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Research article

From phytoaccumulation to post-harvest use of water fern for landfill management

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ABSTRACT

We examined the potential of *Azolla japonica* as a remediating plant for leachate channels and post-accumulation use as fertilizer for landfill slope. The harvested biomass of *Azolla* after one month grown in leachate was 254% that of the initial biomass and the predicted annual harvestable biomass of *Azolla* using a growth model was 32 times that of the initial biomass. Na, Fe, Mn, Mg, and P were accumulated in *Azolla* at very high concentrations. Such rapid increase of biomass and high accumulation rates suggest that this plant could be an excellent remediating plant. The post-harvest use of *Azolla* as compost was studied for the management and use of phytoaccumulating *Azolla*. Metal contents of *Azolla* compost were below permissible limits for co-composting material. Nitrogen, organic matter, P, and Mg content of the *Azolla* compost improved the soil condition of the landfill and enhanced ecophysiological responses of the plants. The application of *Azolla* compost can improve management of sanitary landfills, including the restoration of vegetation. Considering its ease of harvesting, high accumulation rates, harvestable biomass and suitability for composting, *Azolla* can provide a suitable solution for sustainable management of leachate channels and landfill slopes.

1. Introduction

Human population growth, economic development and industrialization generate increasing amounts of waste that demand the development of efficient disposal technologies. As precipitation percolates though decomposing organic waste (Justin and Zupancic, 2009), leachate is produced. Leachate is a cocktail of heavy metals, other toxic materials, and nutrients that constitute a major environmental hazard (Jones et al., 2006) and cause eutrophication (Jokela et al., 2002). Leachate should be processed before being discharged to avoid its migration into the surrounding environment. Physicochemical processing (Deng, 2007; Kurniawan et al., 2006) together with application of microorganisms (Kargi and Pamukoglu, 2003; Kettunen et al., 1996) to facilitate decomposition offers the promise of effective treatment to ameliorate the risk of environmental contamination. However, these processes are usually expensive and are constrained by limited capacities. Substantial resources (~USD $56 million) have been invested in the development and maintenance of leachate treatment facilities in the Sudokwon landfill, the largest in South Korea and among the largest in the world, where we conducted our study. Until now, studies on the efficacies of the treatment processes used have focused on heavy metal remediation and particulate matter (SLMC, 2010). Other consequences of processing, such as eutrophication, thus remain to be considered (Cho, 2008). Furthermore, the complexities and consequence high expense associated with currently used technologies serve as an impetus to develop lower cost alternative processing methods (Mohan and Gandhimathi, 2009).

Constructed wetland treatment systems (Sindilariu et al., 2009) represent one potential low-cost solution. However, every wetland has a limited service lifetime (Sindilariu et al., 2009), and in periods lacking active aeration, removal efficiencies can be inconsistent (Nivala et al., 2007). Therefore, aeration (Nivala et al., 2007), dredging, and management programs (Lee et al., 2009) are essential for successful remediation. Phytoremediation systems are used in constructed wetland treatment systems to detoxify, degrade, and inactivate potentially toxic elements in leachate (Jones et al., 2006). Phytoremediation, as an alternative to conventional engineering-based remediation methods, is both cost-effective and

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environmentally friendly (Kim and Owens, 2010). Some information already is available on the dynamics of phytoaccumulation, the uptake of contaminants by plant roots and the translocation/accumulation of contaminants into shoots and leaves (FRTR, 2010), in landfill leachate (Jones et al., 2006; Kim and Owens, 2010; Zalesny et al., 2006). Leachate treatment and phytoaccumulation is used in the Sudokwon landfill, where reed (*Phragmites australis*), planted in leachate channels (Lee, 2008), increases the uptake of contaminants (Cho, 2008).

Phytoremediation may be costly, but investment in this developing technology should yield important environmental, economic and societal benefits (Cundy et al., 2016) such as reducing air pollution and mitigating urban heat island effects. To ensure its effectiveness, however, phytoremediation must overcome inherent technical limitations (Cunningham and Ow, 1996). For example, the efficiency of the process depends on properties of water, depth and nutrients, as well as ambient atmospheric, physical, and chemical environmental conditions. Also, phytoremediation is only appropriate for sites that are large enough to allow appropriate farming areas and techniques, and harvesting costs of plants after remediation are high. Plants must be harvested before they decompose in the field to prevent the release of uptaken pollutants. Still, the release of contaminants from the vegetation may limit the wetland's total biodegradation capacity (Helfield and Diamond, 1997). To date, no guidelines exist for the after-use or management of phytoaccumulating plants. Harvested biomass might be used for energy production (Vigil et al., 2015), in attached-growth systems to develop detoxifying biofilms (Valipour et al., 2014), or as compost after remediation (Cundy et al., 2016).

