

# Study of the Enhanced Phytoextraction of Cadmium in a Calcareous Soil

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**ABSTRACT:** Heavy metals such as cadmium mainly enter in the environment and ecosystem as a result of human activities. This study was conducted in order to evaluate the ability of bacterial inoculants to enhance efficiency of phytoextraction in a calcareous soil. Three plants (*Amaranthus retroflexus*, *Helianthus annuus* and *Medicago sativa*), along with three levels of inoculants and four levels of Cd were evaluated. The experiment design was factorial with three replications. The ANOVA results showed that application of inoculants led to significant increase ( $P < 0.01$ ) of cadmium concentration in plant's root and shoot. Furthermore, as cadmium concentration increased in different treatments, fresh weight of plant's root and shoot decreased in all treatments but, weight reduction of plant's organs was less in treatments with inoculant application. The amaranthus highest amount of absorption, translocation of Cd to shoot, Translocation factor (TF) and Cd phytoextraction. Thus, amaranthus among studied plants is more appropriate for phytoextraction of cadmium.

**Key word:** Phytoextraction, Cadmium, Soil pollution, Inoculant, Translocation factor

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## INTRODUCTION

In recent years, heavy metal contamination in soil has become a major problem which their accumulation in plants can directly or indirectly influence animals and humans (Pal *et al.*, 2006). The highest amount of cadmium enter into the soil through application of sewage sludge and waste in agricultural soils and also as a result of industrial activities such as dye making, rubber making, production of fertilizer from phosphate rock, automobile fuel and metal melting industry (Ansari & Malik, 2007; pal *et al.*, 2006). Phytoextraction serves as one of the phytoremediation technologies and it is the technique of using plants for decontamination of the environment. Soil remediation by phytoextraction method have many advantages and

disadvantages (Erakhrumen, 2007; Ghosh & Singh, 2005). Low biomass production in hyperaccumulator plants and susceptibility of other plant' root to high metal concentration lead to extension of researches on use of microorganisms in order to develop application of phytoextraction and to make this method economical (Glick, 2003; Ansari & Malik, 2007).

In the present study, effect of the application of inoculants of two strains, resistant to heavy metals was tested on cadmium absorption in greenhouse experiment with amaranthus, sunflower and alfalfa. The objective was to provide a practical solution for decreasing and diminishing of heavy metal contamination from different sources such as fertilizers, industries and mines to agricultural lands using native plant growth promoting rhizobacteria (PGPR) separated from soil around lead and zinc mines in Haft Emarat, Arak, at central province of Iran.

## **MATERIALS & METHODS**

First, composite soil sample was collected from depth of 0-30 cm from the Campus of Agriculture and Natural Resources of University of Tehran located in Karaj with coordinates of latitude of northern 35°48' 35" and longitude of eastern 50° 58' 18" and 1315.5 meters above sea level . The soil was classified as *Xeric Haplocambids, Fine Loamy, Mixed, Super Active thermic*. Samples were air dried and passed through 2-mm sieve and mixed uniformly. Physical and chemical properties and concentrations of elements in samples were measured: Measurements of the soil N was done by Kjeldal method, (Bremner, 1996), available phosphorus by Olsen Method (Kuo, 1996), available Potassium by normal acetate ammonium method (Hemke and Sparks, 1996). Measurements of the soil pH was done on saturated extract (Thomas, 1996) and electriced conductivity by Rhoades method (1996), Equal Calcium Carbonate by Bouyoucos method (Bouyoucos, 1962), organic carbon percentage by Walkly Black (Nelson and Sommers, 1982) and texture of the soil by hydrometric method (Bouyoucos, 1962) and cation exchange capacity by Bower method (Sumner and Miller, 1996). Available concentration of cadmium, lead and zinc were extracted by DTPA method (Linsay and Norvell, 1978) and measured by Atomic Absorption Spectrometry (AAS). Results are given in Table 1. The methods for bacteria isolation and purification and their PGPR characteristics has been reported previous in Moteshare Zadeh *et al.* (2008). The effects of three levels of inoculants [control (B0), *Bacillus mycooides* M1 (B1), *Micrococcus roseus* M2 (B2)] and four levels of Cd [control (Cd0), Cd50, Cd100 and Cd200 mg/kg CdCl<sub>2</sub>.H<sub>2</sub>O ] on three plant cultivars (*Amaranthus retroflexus* , *Helianthus annuus* and *Medicago sativa*), were studied under controlled conditions in a factorial experiment with randomized complete block design (RCBD) with three replicates of each treatment. After 70

