J. Plant Biol. (2012) 55:381-389
DOI 10.1007/s12374-012-0010-5

ORIGINAL ARTICLE

Ecological Responses and Remediation Ability of Water Fern (*Azolla japonica*) to Water Pollution

Uhram Song[1], Hun Park[2] and Eun Ju Lee[1,*]

[1]*School of Biological Sciences, Seoul National University, Seoul 151-742, Korea*
[2]*Center for Energy and Environmental Policy, University of Delaware, Newark, DE, USA*

Received: January 5, 2012 / Accepted: March 23, 2012

Abstract The ability of the water fern *Azolla japonica* to remediate phosphorus (P), nitrogen (N), and iron (Fe) contamination, and its physiological responses to three common sources of water pollution (landfill leachate, swine lagoon sewage, and fish farm sewage) and standard solution were investigated. The biomass, water content, and chlorophyll content of *Azolla japonica* in each solution were measured, and the concentrations and accumulation rates of polluting elements in the solutions were determined. *A. japonica* showed over eight-fold increase in biomass within only 20 d in every solution except in swine lagoon sewage, extremely high in N concentration. Consistent chlorophyll and water contents of the plant in most solutions showed that *A. japonica* can adapt to highly concentrated solutions. N, P, and Fe concentrations of the solutions decreased significantly within the 20 d. In most treatments, *A. japonica* showed high N accumulation and also showed total uptake of P and Fe from the solutions. In reference to this result, using this species as a phytoremediator plant would have additional benefits of helping maintaining endangered populations of *A. japonica*. Therefore, the plant's fast growth, good element remediation efficiency, and conservation needs makes *A. japonica* a suitable plant species for pollution remediation.

Keywords *Azolla japonica*, Bioaccumulation, Iron, Nitrogen, Phosphorus, Wastewater

Introduction

Water shortages are already a threat to more than 1 billion people, owing to the resulting consumption of polluted water (Pimentel et al. 2004). However, wastewater engineering systems are costly and reduce nutrient levels (Park 2005). Therefore, phytoremediation has become one of the most attractive solutions for water pollution problems, based on its efficiency and ability to recycle nutrients (Grady et al. 1999). Since the development of separate garbage claim and wastewater treatment facilities, the heavy metal content of wastewater has decreased (Song and Lee 2010). However, organic pollutants and nutrients from agricultural areas and disturbed fields are becoming a greater concern, and are even polluting soil (Schnoor et al. 1995). Phytoremediation is a very suitable means of treating nutrient-enriched water (Alker 1999), and many remediation systems are developing rapidly that focus on phytoremediation and the recycling of nutrients from wastewater, in combination with biomass production (Weih and Nordh 2002). The phytoremediation ability of plants is a very interesting subject (Park 2005), and several investigations using water ferns have been reported (Vermaat and Khalid Hanif 1998; Forni et al. 2001; Rai 2007). However, these studies did not use *Azolla japonica* Franch. et Savat, which is the native species in Korea. As *A. japonica* is a free-floating species, there is no water depth limit for its growth. As constructed wetlands have become the focus for the final stage of the remediation process (Song 2010), using free-floating water ferns to extend the limits (in terms of depth) of other macrophytes (reeds, cattails, etc.) would be an efficient management method. Two *Azolla* species (*A. japonica* and *Azolla imbricata*) were previously reported in Korea (Park 1961), but only *A. japonica* has been reported recently (Park 2005). Some *Azolla* species have recently become extinct, and many species have disappeared in some countries (Metzgar et al. 2007). Therefore, using *Azolla* species for phytoremediation would help to prevent further extinction. Also, since the growth rate of *Azolla* species is very high, with a doubling time of 3-5 d (Reddy and DeBusk 1985), *Azolla* species have huge potential for use in remediation with high harvestable biomass possibilities. As *Azolla* species has shown high tolerance to pollutants

*Corresponding author;
Tel : +82-2-880-6673
E-mail : ejlee@snu.ac.kr

(Sarkar and Jana 1986) and great potentials for phytoremediation (Vermaat and Khalid Hanif 1998; Forni et al. 2001), the remediation research of the native species would be important for further research in fields and also for the validity of application.

In this study, the remediation properties and physiological reactions of the water fern *A. japonica* upon exposure to 3 common sources of water pollution (leachate from a landfill, swine lagoon sewage, and fish farm sewage) were investigated. The ability of *A. japonica* to accumulate 2 major nutrients (phosphorus; P, and nitrogen; N), and iron (Fe), which is known to be one of the most abundant metals in leachate (Park et al. 1999), was analyzed. The biomass, root length, water content, and chlorophyll content of *A. japonica* in each solution were measured to characterize its physiological responses.

Materials and Methods

Preparation of *Azolla japonica*

A. japonica was collected from a lake in Muan, Jeollanam-do province, Korea, and grown in a greenhouse at Seoul National University, Seoul, Korea, with piped water for a few months before the experiments. The geographic coordinates of the lake are 34° 51 44" N and 126° 31 30" E.

Preparation of treatment solutions

Treated landfill leachate was collected from the Sudokwon landfill site in Seo-gu, Incheon, Korea. This landfill site has one of the biggest leachate processing systems in the world (Song 2010). Fish farm sewage was collected from a fish farm in Paju, Gyeonggi-do province, Korea. Swine lagoon sewage was collected from a pig farm in Gongju, Chungcheongnam-do province, Korea. The maximum concentration of each solution for survival of *A. japonica* was tested for a week before the experiments (Zimmels et al. 2004). One gram *A. japonica* (fresh weight) were grown in diluted (100%, 50%, 20%, 10%, 5% of original solutions) solutions (3 replicates per each) and after a week, the highest concentrations of each solutions showing no significant differences of *A. japonica* biomass between *A. japonica* grown in a culture medium (standard solution) (Ito and Watanabe 1983) were selected for final concentrations.

