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Effects of Macrophytes on Biological Treatment of Processed-Leachate from Sanitary Landfill Sites

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ABSTRACT: Three macrophytes species, that are, *Phragmites australis*, *Zizania latifolia* and *Typha angustifolia were* grown in mono culture in order to compare growth (relative biomass increase rate), variation of photosynthetic pigment (total chlorophyll, Chl *a*, Chl *b* and Chl *a*/Chl *b*) and effectiveness of nutriment removal in 15‰ NaCl-salt solution or processed-leachate (salinity 19.6‰) from sanitary landfill sites. The relative biomass increase rate of *P. australis* was significantly higher than *Z. latifolia* and *T. angustifolia*. In the case of processed-leachate treatment, the relative biomass increase rates of above-part, rhizome and root of *P. australiswere* 178 %, 148 % and 157 %, respectively. Also, in 15 ‰ NaCl-salt solution treatment, the relative biomass increase rates of *P. australis* increased as follows; 161 % (above-part), 183 % (rhizome) and 112 % (root). Total chlorophyll contents increased significantly in the leaves of *P. australis* and *Z. latifolia* grown in 15 ‰ NaCl-salt solution and processed-leachate. Among three macrophytes, *P. australis* was evaluated as most effective macrophyte for the biological retreatment of processed-leachate from sanitary landfill sites.

Key words: Leachates, Macrophyte, Phytoremediation, Photosynthetic pigment, Sanitary landfill site

INTRODUCTION

The leachate from sanitary landfill sites is difficult to characterize because their compositions and contents depend on the variety of factors, such as types of landfill refuse, waste composition and amount, precipitation, age of landfill sites, moisture contents, and landfill methods (Im et al. 2001). Landfill leachate is wastewater that can contaminate hydrospace ecosystems, therefore the need for efficient collection and treatment of leachate from solid waste landfill sites to avoid surface-water and groundwater contamination is widely recognized. Recently many countries have researched several aquatic plants for biological treatment of domestic sewage and wastewater as ecologically stable method and green technology. For this purpose, they have investigated pollutant removal mechanisms in wetlands (Kadlec and Knight 1996), the usefulness of aquatic plants to treat wastewater (Brix 1997, Carmen and Crossman 2001) and the bio-retreatment of domestic effluent (Steer et al. 2002).

The SUDOKWON landfill site located in Incheon City is a sanitary landfill site, and has reclaimed solid waste over the past 14 years from 1992. Because leachate from the SUDOKWON landfill site is high in soluble or insoluble organic matters containing nitrogen, phosphorus and salt contents, a leachate has been processing by various construction progresses (physicochemical processes). Nevertheless, this processed-leachate is very high strength wastewater as usual, and the characteristics of processed-leachate from the SUDOKWON landfill site were as follows: pH, 7.12; total nitrogen, 127.42 mg/L; total phosphorus 0.278 mg/L; COD_{Mn}, 44.2 mg/L; BOD_5, 4.0 mg/L; DO, 1.78 mg/L; total dissolved substrate (TDS), 18,900 mg/L and chromaticity, 105.1 degree. Especially, salinity of processed-leachate is very high (19.6‰) because raw leachate is flowed out from garbage filled in the SUDOKWON landfill site.

The objective of this article was to assess the biological treatment potential of processed-leachate from sanitary landfill sites. For this experiment, we selected three kinds of macrophytes species, that is, *Phragmites australis*, *Zizania latifolia* and *Typha angustifolia*. The reason that chose these macrophytes is that they have been used in biological treatment of wastewater and domestic effluent, and planted in constructed wetland for the water quality improvement (Gersberg et al. 1986, Coleman et al. 2001, Steer et al. 2002). In this research, we present the effects of three kinds of macrophytes on processed-leachate from the sanitary landfill site and NaCl-salt solution, and assess the merit rating, the adaptable potential and the usefulness in the aspect of biological remediation of plant species to processed-leachate.