Azolla (water fern) is a free-floating aquatic fern found across the world in freshwater ecosystems that offers significant promise in phytoremediation. The fern has a symbiotic association with the nitrogen-fixing alga *Anabaena azollae* (Arora and Singh, 2003) and accumulates high concentrations of heavy metals from aqueous media (Sela et al., 1989). Because of their depth, leachate channels typically remain largely uncovered by plants (Lee, 2008; however, the distribution of free-floating *Azolla* is not limited by site topography. In addition, *Azolla* growing over the water surface can be easily harvested using a net, in contrast to much more problematic harvesting of macrophytes that often requires use of a scythe. Ordinary harvesting machinery such as combines cannot be used on phytoaccumulating plants in leachate channels due to water depth and poor accessibility. Because it represents an environmentally friendly phytoremediation system, the use of *Azolla* for phytoremediation at landfills may be acceptable to communities and thus facilitate planning efforts. As an added benefit, fertilization by *Azolla* can improve soil conditions and plant growth at landfills. The plant can replenish nutrients that are depleted in landfill sites as poor-quality soil is added which otherwise would reduce the growth of vegetation (Ettala et al., 1988). As sanitary landfills including our study sites usually aim for environmental restoration (SLMC, 2010), restoration of soil conditions is an important objective.

In this study, we used *Azolla* species as a model bioaccumulating species for phytoremediation, with special emphasis on its potential applications in phytoremediation and subsequent landfill management by examining potential uses of *Azolla* after phytoaccumulation. We investigated the phytoaccumulation capacity of *Azolla* on landfill leachate. We evaluated increases in plant biomass, accumulation of five elements (Na, Mg, P, Fe, and Mn), and bioaccumulation ability of *Azolla* in different concentrations of leachate. In addition, we studied effects of the application of harvested *Azolla* after composting on plants growing on reclaimed land.

2. Materials and methods

2.1. Azolla preparation

Azolla japonica Fr. et Sav. (water fern) was collected in 2004 from its native habitat in Muan, South Korea. This culture was grown in a greenhouse water tank at the Seoul National University for use in this study.

2.2. Study site

The Sudokwon landfill is located in Incheon city, Korea (37° 34′ 52″ N and 126° 37′ 29″ E). The landfill, about 20,000,000 m^2 in size, produces 3500 tons of leachate per day (SLMC, 2010). About 5 km of leachate channels surround its reclamation sites. A wetland has been constructed at the end of the channel as a buffer zone.

2.3. Leachate preparation

Leachate produced from the landfill was collected every month between September 2006 and August 2007. All leachate samples used for our experiments were processed at a leachate-treatment facility before application.

2.4. Experimental design

We conducted phytoaccumulation experiment using leachate in water bowls, and used the results to model *Azolla* growth and harvestable yield prediction. Separately, we grew *Azolla* in 25% of leachate for composting and applied the product on the landfill slope to examine the feasibility of using it as compost.

2.4.1. Phytoaccumulation by Azolla

The collected processed leachate was diluted with piped water to four concentrations (25%, 50%, 75%, and 100%) and used, along with a control of piped water (0%) in our studies. Once a month, 5 g of *Azolla* was placed into a plastic (polypropylene) water bowl (diameter, 30 cm; height, 14 cm) filled with 4 L of water (6 replicates for each treatment). Moisture on the plant samples was carefully removed using paper towels without damaging fine roots before measuring their biomasses. *Azolla* was grown in a greenhouse for three weeks before the final harvest. Water lost by evaporation in each bowl was supplemented by the addition of distilled water to the bowl every week.

2.4.2. Post-harvest experiment

Azolla for post-harvest experiments were grown separately in a greenhouse between March and September 2007. *Azolla* were grown in water bowls (20 L) with 25% leachate collected from the landfill every month. We harvested 50% of *Azolla* when they covered the water surface of the bowls by over 80% (80% coverage). Harvested *Azolla* were dried at room temperature. Composting of *Azolla* was performed using effective microorganisms (EM) as per the instructions in the EM composting manual (EM center, 2003). EM solution includes *Saccharomyces* sp. (4.3×10^2 cfu/g dry slurry), *Rhodopseudomonas* sp. (4.8×10^2 cfu/g), and *Lactobacillus* sp. (1.9×10^7 cfu/g). These inoculants were isolated from natural sources of fermentation. We mixed 1.5 kg of dried *Azolla* with 0.5 L of distilled water, 0.1 L of inoculation solution (EM center, Jeju, Korea), and 5 g of sucrose; the mixture was kept in a sealed container (50 L). The container was covered with a thick blanket to prevent heat loss. After 25 days of composting, the container was held at room temperature (20 °C) until further application.

In May 2008, we planted 2-year old seedling chestnut trees (*Castanea crenata* Sieb. et Zucc.) in pots (19 cm diameter, 16 cm high), filled with 3100 cm^3 of either reclaimed or *Azolla*-fertilized soil. We conducted 10 replicates of each treatment (control, reclaimed or treated soil)). Reclaimed soil was collected from planted areas of the landfill in April and sieved (2 mm) to remove gravel. We used 100% reclaimed soil obtained from the Sudokwon landfill for the control treatment. In the *Azolla* treatment, 20% (by mass) composted *Azolla* was mixed with reclaimed soil. The mixing ratio was determined by previous studies using composts obtained from the same landfill (Song and Lee, 2010). The mean height of the chestnut trees before the experiment was 36.3 cm. The chestnut trees were harvested in October 2008.

