

PHYTO-PURIFICATION OF POLLUTED WATER BY USING POACEAE CASE OF LAKE REGHAIA

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Abstract

This work has two parts as a first part, we are interested in eliminating the heavy metals by two plants that belong to the family of Poaceae (*Arundo donax* and *Phragmites australis*). It is the treatment via planted filters. Secondly, the comparison has been made of the tolerance and accumulation by these two plants. A purification system is set up which consists of two vats: one located in height and the second in low to receive the water that flows from the first vat, in which were planted macrophytes. The results obtained, show a significant elimination of the heavy metals on the plants filter of *Phragmites australis* compared to *Arundo donax* after a stay of 30 days. The yields of eliminations are respectively 84.45% and 75.00% for *Phragmites australis* and *Arundo donax*. The results of this study also showed a significant reduction in COD, BOD₅, Conductivity, Phosphate and Nitrate. The greatest accumulation of heavy metals is produced in the roots followed by leaves for both plants. The calculated values of biological transfer coefficient (BTC) and bioconcentration factor (BCF) showed that *Phragmites australis* and *Arundo donax* are effectively acting as species of phytoremediation for some heavy metals.

Key words: Phyto-purification, Poaceae, Heavy metals, Reghaia lake.

Introduction

Loaded liquid wastes with heavy metals are extremely dangerous as the water enters into the composition of any entity; living or not, and, thus, constitutes a matrix that can transport these polluted objects everywhere. Also, it is clear that this industry is responsible for almost all heavy metals discharged into water. Hence, there is a need to minimize the concentration of metals in the waste water. Achieving such an objective has become possible thanks to special techniques for the elimination and recovery of these metals in water (Crine *et al.*, 2008 and Peter *et al.*, 2008). To overcome the main limitations of the methods that are currently available in the decontamination strategies, researches have been, for years, directed towards the use of the plants (Ellis *et al.*, 1994). A number of plants from humid environment have been found, more and more vigorously in different sites, contaminated with the metals indicating that these species have a certain degree of tolerance to the metals. *Phragmites australis* (common Reed) and *Arundo donax* are rhizonated plants. They belong to Poaceae family with cosmopolitan distribution (Massacci *et al.*, 2001). The natural properties of these plants are exploited in the field of phytoremediation, which is a set of techniques allowing removing the soil, and purifying waste water by using plants (Hall *et al.*, 2003 and Callahans *et al.*, 2006). They usually grow in wet areas with many habitats, they can also flourish and become a dominant species. These plants can resist adverse edaphic conditions, including the presence of toxic heavy metals, such as Zn, pb, Cu and Cd (Schierup *et al.*, 1981). *Arundo donax* and *Phragmites australis* have useful characteristic for the on to phyoremediation of wetlands (Massacci *et al.*, 2001). Numerous phyoremediation studies aim to increase the collection of metals by plants from polluted soils (Yang *et al.*, 2008). The use of constructed wetland (artificial swamps) to treat waste water is a widely accepted ecological engineering

approach throughout the world (Brix, 1993 & Mitsch *et al.*, 2004, Vymazal, 2009 and Memon *et al.*, 2017).

The objective of this study is to eliminate the heavy metals using two plants, which belong to the family of Poacées (*Arundo donax* and *Phragmites australis*). It is the treatment by means of planted filters the choice of waters of Reghaia Lake due to its pollution by industrial waste water, domestic and agricultural irrigation of effluents from the valley of Reghaia and valley of El Abiar, a comparative evaluation of tolerance and accumulation of the heavy metals, by these two plants has been realized.

Material and Methods

Description of the purification system: In the present study, we have built a standard purification filter system, the latter consisting of two vats connected to each other by a pipe, in which are implanted the young plants of macrophytes. The filter is 65 cm long and 40 cm wide, with three layers of gravel of different diameter and thickness. The lining of the floors is divided into three layers. Each tank has a top mounted at the base, in the zones the humid air was supplied through air diffusing pipes located 20 cm below the surface of the vats (Fig. 1).

Preparation of biological materials: Two species of the Poaceae family are used: *Phragmites australis* and *Arundo donax*, they are macrophytes which belong to the group of helophytes and they are particularly characterized by their very active root systems and able to withstand adverse conditions. The plant species were extracted from their initial places at a medium stage of their growth during in December 2016, and then the plants were left in the water for 15 days, until the remarkable development of the roots, then implanted in the filters. The plants were implanted in six plastic pots, three pots are planted with young stems of *Phragmites australis*, and the other three are planted with young

stems of *Arundo donax*. The monitoring of their adaptation has been meticulously followed, first irrigated with tap water not to change the quality of the water, then they were irrigated by the water of Lake Reghaia with watering every three days.



Fig. 1. Purification system by rushes.

Plant sampling and heavy metal analysis: Rapid rinsing of the plants with water to remove dust, then cut it in pieces and separate the parts of the plants (roots, leaves and stems). Plant samples were put in clean plastic bags and labeled carefully with permanent marker. All the collected plant samples were placed in newspapers for the absorption of excessive water and put in oven at 105°C for 5 h. The dried plant samples were ground with a pestle and mortar so that their size was 2mm after grinding.