days, at the beginning of reproductive period, shoot and root of sunflower and aramanthus and after three cuts of alfalfa (mean of three cuts in calculations and comparing it with other plants) in alfalfa, plant material was washed with distilled water and fresh weight determined, then dried in oven at 70°C and dry weight was recorded. Then, samples were milled and extract was prepared by dry ash, and concentration of cadmium, iron, copper, zinc, and manganese were measured using ICP- OES, CAP-6500 model. (Madejon *et al.*, 2003). In order to assess amount of metal transfer from root to shoot, translocation factor was determined by dividing metal concentration at shoot by its concentration at root ( Marchiol *et al.*, 2004). The analysis of variance of the data, was done in a factorial design with random blocks basic design with three replications by means of SAS software. The comparison of means was done with LSD test at 1% level and also figures were drawn by Excel software.

**Table 1:** Physical and chemical properties of soil before adding cadmium

<b>Characteristic</b>	<b>Quantity</b>	<b>Characteristic</b>	<b>Quantity</b>
Soil texture	Loam	Total N (%)	0.08
Clay (%)	25.00	Available P (mg/kg)	17.10
Silt (%)	36.00	Available K (mg/kg)	247.00
Sand (%)	39.00	SO <sub>4</sub> (meq/l)	40.60
pH	7.90	Fe(mg/kg)*	4.28
EC(dS/m)	4.31	Cu(mg/kg)*	4.061
%CaCO <sub>3</sub>	8.90	Mn(mg/kg)*	8.244
%OC	0.84	Zn(mg/kg)*	0.812
%SP	35.6	Pb(mg/kg)*	2.023
CEC(Cmol/Kg)	26.00	Cd(mg/kg)*	0.10

DTPA-Extractable \*

### Results and discussion:

The soil used in greenhouse test (table 1) was selected based on metal toxicity limits and study of sources and doubling concentration of each treatment in comparison with the previous treatment, contaminated with cadmium at rates (0, 50, 100 and 200 mg Cd /kg) (Alloway, 1990; Purohit and Agrawal, 2006). Results of the analysis showed that the intended soil have suitable physical and chemical properties for greenhouse culture and no heavy metal contamination was induced due to the treatments. Results of cadmium test for three plants: amaranthus, sunflower and alfalfa: The results of triplet effects of bacterium, cadmium level and plant in root and shoot are illustrated in figure 1 to 14.

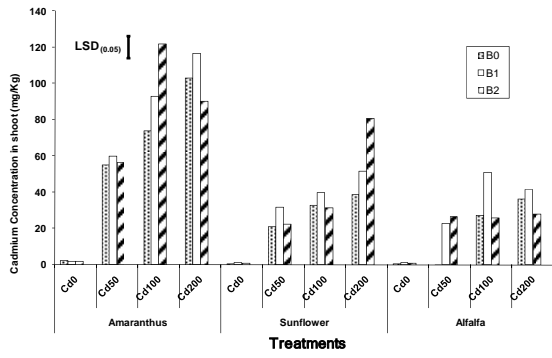


Fig. 1. The effect of treatments on the Cd concentration in different treatments of three plants

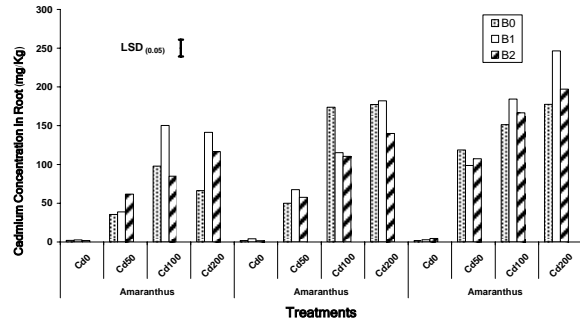


Fig. 2. The effect of treatments on the Cd concentration in different treatments of three plants

