

Synergistic Effect of Plant Diversity on Metal Removal in Lab-scale Constructed Wetland

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Citation: Usma azad, Rabia Shehzadi, Moneeza Abbas (2021) Synergistic Effect of Plant Diversity on Metal Removal in Lab-scale Constructed Wetland 1: 1-10

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ABSTRACT

In Current research work, the efficacy of plant diversity in lab-scale constructed wetland to remove Cr(III) from aqueous solution was evaluated. Lab-scale wetland, facilitating the vertical flow, was constructed by planting four metal excluder plant species *A. helianthus* (sunflower), *B. ciebica* (simbal), *G. jamesonii* (gerbra) and *E. milii* (euphorbia) on soil layer with thin layer of gravel spread underneath in plastic container. Efficacy of wetland for metal removal from aqueous solution of Cr(III) was analyzed by determining the translocation factor and bioaccumulation factor of all plant species and soil. According to the results, soil became more alkaline with the uptake of Cr(III) from aqueous solution and sunflower showed highest translocation factor (0.529) and bioaccumulation factor (0.095) than other plant species in lab-scale wetland. While overall plant diversity in lab-scale wetland showed profound cumulative effect on metal excluding rate and Cr(III) in treated water was found below the WHO guideline after 99% removal of metal content.

Keywords: Aqueous Solution; Cr(III); Lab-scale Constructed (LSC); Metal Excluder; Wetland

Introduction

Since the start of the industrial revolution, soil contamination by dangerous metals quickened significantly and the major responsible factors for the substantial metals originations were mechanical exercises like mining, purifying, refining and assembling forms [1] that discharge the poisonous substances directly into the nature and add to an assortment of harmful consequences for living beings in evolved way of life [2]. Like other heavy metals, chromium is also emerging a devastating threat to environmental quality. Because chromium is a critical component particularly in metallurgical/steel or color industry. Both of its oxidation shapes (+3 and +6) in the synthetic are utilized basically in shades, metal completing, and wood additives [3]. The fundamental wellspring of Cr contamination is thought to be from color stuffs and calfskin tanning when squanders are released specifically into squander streams. These heaps of dirt have been generally the site for transfer for the greater part of the overwhelming metal squanders which should be dealt with. The satisfactory admission (AI) was set up for Cr(III) as 25– 35 $\mu\text{g}/\text{day}$ (female-male); couple of genuine antagonistic impacts have been related with overabundance admission of Cr from nourishment [4]. Currently, conventional remediation methods of heavy metal contaminated soils are expensive and environmentally destructive [1]. But the phytoremediation costs just about one-fourth of the other physical and compound techniques for contamination treatment. The significant preferences of the procedure include change of the pollutant quality, as phytoremediation is driven by sun based vitality, thus appropriate to most locales and atmospheres, financially savvy and actually achievable process. Plants used in are thought to be adequate biomass for fast remediation due to the advance high rhizosphere action that complete the reclamation of soil in a sensible time span of 2 to 3 years [5] and also keep metal from entering their elevated parts or keep up low and consistent metal fixation over an expansive scope of metal focus in soil. Plant use different ways such as rhizofiltration, phytostabilization, phytovolatilization, phytodegradation and phytoextraction for the remediation of contaminants from medium (Kathal et al., 2016). But these functions of plants are used to Phyto-remediate the polluted soil. To extend the use of these characteristic of plants for the clean-up of effluents and drainage waters, trend to construct artificial wetland is emerging [6]. But in constructed wetlands mostly the wetland vegetation is used to treat wastewaters that restrict their use to specific environmental conditions. The use of terrestrial plant in constructed wetland may broader its environmental niche domain. because these plants can accumulate increasing amounts of toxic heavy metals by their roots and transport/translocate them to various plant tissues where these metals can be metabolized, sequestered, and volatilized. Even some plant principally confine metal in their underlying foundations and are called metal excluders while other can ingest abnormal amounts of contaminants amassed either in their foundations, shoots or in their leaves and are hyperaccumulators [7]. Therefore, we used diversity of metal excluding plants to study their efficacy in the removal of metal from aqueous solution in lab scale constructed wetland.

