Role of the macroalgae Corallina officinalis in alleviating the toxicity of hexavalent chromium on Vicia faba L.

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ABSTRACT
The accumulation of heavy metals (Cd, Pb, Cr, Li...) in soils, especially agricultural soils, can cause serious problems for the environment and organisms. The accumulation of these Heavy Metals is mainly due to intense human and industrial activities. These soil contamination problems can be partially solved by the application of phytoremediation technologies or phycoremediation using algae to complex and remove pollutants from the environment. In this study we evaluated the role of a macroalga Corallina officinalis on the mitigation of chromium toxicity in the bean crop (Vicia faba L.). Our results showed that the addition 5% of Corallina officinalis improved growth parameters and chlorophyll content in bean leaves, and that in the presence of chromium, and reduced chromium toxicity on faba bean crop.

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1. Introduction:
Chromium (Cr) is one of the most dangerous environmental pollutants, and as a result of human and industrial activity, contaminated soil is becoming a global problem, because soil pollution has very dangerous consequences on the health of living beings such as plant and their environment. The increasing contamination of agricultural soils by heavy metals has serious consequences for the crops grown in these soils, so heavy metal stress in plants is characterized by decreased photosynthesis, absorption of nutrients, damage to the roots and ultimately plant death [1-3]. However, plant growth is reduced under high levels of Cr stress and has induced physiochemical and ultrastructural changes in plants depending on the species [4]. Higher concentrations of Cr in plants can lead to alterations in plant morphology and physiology due to the overproduction of reactive oxygen species (ROS) from hydroxyl (OH) and superoxide (O₂) radicals and hydrogen peroxide (H₂O₂) which cause oxidative damage in plants [5-6].

Previous studies have described that heavy metals cannot be completely eliminated from the environment, but they can be effectively neutralized or converted to a less toxic form to reduce their harmful effect on the environment. Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless [7-10]. Phytoremediation is the extraction of heavy metals from soil and water by plants and it can be used to decontaminate polluted soils and water bodies [11-12]. Phytoremediation is a simple, cost-effective, environment friendly, economically suitable, and self-sustaining substitute to traditional remediation methods. Previous studies have used hyper-accumulators to extract heavy [13]. Thus, the use of plants and algae for the depollution of heavy metals has attracted increasing attention due to several problems associated with the remove and complexing of pollutants by conventional methods. Bioremediation strategies have been proposed as a solution because of their low cost and high efficiency [14].

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This study was conducted to investigate the toxicity of Cr(VI) on faba bean (Vicia faba L.) crops and to explore the role of Corallina officinalis in alleviating the toxicity of Cr(VI). For this purpose, faba bean seedlings were treated with Cr(VI) alone and Cr(VI) with powder of Corallina officinalis (CO5%) for 30 days. Shoot and root length, dry biomass, and chlorophyll content (Chl), were also studied during the process.

2. Material and methods:

2.1. Sampling:
The harvest of Corallina officinalis was done during the autumn 2019 at low tide in El Oualidia, Morocco (32°45’N, 9°30’W). The sample was washed, placed in plastic bags and transported immediately to the laboratory.

2.2. Experimental procedures:
In the laboratory, we washed the algal material several times to remove sand and algae debris. Drying of samples was done in the laboratory in the shade and in the open air. The algae were reduced to a fine powder in a mechanical grinder.

2.3. Growth conditions and treatments:
Seeds of faba bean (Vicia faba L.) were sterilized with 1.5 % (v/v) sodium hypochlorite solution for 15 min and soaked in distilled water at room temperature for 24 h. They were planted in 30 cm diameter earthen pots containing 1 Kg of soil with Chromium (500ppm) or algal powder 5% (CO 5%) or the combination of Cr (500ppm) and Algal powder 5%, while uncontaminated pots served as control.

2.4. Measurement of growth parameters:
After 30 days, shoot and root length, plant dry weight and chlorophyll content were recorded. The plants were oven dried at 80°C for 24 h to determine the dry matter [15].

2.5. Chlorophyll contents:
The chlorophyll a and b contents were determined in the fresh leaves of Faba bean (Vicia faba L) by following the method by Husen et al. (2017) [16]. Final calculations were made according to the coefficients and equations:

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\begin{align*}
\text{Chl a (mg/g)} &= 11.24 \times D_{662} - 2.04 \times D_{645} \\
\text{Chl b (mg/g)} &= 20.13 \times D_{645} - 4.19 \times D_{662} \\
\text{Total Chlorophyll} &= \text{Chl a} + \text{Chl b}
\end{align*}
\]

2.6. Statistical analysis:
All presented experiments were performed in triplicate. The statistical analyses were performed with one-way analysis of variance (ANOVA) using SPSS version 24, followed by Tukey’s post-hoc test between the means to determine the significant difference.

