RESEARCH ARTICLE

RHOEO DISCOLOR, A MEDICINAL PLANT WITH PHYTOREMEDIATION POTENTIAL.

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Abstract

Rhoeo discolor is a plant used in traditional medicine mainly due to its anticancer properties. The present work studied, for the first time, its use as a phytoremediation plant. Samples of R. discolor were collected in the gardens of the Universidad Juárez Autónoma de Tabasco. Chrysopogon zizanioides (vetiver), a phytoremediation plant, was used as positive control. Both plants were exposed for 144 h to leachates from a sanitary landfill for urban waste. Afterwards, they were washed, dried and pulverized. Specimens of each species were left unexposed to leachates to use as negative controls. Elements were identified and quantified by X-rays and by ICP. The elements identified in R. discolor exposed to leachates were quartz, CaCO₃ and thiocyanate. Arsenic, lithium, Lead, and Thallium were identified at concentrations that were only 1.4 times lower than those found in vetiver, except for Tl (445.71 and 326.33 mg/kg in R. discolor and vetiver, respectively). In both evaluated species, exposure to leachates was associated with an increase in the concentration of Cu, K, Mn, Ni and S. In R. discolor and vetiver, the concentration of S increased 25 and 6 times, respectively. It was concluded that R. discolor has potential as a phytoremediation plant.

Introduction:

Rhoeo discolor [syn. Tradescantia spathacea Swartz, Rhoeo spathacea (Swartz) Stearn] is a plant used in traditional medicine in Mexico. R. discolor belongs to the family Commelinaceae and is native to the Caribbean and Central America. In the Mexican southeast, it is known as "purple maguey". Its use as medicinal plant goes back to the 1930s [1], and its anti-cancer activity has been reported since 1963 [2] and as recently as 2016 [3]. Furthermore, this plant contains compounds with antioxidant [4] and antimicrobial [5] activity.

Plants can transform and mineralize a wide variety of organic complexes [6] as part of their natural process and as a function of their autotrophic nature. Plants constitute the biological group with the greatest biosynthetic capacities.
According to Bragato [7], plants sequester nutrients and store them in their roots and shoots, or accumulate them in other tissues, as in the case of heavy metals, which makes them useful for bioremediation processes.

Phytoremediation is an effective method for the removal of different compounds that can damage or alter the environment. Phytoremediation occupies an important place among the different remediation alternatives because it is an emerging technology based on the combined action of plants (terrestrial or aquatic) and native microbial communities [8]. Phytoremediation takes advantage of the capacity of certain plants to absorb, accumulate, metabolize, volatilize or stabilize contaminants such as heavy metals, radioactive metals, organic compounds and oil products that are present in soil, air, water or sediments. When dealing with organic pollutants, the goal of phytoremediation is the mineralization of substances into non-toxic components (phytodegradation or phytotransformation). Plant enzymes can degrade aromatic rings, polychlorinated biphenyls, phenols and trichloroethylene [8]. The objective of this study was to identify exogenous molecules in the leaves of Rhoeo discolor to demonstrate its potential as a phytoremediation plant.

Materials and Methods:-
Sample collection and processing:-
Specimens of *Rhoeo discolor* were collected in the gardens of the Juárez Autonomous University of Tabasco (UJAT), Villahermosa, Tabasco, Mexico. They were identified in the herbarium of the UJAT. A total of three individuals (*Tradescantia spathacea*) were collected and their leaves separated. *Chrysopogon zizanioides* was used as positive control; it was collected from a sanitary landfill of the company Promotora Ambiental located on the Villahermosa-Teapa road, Tabasco, Mexico.

After an acclimatization period of 168 hours, both plants were exposed for 144 hours to leachates taken from the sanitary landfill, which also receives urban waste. The plants were then washed with distilled water and about 15 g of each individual were air-dried in the shade at room temperature for two weeks. The dry material was pulverized, yielding approximately 5 g of each plant. A control plant (unexposed to leachates) was used for each species.

