigated by leachate depends on an appropriate dose rate, especially in the initial phase of plant growth. In the next section measurements of biomass growth and transpiration of three species of willow are used to determine landfill leachate phytotoxicity.
3.2 Materials and methods
The experiment was designed to determine the optimum duration of testing of willow tolerance to landfill leachate, using two easily measured physiological parameters, as indicators of plant response: stem elongation and transpiration. Three willow species marked as W₁, W₂ and W₃ were tested in the greenhouse located at the University of Warmia and Mazury in Olsztyn, Poland. Individual willow cuttings were placed in 90 transparent plastic bottles with volume 1 dm³ (30 bottles for each willow species). Bottles containing willow cuttings (Fig. 5), were filled with the following solutions of landfill leachate, clean tap water and water taken from a river: 0% (clean tap water), 0% (river water), 12.5 %, 25 % 50 % and 100 % concentrations of leachate. Tap water and river water were used as a reference. The bottles were filled to the same level, and the volume of solution used was in the range 955 to 990 cm³ per bottle. Every variant of the experiment was replicated 5 times. Figure 6 shows the experimental configuration.

The willow cuttings were taken from an experimental plantation owned by UWM. The diameter and length of each cutting was in the range 0.8 to 1.9 cm and 31 to 33 cm. Three quarters of the cutting’s length was inside the bottle.

Fig. 5. The construction of experimental stand.
Fig. 6. Configuration of the experiment.

Tap water used in the experiment is typical for water supplied in Olsztyn municipality. The river water was taken for Kortowka River passing through UWM campus. Landfill leachate came from landfill in Zakurzewo near Grudziadz, Poland. The chemical characteristics of landfill leachate and its dilutions are shown in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Leachate solutions [%]</th>
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<tr>
<td></td>
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<td>100.0</td>
</tr>
<tr>
<td>Reaction</td>
<td>pH</td>
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<tr>
<td>Conductivity</td>
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<tr>
<td>COD</td>
<td>mgO₂/dm³</td>
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<tr>
<td>Chlorides</td>
<td>mg/dm³</td>
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</tr>
<tr>
<td>Kjeldahl nitrogen</td>
<td>mg/dm³</td>
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</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>mg/dm³</td>
<td>576.0</td>
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<tr>
<td>Dissolved solids</td>
<td>mg/dm³</td>
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<tr>
<td>Residual after ignition</td>
<td>%</td>
<td>79.5</td>
</tr>
<tr>
<td>Lost at ignition</td>
<td>%</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Table 2. The chemical characteristics of landfill leachate and solutions used in the experiment
The duration of the experiment was 35 days (5 weeks), during which time the average temperature inside the greenhouse was 22.5 °C.

The tolerance of plants to the prepared leachate concentration was determined by measurements of biomass growth and transpiration rate. Biomass growth was determined by stem elongation measured with 1 mm accuracy. Transpiration was measured as the decrease of water in the bottles, determined gravimetrically with 0.1 g accuracy. Measurements were made every week.

The minimum duration for future experiments to indicate the tolerance of willows to leachate was assessed on the basis of ANOVA analysis of mean values of stem length (significance p<0.05). The toxic effect of leachate on willows was evaluated by analysis of plant biomass growth curves (stem length) and transpiration cumulative curves. Coefficients of growth and transpiration were determined by regression analysis (significance p<0.05). The no-effect concentration of leachate on plants was calculated using DEBTox software tool (body growth model).

### 3.3 Results and discussion

Optimal duration for the toxicity test (long enough to obtain significant differences between variants), was evaluated using ANOVA with post-hoc Tukey’s test (significance level p<0.05) (Fig. 7).

It is clear that during two weeks in most cases there is lack of differences between variants. After a third week differences started to be observed and from the 28th day of experiment differences had stabilized. Extending the experiment beyond 28 days is not necessary, because it did not change the configuration of differences (Fig. 7).

Linear regressions of biomass growth rate during the experiment are shown as cumulative curves of stem elongation (Fig. 8), and are described by equation 1:

\[ y = a \cdot t - b \]  

where:
- \( y \) - stem length [cm],
- \( t \) - time [days],
- \( a \) - regression coefficient (the stem elongation ratio) [cm/day],
- \( b \) - initial stem length [cm].

The calculated regression coefficients (\( a \) and \( b \)) and also the determination coefficients (\( R^2 \)) are shown in table 3.