As a result of this pre-test, the treated landfill leachate was diluted to 10%, and the swine lagoon sewage was diluted to 5% before application. For the control, a culture medium (standard solution) suggested for culturing of *Azolla* species (Ito and Watanabe 1983) was used for growth and accumulation of *A. japonica*.

Experimental Design

One-gram samples of *A. japonica* (fresh weight) were grown in standard solution for a week and then transferred into 4.5 cm diameter and 12 cm height beakers containing 500 mL of the test solutions. Twenty beakers were prepared for each solution. The beakers were placed in a controlled growth chamber. Growth chambers were set on a 16-h light and 8-h dark photoperiod, 24°C/18°C temperature cycle, and 60% humidity. The water level (decreased by vaporization and absorption) was maintained by addition of distilled water every 2 d. *A. japonica* was harvested from 5 beakers of each test solution every 5 d (4 times over 20 d) after washing thoroughly by distilled water.

Ecological Analysis

Maximum root length was measured during harvesting, and 0.1-g plant samples were used to determine chlorophyll content by dimethyl sulfoxide (DMSO) extraction methods (Hiscox and Israelstam 1979). Plants were dried at 60°C to determine their water content and dry weight. The means of these parameters were calculated after excluding the maximum and minimum values.

The pH of solutions was measured using a portable pH meter (YSI 60/10 FT: YSI Inc.), and EC (electrical conductivity) was measured using a portable EC meter (YSI 30/10 FT: YSI Inc.).

Analyses of Nitrogen, Phosphorus, and Iron Content

Dried plant samples were digested using H_2SO_4. Specifically, 0.05-0.1 g (as some samples were less than 0.1 g dry weight) of each plant sample was digested in 3.5 mL H_2SO_4, with 63 mg of $CuSO_4$ and 750 mg of K_2SO_4 as accelerators (Moraghan et al. 1983). The samples were heated at 160°C for 1 h and at 375°C for 2 h. After digestion, samples were mixed with 10 mL distilled water and filtered through Whatman No. 44 filter papers.

The N content of digested samples and solutions was determined using a QuikChem AE Automated Flow Injection Analyzer (Lachat, Colorado). The P and Fe contents of digested samples and solutions were determined using an ICPS-1000IV Inductively Coupled Plasma Emission Spectrometer (Shimadzu, Kyoto) (3 replicates).

Statistical Analyses

One-way ANOVA was performed to identify significant differences between treatments, and when a significant difference was detected, a post hoc Tukey's studentized range (HSD) test was performed using SAS 9.1 software (SAS

Institute Inc.). Differences were considered significant when $p < 0.05$.

Results and Discussion

Table 1 shows the element concentrations and chemical characteristics of each solution (after dilution). Swine lagoon sewage has a very high total nitrogen (TN) and total phosphorus (TP) content, and therefore would contaminate freshwaters if it is released without any processing. Also, its high pH value would increase the pH of the freshwater systems into which it is released, which may cause environmental problems from inhibition of microorganism growth (Moss 1973) to biological breakdown (Schindler 1988). Though the majority of swine lagoon sewage is processed before release (Park 2005), most of the nutrients in the sewage are not recycled. Fish farm sewage and landfill leachate are released into the environment without any further processing. However, these 2 solutions even after dilution still contain high TN and TP concentrations, compared to limits of national standard of Environmental Water Quality Standards of P and N concentration (P: 0.05 mg/L and N: 0.6 mg/L) for water source protection area (WAMIS, 2011), which can cause environmental problems such as eutrophication (Xie et al. 2003). All of these solutions contain Fe, which is a micronutrient (Brown and Jones 1975) and can be deficient in plants (USEPA 2003), but can also cause toxicity when it accumulates (USEPA 2003). High EC values of solutions indicate a high ion (salt) content, which may cause environmental problems. Therefore, to recover nutrients and prevent pollution, these solutions should be processed before release.

Fig. 1A shows that *A. japonica* grew rapidly in most solutions except swine lagoon sewage. The biomass of *A. japonica* in standard solution, fish farm sewage, and landfill leachate increased from 0.5 g to around 4.0 g, showing an increase of more than 8-fold in only 20 d. These results of rapid growth indicate that *A. japonica* has a very high potential for accumulation (Zhang et al. 2010). In addition, harvesting *A. japonica* would be easy, in that it can be simply collected from surface water, which further increases its potential value. As the P and Fe concentration of the culture medium (standard solution) is over the national standards (P: 2 mg/L and Fe: 2 mg/L) of emission to clean areas (WAMIS,