Pre-treatment:-
Zero point five grams (0.5 g) of each plant sample were placed in jars together with boiling beads for further analysis. Seven ml of HNO$_3$ and 3 ml of reagent water were added and heated to a temperature of 85 °C for 3 h or until the volume decreased to 4-2 ml. Given the volume of the samples used in this work, 10 ml of a digestive solution of HNO$_3$ (70%) were added to each sample, a volume equivalent to the volume of acid and reagent water that was added before. The samples were allowed to temper and then adjusted to a volume of 25 ml [9].

The wet digestion method involves decomposition by mixed and unmixed acids in teflon cups placed on aluminum blocks or hot plates [10], or, usually, in precipitate glasses placed on heating plates [11].

Chemical analysis:-
Chemical elements were identified and quantified with an X-ray powder diffractometer (SIEMENS D500). The diffractometer was connected to a computer for data and phase analysis using the Diffrac-AT software (version 3.2, Livermore, CA, United States of America, 1995-2000) [12]. An inductively coupled plasma-optical emission spectrophotometer (ICP-OES; IRIS Advantage, Thermo Jarrell Ash Corporation) and the multielement standard solution 4 for ICP (Sigma-Aldrich, St. Louis, United States) were also used.

The data were processed using ANOVA statistical analysis and Fisher's Least Significant Difference (LSD) method with the software STATGRAPHICS Centurion XVI, version 16.1.02.

Results and Discussion:-
X-ray diffraction:-
X-ray diffraction makes it possible to identify the compounds that give natural products certain interesting properties [13].
Figure 1: shows the results obtained from X-ray diffraction for purple maguey leaves. Three molecules were identified: quartz, calcium carbonate and iron (II) tetrapyridine dithiocyanate.

Figure 1. Powder X-ray diffraction pattern at 25 °C. The following compounds: □ Quartz, low alpha SiO₂; ▲ Calcium carbonate CaCO₃; △ Iron tetrapyridine dithiocyanate C₉H₆N₄·Fe(SCN)₂/Fe(SCN)₂·4(C₃H₈N).

Plants obtain calcium carbonate, an essential compound, from the soil. Calcium is needed for plant growth and for processes of cell division and elongation. This element improves the resistance of plants against pathogens and participates in the development of new leaves, flowers, roots and fruits. A previous study [12] identified ferrocyanide and a derivative of it, sodium nitroprusside, in purple agave. Sodium nitroprusside is a potent vasodilator agent that is used to treat severe cases of arterial hypertension; it is probably responsible for the medicinal properties attributed to purple agave in traditional medicine. Furthermore, the enzyme rhodanese catalyzes the reaction of sodium nitroprusside with thiosulfate to form the metabolite thiocyanate. At the beginning of the 20th century, thiocynate was used in the treatment of hypertension, but its use was abandoned due to the toxicity associated with it [14].

Cyanide is produced by the human body and exhaled with each breath in small concentrations; it is also produced by more than a thousand plant species such as sorghum, bamboo and cassava. Relatively low concentrations of cyanide can be toxic to humans, flora and fauna [15]. A human oral LD₅₀ of 1.1-1.5 mg CN⁻/kg body weight has been estimated for the ingested NaCN and KCN [16]. In contact with normal skin, the LD₅₀ value is 100 mg/kg of weight [17].

Thiocyanate (also known as sulfocyanate, sulphocyanide or rhodanide), which was identified in purple maguey, is the anion [SCN]⁻ and the conjugate base of thiocyanic acid. This compound was previously known as rhodanide
(from the Greek word for rose), because of the red color of its complexes with iron. It is produced by the reaction of elemental sulfur or thiosulfate with cyanide:

$$8 \text{CN}^- + S_8 \rightarrow 8 \text{SCN}^-$$

$$\text{CN}^- + S_2O_3^{2-} \rightarrow \text{SCN}^- + SO_3^{2-}$$

The last reaction is catalyzed by the enzyme sulphotransferase, also known as rhodanasa or rhodanese, and may play a role in the detoxification of the body from cyanide.