2.5. Modeling of Azolla growth

We used a simple exponential growth model [dA/dt = g(t)] and the Runge-Kutta 4th order method for modeling the predictions of monthly harvest and daily relative growth rate of *Azolla*. We've considered detailed conditions such as harvesting condition (Variation in monthly harvest and harvesting *Azolla* before coverage became 100% during experiment). Detailed modeling methods are shown in Appendix A (supplementary data).

Also, by using predicted daily relative growth rates, we calculated the annual yield (harvest) of *Azolla* within bowls under three assumptions. First, the biomass of *Azolla* with 100% coverage of the bowl was estimated at 30 g. This condition was obtained by taking the mean of four actual measurements. Second, half of the *Azolla* was harvested when it reached 100% coverage. Third, the initial biomass was taken to be 15 g (50% coverage).

2.6. Soil, compost, and plant analyses

2.6.1. C and N analysis and moisture

C and N contents of the soil and plants were determined using an elemental analyzer (Flash EA 1112; Thermo Electron Co.). The soil was dried for 48 h at 105 °C to measure the moisture content.

2.6.2. Pollutant analysis

Plants were dried at 60 °C for 2 weeks. Soil and compost were dried at room temperature without exposure to light and dried again for 48 h at 60 °C. Each sample of dried and milled soil, compost, and plants (1 g) was pretreated with 60% HNO_3 for 24 h and heated to 80 °C for 2 h. Then, 10 ml of 70% perchloric acid was added, and the solution was heated to 200 °C until it became clear. The leachate was acidified with nitric acid until pH became less than 2. Then acidified samples of plant, soil and leachate were filtered using Whatman 44 filter paper. The samples were diluted with same volume of distilled water before actual analysis to prevent damage of instruments by acidified solution. Element contents of the above solutions were analyzed by using an inductively coupled plasma (ICP) emission spectrometer (ICPS-1000IV; Shimadzu, Japan).

2.6.3. Photosynthesis

Photosynthesis was measured using a portable photosynthesis-measurement system (Li-6400; Li-cor Biosciences, USA) in early September under controlled conditions (30 °C, 400 ppm CO_2).

2.6.4. Plant biomass

Plant leaves of chestnut trees were harvested five months after planting. Harvested leaf samples were used for biomass and element analyses.

2.6.5. Temperature, pH, and electrical conductivity (EC)

The temperatures of the greenhouse and compost were measured using a TR-71S Thermo recorder (T & D, Japan). The temperature of the solution was measured using a portable EC meter (YSI 30/10 FT meter; YSI, USA) around 2 p.m. The EC of the leachate was measured using the same portable EC meter. The pH of leachate was measured using a portable pH meter (YSI 60/10 FT meter; YSI, USA).

2.7. Statistical analysis

Differences between two groups were evaluated using Student's t-test for normally distributed variables (photosynthetic rates of trees and characteristics of soil, compost and plant leaves) or the Wilcoxon two-sample test when normality assumptions were violated (Zn contents of chestnut trees). For comparing multiple groups with normally distributed variables, data were analyzed by one-way analysis of variance and when a significant treatment effect was detected, post hoc comparisons of the means were made with Duncan's multiple range test (Mg contents of *Azolla*). For comparing multiple independent groups with non-normally distributed variables, data were analyzed by the Kruskal-Wallis test (Na, Fe, Mn and P contents of *Azolla*). Statistical tests were conducted with SAS 9.1 (ANOVA and NONPAR1WAY procedures, SAS Institute, USA). Statistical significance was inferred when $P < 0.05$. Data are presented as mean ± SE.

3. Results and discussion

3.1. Phytoaccumulation of Azolla

3.1.1. Leachates and environmental conditions

Leachate characteristics for each month are shown in Table A1 (Appendix B of supplementary data). Phosphorus (P), magnesium (Mg), and N were analyzed for nutrient contents. Zinc (Zn), sodium (Na), and iron (Fe) were analyzed because they are major elements in leachate of the Sudokwon landfill (Cho, 2008). Manganese (Mn) was analyzed for the first time in the Sudokwon landfill. Leachate characteristics varied among months of our study, possibly in response to precipitation and temperature at the study site (Lee and Ahn, 2000). As the landfill is very well managed, metal contents of the leachate were only present in low concentrations. However, the Na content of leachate (average 2398 mg/L) was relatively high, potentially increased by food wastes (Lee and Ahn, 2000). The P (average 0.6 mg/L) and Mn (average 1.4 mg/L) contents of leachate were higher than the national standard (P: 0.2 mg/L and Mn: 1 mg/L) for release into clean areas (Yum, 2007), but they were lower than the national standard for leachate emission (P: 1 mg/L and Mn: 10 mg/L). The Zn content of leachate (average 0.003 mg/L) was lower than the national standard (1.0 mg/L) for emission. However, nutrients and metals that may produce environmental problems including eutrophication and bioaccumulation were detected, and therefore the treatment of the leachate is required.

The average temperature of the greenhouse during the experiment was 16.1 °C and 26.7 °C for solutions in the water bowl (Fig. A. 2 in Appendix B of supplementary data). During winter, greenhouse temperatures were about 5 °C higher than ambient. Temperatures of solutions were much than ambient air temperatures, as they absorbed solar energy.