Material and Methods

In this research work, locally available metal excluding plant species were used for the removal of Cr(III) from aqueous solution in lab scale constructed wetland. Those species were sunflower (*Helianthus annuus*), simbal (*Bombax ceiba*), Gerbra (*Gerbera jamesonii*) and Euphorbia (*Euphorbia. milii*).

Experimental set-up

For the construction of lab-scale wetland, a plastic container of 35cm internal diameter and 40cm height was used. A tap was fixed at a height of 4cm from its base for the collection of water sample. To allow vertical subsurface flow of water in Container, 5cm thick layer of 25mm gravel on the base followed by 10cm thick layer of 10mm gravel above followed by 05 inches thick layer of soil was placed. Selected plant species were grown on soil layer in container and was applied with Long Ashton's nutrient solution for one week to allow the plants to establish in lab scale wetland and for a healthy biofilm to develop on the gravel and roots. After one week, the LSC wetland was irrigated with 07 litre aqueous solution (225.979 ppm) of Cr (III). Aqueous solution of Cr(III) was prepared from the stock solution of Chromium sulphate because this salt is used in tanning process [8, 9].

Collection and physicochemical analysis of treated water samples

After applying the Cr(III) aqueous solution, 10 water samples were collected from the LSC wetland, at regular interval of time (30 mins) from tap fitted on the base. The treated water samples collected from LSC wetland were analyzed for Cr(III) concentration and pH level. The obtained results were compared with the Permissible Limits of World Health Organization Guideline (WHO,2005).

For metal analysis, Acid Digestion of treated water samples was carried out. 10 ml nitric acid was added in 10 ml water sample and left for 24 hours at room temperature. After that, sample solution was heated for 4 h at 120°C to 140°C on hot plate until volume of acid remained 1ml. sample solution was left to Cool and diluted to 20 ml with 1% V/V nitric acid [10] and analyzed on atomic absorption spectrophotometer (AAS) to analyze chromium content

Cr(III) Content in soil

1g of soil was collected from different points in LSC wetland before and after watering the plants with the chromium solution. Soil sample was taken in petri dishes and dried in oven for 24 hours at 70°C. Dried soil samples were ground using pastel and mortar and sieved to get smooth homogenized soil particles and further subjected to wet digestion. For wet digestion, 1g of ground dried soil sample was mixed with 10ml conc. HNO₃ and was heated on hot plate at 150°C until the acid evaporated completely and the soil became dry. 10ml of 2M HCl was further added in soil sample to dissolve metal salts and left for few minutes. 25ml of distilled water was added in solution and was filtered through filter paper. The extracts were analyzed at atomic absorption spectrophotometer (AAS) for chromium content in soil (Pequerul et al., 1993).

Cr(III) content in plant samples

The root, shoots and leaves were harvested from each plant specie at fixed height before and after adding chromium solution in constructed wetland. All collected parts of plant species were washed and dried in oven at 70 degree Celsius for 3 days. Dried samples were further subjected to wet digestion. For the wet digestion of leaves samples, nitric acid (HNO₃), hydrogen peroxide (H₂O₂) and distilled water with ratio 6:2:2 were used as solution. 1 g of dried leave samples were added in 10 ml of solution and sample was heated on hot plate inside the fume chamber for 2 hours at 180 °C and each hour 10 ml solution was added because the sample get dried. After digestion process, samples were cooled down and mixed with 25 ml distilled water, followed by filtration. Extract was analyzed on atomic absorption spectrophotometer (Thermo fisher MK-11-6) to determine chromium concentration in leave samples (Pequerul et al., 1993). For wet digestion of root and shoot samples, 10 ml nitric acid (HNO₃) was added to 1.0 g dried ground plant sample, and was heated for 45 min at 90°C. After cooling, the solution was diluted to 20 ml with 1% V/V nitric acid [10] and was analyzed on atomic absorption spectrophotometer (Thermo fisher MK-11-6)

Calculations

Translocation Factor (TF) and Bioaccumulation Factor (BAF) of all plants was calculated using following equations (Shen et al., 2017)

$$\text{Bioaccumulation Factor} = C_{\text{shoot}} / C_{\text{soil}}$$

where: C_{shoot} – metals concentration in the shoot of tree [mg kg⁻¹],

• C_{soil} – metals concentration in the soil (rhizosphere zone) [mg kg⁻¹].

$$\text{Translocation Factor} = C_{\text{shoot}} / C_{\text{root}}$$

where: C_{shoot} – metals concentration in the shoot of tree [mg kg⁻¹],

• C_{root} – metals concentration in the root of tree [mg kg⁻¹].