MATERIALS AND METHODS

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Plant Samples and Sampling Site

Plant samples, *Phragmites australis*, *Zizania latifolia* and *Typha angustifolia*, are the major components of the marsh, wetland and riverside ecosystem in Korea, and they are typical emergent plants, namely, macrophytes (Choi 1985, Cronk and Fennessy 2001). All plant samples were selected and collected for the uniformity of size in plant's natural habitat on May, 2003. The sampling site of these plant samples was located at the place that is flowed in from Kyungan stream to Paldang Reservoir, and its geographical position is 37°44′34″ N, 127°30′29″ E (Yangpyung-gun, Gywonggi Province, Korea). We choose this area as sampling site because various hydrophytes inhabit, and it is no possibility of organic pollutant and salt input.

Culture Conditions

To transplant *P. australis*, *Z. latifolia* and *T. angustifolia*, we left rhizome, roots and 5～10 cm above-ground part, and removed residues. Three individuals of each plant species were transplanted to an experiment pot, and acclimated using underground water for one month before the treatment of processed-leachate from the SUDOKWON landfill site or 15‰ NaCl-salt solution. Soil mixtures (4 kg) in each pot were composed of riverside sand (60 %; 2.4 kg) and soil conditioner (40 %; 1.6 kg). The acclimated plant samples (rhizome-grown plants) were cultured in Horgland's nutrient solution (pH 5.7) with underground water (control, C), Hoagland's nutrient solution (pH 5.7) with 15‰ NaCl-salt (S) or processed-leachate (salinity 19.6‰) from the SUDOKWON landfill site (PL). Water table was maintained in 3～5 cm. Each solution was periodically supplied one time per week to pot during 5 months experiment period. The reason that we treat plant samples with 15‰ NaCl-salt solution is to compare with growth and response of each plant species that are represented in processed-leachate treatment. All plant samples were cultured in the greenhouse, and each treatment group was set up three replicates.

Determination of Relative Increase Rate of Biomass

To determine the relative increase rate of the each part biomass of *P. australis*, *Z. latifolia* and *T. angustifolia*, we collected all plant samples from each experiment pot after five months culture, washed by tap water, and divided each plant sample into above-ground part, rhizome and root, and measured the weight of each plant part. And, the relative increase rate of biomass was calculated the increased-biomass in each plant pot.

Estimation of Chlorophyll Contents

We used two methods to determine chlorophyll contents in the leaf of *P. australis*, *Z. latifolia* and *T. angustifolia*. One method was using a chlorophyll fluorescence meter (Hoel and Solhaug 1998, Johnson and Saunders 2003), and the other method was determining chlorophyll contents by spectrophotometric method (Porra et al. 1989). For the measurement by SPAD 502 chlorophyll fluorescence meter (Minolta Co. Ltd., Japan), we selected the fully expanded leaf below the terminal of each plant sample, and measured three replicate. For the determination of chlorophyll content by spectrophotometric method, four leaf-discs were cut off the nourished leaves of *P. australis*, *Z. latifolia* and *T. angustifolia* by an 8 mm diameter punch (approx. 50 mm^2 surface areas). These discs were placed immediately into 5 mL of 100 % methanol, and total leaf-pigments were extracted at 4℃ for 24 h. The extracts were centrifuged at 1,200 ×g for 15 min. The supernatants were collected and absorption spectra at 652.0 and 665.2 nm were recorded using UV-V is spectrophotometer (Spectronic GENESIS 5, Milton Roy, USA) for estimation of Chl *a*, Chl *b* and total chlorophyll contents following the procedure of Porra et al. (1989). The estimation of chlorophyll contents in leaves of each plant sample was done after cultivate four months.

Measurement of Nitrogen and Phosphorus Contents in Experiment Soil

For assessment of bio-treatment potential of each plant species, total nitrogen and phosphorus contents in experiment soils were analyzed and the removal rate of nitrogen and phosphorus were calculated.