The high values of \( R^2 \) obtained (significance p<0.05) indicate a good fit of the estimated regression parameters to the results. Comparing between species, stem elongation ratios obtained as regression slopes (\( a \)) in leachate concentration 0% solution (control) the highest was in willow W1 2.53 cm/day, and the lowest in willow W3 1.46 cm/day. Increasing the leachate concentration to the 12.5 % level did not affect stem elongation ratio. Further increase in leachate concentration caused a slight decrease (of 22 to 53 %) in the stem elongation ratio. Increasing leachate concentration to 50 and 100 % caused stem elongation ratio to decrease dramatically to values in the range 0.02 – 0.31 (Tab. 3). In the case of willow W2 with concentration of leachate 100 % biomass growth did not occur.

Values of stem elongation ratio obtained for willows supplied with river water (similar to values for plants growing in tap water), show no negative effect of its constituents on biomass growth.
Fig. 7. Mean stem length differentiation during experiment. l.o.d. – lack of significant (p<0.05) differences between means. Letters (a, b, c and combinations) show significant (p<0.05) differences between means.

Table 3. Linear estimation parameters of the willow stem elongation during experiment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experiment variants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W1</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>a</td>
<td>2.53</td>
</tr>
<tr>
<td>b</td>
<td>13.4</td>
</tr>
<tr>
<td>R²</td>
<td>.913</td>
</tr>
</tbody>
</table>

The no-effect concentration of leachate on plants (NEC) is the concentration of toxicant in the medium that will not induce effects after prolonged exposure. NEC values, calculated using DEBTox software tool (body growth model), are in range 20.74 (W1) – 24.21 (W2) % (Tab. 4). Those results are similar to observations of changes of stem elongation ratio. This indicates the usefulness of the DEBtox tool for selection of leachate dose for treatment by plants.

Bialowiec et al. (2003) in earlier research on leachate with a lower concentration of nitrogen, 148 mg/dm³, observed that increasing the leachate concentration up to 50 % stimulated biomass growth, but further increase in leachate concentration to 100 % slowed it down.
Fig. 8. Stem elongation cumulative curves during experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC</td>
<td>%</td>
<td>20.74</td>
<td>24.21</td>
<td>23.04</td>
</tr>
<tr>
<td>The ultimate length*</td>
<td>cm</td>
<td>217.9</td>
<td>189.3</td>
<td>141.3</td>
</tr>
<tr>
<td>Mean deviation</td>
<td>cm</td>
<td>10.2</td>
<td>9.1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Table 4. The parameters of NEC calculation. * The ultimate length is the body length after a very long time without distortion of the growth process.

This shows the necessity of performing individual tests for each kind of leachate, because of different possible responses of plants to variable leachate properties.

There is an influence of leachate concentration on transpiration. Non-linear regression of transpiration during the experiment is shown as cumulative curves of the mass of transpired water [g H$_2$O per plant] (Fig. 9).
Fig. 9. Transpiration cumulative curves during the experiment.

This dependence is described by equation 2:

$$T = T_0 \cdot e^{kt}$$  \hspace{1cm} (2)

where:
- $T$ – transpiration [g H$_2$O per plant],
- $T_0$ – initial transpiration [g H$_2$O per plant],
- $t$ – time [days],
- $k$ – regression coefficient (transpiration rate constant) [1/day].

The calculated regression coefficients ($k$ and $T_0$) and also the determination coefficient ($R^2$) are shown in table 5.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experiment variants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W1 12.5 25 50 100 Riv.</td>
</tr>
<tr>
<td>$k$</td>
<td>.131 .145 .090 .047 .046</td>
</tr>
<tr>
<td>$T_0$</td>
<td>5.89 3.49 7.29 12.0 9.00</td>
</tr>
<tr>
<td>$R^2$</td>
<td>.998 .999 .997 .901 .896</td>
</tr>
</tbody>
</table>

Table 5. Non-linear estimation parameters of the willow transpiration during experiment.
The high values of $R^2$ obtained (significance $p<0.05$) indicate a good fit of the estimated regression parameters to the results. Comparing between species, transpiration rate constants obtained as regression slopes ($k$) in leachate concentration 0% solution (control) the highest was in willow W2 0.144 1/day, and the lowest in willow W3 0.103 1/day. Increasing the leachate concentration to 12.5 % did not affect transpiration. Similarly, as in the case of stem elongation ratio, increasing leachate concentration from 12.5 to 25 % caused a slight decrease in transpiration rate constant. Increasing leachate concentration to 50 and 100 % caused intense decrease of transpiration rate constant to values in the range 0.041 – 0.048 (Tab. 5).

