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BIOACCUMULATION POTENTIAL AND TOXICITY OF ARSENITE USING ROOTED-SUBMERGED *VALLISNERIA SPIRALIS* IN A HYDROPONIC CULTURE AND ITS CHARACTERIZATION STUDIES

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ABSTRACT

In this study, the bioaccumulation technique was used to removal of arsenite from water using naturally occurring macrophyte plant *Vallisneria spiralis*. Plants were treated in deionized water with nutrient solution at pH 6.8. Experiments were conducted at different concentration of arsenite solution with 0, 0.10, 0.25, 0.5 and 1 mg As/L as arsenic trioxide (As_2O_3). After 20 days experiment the highest accumulation arsenite concentrations in roots 9.52 mg kg-1, dry weight and shoots 36.52 mg kg-1, dry weight treated with 1 mg/L arsenite solutions. *Vallisneria spiralis* shoot biomass was characterized using SEM-EDX and FTIR. Arsenite translocation and detoxification processes in plant cells in presence of bacterial arsenate reductase enzyme and glutathione. The arsenite accumulation and relative growth of plants on differential concentration of arsenite solutions were significantly increased with the passage of time.

Keywords: Bioaccumulation; *Vallisneria spiralis*; Arsenite; Scanning electron microscope.

1. INTRODUCTION

In the present scenario different industries and mine sectors are main participators for environmental pollution. Heavy metals/metalloids pollution by an anthropogenic activity is a serious significant threats to the living environment [1, 2].Arsenic is a most important toxic element for living organisms which are enter into streams, lakes, rivers and ground waters by natural and man-made processes [3, 4].Potential sources of arsenic are physical weathering, biological decomposition, geochemical action, magma eruption, and combustion of fossil fuels [5, 6]. Some researchers have been reported in different countries arsenic contain at high level in drinking water [7], where a large proportion of arsenic in groundwater at levels from 100 to over 2000 μg L^{-1} [8]. Inorganic arsenic exists in different form in an aqueous solution e. g. metalloid arsenic, arsenite, and arsenate. Arsenite is predominating, more mobile and 25-60 times more toxic than arsenate [9]. Intake of higher doses of arsenic by human beings shows delirious effects on human health like stomach ulcer, hepatic & renal damage, irritation of central nervous system and gastrointestinal region and possible carcinogenic effect on liver and kidney [10, 11]. The maximum permissible limit of arsenic ions in drinking water is 10 μg L^{-1} recommended by World Health Organization (WHO) [12, 13].

Various physiochemical techniques have been used to remediation of arsenic ions from aqueous solution [14, 15], but bioaccumulation technique is a cost effective and efficient method for removal of heavy metals/metalloids ion from aqueous solution using micro and macrophytes. Toxic pollutants removal from aqueous solution by aquatic plant using two uptake processes such as (i) biosorption of metal binding and reversible, (ii) phytoaccumulation of ion-sequestration and irreversible [16-18]. Aqueous solution treatment process is an easy observation, quick screening, eco-friendly, costless, and technologically feasible techniques. *Vallisneria spiralis*is is a macrophyte plant that prefers a good light and a nutrient rich substrate. It has narrow linear leaves that range in colour from a pale green to reddish up to 3 feet long and 0.75 inches broad. The objective of the present study was (i) to estimate the total absorption of arsenite from different aqueous solution by *Vallisneria spiralis*. (ii) to evaluate accumulation potential and relative growth of treated plants. (iii) to characterize *Vallisneria spiralis* shoot biomass using SEM-EDX and FTIR techniques to know the uptake of arsenic ions.

2. MATERIAL AND METHODS

2.1. Experimental procedure

Vallisneria spiralis plants were collected from University campus, Nuapadhi, Balasore. The collected young plants were washed with running water very carefully and

placed in circular plastic container under natural atmospheric conditions for 20 days. These plants were treated in deionized water with differential concentration of 0.1, 0.25, 0.5 and 1.0 mg As/L as arsenic trioxide (As_2O_3; Merck Chemicals, Germany). The experiments were conducted in a plastic circular container containing modified 0.25 M Hoagland nutrient solutions prepared with deionized water. Each plastic circular container was kept with one liter of double distillation water and selected four numbers of healthy *Vallisneria spiralis* plants in a culture room at room temperature 30± 2°C with fluorescent lamp (350 µmol.m^{-2} s^{-1}) under 16 h photoperiod. The treated solutions of pH (Systronics, Digital pH meter) was adjusted 6.8 to 7.2 using required amount of reagent grade dilute 1 M HNO_3 and 1 M NaOH solutions. Treated plants were harvested after 10, 20 and 30 days of the same variation in size of root and shoot length selected for experimental work. After the completion of each test duration, *Vallisneria spiralis* plants were separated roots and shoots and its keep in separately freeze dried atmosphere. Formed fine powder using mortar and pestle separately roots and shoots part for analysis of arsenite absorption, relative growth, toxicity and bio-concentration factor. All experiments were performed in three replicates.