3.1.2. Growth of Azolla

Azolla biomass increased significantly in treatments of 50% leachate and above (Fig. 1). The mean harvested biomass of *Azolla* was 12.7 ± 0.7 (g).

Masses ($\overline{X}$±SE; n = 6) were determined from fresh samples.

From early spring to late autumn, *Azolla* biomass rapidly increased in mass, but growth was slower in winter (Fig. 1). The growth rate in April, compared to preceding and following months,

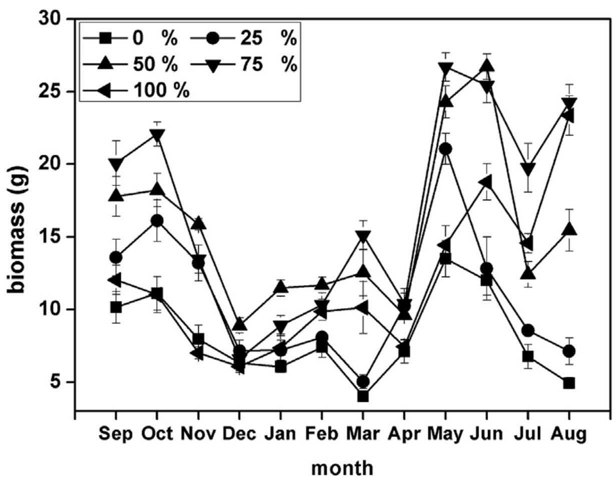

Fig. 1. Monthly harvested biomass of *Azolla japonica* in leachate treatments of different concentration.

unexpectedly dipped. This coincided with high metal contents in leachate that we sampled that month (Table A.1 in Appendix B). The rate also decreased in July, perhaps because of reduced light intensity on cloudy days accompanying extensive rainfall. Despite being the hottest and sunniest month, the rate in August was not the highest. As the temperature of the solution reached 40 °C in August, growth of *Azolla* apparently was adversely affected. Temperatures above 40 °C can decrease growth rates of *Azolla* species (Uheda et al., 1999). Furthermore, rapid evaporation at high temperatures and under high light intensity may have concentrated solutions to which *Azolla* was exposed. Nevertheless, *Azolla* biomass increased substantially. These rapid growth rates demonstrate that *Azolla* can yield substantial amounts of total harvestable biomass, which is essential for remediating plants.

Predicted monthly harvestable biomass (Fig. 2 A–E) and daily relative growth rate (Fig. 2 F) are shown in Fig. 2.

A is the predicted and harvested monthly mean biomass of 0% *Azolla japonica* in leachate treatments. B is 25%, C is 50%, D is 75%, and E is 100% leachate treatments. The symbols represent the predicted and harvested biomass of *Azolla japonica* of every month. Results are shown for a full year, beginning on the 1st January.

Measured harvestable biomass measures (9.94 ± 1.43%; mean ± SE of 60 replicates) fell within 10% error of predicted values (r values: 0% treatment = 0.89; 25% treatment = 0.85; 50% treatment = 0.89; 75% treatment = 0.90; and 100% treatment = 0.87). Based on these conditions, the annual yield of *Azolla* in a bowl was calculated as: 185.73 g in 100% leachate treatment, 291.90 g in 75% leachate treatment, 460.80 g in 50% leachate treatment, 481.19 g in 25% leachate treatment, and 345.85 g in the control treatment. Extrapolating from the 15 cm bowl radius, the annual yield per m^2 should be 14.2 times higher. The calculated annual *Azolla* yield per m^2 of the 25% leachate treatment, 6810.90 g, would result in an annual harvest of more than 32 times the initial biomass of phytoaccumulating *Azolla*. The detailed model results, including those for continuous rather than monthly harvesting, are presented in Fig. A1 (Appendix B of supplementary data).

3.1.3. Accumulation of Azolla

Accumulated elements in *Azolla*, by treatment, are shown in Table 1. Na was accumulated in *Azolla* at very high concentrations (22,000 mg/kg in 100% treatment). These Na concentrations concur with results from other *Azolla* species (Cohen-Shoel et al., 2002; Sela et al., 1989). Thus, *Azolla* is very effective for biofiltration of Na in solutions. Wetlands are prone to salinization due to anthropogenic changes to the hydrological cycle (Jolly et al., 2008) such as leachate inflow. Therefore, the ability of *Azolla* to tolerate and accumulate Na from leachate (Arora and Singh, 2003) will be very important as leachate contains high salt concentrations.

The Fe accumulation in *Azolla* was very high considering the concentrations of leachate. Fe can reduce growth rates and physiological activities of plants (Rai and Chandra, 1992) and Fe toxicity is associated with decreased leaf strength and increased susceptibility to diseases (USEPA, 2003). As Fe is the most widely used industrial metal in the world today, the ability of *Azolla* to accumulate Fe should be advantageous to treatment programs.

Mn levels in leachate have not been studied in detail in the landfill. Mn is essential for normal growth and development, but overexposure to Mn has been reported to cause neurological symptoms in many organisms (Roth and Garrick, 2003). High concentrations can reduce growth and decrease chlorophyll contents of plants (Rai and Chandra, 1992). We recorded high levels of Mn accumulation in *Azolla*, but these varied from month to month more than other elements, presumably in response to variable Mn levels in the leachate. Organic matter of constructed wetlands typically contains much Mn (Obarska-Pempkowiak and Klimkowska, 1999) so its biofiltration capabilities make *Azolla* a potentially useful tool to reduce Mn accumulation.