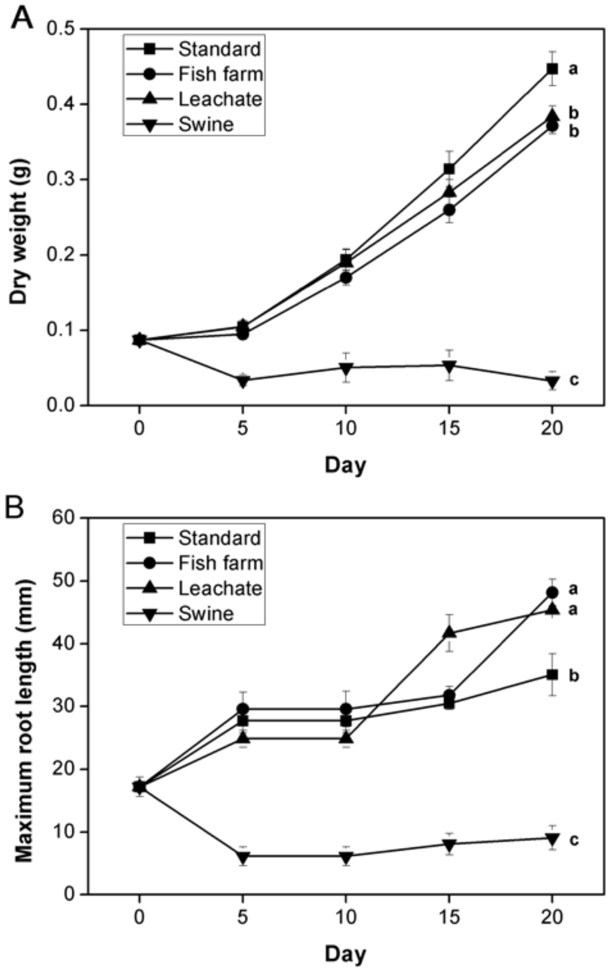

Fig. 1. (A) Dry weights and (B) maximum root lengths of *A. japonica* grown in each solution.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols having the same letter are not significantly different at the 0.05 level. ※Standard: Standard solution (culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

Table 1. Key element concentrations and chemical characteristics of each solution

	Standard solution (Culture medium)	Fish farm sewage	Treated landfill leachate	Swine lagoon sewage
Total nitrogen (mg N/L)	0.272 ± 0.134	3.889 ± 0.145	2.599 ± 0.113	36.269 ± 0.479
Total phosphorus (mg P/L)	15.382 ± 0.386	0.171 ± 0.001	0.069 ± 0.001	1.280 ± 0.028
Iron (mg Fe/L)	2.718 ± 0.062	0.312 ± 0.015	0.156 ± 0.015	0.359 ± 0.017
pH	5.07 ± 0.02	6.52 ± 0.02	6.62 ± 0.01	8.33 ± 0.03
Electrical conductivity (μS/cm)	743.3 ± 7.3	262.1 ± 1.0	1816.0 ± 10.1	558.7 ± 4.1

Values for treated landfill leachate and swine lagoon sewage are after dilution.
Values represent the mean ± SE of 3 replicates.

2011; Song 2010), *A. japonica* would have great effects in actual remediation sites (Such as leachate channel and buffer wetlands) where polluted water will be released with concentrations lower than the national standards (The concentration of N in the standard solution is much lower than the national standard, 30 mg N/L because the *A. japonica* is a N fixing plant.). And as the most of the actual waste water emission cases would be under national standards, the concentration values would be more similar to values of solutions after dilution (applied solutions).

However, in swine lagoon sewage, *A. japonica* did not show any significant increase in biomass. Since only 1 g of plant tissue was added to each beaker, and the plant surfaces were wiped with tissues before application, some of the plants suffered stress and root damage that interfered with early growth. Therefore, some beakers showed much lower biomass values that do not represent the actual underlying phenomena. Therefore, the maximum and minimum values were excluded when calculating means (Yauch et al. 2005). Fig. 1A and Fig. 1B shows that the biomass and the root length of *A. japonica* in swine lagoon sewage significantly decreased, indicating that plants did not survive well in this solution. Though *A. japonica* in 5% swine lagoon showed no significant differences of biomass compared to culture solution in pre-test, the results showed that 5% of swine lagoon was over suitable concentration for *A. japonica*. As pre-test was held for a week, responses of *A. japonica* for 20 d were different from the result of the pre-test. Also as 5% swine lagoon showed no differences (compared to culture medium) in pre-test and showed differences in the experiment, the concentration would be around the boundary concentration (However, more segmentalized treatments such as 3% and 4% were not tested because the pre-test had to cover wide range of concentrations). Previous studies also reported significant growth inhibition of *Azolla* species in 5% swine lagoon sewage (Costa et al. 1999), and suggested that its high N content and pH affects growth. As swine lagoon sewage has extremely high TN levels and pH values (considering the values of Table 1 is after dilution), it is not surprising that it would have negative effects on plants. However, plants in the other solutions showed significantly greater root lengths compared to those in swine lagoon sewage, and even compared to initial root lengths. (Root length is discussed further below). The measurements of water and chlorophyll content (Fig. 2) show that *A. japonica* in swine lagoon sewage did not grow well and began to fade. However, in the other treatments, *A. japonica* survived in highly concentrated solutions and did not lose water by osmosis. In addition, the chlorophyll content was significantly increased, probably due to the higher concentrations of nutrients in these solutions (Johnson et al. 1966). Since *Azolla* species under stress show decreased chlorophyll levels (Sarkar and Jana 1986), this result would indicate that *A. japonica* grew well in the highly concentrated solutions, except for swine lagoon sewage. Since the plants were grown in piped water for 1 week before the experiment, an increase in chlorophyll content was observed even in the standard solution.

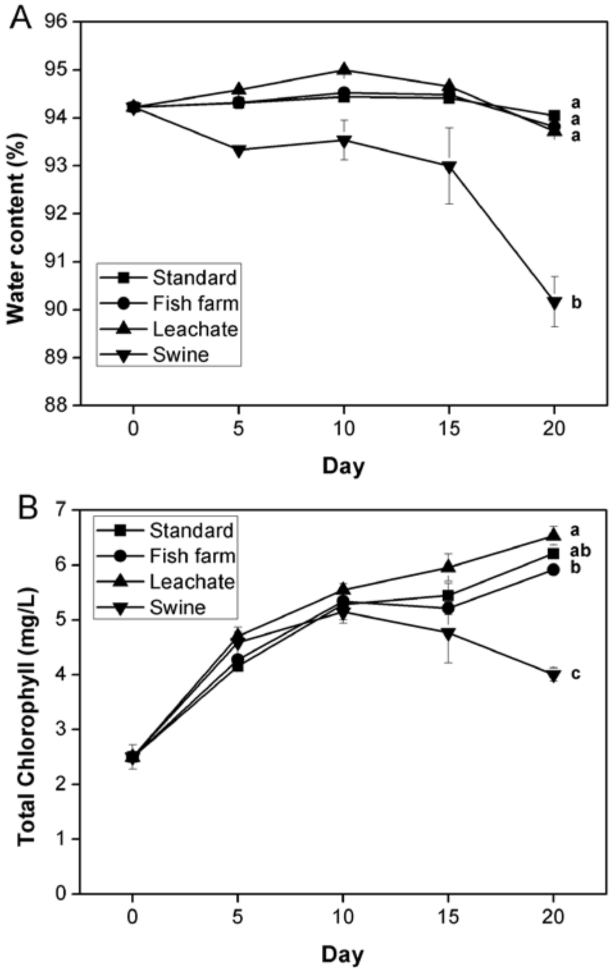

Fig. 2. (A) Water content and (B) total chlorophyll content of *Azolla japonica* grown in each solution.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols having the same letter are not significantly different at the 0.05 level. ※Standard: Standard solution (culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

Changes in element concentrations in solutions (indicating the remediating effects of *A. japonica*) are shown in Fig. 3. Element accumulation rates will provide an indication of the overall remediation potential of *A. japonica*. However, since the specific changes in solutions are important to understand, these results are shown first. Solutions with high N concentrations (swine and fish farm sewage) showed significant decreases in N concentration over the 20-d experiment, while the other solutions did not show any differences (Fig. 3A). As *Azolla japonica* is known for its ability to fix N

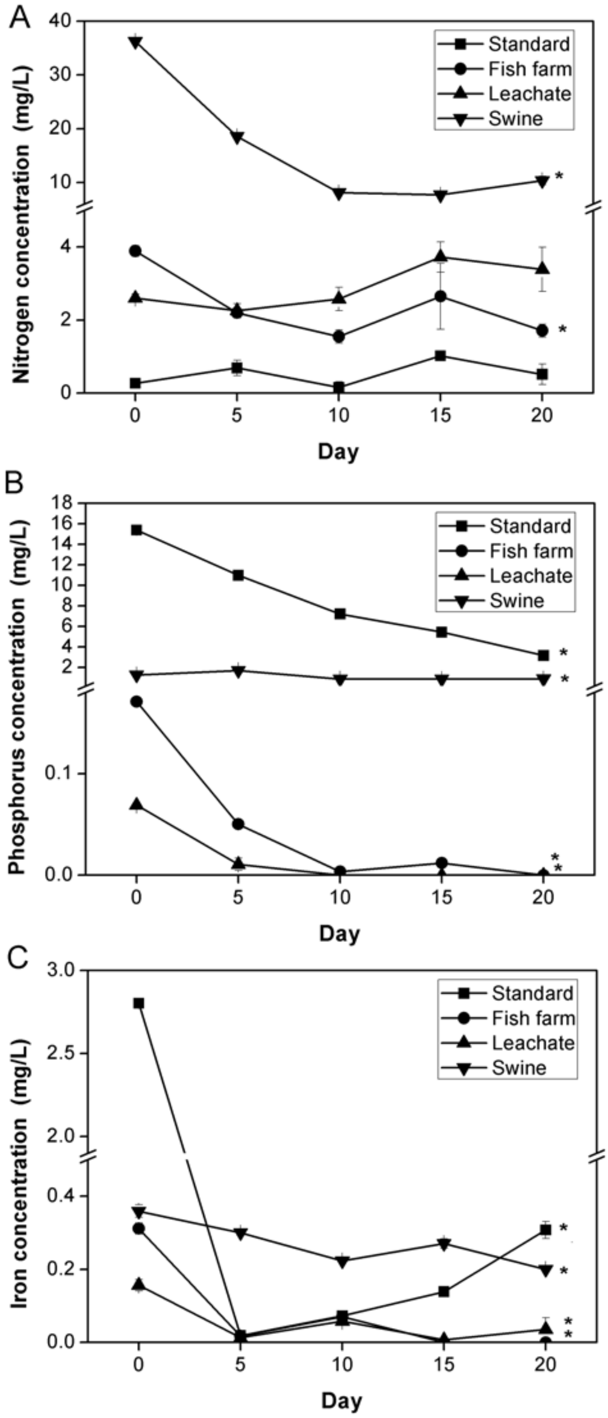

Fig. 3. Changes in (A) nitrogen concentration, (B) phosphorus concentration and (C) iron concentration of each solution with Azolla japonica growth.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols marked with an asterisk (at day 20) are significantly different from initial values (at day 0) at the 0.05 level.
※The phosphorus concentration of swine sewage solution in B) is significantly decreased (from 1.3 to 0.6), although this may be unclear due to the break in this figure between 0.1 and 2 on the vertical scale. ※Standard: Standard solution (culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

(Wagner 1997), the N levels of the solutions in which it grows will not necessarily change when N accumulates in the plant. However, since *A. japonica* was found to decrease the N concentration of solutions with high N levels to less than half of their original value, we believe that it would be a useful N remediation agent. Fig. 3B shows that the P concentrations of the solutions significantly decreased after 20 d, except in the case of swine lagoon sewage, which showed scant plant growth. The standard solution showed less than 20% of the original P concentration, and fish farm sewage and leachate solutions showed complete absence of P after 20 d. Nitrogen fixing plants tend to require more P than other plants (Vitousek et al. 2002), so *A. japonica* is expected to have a high P absorption rate. These results indicate that *A. japonica* is a very effective P accumulator, and as P is an essential element for agriculture, and phosphorus mines are being depleted, recycling P is very important (Weikard and Seyhan 2009). The concentrations of P and N also may explain the rapid root growth after 10 d (Fig. 1B), even when these nutrients became depleted in the solutions (Fig. 3). Since P deficiency (Cassman et al. 1980) and low concentrations of N in growing solutions (Landolt 1986) enhance root elongation, *A. japonica* may have grown roots in an attempt to find sources of N and P after consuming them. Every solution showed a significantly decreased Fe concentration after 20 d (Fig. 3C). Iron is rapidly taken up by *A. japonica*, since N fixing plants require Fe for fixation (Bazhenova et al. 2002). Therefore, *A. japonica* is a good plant for Fe pollution remediation. Because iron (Fe) is known to be one of the most abundant metals in leachate (Park et al. 1999) and it is toxic on accumulation (USEPA 2003), *A. japonica* would be an efficient and environmentally friendly tool for removing Fe from wastewater.

Fig. 4A shows the N content of *A. japonica* in each solution. In leachate, *A. japonica* showed significantly higher N levels than in other solutions and other treatments also showed significantly higher N accumulation than swine treatment. The concentration of N in swine sewage showed highest decrease in solution (Fig. 3A), while the N contents and biomass of the *A. japonica* in the swine sewage showed the lowest value. As the plants were floated over 20 d in the solution, plants in the highest N concentration would have more elements deposited in the surface and removed during washing by distilled water (Marques et al. 1993). As bottom of the solutions are turned over during the addition of distilled water to maintain the water level, particles containing N would have more chance to deposit in the plant's surface. Also as the roots of the *A. japonica* in swine sewage is lost during the research (Fig. 1B) and sunk in the bottom of the solution, it also may have deposited N. And as Fig. 2 shows that *A. japonica* in the swine sewage is under stress, it would

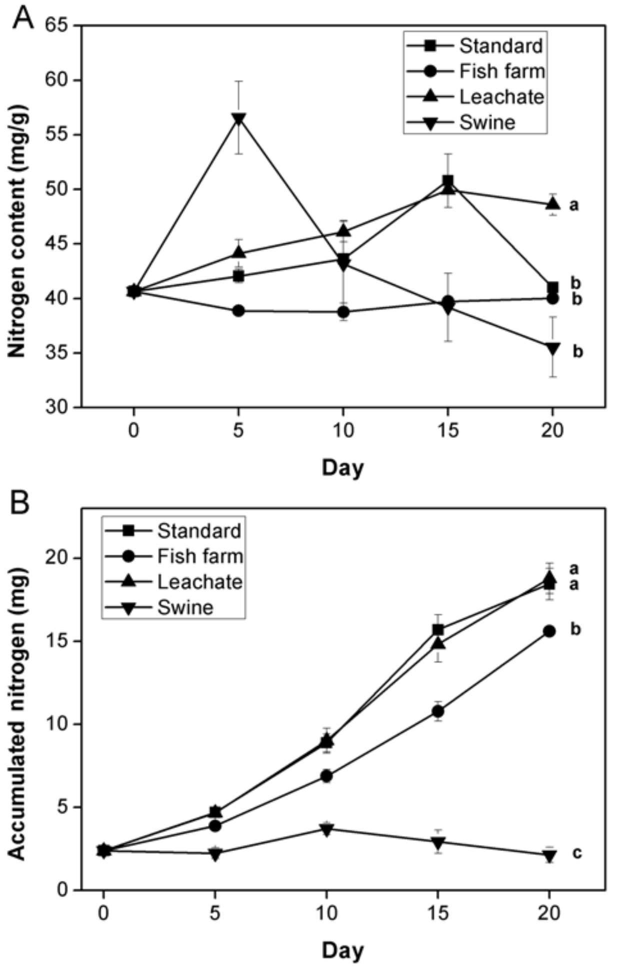

Fig. 4. (A) Nitrogen content and (B) total accumulated nitrogen in Azolla japonica grown in each solution.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols having the same letter are not significantly different at the 0.05 level.
※Standard: Standard solution (culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

