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Heavy Metal Contamination of Zn, Cu, Ni and Pb in Soil and Leaf of *Robinia pseudoacacia* Irrigated with Municipal Wastewater in Iran

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1. Introduction

The economic development of the society towards large-scale urbanization and industrialization is leading to production of huge quantities of wastewaters (Singh & Agrawal, 2008). Wastewaters can be used for the restoration of degraded land (Madejo´n et al., 2006), and the growth of vegetation having commercial and environmental value (Aggeli et al., 2009). Establishment of tree plantations following wastewater irrigation has been a common practice for many years (Kalavrouziotis & Arslan-Alaton, 2008). Several researches of wastewater irrigated plantations in many countries such as India (Bhati & Singh, 2003; Singh & Bhati, 2005), Australia (Sharma & Ashwath, 2006), New Zealand (Guo et al., 2002; Kimberley et al., 2003), Sweden (Hasselgren, 2000), Canada (Cogliastro et al., 2001), Hungary (Vermes, 2002), etc. are available.
In Iran, huge section of useful water of major metropolitan cities converts to the municipal wastewater (Tajrishi, 1998). Since the deficiency of access to adequate water for irrigation is a matter of increasing concern and limiting factor to develop plantation, therefore municipal wastewater could be utilized as an important source of water for expansion of tree plantations in and around the city and industrial complexes (Al-Jamal et al., 2000; Kalavrouziotis & Apostolopoulos, 2007; Salehi et al., 2007). This practice not only reduces the toxicity of soil and plays an important role in safeguarding the environment, because woody species may utilize wastewater and uptake heavy metals through extensive root systems and retain them for a long time (Madejo´n et al., 2006), but it also creates opportunities for commercial biomass production and sequestration of excess minerals in the plant system (Sharma & Ashwath, 2006).
Again, wastewaters may contain amounts of potentially harmful components such as heavy metals and pathogens (Rattan et al., 2005; Toze, 2006). The effects of microbial pathogens are usually short term and vary in severity depending on the potential for human, animal or environmental contact (Toze, 2006), while the heavy metals have longer term impacts that could be a source of contamination and be toxic to the soil (Sharma et al., 2007) and plant (Gasco´ & Lobo, 2007). Hence if wastewater is to be recycled safely for irrigation, the problems associated with using it need to be known (Emongor & Ramolemana, 2004).

According to differences in climatic, vegetation, socio-economic conditions and also in quality of soil and wastewater between different regions and even within different time periods in one region, utilizing only the applicable guidelines to other regions of the world would be a mistake and in long-term would damage the soil and water resources, therefore local researches need to be carried out (Kalavrouziotis & Arslan-Alaton, 2008).

Robinia pseudoacacia L. (black locust) is native to the southeastern United States, but has been widely planted and naturalized elsewhere. *R. pseudoacacia* trees have nitrogen- fixing bacteria on its root system, for this reason it can grow on poor soils, therefore it can improve fertility of soil. In Iran it often planted alongside streets, in green space and parks, especially in large cities, because it tolerates pollution well (Mossadegh, 1993). The use of municipal wastewater in growing *R. pseudoacacia* in suburban areas could be beneficial for the economic disposal of wastewater, defers ecological degradation by containing the pollutants in the soil and growth of vegetation having aesthetic and environmental value. The present study was carried out around Tehran, Iran, where wastewater has been commonly used for irrigation of peri-urban crops for many years. The objective of the present report is to quantify concentration and contamination of Zn, Cu, Ni and Pb in irrigation water, soil and leaves of *R. pseudoacacia* trees from site having long-term use of wastewater for irrigation of land.

2. Materials and methods

2.1 Site description

The study site is located in Shahr-e Rey, 5 Km south of Tehran-Iran (Latitude 35° 37' N, Longitude 51° 23' E, 1005 m above sea level). The climate of the area is semi-arid with mild-cold winters and 7 months (Mid April-Mid November) dry season. Average annual rainfall and average annual temperature are 232 mm and 13.3° C, respectively. The highest rainfall is in March (41.32 mm) and the lowest in August (0.89 mm). The warmest month occurs in August and the coldest in January.

Experiments were conducted at two even-aged (15 years) artificial stands of black locust in October 2006. The first stand was irrigated with municipal wastewater and the second with well water since they were planted. Durations of irrigation were based on tree water-use and the potential evapo-transpiration, which varied seasonally in response to the climate and on an average the irrigations were carried on 8 day durations for 8 months/year (April-November). The soil of two stands were both clay-loam (according to US soil taxonomy) with 29.25% clay, 36.20% silt and 34.55% sand in the stand irrigated with municipal wastewater and 27.14% clay, 37.86% silt and 35% sand with well water (Table 2).

2.2 Plant and soil sampling

For the sampling of leaf and soil, four plots were randomly identified in each stand. Plots were 30 m × 30 m, with tree spacing of 3 m × 4 m. In each plot, four trees were selected and in the growing season leaf samples of *Robinia pseudoacacia* trees taken from the top of crown and the part affected by sunlight (Habibi Kaseb, 1992). This collection provided 16 leaf samples in each stand. At the end of the sampling, one representative leaf sample from each plot (by mixing of four samples of each plot) was taken (decreasing of sample quantity for chemical analysis). Soil samples were taken under each selected tree from the root zone at a depth interval of 15 cm down to 60 cm by digging profiles. This collection provided 48 soil samples in each stand from three depths (0-15, 15-30 and 30-60 cm). At the end of soil sampling, three representative soil samples of three depths from each plot were taken by mixing of samples of each layer in each plot (decreasing of sample quantity for chemical analysis) according to Habibi Kaseb (1992). Municipal wastewater and well water were sampled daily (3 days in each month) from early June to late November at three-hour intervals (7 am, 13 pm and 19 pm) to make a composite sample of each day.