2.2. Preparation of standards and arsenite analysis

Arsenite stock solution of 1,000 mgL^{-1} was prepared by dissolving 1.320 g of arsenite (As_2O_3; Merck Chemicals, Germany) in distilled water containing 4 g NaOH in 1 L of double-distilled water (Greenberg et al. 2005). Subsequently, different working arsenic solutions of required concentrations were prepared by proper dilution from stock solutions. The 500 mL $NaBH_4$ solution was prepared by dissolving 2.5 g NaOH and 2.0 g $NaBH_4$, in double-distilled water and diluting up to mark. The $NaBH_4$ reagent was always prepared immediately before use. Sodium tetrahydroborate solution was dispensed into the acidic test sample solution.

Half (0.5) g of freeze dried fine powder each root and shoot samples were separately weighed into a 50 mL conical flask to which 10 mL of concentrated nitric acid was added. Digestion of samples in this study was performed according to the standard method [19]. The mixture was heated at 50°C for 30 min, and then the temperature was further increased to 70 °C. To determine arsenite concentration in above digested samples, 1 mL of digest was mixed with 9 mL of reducing solution consisting of 1.5% (w/v) potassium iodide, 1.5% (w/v) ascorbic acid and 10% (v/v) hydrochloric acid. This solution was heated at 50 °C for 1h. The carrier solution was 5% (v/v) nitric acid, and the reductant solution consisted of 0.2% (w/v) sodium borohydride and 0.05% (w/v) sodium hydroxide. The final results were made up with double distillation water and arsenite analyses carried out by using hydride generation atomic absorption spectrophotometry (HG-AAS) (Perkin-Elmer P 200, USA).The results of the arsenite accumulation were reported as concentration (mg kg^{-1}), dry weight of arsenic ions in plants. The arsenite ions removal efficiency is calculated as follows:

$$\text{Efficiency } (\%) = \frac{C_i - C_f}{C_i} \text{ x } 100 \quad (1)$$

Where, C_i and C_f are the initial and final concentrations of the arsenite in the aqueous solution (mg L^{-1}), respectively. To ascertain the statistical significant differences of various experimental parameters analysis were using a MINITAB 15 for windows software program [20]. The before and after absorption of arsenite ions was investigated using a scanning electron microscope (SEM-EDX; JOEL model JSM-6480 LV, Japan). FT-IR spectra of the samples with and without arsenite ions were obtained by using PerkinElmer Spectrometer Spectrum RX-I.

2.3. Relative growth and bio-concentration factor

Relative growth of plants of before and after experiments was calculated and the results of biomass expressed per unit mass per day (g / g. d) [21].

Relative growth = Final fresh weight (FFW) / Initial fresh weight (IFW)

The bio-concentration factor referred to as metal concentration in the plant body with respect to initial concentration of metal in aqueous solution. The result of BCF was calculated as follows [22].

BCF= Concentration of metal in plant tissue (mg/kg) / Initial concentration of metal in external solution (mg/L).

3. RESULTS AND DISCUSSION

3.1. Characterization of *Vallisneria spiralis* biomass.

3.1.1. SEM-EDX and FTIR analysis

The surface morphology of the *Vallisneria spiralis* biomass without and with sorption of arsenite ions at pH 6.8 during absorption process was measured with the help of SEM-EDX (JEOL JSM- 6480 LV) and presented in

Figure 1(a) and 1(b). Without treatment of plant biomass were clearly shows the surface structure and porosity in the samples. **Figure 1(b)** represented after treatment of plant biomass were clearly shows the white shaped particles over the surface but in absent of the without treatment biomass. The Energy-dispersive X-ray spectra was another evidence of arsenite absorption with and without treated shoot biomass of *Vallisneria spiralis* as shown in **Figure1 (a) and (b),** respectively. So, concluded that, arsenite ions were absorbed on the surface of the biomass. These results were further confirmed with the results of FTIR spectra analysis.

