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Eradication of Chromium (VI) by Employing (*Equisetum hyemale*) Biomass

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MJGP and ARP did the experimental work. Authors JFCG and IAR wrote the protocol and the first draft of the manuscript. Authors MJGP and IAR managed the analyses of the study. Authors AGP and JFCG designed the study, managed the literature searches. Author IAR did the formal analysis, writing-review, editing and supervision. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Currently, a great alternative to try to bioremediate contaminated sites, mainly of heavy metals, is the use of natural biomass, with highly satisfactory results, among which are: fungi, bacteria, algae, agricultural waste of plants, and others. Our objective was analyzing the removal of Chromium (VI) by the *Equisetum hyemale* biomass, by a colorimetric method.

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Place and Duration of Study: Sample: Faculty of Chemical Sciences. Autonomous University of San Luís Potosí, S.L.P., between March and June 2024.
Methodology: The biomass was obtained from the horse tail plant (*E. hyemale*), acquired in the Republic market of the city of San Luís Potosí, during the month of February 2024. For obtain the biomass, the plant was washed during 24 hours in EDTA at 10% (p/v), and after 1 week with trideionized water, with water changes every 12 hours, and we analyze the removal capacity for Chromium (VI) (100 mg/mL) under different conditions. The pH of the solution was adjusted with 1 M H_2SO_4 and/or 1 M NaOH, before adding it to the biomass.
Results: The most bioadsorption was at 8 hours, at pH 1.0, 1 g of biomass, 100 rpm, and 28°C. At a higher incubation temperature, the removal capacity increases, since at 60°C, 100% is removed after 4 hours in the same conditions. With respect to the heavy metal concentration, no differences were found in metal removal at 60°C, although a shorter incubation time was observed at 200 mg/L of the heavy metal, while at 28°C, to lower concentrations, is better the removal of the contaminant. Too, if we increase the biomass concentration, increase the efficiency of removal. Finally, this natural biomass, efficiently removal the metal of wastewater, after 10 days of incubation, 28°C, 5 g of biomass, because 86% of soil and 70% of wastewater, were eliminated from contaminated with 100 mg/g earth, and 100 mg/L of wastewater.
Conclusion: This natural biomass obtained from agricultural waste, eliminate the heavy metal in solution, and can be a good alternative for their removal from industrial wastewater and contaminated soil.

Keywords: Removal; horsetail biomass; chromium (VI); wastewater.

1. INTRODUCTION

"Heavy metals are naturally occurring substances with high molecular weight, widespread and in many cases very useful, such as chromium, lead, and mercury, and they are of the main pollutants of the environment. Its effects on the environment are quite serious, like: it changes the alkalinity of the soil, obviously, it depends a lot on the concentration. They also contaminate water and crops. In these, if there is an excessive amount of a heavy metal, some alterations can occur in the plants, it also degrades the soil, which reduces its productivity, if the contamination is excessive, it can lead to desertification. At the level of rivers and lakes, it also mainly affects fauna. The problem with contamination of the environment by heavy metals is that their effect is silent, it is not seen, and when we realize the damage they cause, it is already too late and, above all, they are dangerous to health" [1]. Heavy metals can enter the body through ingestion of contaminated food and water, tobacco use, exposure to the workplace, and inhalation of dust and airborne particles. Symptoms of heavy metal poisoning can include headache, nausea, fatigue, muscle weakness, skin irritation, and in severe cases, brain and kidney damage, and heart problems, which can be irreversible in some cases. Long-term exposure to heavy metals has also been shown to increase the risk of chronic diseases such as diabetes, cardiovascular disease, and some types of cancer. In addition, exposure to heavy metals during pregnancy can increase the risk of birth defects and developmental delays in the fetus [1,2, and 3]. These contaminants are not eliminated from the human body, and are eliminated in a very low quantity, so their effect is rather cumulative, and they are acquired and accumulate in certain parts of the body, are not metabolized, and some are diluted in the blood, but they are always found somewhere causing damage, although can be eliminated by the kidney and by other means. If the contamination is very high, they accumulate in the hair, making it brittle [2]. In Mexico, the main states with the highest levels of heavy metal contamination are: Zacatecas, Veracruz, San Luis Potosí, Queretaro, Hidalgo, and Chihuahua, which is mainly due to mining activities, because this activity has an impact considerable on the environment since it generates large quantities of waste that can become in heavy metal sources, with the consequent damage to health and the environment [3].

"On the other hand, chromium can exist in different forms, depending on its oxidation state, it can be found in a liquid, solid, or gaseous state. The most common chemicals forms are Cr (0), Cr (III) and Cr (VI), the latter being its most toxic form" [4]. In Mexico, the tanning industry contribute mainly for the most of the pollution due to this element, mainly, for the use of chromium salts for the skin processing. Ingestion of water

with high amounts of chromium (VI) can cause intestinal problems, gastric and liver diseases, too is considered a genotoxic and cytotoxic element for bacteria and eukaryotic organisms [3], while chromium (III), because it precipitates at neutral pH, it is much less toxic than hexavalent chromium [3], which is a very toxic and very dangerous for the human health, because at the industrial level, it is used in the tanning of skins, the production of pigments, preservatives of the textile and wood industry, alloys, antifouling paints, catalysts, anticorrosive agents, drilling muds, high temperature batteries, fungicides, metallic and electrogalvanized coatings, which are an important source of contamination by this heavy metal. For the above, different strategies have been proposed to contribute its solution, focusing on the use of biological alternatives that result in less alteration of the environment, specifically through use of phytoremediation, waste plants and shell biomasses for the removal of heavy metals [2], like the horsetail (*Equisetum hyemale)* or "equiseto", which in different forms, it has been used for the removal of heavy metals from contaminated sites, for example: silico nanoparticles for removing chromium (VI) from aqueous solutions [5], a assay simultaneous of electrocoagulation and electro-assisted phytoremediation [6], the removal of some heavy metals by carbonaceous materials [7], the phytorremediation of iron, lead and chromium [8,9], and plant-derived silica [10], with good results, which suggest their potential applicability for the remediation of this heavy metal from polluted soils, and the objective of this work was analyze the Chromium (VI) removal capacity in aqueous solution by the Horsetail (*E. hyemale*) plant biomass, to obtain efficient methodologies for the elimination of this pollutant from contaminated sites, to contribute to the improvement of the quality of the environment

2. MATERIALS AND METHODS

2.1 Bioadsorbent used

For the obtained of the biomass, the plant was acquired in the Republic Market of the capital city of San Luis Potosí, S.L.P., México, in the month of February 2024. It was washed 24 hours with EDTA at 10% (w/v) in trideionized water, and later one week with trideionized water at 100 rpm, and water changes every 24 hours, and it was boiled for 1 hour to removal the dust and adhering organic components, and it was washed again under the same conditions for 24 hours. It was dried at 80°C for 48 hours in a bacteriological oven, ground in a blender and stored in an amber bottle until use [11].

2.2 Chromium (VI) Removal Studies

A different chromium (VI) solutions ($K_2Cr_2O_7$) (100 mg/L), the pH of the solution to be analyzed was adjusted with H_2SO_4 1 M and/or 1 M NaOH, before adding it to 1 g of biomass/100 mL, and were incubated to 28°C to 100 rpm. Subsequently, were take samples at certain times, the biomass is removed by centrifugation (3000 rpm/5 min) and the supernatant was analyzed to determine the concentration of the metal ion by the Diphenylcarbazide colorimetric method, with which a complex of purple color is formed, and read at a 540 nm absorbance [12]. The removal of the metal was carried out at different conditions.

2.3 Determination of the Optimum time and pH for the Chromium (VI) Removal

For this determination, were prepared different standard solutions of the heavy metal (100 mg/L) of water trideionized; adjusted to different values of pH (1.0, 2.0, 3.0, and 4.0). Later it was added to each of the Erlenmeyer flasks of 250 mL which contain 1 g of sterile biomass of *E. hyemale*, and 100 mL of heavy metal solution, and were incubating them at 28°C in a metabolic bath at 100 rpm, and were taken 5 mL aliquots of the samples at different incubation times The bioadsorbent was removed by centrifugation (3000 rpm/5 min), and in the supernatant was determined the percentage of chromium (Vl) not adsorbed, and by difference determines the amount adsorbed by the biomass. The optimal pH and incubation time determined by comparing the percentage metal removal rate measured every given time and pH analyzed.

2.4 Determination of the Optimum Temperature for the Chromium (VI) Removal

A standard solution of Chromium (VI) was prepared (100 mg/L) in trideionized water and adjusted to pH optimum obtained from the previous test. Subsequently, 100 mL of this solution, were added to each of 250 mL Erlenmeyer flasks, containing 1 g of the *E. hyemale* biomass, and incubated at 28°C, 40°C, 50°C, and 60°C, in a metabolic bath at 100 rpm.

Aliquots of 5 mL were taken at different incubation times, and the biomass was removed by centrifugation (3000 rpm/5 min). In the supernatan was determined the percentage of chromium (VI) not adsorbed. The optimal incubation temperature was determined with the comparison of the percentages of the heavy metal, in the different temperatures at which were analyzed.

2.5 Effect of Initial Concentration of the Metal on the Bioadsorption of Chromium (VI)

Ten standard solutions of the heavy metal were prepared (200, 400, 600, 800 and 1000 mg/L) in water trideionized, which were adjusted to optimal pH of removal obtained in the tests before made. Next, 100 mL of each chromium (VI) standard solution were added to each of the 250 mL Erlenmeyer flasks containing 1 g of the biomass, and incubated at 28°C and 60°C in a metabolic bath at 100 rpm, were taken aliquots of 5 mL at different times, removing the bioadsorbent by centrifugation (3000 rpm/5 min) and the supernatant was determined the concentration of chromium (VI) not adsorbed.

2.6 Effect of Initial Concentration of Biomass on the Bioadsorption of Chromium (VI)

Different standard chromium (VI) solutions (100 mg/L), in 100 mL of trideionized water and adjusted to pH optimum of removal were placed in 250 mL Erlenmeyer flasks, with different concentrations of the biomass under study, and were incubated at 28°C, in a bath with constant agitation at 100 rpm, taking aliquots of 5 mL at different times, removing the cellular bioadsorbent by centrifugation (3000 rpm/5 min), and in the supernatant the concentration of chromium (VI) not adsorbed was determined.

2.7 Bioremediation Assays

To two 250 mL Erlenmeyer flasks containing each 5 g of biomass, 95 mL of water and/or 5 g of earth contaminated with 100 mg/L of chromium (VI) (adjusted), from effluent of a Tannery of the city of Celaya, Guanajuato, Mexico. The mixture was incubated at 28˚C with constant stirring (100 rpm), taking 5 mL aliquots every 24 hours, removing the biomass by centrifugation (3000 rpm/5 min), and in the supernatant the concentration of chromium (VI) not adsorbed was determined.

2.8 Determination of the Concentration of Chromium (VI)

The concentration of the metal ion was determined by the Dhiphenylcarbazide colorimetric method, with which a complex of purple color is formed, and is read at a 540 nm absorbance [12]:

1. Place 5.0 mL of sample in a 50 mL volumetric flask. The sample should have a concentration in the range of 0.154 to 0.616 mg/L. If the concentration is higher, appropriate dilutions should be made.
2. Add 0.5 mL of a 1:1 sulfuric acid solution, and stir for 1 minute.
3. Add 0.1 mL of 85% phosphoric acid and stir for 30 seconds.
4. Add 1.0 mL of the diphenylcarbazide solution, stirring for 1 minute, and add to 50 mL with trideionized water.
5. Allow to stand for 10 minutes for the color to fully develop.
6. The absorbance of the sample is measured in a UV-Visible Spectrophotometer (Shimadzu model 160-A) at a wavelength of 540 nm, using as reference a blank prepared with trideionized water according to the previous procedure. The concentration of the sample is determined by means of a calibration curve.

3. RESULTS AND DISCUSSION

3.1 Effect of Incubation Time and pH

With respect for this parameter, we observe the most removal of 100 mg/L of chromium (VI) at 8 hours, pH 1.0, 28°C, 1 g/100 mL of natural biomass, and 100 rpm (Fig. 1), using a Corning Pinnacle 530 model pH meter and 1 M HNO_3 to keep the pH value constant, since the capture speed is controlled by the time at which the adsorbate it is transported from the outside to the inside of the bioadsorbent particles [13], and this are similar for the *Cucurbita maxima* biomass, with the same incubation time with 50 mg/L of the heavy metal, pH 1.0 and 28°C [14]. But, this results are different for *M.piperita* biomass, which the most removal of the same metal was in 3 hours, in the same conditions [11], for commercial tobacco biomass, which 1 g of this removal 72 mg/L of the metal at 24 h, pH 2.0,

28°C and 100 rpm [15], for the seaweed biomass of *Kappaphycus alvarezii*, with an incubation time of 150 minutes and 64.8%-85.2%, pH 3.0 [16], 30 minutes for the removal of 25 mg/L of the same metal with nanobiochar artichoke leaves [17], 168 hours for the elimination of 50 mg/L of this metal at pH 5.0, 28°C with *Lemna minor* L., in synthetic water [18], 24 hours for the removal of 50 mg/L with 1 g of coconut coir biochar [19], 20 hours for the removal of 20 mg/L, pH 2.0, 28°C, and 10 g of *Coptotermes formosanus* and *Odontotermes formosanus* nestes, for the elimination of chromate in *Brassica chinensis* L. [20], 2 hours for the removal (54%) of 50 mg/L of chromium (VI), pH 5.0, and 5 g/L of *Jatropha curcas* seedcake like bioadsorbent [21], 4 hours for the bioasorption of 25 mg/L (97.7%), of this heavy metal, pH 2.0, 28°C, and 2.4 g/L of natural biomass of *Euryale ferox* Salisbury seed coat [22], and 10 minutes with *Nephelium lappaceum* L. peel extract, for the removal of 5 mg/L of the contaminant, pH 3.0, and 0.15 g/100 mL of biomass [23]. On the other hand, the highest metal adsorption was observed at a pH of 1.0 with the analyzed biomass (Fig. 1), and this is probably what the dominant species (CrO_4^{2-} and $Cr_2O_7^{2-}$) of Cr ions in solution, interact more strongly with the ligands carrying positive charges [24], and this is like that reported for the *M. piperita* and *C. maxima* biomasses, with 50 mg/L of the heavy metal, pH 1.0 and 28°C, at the same pH was the most removal [11,14], but, are different for commercial tobacco biomass, which the optimum pH reported was of 2.0, with 72% of removal in the same conditions [15], for modified sawdust with a value pH of 3.0 [25], for the seaweed biomass of *K. alvarezii*, with a pH of incubation of 3.0 in 150 minutes [16], a pH value of 2.0, for the removal of 10 mg/L of the same metal, in 30 minutes with nanobiochar artichoke leaves [17], a pH of 2.7 for the removal of 539 mg/g of same heavy metal with hidrochar of Argan nut shells [26], a pH of 6.68 with *Lysinibacillus cavernae* CR-2 Isolated from chromite-polluted soil, for the removal of 500 mg/L of chromium (VI) [27], a pH of 5.0 for the removal of 50 mg/L of this metal at pH 5.0, 28°C with *L. minor* L [18], an pH of 2.0 for the removal of 20 mg/L, 20 hours, 28°C, and 10 g of *C. formosanus* and *O. formosanus* nestes, for the elimination of chromate in *B. chinensis* L. [20], an pH of 5.0 for the removal of 54% of an initial concentration of 50 mg/L of this metal with *J. curcas* seedcare [21], a value of optimum pH of 2.0 for the bioasorption of 25 mg/L, with 2.4 g/L of *E. ferox* biomass [22], an pH 3.0 for the removal of 5 mg/L of chromium (VI) with *N. lappaceum* biomass [23], and a pH of 2.5 for the elimination of 100 mg/L of chromium (VI) with *Elaeagnus angustifolia* L. [28].

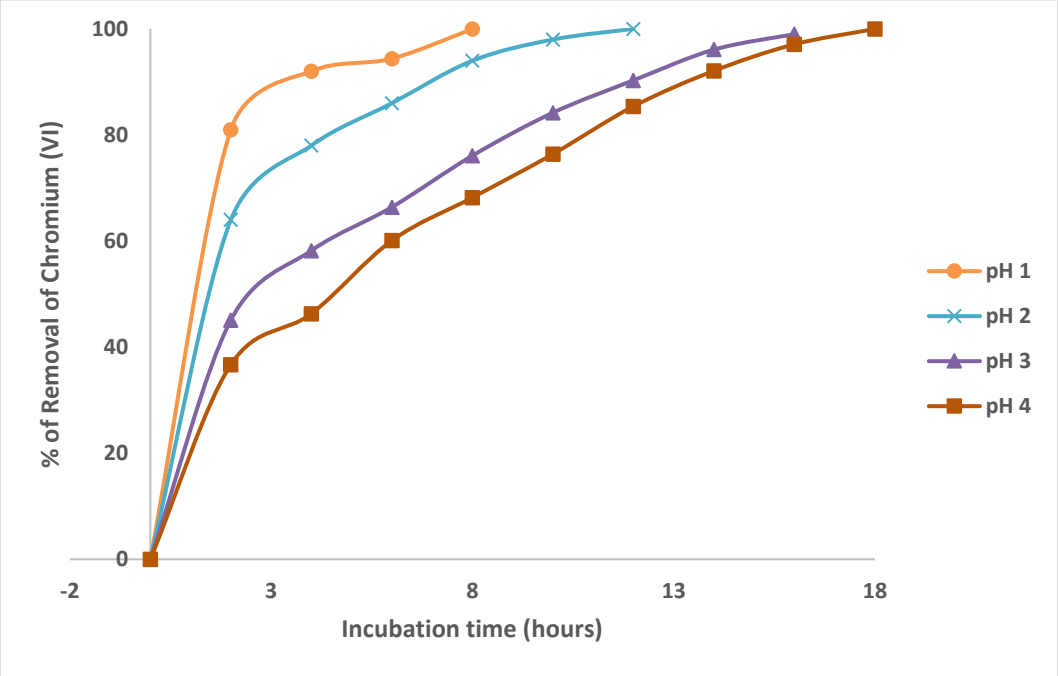


Fig. 1. Effect of pH and incubation time on the bioadsorption of Chromium (VI) in aqueous solution by the biomass of *E. hyemale* 100 mg/L. 1 g of biomass, 28°C, 100 rpm

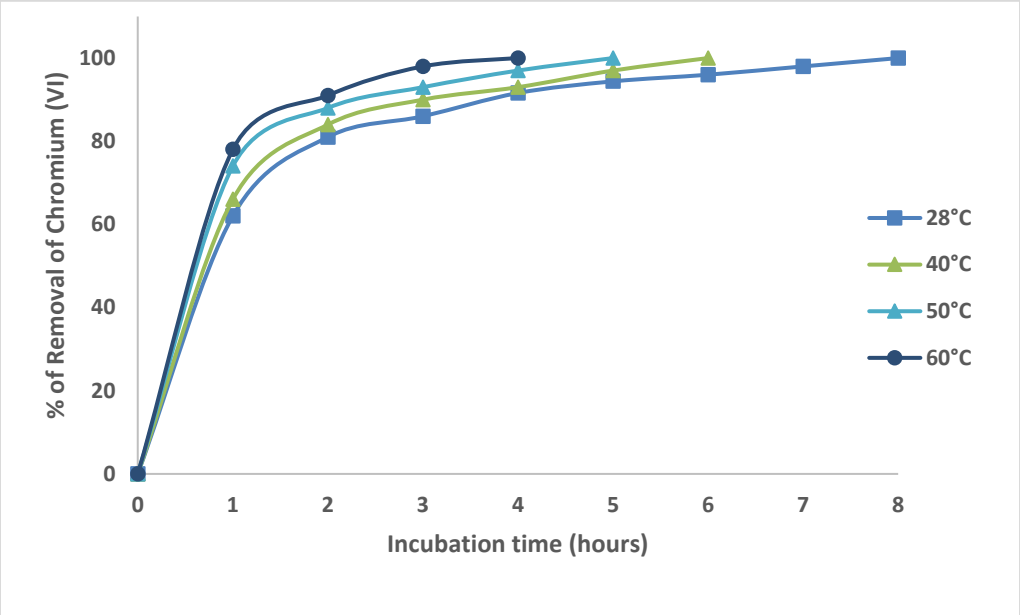


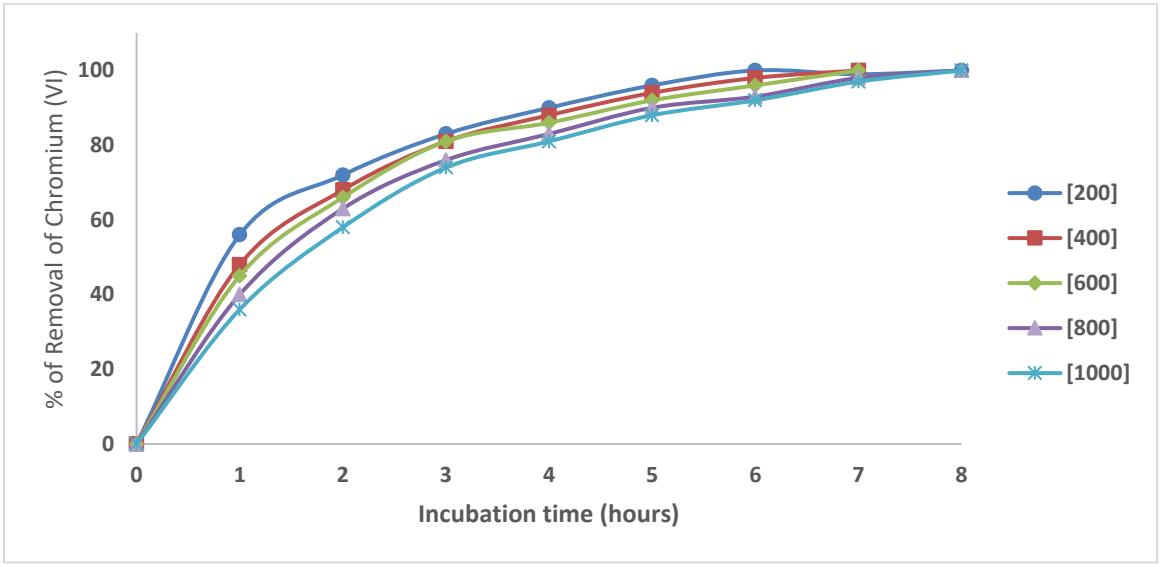
Fig. 2. Effect of incubation temperature on the bioadsorption of Chromium (VI) in aqueous solution by the biomass of *E. hyemale* 100 mg/L. pH 1.0. 1 g of biomass. 100 rpm.

3.2 Effect of Incubation Temperature

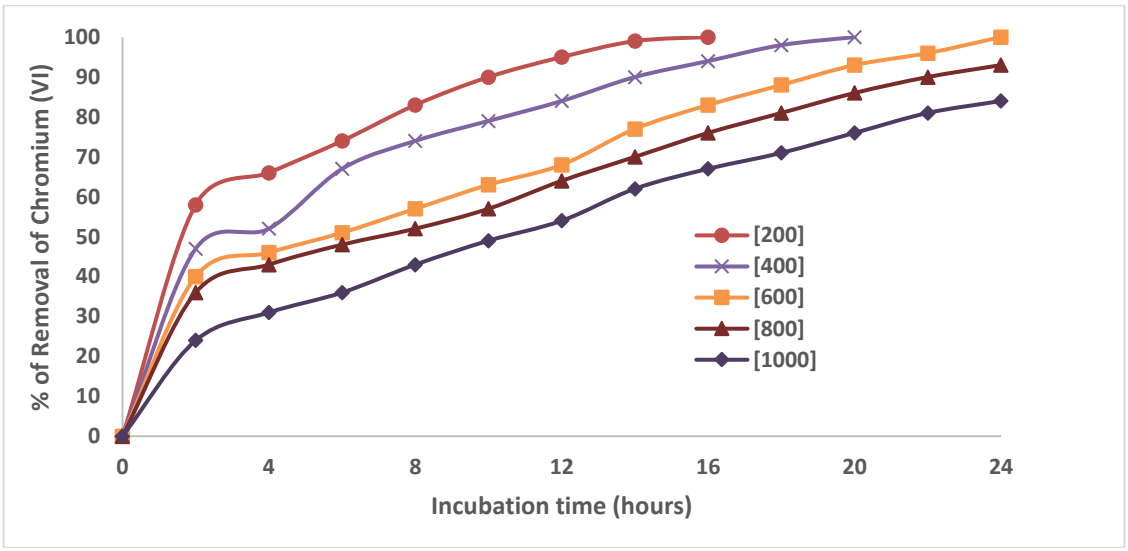
At a higher temperature, the efficiency of removal is better, since the temperature increases the active surface charge activity and the kinetic energy of the bioadsorbent, thus improving the removal of metal ions [1]. At 60°C the total removal was to 4 hours of incubation, and to 28ºC, the optimum time was in 8 hours (Fig. 2). These results are similar for *N. tabacum* biomass, where the highest removal (100%) was observed at 60°C, 24 h, and 100%, while at 28°C, 66.54% is removed at the same time [15], similar results with nanobiochar artichoke leaves, for the elimination of 10 mg/L of chromium (VI) [17]. But, in comparison with *M. piperita* biomass, these results are least efficient, because, the most efficient removal, was between 40°C and 60°C, with a total removal after 75 and 90 minutes, respectively [11], for modified sawdust, with a temperature of incubation of 25°C [25], too, this parameter not influence in the elimination, since 100% of it is eliminated after 150 minutes at 40°C, 50°C, and 60°C [14], an optimum temperature of 28.9°C for the removal of the same heavy metal with *L. cavernae* CR-2 [27], 28°C with *C. formosanus* and *O. formosanus* nestes, for the elimination of chromate in *B. chinensis* L. [20],

3.3 Effect of Initial Concentration of the Metal on the Bioadsorption of Chromium (VI)

With respect to effect of different concentrations of the metal in solution, at pH 1.0, with 1 g of horsertail biomass, at 60°C and 100 rpm, it was found a major removal, with a 100% of removal at 8 hours at the metal concentration analyzed (Fig. 3A), while to 28°C, the total removal was 16 hours for 200 mg/L, and 24 hours for 1 g/L of heavy metal, with100% and 84% of elimination, respectively (Fig. 3B). These results are similar for the *M. piperita* biomass, in which the removal of metal was 100% at 14 and 24 hours, at 28°C, for 200 and 1000 mg/L, respectively, while at 60°C, the removal is total between 5 and 9 hours of incubation, for the same metal [11], too for *N. tabacum* biomass, at 28°C the maximum removal (89.36%) was at 600 mg/L, decreasing with 800 mg/L (82.02%) and 1000 mg/L (64.72%), while to 60°C, the total removal of 800 mg/L and 1000 mg/L, was at the 8 h of incubation in the same conditions [15], a slight decrease in the removal of chromium (VI), by increasing their concentrations from 50 to 200 ppm [21]. But, our results are different for modified sawdust, since increase the metal concentration, too increase the efficiency of elimination, at pH 3.0 and 28°C [25], for *C. maxima*, the heavy metal concentration, has no effect on the removal of chromium (VI), although efficiency is greater at 40°C [14].



A



B

Fig. 3. Effect of initial concentration of Cr (VI) on the bioadsorption of Chromium (VI) in aqueous solution by the biomass of *E. hyemale*. 1 g of biomass. 100 rpm. pH 1.0. 100 rpm. A.- 60°C B.- 28°C.

3.4 Effect of Initial Concentration of *E. hyemale* Biomass on the Bioadsorption of Chromium (VI)

The effect of the biomass concentration on the chromium (VI) removal capacity is shown in Fig. 4, in which it is observed that if the biomass concentration increases, the elimination of the metal in solution too increase, since with 5 g of biomass eliminate 100% of the metal in 3 hours, while 1 g eliminate the metal at 8 hours, because there are more bioadsorption sites of it, since the amount of bioadsorbent added determine the number of binding sites available for the biosorption of heavy metals [29], and these results are like those reported for *M. piperita* biomass, because with 1 g of the analyzed biomass, 100% of the metal is removed after 180 minutes, while with 4 and 5 g, the removal is total after 75 minutes [11], for modified sawdust, with an increase of 0.5-1.5 g/L at 25°C, too increase the efficiency of elimination [25], similar results with nanobiochar artichoke leaves, for the elimination of 10 mg/L of chromium (VI) [17], for *C. maxima* biomass, the removal of the heavy metal in solution increase significantly, because

with 1 g of the analyzed biomass, 100% of the metal is removed after 480 minutes, while with 5 g, the removal is total after 25 minutes [14]. Too, these results are different for *N. tabacum* biomass, which if the concentration of this is increased, the elimination of the metal in solution is not affected because at the concentrations of biomass analyzed, the removal is similar after 24 h of incubation [15], 1 g of coconut coir-biochar for the removal of 50 mg/L [19], 10 g of *C. formosanus* and *O. formosanus* nestes, for the elimination of 20 mg/L of chromate in *B. chinensis* L. [20], 5 g/L of *J. curcas* seedcake dose, with a removal of 50.18% of the heavy metal, and further increase in the biosorbent decreased the efficiency of the removal [21], and 2.27 g/L of *E. angustifolia* for the elimination of 100 mg/L of chromium (VI) [28].

3.5 Bioremediation of Chromium (VI) from Earth and Water Contaminated with the Heavy Metal

We adapted a water-phase bioremediation assay to explore possible usefulness of this biomass for eliminating chromium (VI) from industrial wastes. The biomass (5 g), was incubate with 5 g of non-sterilized contaminated soil 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, and after 10 days of incubation, 28°C, 5 g of plant biomass, well 86% of soil and 70% of wastewater, were eliminated from contaminated earth and wastewater. The metal removal capacity from earth and wastewater by the biomass is equal or better than others analyzed, for example: The removal of the same metal by a assay simultaneous of electrocoagulation and electro-assisted phytoremediation with rough horsetail (*E. hyemale*) [6], the use of wetlands reactors of the same plant for reducing the contamination of iron in wastewater, and the lead and chromium removal from leachate [7,8], for the elimination of the same metal with *M. piperita* and *N. tabacum* biomasses, [11,15], similar results with nanobiochar artichoke leaves, for the removal of 10 mg/L of chromium (VI), from tap, waste, and sea water [17], for the removal of 50 mg/L of the same heavy metal by *C. maxima* biomass, It was observing that in 7 days of incubation, the chromium (VI) concentration of earth and water samples decrease 93% and 94.5% in both samples [14], and the phytoremediation with *Hydrocotyle Ranunculoides*, in wastewater contaminated with heavy metals [30].

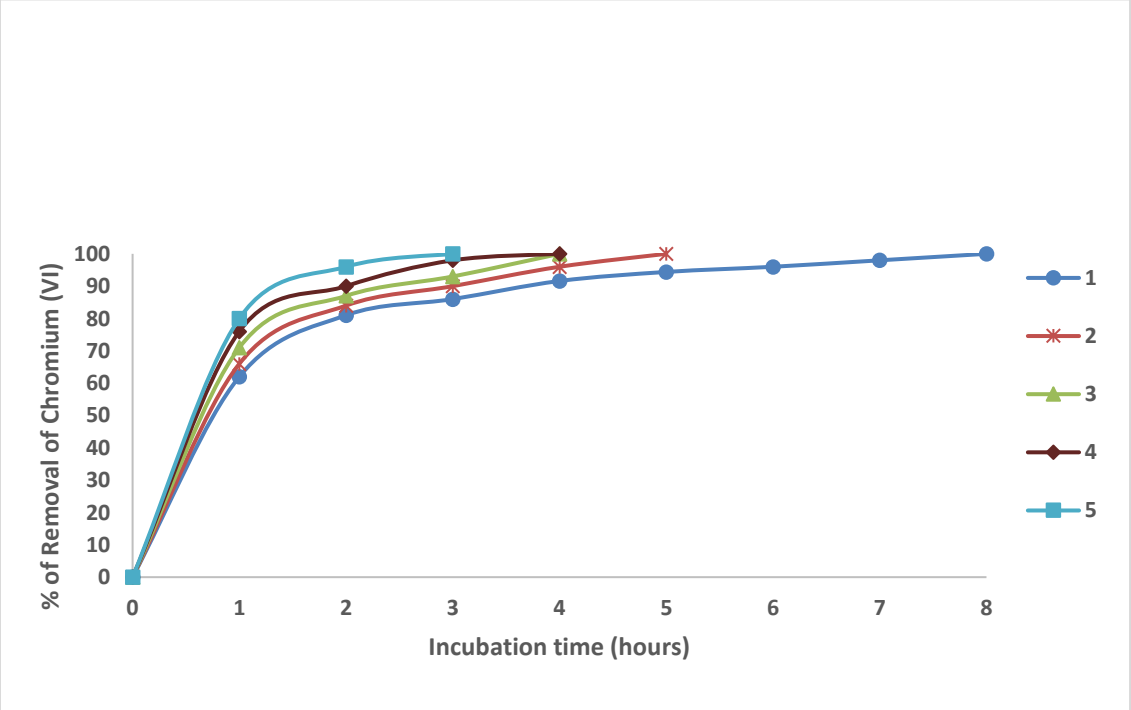


Fig. 4. Effect of initial concentration of the *E. hyemale* biomass on the bioadsorption of Chromium (VI) in aqueous solution. 100 mg/L of Cr (VI). 100 rpm. pH 1.0.

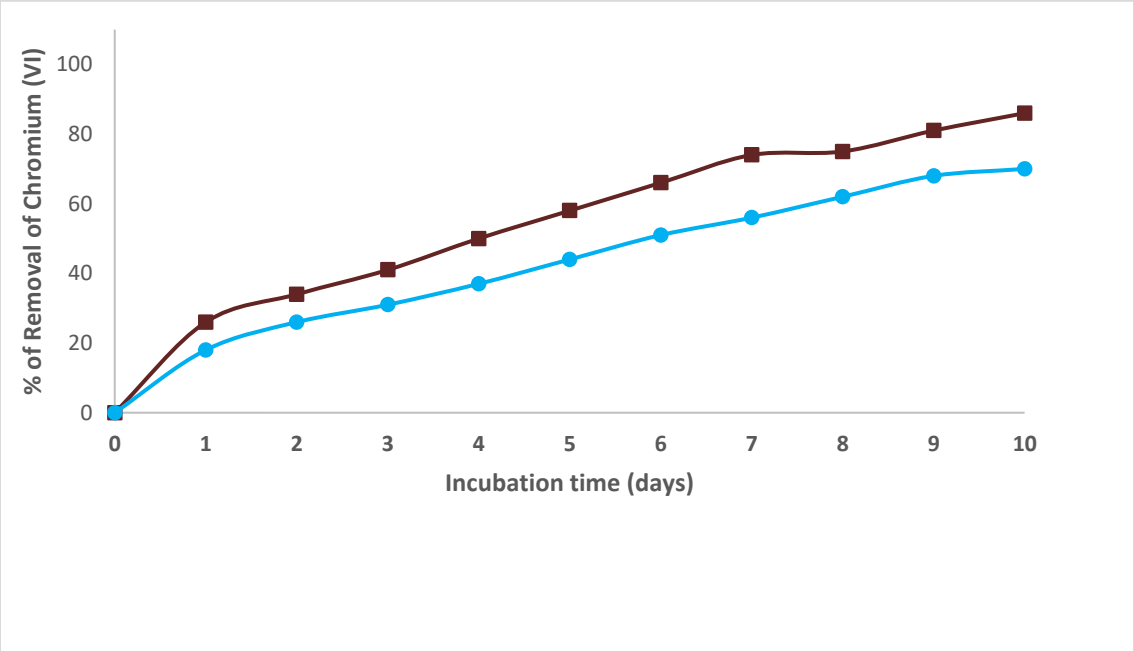


Fig. 5. Bioremediation of Chromium (VI) from earth and water contaminated with 100 mg/g soil (pH 6.8), and 100 mg/L Chromium (VI) (pH 8.2) (5 g of *E. hyemani* biomass. 28ºC, 100 rpm

4. CONCLUSION

Different methods have been analyzed for wastewater decontamination, and some of this are expensive, complicated, require time and energy consuming, etc. Actually, the phytoremediation is a good alternative, because it is most efficient and applied in wastewater treatment, and a great number of different adsorbents from various sources have been utilized to removal a great variety of contaminants, in particular, the use of waste biomass from invasive plants as adsorbent materials for the removal of heavy metals from aqueous streams could decrease their threat [2, 3, and 24]. In this work, the biomass of a commercial plant *E. hyemani* was studied for the removal of chromium (VI) in aqueous solution, with the following conclusions:

1. The natural biomass of *E. hyemani* removal 100 mg/L of the heavy metal at 8 hours of incubation, with 1 g of biomass, pH 1.0 and 100 rpm.
2. If the temperature is increased, the removal efficiency is not affected.
3. To lower metal concentration, is better the removal efficiency.
4. To higher biomass concentration, the removal efficiency increases.
5. In bioremediation tests, it was found that biomass eliminate efficiently the metal from contaminated soil and wastewaters with chromium (VI).
6. The application of this methodology is viable for the elimination of this and other heavy metals from contaminated sites, in addition, the biomass analyzed very efficient, of easy obtained, handling, and low cost.
7. Therefore, this type of methodology presents additional advantages over traditional removal methods, in addition to the fact that it can be used during different removal cycles, with a slight loss in the efficiency of this method.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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