Use of phytoremediation for pollution removal of hexavalent chromium-contaminated acid agricultural soils

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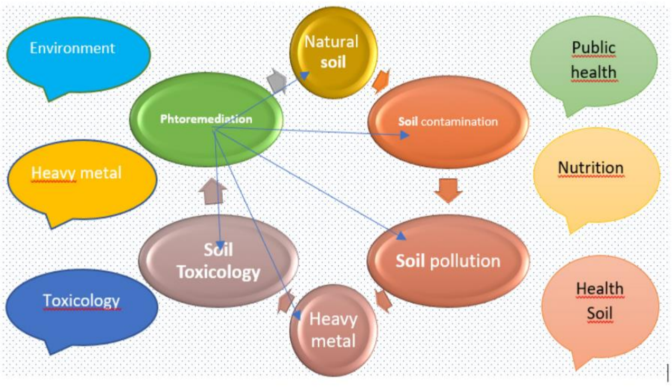
GRAPHICAL ABSTRACT



Abstract

Chromium is a common heavy metal pollutant found in industrial wastewaters which may pollute agricultural soils through groundwater and watering. Phytoremediation is an economical and highly applicable method for removal of pollutants from agricultural soils. This research was carried out for the removal of hexavalent chromium (Cr (VI)) contamination from the soil with the phytoremediation method. For this purpose, only 30 mg kg^{-1} hexavalent chromium (Cr (VI) as Chromium CrO_3, only 10 mL bacteria *Rhodobacter capsulatus* DSM1710 and chromium plus bacteria applied to the pots and Malabar spinach (*Basella alba* L.) grown in the pots. At the end of experiment the results showed that side branching, leaf width, plant dry weights were the highest agro-morphological traits when bacteria were applied to chromium polluted soil. Some macro and micro nutrient elements which are essential for plant nutrition were analyzed (N, P, K, Ca, Mg, Fe, Cu, Mn and Zn). Among them, N, P, Fe, Cu, Mn and Zn were found to be

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statistically significant at the level of 5%. The Cr content of Malabar spinach in control soil was 0.31mgkg^{-1}, but it was 2.33mgkg^{-1} when the soil was contaminated with Cr at the end of experiment. Moreover, when bacteria were additionally applied the Cr content increased to 4.02 mgkg^{-1} of Malabar spinach. Chromium pollution antagonistically affected both some nutrient element (P, K, Ca; Mg) and some heavy metals (Fe, Cu, Zn, Mn) in the soil. This study shows that phytoremediation can be used to remove the soil pollution caused by containing high hexavalent chromium. For this reason, the nitrogen fixing bacterium *Rhodobacter capsulatus* and the hyperaccumulator Malabar spinach plant can be used. It is the first study where Malabar spinach was used a hyperaccumulator plant for chromium pollution in the soils.

Keywords: Toxicity, Phytoremediation, macro and micro-elements, Cr (VI), *Rhodobacter capsulatus*