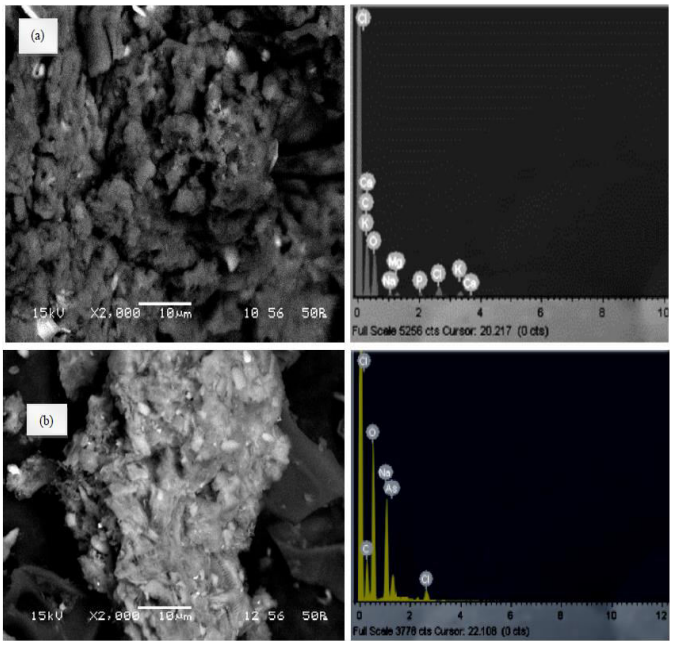

Fig. 1: SEM-EDX of *Vallisneria spiralis* biomass control at 2000 x. (b) SEM-EDX of arsenite exposed *Vallisneria spiralis* biomass at 2000 x.

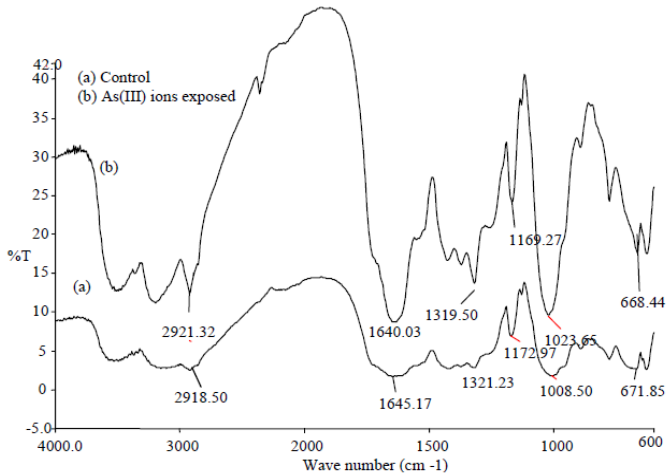

Fig. 2: FTIR spectra of the *Vallisneria spiralis* biomass without (control) and with (0.100 mg/L) loaded arsenite ions at pH 6.8.

The FTIR spectra of the *Vallisneria spiralis* biomass loaded and unloaded arsenite ions were obtained to determine which functional groups may have attributed to the treated biomass**.** The Infrared spectra of the without arsenite treated biomass shows number of absorption peaks, indicating the complex nature of the biomass, using Perkin Elmer FTIR spectrophotometer SPECTRUM RX-I as shown in Figure 2**.** After treatment of biomass reveals a significant shift of few absorption peaks indicating the coordination of arsenite ions with biomass and results are represented in **Table 1**. The above results show changes in the spectra may be interaction of arsenite ions with the carboxyl, hydroxyl and amino groups present on the surface of the *Vallisneria spiralis* shoot biomass [23-28].

3.1.2. Relative growthand bio-concentration factor

The relative growth and bio-concentration factor of treated plants with different times were represented in Table 2. The relative growth of treated plants significantly increased with the test duration. In the present study, the relative growth increased in treated plants with concentrations of As_2O_3 of 0.10, 0.25 and 0.5, but decreased in 1.0 mg/L. The highest relative grows that the concentration of 0.50 mg/L after 30 days experiments were 1.30 g/g d. At the low concentration level arsenite ions has stimulate plant growth and development. Due to the higher concentration of arsenite ions having inhibitory effects on plant physiology system, alternatively reducing plant metabolic activity. The total dissolved solids and dissolved oxygen also effects on plant growth and development. It may be due to the excess amounts of cations and anions have influencing effect on the nutrient uptake and the molecular oxygen enhancing the binding to the site of arsenite ions. Bio-concentration factor is an important parameter to evaluate accumulation potential of the plants and the result was calculated on a dry weight basis. The highest bio-concentration factor result for arsenite ions was 345±1.2 at 1 mg/L arsenite solution after 30 days' experiment and shown in **Table 2.**

Arsenite accumulation and detoxification

Arsenite uptake by *Vallisneria spiralis* at different concentrations of synthetic arsenite solution and different times were separately presented in Table 3. After 30 days experiments reveals that absorption of arsenites gradually increases with respect to increase in time periods.