Similarly, Białowiec et al. (2003) in earlier research, determined that leachate concentrations exceeding 25 % had a negative influence on willow transpiration. Leachate used in the cited experiment was characterized by a much higher concentration of chlorides (1540 mg/dm$^3$) than in the results presented here. This may show that different pollutants influence each physiological process in different ways. The presence of dissolved compounds in water may affect transpiration in two ways; (i) it may pose toxic threats to plants, (ii) it may decrease the difference between soil water tension and soil water tension at the point of air entry, and hence decrease water flux (Persson, 1995). It may shown that transpiration is a more sensitive physiological process than biomass growth in the presence of toxicants. This parameter (qualitative differences between toxicants), should be included in the test procedure, because lack of knowledge about the influence of pollutants on transpiration may lead to further mistakes during dose selection of landfill leachate for treatment by plants. A possible mistake is to obtain good biomass growth but with low transpiration. This should be taken into consideration because the main aim of leachate treatment in soil-plant systems is not biomass production but leachate volume decrease due to evapotranspiration.

4. Summary

Early operational results of landfill leachate treatment in soil-plant systems show that this method may be used as an alternative technology in contrast to treatment in a wastewater treatment plant. In the initial phase (July 2006 to the end of November 2006), about 1100 m$^3$, and during the year 2007, 4241 m$^3$ water were lost by evapotranspiration.

Reeds growing in a soil plant system had higher resistance to pollutants present in leachate than plants exposed to leachate in the laboratory. Despite high concentration of ions in water collected from the soil-plant system (10.78 mS/cm] reeds showed high transpiration rate, about 250 mmol m$^{-2}$ s$^{-1}$. During the phytotoxicological tests the same transpiration rate value was observed with plants watered by a solution with 10-fold lower ionic concentration (1.10 mS/cm).

Experiences with the first pilot scale evapotranspirative system for landfill leachate allowed us to propose a formula to calculate the minimum size of such zero discharge systems (Białowiec, 2009):

$$A_s = \frac{-A_l (Q_p - E_v)}{(E_v - E)}$$  \hspace{1cm} (3)

where:
Evapotranspiration

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Qp – annual precipitation [m/y],
Ev – annual evaporation [m/y],
E – annual evapotranspiration [m/y],
Al – landfill site area (sealed) [m²],
As – evapotranspirative system area (located within the landfill site) [m²]

Before implementation of a soil-plant system for evapotranspiration of landfill leachate the LL dose rate should be assessed on the basis of phytotoxicological test. The proposed procedure to determine willow tolerance to landfill leachate is easy to perform, proposed indicators of plant response to leachate (stem elongation and transpiration) are easy to measure and allow information about possible leachate dose for willow irrigation to be obtained. The duration of tests does not need to be longer than 28 days (4 weeks). Both stem elongation and transpiration should be measured during the experiment, because of different sensitivity of those physiological processes to leachate constituents. Because of different leachate chemical properties and landfill conditions the proposed testing procedure for the assessment of leachate dose for willow irrigation should be repeated every time a new plantation is planned. Using data from another experiment with different leachate may lead to failure of the whole system. Experiments performed here showed that the willow plantations should not be irrigated by solutions of this kind of leachate with a share higher than 25 % of the total amount of water supplied to the plantation (leachate + precipitation).

5. Acknowledgements

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6. References


Evapotranspiration is a very complex phenomenon, comprising different aspects and processes (hydrological, meteorological, physiological, soil, plant and others). Farmers, agriculture advisers, extension services, hydrologists, agrometeorologists, water management specialists and many others are facing the problem of evapotranspiration. This book is dedicated to further understanding of the evapotranspiration problems, presenting a broad body of experience, by reporting different views of the authors and the results of their studies. It covers aspects from understandings and concepts of evapotranspiration, through methodology of calculating and measuring, to applications in different fields, in which evapotranspiration is an important factor. The book will be of benefit to scientists, engineers and managers involved in problems related to meteorology, climatology, hydrology, geography, agronomy and agricultural water management. We hope they will find useful material in this collection of papers.

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