

APPLICATION OF SPINACH BIOMASS (*Spinacia oleracea*) FOR THE REMOVAL OF CHROMIUM (VI) IN AQUEOUS SOLUTION

Data de aceite: 03/07/2023

Ismael Acosta Rodríguez

Laboratorio de Micología Experimental
Facultad de Ciencias Químicas
Universidad Autónoma de San Luis
México
ORCID: 0000-0001-8620-2727

Juana Tovar Oviedo

Laboratorio de Microbiología
Facultad de Ciencias Químicas
Universidad Autónoma de San Luis Potosí
San Luis Potosí, México

Adriana Rodríguez Pérez

Universidad Autónoma de San Luis Potosí.
Centro de Investigación y Extensión de la
Zona Media. San Luis Potosí
ORCID: 0000-0002-6570-6579

Juan Fernando Cárdenas González

Universidad Autónoma de San Luis Potosí.
Centro de Investigación y Extensión de la
Zona Media. El Balandran
San Luis Potosí, México
ORCID: 0000-0002-3502-5959

Víctor Manuel Martínez Juárez

Área Académica de Medicina Veterinaria
y Zootecnia. Instituto de Ciencias
Agropecuarias. Universidad Autónoma del
Estado de Hidalgo. Hidalgo, México
ORCID: 0000-0002-7426-6835

Dalila del Socorro Contreras Briones

Laboratorio de Micología Experimental
Facultad de Ciencias Químicas
Universidad Autónoma de San Luis Potosí
San Luis Potosí, México
ORCID: 0000-0001-8620-2727

ABSTRACT: Spinach it is a low-calorie food with a low content of fat, relatively low protein and good contributor of fiber and micronutrients such a vitamin A and C, and minerals specially iron. Its production is exposed to contaminating factors such as toxic metals from irrigation water, so it can accumulate them, and when ingested by humans, they can be poisoned with these heavy metals, and this accumulation characteristic can be used to try to eliminate these contaminates from soil and water contaminated with them. So, the objective of this work was analyzing the Cr (VI) removal capacity in aqueous solution by the spinach biomass, by the colorimetric method of Diphenylcarbazide, to evaluate the metal concentration. Biosorption at different pH was evaluated for 90 minutes. We too studied the effect of temperature in the range of 28 to 60°C, the removal at different initial concentrations of Cr (VI) and

of biomass, and in contaminated niches. Therefore, the highest biosorption of the metal (100 mg/L) occurs within 90 minutes, at pH of 1.0, 1.0 g of natural biomass, and 28°C. According to temperature, the highest removal was observed at 60°C, in 30 minutes, when the metal is completely adsorbed. It was observed that higher concentration of the metal, the removal is less, and if the biomass concentration increases, the removal of the metal in solution is better. Besides its removal efficiently the metal *in situ* (100% in soil and water contaminated, after 24 and 40 hours of incubation, with 5 g of biomass and 28°C; so, it can be used to eliminate it from industrial wastewater.

KEYWORDS: Chromium (VI), Removal, Shell Biomass, Detoxification.

1 | INTRODUCCIÓN

Spinach is an excellent natural resource of vitamins, fibers, and minerals, which compared to meats, provides few calories, and does not contain fats. It is also rich in phytonutrients, especially beta-carotene and lutein, making it a vegetable with antioxidant properties that protect us from cell damage [1]. Its stems are richer in fiber than the leaves. The spinach (*Spinacea oleraceae*) belongs to the Amaranthaceae family, quenopodiodeáceas, as well as beets, quinoa and chard. They grow very well in temperate climates, being today the United States and China, among others, the most important producing countries. They are available throughout the year [2]. Spinach is mostly water. The amount of fats and carbohydrates is very low, but it is one of the vegetables that contains more protein. It is rich in fibers, especially stems, proving very beneficial for our health, and is an excellent natural source of vitamins and minerals [3], and this include calcium, iron, potassium, magnesium, manganese, and phosphorus. Regarding the vitamin content, spinach is rich in vitamin A, vitamin C, vitamin E, vitamin K, as well as vitamins of group B (B6, B2, B1) and folic acid (vitamin B9). It also contains antioxidant substances such as flavonoids, and carotenoids (lutein and zeaxanthin, neoxanthin). It is also a good source of Omega-3 fatty acids [4]. Also, other properties have been described as: Promotes the transport and deposition of oxygen in tissues, increase muscle strength, it helps you lose weight, help prevent diseases, benefits pregnant women and children, improves vision, and keeps blood pressure balanced [5].