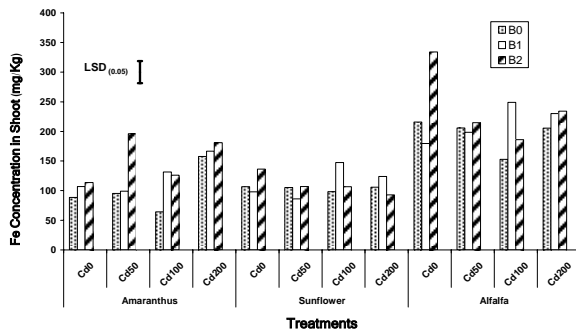


Fig. 3. The effect of treatments on the Fe concentration in different treatments of three plants

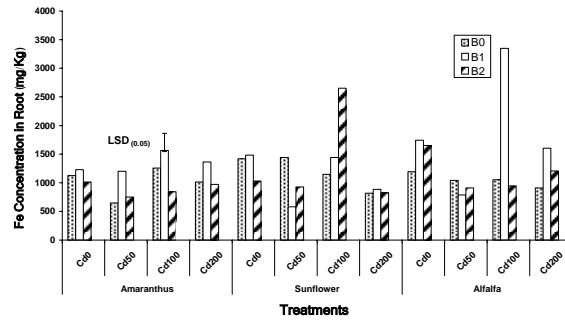


Fig. 4. The effect of treatments on the Fe concentration in different treatments of three plants

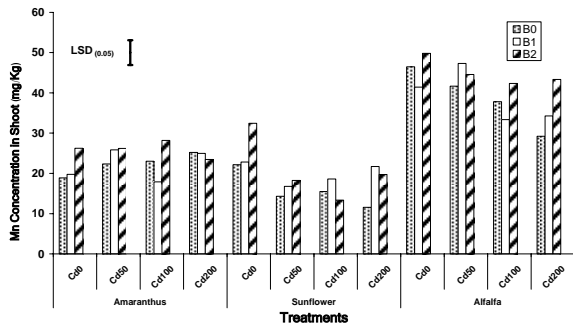


Fig. 5. The effect of treatments on the Mn concentration in different treatments of three plants

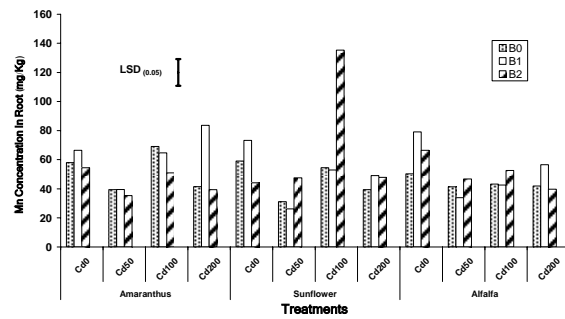


Fig. 6. The effect of treatments on the Mn concentration in different treatments of three plants

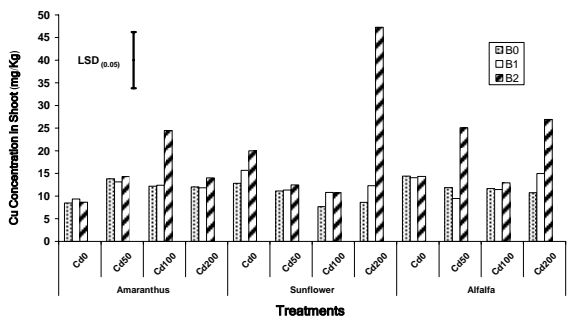


Fig. 7. The effect of treatments on the Cu concentration in different treatments of three plants

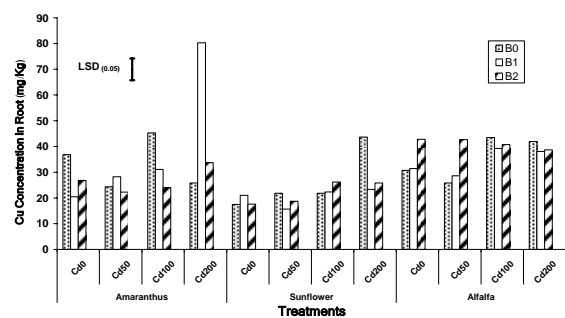


Fig. 8. The effect of treatments on the Cu concentration in different treatments of three plants