Mg accumulation in *Azolla* was about 5000 mg/kg in the 100% leachate treatment (Table 1). *Azolla* absorbs Mg at a high rate (Cohen-Shoel et al., 2002). As Mg is considered an essential mineral (Kaiser et al., 2009) rather than a toxic heavy metal, Mg accumulation in *Azolla* can be useful for post-harvest usage.

P accumulation in *Azolla* was about 2000 mg/kg in 100% treatments. Even in the 0% treatment, however, *Azolla* accumulated high P levels due to its presence in the piped water (P: 0.6 ± 0.2 mg/kg). P accumulation in *Azolla* with similar P levels in the media (Arora and Saxena, 2005) was about twice that observed in our study. Overall, the P accumulation levels that we recorded were quite high in comparison to previous findings (Arora and Saxena, 2005). As P is one of the major causes of eutrophication in wetlands (Lowe and Keenan, 1997), biofiltered P will decrease the impact of leachate on wetlands. In addition, absorbed P should enhance the use of leachate as a soil fertilizer.

When considering predicted annual biomass yield (Fig. 2) and average uptake of plants (Table 1), the 50% treatment showed maximum accumulation of every analyzed element. Although the accumulation rate of the 50% treatment was only slightly more than half of that of the 100% treatment (Table 1), the expected biomass yield of 50% (461 g of annual yield of *Azolla* in a bowl) was more than twice that of the 100% treatment (186 g). Therefore, 50% appears to be the most effective concentration for bioaccumulation.

The high accumulations shown in the 0% treatment raise the possibility that distilled water might have served as a better control but the quantities of water needed made this impracticable. The average elemental contents (mg/L) of piped water were Na: 45.5 ± 8.1, Mg: 4.5 ± 0.1, P: 0.6 ± 0.2, Mn: 0.2 ± 0.0, and Fe: 0.02 ± 0.01 (collected in every season, n = 4). The Na and Mg contents of *Azolla* grown in 0% treatments were high in comparison to the Na and Mg levels of piped water. As there were small amounts of nutrients in the media, plants would take up more water to compensate for their nutrient requirement. *Azolla* species showed a higher number of roots in nutrient-deficient media, probably reflecting this increased accumulation of elements. Overall, in both low and high concentration treatments, *Azolla* was excellent in accumulating metals.