Table 1. IR absorption bands and corresponding possible functional groups observed on after and before treated arsenite ion

Vallisneri aspiralis		Assignments	Probable site for functional group
without treated of arsenite ions wave number (cm^{-1})	with treated of arsenite ions wave number (cm^{-1})		
2918.50	2921.32	-CH stretching vibration of alkyl chains	Complexation of –OH groups with arsenite ions [24]
1645.17	1640.03	C=O stretching vibration of carboxylic or ester groups	Complexation of carboxylic group with arsenite ions [25].
1321.23	1319.50	Attributed to N-H stretching vibration, $-CH_2$ scissoring or $-CH_3$ anti-symmetrical bending vibration and O-H deformation	Corresponding to the complexation of nitrogen with arsenite from the N-H group [26].
1172.97 and 1008.50	1169.27 and 1023.65	Attributed to C-N stretching vibration of amino group	May be due the interaction of nitrogen from amino group with arsenite ions [23].
671.85	668.44	Attributed to O-C-O scissoring vibration of polysaccharide such as chitin or similar compound	Corresponding to the O-C-O scissoring vibration of polysaccharide [27-28].

Table 2. The relative growth and bio-concentration factor of Vallisneria spiralis at different concentrations of arsenite and exposure times. (Mean ± S.D.).

Relative growth ($g.g^{-1}.d^{-1}$)	Mean ± S.D.		
As_2O_3 concentration (mg/L)	Days		
	10	20	30
0.10	1.04 ± 0.01	1.12 ± 0.05	1.17 ± 0.01
0.25	1.10 ± 0.03	1.24 ± 0.03	1.28 ± 0.04
0.50	1.23 ± 0.02	1.26 ± 0.04	1.30 ± 0.03
1.0	1.22 ± 0.21	1.24 ± 0.02	1.28 ± 0.46
Bio-concentration factor			
0.10	186 ± 1.03	206 ± 1.44	223 ± 1.13
0.25	208 ± 1.11	229 ± 1.21	231 ± 1.01
0.50	220 ± 1.24	232 ± 1.18	234 ± 1.04
1.0	298 ± 1.01	328 ± 0.05	345 ± 1.12

The maximum accumulation of arsenite ions in shoot was36.52 mg/kg and root is 9.5 mg/kg after 20 days' experiments [29, 30]. In the present study, maximum absorption of arsenite ions by treated plants was86% after 20 days' treatments**.** Metal ions penetrated plants by passive processes, mostly by exchange of cations which occurred in the cell wall. The high reactivity of metal ions with thiol, amino or hydroxyl groups makes the molecules carrying these functional groups as metal chelators.

Table 3. The accumulation of arsenite ions at pH 6.8. in (a) shoots and (b) roots of *Vallisneria spiralis* at different arsenite concentration and exposure time.

	(Mean ± S.D.)		
As_2O_3 concentration (mg/L)	Days		
	10	20	30
	Shoot (mg/kg)		
0.10	1.42 ± 0.04	1.61 ± 0.03	1.74 ± 0.12
0.25	3.05 ± 0.08	4.47 ± 0.11	5.47 ± 0.06
0.50	9.58 ± 0.13	10.21 ± 0.06	12.21 ± 0.22
1.0	19.53 ± 0.24	36.52 ± 0.28	32.12 ± 0.05
	Root (mg/kg)		
0.10	0.43 ± 0.02	0.46 ± 0.01	0.52 ± 0.03
0.25	1.11± 0.05	1.25 ± 0.08	1.32 ± 0.05
0.5	1.82 ± 0.11	2.52 ± 0.04	3.18 ± 0.14
1.0	4.21 ± 0.15	9.52 ± 0.02	8.22 ± 0.12

Effective metal chelators are small Cys-rich proteins metallothione ion (MTS), Cys-containing peptides glutathione (GSH), and Phytochelatins (PCs). Arsenite translocation and detoxification mechanisms inside root cells are responsible for the phytochelatins. Arsenite is oxidized in presence of phytochelatins to form arsenate. In presence of arsenate reductase and non-enzymatically by glutathione (GSH) again arsenate reduced to form arsenite.The synthesis of glutathione and phytochelatin in presence of the bacterial γ-glutamylcysteine synthetase (γ-ECS) catalyzes the formation of γ-glutamylcysteine (γ-

EC) from the amino acids glutamate and cysteine and detailed presented in **Fig. 3** [31, 32, 24].