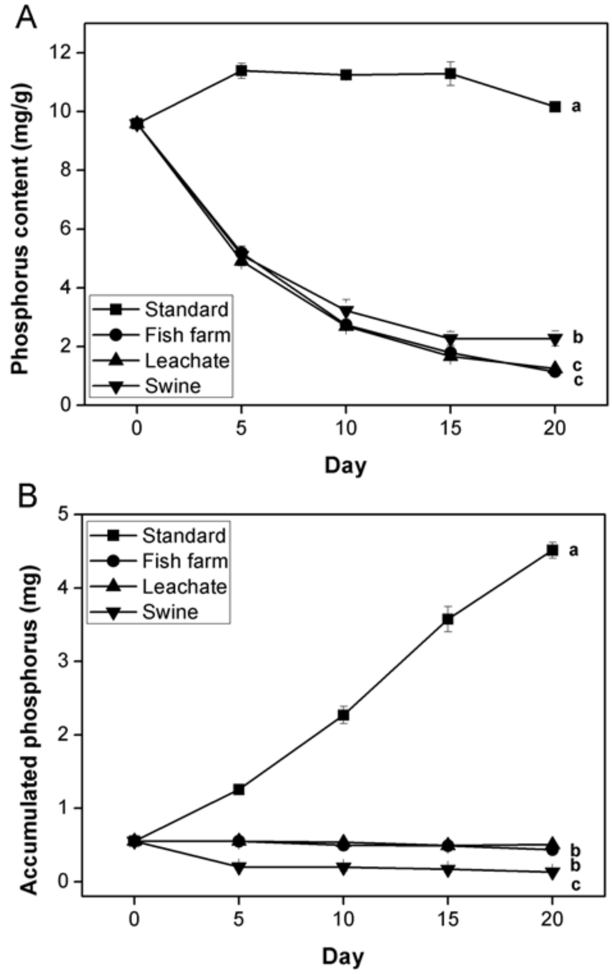

Fig. 5. (A) Phosphorus content and (B) total accumulated phosphorus in Azolla japonica grown in each solution.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols having the same letter are not significantly different at the 0.05 level.
※Standard: Standard solution (culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

have fixed lowest N among other treatments (Standard solution in Fig. 3A shows that fixed N increases N concentration of the solution). Also *A. japonica* tends to fix N less in solutions with high N contents but uses more fixed N in solutions with lower N (As the N concentration of solutions with higher N showed decrease of N, while solutions with lower N showed no decrease of N indicating that plant has used fixed N for its own growth). Nevertheless, the N contents of are *A. japonica* (Fig. 3A) was higher than typical average N contents (15-40 mg/g) for floating plants as water lettuce (*Pisitia stratiotes*) and water hyacinth (*Eichhornia crassipes*), indicating high accumulation (Lu et al. 2010).

By multiplying by the plant mass, the total accumulated nitrogen was calculated (Fig. 4B). Except in the case of swine sewage, *A. japonica* showed high N accumulation in all solutions. Considering that only 1 g of *A. japonica* was used for each test, and the plants were only grown for 20 d, the potential of *A. japonica* for N accumulation and remediation is therefore enormous. However, as *A. japonica* is known for its ability to fix N (Wagner 1997), the exact amount of N absorbed from solutions would vary. Nevertheless, *A. japonica* showed a significant ability to remove N from solutions (Fig. 3A). Thus, as it is an N fixing plant that also rapidly accumulates N from solutions, it has a high (over 4%) N content and could be used as a fertilizer. Therefore, after accumulating nutrients from wastewater, *A. japonica* would have great potential as a biofertilizer.

A. japonica in standard solutions showed significant P accumulation (Fig. 5). As P is becoming increasingly depleted in soils (Weikard and Seyhan 2009), the high P accumulation potential of *A. japonica* is very important. However, since the P in the treated landfill leachate and fish farm sewage was almost completely absorbed (Fig. 3B), and *A. japonica* did not grow well in swine lagoon leachate (Fig. 1A), only the plants in the standard solution, which had enough P, showed significant P accumulation (Fig. 5B). However, if the other solutions had higher P concentrations, we predict that they would also cause significant P accumulation in *A. japonica*. Nevertheless, the P contents of *Azolla* (Fig. 4A) in culture medium (with enough P) were higher than typically average P contents (4-10 mg/g) for floating plants as water lettuce (*Pisitia stratiotes*) and water hyacinth (*Eichhornia crassipes*), indicating high accumulation (Lu et al. 2010).

A similar pattern was observed for iron accumulation (Fig. 6). As the Fe in the solutions was rapidly depleted (Fig. 3C) it did not accumulate significantly in plants grown in landfill

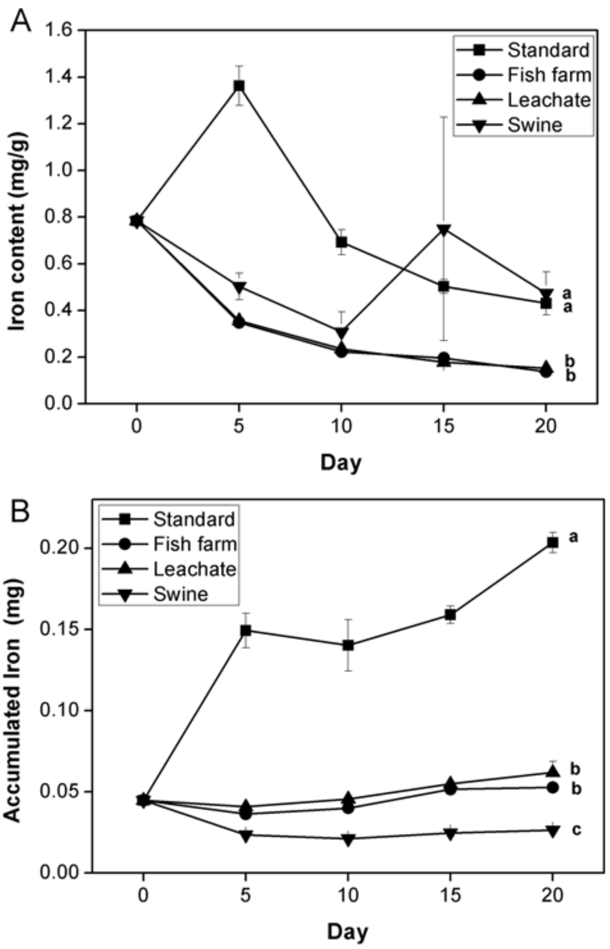

Fig. 6. (A) Iron content and (B) total accumulated iron in Azolla japonica grown in each solution.
Symbols and bars represent the mean ± SE of 3 replicates.
Symbols having the same letter are not significantly different at the 0.05 level.
※Standard: Standard solution (Culture medium), Fish farm: Fish farm sewage, Leachate: Treated landfill leachate, Swine: Swine lagoon sewage

leachate or fish farm sewage (Fig. 6B). However, plants in the standard solution, which contained enough Fe, showed significant accumulation (Fig. 6B). As other experiment using floating plants (water lettuce and water hyacinth) with higher initial biomass rate (100g plant/2L) showed less Fe reduction percentage (about 80%) in 5 mg Fe/L and 1 mg Fe/L solutions (Dixit and Dhote 2010), uptake rate of *A. japonica* is interesting. As N fixing plants such as *A. japonica* rapidly take up Fe for N fixation (Bazhenova et al. 2002), *A. japonica* would have a higher potential for Fe remediation than other plants. However, these results (Fig. 5A and Fig. 6A) also indicate that if not harvested, *A. japonica* would release P and Fe again to the surrounding environment. Therefore, research on the harvesting and utilization of *A. japonica* is needed.

Using *Azolla* species as a remediation plant would be advantageous for reasons other than its ability to accumulate elements. Several *Azolla* species recently disappeared in some countries (Metzgar et al. 2007), and Korea also faces this crisis. Only 3 sites were previously reported to be inhabited by *A. japonica*: Baekryeonji in Muan, Jeollanam-do province (Park 2005), Juamho in Boseong-gun, Jeollanam-do province (YERC, 2000), and Munsanchun in Paju, Gyeonggi-do province (Choi and Kim 2001). However, more recently, researchers were unable to locate *A. japonica* at Juamho (2009) or Munsanchun (2011). Only Baekryeonji (2009) showed inhabitation by *A. japonica* in our recent research. Therefore, this species is under serious threat of extinction. Thus, using *A. japonica* as a means of phytoremediation would help to maintain communities of this species and prevent its extinction. It may not help to conserve the species in the natural habitat, but if use of *A. japonica* species as remediating plant became more popular by our and further research, it may induce cultivation and would help to prevent total extinction. Also increased practical use would help to gather public attentions and may help to start conservation activities (Maunder 1992).

Conclusions

A. japonica showed rapid growth rate, high accumulation rate, and remediation potential in highly concentrated solutions. Except in swine lagoon sewage, which had an extremely high N concentration, *A. japonica* showed an increase in biomass of more than 8-fold within only 20 d. Measurements of water and chlorophyll content showed that *A. japonica* has the ability to adapt to highly concentrated solutions and does not lose water by osmosis. Solutions with high N concentration (swine and fish farm sewage) showed a significant decrease in N concentration in only 20 d, and the P concentrations of solutions were also significantly decreased. All solutions showed significantly decreased Fe concentrations after 20 d, since *A. japonica*, an N fixing plant, absorbs Fe rapidly for use in N fixation. Except in the case of swine sewage (which gave limited plant growth), *A. japonica* showed high N accumulation in all of the solutions. *A. japonica* in standard solutions showed significant P and Fe accumulation, and plants in the other treatments also showed increases in P and Fe until depletion of these elements in the solutions (by uptake). As *A. japonica* is under serious threat of extinction, using this species as a means of phytoremediation would help to maintain populations. Therefore, based on its fast growth, element remediation (accumulation) efficiency, and ecological circumstances (endangered), *A. japonica* would be an adequate choice of plant for pollution remediation.

References

Alker G (1999) Phytoremediation of nutrient rich wastewaters and leachates using *Salix*. Imperial College of Science Technology and Medicine, University of London, London

Bazhenova MA, Bazhenova TA, Petrova GN, Mironova SA (2002) Mutual effects of substrates and inhibitors in reactions catalyzed by isolated iron-molybdenum cofactor of nitrogenase. Kinet Catal 43:592–602

Brown JC, Jones WE (1975) Heavy-metal toxicity in plants 1. A crisis in embryo. Commun Soil Sci Plant Anal 6:421–438

Cassman KG, Whitney AS, Stockinger KR (1980) Root growth and dry matter distribution of soybean as affected by phosphorus stress, nodulation, and nitrogen source. Crop Sci 20:239–244

Choi HG, Kim CG (2001) National investigation of inland wetlands. Korean Ministry of Environment, Seoul

Costa ML, Santos MC, Carrapiço F (1999) Biomass characterization of *Azolla filiculoides* grown in natural ecosystems and wastewater. Hydrobiologia 415:323–327

Dixit S, Dhote S (2010) Evaluation of uptake rate of heavy metals by *Eichhornia crassipes* and *Hydrilla verticillata*. Environ Monit Assess 169:367–374

Forni C, Chen J, Tancioni L, Grilli Caiola M (2001) Evaluation of the fern *Azolla* for growth, nitrogen and phosphorus removal from wastewater. Water Res 35:1592–1598