1. Introduction

Continuously changing and evolving technology affect agriculture directly. Various applications have been carried out in agriculture such as chemical fertilizers, hormones, soil regulators, pesticides, sludge and wastewater usage for watering in order to obtain the highest efficiency from unit area. On the other hand, fast and unbalanced increase in population, urbanization and industrialization cause environmental problems. Among these problems resides the heavy metal pollution of the soil and water sources (Adiloğlu 2016; Adiloğlu *et al*., 2016). Metals such as Ag, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb and Zn are soil polluters which can be uptake by the plants from the soil. Chromium is one of the common heavy metals that pollute the agricultural soils as a result of industrial activities. It is a transition element in VI-B group with proven toxic effect. It can take various values ranging from $^{+1}$ to $^{+6}$. Chromium is found as chromium oxide in the soil and has two oxidation states: Cr^{+3} and Cr^{+6} (Bebek 2001; Türsün 2017) and Cr^{+6} is more toxic than Cr^{+3}. The total chromium level allowed in the agricultural soils is 100 mg/kg, while the extractable level is only 1 mg/kg (Adiloglu 2013). Generally, chromium in the soil varies between 7-760 mg/kg depending on the source (Demir 2008). Some plants are reported to be highly efficient in terms of heavy metal remediation from the soil and they are tolerant to heavy metal toxicity. Using plants for removal of pollutants from the soil is called phytoremediation which can be applied easier than physicochemical technologies. Advantages of phytoremediation are being effective both on organic and inorganic pollutants and lower expenses of system set up and amelioration. The system can be used in both natural and artificial environments and the dimensions of the contaminated area are not disadvantageous in phytoremediation using plants. Phytoremediation using plants is a cheap option in these circumstances (Sadowsky 1999; Yinanç and Adiloğlu, 2017). Being economical is indeed one of the important advantages of phytoremediation. The cost of cleaning of a Pb contaminated area was calculated to be 6 times lower by phytoextraction than engraving the soil over 30 years of time (Cunningham, 1996). The economic value of phytoremediation for farmers was calculated to be around 15,000 € over a period of 20 years (Lewandowski et al. 2006). The primary factor affecting the success of phytoremediation is the heavy metals in the soil becoming available for uptake by the plant roots. For this reason, complex forming chelates are used which increase the uptake of metals by plants. There are various phytoremediation methods depending on the plant to be used and the pollutant. The choice of the method therefore depends on the uptake and removal mechanisms of the plants, chemical and physical properties of the pollutant, the suitability of the phytoremediation method to the pollutant, the concentration and the depth of the pollutant in the soil and climate (EPA 2004). Malabar spinach belongs to the *Basellaceae* family (Deshmukh and Gaikwad, 2014). There are two taxonomic varieties: *Basella rubra* L. ve *Basella alba* L. They are differentiated by their leaf properties and stem color (Adhikari *et al.*, 2012; Cook 2010; Deshmukh and Gaikwad, 2014; Ray and Roy, 2007). The origin of Malabar spinach is in India and Indonesia and can be naturally grown in tropical Asia (Saroj *et al.*, 2012). It is known that green plants respond to nitrogen containing inorganic fertilization, but nitrogen application can affect yield up to a certain point. Nitrogen plays an important role in vegetative development and yield quality. Increased nitrogen application can positively or negatively affect some agro-morphological properties, macro and micro nutrition contents and quality of the product. (Lemaire and Gastal, 2009). *Rhodobacter capsulatus* is a photosynthetic Gram negative purple non-sulfur bacterium (PNSB) that lives in soil and fresh water. It possesses nitrogenase enzyme through which it can fix free nitrogen from the air. This bacterium has been subject of many researches due to its versatile metabolism, hydrogen production and nitrogen fixation abilities (Weaver *et al*., 1975). It can utilize many organic substances therefore can be used for wastewater and sewage treatment and bottom mud contaminated with organics. (Nagadomi *et al.*, 2000; Sasaki *et al*., 1998). It is known that heavy metal contamination decreases the bacteria amount in soil and this affects the soil vitality (Ding *et al.*, 2017). *R. capsulatus* and other some PNSB have been shown to be used for bioremediation of certain heavy metal contaminations in soil (Ge *et al.*, 2017; Kis *et al.*, 2015). Moreover, *R. capsulatus* was shown to reduce hexavalent chromium to less toxic trivalent chromium (Merugu *et al.*, 2013; Rajyalaxmi *et al.*, 2017). Bahadur *et al*. (2017) and Polti *et al.* (2011) studied different bacteria (rhizobacteria and *Streptomyces* sp. MC1) for the removal of chromium pollution from the soils. According to the researchers, different bacteria positively affected and decreased the chromium pollution in the soils. The aim of this study is using phytoremediation, a cheap and efficient biological method for cleaning soil contaminated with chromium of low mobility. It was revealed that the Malabar spinach *Basella alba* L. is a hyperaccumulator plant together with the applied bacterium *R. capsulatus*. To the best of our knowledge, this study is the first example of employing *R. capsulatus* to enhance the hexavalent chromium phytoremediation capacity of the Malabar spinach.