Thiocyanate shares its negative charge almost equally between sulfur and nitrogen. It is an ambiguous ligand and can thus act as a nucleophile in both sulfur and nitrogen. $[\text{SCN}]^-$ can also act as a bridge between two or even three metals ($M-\text{SCN}-M; >\text{SCN}-$ or $-\text{SCN}<$)\(^{(17)}\).

In the present study, thiocyanate may have come from the leachates or may have originated in the reaction catalyzed by rhodanese in the presence of cyanide\(^{(12)}\). Whichever is true, the results confirmed the initial hypothesis that \textit{R. discolor} has the capacity to absorb and translocate contaminating elements and can thus be considered a phytoremediation plant. This is the first such study on this species; however, further research is required to fully characterize it and improve its economic value. The results of this study can also provide important information to rural populations where this species is consumed frequently, so that people take into consideration the places from which \textit{Rhoeo discolor} plants are obtained for medicinal use. Furthermore, this study could be used to provide advice for the development of backyard plantations with well-characterized species.

### Optical spectroscopy:

#### Table 1: Analysis of elements in two species exposed to leachates

| Elements | Leaves of contaminated purple maguey | Leaves of uncontaminated purple maguey | Leaves of uncontaminated vetiver | Uncontaminated vetiver leaves | Total levels (leaves and roots) and thresholds in vetiver |
|----------|-------------------------------------|---------------------------------------|---------------------------------|-----------------------------|---------------------------------------------------------|
| Ace      | 42.686                              | ND                                    | 59.715                          | ND                          | 21-72 \(^{(18)}\)                                       |
| Cu       | 0.546                               | 0.23                                  | 0.512                           | 0.895                       | 13-15 \(^{(18)}\)                                       |
| K        | 4086.939                            | 845.33                                | 1310.91                         | 919.81                      | 35-45 \(^{(19)}\)                                       |
| Li       | 0.913                               | ND                                    | 1.329                           | ND                          | ND                                                      |
| Mn       | 32.037                              | 11.23                                 | 42.094                          | 13.96                       | 4-13 \(^{(20)}\) ND                                     |
| Ni       | 238.686                             | 103.43                                | 204.39                          | 114.41                      | 347 \(^{(18)}\)                                        |
| Pb       | 0.417                               | ND                                    | 0.443                           | ND                          | >78 \(^{(18)}\)                                         |
| S        | 36.463                              | 1.45                                  | 68.523                          | 7.605                       | ND                                                      |
| Ti       | 445.713                             | ND                                    | 326.33                          | ND                          | ND                                                      |

Units are shown in mg/kg.

ND. Not available.

Truong et al., 2008 \(^{(18)}\). Roongtanakiat, 2009 \(^{(19)}\). Banerjee et al., 2016 \(^{(20)}\).

Truong\(^{(21)}\) reported Vetiver plants with high tolerance to metals such as As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn. Truong\(^{(22)}\) reported As levels of 11.2 mg/kg in the leaves of vetiver. The levels of arsenic detected in this study in the leaves of vetiver were higher in the presence of leachates than those reported by Truong. Vetiver is plant that has been widely studied due to its phytoremediation capacity, in contrast with \textit{Rhoeo discolor}, which has not been reported to have phytoremediation properties in soils contaminated with metals. A concentration of arsenic of 20.13 mg/kg has been reported in Artemisia abrotanum.

Copper (Cu) was present in low concentrations, at the threshold levels reported by Truong\(^{(23)}\), as is shown in Table 1.

Potassium (K) is an alkaline metal that is transported in cationic form. This is the most abundant cation in vacuoles and the main osmolyte in vetiver. It can reach concentrations between 2,000 and 5,000 ppm \(^{(24)}\), but the concentration obtained in this study was 4086.939 mg/kg, although higher concentrations have been reported in other studies. For example, Zhang\(^{(25)}\) reported that, under controlled conditions, the concentration of potassium in
Pennisetum purpureum K. Schumach x P. thyphoideum Rich was 4000 and 20000 mg/kg in roots and leaves, respectively.