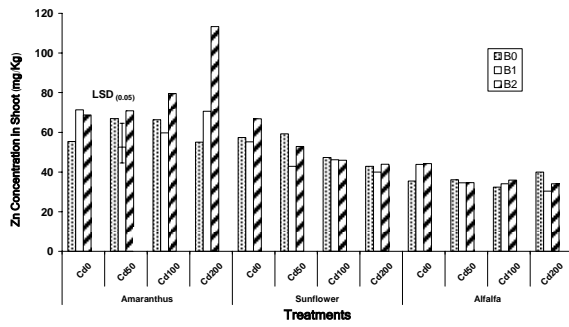


Fig. 9. The effect of treatments on the Zn concentration in different treatments of three plants

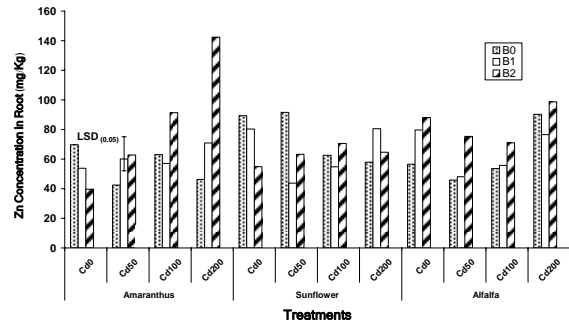


Fig. 10. The effect of treatments on the Zn concentration in different treatments of three plants

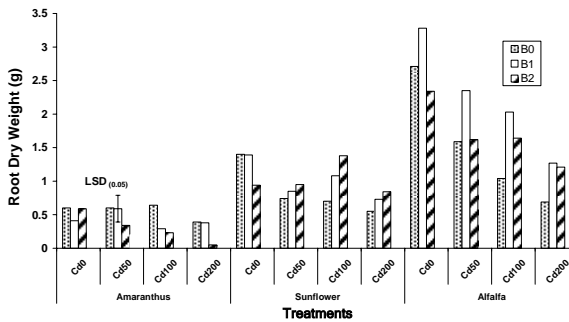


Fig. 11. The effect of treatments on the Root Dry Weight in different treatments of three plants

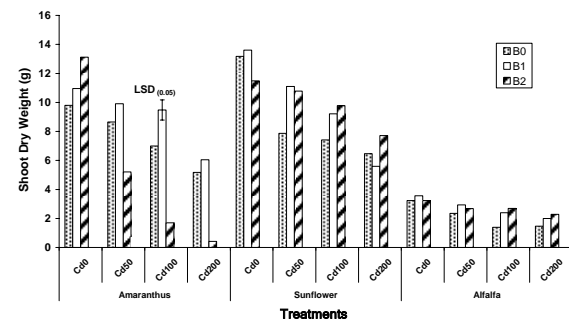


Fig. 12. The effect of treatments on the Shoot Dry Weight in different treatments of three plants

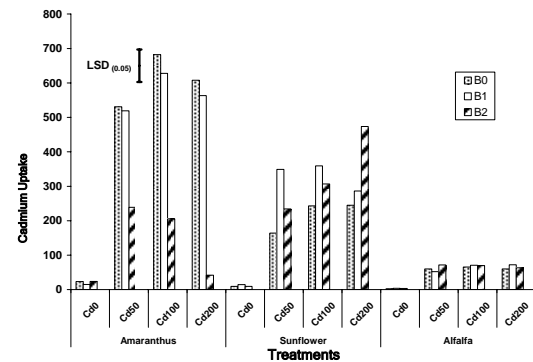


Fig. 13. The effect of treatments on Cadmium Uptake ( $\mu\text{g/Pot}$ ) in different treatments of three plants

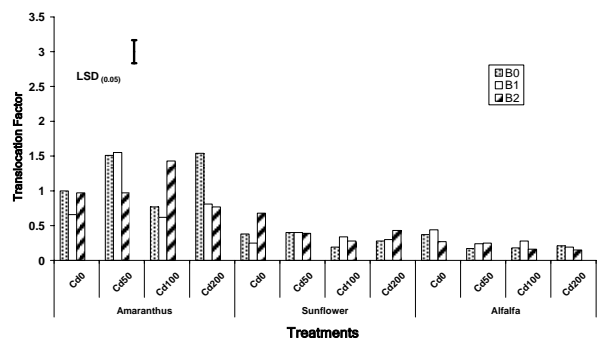


Fig. 14. The effect of treatments on the Translocation Factor in different treatments of three plants

Strains of *bacillus mycoides* M1 (B1), among two strains used at this study, was individually identified resistant to four metals: lead, zinc, cadmium and nickel (B. Moteshare Zadeh *et al.*, 2008). According to Yan- de *et al.*, (2007), multiple metal resistance (MMR) have more effects in bacteria than the resistance to one metal, thereby it is possible that in treatments with application of this inoculant, better results will be achieved in terms of plant growth and phytoextraction. In a similar study at India (Malik & Jaiswal, 2000), 45 *Pseudomonas* strains were separated from

soils of contaminated lands with industrial sewage and also noncontaminated lands, and their biochemical and morphological characteristics were determined. The research's results of these researchers showed that 80% of strains were resistant to copper, 73% resistant to cadmium, 71% to zinc and 48.8% to mercury. The applied concentration in the study were 3.12 to 3200 mg/ml. Aleem *et al.*, (2003), in the study on noncontaminated soils and soils of grain fields which were irrigated by industrial sewage for a long time, 57 strains of *Azotobacter chroococcum* isolated from rhizospheric soil and assessed their chemical and superficial characteristics. Among 36 identified strains from rhizosphere of wheat and 94.4% strains were resistant to lead and mercury and 63.8%, 77.5% and 86.1% resistant to zinc, Cr<sup>+6</sup>, Cr<sup>+3</sup>. According to obtained results, this researchers declared that identified bacteria from soil irrigated by industrial sewage had significant difference in terms of resistance to metal, with noncontaminated soil. According to the report of Hada and Sizemone (1981), although bacterium in noncontaminated area may be compatible to high concentration of metal, but evidences indicated that there were more resistant strains in contaminated area than in noncontaminated area. Also, it was reported that amount of metal in soil was associated with the rate of bacterium resistance to metal. The gram positive and negative bacteria could be resistant to heavy metals (Siler & Misra, 1998).

With regard to results provided in figure 2, application of inoculant led to cadmium concentration increase in roots of three plants also, iron concentration have increased in similar treatments (figure 3). Glick (2003) declared that growth stimuli bacterium can effect on plant growth both directly and indirectly. In the direct effect, plant growth and consequently absorption and nutrition synthesis improvement will increase. Growth stimuli bacterium may stabilize nitrogen of atmosphere and increase availability of this element for plants or by siderophore production, increase the solubility of iron in soil and facilitate iron absorption by plants. The synthesis of different plant hormone such as auxin, considered as one method for stimulation and influence on plant growth by growth stimuli bacterium. By synthesis of ACC Deaminase enzyme, tension ethylene decrease and plant growth will stimulate. Moreover, in the indirect stimulation, useful bacteria prevent the influence of phytopathogens (Glick, 2003). This

researcher expressed that high heavy metal concentration have negative influence on plant growth by generating two problems: tension ethylene and limited iron concentration. The growth stimuli bacteria have useful characteristics such as ability of ACC deaminase enzyme production and siderophore production which can help to hinds plant growth in confronting with high heavy metal concentration. The close identification and analysis of these characteristics can be useful for understanding of heavy metal phytoextraction and phytoremediation mechanisms. Kuffnet *et al.*, (2008) studied on effects of rhizosphere bacteria on absorption and metal concentration in willows and identified 10 strains from contaminated soils of lead mine. Among identified bacteria, bacteria of six type were as follow: pseudomonas, agromyces, streptomyces, flavobacterium, servatia and janthinobacterium. Among strains, four strains (from pseudomonase, serratia type) and two strains (from streptomyces type) have the ability of siderophore production and three strains (two from janthinobacterium type and one from serratia type) have the ability of auxin production. Also non of the ten strains have the ability of ACC deaminase enzyme production. The resistance of these bacteria to zinc, lead and cadmium were assessed too. Yan-de *et. al.*, (2007), provided the list of growth stimuli strains and their effects on plant including control of phytopathogen, growth and nutrition absorption improvement, resistant once to cadmium, zinc, copper, lead, nickel, cobalt, chrome contamination, root production and also resistant once to salinity and drought tension, however, rhizobacteria in addition to metal transmission from soil to plant can increase phytoremediation efficiency by effects on soil pH, iron supply through siderophore production, phosphorus solubility increase, effects on phytopathogen, resistant once to salinitary and drought tension, and indole acetic acid (IAA) production.

