Plant Archives Volume 20 No. 2, 2020 pp. 6051-6056 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

LEAD (PB) PHYTOREMEDIATION POTENTIAL ASSESSMENT OF *BRACHIARIA MUTICA* L. (PARA GRASS) AND *CYPERUS ROTUNDUS* L. (NUT GRASS) FROM AQUEOUS SOLUTION

Sunil Kumar[1]*, Jatin Kumar[1], Sonu Singh[1], Sandeep Kumar[1] and Sunder Singh Arya[2]

[1]Department of Environmental sciences, Maharshi Dayanand University, Rohtak (Haryana), India.
[2]Department of Botany, Maharshi Dayanand University, Rohtak (Haryana), India.

Abstract

The phytoremediation efficiency of two native species *viz. Brachiaria mutica* L. (Para grass) and *Cyperus rotundus* L. (Nut grass) were assessed for lead elimination from aqueous solution in the current study. Both the species were collected from the wild and acclimatized in the 1/10 Hoagland culture solution. The soil-plants system experiments were conducted at 10, 20 and 30 mg/L lead (Pb) concentration in plastic pots. The experiments were carried out for 30 days. The water samples from the pots were collected at the interval of 5 days. The biomass of plant species was harvested after completion of the experiment (30 days) and Pb content evaluated in roots and shoots. The Pb concentration in water samples and plant parts was determined by using the atomic absorption spectrophotometer (AAS). The *Brachiaria mutica* showed the 92, 95 and 95% Pb removal, while *Cyperus rotundus* showed the 95, 96 and 94% removal from the 10, 20 and 30 mg/L Pb solutions. The roots Pb concentrations were varied from 2155 to 3942 and 974 to 1408 mg/kg in *Brachiaria mutica* and *Cyperus rotundus*, respectively at various concentrations (10, 20 and 30 mg/L). The 60 % biomass reduction in comparison to control was observed in *Brachiaria mutica* in 30 mg/L concentration, which indicates the toxic effects on the biochemical and physiological properties increased with increasing Pb concentration. The present study recommended that both the plants can be efficiently used as low-cost phytoremediation process for removal of Pb metal.

Key words: Phytoremediation, *Brachiaria mutica, Cyperus rotundus,* reduction, toxic effects.

Introduction

The toxic heavy metals contamination of water is an environmental problem at a global level. The various industries activities namely, battery manufacturing processes, metal plating facilities, the production of paints and pigments, glass production industry and mining operations are produced heavy metals containing wastewaters (Ong *et al*., 2007). From the thousands of years, heavy metals have been used for various kinds of purposes. The ancients activities like pigments for glazing ceramics, building materials and pipes for transporting water have been using lead (Järup, 2003). The predominant toxic symptoms of lead (Pb) are irritability, abdominal pain, headache and various problems associated with the nervous system. The neurological disorder associated with lead is characterized by sleeplessness and restlessness. The learning and concentration difficulties and behavioural disturbances are also reported in children (WHO, 1995). Therefore, it is very important to remove lead from soil and industrial wastewaters by proper treatment. The reverse osmosis, ion exchange, chemical precipitation and adsorption are some conventional treatment process for removal of lead (Pb) from wastewaters. The main disadvantages of these treatment processes are ineffective and production of huge quantity sludge. The phytoremediation is simple, effective and eco-friendly technology and provides the best solution to overcome the drawbacks of conventional treatment for the removal of toxic heavy metal from wastewater (Mishra and Tripathi, 2009; Singh *et al*., 2012).

The phytoremediation of water contaminated with heavy metals by aquatic macrophytes have great potential. These plants are convenient in ecotoxicological experiments because they are easy to culture in the