2. Materials and methods

2.1. Setting up and running the experiment

This study was carried out in pots under controlled conditions in November 2017- March 2018 in Namık Kemal University Faculty of Agriculture Soil Science and Plant Nutrition Department (40°98'N, 27°48'E). The study was designed in triplicates according to randomized block design. A total of 12 pots were used in the study. For each trial, 3 pots were used: 3 control pot, 3 pots for only bacteria application, 3 pots for chromium application, and 3 pots for the application of chromium and bacteria together. The day temperature was around 27°C and night temperature was 21-22°C with humidity not less than 85%. The plants obtained light for twelve hours per day during the experiment. No pesticides were used during the experiment. The standard variety of Malabar spinach (Zengarden Firm) was used for the research. Seeds were sown in multi-celled trays filled with peat (Klasmann- Deilmann, Potground H, Germany) in November 2017. Chromium (VI) oxide (CrO_3) (Sigma-Aldrich No: 232653) was used as the heavy metal. It was dissolved in distilled water to have the concentration of 30mgkg^{-1} CrO_3. Later this water was applied to the pot soil 30 days before transplantation of the plants from the multi-celled trays. After the germination and generation of 2-3 leaves (19 days after germination), the plants were transplanted to pots of 4 kg as 1 plant/pot.

Rhodobacter capsulatus DSM1710 was grown in modified Biebl Phennig medium (Biebl and Pfennig, 1981) containing 20 mM acetate and 10 mM glutamate as carbon and nitrogen sources, respectively. Bacteria were grown anaerobically in fully filled glass bottles under constant illumination with 2000 lux light intensity at 30°C. In the mid exponential phase of growth, bacteria were collected by centrifugation at 4100 rpm for 20 min. The bacterial pellet was washed twice with sterile saline solution. The pellet was re-suspended in sterile distilled water to reach a final concentration of 10^7 CFU/mL and 10 mL bacteria were applied to the root area of the plants in pots. The applications in this study were as follows: Control, only bacteria, only chromium, chromium + bacteria. The peat used for germination contained 160-260 mgl^{-1} N, 180-280 mgL^{1} P_2O_5, 200-150 mgL^{-1} K_2O, and 80-150 mgL^{-1} Mg. The pH of the turf was 6. The organic matter of the peat was 70% and the C content was 35%.

2.2. Soil and plant analysis

The soil used in the study contained 3.9% organic matter, and 5.2% lime. The EC x10^6 of the soil was 700. The changeable potassium (K_2O) in the soil was 128 kg da^{-1}, while available phosphorous (P_2O_5) amount was 9.25 kg da^{-1}. The pH value of soil samples was determined in 1:2.5 soil: water using a pH meter, the lime contents of soil samples were determined by a calcimeter, organic contents were determined by Smith-Weldon method, and phosphorus contents were determined by $NaHCO_3$ method (Sağlam, 2012). The salt contents of soil samples were determined with EC meter (U.S. Soil Survey Staff, 1951). The texture of soil samples was evaluated according to Bouyoucos method (Bouyoucos 1955; Tuncay, 1994). The available Zn, Cu, Fe, Mn and extractable chromium contents of the soil samples were analysed with ICP-OES using a buffer solution (DTPA method:0.005 M DTPA+0.01M CaCl2+0.1 M TEA (pH:7.3)) (Lindsay and Norvell, 1978). The pH of the soil, $CaCO_3$ content, electrical conductivity, organic matter content, available P, exchangeable K, available Zn, Fe, Mn, Cu, and extractable Cr and texture given in Table 1 (Bouyoucos 1955; Jackson 1967; Kacar 1995; Lindsay and Norvell, 1978; Olsen and Sommers, 1982; Sağlam 2012).

Table 1. Some physical and chemical properties of the experimental soil

Physical and chemical properties	Values	Reference
pH (1:2.5)	6.43	Sağlam, 2012
EC (µs/cm)	1533	U.S. Soil Survey Staff, 1951
$CaCO_3$ (%)	5.84	Sağlam, 2012
Organic matter (%)	1.91	Sağlam, 2012
Texture	Clay loam	Bouyoucos 1955; Tuncay, 1994
Available P ($mgkg^{-1}$)	41.39	Olsen and Sommers, 1982
Exchangeable K ($mgkg^{-1}$)	262.75	Kacar, 1995
Available Mn ($mgkg^{-1}$)	0.81	Lindsay and Norvell, 1978
Available Cu ($mgkg^{-1}$)	1.79	Lindsay and Norvell, 1978
Available Fe ($mgkg^{-1}$)	0.37	Lindsay and Norvell, 1978
Available Zn ($mgkg^{-1}$)	0.79	Lindsay and Norvell, 1978
Extractable Cr ($mgkg^{-1}$)	0.45	Lindsay and Norvell, 1978

Plants were harvested after 120 days after germination. Plant height, number of plants, leaf width, leaf length, number of side branches, wet and dry weights of the Malabar spinach were measured. Some macro and micro nutrition element contents (P, K, Ca, Mg, Fe, Cu, Mn, Zn, and Cr) of plants were determined with ICP-OES (Agilent 700 series) after wet decomposition and N content was determined with Kjeldahl method in Namık Kemal University Central Research Laboratory (Kacar and Inal, 2010; EPA 1996).

2.3 Statistical analysis

Statistical analysis of the results was carried out with Analysis of variance (ANOVA) and Duncan's Multiple Range Test using Statistical Package for Social Sciences (SPSS) Version 21 (IBM 2012).

3. Results and discussion

The images from experimental setup and Malabar spinach during experiment and after the harvest can be seen in Fig. 1. The plants in the pots at the start of the experiment (Fig. 1. a) and at the end of the experiment (Fig. 1. b) were photographed. The Malabar spinach of the control condition can be seen in Fig. 1. c, while the comparison of the control with a plant of bacterial application was given in Fig. 1. d (left: control, right: bacterial application). The improvement of plant growth by addition of *R. capsulatus* is quite noticeable; the bacterial addition has stimulated plant growth in this experiment. The effects of chromium and bacteria applications on the Malabar spinach were given in Table 2.

Figure. 1. Images of experimental process and harvest of the Malabar spinach. a) Start of the experiment, plants in pots. b) Plants on the day of harvesting. c) Overall image of Malabar spinach (control experiment pot). d) Malabar spinach of control (left) and bacteria application (right).

Table 2. Effects of chromium and bacteria treatments on agro-morphological traits of Malabar spinach

Treatments	***Plant height (cm)***	***Number of leaves (unit)***	***Leaf height (cm)***	***Leaf width (cm)***	***Number of Side branch (unit)***	***Wet weight (g)***	***Dry weight (g)***
Control	148 ± 36.3^{ns}	40 ± 9.5^{ns}	12 ± 0.0^{ns}	8.6 ± 0.2^{b}	10.6 ± 4.0^{a}	44 ± 11.4^{ns}	4.94 ± 0.0^{a}
Bacteria	185 ± 27.5^{ns}	31 ± 4.6^{ns}	12 ± 0.7^{ns}	9.1 ± 0.6^{ab}	7.0 ± 1.5^{a}	47 ± 5.6^{ns}	3.11 ± 0.0^{c}
Chrome (Cr^{+6})	149 ± 16.8^{ns}	30 ± 4.0^{ns}	11 ± 0.6^{ns}	8.4 ± 0.4^{b}	2.0 ± 1.0^{b}	35 ± 6.0^{ns}	3.58 ± 0.0^{b}
Chrome and bacteria	135 ± 5.0^{ns}	33 ± 6.3^{ns}	12 ± 0.8^{ns}	10.3 ± 1.0^{a}	5.6 ± 0.8^{ab}	45 ± 4.8^{ns}	3.52 ± 0.0^{b}

All the values are mean ± standard error (SE), n=3. Different letters (a, b, c) indicate significances at p≤0.05, ns: non-significant

Although there have been increase and decrease in plant height, number of leaves, leaf height and wet weight of the Malabar spinach by applications of bacteria and chromium alone and together, they were found to be statistically insignificant. However, changes in leaf width, number of side branches and dry weight were statistically significant. The negative effects of heavy metal application were obvious on these biological traits when compared to the control condition. The addition of *R. capsulatus* bacteria in heavy metal contamination showed the most significant effect on the width of Malabar spinach leaves. The decrease in the leaf widths by heavy metal application were reversed by bacterial activity.

Table 3. Effects of chromium and bacteria treatments on macro nutrition elements content of Malabar spinach shoot

Treatments	***N (%)***	***P (%)***	***K (%)***	***Ca (%)***	***Mg (%)***
Control	4.57 ± 0.4^{c}	0.47 ± 0.02^{b}	2.87 ± 1.22^{ns}	1.47 ± 0.17^{ns}	0.92 ± 0.05^{ns}
Bacteria	5.07 ± 0.3^{b}	0.58 ± 0.01^{a}	5.03 ± 0.05^{ns}	1.87 ± 0.04^{ns}	1.04 ± 0.01^{ns}
Chromium (Cr^{+6})	5.56 ± 0.7^{a}	0.37 ± 0.02^{c}	2.51 ± 1.08^{ns}	1.66 ± 0.24^{ns}	0.88 ± 0.07^{ns}
Chromium and bacteria	5.15 ± 0.17^{b}	0.43 ± 0.01^{b}	4.03 ± 0.05^{ns}	1.69 ± 0.04^{ns}	0.96 ± 0.02^{ns}

All the values are mean ± standard error (SE), n=3. Different letters (a, b, c) indicate significances at p≤0.05, ns: non-significant, each element was evaluated individually

The effects of heavy metal and bacteria applications on macro nutrient elements of Malabar spinach were given in Table 3. The nitrogen content of the Malabar spinach increased with application of heavy metal compared to the control condition. The highest nitrogen content was obtained when heavy metal was applied alone. The reason may be a synergistic effect between chromium and nitrogen. Moreover, there is an increase in nitrogen content in heavy metal and bacteria application together. Higher acquisition of nitrogen from the soil was suggested to be a mechanism of stress avoidance (Blaudez *et al*., 2000). On the other hand, the lowest nitrogen content amount all the conditions were observed in bacteria only application. The reason can be that bacteria may also utilize the nitrogen in the soil. *Rhodobacter capsulatus* has many different metabolisms. It can fix nitrogen form the soil, but can also shift its metabolism and can consume the nitrogen available in the soil for growth and maintanance. The experiment period was 2 months, and for better evaluation of nitrogen contents, the experiment duration should be increased and field researches should be conducted. As the pot experiments took only two months, in order to see the maximum bacterial utilization process, the experiments should continue in greenhouse and field. Nitrogen plays an important role in biological properties, yield and quality of the plants. Nitrogen deficiency negatively affects the vegetative development of the plant as nitrogen is a crucial element for green parts of the plants (Lemaire and Gastal, 2009; Özer 2003). In case of deficiency, leaf and stem structures would be weak and vegetative development period would be short (Güneş *et al*., 2007; Karaman *et al*., 2012; Smith and Cassel, 1991). In a previous study done with komatsuna, as the nitrogen was applied as 10 kg da^{-1}, its effect on plant length, dry and wet weights was higher than other doses of no nitrogen, 15 g da^{-1} and 20 kg da^{-1} nitrogen (Acikgoz *et al.,* 2014). This showed that as well as deficiency, over application of nitrogen can have negative effects. Phosphorus content was the highest under bacteria only application. Phosphorus is an element known for its generative developmental effect on the plants. Besides, phosphorus negatively affects plant vegetative growth (Adiloğlu *et al*., 2011; Güneş *et al*., 2007; Karaman *et al*., 2012). Potassium, calcium and magnesium contents were not significantly affected by heavy metal and bacteria applications. Potassium amount in plants has an impact on resistance against diseases and pests. In case of potassium deficiency, the opening and closing metabolism of the stoma are disrupted. This increases the chance of bacterial and fungal infections in the plant (Öktüren Asri and Sönmez, 2005). Calcium is vital for plant development and cell wall synthesis as 90% of the calcium take place in the cell wall. Magnesium has an active role in energy metabolism in the roots (Karaman *et al.,* 2012; Mikkelson 2010). The chromium heavy metal contamination did not negatively affect the amounts of these elements, which enhanced the phytoremediation capacity of the Malabar spinach.

Table 4. Effects of chromium and bacteria treatments on micro nutrition elements content of Malabar spinach shoot

Treatments	***Fe (mgkg^{-1})***	***Cu (mgkg^{-1})***	***Mn (mgkg^{-1})***	***Zn (mgkg^{-1})***	***Cr(mgkg^{-1})***
Control	82.70 ± 2.01^{a}	9.63 ± 0.26^{b}	116.60 ± 5.69^{b}	32.17 ± 1.52^{ab}	**0.31 ± 0.10^{c}**
Bacteria	81.57 ± 1.73^{b}	9.90 ± 0.06^{b}	164.97 ± 2.81^{a}	36.53 ± 0.52^{a}	**0.39 ± 0.03^{c}**
Chromium (Cr^{+6})	43.57 ± 2.92^{c}	8.13 ± 0.17^{c}	126.63 ± 9.70^{b}	31.20 ± 2.0^{b}	**2.33 ± 0.03^{b}**
Chromium and bacteria	59.27 ± 0.79^{bc}	11.43 ± 0.09^{a}	162.47 ± 3.38^{a}	36.13 ± 0.47^{a}	**$4.02 \pm 0.0.48^{a}$**

All the values are mean ± standard error (SE), n=3. Different letters (a, b, c) indicate significances at p≤0.05, each element was evaluated individually

An antagonistic effect between Fe and Cu contents was observed in the chromium heavy metal applied pots. This situation is obvious from the statistically different groups in the analysis results. The change in the contents of Mn and Zn were found to be nonsignificant. The contents of Fe and Mn were found to be statistically different at 5% level in the pots with bacteria application. When evaluated with increased N contents in soil upon bacteria application, the nitrogenase of this bacterial species can be suggested to be active in this study. This enzyme contains Fe in the structure. Therefore, it may be suggested that Fe was consumed by bacteria, and there may be synergistic effect between Fe and Mn. In the pots with both chromium and bacteria application it was observed that bacteria could compensate the negative effects of chromium and also positively affected Cu, Mn and Zn plant nutrient elements. The dual application of chromium and bacteria also increased the accumulation of chromium in the plant.

The Malabar spinach was shown to accumulate the heavy metal chromium (Table 4). The chromium content in the control was 0.31 $mgkg^{-1}$, this was the chromium content of the Malabar spinach accumulated from the soil without any additional heavy metal application. The addition of the bacteria to the soil, again without additional heavy metal, did not significantly enhance chromium uptake of the Malabar spinach from the soil.

However, when 30 $mgkg^{-1}$ chromium was applied to the soil, Malabar spinach could accumulate 2.33 $mgkg^{-1}$ chromium according to the control. This shows that Malabar spinach can act as a heavy metal hyper accumulator plant. Its accumulation of the heavy metal chromium was even enhanced with the addition of the bacteria to the soil and increased to 4.02 $mgkg^{-1}$. This proves that application of *R. capsulatus* increased the phytoremediation of chromium from the soil by the Malabar spinach. Moreover, Cu, Mn and Zn, but not Fe contents were improved compared to the control when chromium was applied together with the bacteria. Chromium and bacteria have antagonist effect on Fe but synergistic effect on Cu, Mn and Zn contents of Malabar spinach. Similar results were obtained earlier researches with sunflower (*Helianthus annuus* L.), spiny chicory (*Cichorium spinosum*), black gram (*Vigna mungo*) different plants (Antoniadis *et al*., 2017; Bahadur *et al.,* 2017; Saravanan *et al*., 2019).

Table 5. Effects of chromium and bacteria treatments on macro nutrition elements content of soils

Treatments	***N (%)***	***P (%)***	***K (%)***	***Ca (%)***	***Mg (%)***
Control	0.30 b	0.015 a	0.548 a	0.301 a	0.247 a
Bacteria	0.68 a	0.012 b	0.486 b	0.278 c	0.194 b
Chromium (Cr^{+6})	0.69 a	0.013 ab	0.496 b	0.271 d	0.172 c

Chromium and bacteria	0.68 a	0.015 a	0.538 a	0.291 b	0.205 b

All the values are mean ± standard error (SE), n=3. Different letters (a, b, c) indicate significances at p≤0.05, each element was evaluated individually

Nitrogen content of the soil increased according to control with bacteria, bacteria and chromium and only chromium applications (Table 5). Available phosphorus content of the soil increased with only bacteria application. But exchangeable K content of the soil decreased with only bacteria and chromium application. Exchangeable Ca and Mg changed in the same way. These increases and decreases were found to be statistically significant at the level of 5 %. These values were obtained after the experiment (Table 5).

Table 6. Effects of chromium and bacteria treatments on micro nutrition elements content of soils

Treatments	***Fe (mgkg^{-1})***	***Cu (mgkg^{-1})***	***Mn (mgkg^{-1})***	***Zn (mgkg^{-1})***
Control	2.69 a	0.22 ab	6.953 b	0.47 a
Bacteria	2.40 c	0.21 bc	6.233 d	0.47 a
Chromium (Cr^{+6})	2.36 c	0.20 c	6.406 c	0.45 b
Chromium and bacteria	2.59 b	0.24 a	9.086 a	0.45 b

All the values are mean ± standard error (SE), n=3. Different letters (a, b, c) indicate significances at p≤0.05, each element was evaluated individually

Generally, available Fe, Cu, Mn and Zn contents of the soil decreased with bacteria and chromium applications according to control (Table 6). Iron nutrient element were lower in all the trials compared to the control pots most probably due to the consumption of Fe by bacteria. Because of the antagonist relationship between chromium and copper, the lowest Cu was observed in the pot with only chromium application. This decreased was shown to be compensated by the bacterial presence. All the micro nutrients except Zn decreased in the only bacterial application. The reason may be the uptake and use of these elements by the additional bacteria. However, Zn content did not significantly change but it has a synergistic relationship with chromium. But these element values decreased with bacteria plus chromium applications except Mn contents. These increases and decreases were found to be statistically significant at the level of 5%. Chromium application (30 mgkg^{-1}) decreased Fe, Cu, Zn and Mn contents of the soil. Chromium pollution negatively affected some micro nutrient element contents in the soil.

But the negative effects of chromium were decreased with bacteria plus chromium application. All heavy metal values were determined after the harvest of the plants (Table 6).

4. Conclusions

Industrial and agricultural activities result in pollution of water and soils which are important environmental parameters. Heavy metal pollution is one of the leading causes of water and soil pollution. It was shown that phytoremediation can be easily and economically applied to accumulate chromium from the soil which was polluted with chromium contaminated soil. The nitrogen fixing *R. capsulatus*, which belongs to an important group of bacteria for soil biological activity and plant nutrition, increased phytoremediation capacity of the Malabar spinach. Different treatments in this study significantly affected the nitrogen content positively, phosphorous content negatively; and other macro nutrient elements were not significantly affected in Malabar spinach shoot. Similarly, among the micro nutrient elements, Fe content was negatively affected while Cu, Mn and Zn contents were positively affected by heavy metal and bacteria treatments in Malabar spinach. It can be seen that the applied *R. capsulatus* bacterium is especially effective on acquisition of these elements.

The results of the study revealed that Malabar spinach can be used in phytoremediation of heavy metal contaminated soil together with soil application of the bacterium *Rhodobacter capsulatus*. Yet, field experiments should be carried out for more certain inference about their use in large scale. However, the results may indicate that *R. capsulatus* may be a resistant bacterium for chromium contamination in soil, and can be added to the soil contaminated with chromium for remediation purposes. This study suggests that soil contaminated with chromium cleaned by Malabar spinach, and *R. capsulatus* was shown to increase the phytoremediation efficiency of Malabar spinach. In order to have an information on *R. capsulatus* growth in the soil, *R. capsulatus* counting in a soil sample can be conducted in the future. Moreover, a detailed analysis on how the interaction of addition of this bacterium to soil with other bacteria can be done by a high throughput microbiome study.

The Malabar plant has been used for the first time in the literature to remove chromium contamination from soils. This plant can be a hyper accumulator for chromium. Malabar spinach a hyper accumulator plant in such cases may take up the heavy metals from the soils, and hence clean the soils polluted with some heavy metal contaminated soils.

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