Whereas statistical analysis of data was carried out using IBM SPSS statistics 24 and Microsoft Excel 2016, standard deviation, linear regression and Pearson correlation were applied on the data to determine variance.

Results and Discussion

To evaluate the cumulative effect of plant diversity on metal removal in LSC wetland, samples of water, soil and different parts of plants were analyzed for Cr (III) Content and obtained results were scrutinized to determine the bioaccumulation factor and translocation factor of plant species in LSC wetland.

Effect of LSC wetland on the pH of aqueous

The contact time of Cr (III) solution with lab scale wetland (Figure 1) showed a profound effect on the pH of treated water. According to the results, pH of treated water samples increased, and treated water became more alkaline (pH:7.8) but remained in compliance with the limits prescribed by WHO guidelines. Reason for high pH is attributed to various chemicals e.g., carbonates, bicarbonates or hydroxide compounds that get dissolved in water after coming in contact with soil. Minerals diabasic in nature may increase the pH of water samples [11].

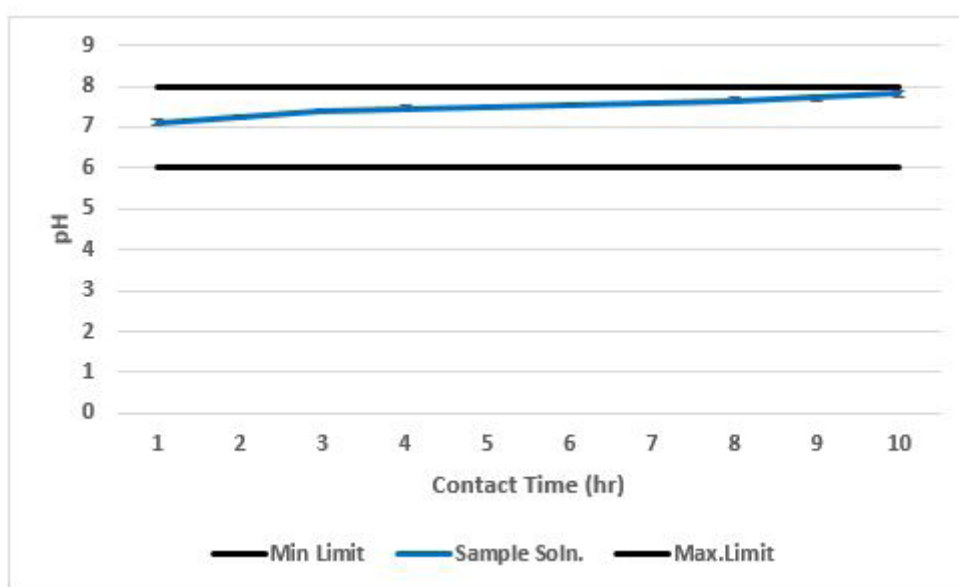


Figure 1: Effect of LSC wetland on the pH of aqueous solution and its compliance with the standard (6-8) prescribed by WHO guidelines

Effect of pH on Cr(III) uptake by soil

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Kabata-Pendias, 2011). As results showed (Figure 2) that soil became more alkaline (pH:8.098) with the increase in contact time with aqueous solution that also increased the Cr(III) uptake by soil upto 25ppm . The reason for the increase in alkalinity of soil and uptake of Cr(III) is attributed to the fact that the presence of metal in the aqueous solution increased the number of cations with the increased dissolution of $\text{Cr}_2(\text{SO}_4)_3$ in the soil and at higher pH chromium get oxidized and decrease the proportion of Cr(III) in exchangeable fractions that cause the high retention of Cr(III) in pH8 soil [12].