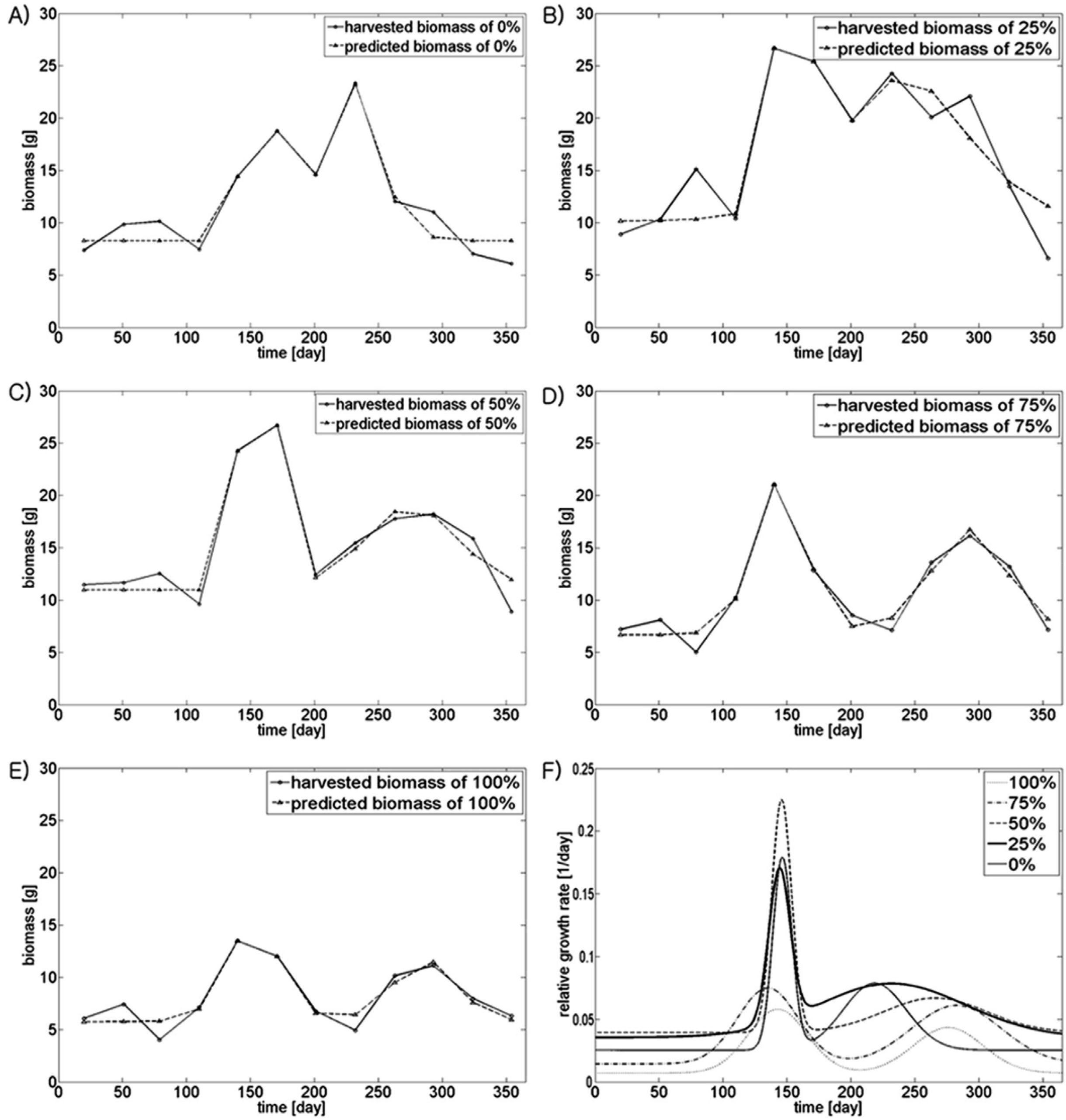

Fig. 2. Predicted and harvested monthly mean biomass (A, B, C, D, and E) and predicted daily relative growth rate (F) of *Azolla japonica* in leachate treatments of different concentrations.

3.2. Post-harvest experiments

3.2.1. Azolla as a biofertilizer

Management of plants after remediation requires careful planning because the plants serve as reservoirs of toxic materials that, if they remain unharvested, ultimately will be re-released as they decompose. During our study, reed plants were in the process of decomposing in leachate channels of our study sites. Harvesting of remediation plants may be labor-intensive and costly, which is one of the major disadvantages of phytoremediation. Our pilot study of post-harvest use of *Azolla* was designed to determine requirements for effective processing. *Azolla* can be used as a source of N biofertilizer for paddy fields (Arora and Saxena, 2005), so the composted *Azolla* plants were used to fertilize soil. The ease with which *Azolla* can be harvested by net from the water surface represents a major advantage of using it for phytoremediation. Because *Azolla* has a symbiotic association with the nitrogen fixing alga *Anabaena azollae* (Arora and Singh, 2003), *Azolla* appears to be more useful as an N source. However, as *Azolla* accumulates salt and heavy metals, its application to clean agricultural areas requires some caution.

Thus, we investigated effects of the application of *Azolla* to a disturbed area, the Sudokwon landfill. The application of *Azolla* compost to already contaminated landfills would be unlikely to face objections by members of the public because the environmental impact would be low with little possibility of food chain contamination. In addition, the application of *Azolla* to landfills holds the potential to effectively reclaim poor landfill soil. Meanwhile, the application of phytoaccumulating *Azolla* should retain toxic

Table 1
Phytoaccumulated elements of *Azolla* grown in leachate treatments.

Items	%	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Mean	F(Chi-Square) value (*P*)
Na (mg/g)	0	2.5	2.5	1.8	0.6	0.5	1.9	1.6	1.3	1.5	2.0	1.8	2.0	**1.7**	**41.2*****
	25	12.6	8.5	6.5	2.5	1.2	9.2	7.7	8.5	7.8	6.7	9.2	14.6	**7.9**	
	50	15.7	11.5	8.5	3.6	8.5	13.2	33.4	11.2	8.0	18.0	15.4	16.3	**13.6**	
	75	13.6	14.3	10.3	5.2	12.3	15.0	14.0	15.2	13.9	20.3	32.7	18.2	**15.4**	
	100	21.3	15.2	13.7	9.9	24.4	17.6	20.1	26.2	16.9	28.4	33.5	37.3	**22.0**	
Fe (mg/kg)	0	108	125	155	124	155	123	142	192	119	162	190	114	**142**	
	25	310	345	270	256	453	651	359	390	403	1431	974	534	**531**	**38.8*****
	50	370	634	584	384	463	696	623	503	440	1056	1254	626	**636**	
	75	857	1383	695	584	495	705	603	584	1361	1460	1466	754	**912**	
	100	1627	1425	785	623	1379	815	556	611	846	1628	1688	864	**1071**	
Mn (mg/kg)	0	114	86	106	57	84	201	125	102	77	146	180	192	**123**	
	25	703	588	399	128	240	240	165	590	105	1036	2103	491	**566**	**34.4*****
	50	1006	731	459	226	452	274	437	1007	169	3323	2562	880	**961**	
	75	1635	2987	590	435	683	285	243	1546	1353	4696	2676	991	**1510**	
	100	2563	3563	888	685	1310	1662	262	2034	1426	5956	3543	1470	**2114**	
Mg (mg/kg)	0	519	1024	856	242	322	937	843	529	890	764	542	580	**671**[c]	
	25	3595	3525	1256	1086	1356	3462	1879	3524	2453	5524	4145	2173	**2832**[b]	**15.4*****
	50	3394	5101	1355	1125	1860	3692	3318	4353	2661	4131	4456	2254	**3142**[b]	
	75	3482	4655	1523	1563	3457	4992	3284	4453	4821	4416	4695	2390	**3644**[ab]	
	100	3643	5621	1598	1623	6696	6389	3679	4629	6814	4525	4956	4652	**4569**[a]	
P (mg/kg)	0	955	964	854	1054	1024	991	543	897	942	778	547	763	**859**	
	25	966	987	888	1124	1345	1118	1319	846	1255	968	869	782	**1039**	**34.4*****
	50	1036	1325	982	1543	1542	906	827	577	1338	1013	1256	846	**1099**	
	75	1317	1207	1542	1654	1852	1411	1494	1843	1162	1487	1517	578	**1422**	
	100	1626	1453	1637	1842	2313	1928	2886	1681	1356	4687	1875	1288	**2048**	

The means within a column followed by the same letter are not significantly different at 0.05 level.
※ Na, Fe, Mn and P values are statistically analyzed by Kruskal Wallis test: Chi-Square (*P*). (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).
Mg values are statistically analyzed by Duncan's multiple range test: F value (*P*).

materials within the landfill ecosystem and prevent diffusion to other areas by decomposition of phytoaccumulating plants.

As expected, the N contents of *Azolla* were very high (Fig. A. 3 in Appendix B). The N content of *Azolla* grown in the 0% leachate treatment increased by 2 percent every month. Other treatments showed increases of almost 3 percent in mean N contents. This N content of the harvested *Azolla* makes it suitable as co-composting material. The 25% leachate concentration was selected for the composting and application experiment because it produced substantial increases in biomass. The Korean national standard for the Na content of compost (KEI, 2003) is 1%, and only *Azolla* at the 25% concentration met this standard, containing less than 1% Na. The overall N content of collected *Azolla* of 25% concentration grown in water bowls (20 L) in 2007 was 2.63 ± 0.03% (mean ± SE of triplicates).

The temperature of the compost increased to over 40 °C after 7 days during composting and remained at this temperature for the following week. The maximum temperature during composting, beginning 10 days after inoculation, was 43.6 °C, and dropped to 26.4 °C after 20 days. These temperature regimens are consistent with those expected.

The nutrient and metal contents in soil, *Azolla* compost and chestnut trees after application are shown in Table 2. The metal values were lower than those of North American compost standards (Hogg et al., 2002), as reflected in specifications of the EPA Part 503 Biosolids Rule as well as the corresponding Korean standards (KEI, 2003). The Na content of *Azolla* compost was relatively high (6400 mg/kg), but the Na content of *Azolla* compost was below the Korean compost standards (10,000 mg/kg) (KEI, 2003), so *Azolla* compost should be acceptable for application.

No prescribed acceptable levels for Fe and Mg have been determined by the US EPA or by the Korean government. On the other hand, *Azolla* compost showed much higher N contents than untreated landfill soil (Table 2). The final N content of the reclaimed soil significantly increased to 0.40± 0.02% (n = 3) five months after application of *Azolla* compost (before harvest). The moisture content of the soil significantly increased from 11.3% (control) to 18.4% (*Azolla* compost treatment), five months after treatment (n = 3). As the landfill uses mined soil for reclamation work, the low organic matter content and high heavy metal content of the soil prevents plants of the landfill from becoming well established (Lee et al., 2004).

Although *Azolla* compost contains accumulated metals, their concentrations, except for Mg, were significantly lower than those

Table 2
Characteristics of reclaimed soil, *Azolla* compost, and chestnut tree leaves after the experiments.

		N (%)	C (%)	Na (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Soil & Compost	Control	0.03 ± 0.00	1.02 ± 0.04	38.0 ± 3.4	423 ± 23	22,274 ± 793	765 ± 52	70.2 ± 3.7
	Azolla	2.57 ± 0.02	42.8 ± 0.93	6400 ± 240	854 ± 25	520 ± 26	412 ± 14	34.4 ± 5.4
	T value	−82.14***	−39.01***	−26.45***	−12.44***	27.39***	6.57**	5.64**
Chestnut tree	Control	1.32 ± 0.02	47.3 ± 0.89	288 ± 35	127 ± 4	3510 ± 176	141 ± 22	2.5 ± 0.1
	Azolla	2.10 ± 0.07	47.4 ± 1.02	1345 ± 49	130 ± 5	2563 ± 76	121 ± 2	2.6 ± 0.1
	t value	−10.89***	−0.07	−17.61***	−0.45	4.95**	0.91	0.89

The data presented are mean ± SE (n = 3).
Means within a column followed by the same letter are not significantly different at the 0.05 level (*t*-test).
※ Zn contents of chestnut trees are statistically analyzed by Wilcoxon Two-Sample Test (Chi-Square value).
(*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

of reclaimed soil. Since Mg is considered an essential mineral (Kaiser et al., 2009), Mg accumulation in *Azolla* may be potentially useful. *Azolla* grown in 25% leachate contained on average of 1039 mg/kg of P (Table 1), the most important elements of fertilizer; thus, *Azolla* compost would provide low levels of P to the soil. In addition, the N content and the moisture content of the soil were significantly increased five months after treatment. These results indicate that *Azolla* compost worked as an effective and safe fertilizer.