For the analysis of the metals, 0.5 g of sample of leaves, stems and roots were taken. 5 ml of nitric acid (65%) and 1 ml of perchloric acid (70-72%) were added (Wang *et al.*, 2003). Digest with gently boiling on a hot plate for 10 minutes. After cooling, add 25 ml of distilled water, then filtered into a 50 ml flask and filter paper. Volume was adjusted. This extract is used for the determination of heavy metals (Pauwels *et al.*, 1992). These steps are used for all the ashes of (leaves, stems and roots) of two tested plants. The physicochemical analyzes were carried out in the research laboratory of M'Hamed Bougara-Boumerdes University and the ONA of Djelfa, Algeria.

Monitoring physico-chemical parameters and the pollution parameters (BOD5 and COD) was carried out according to the technique of Rodier (1996). All prepared samples were analyzed for heavy metals by flame atomic absorption spectrophotometer Model ICP Perkin Elmer AA400 in Baraki ONA laboratory, Algeria.

Determination of chlorophyll of helophytes: The extraction of chlorophyll realized according to the method of Holm-Hansen (1965). One g leaves of plant samples cut into small pieces and ground in a mortar with 20 ml of 80% acetone and a pinch of calcium carbonate (CaCO₃). The assay was realized by taking 3 ml of the solution in the spectrometer tube. The reading is done at two wavelengths 645 nm and 663 nm.

Phytoremediation: Phytoremediation was defined by two biological methods. The biological transfer coefficient (BTC) has been defined as the concentration of a metal accumulated in the shoot on it in the root (Khan *et al.*, 2013). $BTC = \frac{[Metal] \text{ shoot}}{[Metal] \text{ root}}$.

The bio-concentration factor (BCF) was the concentration of heavy metals accumulated in plant roots divided by the concentration of the same metal obtained in their respective soil (Khan *et al.*, 2013). $BCF = \frac{[Metal] \text{ root}}{[Metal] \text{ soil}}$. (Khan *et al.*, 2013).

Results and Discussions

The result of ANOVA described the significance level of these parameters among treatments. The xenobiotics in the water used decrease after two purifications with the reeds.

Physico-chemical parameter changes in lake water before and after biofiltration:

The pH values are illustrated in Fig. 2(a), a slight decrease in the pH after phytopurification, in general the pH value of the water filtered by *Phragmites australis* are invariably greater than that in the water filtered by *Arundo donax* with mean pH value was (6.6, 7.5. respectively). The pH of 7.5 was the most ideal for successful implementation of a water phytoremediation system and optimal basin performance. (Irami *et al.*, 2012). pH plays a very important role in the absorption and accumulation of trace elements because the more acidic the pH of the milieu, the more vegetation accumulates. Thus, according to Bryan (1979), the pH has a direct action on the availability of metal ions in the marine environment and thus on the uptake by the organisms while modifying the form of the metal and by acting on the physiology of the organisms.

Fig. 2(b) showed the variations in the conductivity of the water in the treatment vats, a very apparent reduction in the electric conductivity of water is noted with a clear predominance of *Phragmites australis* plant compared to *Arundo donax* the conductivity decreases by about 60% for the first plant and 15% for the second plant. Its reduction after passing through both vats can be explained according to Coulibaly *et al.*, (2008), by a retention of salt in the filter the solid mass. A similar result is obtained by Finlayson & Chick (1983) for a *Typha Latifolia* plantation, and oppose those obtained by Coulibaly *et al.*, (2008). This difference in results is linked to the type of plant used (*Amaranthus hybridus*, *Corchorus oliterius*).

NO₃⁻ values in the filters planted during the purification illustrated in Fig. 2(c). NO₃⁻ values after the passage of lake water in both vats decreased by 35.89% for *Phragmites Australis* and 22.53% for *Arundo donax*. A significant decrease of phosphate after passing through the filters planted (Fig. 2(d)) with a clear predominance of *Phragmites australis* (99.81%) compared to *Arundo donax* (51.12%).

The results of ANOVA showed highly significant increases $p < 0.001$ of O₂ dissolved in water filtered by *Phragmites Australis* and *Arundo donax* to that of the control with a yield of 93.24% and 90.32% respectively (Fig. 2 (e)). Plants can contribute to purification processes by absorbing nutrients and trace elements that allow the release of oxygen (Kadlec *et al.*, 2000).