Grady Jr. LCP, Daigger GT and Lim HC (1999) Biological wastewater treatment. 2nd edition. Basel

Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Botany 57:1332–1334

Ito O, Watanabe I (1983) The relationship between combined nitrogen uptakes and nitrogen fixation in *Azolla anabaeba* symbiosis. New Phytol 95:647–654

Johnson GV, Mayeux PA, Evans HJ (1966) A cobalt requirement for symbiotic growth of *Azolla filiculoides* in the absence of combined nitrogen. Plant Physiol 41:852–855

Landolt E (1986) The family of Lemnaceae – a monographic study. Veröffentlichungen des Geobotanischen Institutes der ETH, Zürich. pp 70–150

Lu, Q, He Z, Graetz D, Stoffella P, Yang X (2010) Phytoremediation to remove nutrients and improve eutrophic stormwaters using water lettuce (*Pistia stratiotes* L.). Environ Sci Pollut Res 17:84–96

Marques MI, Carvalho ML, Öblad M, Amorim P,Ramos MT (1993) EDXRF analysis of trace elements in Nerium oleander for pollution monitoring. X-Ray Spectrometry 22:244–247

Maunder, M (1992) Plant reintroduction: an overview. Biodivers Conserv 1:51–61

Metzgar JS, Schneider H, Pryer KM (2007) Phylogeny and divergence time estimates for the fern genus *Azolla* (*Salviniaceae*). Int J Plant Sci 168:1045–1053

Moraghan JT, Rego TJ, Sahrawat KL (1983) Effect of water pretreatment on total nitrogen analysis of soils by the Kjeldahl method. Soil Sci Soc Am J 47:213-217

Moss B (1973) The influence of environmental factors on the distribution of freshwater algae: An experimental study: II. The role of pH and the carbon dioxide-bicarbonate system. J Ecol 61:157–177

Park H (2005) General growth charateristics and water treatement capacity of waterfern *Azolla japonica* Franch. et Savat. MD thesis. Seoul National University, Seoul

Park MG (1961) Pteridophyta of Korea. Kyo-Hac press, Seoul. pp 234–235

Park S, Joe KS, Han SH, Eom TY, Kim HS (1999) Characteristics and distribution of metallic elements in landfill leachates. Environ Technol 20:443–448

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S (2004) Water resources: Agricultural and environmental issues. Bio Sci 54:909–918

Rai PK (2007) Wastewater management through biomass of *Azolla pinnata*: An eco-sustainable approach. AMBIO: J Human Environ 36:426–428

Reddy K, DeBusk W (1985) Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: II. Azolla, Duckweed, and Salvinia. Econ Bot 39:200–208

Sarkar A, Jana S (1986) Heavy metal pollutant tolerance of *Azolla pinnata*. Water, Air, and Soil Pollut 27:15–18

Schindler DW (1988) Effects of acid rain on freshwater ecosystems. Science 239:149–157

Schnoor JL, Light LA, McCutcheon SC, Wolfe NL, Carreia LH (1995) Phytoremediation of organic and nutrient contaminants. Environl Sci Tech 29:318–323

Song U (2010) Ecological monitoring and management of plant, soil and leachate channel in the Sudokwon landfill, Korea. Ph. D thesis. Seoul National University, Seoul

Song U, Lee EJ (2010) Environmental and economical assessment of sewage sludge compost application on soil and plants in a landfill. Resources, Conservation and Recycling 54:1109–1116

United States Environmental Protection Agency (USEPA) (2003) Ecological soil screening level for iron. URL http://rais.ornl.gov/documents/eco-ssl_iron.pdf [Accessed June, 27th, 2011]

Vermaat JE, Khalid Hanif M (1998) Performance of common duckweed species (Lemnaceae) and the waterfern Azolla filiculoides on different types of waste water. Water Res 32:2569–2576

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochem 57-58:1–45

Wagner G (1997) *Azolla*: A review of its biology and utilization. Bot Rev 63:1–26

Water Management Information System (WAMIS) (2011) Environmental water quality standards. URL http://www.wamis.go.kr/WKE/WKE_WQBASE_LST.ASPX [Accessed February, 10th, 2012]

Weih M, Nordh N-E (2002) Characterising willows for biomass and phytoremediation: growth, nitrogen and water use of 14 willow clones under different irrigation and fertilisation regimes. Biomass and Bioenergy 23:397–413

Weikard H-P, Seyhan D (2009) Distribution of phosphorus resources between rich and poor countries: The effect of recycling. Ecological Econ 68:1749–1755

Xie L, Xie P, Li S, Tang H, Liu H (2003) The low TN:TP ratio, a

cause or a result of Microcystis blooms? Water Res 37:2073–2080
Yauch RL, Januario T, Eberhard DA, Cavet G, Zhu W, Fu L, Pham TQ, Soriano R, Stinson J, Seshagiri S, Modrusan Z, Lin C-Y, O'Neill V, Amler LC (2005) Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. Clin Cancer Res 11:8686–8698
Yeongsanriver Environment Research Center (YERC) (2000). Fate of pollutants and ecological changes of Juamho. Annual almanac of Korea National Institute of Environ Res 22:459–476
Zhang X, Xia H, Li Z, Zhuang P, Gao B (2010) Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresource Technol 101:2063–2066
Zimmels Y, Kirzhner F, Roitman S (2004) Use of naturally growing aquatic plants for wastewater purification. Water Environ Res 76:220–230