3. Results and discussion:
The faba bean plant grown in contaminated soil was severely affected by the chromium, unlike to the plant grown in medium with algal powder, the results showed that Corallina officinalis powder improves the growth parameters (length and Dry matter), as compared to the control plant.
The length of shoot and root (Figure 1) of Vicia faba was affected by the chromium, the metal only caused a reduction of about 41.02%, 35.48% and by 88.57%, 0.80% when we coupled chromium with algal powder respectively, as compared to control plant. Despite, addition the algal powder to growth medium increased the length of shoot and root by 113.78% and 106.95% respectively.
The dry weight of shoot and root biomass (Figure 2) of Vicia faba was affected by the chromium, the metal only caused a reduction of about 52.87%, 63.19% and by 39.64%, 54.65% when we coupled chromium with algal powder respectively, as compared to control plant. Despite, addition the algal powder to growth medium increased DW of shoot and root by 115.04% and 116.95% respectively.
The chlorophyll content in leaves of Vicia faba was also affected by chromium (Figure 3), we observed a decrease by 23.02% and 15.36% in presence of Cr (500ppm) and Cr+CO, respectively as compared to control plant. On the other hand, in the presence of CO 5% without chromium we noticed that there is a slight increase in chlorophyll content but the difference is not significant compared to the control.

Our results showed that chromium have a negative impact on growth parameters and content chlorophyll. Similar results are found by Farid et al. (2018) [17] who showed that Cr suppressed the overall growth, biomass, gas exchange attributes and chlorophyll content of sunflower plants. Moreover, lower levels of Cr (5 and 10 mg kg⁻¹) increased the production of reactive oxygen species (ROS) and electrolyte leakage (EL) along with the activities of antioxidant enzymes i.e., superoxide
dismutase (SOD), guaiacol peroxidase (POD), ascorbate (APX), catalase (CAT). But at higher concentration of Cr (20 mg kg⁻¹), the activities of these enzymes presented a declining trend. However, Cr(VI) interferes with various physiological processes of plants and finally leads to reduction in growth, photosynthesis, and induced changes in leaf protein profiles and root micro RNA expression [18-19]. Previous studies showed that Cr(VI) toxicity symptoms in plants included inhibition of seedling growth and development, root growth, and biomass [20]. Adrees et al. (2015) [4] noted that Growth parameters of wheat plants like plant height, root length, kernel length, number of tillers per plant, Total chlorophyll and carotenoids contents were significantly lower and affected under Cr stress than those in control plants and gradually decreased with increasing Cr levels. This reduction in growth under Cr stress might be due to higher Cr accumulation and/or due to ultrastructural damages of leaf mesophyll cells and oxidative stress [21].

In the present study, the results showed that Corallina officinalis have a very good biofertilizing effect on the faba bean crop. The results of Kholssi et al. (2019) [22] showed that plant length was increased by 30% , total dry biomass of aboveground and belowground parts were improved by 22% and 51%, respectively, in treatments with filtrate of Chlorella sorokiniana, as compared to the control, indicating that nutrients and extracellular substances excreted by algae in the filtrate were pertinent to the beneficial effects on plant growth. In the other hand Uthirapandi et al. (2019) [23] noted that Sargassum wightii, Turbinaria ornata and Caulerpa racemosa have a positive effect on growth and biochemical parameters of Andrographis paniculata. However, Al-Saman et al. (2015) [24] who study the effect of three species of red marine algae Laurencia obtusa, Corallina elongates and Janiarubens of maize (Zea mays L.) observed that the application of Janiarubens increased both of whole plant nitrogen content and protein content by 129.2% while application of Janiarubens with Corallina elongate increased the sugar contents by 32.4% and it was the superior treatment in increasing shoot polyphenolic contents and antioxidants content while the treatment of Corallina elongate was the superior one in increasing the shoot polyphenolic content, shoot antioxidants and root tannic acid content.

In this study we noticed that the addition of corallina officinalis at 5% limited the negative effect of chromium. Previous researchers study the chromium bioabsorption by algae, Biosorbents had been developed and evaluated for their Cr removal efficiency by implementing biomass of Euglena [25], Cladophora sp. [26], Scenedesmus [27], Selenastrum [28], Spirogyra sp. [29], Ceramium virgatum [30], Nostoc linickia [31], Spirulina sp. [32] and Chlorella vulgaris [33].

![Figure 1. Shoot and Root length of Faba bean plant.](image1)

![Figure 2. Shoot and Root Dry matter of Faba bean plant.](image2)
4. Conclusion:
In this study, the toxicity of Cr(VI) on the faba bean and the role of Corallina officinalis against Cr(VI) stress were analysed. Under Cr(VI) stress, a decrease in the growth parameters and Chlorophyll levels demonstrated the toxicity of Cr(VI) on the crop. However, treatment with Corallina officinalis powder improved the morphological and photosynthetic behaviour of Vicia faba L. against Cr(VI) stress. According to our results we can suggest the use of Corallina officinalis for a good remediation, and to study other physiological parameters such as proteins, total sugars, antioxidants and the accumulation of heavy metals in plants to confirm the effectiveness of this macro-algae in the decontamination of soil and water.

References:


