1. Introduction

Chromium (Cr) toxicity is one of the major causes of environmental pollution emanating from tannery effluents. This metal is used in the tanning of hides and leather, the manufacture of stainless steel, electroplating, textile dyeing and as a biocide in the cooling waters of nuclear power plants. Consequently, these industries discharged chromium (VI) bearing effluents which are of significant environmental concerns [1]. Cr exists in nine valence states ranging from -2 to +6. From these, only the hexavalent [Cr (VI)] and trivalent chromium [Cr (III)] have primary environmental significance since they are the most stable oxidized forms in the environment.

Both are found in various bodies of water and wastewaters [2]. Cr (VI) typically exists in one of these two forms: chromate (CrO$_4^{2-}$) or dichromate (Cr$_2$O$_7^{2-}$), depending on the pH of the solution [2]. These two divalent oxyanions are very water soluble and poorly adsorbed by soil and organic matter, making them mobile in groundwater. Both chromate anions represent acute and chronic risks to animals and human health, since they are extremely toxic, mutagenic, carcinogenic and teratogenic [3]. In contrast to Cr (VI) forms, the Cr (III) species are predominantly hydroxides, oxides and sulphates, less water soluble, less mobile, 100 times less toxic [4] and 1,000 times less mutagenic [5]. The principal techniques for recovering or removing Cr (VI), from wastewater are: chemical reduction and precipitation, adsorption on activated carbon, ion exchange and reverse osmosis [6]. However, these methods have certain drawbacks, namely high cost, low efficiency, generation of toxic sludge or other wastes that require
disposal and imply operational complexity [7]. In this context, considerable attention has been focused in recent years upon the field of biosorption for the removal of heavy metal ions from aqueous effluents [8].

The process of heavy metal removal by biological materials is known as biosorption. Biomass viability does not affect the metal uptake. Therefore any active metabolic uptake process is currently considered to be a negligible part of biosorption. Various biosorbents have been tried, which include seaweeds, molds, yeast, bacteria, crab shells, agricultural products such modified corn stalks, [9], hazelnut shell [10], orange waste [11] and tamarind peel [12]. It has also been reported that some of these biomass can reduce chromium (VI) to chromium (III), like Litchii cinensis Sonn peel [13], tea fungal biomass [14], Mesquite [15], Eucalyptus bark [16], red rose’s waste biomass [17] and Yohimbe bark [18]. The present study is undertaken with following objective: Investigate the use of different natural biomasses for the biosorption and reduction of Chromium (VI) in aqueous solution, and their elimination from contaminated sites.

2. Material and methods

2.1. Biosorbents used

Litchi chinensis Sonn, Citrus limonium, Mammea americana, Tamarindus indica, Citrus sinensis Osbeck, Citrus reticulate, Cucumis melo L., and Musa cavendishii shells were obtained from the fruits harvested between the months of June-September 2010, in the marketplace Republic of San Luis Potosi, SLP. Mexico. To obtain the natural biomass, shells rind were washed with trideionized water 72 h under constant stirring, with water changes every 12 h. Subsequently, boiled for 1 h to remove traces of the fruit and was dried at 80°C, for 12 h in the oven, ground in blender and stored in amber vials until use.

2.2. Preparation of stock solution

An aqueous stock solution (1000 mg/L) of Cr (VI) ions was prepared using K₂Cr₂O₇ salt. pH of the solution was adjusted using 0.1 N HCl or NaOH. Fresh dilutions were used for each study.

2.3. Biosorption studies

The biosorption capacity of shells biomasses was determined by contacting 100 mL of solution containing different concentration of Cr (VI) (100-1000 mg/L) in 250 mL Erlenmeyer glass flasks, with 1 g of biomass. The mixture was shaken in a rotary shaker at 120 rpm followed by filtration at different times (covering minutes, days and weeks). The filtrate containing the residual concentration of Cr (VI) was determined spectrophotometrically at 540 nm after complexation with 1, 5 Diphenylcarbazide, these method have a detection limit between 0.02-0.5 mg/L of Cr (VI) [19], Cr (III) with Chromazurol S [20], and Cr total by Electrothermal Atomic Absorption Spectroscopy [19]. For the determination of rate of metal biosorption by biomasses from 100 mL (at 100, 200, 300, 400, 500, and 1000 mg/L), the supernatant was analyzed for residual Cr (VI) after the contact period of 1-12 hours. The effect of pH and
temperature on Cr (VI) sorption by natural biomass, was determined at pH values of 1, 2, 3, and 4, 28°C, 40°C, and 50°C, respectively. The effect of different doses of biomass ranging from 1 to 5 g/L, with 100 mg/L of Cr (VI) concentrations was determined. The values shown in the results section are the mean from three experiments carried out by triplicate.

2.4. Bioremediation assay

Four 250 mL Erlenmeyer glass flasks, with 5 g of shell biomass, were added with 20 g of contaminated earth and water with 297 mg Cr (VI)/g earth or 373 mg Cr(VI)/L water, of tannery (Celaya, Guanajuato, México), and the volume was complete to 100 mL with trideionized water. The mixture was shaken in a rotary shaker at 120 rpm followed by filtration using Whatman filter paper No. 1. The filtrate containing the residual concentration of Cr (VI) was determined with 1, 5 diphenylcarbazide [19].

2.5. Determination of hexavalent, trivalent and total Cr:

Hexavalent and trivalent chromium were quantified by a spectrophotometric method employing diphenylcarbazide and chromazurol S, respectively [19, 20], and total Chromium by Electrothermal Atomic Absorption Spectroscopy [19].

3. Results and discussion

3.1. Effect of incubation time and pH

Figure 1 shows the effect of the incubation time and pH on Cr (VI) removal by *L. chinensis* Sonn shell. The optimum time and pH for Cr (VI) removal are 10 min and pH 1.0, at constant values of biosorbent dosage (1 g/100 mL), initial metal concentration (100 mg /L) and temperature (28°C). The literature [11], report an optimum time of 60 min., for the removal of lead by orange waste, 30 min and 60 for the removal of Cr (VI) by the tamarind peel and eucalyptus bark [12, 16]. Changes in the permeability of unknown origin, could partly explain the differences found in the incubation time, providing greater or lesser exposure of the functional groups of the cell wall of biomass analyzed. Adsorption efficiency of Cr (VI) was observed maximum at pH 1.0 with Litchi shell. This was due to the dominant species (CrO$_4^{2-}$ and Cr$_2$O$_7^{2-}$) of Cr ions in solution which were expected to interact more strongly with the ligands positively charges [21]. These results are like for tamarind peel [10], but the most of authors report an optimum pH of 2.0 like tamarind seeds [10], eucalyptus bark [16], bagasse and sugarcane pulp, coconut fibers and wool, [22], for the tamarind fruit shell treated with oxalic acid [23], at pH of 2.0 and 5.0 for the mandarin bagasse [24] and almond green hull [25].

3.2. Effect of temperature on Cr (VI) removal by *L. chinensis* Sonn shell

Temperature is found to be a critical parameter in the bioadsorption of Cr (VI) by *L. chinensis* Sonn shell (Figure 2). The highest removal was observed at 40 and 50°C. At this point the total removal of the metal is carried out. The results are coincident for tamarind seeds with 95% of
removal at 58°C and 3 h [26], for the adsorption of cadmium (II) from aqueous solution on natural and oxidized corncob (40°C and 5 days) [27], but this are different for the mandarin waste [24], Caladium bicolor (wild cocoyam) biomass [29] and Saccharomyces cerevisiae [30]. The increase in temperature increases the rate of removal of chromium (VI) and decreases the contact time required for complete removal of the metal, to increase the redox reaction rate [26].