After the culture of each sample plant in processed-leachate or 15‰ NaCl-salt solution, experiment soils were collected and dried at 60℃ for 12 h. Dried-soils were passed through a 1mm sieve. To analyze nitrogen (total nitrogen; TKN) in soil samples, 0.5 g dried-soil samples were digested with 5 mL sulfuric acid and 1.83 g catalyst (a mixture of 1.64 g K_2SO_4 and 0.19 g $CuSO_4$) at 370℃ for 4 h. Nitrogen was analyzed by Automatic Kjeldahl Protein/Nitrogen Analyzer (Kjeltec Auto 1035/1038 System, Tecator AB, Sweden). To analyze phosphorus (total phosphorus; TKP) in soil samples, 0.5 g dried-soil samples were pretreated with 3 mL nitric acid at room temperature for 12 h, and added on 3 mL perchloric acid and digested at 200℃ for 3 h. Phosphorus contents were analyzed by Inductively Coupled Plasma Emission Spectrophotometer (ICPS-1000 IV, Shimazu, Japan).

RESULTS AND DISCUSSOIN

Comparison of Relative Biomass Increase Rate

P. australis showed positive without obvious symptoms of toxicity or nutrient deficiency during five months growth in processed-leachate or 15‰ NaCl-Salt solution. The relative biomass (wet weight) increase rate of *P. australis* was significantly higher than *Z. latifolia* and *T. angustifolia*. In the case of processed-leachate treatment, the relative biomass increase rates of above-part, rhizome and root of *P. australis* were 178 %, 148 % and 157 %, respectively. Also, in 15 ‰ NaCl-salt solution treatment, the relative biomass increase rates of *P. australis* increased as follows; 161 % (above-part), 183 % (rhizome) and 112 % (root). However, compared with control, the relative biomass increase rate of *Z. latifolia* remarkably decreased, and in the case of *T. angustifolia*, only rhizome biomass was reduced (Table 1). Especially, in *Z. latifolia* and *T. angustifolia*, under-ground biomass was strongly influenced by salt contents. Moreover, *P. australis* exhibited rapid initial shoot development and steady increase in shoot density over the experiment period in all processed-leachate and 15‰ NaCl-salt solution treatment pots (data not shown). On the other hand, *Z. latifolia* and *T. angustifolia* were not reproduced new shoots. The biomass increase patterns among these plant sample species in salt stress are similar with the results of Hootamans and Wiegman (1998). According to Hootsmans and Wiegman, biomass increased and relative growth rate of *P. australis* were higher than *T. latifolia*, *Scirpus lacustris* and *S. maritimus* in 1.8‰ or 18‰ NaCl-salt solution, and in 18‰ NaCl-salt solution, all plants of *T. latifolia* and *S. lacustris* died, while mortality was 60 % in *P. australis* and *S. maritimus*. Therefore, this result showed that *P. australis* can grow well in processed-leachate than *Z. latifolia* and *T. angustifolia*.

Changes in Chlorophyll Contents

Total contents of chlorophyll increased significantly in the leaves of *P. australis* and *Z. latifolia* cultured in processed-leachate and 15 ‰ NaCl-salt solution. However, in the case of *T. angustifolia*, total chlorophyll contents increased only by processed-leachate, and decreased by 15‰ NaCl-salt solution (Fig. 1). For both species, *P. australis* and *Z. latifolia*, Chl *a* content significantly increased in 15 ‰ NaCl-salt solution and salt containing solution (processed-leachate), and the Chl *a*/Chl *b* ratio increased as follows; in the case of *P. australis*, 1.6 to 2.3 in 15‰ NaCl-salt solution and to 2.0 in processed-leachate, and in the case of *Z. latifolia*, 1.9 to 2.1 in 15‰ NaCl-salt solution and to 2.5 in processed leachate. But, in the case of *T. angustifolia*, the Chl *a*/Chl *b* ratio remained unchanged in leaves as follows; 1.9 in control to 1.8 in 15‰ NaCl-salt solution, and to 1.9 in processed-leachate. Many researchers have been demonstrated the effect on photosynthetic pigments by salt stress in plants. Ma et al. (1997) reported that Chl *a* concentration, the total amount of Chl *a* + *b* and Chl *a*/Chl *b* ratio increased, while Chl *b* and carotenoid concentration decreased with increasing salt level in *Populus euphratica*. Similar tendency was showed in *P. australis* and *Z. latifolia*. On the other hand, Parida et al. (2004) reported that total contents of chlorophyll and carotenoid were decreased significantly with NaCl treatment, and high salt concentration (250 mM NaCl) did not affect Chl *a*/Chl *b* ratio even though the total chlorophyll content decreased in *Aegiceras corniculatum*. The decrease of chlorophyll contents may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis, and during the process of chlorophyll degradation, Chl *b* is converted in Chl *a* (Fang et al. 1998). This may explain the increase of the Chl *a*/Chl *b* ratio in the leaves of *P. australis* and *Z. latifolia* cultured in 15‰ NaCl-salt solution or processed-leachate (19.6‰ salinity). It is well known that chlorophyllase activity increases with time in unstressed plants grown under photoperiod. However, about chlorophyllase activity in plant treated with NaCl salt solution, Santos (2004) reported that mild stress of NaCl (25 mM NaCl) stimulates chlorophyllase activity, while more severe NaCl concentrations (50 and 100 mM NaCl) inhibit the activity of this enzyme. Therefore, from results in this experiment, that were, the increase of total chlorophyll content in the leaves of *P. australis* and *Z. latifolia* cultured in 15‰ NaCl-salt solution or processed-leachate (19.6‰ salinity), we consider that chlorophyll degradation process is not dependent at 15‰ or 19.6‰ salt stress on the activity of chlorophyllase, and other alternative pathways in *P. australis* and *Z. latifolia* must be involved.

Assessment of Bio-Treatment Potential

Table 1. Relative biomass increase rate of above part, rhizome and root for *Phragmites australis*, *Zizania latifolia* and *Typha angustifolia* after five months growth (C; control, S; 15‰ salt solution, PL; processed-leachate, unit; %)

	P. australis			*Z. latifolia*			*T. angustifolia*		
	Above	Rhizome	Root	Above	Rhizome	Root	Above	Rhizome	Root
C	100	100	100	100	100	100	100	100	100
S	161	183	112	64	61	63	134	67	102
PL	178	148	157	61	61	60	98	55	89

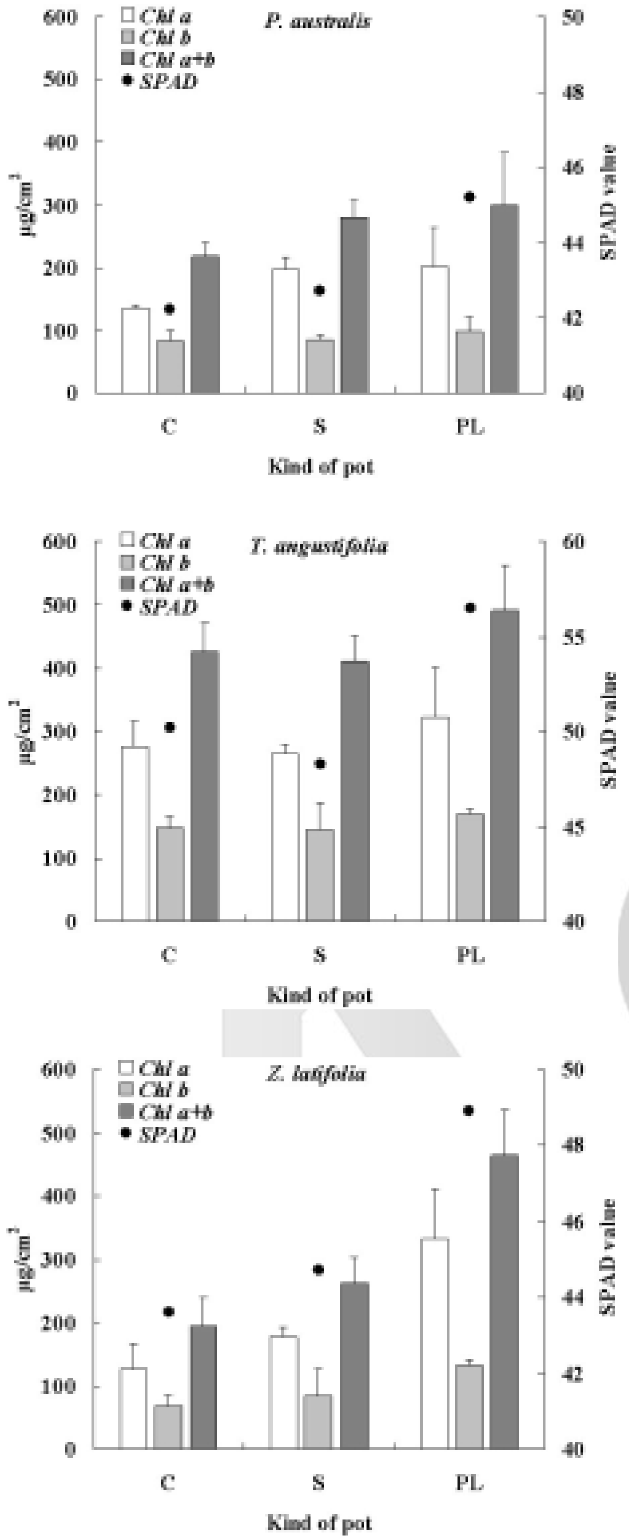

Fig. 1. Comparison of chlorophyll contents in the leaves of plants (C, control; S, 15‰ salt solution treatment; PL, processed-leachate treatment).

After cultivation in 15‰ NaCl-salt solution or processed-leachate, a comparison of total nitrogen (T-N) and phosphorus (T-P) contents in experiment soils are shown in Fig. 2. Table 2 gives the nitrogen and phosphorus removal rate by *P. australis*, *Z. latifolia* and *T. angustifolia* from soils treated with processed-leachate. After five months, the variation of total nitrogen and phosphorus contents in non-planted pot (only treated with 15 ‰ NaCl-salt solution or processed-leachate) was as follows; for 15 ‰ NaCl-salt solution treatment, 7.8 % increase (89.22 mg T-N/L) and 1.2 % increase (5.71 mg T-P/L), and for processed-leachate treatment, 50.3 % increase (124.37 mg T-N/L) and 14.9 % increase (6.48 mg T-P/L) than non-planted control pot (only treated with underground water; 82.75 mg T-N/L and 5.64 mg T-P/L). As showed in Table 2, for processed-leachate treatment, the nitrogen removal rate of *Z. latifolia* (44.3 %) was higher than that of *P. australis* (29.6 %), but, the phosphorus removal rate of *Z. latifolia* (20.8 %) was lower than that of *P. australis* (29.2 %). On the other hand, for 15 ‰ NaCl-salt

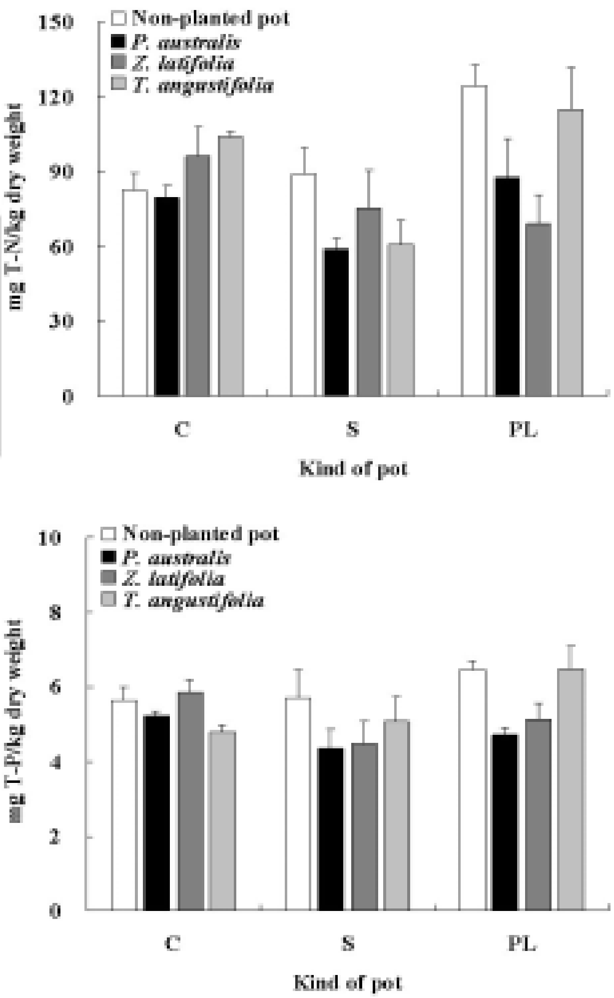

Fig. 2. Comparison of total nitrogen (T-N) and phosphorus (T-P) contents in soil (C, control; S, 15‰ salt solution treatment; PL, processed-leachate treatment).

Table 2. Removal rate of nitrogen and phosphorus from soil by *Phragmites australis, Zizania latifolia* and *Typha angustifolia* after five months growth (C; control, S; 15‰ salt solution, PL; processed-leachate, unit; %)

	P. australis		*Z. latifolia*		*T. angustifolia*	
	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrogen	Phosphorus
C	3.9	7.4	−16.4*	−3.9	−25.7	15.1
S	33.5	23.8	15.4	21.7	31.4	11.2
PL	29.6	27.2	44.3	20.8	7.4	−0.2

* A minus quantity means the increase of total-nitrogen and -phosphorus contents.

solution, the removal rates of nitrogen and phosphorus were higher *P. australis* (33.5 % nitrogen and 23.8 % phosphorus) than *Z. latifolia* (15.4 % nitrogen and 21.7 % phosphorus). According to Fraser et al. (2004), total nitrogen and phosphorus from the soil leachate were effectively reduced by the constructed wetland consisted of four wetland plants, that are, *Scirpus validus*, *Carex lacustris*, *Phalaris arundinacea* and *Typha latifolia*, and the reduction of nitrogen and phosphorus in the soil leachate was 10 times greater in vegetated microcosms than unvegetated microcosms. Gersberg et al. (1986) investigated constructed wetlands that were planted by *Scripus validus*, *Phragmites communis*, or *Typha latifolia* or that were unplanted in order to determine the efficiency among the treatments at reducing nitrogen. In all cases unplanted wetlands were less effective than any of the planted wetland. And, among species, *S. validus* and *P. australis* were more effective and *T. latifolia* was the least effective. These results were similar to our results on *P. australis*, *Z. latifolia* or *T. angustifolia* in processed-leachate. The artificial wetlands for biological treatment need to be organized on various macrophytes and microhabitat for microorganisms. With these results, we estimated that Typhaceae (*T. latifolia* and *T. angustifolia*) was less effective on the biological treatment of domestic wastewater or various leachates than other macrophytes species (*P. australis* or *Z. latifolia*) because of its shallow rooting zone, the inability to create an effective environment for various microbial communities and the intolerability on severe water pollutants such as high level-salt or -organic matter.

From present results, we confirmed that improvement of processed-leachate quality made by *P. australis* or *Z. latifolia*, and it was more clearly demonstrated by the removal rate of nitrogen and phosphorus from *P. australis*- and *Z. latifolia*-planted pot than that from *T. angustifolia*-planted pot. Also, we verified that *P. australis* was most effective macrophyte for the biological retreatment of wastewater containing high salt and much organic matter such as processed-leachate from sanitary landfill sites.

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