***Author for correspondence :** E-mail : sunilevs@yahoo.com

laboratory. Mishra and Tripathi (2008) demonstrated the removal mechanism of heavy metals by aquatic macrophytes. They suggested the three patterns (a) The excluders plants restricted the heavy metals entering to the plant's cells to survive against excess metals concentrations (b) The phytostabilizing plants accumulated the metals in the roots, but limited translocation to the shoot is occurred (c) The hyperaccumulation plants, accumulated the metals in all plant parts. The aquatic macrophytes hyper accumulative capacities are useful for the treatment of heavy metals. The large contaminated area cleaning through phytoremediation using the aquatic ecosystem is a cost-effective technique (Ali *et al*., 2020). Contaminants and heavy metals are naturally absorbed by aquatic plants (Pratas *et al*., 2014). The application of aquatic plants in the elimination of various heavy metals and other contaminants are the most proficient and profitable method (Ali *et al*., 2013; Guittonny-Philippe *et al.,* 2015). Recently, various aquatic plants have been used by many researchers for the removal of heavy metals from the aqueous solution. Hussain *et al.,* (2010) used *Eichhornia crassipes* for removal of arsenic, zinc, mercury, nickel, copper and lead from industrial and domestic wastewater. The *Pistia stratiotes* showed the greater removal capacity for the zinc and mercury from the industrial wastewater stream (Skinner *et al*., 2007). The Pb, Ni and Cr (VI) were efficiently removed by *Lemna minor* from wastewaters (Uysal, 2013; Ansari *et al*., 2020).

The para grass scientifically knows as *Brachiaria mutica* L. is found in tropical countries and native to Brazil and Africa. But recently, it is abundantly grown in southern Asian countries and in many Pacific islands, it dominantly present in poorly drained habitats and swampy land. Para grass flourished in humid and hot temperatures and mainly grows in summer (Van Thi Thanh Ho *et al*., 2020). Another plant named as nut grass and scientifically knows as *Cyperus rotundus* L belongs to the sedge family, Cyperaceae is a perennial herb, 7–40 cm tall and reproduces largely by rhizome and tubers. The leaves size are varies from 5 to 12 mm wide, 50 cm long, dark green, shiny, narrow and grass-like (Kamala *et al*., 2018). The present work assessed the phytoremediation potential of *Brachiaria mutica* L. (para grass) and *Cyperus rotundus* L. (nut grass) for removal of lead (Pb) heavy metal from aqueous solution in pot laboratory experiments.

Materials and Methods

Plants collection and experimental setup

Plant samples were collected from the Tilyar Lake and Omaxe city of Rohtak. *Brachiaria mutica* was collected from the Tilyar lake and its common name is para grass. *Cyperus rotundus* was collected from the Omaxe city of Rohtak and its common name is nut grass. From the collection sites these plant species were transported to the laboratory in the plastic container. The plants were identified from the Botany department of the M.D. university, Rohtak using plants herbarium. The plants were washed under tap water and kept in1/10 Hoagland culture solution under the controlled conditions for acclimatization (Hoagland and Arnon, 1950). For phytoremediation experiments, plastic pots of 10 liters were prepared by putting a 5 cm gravels layer in the bottom, followed by a 5 cm soil layer. Plants were established in soil layers of pots and acclimatization for one week by Hoagland media. After one week of cultivation, 5 liters synthetic solution of Pb was added to the pots in concentrations of 10, 20 and 30 mg/L for 30 days. Simultaneously, the control experiment was performed by using 5 liters distilled water. The treated water samples were collected from the bottom of the pots by the outlet at 5, 10, 15, 20, 25 and 30th day. The biomass of plants was harvested, collected and washed after 30 days of heavy metal exposure. The collected biomass stored in a deep freezer for subsequent steps of the chemical analyses. Both controls and heavy metal treated experiments were performed and analyzed in triplicate. The evaporation losses in plastic pots during experiments were overcome by using distilled water.

Biomass measurement

The biomass of the plants was measured by the electric weighing balance. The initial weight of plants was determined at the starting of the experiment for each pot. The final weight of plants was determined after completion of the experiment (30days) for each pot.

Preparation of lead solution

The 1000 mg/L stock solution of lead was prepared by dissolving 1.615 g analytical grade lead nitrate $Pb(NO_3)_2$ in 1000ml of distilled water. The stock solution was diluted by 100, 50 and 33 times for preparation of a lead working solution of 10, 20 and 30mg/L.

Heavy metal analysis in aqueous solution and plant parts

The treated water from each pot was collected from the bottom of the plastic pot through the outlet at the interval of 5days (5, 10, 15, 20, 25 and 30th day). The Pb concentration was measured by AAS as prescribed by the Standard Method of Water and Waste Water Analysis (APHA, 1998). The percentage removal of Pb was computed by using equation 1.

$$\mathrm{Re}\,moval = \frac{(C_i - C_{eq})}{C_i} \times 100 \qquad (1)$$

The lead (Pb) initial and the final concentrations are denoted by C_i and C_{eq}.