On the other hand, Industrial development, agricultural exploitation and population expansion lead to the emergence of emissions and waste that constitute a growing threat to the environment. These damages translate into changes that affect the quality of life and health of the beings that inhabit the earth, due to alterations in the air, soil, water and all urban and rural environments [6]. Among the contaminants are metals, whose presence is important to be considered in food, both of vegetable and animal origin [7]. Metals can accumulate in crops, either through their absorption by contaminated irrigation water, by the soil through the roots or by deposition in the foliage of airborne particles. The ability of plants to bioaccumulation metals and other possible contaminants varies depending on the plant species and the nature of the contaminants. Heavy metals can be absorbed by plants

depending on their availability in the soil and the selectivity mechanisms of each species, variety and genotype [8]. In Mexico, plant species with metal accumulative capacity have been identified. These include water lily (*Eichhornia crassipes*) [9], onion (*Allium cepa* L., Liliaceae), beetroot (*Beta vulgaris* L., Chenopodiaceae), rice (*Oriza sativa* L., Poaceae), radish (*Raphanus sativus* L., Brassicaceae) [10], and *Ananas comosus* [11]. Thus, there is a need to develop or find innovative low cost adsorbents with an affinity towards metal ions for the removal of Cr (VI) from aqueous solution, which leads to high adsorption capacity [12]. The objective of this study was to analyze in vitro biosorption of Cr (VI) by spinach (*Spinacea oleraceae*) biomass.

2 | EXPERIMENTAL

A. Biosorbent used

The spinach biomass was obtained from the fruits harvested and offered in the marketplace Republic, in the month of May in 2019, of the capital city of San Luis Potosí, S.L.P. México. To obtain the biomass, the leaves was washed with EDTA (10%) in trideionized water, and then with trideionized water during 3 days at constant stirring, with water changes every 12 hours. Subsequently, it was boiled 1 hour to removal traces of the color, dust and were dry at 80°C for 12 hours in an oven, ground in blender and stored in amber vials until use.

B. Biosorption studies and determination of Cromium hexavalent, trivalent and total chromium

For these studies, was used 1 g of dried biomass mixed with 100 mL of trideionized water containing 100 mg/L of the metal, in an Erlenmeyer flask at the desired temperature and pH. The flasks were agitated on a shaking bath Yamato BT-25 model. Samples of 5 mL were taken at different times, and centrifuged at 3000 rpm for 5 min. The supernatant liquid was separated and analyzed for Hexavalent chromium, which was quantifying by a Spectrophotometric method employing Diphenylcarbazide [13]. The information shown in the results section are the mean from three experiments carried out by triplicate.

3 | RESULTS AND DISCUSSION

A. Effect of incubation time and pH

The optimum time and pH for Cr (VI) removal for *B. vulgaris* biomass was 90 minutes and pH 1.0, at constant values of biosorbent dosage (1 g/100 mL), with an initial metal concentration of 100 mg/L, and a temperature of 28°C (Figure 1). It was used a pH meter Corning Pinnacle 530 model and we use nitric acid 1M to maintain the pH. The literature [9],