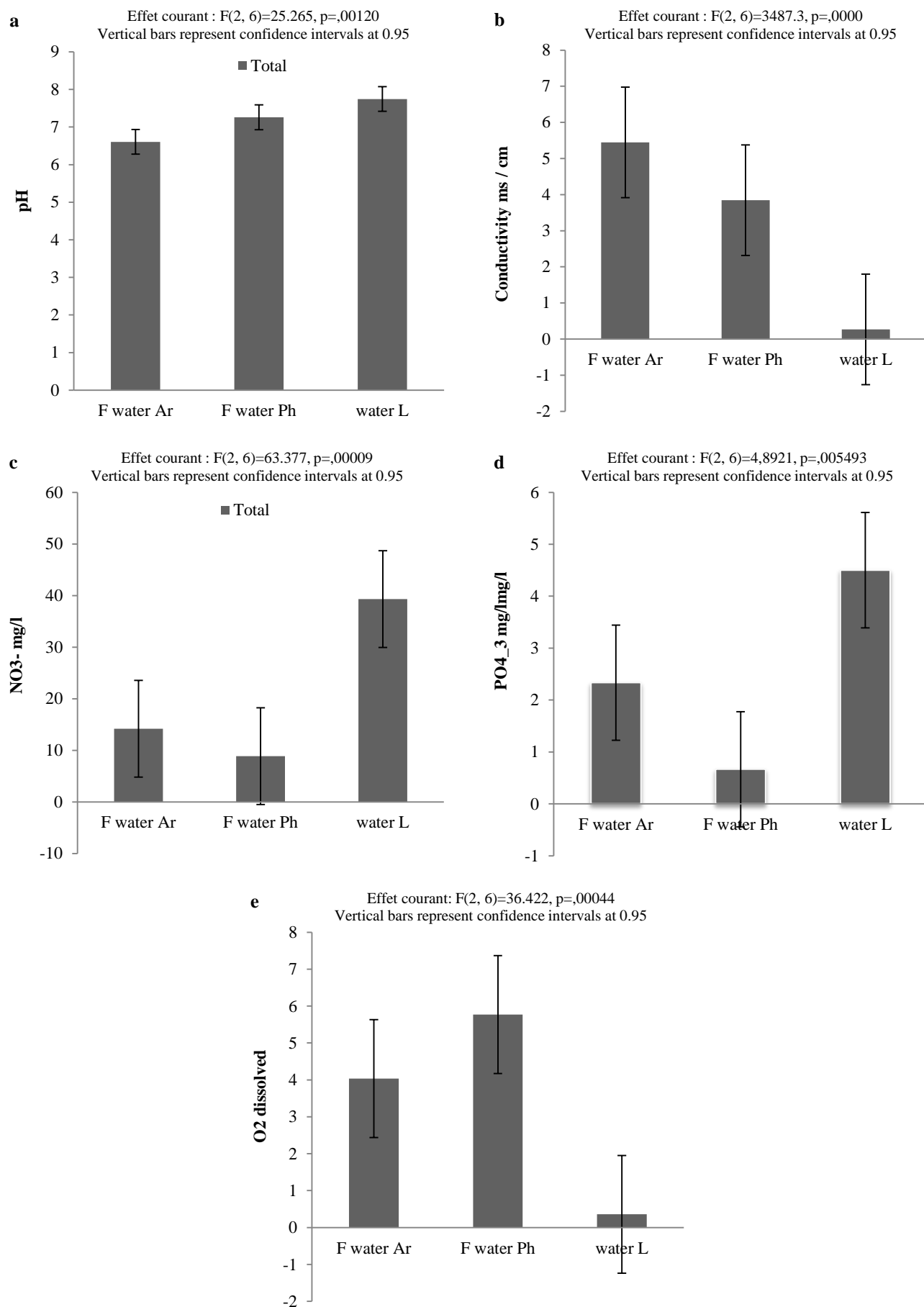


Fig. 2. Physico-chemical parameter changes in lake water before and after biofiltration by *Phragmites australis* and *Arundo donax* in the water of Reghaia lake.

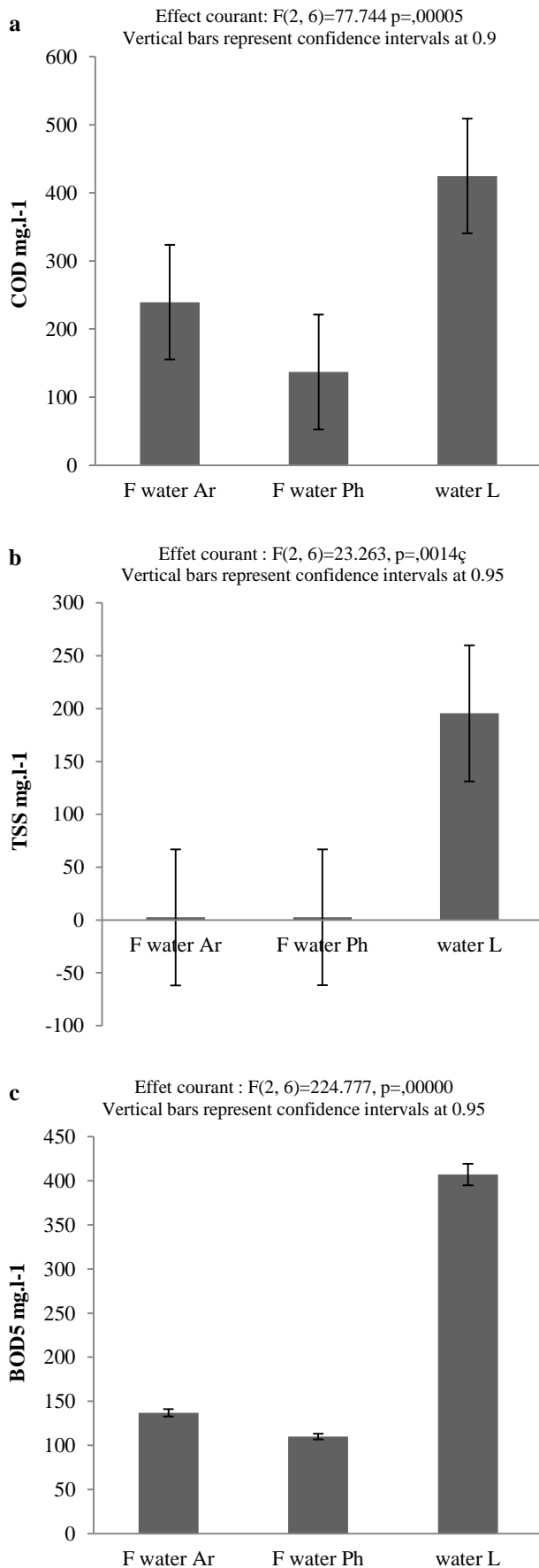


Fig. 3. Variations of pollution indicators, before and after biofiltration stay by helophyte *Phragmites australis* and *Arundo donax* in Reghaia lake water.