The plants were harvested after completion of the experiment (30 days), roots and shoots separated by using a knife. The plant parts were dried under sunlight for 12 hours, followed by oven dryness at 100°C for 72 hours. The dried plants parts were ground into the powder. One gm of fine powder of the plant parts shoot, roots were the first acid digested with a di-acid mixture (conc. HNO_3:$HClO_4$ = 3:1). The plant samples with 20 di-acid mixture in the flask were placed on an electric hot plate for heating at 80-90°C temperature until the solid particles disappeared till the destruction of organic matter and white fumes were evolved from the flask. Then, it was cooled at room temperature, washed with distilled water and filtered into 100 ml volumetric flask through filter paper (Whatman No.1). Finally, the volume was made up to 50 ml with distilled water. The concentrations of Pb in the extract samples were determined by using an AAS (HITACHI, Polarized Zeeman Atomic Absorption spectrophotometer and Model No. Z-6100).

Results and Discussion

The present study was conducted to assess the phytoremediation capacity of two aquatic plant species growing locally namely, *Brachiaria mutica and Cyperus rotundus* for lead (Pb). To conduct the phytoremediation experiment, 5 liters of different concentrations of Pb (10, 20, 30 mg/L) were used in plastic pots. The experiments were conducted for 30 days. The Pb concentrations were measured on 5, 10, 15, 20, 25 and 30 days.

The removal capacities of both the aquatic species for the lead from day 5th to 30thday shown in table 1. About 82 to 89 % and 85 to 91 % of Pb was removed by *Brachiaria mutica* and *Cyperus rotundus* from 10 and 20 mg/L solution on 5th day of the experiment. While in the case of 30 mg/L Pb solution both aquatic species were showed a slightly lesser removal rate. On the 15th day of the experiment removal rate slightly inclined to 93 % in 20 and 30 mg/L for both the species. The experiment was carried out for 30 days. The %ages removal at the end of the experiment was found between 92% in 10 mg/L to 95% in 20 and 30mg/L by *Brachiaria mutica* and 94% in 30 mg/L to 96% in 20 mg/L by *Cyperus rotundus,* respectively. A slightly irregular pattern of removal %age has been shown by both the species, *Brachiaria mutica* showed slightly higher removal in 30 mg/L Pb solution, while *Cyperus rotundus* showed slightly higher removal in 10 and 20 mg/l at the end of the experiment. The removal amount became more or less constant towards day 30th of the experiment. Similarly, remaining/residual concentrations of lead at the end of *Brachiaria mutica* aquatic plant experiments were found 0.8, 1.0 and 1.6 ppm in 10, 20 and 30 mg/L, respectively. While, 10, 20 and 30 mg/L Pb solutions were reduced to 0.5, 0.6 and 1.9 mg/L in *Cyperus rotundus* experiments, respectively.

The results of the present study are consistent with the finding of Naghipour *et al.,* (2018). They utilized the *Azolla filiculoides* for removal of Pb from aqueous solution. The concentrations of Pb used in their 15 days experiments were 5, 10 and 25

Table 1: Removal of lead (Pb) by *Brachiaria mutica* and *Cyperus rotundus* (all values in mg/L).

Concen-tration of Pb in solution	PLANTS					
	Brachiaria mutica			*Cyperus rotundus*		
	Removal Conc.	Remaining Conc.	Removal %	Removal Conc.	Remaining Conc.	Removal %
DAY 5th						
10ppm	8.1±0.8	1.8	82%	8.4±1.2	1.5	85%
20ppm	17.8±2.0	2.2	89%	18.1±1.6	1.8	91%
30ppm	26.3±0	3.6	88%	26.0±0.41	3.9	87%
DAY 10th						
10ppm	8.6±1.0	2.4	86%	8.8±1.0	1.1	89%
20ppm	18.4±0.5	2.6	92%	18.4±1.7	1.5	92%
30ppm	27.0±2.7	3.9	90%	26.7±1.1	3.1	89%
DAY15th						
10ppm	8.8±1.0	2.2	88%	9.0±1.7	1.0	90%
20ppm	18.5±1.3	1.4	93%	18.5±2.0	1.4	93%
30ppm	27.9±1.1	2.1	93%	27.3±2.7	2.7	91%
DAY20th						
10ppm	9.0±0.3	1.0	90%	9.0±0	1.0	90%
20ppm	18.7±1.0	1.2	94%	18.7±0.3	1.1	94%
30ppm	28.2±0.6	1.7	94%	28.0±0.3	2.1	93%
DAY25th						
10ppm	9.1±0.3	0.9	91%	9.3±0.2	0.7	93%
20ppm	18.7±2.2	1.2	94%	19.0±1.8	1.0	95%
30ppm	28.1±0.7	1.8	94%	28.1±0.7	1.8	94%
DAY30th						
10ppm	9.2±0.0	0.8	92%	9.4±1.3	0.5	95%
20ppm	19.0±1.2	1.0	95%	19.4±0.8	0.6	96%
30ppm	28.5±1.1	1.6	95%	28.1±0	1.9	94%