Another essential nutrient is nickel (Ni); plants cannot complete their life cycle in its absence and it cannot be replaced by any other nutrient [2,3]. The concentrations observed in other plants are generally between 0.05-5 mg/kg. Symptoms of toxicity appear at concentrations between 25 and 50 mg/kg plant dry weight [26,27]. However, C. zizanioides accumulated 204.39 mg Ni/kg dry weight from an initial concentration of 41.012 mg/kg (control sample). The concentration observed in the contaminated species approached the threshold level of Ni (347 mg/kg) for vetiver that was reported by Truong [23].

The concentration of Mn in both contaminated species ranged between 32 and 42 mg/kg, within the range reported in other studies (20-300 mg Mn/kg) [28]. Due to the high amount and availability of this metal in the soil, it may have replaced essential elements for plants such as Ca and Magnesium (Mg) [29].

The concentration of sulfur in the control samples was significantly lower than in the contaminated samples, ranging between 36-58 mg/kg.

Regarding thallium, the norm NOM-147-SEMARNAT-SSA1-2004 [33] indicates that the reference concentration of Tl in soil under industrial use is 67 mg/kg. This value was exceeded in the samples of soil contaminated by leachates from the sanitary landfill due to the amount of Tl in the leachates (data not shown). The vetiver plant was able to absorb Tl at a concentration of 326.33 mg/kg, while maguey absorbed it at a concentration of 445.713 mg/kg. Although little is known about the accumulation of Tl in C. zizanioides, concentrations of 65 mg/kg [34] and 251 mg/kg have been reported in other species [35].

Thallium is a heavy metal that is highly toxic to plants, animals and humans. Zitko [36] reported a LD50 of 0.03 mg/L for Atlantic salmon. In plants, it inhibits the germination of seeds and the formation of chlorophyll. Furthermore, the human body absorbs Tl very efficiently, especially through the skin, the respiratory organs and the digestive tract. Thallium poisoning is usually caused by the accidental intake of rat poison, which contains large amounts of thallium sulphate. Thallium can also be used for ant control, and it has uses in the electronics industry for the manufacturing of semiconductors switches and fuses. This metal is soluble in water and mobile in soil, which explains its bioavailability in soil and leachates.

Chrysopogon zizanioides is a plant species native to India that is used for the phytoremediation of metals [37] due to its capacity to bioaccumulate metals such as cadmium and copper [38]. There are also reports of the translocation of mercury through phytoextraction mechanisms [39]. Abaga et al. [40] reported the effectiveness of vetiver in the absorption and elimination of endosulfan (an insecticide and organochlorine acaricide). Some studies report the use of this plant for the treatment of wastewater [41]. Other important studies on this species have found evidence of the
absorption of tetracycline (antibiotic) and mercury using liquid chromatography-tandem-mass spectrometry, with complete elimination of these elements from roots and shoots in forty days [42].

Vetiver has also been reported to accumulate arsenic in soils contaminated with pesticides with different physicochemical properties. The results obtained by Datta et al. [43] show that vetiver is able to tolerate moderate levels of arsenic, up to 225 mg/kg, and can be efficiently used for the removal of arsenic. There are reports of this plant as a "hyperaccumulator" of lead and zinc [44]. It has also been used in phytoremediation tests with hydrocarbons [45].

Chrysopogon zizanioides tolerates extreme climatic variations such as prolonged droughts, floods, submersion and extreme temperatures (-15 to 55 °C). It also tolerates soils high in acidity and alkalinity (pH 3.3 to 9.5), high levels of Al (saturation percentage of 85%), Mn (578 mg/kg), soil salinity (ECSE 47.5 ds/m), and sodicity (ESP 48%) [46,47], as well as a wide range of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn), especially lead and zinc. Chrysopogon zizanioides can also absorb and promote the biodegradation of organic waste (2,4,6-trinitrotoluene, phenol, ethidium bromide, benzo [α] pyrene, atrazine) [48].

This shows that the control species (vetiver) used in the present study has been widely characterized. In contrast, this is the first time that metals absorbed by Rhoeo discolor are identified and quantified. The most important pollutants, in terms of their toxicity, that were observed in purple maguey leaves were arsenic, lead and thallium.