report an optimum time of 8 h for the removal of Cr(VI) by porous carbon derived from corn straw, 150 and 180 minutes for Cr (VI) removal using oleaster (*Elaeagnus*) seed and cherry (*Prunus avium*) stone biochar [10], 10 hours by *Ananas comosus* biomass shell [11], and 120 minutes, 150 mg/L of the metal and 2.5 g/L of natural biomass with *Macadamia* nutshell powder oxidized by hydrogen peroxide solutions [12]. Changes in the cell permeability of unknown origin, could partly explain the differences founded in the incubation time, providing greater or lesser exposure of the functional groups of the cell wall of the biomass analyzed [4, 5, and 6]. Adsorption efficiency of Cr (VI) was observing a maximum at pH 1.0 and 180 minutes with the biomass analyzed. The results showed that the increase in pH resulted in decrease in the removal of the metal. It was reported an optimum pH 1.5, for the Cr (VI) removal using oleaster (*Elaeagnus*) seed and cherry (*P. avium*) stone biochar [10], a pH of 1-3, for the removal of Cr (VI) by porous carbon derived from corn straw [9], an optimum pH of 1.0 by *A. comosus* biomass shell [11]. Although other authors report an optimum pH 2.0 for epicarpio of *Vitis vinifera* L. [13], a pH of 3.0 by hydrothermal carbon-sphere- Fe_3O_4 [14], pH of 2.0 by modified Russian knapweed flower powder [15]. This was due to the dominant species (CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$) of Cr ions in solution, which were expected to interact more strongly with the ligands carrying positive charges [6, 16].

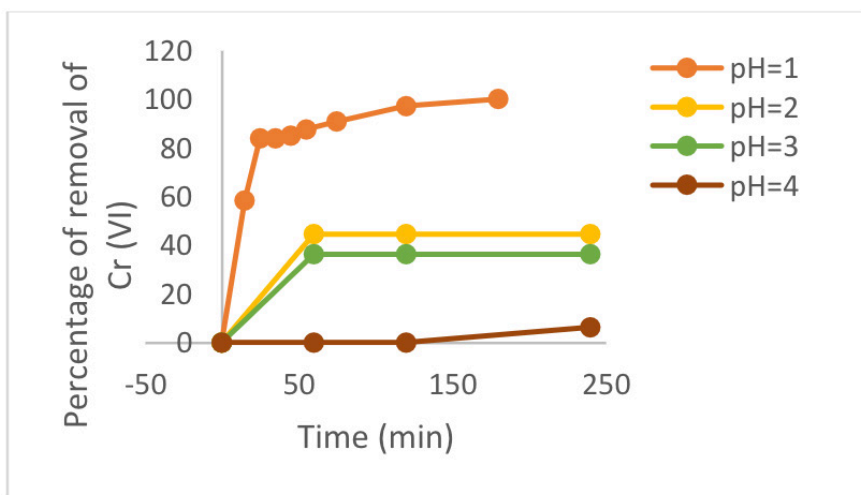


Figure 1. Effect of incubation time and pH on Chromium (VI) removal by *B. Vulgaris* biomass. 100 mg/L Cr (VI), 100 rpm, 28°C. 1.0 g of biomass.

B. Effect of the temperature

On the other hand, temperature was found to be a critical parameter in the bioadsorption of Cr (VI) (Figure 2). To maintain constant the temperature in all experiments, we use a shaking bath Yamato BT-25 model. The total removal was observed at 12 and 180 minutes of incubation, for 60°C and 28°C, respectively. These results are coincident

for power of orange peel biosorbent for the removal of Pb (II) and Zn (II) [17], *A. comosus* biomass shell [11], and residue of the *Persea Americana* Shell [18], by a reusable chitosan-modified multi-walled carbon nanotube composite [19], by dried twigs of *Melaleuca diosmifolia* [20]. The increase in temperature increases the rate of removal of Cr (VI) and decrease the contact time required for complete removal of the metal, to increase the redox reaction rate [21].

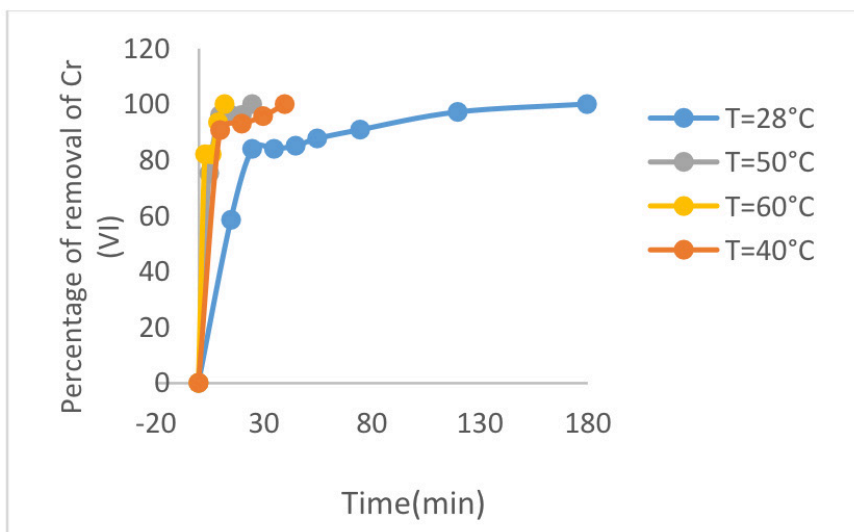


Figure 2. Effect of the temperature on Chromium (VI) removal by *B. vulgaris* biomass.

1/L Cr (VI), pH 1.0, 100 rpm. 1.0 g of biomass.

C. Effect of initial metal concentration

We observe that the removal of metal was 100% at 5 and 24 hours, at 28°C, for 200 and 1000 mg/L, respectively (Figure 3). In addition, we observe the development of a blue-green and white precipitate, which changes more rapidly at higher temperatures (date not shown), and we determined this precipitate how Cr (III) [8]. The results are coincident for the removal of Pb (II) by dried green algae collected from Jeddah coast [22], the removal of Cr (VI) by residual biomass of eucalyptus leaves (*Globulus labill*) [23], and the removal of the same metal by coffee ground and mixed waste tea [16], and are different for the chromium removal using *Platanus x hispánica* aquenium [24], in it was found that the increase in the initial concentration led to an increase in the removal of the contaminant [24]. The increase in initial concentration of Cr (VI), results in the increased uptake capacity and decreased in the percentage of removal of the metal. This was due to the increase in the number of ions competing for the available functional groups on the surface of biomass [6, 21].

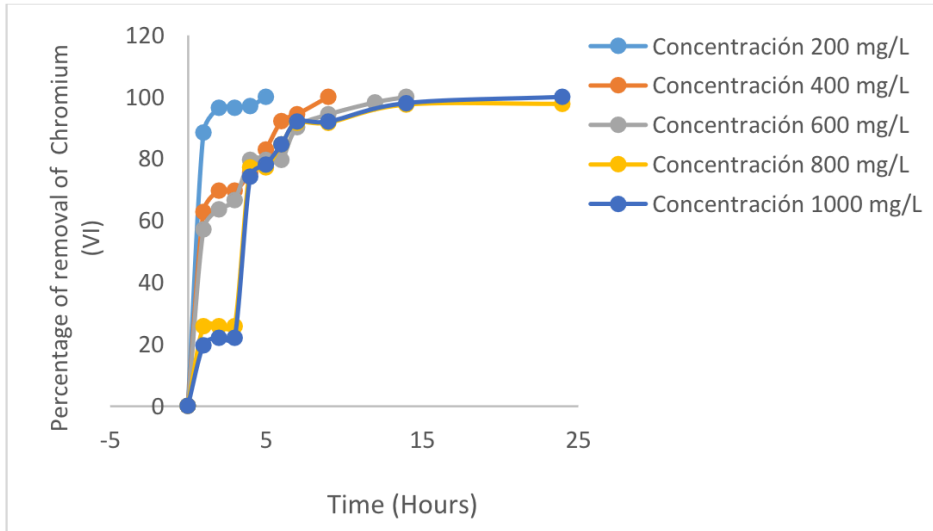


Figure 3. Effect of initial metal concentration on Cr (VI) removal by *B. Vulgaris* biomass. pH 1.0, 100 rpm, 28°C.

D. Effect of biosorbent dose

The influence of biomass concentration on the removal capacity of Cr (VI) is depicted in Figure 4. If we increase the amount of biomass, the removal of the metal in solution decreased slightly, well the removal obtained was observed between 100%-96.2%, with 1-4 g of natural biomass, although it has been reported what with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption [26]. Different results have been reported for almond green hull [27], with a metal concentration of 10 mg/L, with 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, y 5 g de biomass. Too was reported a efficient removal of the metal if the biomass concentration was increased for oleaster (*Elaeagnus*) seed and cherry (*P. avium*) stone biochar [10], using modified Russian knapweed flower poder to initial concentrations of Cr (VI) of 2, 10 and 15 mg/L with pH 2.0 [15], with coffee grounds, where there is an increase in the removal of 39% to 97%, if the concentration of the bioadsorbent is increased of 0.05 to 0.3 g/100 mL [28], as well as for newspaper, where by increasing the concentration of 2 to 6 g/L, increase the removal efficiency of 43.4% to 98.3% [29].

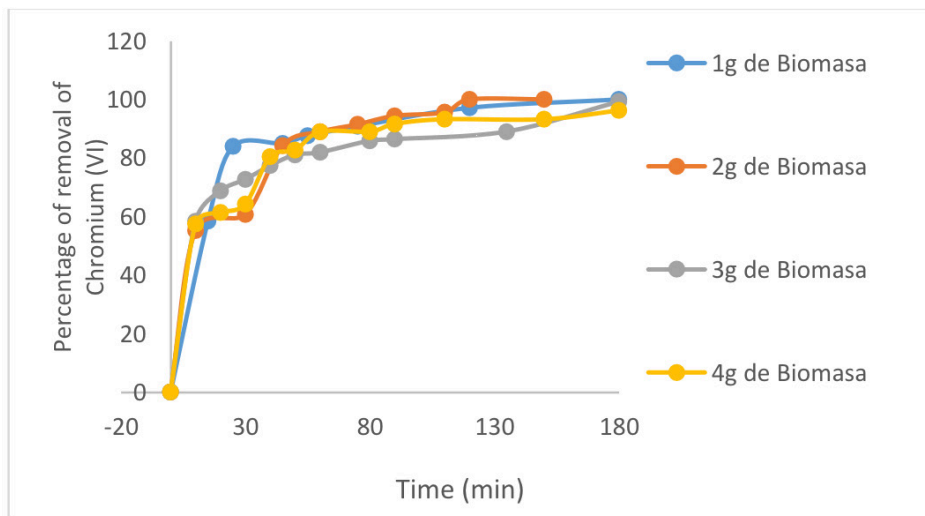


Figure 4. Effect of biomass concentration of *B. vulgaris* biomass, on the removal of 100 mg/L Cr (VI), 28°C, pH 1.0, 100 rpm.

E. Removal of Cr (VI) in industrial wastes with *B. vulgaris* biomass.

We adapted a water-phase bioremediation assay to explore possible usefulness of this biomass for eliminating Cr (VI) from industrial wastes, the biomass (5 g), was incubate with 10 g of non-sterilized contaminated earth with 100 mg/g, and wastewater containing 100 mg/L of Cr (VI) (adjusted), suspended in trideionized water to a final volume of 100 mL. It was observing that in 24 hours of incubation, the Cr (VI) concentration of earth and water samples decrease fully in both (Figure 5), and the decrease level occurred without change significant in total chromium content during the experiments. In the experiment carried out without absence biomass, the Cr (VI) concentration of the earth samples decreased by about of 18% (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil [5, 6, 11, and 18]. This results coincides with the literature reports for other natural biomass, such as seeds of *Moringa oleifera* with 1 g of biomass and concentrations of 10 to 150 ppm of Cu (II), Ni (II), and Cr (VI), with percentages of removal between 37-53%, 39-76%, and 11-33%, respectively [30], for a wastewater sample with Cr (III) (4 mg/L), Zn (II) (1 mg/L), Fe (II) (6 mg/L), Ni (II) (2 mg/L), and Cu (II) (31 mg/L) by *Macadamia* nutshell powder oxidized by hydrogen peroxide solutions [12], the Cr (VI) biosorption, in aqueous solutions and in effluents industrial tannery, using biosorbent of eucalyptus tree waste leaves (*G. labill*), activated in acidic medium [23].

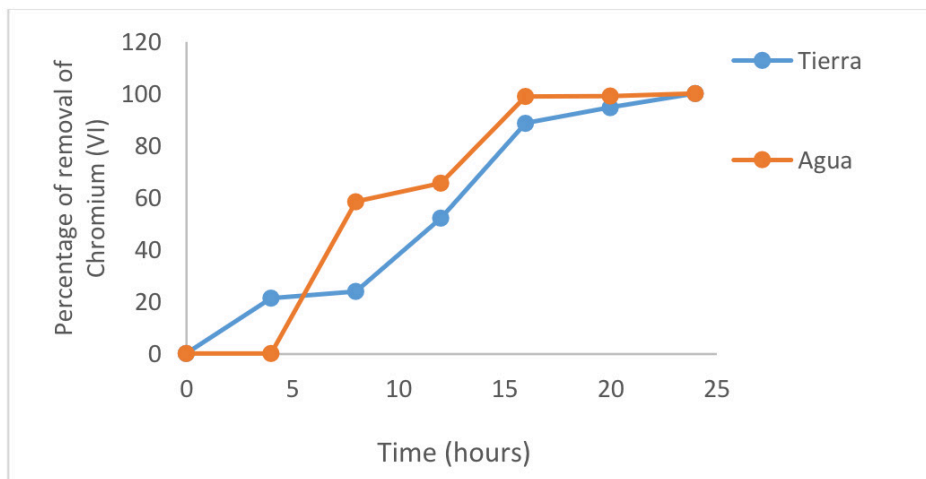


Figure 5. Removal of Cr (VI) in industrial wastes incubated with 5 g of *B. Vulgaris* biomass. 28°C, 100 rpm, 10 g of contaminated earth with 100 mg/g and 100 mL of contaminated water with 100 mg/L (100 mg Cr (VI)/L (adjusted)).

4 | CONCLUSIONS

The biomass analyzed, showed complete capacity of biosorption of 100 mg/L Cr (VI) in solution at different time of incubation, at 28°C, 100 rpm with 1 g of natural biomass, besides this removal the metal *in situ* (24 hours of incubation, with 5 g of biomass), in earth and water contaminated. These results suggest their potential applicability for the remediation of Cr (VI) from polluted soils in the fields.

REFERENCES

- [1] A.F. Pighín, and A.L. Rossi. “Espinaca fresca, supercongelada y en conserva: Contenido de vitamina C pre y post cocción”. *Rev. Chil. Nutr.* Vol. 37, No. 2, pp. 201-207. 2010.
- [2] J.F. Paz León. “Elaboración y control de calidad de pan enriquecido con fibra de cutícula de tomate (*Solanum lycopersicum*) y espinaca (*Spinacea oleracea*)”. Tesis de grado. Bioquímico Farmacéutico. Escuela Superior Politécnica de Chimborazo. Facultad de Ciencias. Escuela de Bioquímica y Farmacia. Riobamba, Ecuador. 2014.
- [3] V. Hoyos, M. Rodríguez, J.F. Cárdenas-Hernández, and H.E. Balaguera-López. “Análisis del crecimiento de espinaca (*Spinacia oleracea* L.) bajo el efecto de diferentes fuentes y dosis de nitrógeno”. *Rev. Col. Ciencias Hortic.* Vol. 3, No. 2. pp. 175-187. 2009.
- [4] M.T. Martínez-Damián y M.C. Cantwell de Tejo. “Cambios de calidad en espinaca almacenada en atmosferas controladas”. *Rev. Chapingo Serie Hortic.* Vol. 8, No. 1. pp. 49-62, 2002.
- [5] S.B. Pérez-Cabeza, N. Morón Angarita, M. Cervantes, and M. A. Barón-Rodríguez. “Evaluación del potencial antioxidante en extracto de espinaca por voltamperometría cíclica”. *Rev. Ion.* Vol. 30, No. 2. pp. 99-105. 2017.

- [6] S.A. Covarrubias and J.J. Peña Cabriales, "Contaminación ambiental por metales pesados en **México**: Problemática y estrategias de fitorremediación". RICA. Vol. 33. pp. 7-21. 2017.
- [7] M.C. Fiallos Morales. "Cuantificación de metales pesados y calidad microbiológica de frutas y vegetales que se expenden en el mercado mayorista de la ciudad de Ambato". Tesis de Ingeniería Bioquímica. Facultad de Ciencia e Ingeniería en Alimentos. Universidad Técnica de Ambato. Ecuador. 2017.
- [8] M.L. Garrido, S.A. Veitia, T. de Armas Guillen, O. Collazo García, J. Jiménez Chacón, D. Castro Espín, M. Álvarez Prieto, A. Rodríguez Nodals y R. González Bayón. "Procedimiento analítico para la determinación de metales pesados en zanahoria y espinaca cultivadas en organopónicos urbanos". Rev. Cien. Téc. Agropec. Vol. 22, No. 1. pp. 20-26. 2013.
- [9] C. Carrión, C. Ponce-de León, S. Cram, I. Sommer, M. Hernández, y C. Vanegas. Aprovechamiento potencial del lirio acuático (*Eichhornia crassipes*) en Xochimilco para fitorremediación de metales". Agrociencia. Vol. 46. pp. 609-620. 2012.
- [10] J. Prieto Méndez, C.A. González Ramírez, A.D. Román Gutiérrez y F. Prieto García. "Contaminación y fitotoxicidad en plantas por metales provenientes de suelos y agua". Trop. and Subtrop. Agroecosyst. Vol. 10. pp. 29 – 44. 2009.
- [11] I. Acosta-Rodríguez, J.F. Cárdenas-González, K. Galaviz-Orocio, A. Rodríguez-Pérez, C. Michel-Cuello, and A. Muñoz-Morales, "Biosorption of Chromium (VI) in aqueous Solution by *Ananas comosus* biomass shell". Modern Concepts & Developments in Agronomy, Vol. 4, No. 1 pp. 1-6. 2019.
- [12] S. Xia, Z. Song, P. Jeyakumar, S. M. Shaheen, J. Rinklebe, Y. Sik Ok, N. Bolan, and H. Wang. "A critical review on bioremediation technologies for Cr(VI)-contaminated soils and wastewater". Crit. Rev. in Environ. Science and Technol. Vol. 49, No.12 pp. 1027-1078, 2019.
- [13] A.E. Greenberg, L.S. Clesceri, and A.D. Eaton, "Standard Methods for the Examination of Water and Wastewater", American Public Health Association, Washington, DC, USA, 18th edition, 1992.
- [14] F. Liu, W. Zhang, L. Tao, B. Hao, and J. Zhang, "Simultaneous photocatalytic redox removal of chromium (VI) and arsenic (III) by hydrothermal carbon-sphere@nano-Fe₃O₄". Environ. Sci. Nano, Vol. 6. pp. 937-947. 2019.
- [15] M. Taghi Ghaneian, M. Dehvari, and B. Jamshidi. "A batch study of hexavalent chromium removal from synthetic wastewater using modified Russian knapweed flower powder". Int. J. of Environ. Health Eng. Vol. 2, No. 5. pp. 38-46. 2013.
- [16] C.E. Barrera-Díaz, V. Lugo-Lugo y B. Bilyeu, "A review of chemical, electrochemical and biological methods for aqueous Cr (VI) reduction", J. Hazard. Mat. Vol. 223– 224, pp. 1– 12, 2012.
- [17] A.F. Cardona Gutiérrez, D.D. Cabañas Vargas, and A. Zepeda Pedreguera. "Evaluación del poder biosorbente de cáscara de naranja para la eliminación de metales pesados, Pb (II) y Zn (II)". Ing. Vol. 17, No. 1. pp. 1-9. 2013.
- [18] I. Acosta-Rodríguez, V. Hernández-López, M.M. Nájera-Pérez, V.M. Martínez-Juárez, M.F. Avalos-Sekeres, C. Michel-Cuello, A. Muñoz-Morales, A. Rodríguez-Pérez, and J.F. Cárdenas-González, "Removal of Cr (VI) Through the Use of the Agroindustrial Residue of the *Persea Americana* Shell". Modern Concepts & Develop. in Agronomy. Vol. 3, No. 4. pp. 1-6. 2019.