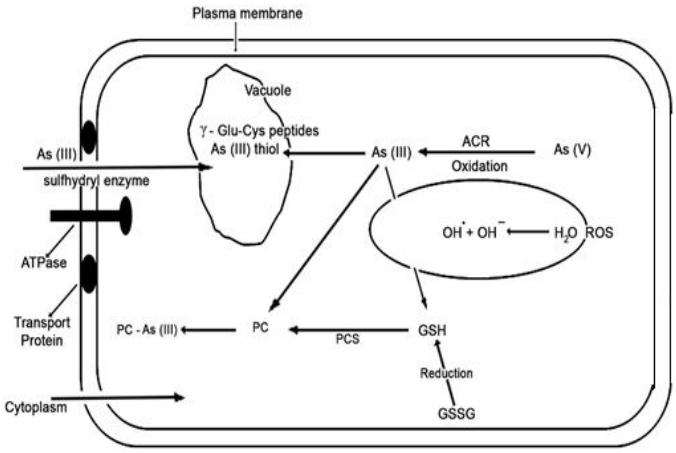

Fig. 3: Hypothetical model of arsenic ions transport mechanisms in *Vallisneria spiralis* root.

Detoxification of arsenite is accomplished by the formation of thiol-rich peptides. The different enzymes are involved in the detoxification of reactive oxygen species (ROS) include superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase [25, 28]. The antioxidative enzymes are generally electron donors and react with reactive oxygen species to form a neutral and nontoxic form. Superoxide dismutase (SOD) is an important defense function against reactive oxygen species in plants. Arsenic induced increase in the activity of superoxide dismutase can be enhanced level of oxygen [33-35]. Phytochelatins are enzymatically synthesized to form cysteine-rich peptides known to bind arsenate in plant cells. Plasma membrane plays a significant adaptation of arsenic ions and it is regulated at the molecular and biochemical levels reactions as [36]:

$$Na_2HAsO_4.7H_2O \longrightarrow H_3AsO_4 + 2NaOH + 5H_2O$$

$$H_3AsO_4 + 2H^+ + 2e^- \longrightarrow H_3AsO_3 + H_2O$$

$$H_3AsO_3 \longrightarrow H_2AsO_3^- + H^+$$

$$H_2AsO_3^- \longrightarrow HAsO_3^{2-} + H^+$$

(Sulfhydryl enzymes)

$$HAsO_3^{2-} \longrightarrow AsO_3^{2-}\text{-SH (Thiol complex)}$$

4. CONCLUSION

Removal of contaminants from aqueous solution using water hyacinth is an efficient technique. The highest relative growth and bio- concentration factor of treated plants were 1.30 ± 0.03 g/g.d and 345 ± 1.12 after 30 days experiment. Maximum absorption of arsenite was 86% after 20 days' treatments at the concentration of 1 mg/L. The arsenite accumulation by *Vallisneria spiralis* biomass was characterized using SEM-EDX and FTIR techniques. The FTIR spectroscopic analysis confirmed the C=O stretching vibration of carboxylic, Complexation of –OH andattributed to C-N stretching vibration of amino groups were involved in the treated surface of the biomass. Great removal efficiency and high arsenite ions accumulation capacity make *Vallisneria spiralis* a best for phytoaccumulation processes.