The results obtained here showed that R. discolor is a plant with potential for use in the phytoremediation of leachates, in addition to its use in traditional medicine and gardening. This plant may be more efficient than Chrysopogon zizanioides; however, a possible disadvantage is that it has a lower biomass than vetiver.

References:
1. Kato K. Cytological Studies of Pollen Mother Cells of Rhoeo discolor, Hance with Special Reference to the Question of the Mode of Syndesis. Botanical Institute, Science Department, Kyoto Imperial University, 1930. Memoirs of the College of Science, Kyoto Imperial University. Series B. 5:139-161
2. Freytag A. Contribution on the development of a new cancer test. (i). On the influence of carcinomatous mouse ascites on resting plant cells (Kohlrabi, Rhoeo discolor). Z Gesamte Inn Med. 1963;18:1018-24.
3. García Varela R, Fajardo Ramírez OR, Serna-Saldivar SO, Altamirano J, Cardineau GA. Cancer cell specific cytotoxic effect of Rhoeo discolor extracts and solvent fractions. J Ethnopharmacol. 2016;190:46-58. DOI: 10.1016/j.jep.2016.05.051.
4. González-Avila M, Arriaga-Alba M, De la Garza M, Hernández-Pretelín MC, Domínguez-Ortúz MA, Fattal-Fazenda S, Villa-Treviño S. Antigenotoxic, antimutagenic and ROS scavenging activities of a Rhoeo discolor ethanolic crude extract. Toxicology in vitro. 2003;17:77-83.
5. García-Varela R, García-García RM, Barba-Dávila BA, Fajardo-Ramírez OR, Serna-Saldivar SO, Cardineau GA. Antimicrobial activity of Rhoeo discolor phenoic rich extracts determined by flow cytometry. Molecules. 2015;20:18685-18703.
6. Saad I, Castillo JI, Rebolledo D. Fitorremediación: estudio de inteligencia tecnológica competitiva. In Memorias del 4 Congreso Internacional de Sistemas de Innovación para la Competitividad: Hacia la Inteligencia Competitiva. León, Guanajuato, México. 2009.
7. Bragato C, Brix H, Malagoli M. Accumulation of nutrients and heavy metals in Phragmites australis (Cav.) Trin. ex Steudel and Bolboschoenus maritimus (L.) Palla in a constructed wetland of the Venice lagoon watershed. Environ Pollut. 2006;144(3):675-75. DOI: 10.1016/j.envpol.2006.06.046
8. López Martínez S, Gallegos Martínez ME, Pérez Flores LJ, Gutiérrez Rojas M. Mecanismos de fitorremediación de suelos contaminados con moléculas orgánicas xenobióticas. Rev Int Contam Ambie. 2005;21(2):91-100.
9. Edgell K. Method study 37 SW-846 method 3050 acid digestion of sediments, sludges, and soils. US Environmental Protection Agency, Environmental Monitoring Systems Laboratory. 1989.
10. Norma Mexicana. NMX-051-SCFI-2001. Determinación de metales por absorción atómica en aguas naturales, potables, residuales y residuales tratadas.
11. Mendoza Salgado P, López Trujillo V. Estudio de la calidad del lixiviado del relleno sanitario La Esmeralda y su respuesta bajo tratamiento en filtro anaerobio piloto de flujo ascendente [dissertation], Manizales (Colombia): Universidad Nacional de Colombia; 2004.
12. López-Martínez S, López Y Celis I, Lara-Corona VH, Velázquez-Martínez JR. Chemical composition of the leaves Rodeo discolor medicinal plant distribute in Central America and Mexico using X-ray diffraction spectroscopy. J Sylwan. 2016;160:165-177.

13. Bruno-Colmenaré J, Usbullaga A, Khouri N, Díaz de Delgado G. Cubebin: a lignan isolated from Aristolochia odoratissima L. Acta Cryst E. 2007:63:o2046-o2047. DOI: 10.1107/S1600536807013487

14. German WF, Messinger E, Herman M. Toxicity of thiocyanates used in treatment of hypertension. Ann Intern Med. 1949;30(5):1054-9. DOI: 10.7326/0003-4819-30-5-1054

15. Ramírez AV. Toxicidad del cianuro. Investigación bibliográfica de sus efectos en animales y en el hombre. Rev Med Peru. 2010;71(1):54-61. DOI: http://dx.doi.org/10.15381/anales.v71i1.74

16. Shifrin NS, Beck BD, Gauthier TD, Chapnick SD, Goodman G. Chemistry, toxicology, and human health risk of cyanide compounds in soils or former manufactures gas plant sites. Refulatory toxicology and Pharmacology. 1996;23:106-116.

17. Quiroga A N, Olmos V. Revisión de la toxicocinética y la toxicodinamia del ácido cianhídrico y los cianuros. Act Tox Ar. 2009;17(1):20-32.

18. Truong P, Van TT, Pinners E. Vetiver System Applications: Technical Reference Manual. The Vetiver Network International. 2008; pp. 88,107.

19. Roogtanakit N. Vetiver phytoremediation for heavy metal decontamination. PRVN Tech. Bull, 2009; pp. 1.

20. Banerjee R, Goswami P, Pathak K, Mukherjee A. Vetiver grass: an environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecological Engineering. 2016;90:25-34. DOI:10.1016/j.ecoleng.2016.01.027

21. Truong PNV. Vetiver Grass Technology for mine tailings rehabilitation. Ground and Water Bioengineering for Erosion Control and Slope Stabilisation. Published by Science Publishers Inc. NH, USA. 2004.

22. Truong PNV. Vetiver grass technology for mine rehabilitation. Office of the Royal Development Projects Board, 1999.

23. Truong PNV. Vetiver grass technology for environmental protection. Paper presented at: The 2nd Int. Vetiver Conf:Vetiver and the Environment; 2000 Jan; Thailand.

24. Azcón Bieto J, Talón M. Fundamentos de fisiología vegetal. Madrid. McGraw-Hill Interamericana. 2008.

25. Zhang X, Gao B, Li Z, Xia H, Li H, Li J. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (Pennisetum americanum × P. purpureum). Biomass and Bioenergy. 2014; 67, 179–187 DOI:10.1016/j.biombioe.2014.04.030

26. Malavolta E. Avaliação do estado nutricional das plantas: princípios e aplicações. Brasil: Ed. Potato; 1997.

27. Malavolta E, y Moraes MF. Orelha de rato. Informe Laboratorio de Nutrición Mineral de Plantas. Brasil: CENA-USP; 2010-2.

28. Davies B. Trace element pollution. New York: USA; 1980.

29. Kabata Pendías A, Mukherjee AB. Trace elements from soil to human. Springer Science & Business Media, 2007.

30. Hettiarachchi GM, Pierzynski GM. In situ stabilization of soil lead using phosphorus and manganese oxide: influence of plant growth. J Environ Qual. 2002;31(2):564-72. DOI: 10.1011/es001228p

31. Salido AL, Hasty KL, Lim JM, Butcher DJ. Phytoremediation of arsenic and lead in contaminated soil using Chinese brake ferns (Pterisvittata) and Indian mustard (Brassica juncea). Int J Phytoremediation. 2003;5(2):89-103. DOI: 10.1080/713610173

32. Bonilla I. Introducción a la nutrición mineral de las plantas. Los elementos minerales. In: Azcón Bieto, J, Talón M, Editors. Fundamentos de fisiología vegetal. Madrid, Mc Graw Hill Interamericana, 2008. p. 103-121.

33. Mexicana, N. O. PROY-NOM-147-SEMARNAT/SSA1-2004. Que establece Criterios para Determinar las Concentraciones de Remediaci6n de Suelos Contaminados por Arsénico, Berilio, Cadmio, Cromo Hexavalente, Mercurio, Níquel, Plomo, Selenio, Talio y Vanadio, Publicada en el Diario Oficial de la Federación el, 11.

34. Habashi F. Thallium. Physical and Chemical Properties. In Encyclopedia of Metalloproteins. New York; Springer; 2013.

35. Augustynowicz J, Tokarz K, Baran A, Plachno BJ. Phytoremediation of water polluted by thallium, cadmium, zinc, and lead with the use of macrophyte Callitriche cophocarpa. Arch Environ Contam Toxicol. 2014;66(4):572-81. DOI: 10.1007/s00244-013-9995-0.

36. Zitko V. Toxicity and pollution potential of thallium. Sci Total Environ. 1975;4(2):185-92. DOI: 10.1016/0048-9697(75)90039-X

37. Adams RP. Comparison of vetiver root essential oils from cleansed (bacteria- and fungus-free) vs. non-cleansed (normal) vetiver plants. Biochem Syst Ecol. 2008; 36(3), 177-182. DOI:10.1016/j.bse.2007.10.004
38. Ondo Zue AN, Dousset S, Mbengue S, Munier-Lamy C. Is vetiver grass of interest for the remediation of Cu and Cd to protect marketing gardens in Burkina Faso. Chemosphere. 2014;113:42-7. DOI: 10.1016/j.chemosphere.2014.04.010

39. Lomonte C, Wang Y, Doronila A, Gregory D, Baker AJ, Siegle R, Kolev SD. Study of the spatial distribution of mercury in roots of vetiver grass (Chrysopogon zizanioides) by micro-pixe spectrometry. Int J Phytoremediation. 2014;16(7-12):1170-82. DOI: 10.1080/15226514.2013.821453

40. Abaga NO, Dousset S, Munier-Lamy C, Billet D. Effectiveness of vetiver grass (Vetiveria zizanioides L. Nash) for phytoremediation of endosulfan in two cotton soils from Burkina Faso. Int J Phytoremediation. 2014;16(1):95-108. DOI: 10.1080/15226514.2012.759531

41. Kantawanichkul S, Sattayapanich S, van Dien F. Treatment of domestic wastewater by vertical flow constructed wetland planted with umbrellasedge and Vetiver grass. Water Sci Technol. 2013;68(6):1345-51. DOI: 10.2166/wst.2013.379.

42. Datta R, Das P, Smith S, Punamiya P, Ramanathan DM, Reddy R, Sarkar D. Phytoremediation potential of vetiver grass [Chrysopogon zizanioides (L.)] for tetracycline. Int J Phytoremediation. 2013;15(4):343-51. DOI:10.1080/15226514.2012.702803

43. Datta R, Quispe MA, Sarkar D. Greenhouse study on the phytoremediation potential of vetiver grass, Chrysopogon zizanioides L., in arsenic-contaminated soils. Bull Environ Contam Toxicol. 2011 Jan;86(1):124-8. DOI: 10.1007/s00128-010-0185-8

44. Antiochia R, Campanella L, Ghezzi P, Movassaghi K. The use of vetiver for remediation of heavy metal soil contamination. Anal Bioanal Chem. 2007;388(4):947-56. DOI:10.1007/s00216-007-1268-1

45. Brandt R, Merkl N, Schultzze-Kraft R, Infante C, Broll G. Potential of vetiver (vetiveria zizanioides (L.) Nash) for phytoremediation of petroleumhydrocarbon-contaminated soils in Venezuela. Int J Phytoremediation. 2006;8(4):273-84. DOI:10.1080/15226510600992808

46. Truong P, Baker D. The role of vetiver grass in the rehabilitation of toxic and contaminated lands in Australia. Paper presented at: International Vetiver Workshop; 1997 October. Fuzhou, China.

47. Truong P, Baker D. Vetiver grass system for environmental protection. Paper presented at: Pacific Rim Vetiver Network, Office of the Royal Development Projects Board; 1998.

48. Danh LT, Truong P, Mammucari R, Tran T, Foster N. Vetiver grass, Vetiveria zizanioides: a choice plant for phytoremediation of heavy metals and organic wastes. Int J Phytoremediation. 2009;11(8):664-91. DOI: 10.1080/15226510902787302.