Cr (III) content accumulated in different parts of plants grown in LSC wetland

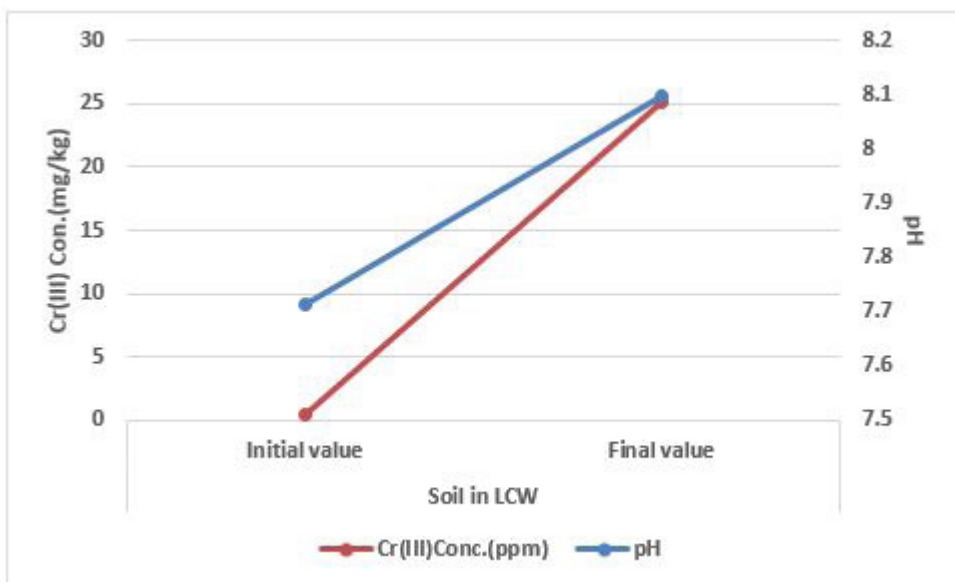


Figure 2: Effect of pH on Cr(III) uptake by soil in lab-scale constructed wetland

Although all plants grown in the same container, under same conditions, were irrigated with the Cr(III) solution of same concentration, but the total metal accumulation rate was different in all parts of all plant species. According to the analysis (Figure 3), the roots of all plant species in LSC wetland retained higher level of Cr(III) content as compared to shoots and leaves. Reason is attributed to the fact that Gravel bed performed as hydroponic systems in LSC wetland that contain a wide variety of partitioned micro-environments in the form of biofilms on the root surfaces and on the gravel that enhanced the Cr(III) uptake and transportation from aqueous solution to rhizosphere (Williams, 1993), while the shoots of sunflower (2.3mg/kg) and leaves of euphorbia(1.5mg/kg) showed higher Cr(III) content than other plant species.

Bioaccumulation factor of plant species grown in LSC wetland

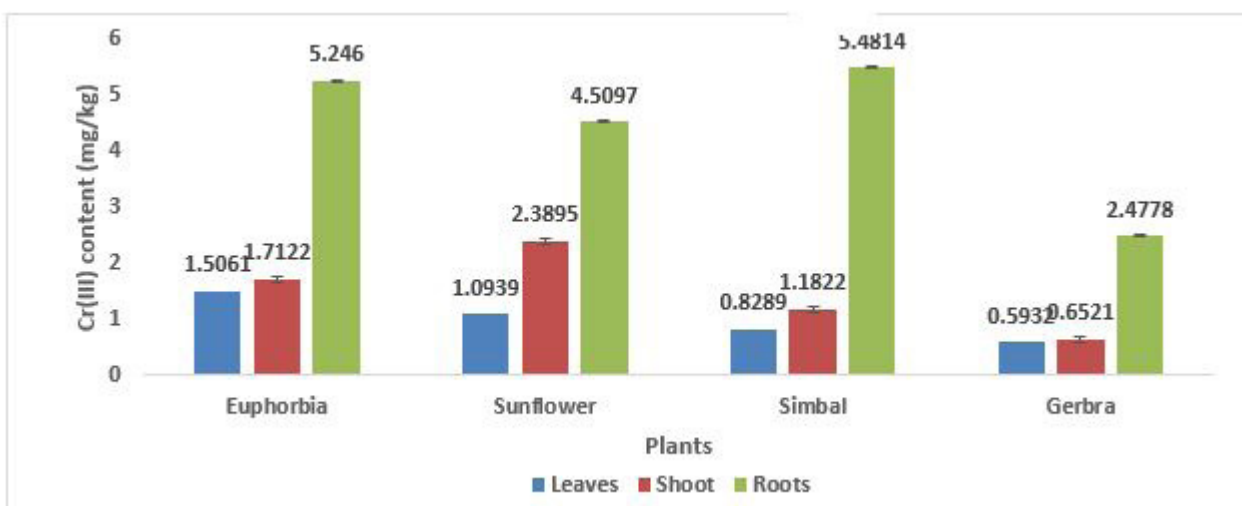


Figure 3: Cr (III) content accumulated in different parts of plants grown in LSC wetland after trial

BAF is the ratio of metal concentration in plant to soil that is greater than 1 for hyperaccumulator (Brooks,1998) and mainly used the first screening parameter for stubborn, bioaccumulative, and lethal substances [13]. Results showed (Figure 4) that the bioaccumulation factor of sunflower was higher than other plant species. Because bioaccumulation and metal accumulation in plant species vary from metal to metal and species to specie (Baker et al., 1981) but bioaccumulation factor of all plant species was far below the prescribed limit (BF: 01) . This proves the fact that heavy metals has specific adsorption to soil colloids. They are taken up by roots only through diffusion when the concentration gradient creates in soil solution. Sequestration rate of metal ion by both passive (diffusion) and active (involving energy expenditure) mechanisms are different in metal excluder and hyper-accumulator plant.

Translocation factor of plant species grown in LSC wetland

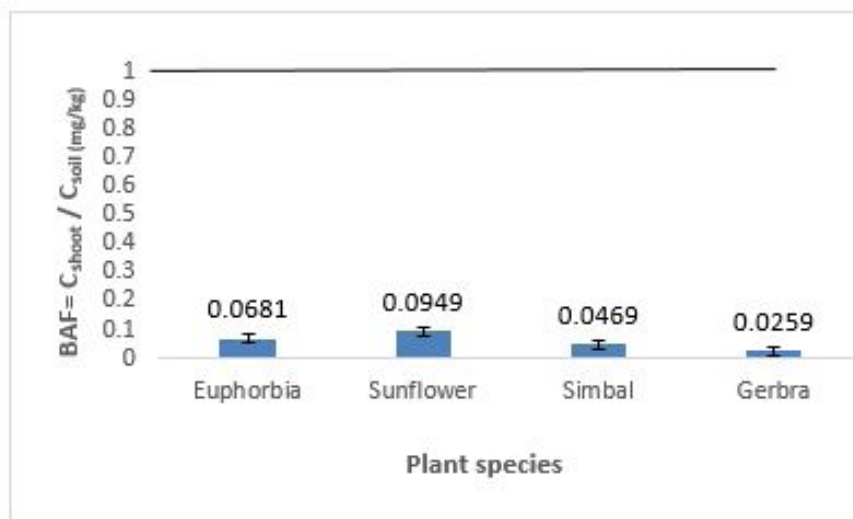


Figure 4: Bioaccumulation factor (BAF) of plant species in LSC wetland

Translocation factor is the ratio of metal in shoots to that in the roots and is a important tool used to assess a plant potential for phyto-remediation (Qihang et al., 2011) Results showed (Figure 5) that the translocation factor of sunflower was higher (TF:0.52) than other plant species. but translocation efficiency was far below the prescribed limit (TF: 1.0) of all plant species. The translocation factor > 1 in our study demonstrates that only a small proportion of Cr has been translocated to the harvestable biomass of the plant. The minimum translocation of metals into above ground tissues might be possible due to the sequestration of metals inside the root vacuoles of the plant where metals are fixed as nontoxic elements (Shanker et al., 2005) and on the base of translocation factor below one(1.0) , plant species can be categorized as metal excluder

Effect of plant diversity on metal removal from aqueous solution in LSC wetland

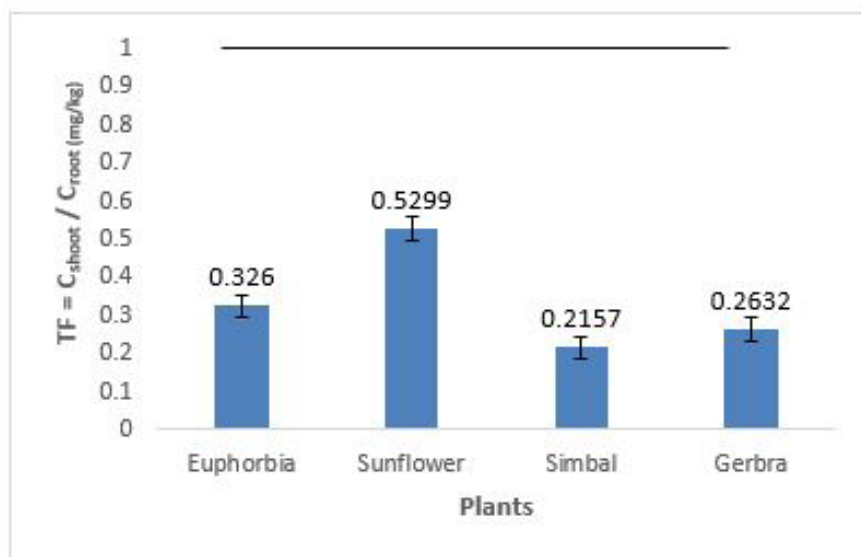


Figure 5: Translocation factor (TF) of plant species in LSC wetland

Result showed (Figure 6) that Cr(III) content in treated aqueous solution decreased from 225.97ppm to 0.043 ppm that was far below the standard limit prescribed by WHO (0.05ppm). Plant diversity showed profound synergistic effect ($R^2=0.9336$) on metal removal rate from aqueous solution in wetland. The wetland system achieved similar percentage of removals (95 - 99%) in 1 hr as work done [14] but the noncompliance of treatment time of 20min with current experimental work was due to the fact that plant was taken from local areas and were already retaining the substantial amount of metals due to being hyper accumulator that caused the slow uptake of metal [15-20].

Conclusion

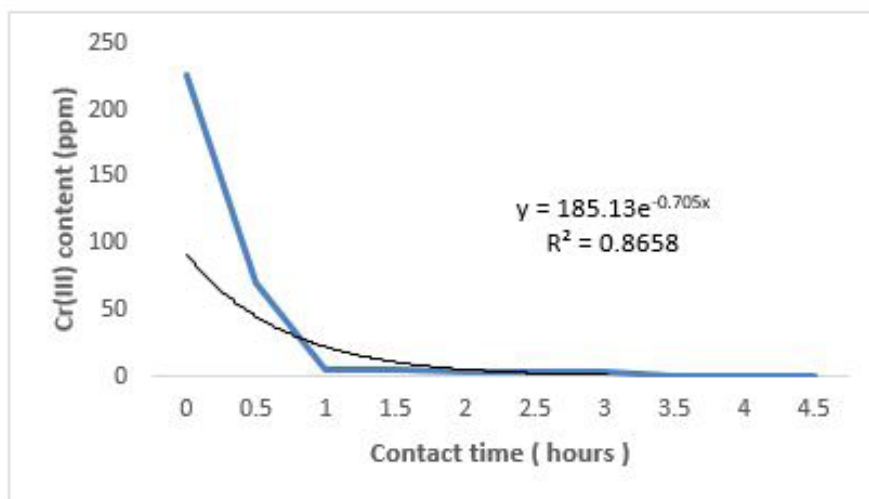


Figure 6: Efficacy of plant diversity in lab scale constructed wetland to remove Cr (III) from aqueous solution

In the Cr(III) content in soil increased from 0.52 ppm (initial) to 25.1 ppm and soil became more alkaline. But the translocation factor and bioaccumulation factor of all plants was found below one (1) and these inferences approve these plants as metal excluder [21-26]. While all plants species cumulatively removed 99% content of Cr(III) from treated aqueous water. Thus this is concluded from current research work that plant diversity cumulatively have profound effect on the removal of Cr(III) from aqueous solution in lab scale constructed wetland.

Conflicts of interests/Competing interests.

We have no interest of conflict to disclose and declare no competing interest.

Availability of data and material

Data is original and would be shared on request.

Funding

Publication funding provided by Lahore College for Women University

Acknowledgements

We acknowledge the support of Environmental Science Department, Lahore College for Women University for the completion of this research work.

Additional information

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Consent for publication

All authors participated in this research work have consented to the submission of this research article and relevant research data

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