ppm at 0.2, 0.4 and 0.8 g *Azolla filiculoides* weight amount. The removal efficiency was inclined from 89 to 95, 68 to 97 and 75 to 79% from 5th to 15th day for 5, 10 and 25 ppm, respectively. In their conclusion, they emphasized that with increasing metal concentration from 5 to 25 mg/L the removal efficiency decreased. The heavy metals concentrations of 5 mg/L on the 10th day showed the highest removal efficiency. In contrast to the present study, lesser Pb removal efficiency by using *Eichhornia crassipes* aquatic plant reported by Adelodun *et al*., (2020) from polluted water. They observed a removal efficiency of 45.81% (reduction of Pb from 3.52 to 1.85 mg/L) and 25.63% (reduction of Pb from 4.32 to 3.21 mg/L) using *Eichhornia crassipes* at the 12th day of experiments.

Concentration of heavy metals in different plant parts

The concentrations of lead (Pb) in roots and shoots after completion of experiments are represented in table 2. The lead (Pb) was found to be maximum concentrated in roots varying from 2155 mg/kg in 10 mg/L to 3942 mg/kg in 30 mg/L in *Brachiaria mutica,* while the shoot had lower levels of lead ranging from 211 to 486 mg/kg as approached the higher concentrations of 30 mg/L. Similarly, Pb concentration in roots and shoots of *Cyperus rotundus* varied from 974 to 1408 and 340 to '1017 mg/kg, respectively. This study highlighted that the Pb concentrations were maximum in roots in comparison to shoots, while roots of *Brachiaria mutica* showed higher concentration than roots of *Cyperus rotundus*. The higher concentration of Pb observed in *Brachiaria mutica* and concentration increased with increasing concentration. The shoots Pb concentration was found higher in *Cyperus rotundus,* indicating higher translocation of Pb in comparison to *Cyperus rotundus*.

The finding suggested that *Brachiaria mutica* and *Cyperus rotundus* did not fall under the hyperaccumulator plants because the maximum concentration found in the roots. A similar observation has been reported by Al Chami *et al.,* (2015). They evaluated the lead (Pb) removal efficiency through phytoremediation using two energy crops, *Sorghum bicolor* and *Carthamus tinctorius* in hydroponic solution spiked with 5 to 100 mg/L Pb concentrations in a growth chamber. The *Carthamus tinctorius* roots showed the highest Pb concentration (36,229 mg/kg) in the Pb 25 mg/L solution. While the shoots of *Carthamus tinctorius* showed the highest Pb concentration (2292 mg/kg). They also concluded that lead was the maximum accumulated in roots during their study. Yoon *et al*., (2006) reported the accumulation of Pb in 17 native plant species of contaminated Florida site. They observed that Pb in most of the plant species roots was much higher in comparison to shoot Pb contents, suggesting low roots to shoots Pb mobility.

Table 2: Concentration of lead in different plant parts (mg/kg).

Concentrat-ionof pb in solution	PLANTS			
	Brachiaria mutica		*Cyperus rotundus*	
	Root	Shoot	Root	Shoot
Control	6±3	3.6±6	11±3	9.5±3
10ppm	2155±86	211±28	974±35	340±6
20ppm	2979±19	352±22	1160±28	669±9
30ppm	3942±64	486±25	1408±38	1017±38

Lead toxic effect on biomass production

The study was also conducted for estimation of plant biomass increase or decrease under Pb treatment. The initial weight was recorded before the plants were potted and final weight was recorded after the completion of the pot experiments to study the effect of heavy metal stress on plants growth. The biomass weight of various concentration treatments was compared with the control biomass. The plant biomass is an indicator of plant productivity in terms of dry matter yield. The increased photosynthetic process is considered as the basis for the building up of organic substances which accounts for 80–90% of the total dry matter of plant. This study demonstrated that with increasing contents of Pb considerable amount of biomass decreased in comparison to control due to the toxicity of heavy metal on biochemical and physiological properties of *Brachiaria mutica* and *Cyperus rotundus* plants. The weight gain by control plants was 250 and 200 gm by *Brachiaria mutica* and *Cyperus rotundus* at the end of experiments (after 30 days), respectively. The biomass was increased by 200, 150 and 100 gm by *Brachiaria mutica* at 10, 20 and 30 mg/L Pb concentration, whereas in case of *Cyperus rotundus* biomass increased by 120, 100 and 100 gm at 10, 20 and 30 mg/L, respectively. The reduction in plants

Table 3: Plant biomass at various lead heavy metal concentration.