According to figure 1, shoot highest cadmium concentration and also the most absorption were observed for amaranthus. It seems that, the result of inoculant application were not identical in three plants and amaranthus did not significantly respond to inoculant application. In terms of root cadmium concentration, the most amount was observed at alfalfa and with B1 inoculant consumption. Also, the highest root and shoot iron concentration was observed at alfalfa and

the same inoculant. As a whole, the most wet weight of root was observed for alfalfa, the most wet weight of shoot for sunflower and the most transmission factor and cadmium absorption for amaranthus. According to these findings, applicability of plants with high biomass production and inoculant effects on growth promotion and plant development was proved. Other researchers including Madejon *et al.*, (2003) and Cheng (2003) and Mathe- Gasper and Anton (2005) have reported the same findings. According to study of Madejon *et al.*, (2003), shoot biomass and root of sunflower were significantly less in contaminated soil than non contaminated soil. But, this effect was not important at maturation, of the product. Oil production was more in contaminated soil. These researchers declared that toxic effect of arsenic, cadmium, copper, lead, thallium was not observed at plants of the soil around mine. In this study, the potential phytoremediation of sunflower was affirmed, and with regard to its lower potential for phytoextraction, there were suggestion on sunflower application for soil protection and oil production for industrial consumption. According to results of this study, total cadmium concentration at sunflower was 125 mg/kg, which including 80 mg/kg at shoot, 13 mg/kg at root and 25 mg/kg at seeds. According to Cheng's report (2003), heavy metal accumulation and distribution in plant depends on different elements such as: environmental elements as plant species, type of element, chemical form, bioavailability, oxidation- reduction potential, pH, cation exchange capacity, solute oxygen, temperature and root distribution. Song *et al.*, (1996), by assessing heavy metal concentration such as cadmium, zinc, lead in spinage organ, has illustrated the relation between metal amount in spinage edible organ and forms of these metal in soil as several equations. These researchers expressed the process of metal accumulation as follow: cadmium > zinc > lead. Products that research's focus on them due to their high biomass production include: sunflower, cotton, oil seed, corn, Indian mustard, cereal (Vassiller *et al.*, 2002). The extent of cadmium concentration at corn in phytoremediation was mentioned as 25 to 150 mg/kg of shoot dry material.

## CONCLUSION



Based on these results, plant type was effective in phytoextraction efficiency increase and also, inoculants application was useful and effective in phytoextraction efficiency increase. For conformation of the results we can point to the reports of Lasat (2002), Glick (2003), Aleem *et al.*, (2003), Yan- de *et al.*, (2007), Ansari and Malik (2007) and Kuffner *et al.*,(2008) which deals with the effect of growth stimuli bacteria through indirect and direct effect on root growth, root growth stimulation through siderophore production (Fig. 3,4) and phosphorous and iron supply and other nutrients (Fig. 7,9,10), control of heavy metal stress through ACC deaminase enzyme production and tension ethylene decrease, ability of auxin (IAA) production, control of salinity and drought stress, absorption stimulation and heavy metal transmission from rhizosphere environment to plant and pathogen element effect decrease and also declares the useful effects of plant growth stimuli bacteria and its synergistic effect with plant in Green remediation.

Alfalfa, as a whole, is considered as an appropriate plant for stabilization of pollutant metals and preserving their distribution, due to its high potential in biomass production. Also, there were no significant risk in terms of its shoot metal concentration. The same results mentioned for sunflower too. So we can successfully use this plant for phytoremediation technology with plant stabilization method. Amaranthus can be considered as a potential option for removing of pollutants and land remediation, due to its resistance to weather conditions and also its ability in metal accumulation and transmission from root to shoot by phytoextraction mechanism and low risk of nutrition in food cycle. As mentioned above, contaminated soil phytoextraction is a relatively new and developing technology with special advantages. Economical use of native and cultivated plants for phytoremediation of moderately polluted soils with heavy metals should be approached with more research and field evaluations.

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