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

**Figure 2.** Effect of temperature on Chromium (VI) removal by *L. chinensis* Sonn shell. 100 mg/L Cr (VI). pH 1.0, 100 rpm. 1 g biomass.
3.3. Effect of initial metal concentration

On the other hand, at low metal concentrations (100 and 200 mg/L), biomass studied, shows the best results for removal, adsorbing 100% at 10 and 20 min. respectively, while 1000 mg/L of metal is removed 100% up to 195 min of incubation at 28°C (Figure 3). Also, we observed the development of a blue-green and a white precipitate (Cr(OH)$_3$), which changes more rapidly at higher temperatures (Figure 4). The results are coincident for tamarind peel and seeds, and C. limonium [10, 26, and 29]. The increase in initial concentration of Cr (VI) results in the increased uptake capacity and decreased the percentage of Cr (VI) removal. This was due to the increase in the number of ions competing for the available functions groups on the surface of biomass [26].

![Figure 3](image_url)

**Figure 3.** Effect of initial metal concentration on Chromium (VI) removal by *L. chinensis* Sonn shell. 28°C, pH 1.0, 100 rpm. 1 g biomass.

4. Time course of Cr (VI) decrease and Cr (III) production

The ability of the *L. chinensis* Sonn shell to decrease the initial Cr (VI) of 1.0 g/L and Cr (III) production in solution are analyzed. Figure 5 shows that the shell exhibited a remarkable efficiency to diminish Cr (VI) level with the concomitant production of Cr (III) as Cr(OH)$_3$ in the solution (indicated by the formation of a blue-green color and a white precipitate (Cr(OH)$_3$) and his determination for Cromazurol S, (Figures 4 and 6) [19, and 20].

Thus, after 1 h of incubation, the shell biomass caused a drop in Cr (VI) from its initial concentration of 1.0 g/L to almost undetectable levels and the decrease level occurred with no significant change in total Cr content. As expected, total Cr concentration remained constant over time, in solution control. These observations indicate that Litchi shell is able to reduce Cr (VI) to Cr (III) in solution. Furthermore, as the *L. chinensis* Sonn shell contains vitamin C and
some carbohydrates [32], we found that vitamin C and Cystine quickly reduce Cr (VI) to Cr (III) and could be a very important part in the metal reduction (Table 1), according to some reports in the literature [2, 12, 13, 31, 35, 36, 37, and 38]. There are two mechanisms by which chromate could be reduced to a lower toxic oxidation state by an enzymatic reaction. Currently, we do not know whether the shell biomass used in this study express Cr (VI) reducing enzyme(s). Further studies are necessary to extend our understanding of the effects of coexisting ions on the Cr (VI) reducing activity of the biomass reported in this study. Cr (VI) reducing capability has been described in some reports in the literature [2, 3, 7, 12, 13, 15, 18, 31, 39, and 40]. Biosorption is the second mechanism by which the chromate concentration could be reduced, because the biomass shell can be regarded as a mosaic of different groups that could form coordination complexes with metals and our observations are like to the most of the reports [2, 3, 7, 12, 15, 18, 39, and 40].
Table 1. Formation of blue-green color by different chromium (VI) concentrations to 28°C and 60°C, in the presence of Litchi shell. pH 1.0. 1 g biomass.

| Cr (VI) Concentration (mg/L) | Time (min) | 60°C | 28°C |
|------------------------------|------------|------|------|
| 0*                           | 10         | 45   |      |
| 200                          | 15         | 60   |      |
| 300                          | 15         | 60   |      |
| 400                          | 15         | 80   |      |
| 500                          | 20         | 120  |      |
| Cystine                      | N.D. **    | 5    |      |
| Vitamin C                    | N.D. **    | 5    |      |

*Control: 100 mL of trideionized water, pH 1.0. There were no variations in color.

**Not determinated.

4.1. Effect of biosorbent dose

The influence of biomass on the removal capacity of Cr (VI) was depicted in Figure 7. If the researchers increase the amount of biomass also increases the removal of Cr (VI) in solution (100% of removal, with 5 g of biomass, 20 minutes), with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption [27]. Similar results have been reported for modified corn stalks [9], tamarind shell [12], and Mucor hiemalis and Rhizopus nigricans, although latter with 10 g of biomass [1, 28], but they are different from those reported for biomass wastes from the mandarin (bagasse), with an optimal concentration of biomass of 100 mg/L [24].
4.2. Cr (VI) Removal in the presence of different heavy metals

The researchers analyzed whether the presence of different metals interfere with the Cr (VI) removal (500 mg/L) at a pH of 1.0, with 1 g of Litchi shell, finding that none of the added metals (salts of cadmium, copper, zinc and mercury) interferes with the Cr (VI) removal, but in the presence of zinc and mercury takes 10-20 min longer to remove 100% of the metal (Figure 8). This is consistent with many reports in the literature [1, 12, 13, 16, 31, 32, and 33].
4.3. Cr (VI) Removal by different biomasses

The researchers studied the Cr (VI) (100 mg/L) removal, with 1 g of different biomass. Litchi shell was the most efficient, because in 10 min at 28°C remove 100% of the metal, followed by xylan and polygalacturonic acid (150 and 300 min at 60 °C, respectively) and starch and cellulose were less efficient (43.6% at 28°C and 300 min of incubation and 21.83% at 60°C, at the same time of incubation, respectively) (Figure 9). With respect to other biomasses used, most authors report lower removal efficiencies of metal, for example: 45 mg/L for eucalyptus bark [16], 13.4 and 17.2 mg/L for bagasse and sugar cane pulp, 29 mg/L coconut fibers, 8.66 mg/L for wool [22], 25 and 250 mg/L of chitin and chitosan [41] and 1 mg/L for cellulose acetate [42].

![Figure 9](image_url)

**Figure 9.** Chromium (VI) removal by different biomasses. 100 mg/L Cr (VI). 1.0 g biomass. pH 1.0. 100 rpm. 60°C, Litchi peel 28°C.

4.4. Removal of Cr (VI) in industrial wastes with *Litchi chinensis* Sonn shell

The researchers adapted a water-phase bioremediation assay to explore possible usefulness of *L. chinensis* Sonn shell, for eliminating Cr (VI) from industrial wastes, the biomass (5 g) was incubated with 20 and non-sterilized contaminated soil containing 297 mg Cr (VI)/g, suspended in trideionized water, and 100 mL of contaminated water with 373 mg/Cr (VI) /L. It was observed that after five and six days of incubation with the biomass, the Cr (VI) concentration of soil and water samples decrease 100% (Figure 10), and the decrease level occurred without change significant in total Cr content, during the experiments. In the experiment carried out in the absence of the biomass, the Cr (VI) concentration of the soil and water samples decreased by about of 18%, and 8%, respectively (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil and water.

The chromium removal abilities of *L. chinensis* Sonn shell are equal or better than those of other reported biomass, for example tamarind shell [12], *M. americana* [38], *Candida maltosa* RR1 [39]. In particular, this biomass was superior to the other biomass because it has the capacity for efficient chromium reduction under acidic conditions. Many of the Cr (VI) reduction studies...
were carried out at neutral pH [43]. Aspergillus niger also has the ability to reduce and adsorb Cr (VI) [41]. When the initial concentration of Cr (VI) was 500 ppm, A. niger mycelium removal 8.9 mg/L of chromium/g dry weight of mycelium in 7 days.

**Figure 10.** Chromium (VI) removal in industrial wastes incubated with the biomass. 100 rpm. 28°C. 5 g Litchi biomass. Contaminated soil and water (297 mg/Cr(VI)/g soil] and 373 mL Cr(VI)/L, respectively.

4.5. Biorremediation assay *in situ*

100 kg of contaminated soil (345 mg Cr(VI)/g soil), with 20 g of natural biomass of *M. Americana* were incubated in a greenhouse at 28°C. After 10 weeks of incubation, the natural biomass removal 83% of the metal from contaminated soil (Figure 11), without change significant in total Cr content. In the experiment carried out in the absence of the biomass, the Cr (VI) concentration of the soil sample decreased by about of 15% (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil, like microorganisms lactic acid producers, which reduce Cr (VI) to Cr (III) (Figure 12). Reports on applications of microorganisms for studies of bioremediation of soils contaminated with chromates are rare. Such study involved the use of *Pseudomona mendocina* for the removal of the metal from cooling tower effluent [44], and soil microcosms [45]. In the first process when carried out in a 20 liter continuous stirred tank reactor removed 25-100 mg chromate/L, in 4.5-8 hours with >99.9% efficiency in the presence of sugarcane molasses as nutrient, and in soil microcosms could immobilize 100 µg (2mM) chromate/g soil in 8 hours by converting into trivalent form, and the chromate contaminated soil, after microbiological treatment, supported growth of wheat seedlings without exerting any toxic effects. Other study involved the use of unidentified bacteria native from the contaminated site, which is used in bioreactors to treat soil contaminated with Cr (VI). It was found that the maximum reduction of Cr (VI) occurred with the use of 15 mg of bacterial biomass/g of soil (wet weight), 50 mg/g of soil molasses as carbon source, the bioreactor operated under these conditions, completely reduced 5.6 mg/Cr (VI)/g of soil at 20 days [46]. In another study using unidentified native bacteria reducing Cr...
(VI) of a contaminated site, combined with *Ganoderma lucidum*, the latter used to remove by biosorption Cr (III) formed. The results showed that the reduction of 50 mg/L of Cr (VI) by bacteria was about 80%, with 10 g / L of peptone as a source of electrons and a hydraulic retention time of 8 h. The Cr (III) produced was removed using a column with the fungus *G. lucidum* as absorber. Under these conditions, the specific capacity of adsorption of Cr (III) of *G. Lucidum* in the column was 576 mg/g [47]. In other studies, has been tested the addition of carbon sources in contaminated soil analyzed in column, in one of these studies was found that the addition of tryptone soy to floor with 1000 mg/L of Cr (VI) increase reduction ion, due to the action of microorganisms presents in the soil, although such action is not observed in soil with higher concentrations (10.000 mg/L) of Cr (VI) [48]. Another study showed that the addition of nitrate and molasses accelerates the reduction of Cr (VI) to Cr (III) by a native microbial community in microcosms studied, in batch or columns of unsaturated flow, under similar conditions to those of the contaminated zone. In the case of batch microcosms, the presence of such nutrients caused reduction of 87% (67 mg/L of initial concentration) of Cr (VI) present in the beginning of the experiment, the same nutrients, added to a column of unsaturated flow of 15 cm, added with 65 mg/L of Cr (VI) caused the reduction and immobilization of 10% of metal, in a period of 45 days [49]. Finally, Cardenas-Gonzalez and Acosta-Rodriguez [40], adapted a water-phase bioremediation assay to explore possible usefulness of strain of *Paecilomyces* sp to eliminate Cr (VI) from industrial wastes, the mycelium biomass was incubated with non-sterilized contaminated soil containing 50 mg Cr (VI)/g, suspended in Lee’s minimal medium [50] pH 4.0. It was observed that after eight days of incubation with the *Paecilomyces* sp biomass, the Cr (VI) concentration (50 mg/g) of soil sample decrease fully (100%).

![Graph showing the removal of hexavalent chromium from solutions and contaminated sites by different natural biomasses.](http://dx.doi.org/10.5772/56152)

**Figure 11.** Bioremediation of Cr (VI) *in situ*, by *M. americana* biomass. 100 Kg of contaminated soil with 345 mg de Cr (VI)/g soil. (20 Kg biomass. 28°C).
4.6. Desorption of Cr (VI) by different solutions

Furthermore, the researchers examined the ability of different solutions to desorb the metal bioadsorbed (250 mg/L) for the Litchi biomass, obtaining high efficiency with 0.1 N NaOH and 0.5 N (80 and 61% respectively (Figure 13), which are less those reported for desorption of Chromium (VI) with alkaline solutions (100%, pH 9.5), 1.0 N NaOH (95%) and a hot solution of NaOH/Na₂CO₃ (90%), respectively, [21, 51], and are higher than that reported (14.2%) using 0.2 M NaOH [52]. This indicates that binding of metal to biomass is not as strong and that it can be used up to 6 desorption cycles of removal, which further lowers the metal removal process of niches contaminated with it.
4.7. Biosorption of Chromium (VI) in solution by different natural biomasses

In Table 2, the researchers show the biosorption of Chromium (VI) by the different biomasses analyzed. It was found that the biomass of *L. chinensis* Sonn, *T. indica*, *M. Americana*, and *C. reticulata*, shells were the most efficient at removing the metal in solution (100% at 20, 40, 50 and 60 minutes, respectively), at pH 1.0 at 50°C, the biomasses reduce the metal in solution, they can remove it from contaminated industrial wastes, and can be used six times efficiently.

| Parameter                          | *Litchi chinensis* | *Citrus reticulata* | *Mammea americana* | *Citrus sinensis* | *Citrus limonium* | *Tamarindus indica* | *Musa cavendishii* | *Cucumis melo L.* |
|-----------------------------------|--------------------|--------------------|--------------------|-------------------|-------------------|--------------------|--------------------|-----------------|
| pH optimum                        | 1.0                | 1.0                | 1.0                | 1.0               | 1.0               | 1.0                | 1.0                | 1.0             |
| Incubation time (100 mg/L, 28°C)  | 10 min             | 40 min             | 50 min             | 120 min           | 80 min            | 60 min             | 60 min             | 230 min         |
| Temperature (50°C, 1.0 g/L)       | 35 min             | 90 min             | 110 min            | 120 min           | 200 min           | 140 min            | 80 min             | 105 min         |
| Biomass concentration (5 g. 1.0 g/L) | 20 min          | 60 min             | 50 min             | 75 min            | 85 min            | 40 min             | 25 min             | 600 min         |
| Presence of different heavy metals (500 mg/L) | Not Interfere | Not Interfere | Not Interfere | Not Interfere | Not Interfere | Not Interfere | Not Interfere | Not Interfere |
| Reduction of Cr VI to Cr III      | Yes                | Yes                | Yes                | Yes               | Yes               | Yes                | Yes                | Yes             |
| Biorremediation of contaminated sites (100%) | Soil: 5 days | Soil: 5 days | Soil: 5 days | Soil: 5 days | Soil: 5 days | Soil: 5 days | Soil: 5 days | Soil: 5 days |
|                                   | Water: 6 days      | Water: 6 days      | Water: 6 days      | Water: 6 days     | Water: 6 days     | Water: 6 days     | Water: 6 days     | Water: 6 days   |
| Desorption (7 days)               | 81.2%              | 80.1%              | 78.3%              | 83%               | 79.3%             | 80%                | 78%                | 78%             |

Table 2. Chromium (VI) removal of 1.0 g/L with different natural biomasses.

5. Conclusion

The use of biomaterials like natural biomasses has demonstrated to be a promising alternative for removal of Chromium hexavalent from aqueous solution. The screening and selection of the most effective biomaterial (biomasses) with sufficiently high metal binding capacity and selectivity for heavy metal ions, in this case, Chromium (VI), are prerequisite for a full process.

The natural biomasses showed complete capacity of biosorption and reduction concentrations of 1.0 g/L Cr (VI) in solution after different incubation times, and *L. chinensis* Sonn, *C. reticu-
*lata*, and *M. americana* shells, were the most efficient, at 28°C, 100 rpm with 1 g of biomass, and after of 10 weeks the natural biomass of *M. americana*, removed 83% of the metal in contaminated soil (100 kg), with 345 mg Cr (VI)/g of soil. These results suggest the potential applicability of these biomasses for the remediation of Cr (VI) from polluted soils and waters in the fields, and this biomasses are naturals, they can be obtained in big amount, cheaper, and could be removal selectively heavy metals from aquatic mediums.

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