Estimation of plant biomass (Weight in grams)						
Concen-tration of Pb	*Brachiaria mutica*			*Cyperus rotundus*		
	Initial weight	Final weight	Weight gain	Initial weight	Final weight	Weight gain
Control	300	550	250	300	500	200
10 ppm	200	400	200	250	370	120
20 ppm	300	450	150	250	350	100
30 ppm	250	350	100	250	350	100

biomass in comparison to control was found a maximum 60 % and 50 % by B. *mutica* and C *rotundus* at 30 mg/L lead concentration on completion of experiments. The biomass reduction percentage increased with increasing concentration. Adelodun *et al*., (2020) reported that Pb concentration above 15 mg/L showed the observable phototoxic damage as the leaves wilted off within 3–6 days before final death by the 12th day in *Eichhornia crassipes* aquatic plant. The Pb toxic effect on the physiological and biochemical properties of *Lemna minor* was assessed by Jayasri *et al*., (2017). The significant toxicity was showed by 8 mg/L Pb concentration, soluble protein reduced by 4 fold and photosynthetic pigments one fold. They revealed that *L.minor* tolerate the Pb concentration up to 4 mg/L without any toxic effect. Similarly, Shi *et al.,* (2020) reported the same trends in the roots and shoots fresh weigh changes of ryegrass. The extremely remarkable inhibition effect of the heavy metal compound contamination has been observed on the growth of plants. The 28.2% decline in shoots biomass after 45 days of experiments, while roots showed a 34.7% decline in comparison to control. The effect of Pb on ryegrass was more in root than shoots, no indication of shrink or turning yellow of ryegrass leaves was seen as compared to control.

Conclusion

This study was conducted for the assessment of the phytoremediation rate of Pb from aqueous solution by using the *Brachiaria mutica* and *Cyperus rotundus* native species in pots experiments. The concentrations of Pb in aqueous solution were 10, 20 and 30 mg/L. Both the species reduced the more than 90% of Pb in 30 days experiments. Both the plant species have been shown the same lead removal pattern during the phytoremediation experiments. The Pb removal rate was higher during the initial phase of experiments, after 15th day removal was gradually slower down and nearly constant at the end of experiment. The higher concentration of Pb was observed in roots in comparison to shoots of both the species. The maximum concentration of Pb was showed by roots *Brachiaria mutica* in 30 mg/L experiment. The Pb translocation rate from roots to shoots was lesser for both the species. The higher concentrations of Pb were showed a greater toxicity in terms of biomass reduction in comparison to control. The results of the present study highlighted that both these species have greater efficiency for the treatment of Pb containing wastewaters. Further, phytoremediation applicability and survival rate of these plant species in industrial effluents need to be evaluated.

References

Adelodun, A.A., N.O. Afolabi, E.F.C. Chaúque and A.S. Akinwumiju (2020). The potentials of *Eichhornia crassipes* for Pb, Cu and Fe removal from polluted waters. *SN Applied Science,* **2:** 1646 https ://doi.org/10.1007/s4245 2-020-03392 -9.

Al Chami, Z., N. Amer, L. Al Bitar and I. Cavoski (2015). Potential use of *Sorghum bicolor* and *Carthamus tinctorius* in phytoremediation of nickel, lead and zinc. *International Journal of Environmental Science and Technology*, **12:** 3957–3970.

Ali, H., E. Khan and M.A. Sajad (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere*, **91:** 869–881.

Ali, S., Z. Abbas, M. Rizwan, I.E. Zaheer, I. Yava, A. Ünay, M.M. Abdel-DAIM, M. Bin-Jumah, M. Hasanuzzaman and D. Kalderis (2020). Application of floating aquatic plants in phytoremediation of heavy metals polluted water: A Review. *Sustainability*, **12:** 1927 doi:10.3390/su12051927.