- [19] Y. Huang, X. Leeb, F.C. Macazo, M. Grattieric, R. Cai, and S. D. Minteerc. "Fast and efficient removal of chromium (VI) anionic species by a reusable chitosan-modified multi-walled carbon nanotube composite". Chem. Eng. J. Vol. 339. pp. 259-267. 2018.
- [20] D. Pradhana, L.B. Suklaa, M. Sawyerb, and P. K.S.M. Rahman. "Recent bioreduction of hexavalent chromium in wastewater treatment: A review". J. Ind. and Eng. Chem. Vol. 55. pp. 1-20. 2017.
- [21] G.S. Agarwal, H. Kumar, and S. Chaudari. "Biosorption of aqueous chromium (VI) by *Tamarindus indica* seeds". Bioresour. Technol., Vol. 97, pp. 949-956, 2006. [22] R.H. Amasha and M. M. Aly. "Removal of dangerous heavy metals and some human pathogens and some humans pathogens by dried green algae collected from Jeddah Coast". Pharmacophore, Vol. 10, No. 3. pp. 5-13. 2019.
- [23] N. Miranda Zea. "Biosorción de cromo Cr (VI) de soluciones acuosas por la biomasa residual de hojas de eucalipto (*Globulus labill*)". Rev. Did. de las Ciencias Nat. Vol. 1 No.1. pp. 20-32. 2019.
- [24] S.I. García, L. Candía, M.I. Frascaroli, and J.C. González. "Sorcción de cromo utilizando aquenio de *Platanus x hispánica*". Av. Cien. Ing. Vol. 9, No. 2. pp. 21-31. 2018.
- [25] W. Cherdchoo, S. Nithettham, and J. Charoenpanich, "Removal of Cr(VI) from synthetic wastewater by adsorption onto coffee ground and mixed waste tea". Chemosphere. Vol. 221. pp. 758-767. 2019.
- [26] C. Cervantes, J. Campos, S. Devars, F. Gutiérrez, H. Loza, J.C. Torres, and R. Moreno, "Interactions of chromium with microorganisms and plants", FEMS Microbiol. Rev., Vol. 25, pp. 335-347, 2001.
- [27] M. Sahranavard, A. Ahmadpour, and M. Reza Doostiet, "Biosorption of hexavalent chromium ions from aqueous solutions using almond green hull as a low-cost boosorbent". European J. of Scient. Res. Vol. 58, No. 3. pp. 392-400. 2011.
- [28] G.V. Krishna Mohjan, A. Naga Babu, K. Kalpana, and K. Ravindhranath, "Removal of chromium (VI) from water using adsorbent derived from spent coffee grounds". J. Environ. Science and Technol. Vol. 16. pp. 101-112. 2019.
- [29] X. Song Wang, and Z. Zhong, "Removal of Cr (VI) from aqueous solution by newspapers. Desalination. Vol. 249. pp. 175–181. 2009.
- [30] A.C. Landázuri, J.D. Cahuasquí, and A.S. Lagos. "Metal adsorption in aqueous media using *Moringa oleifera* Lam. seeds produced in Ecuador as an alternative method for water treatment". Vol. 11, No. 17. pp. 190–205. 2019.
- [31] Y. Cui, A. Masud, N. Aich, and J.D. Atkinson. "Phenol and Cr(VI) removal using materials derived from harmful algal bloom biomass: Characterization and performance assessment for a biosorbent, a porous carbon, and Fe/C composites". J. Hazard. Mater. Vol. 368. pp. 477–486. 2019.