3.2.2. Eco-physiological responses of plants after Azolla compost application

Chestnut trees were selected because the species was used for restoration in the landfill. Chestnut trees were planted on slope during the research period (2006–2007). Improved soil conditions by *Azolla* compost caused plants to show better eco-physiological reactions. The leaves of chestnut trees in the *Azolla* compost treatment contained significantly higher N contents (Table 2). On the other hand, metal concentrations of chestnut trees grown in *Azolla* compost contained lower levels of Mn and Zn than those allowed by the US EPA (450 mg/kg for Mn and 160 mg/kg for Zn) (USEPA, 2007), indicating the compost was safe for application. Therefore, *Azolla* compost potentially could be a useful N source to augment the low N levels characteristic of landfill soil (Table 2). Chestnut trees exposed to the *Azolla* compost treatment showed significantly better photosynthetic performance than controls (Fig. 3).

Symbols with the different letters are significantly different (t value = 5.86, $p < 0.001$).

The harvested biomass of the leaves of plants in the *Azolla* compost treatment (13.3 ± 1.3 g, n = 10) was significantly larger than that of the control treatment (10.3 ± 1.1 g, n = 10). We planned to measure the growth of trees, but were unable to do so, as the growth occurred primarily through budding rather than increasing stem breadth. The leaves of chestnut trees in the *Azolla* compost treatment contained significantly higher N contents, suggesting that the compost can be a useful N source to plants. As moisture, N, and Mg contents of the soil significantly increased in response to application of *Azolla* compost, the physiological responses of the plants also improved (Fig. 3).

Untreated reclaimed soil contained only low levels of nutrients but substantial amounts of heavy metals, which probably caused the photosynthetic rates of chestnut trees growing in the soil to be quite low. In the absence of irrigation, the low moisture content of the soil might limit photosynthesis. However, as the soil conditions were improved by *Azolla* compost, the photosynthetic rates of chestnut trees growing in the soil significantly increased. Specifically, physiological changes induced by *Azolla* compost appear to be responsible for the increased growth that we observed. Overall, ecophysiological responses were significantly improved by *Azolla* compost applications, demonstrating the effectiveness of *Azolla* compost as a fertilizer. Because the metal content of plant leaves did not significantly increase, the use of *Azolla* as compost appears to offer benefits at little cost.

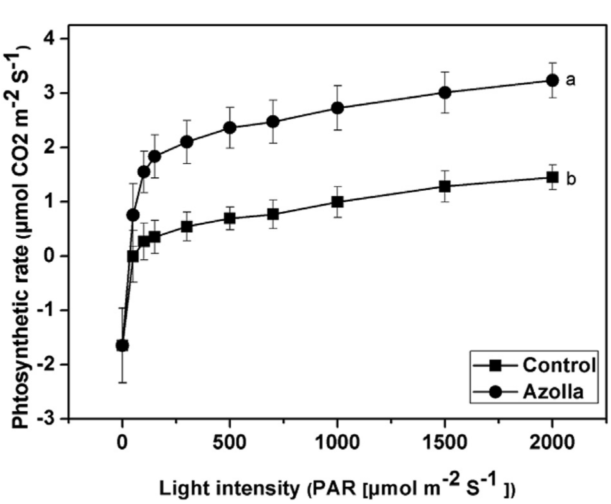

Fig. 3. Photosynthetic performance of the trees in *Azolla japonica* compost treatments (30 °C, 400 ppm CO_2;$\overline{X}$±SE; n = 5).

4. Conclusions

Azolla grown in landfill leachate showed impressive accumulation rates. Moreover, *Azolla* can be harvested easily, thereby preventing re-inflow of the pollutants from decomposing remediation plants to the leachate channel. Considering *Azolla*'s potential harvestable biomass, convenience of harvesting, ability to thrive in varied water depths, high accumulation rates, and long growing season, we suggest that *Azolla* can be used effectively to improve leachate biofiltration programs.

In the post-harvest research, we found metal (Na, Mg, Fe, Mn and Zn) contents of *Azolla* compost to be lower than those set by the US and Korean governments. Therefore, the use of *Azolla* as compost can provide N, C, P and Mg to nutrient-deficient landfill soil without much risk of adding significant contamination of polluting elements, at least not those that we examined. *Azolla* applications also enhanced ecological and physiological responses of plants. As applying fertilizers to landfills would be prohibitively expensive, the use of *Azolla* as fertilizer pshould provide both economic and ecological benefits.

While our study focuses on the use of *Azolla* compost in a single urban landfill, we suggest that our results may be useful in developing landfill remediation plans elsewhere. Application of *Azolla* will improve leachate quality during phytoaccumulation and also improve soil condition - a key problem for all landfill reclamation projects – through post-harvest composting in a variety of landfill ecosystems. Aside from its use in detoxification in landfills, *Azolla* should be considered more generally as a candidate remediating plant for eutrophicated wetlands. Such ecosystems are common around the world, and we believe these might benefit by using these plants for remediation. Wetland preservation additionally can provide a valuable source of high-nutrient fertilizers.

Its ease of harvesting, growth in a variety of water depths, high accumulation rates and harvestable biomass, and utility for composting material after remediation all support our suggestion that the use of *Azolla* for biofiltration in remediation programs merits serious consideration.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2016.07.052.

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