Ansari, A.A., M. Naeem, S.S. Gill and F.M. AlZuaibr (2020). Phytoremediation of contaminated waters: An eco-friendly technology based on aquatic macrophytes application. *Egyptian Journal of Aquatic Research*. https://doi.org/10.1016/j.ejar.2020.03.002.

APHA, Standard methods for the examination of waters and wastewaters. APHA- AWWA-WEF, Washington, DC, 1998.

Guittonny-Philippe, A., M.E. Petit, V. Masotti, Y. Monnier, L. Malleret, B. Coulomb and I. Laffont-Schwob (2015). Selection of wild macrophytes for use in constructed wetlands for phytoremediation of contaminant mixtures. *Journal of Environment Management*, **147:** 108–123.

Hoagland, D.R. and D.I. Arnon (1950). The Water-Culture Method for Growing Plants without Soil. California Agricultural Experiment Station, Circular-347.

Hussain, S.T., T. Mahmood and S.A. Malik (2010). Phytoremediation technologies for Ni^{++} by water hyacinth. *African Journal of Biotechnology*, **9:** 8648–8660.

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin,* **68:** 167–182.

Jayasri, M.A. and K. Suthindhiran (2017). Effect of zinc and lead on the physiological and biochemical properties of aquatic plant *Lemna minor*: its potential role in phytoremediation. *Applied Water Science*, **7:** 1247–1253.

Kamala, A., S.K. Middha and C.S. Karigar (2018). Plants in traditional medicine with special reference to *Cyperus rotundus* L.: a review. *3 Biotech*, **8:** 309. https://doi.org/10.1007/s13205-018-1328-6.

Mishra, V.K. and B.D. Tripathi (2008). Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. *Bioresource Technology,* **99:** 7091–7097.

Mishra, V.K. and B.D.Tripathi (2009). Accumulation of chromium and zinc from aqueous solutions using water hyacinth

(*Eichhornia crassipes*). *Journal of Hazardous Materials*, **164(2):** 1059-1063.

Naghipour, D., S.D. Ashrafi, M. Gholamzadeh, K. Taghavi and M. Naimi-Joubani (2018). Phytoremediation of heavy metals (Ni, Cd, Pb) by *Azolla filiculoides* from aqueous solution: A dataset. *Data in Brief*, **21:** 1409–1414.

Ong, S.A., C.E. Seng and P.E. Lim (2007). Kinetics of adsorption of Cu(ii) and Cd(ii) from aqueous solution on rice husk and modified rice husk. *Electronic Journal of Environment, Agriculture, Food and Chemistry*, **6(2):** 1764-1774.

Pratas, J., C. Paulo, P.J. Favas and P. Venkatachalam (2014). Potential of aquatic plants for phytofiltration of uranium-contaminated waters in laboratory conditions. *Ecological Engineering*, **69:** 170–176.

Shi, G., Y. Yan, Z. Yu, L. Zhang, Y. Chang and W. Shi (2020). Modification-bioremediation of copper, lead and cadmium-contaminated soil by combined ryegrass (*Lolium multiflorum* Lam.) and *Pseudomonas aeruginosa* treatment. *Environmental Science and Pollution Research,* **27:** 37668–37676.

Skinner, K., N. Wright and E. Porter-Goff (2007). Mercury uptake and accumulation by four species of aquatic plants. *Environmental Pollution*, **145:** 234–237.

Singh, D., A. Tiwari and R. Gupta (2012). Phytoremediation of lead from wastewater using aquatic plants. *Journal of Agricultural Technology*, **8(1):** 1-11.

Uysal, Y. (2013). Removal of chromium ions from wastewater by duckweed, *Lemna minor* L. by using a pilot system with continuous flow. *Journal of Hazardous Materials*, **263:** 486–492.

Van Thi Thanh Ho, M.P. Dang, L.T. Lien, T.T. Huynh, T.V. Hung and L.G. Bach (2020). Study on domestic wastewater treatment of the horizontal subsurface flow wetlands (HSSF-CWs) Using *Brachiaria mutica*. *Waste Biomass Valorization,* **2020:** https://doi.org/10.1007/s12649-020-01084 - 4.

WHO. (1995). Lead. Environmental Health Criteria, vol. 165. Geneva: World Health Organization.

Yoon, J., X. Cao, Q. Zhou and L.Q. Ma (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Science of Total Environment*, **368(2–3):** 456–464.