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6. REFERENCES

1. Tripathi RD, Mishra S, Srivastava S, et al. *Proceedings of the National Institute Sciences, India B: Biological Science* 2008; **78:**167-182.
2. Rahman MA, Hasegawa H, *Chemosphere*, 2011; **83:**633-646.
3. Maiti A, Basu JK, De S, et al. *Industrial & Engineering Chemistry Research*, 2010; **49:**4873 - 4886.
4. Mamun AA, Maan FR, Zahiarh AK, Yehya MA, Mohammed ARS, Alam MZ, Muyibi SA, Faris IA, Azin I, et al. *Journal of Applied Sciences*, 2009; **9:** 3180-3183.
5. Wang QA, Li Z, Cheng SP, Wu ZB, et al. *Environmental Earth Sciences*, 2010; **6:**1207-1213.
6. Misbahuddin M, Fariduddin A, *Archives of Environmental Health*, 2002; **57 (6):** 516-519.
7. Mohan D, Pittman Jr CU, *Journal of Hazardous Materials*, 2007; **142:**1-53.
8. Roy P, Mondal NK, Das K, et al. *Journal of Environmental Chemical Engineering*, 2014; **2:**585-597.
9. Guo X, Chen F, *Environmental Science and Technology*, 2005; **39:**6808-6818.
10. Ratna KP, Chaudhari S, Khilar KC, Mahajan SP, *Chemosphere*, 2004; **55:**1245-1252.
11. Basu A, Kumar S, Mukherjee S, et al. *Indian Journal of Environmental Health*, 2003; **45 (2):** 143-150.
12. Mishra VK, Upadhyay AR, Pathak V, Tripathi BD, et al. *Water, Air, and Soil Pollution,* 2008; **192:**303-314.
13. Ranjan D, Talat MH, Hasan SH, et al, *Journal of Hazardous Materials*, 2009; **166:**1050-1059.
14. Smith AH, Lopipero PA, Bates MN, Steinmaus CM, et al. *Science*, 2002; **296 (21):** 2145-2146.
15. Giri AK, Patel RK, *Water Science & Technology*, 2011; **63(5):**899-907.

16. Kamsonlian S, Suresh S, Majumder CB, Chand S, et al. *International Journal of Current Engineering and Technology*, 2012; **2:**153-158.
17. Alvarado S, Guédez M, Lué-Merú MP, Nelson G, Alvaro A, Jesús AC, Gyula Z, *Bioresourse Technology,* 2008; **99(17):** 8436-8440.
18. Miretzky P, Saralegui A, Fernandez, Cirelli A, *Chemosphere*, 2004; **57:**997-1005.
19. APHA. AWW. WEF, *Standard Methods for the Examination of Water and Wastewater.* 20th ed. Washington DC. 1998.
20. Winer BJ, Statistical principles in Experimental Design. *International Student Edition*, London. 1981.
21. James JJ, Drenovsky RE, *Rangeland Ecology and Management*, 2007; **60:**395-400.
22. Snyder KVW, *Removal of Arsenic from Drinking Water by Water Hyacinths* (Eichhornia crassipes). J U S S J W P Volume 1: 2006.
23. Dambies L, Vincent T, Guibal E, et al. *Water Research*, 2002; **36:**3699-3710.
24. Paola C, Margherita S, Stefano E, Pietro M, et al. *Journal of Hazardous Materials*, 2010; **175:**172-178.
25. Jing L, Fenghua Z, Journal *of Geochemistry*, 2009; **28:** 61-99.
26. Giri A K, Patel RK, Mandal S, et al. *Chemical Engineering Journal*, 2011; **178:**15-25.
27. Roddick-Lanzillota AJ, Macquillan AJ, Crow D, et al. *Applied Geochemistry*, 2002; **17:**445-454.
28. Jia Y, Xu L, Wang X, et al. *Demopoulos GP.Geochimicaet Cosmochimica Acta*, 2007; **71:**1643-1654.
29. Thomas SY, Choon TG, Robiah Y, Gregory Koay FL, Azni I, et al. *Desalination*, 2007; **217:**139-166.
30. Meharg AA, Hartley-Whitaker J, *New Phytologist*., 2002;**154:**29-43.
31. Mukhopadhyay R, Shi J, Rosen BP, et al. *Journal of Biological Chemistry,* 2002; **275:**21149-21157.
32. Delnomdedieu M, Basti M, Otvos JD, Thomas DJ, et al. *Chemical Biological Interactions*, 1994; **90:**139-155.
33. Abubacker M N, Sathya C. J, *Environment and Biotechnology Research*, 2017; **6(1):**82-87.
34. Ghulam I A, Behzad I M, Irshad B, MuhammadI S, Niazi NK, Muhammad KI, MuhammadI A, Munawar H, Natasha, et al. *International Journal of Environmental Research and Public Health*, 2018; **15:**2-45.
35. Imran MA, Ch MN, Khan RM, Ali Z, Mahmood T, et al. *International Journal of Physical Sciences*, 2013; **8(17):**840-847.
36. Sneller FEC, Heerwaarden L M, Kraaijeveid, SFJL, Ten BWM, Bookum WM, Koevoets PLM, Schat H, Verleij JCK, et al. *New Phytologist*., 1999; **144:**223-252.