Variation of pollution indicators: Fig. 3 described the results of ANOVA of Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and Biological Oxygen Demand (BOD₅). COD and BOD₅ values were significantly decreased in the waters filtered by *Phragmites australis* and *Arundo donax* by comparison with the controls (***) $P = 0.0044$, 0.0005 respectively), with a yield of 67.74% and of 56.34% for COD and 72.97% and 66.34% for BOD₅ (Fig. 3(a, b)). According to Polprasert & Khatiwada, (1998) the removal of organic matter in artificial wetlands is based on a symbiotic relationship between plants and bacteria in which the bacteria use the oxygen provided by the plants during photosynthesis to degrade organic carbon. These results have been confirmed by (Garcia *et al.*, 2005) who explain that this decrease is ensured by the good functioning of the system. The plants, through photosynthesis, thus favored the aerobic phenomena, and the structure of the filtering mass chosen made it possible to ensure the easy diffusion of the atmospheric oxygen, and consequently the dissolved oxygen in the water; which is a very important factor for good effluent purification. Indeed the significant decrease in BOD₅ and COD shows that the system is well oxygenated.

TSS values showed the a very significant reduction $p<0.015$ of the contents of the suspended material after purification. The difference is respectively 98.73% and 98.66% for *Phragmites australis* et *Arundo donax*. The decrease in the concentration of Total Suspended Solids in the different filtrates would be due to physical filtration that retains coarse surface materials and the finest, either by blocking between the pores, by an interception and a fixation on the grain or chemical interactions of Van Der Waals type (Chachuat, 1998).

Variations of chlorophyll a and b: The results of ANOVA showed a highly significant decrease $p<0.001$ of the chlorophyll a content in *Phragmites australis* and *Arundo donax* irrigated by lake water compared with those in the control irrigated by drinking water or the mean values of chlorophyll a and b in *Phragmites australis* irrigated by lake water was found to be $18.74 \mu\text{g.g}^{-1}$ and $11. \mu\text{g.g}^{-1}$ respectively, with the control is $20.14 \mu\text{g. g}^{-1}$ and $57.14 \mu\text{g. g}^{-1}$ respectively, concerning *Arundo donax* irrigated by lake water, the chlorophyll a, b rate was $15.45 \mu\text{g. g}^{-1}$, $15.24 \mu\text{g.g}^{-1}$ respectively, and $24.17 \mu\text{g.g}^{-1}$ and $37.45 \mu\text{g.g}^{-1}$ of the control.

Phragmites australis appear to be more sensitive to the pollution that is due to the heavy metals present at the water level of the lake.

The decline in chlorophyll content in plants exposed to heavy metal stress is believed to be due to inhibition of important enzymes, such as α -aminolevulinic acid dehydratase (ALA-dehydratase) and protochlorophyllide reductase associated with chlorophyll biosynthesis, so the loss in chlorophyll content can consequently lead to disruption of photosynthetic system (Dhir *et al.*, 2004).

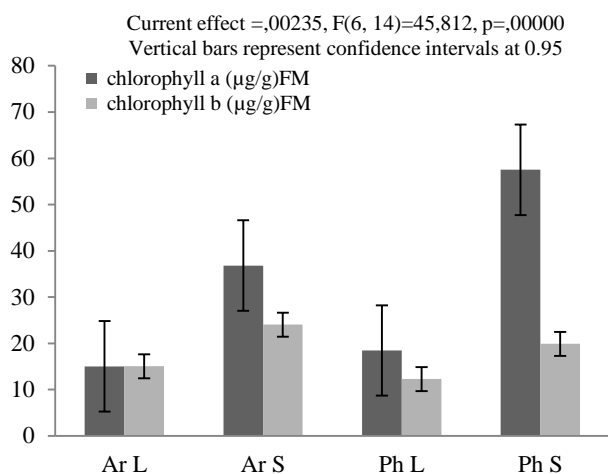


Fig. 4. Variations of chlorophyll a and b before and after biofiltration stay of reed plants *Phragmites australis* and *Arundo donax* in Reghaia lake water.

Heavy metals: The results of one-factor ANOVA showed that a very significant difference with a probability of $p < 0.001$ between the different concentrations of heavy metals before and after biofiltration stay by helophyte *Phragmites australis* and *Arundo donax* in the water of the Reghaia lake for all samples except the Cadmium (Cd).

The values for iron were strongly accumulated at the leaf level for *Phragmites australis* with $450 \text{ mg} \cdot \text{g}^{-1}$ by dry matter (DM) and $340 \text{ mg} \cdot \text{g}^{-1}$ DM for *Arundo donax* roots (Fig. 5(a)). The concentration of Cu and of *P.australis* variation in the leaves from $10 \text{ mg} \cdot \text{g}^{-1}$ DM to $5.1 \text{ mg} \cdot \text{g}^{-1}$ DM in roots, for *A.donax* the accumulation of Cu was in the roots with a mean of $11.6 \text{ mg} \cdot \text{g}^{-1}$ DM followed by the leaves with $6 \text{ mg} \cdot \text{g}^{-1}$ DM. These results show that Zinc (Zn) uptake is highly important in the aerial part, especially in the *Arundo donax* leaves with $40 \text{ mg} \cdot \text{g}^{-1}$, compared with those obtained in the *Phragmites australis* level. The largest fraction of Zn retained by the plant was found in the "treated" part of the limb but this element seems more mobile than copper. In contrast to the latter, there is virtually no retention of zinc in cuticular waxes resulting in a high circulation of this metal in the plant and a high migration rate (Chamel *et al.*, 1979). From Fig. 5(f) it is found that nickel (Ni) is highly accumulated in leaves for *A. donax* with an amount of $2.6 \text{ mg} \cdot \text{g}^{-1}$ DM and $1.7 \text{ mg} \cdot \text{g}^{-1}$ DM for the roots. For example, nickel accumulation and tolerance in the genus *Alyssum* appear to be mainly due to increased synthesis of histidine (Kramer *et al.*, 1996).

P. australis accumulates Lead (Pb) at stems with an average of $2.4 \text{ mg} \cdot \text{g}^{-1}$ DM, however a low accumulation in roots and leaves found and varies between 1.3 and $1.49 \text{ mg} \cdot \text{g}^{-1}$ of. Lead accumulation is strongly important in the aerial part of *P. australis* with $2.7 \text{ mg} \cdot \text{g}^{-1}$ DM for stem and $2.2 \text{ mg} \cdot \text{g}^{-1}$ DM for leaves, whereas accumulation at *A. donax* in roots and leaves with $3.6 \text{ mg} \cdot \text{g}^{-1}$ DM (Fig. 5(e)). The accumulation of Cobalt (Co) ($p < 0.001$) was highly important in the aerial part for both plants, this accumulation was localized at the *Phragmites australis* stems with a rate of $1.3 \text{ mg} \cdot \text{g}^{-1}$ DM and at the of leaves for *Arundo donax* with a rate of $0.94 \text{ mg} \cdot \text{g}^{-1}$ of DM (Fig.

5(f)). The *Arundo donax* showed the same behavior as *Phragmites australis* was a better accumulation of silver (Ag) in the aerial parts at the leaf level with a rate which varies between 0.27 and $0.32 \text{ mg} \cdot \text{g}^{-1}$ of DM, while for the roots the accumulation ranges from 0.24 to $0.29 \text{ mg} \cdot \text{g}^{-1}$ DM for both plants.

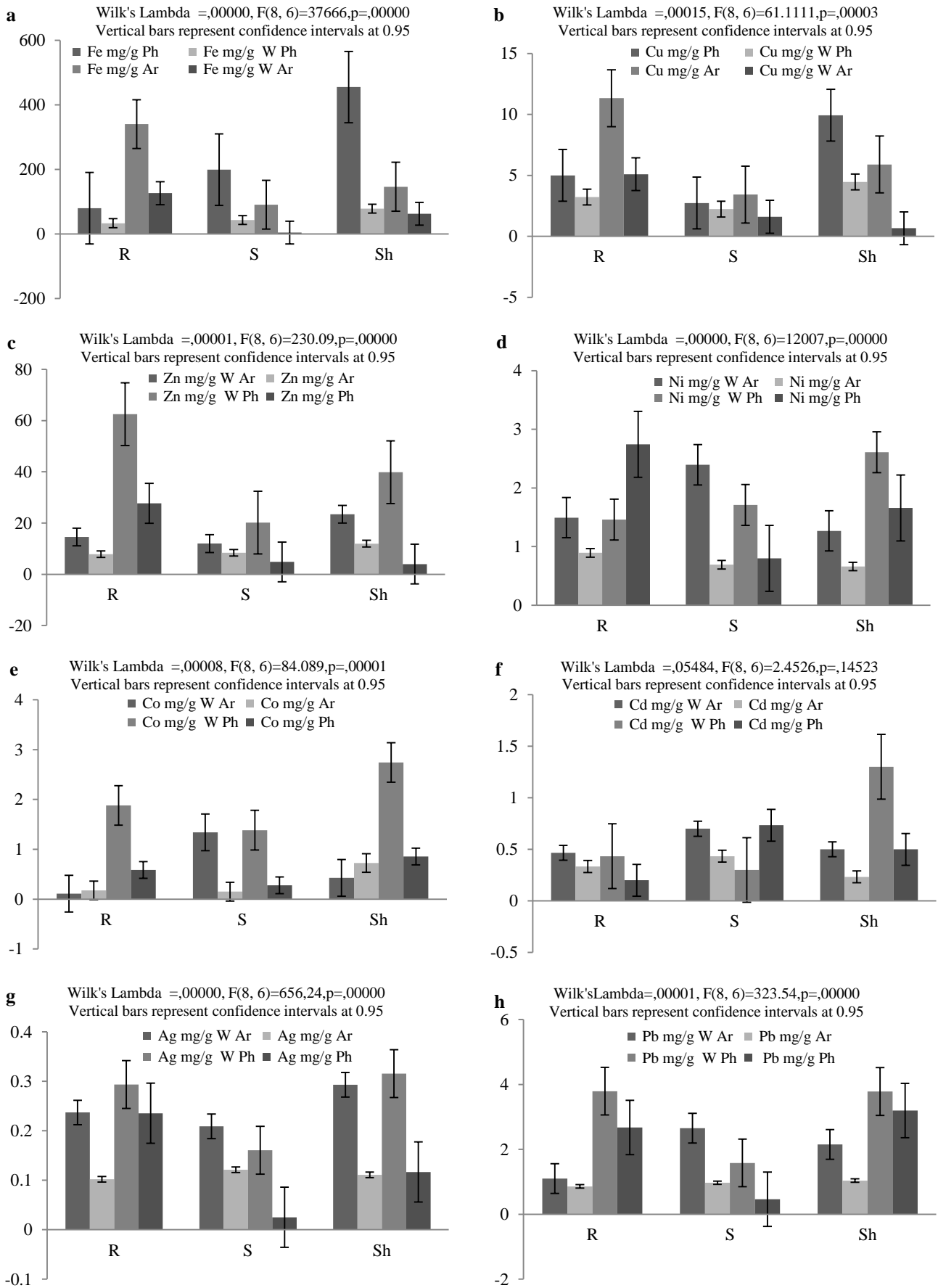
Phytoremediation of heavy metals is a profitable green technology; there are more benefits when it comes to using native plants with natural growth. (Irami *et al.*, 2012) Phytofiltration is the use of plants to absorb or adsorb pollutants, especially metals contained in aquatic environments (Prasad, 2004). The roots of the plants will absorb, precipitate and concentrate the metals present in the water (Dushenkov & Kapulnik, 2000). Mechanisms involved in biosorption include chemisorption, complementation, ion exchange, micro-precipitation, surface adsorption (Gardea *et al.*, 2004).

The presence of the filters ensures a high efficiency for the retention of the heavy metals with the following classifications $\text{Fe} > \text{Cu} > \text{Zn} >$ and Pb minimal absorption for Ni, Co, Cd and Ag for *Phragmites australis*. (Fig. 6) On the other hand, for *Arundo donax* we found a following classification $\text{Fe} > \text{Cu} > \text{Zn} > \text{Ni} > \text{Pb} > \text{Ag}$ and minimal absorption for Co and Cd (Fig. 7).

Phytoremediation: In this study, none of the plant species showed metal concentrations $> 1000 \text{ mg} \cdot \text{kg}^{-1}$ in shoots. None of them are hyper accumulators. However, the ability of these plants to tolerate and accumulate heavy metals may be useful for phytostabilization. BTC and BCF can be used to estimate a potential for phytoremediation purposes (Nazir *et al.*, 2013).

Table 1 evaluates the BTC and BCF results of heavy metal concentration in *Phragmites australis* and *Arundo donax*. The only species with BCF, BTC greater than 1 have the potential process of remediation of metals, Considering the BCF, *Phragmites australis* has the characteristic of hyper accumulator for Fe, Zn, Cu, Cd, Pb, Ni and Ag, while that *Arundo donax* has the characteristic of hyper accumulator for Cd, Co, Ni and Ag. Some plants are able to uptake heavy metals by their roots or absorb them by their leaves and stems (Alloway *et al.*, 1995), as *Arundo donax*.

Accumulative plants are characterized by an ability to efficiently transport heavy metals from the roots to the leaves, whereas in some plants the tendency to remain in the subterranean parts (roots) is greater (McGrath *et al.*, 2002) as *Phragmites australis*. The phytoextraction process usually requires the translocation of heavy metals to the parts of plants, TF values showed a significant percent of root Ni, Ag, Zn and Cu translocated to the shoot was 63%, 46.2%, 11.4% and 9.38% respectively in *Phragmites australis*, on the other hand *Arundo donax* has a better Ni accumulation (2.92) with a significant percent of translocation of Ni, Cu, Ag Zn and, Pb (60.46%, 43.63%, 40.24%, 31.94%, 17.92% respectively). Microorganisms can play a dual role in the mobilization of metals. They can affect their bioavailability by fixing the elements and releasing them by decomposing organic matter (Herman *et al.*, 1995).



Note: a.Fe, b. Cu, c .Zn, d. Ni, e. Co, f. Cd, g .Ag, h. Pb
 Fig. 5. Variations of heavy metals before and after biofiltration by the helophyte *Phragmites australis* and *Arundo donax* in the water of the Reghaia lake.

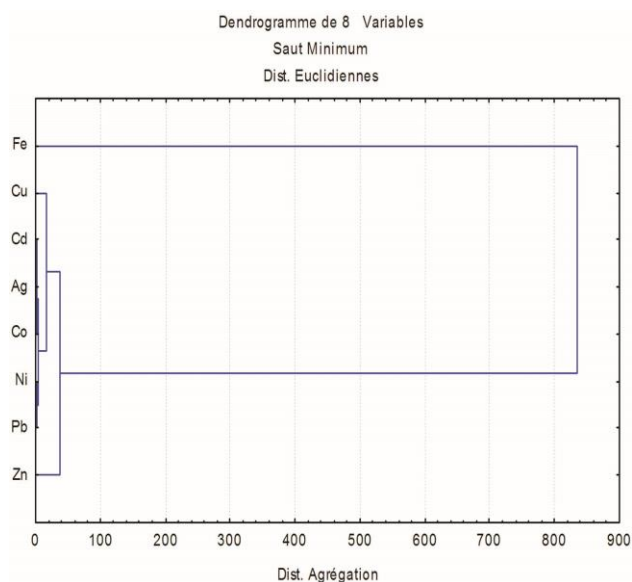


Fig. 6. Dendrogram of absorption of heavy metals by *Phragmites australis*.

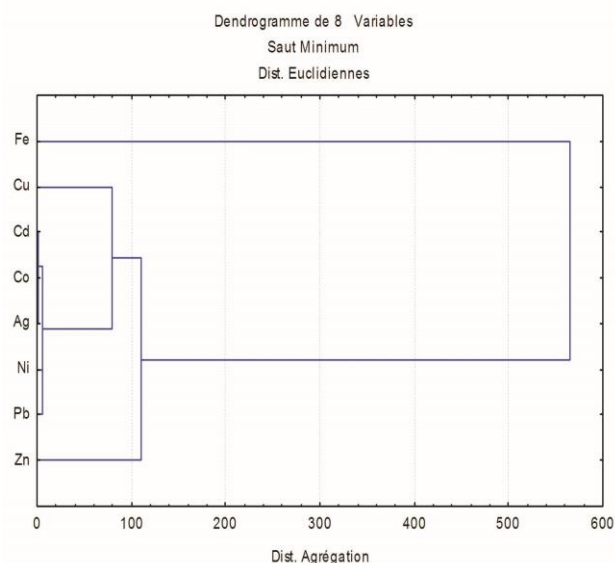


Fig. 7. Dendrogram of absorption of heavy metals by *Arundo donax*.

Table 1. BCF and BTC of *Phragmites australis* and *Arundo donax*.

Metals	Control	<i>Phragmites australis</i>	<i>Arundo donax</i>
Fe	BTC	8,17	0,54
Fe	BCF	8,87	37,62
Zn	BTC	2,28	0,99
Zn	BCF	11,4	31,94
Cu	BTC	2,52	0,825
Cu	BCF	9,83	43,63
Cd	BTC	2,33	2,85
Cd	BCF	1,54	0,3
Co	BTC	15,72	1,52
Co	BCF	0,67	0,51
Pb	BTC	4,35	1,25
Pb	BCF	5,08	17,92
Ni	BTC	1,33	2,92
Ni	BCF	63	60,46
Ag	BTC	2,16	1,63
Ag	BCF	46,2	40,24

Note: BCF: Bioconcentration Factor, BTC: Biological Transfer Coefficient

Conclusion

Phragmites australis is known for their accumulating power, they can respond to pollution in a sensitive and effective way, hence the importance of their use in phytoremediation. The results of this study showed that *Arundo donax* will also have an accumulation capacity, such as *Phragmites australis*, which would reduce water pollution in the Reghaia Lake, especially those loaded with heavy metals. This study showed that *Arundo donax* has the highest ability to accumulate Fe, Zn, Cu, Cd, Pb, Ni and Ag via its roots, while *Phragmites australis* has the hyperaccumulator characteristic for Cd, Co, Ni and

Ag. The results indicate that plant species differ greatly in their ability to accumulate heavy metals in roots and shoots. This hyperaccumulation is probably the cause of their high tolerance at high levels of pollution. The treatment with filters planted macrophytes seems to be an alternative effective and well enough adapted to the waters of the lake with a pollutant load variable. This study will help make a best choice for restoration of lake water; this technology is inexpensive and easy to use.

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References

- Alloway, B.J. 1995. In: *Heavy Metals in Soils*, Second edition, (Ed.): B.J. Alloway, Blackie Academic & Professional, Londres (Royame-Uni), pp. 113-129.
- Brix, H. 1993. Wastewater treatment in constructed wetlands: system design, removal processes and treatment performance, In: Moshiri, G.A. (Ed.), *Constructed Wetlands for Water Quality Improvement*. CRC Press, Boca Raton, pp. 9-22.
- Bryan, G.W.1979. Bioaccumulation of marine pollutants. *Phil. Trans. Re. Soc. Lond. B*, pp.286-204.
- Callahans, D.L., A.J.M. Baker. S.D. Kolev and A.G .Wedd.2006. Métal ion ligands in hyperaccumulating plants. *J. Biol. Inorg. Chem.*, 11: 2-12.
- Chachuat, B. 1998. Effluent treatment concentrated by crops on gravel. DEA Strasbourg, 154 p.
- Chamel, A and B.G. Ambonnet. 1997. Etude, avec des feuilles in situ et des cuticules isolées, du comportement du cuivre et du zinc fournis par voie foliaire. *Proc. Symp. Isotopes and Radiation in Research on Soil-Plant Relationship*, Vienne, pp. 373-391.
- Coulibaly, L., L. Savane and Gourene. 2008. Domestic Wastewater treatment with a vertical completely drained pilot scale construed wetland planted with *Corchorus oliterius*. *Afr. J. Biotechnol.*, 3(9): 587-596.

- Crine, M., J.M. Baldewijnis, M. Schitz and T. Salmon. 1988. Cd, Cu and Zn uptake by sulfate reducing bacteria in an up flow fixed bed reactor. *Inter Biotech Symposium*, Paris, pp. 236-241.
- Dhir, B., P. Sharmila and P.P. Saradhi. 2004. Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. *Aqu. Toxicol.*, 66: 141-147.
- Dushenkov, S. and Y. Kapulnik. 2000. Phytoremediation of metals. In: *Phytoremediation of toxic metals – Using plants to clean-up the environment*. (Eds.): I. Raskin and B.D. Ensley, New York: Wiley, pp. 89-106.
- Ellis, J.B., R.B. Shutes, D.M. Levitt and T.T. Zhanc. 1994. Use of macrophytes for pollution treatment in urban wetlands. *Resour. Conser. Rec.*, 11: 1-12.
- Finlayson, C.M. and A.J. Chick. 1983. Testing the potential of aquatic plants to treat abattoir effluent. *Wat. Res.*, 17: 415-422.
- Garcia, P., J. Aguirre, R. Barragán, V. Mujeriego, Matamoros and J.M. Bayona. 2005. Effect of key design parameters on the efficiency of horizontal subsurface flow constructed wetlands. *Ecol. Eng.*, 25: 405-418.
- Gardea, T.J.L., J.R. Peralta, G. de la Rosa and J.G. Parsons. 2005. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. *Coord. Chem. Rev.*, 249: 1797-1810.
- Hall, J.L. and L.E. Williams. 2003. Transitio metal transporters in plants. *J. Exp. Bot.*, 54: 2601-2613.
- Herman, D.C., J.F. Artiola and R.M. Miller. 1995. Removal of cadmium, lead and zinc from soil by a Rhamnolipid Biosurfactant. *Environ. Sci. & Technol.*, 29: 2280-2285.
- Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes and J.D.H. Strickland. 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Explor. Mer.*, 30: 3-15.
- Irami, S., A. Ifikhar, R. Yousaf and Z. Ayesha. 2012. Treatment of wastewater by *Lemna Minor*. *Pak. J. Bot.*, 44(2): 553-557.
- Kadlec, R.H., R.L. Knight, J. Vymazal, H. Brix, P. Cooper and R. Haberl. 2000. Constructed wetlands for pollution control-processes, performance, design and operation. *IWA Scientific and Technical Report*, London, 8: 141-149.
- Khan, M.U., A. Moinuddin, S.S. Shaikat, K. Nazim and M.A. Qadeer. 2013. Effect of industrial waste on early growth and Phyto remediation potential of *avicennia marina* (Forsk) vierh. *Pak. J. Bot.*, 45(1): 17-27.
- Kramer, W., B. Fartmann and E.C. Ringbeck. 1996. Transcription of *mutS* and *mutL*-homologous genes in *Saccharomyces cerevisiae* during the cell cycle. *Mol. Gen. Genet.*, 252(3): 275-283.
- Massacci, A., F. Pietrini and M.A. Iannelli. 2001. Remediation of wetlands by *Phragmites australis*. *Minerva Biotechnologica.*, 13: 135-140.
- McGrath, S.P., F.J. Zhao and E. Lombi. 2002. Phytoremediation of metals, metalloids, and radionuclides. *Adv. Agron.*, 75: 1-56.
- Memon, S.A., O.S. Raja, B. Kandhro, I. Salim and C.H. Lee. 2017. International Conference on Renewable Energy and Environment (ICREE 2017) OP Conf. Series: *Earth and Environmental Science*. 127(2018): 012014 PP 7.
- Mitsch, W.J. and S.E. Jørgensen. 2004. Ecological Engineering and Ecosystem Restoration. John Wiley & Sons, Inc., New York, pp. 4-11.
- Nazir, A., N. Riffat, M.M. Ajajib and S. Hamayun. 2013. Accumulation of cadmium in soil and plants in vicinity of Koh-E-Noor Textile Mills Rawalpindi, Pakistan. *Biologia (Pakistan)*, 59(2): 197-203.
- Pauwels, J.M., E. van Ranst, M. Verloo and Z.E.A. Mvendo. 1992. Soil laboratory manual. Soil and plant analysis methods, equipment, inventory management of glassware and chemicals. Brussels: General Administration of Development Cooperation (AGCD).
- Peter, S., D. Daubner, M. Heiko, J. Neustifter and D. Reinhard. 2008. Phytoremediation of organic xenobiotics – Glutathione dependent detoxification in *Phragmites* plants from European treatment sites. *Biores. Technol.*, 99: 7183-7191.
- Polprasert, C., N.R. Khatiwada and J. Bhurtel. 1998. A model for the removal of organic matter wet surfaces built on the surface of open water. *Wat. Sci. Tech.*, 38(1): 369-377.
- Prasad, M.N.V. 2004. Heavy metal stress in plants: from biomolecules to ecosystems (2ème édition). *Springer*, pp. 1-27.
- Rodier, J. 1996. Water analysis, natural waters, wastewater, Dunod Bordas, Paris, 7th Edition, pp. 1365-1366.
- Schierup, H.H. and V.J. Larsen. 1981. Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav) Trin. *Aqu. Bot.*, 11: 197-210.
- Vymazal, J. 2009. The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. *Ecol. Eng.*, 35: 1-17.
- Wang, X. and Q.X. Zhou. 2003. Distribution forms of cadmium, lead, copper and zinc in soil and its influences by modifier. *J. Agro. Environ. Sci.*, 22: 541-545.
- Yang, Z., S. Zheng, J. Chen and M. Sun. 2008. Purification of nitrate-rich agricultural runoff by a hydroponic system. *Biores. Technol.*, 99: 8049-8053.

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