2.3 Laboratory analysis

Concentrated HNO_3 was added to the water samples to avoid microbial utilization of heavy metals (Sharma et al., 2007) and then they were brought to the laboratory in resistant plastic bottles to avoid adherence to the container wall. They were filtered through a Whatmann 42 mm filter paper and stored at 4 °C to minimize microbial decomposition of solids (Yadav et al., 2002; Bhati & Singh, 2003). Some parameters were measured separately, pH and EC by the procedure described using OMA (1990) and heavy metals (Zn, Cu, Ni and Pb) of water samples were estimated by the aqua regia method of Jackson (1973) followed by a measurement of concentrations using an Atomic Absorption Spectrophotometer (model-3110, Perkin-Elmer, Boesch, Huenenberg, Switzerland).
The soil samples air-dried, crushed, passed through a 2 mm sieve and were analyzed for various physico-chemical properties. Soil texture was determined using the hydrometer method according to Bouyoucos (1965). Soil pH and electrical conductivity (EC) were determined in 1:2 soil:water suspension by pH and EC meters (Hati et al., 2007). Soil organic carbon (SOC) content was determined by the Walkley–Black method (Nelson & Sommers, 1996). Calcium carbonate ($CaCO_3$) was measured with a calcimeter. The concentration of soil heavy metals (Zn, Cu, Ni and Pb) was extracted after digestion with 3:1 concentrated HCl–HNO_3 and measured by Atomic Absorption Spectrophotometer (Gasco´ & Lobo, 2007).
Leaf samples were washed using tap water, rinsed with distilled water, oven dried at 80 °C for 24 h, ground in a stainless steel mill and retained for chemical analysis (Singh & Bhati, 2005). For determination of heavy metal concentration (Zn, Cu, Ni and Pb), the leaf samples were wet digested as per Jackson (1973) and were measured using an Atomic Absorption Spectrophotometer.

2.4 Statistical analysis

Average leaf heavy metals and soil physico-chemical properties of two stands (irrigated with municipal wastewater and irrigated with well water), compared using independent-samples t-test (Pelosi & Sandifer, 2003). Data of soil heavy metals were analyzed for differences due to depth in the profile using one-way ANOVA. Furthermore, the variations in EC, pH and heavy metals of municipal wastewater and well water were also tested using independent-samples t-test. All the data were analyzed using the SPSS statistical package (Lindaman, 1992).

3. Results and discussion

3.1 Physico-chemical properties of wastewater and well water

The quality of municipal wastewater and well water was assessed for irrigation with respect to their pH, EC, and concentration of heavy metals (Table 1). Results indicated that the waters were alkaline in reaction. The pH of the municipal wastewater in various months ranged from 7.51 to 7.75 and 6.69 to 7.62 for well water. The EC of wastewater ranged from 1.78 to 2.12 dS/m with the greatest value detected in August. The average EC of municipal wastewater exceeded 1 dS/m (1.91 dS/m) indicating that this wastewater was saline in nature (Rattan et al., 2005). The pH and EC of municipal wastewater were significantly ($P < 0.01$) higher than the well water. The concentration of heavy metals (Zn, Cu, Pb and Ni) tended to be higher in municipal wastewater. In water samples, Zn, Cu, Pb and Ni concentrations were 0.43, 0.09, 0.033 and 0.028 mg/l, respectively in well water samples, whereas, corresponding values for wastewater were 3.30, 1.26, 0.106 and 0.081 mg/l. On an average, wastewater contained 7.67, 14, 3.21 and 2.89 times higher amounts of Zn, Cu, Pb and Ni respectively compared to well water. The comparison of measured factors with WHO (World Health Organization) standard showed that water used for irrigation based on pH and EC were in a normal range, however based on heavy metals: Pb and Ni concentration of municipal wastewater and well water was higher than standard range. Zn concentration of municipal wastewater also was higher than the standard but Cu concentration was normal. The concentration of these two elements was lower than the standard in well water (Table 1).

Parameters	Units	Municipal wastewater	Well water	WHO*
pH	---------	7.63 ± 0.01^{a}	7.32 ± 0.05^{b}	6.5 - 8.5
EC	(dS/m)	1.91 ± 0.02^{a}	0.590 ± 0.008^{b}	3
Zn	(mg/l)	3.30 ± 0.06^{a}	0.43 ± 0.07^{b}	3
Cu	(mg/l)	1.26 ± 0.03^{a}	0.09 ± 0.01^{b}	1-2
Pb	(mg/l)	0.106 ± 0.063^{a}	0.033 ± 0.026^{b}	0.01
Ni	(mg/l)	0.081 ± 0.007^{a}	0.028 ± 0.002^{b}	0.02
Different superscripts in row indicate significant ($P < 0.01$) difference. Values are mean of eighteen replications (3 days * 6 months) with ± SE, * Hach, 2002				

Table 1. Characteristics of municipal wastewater and well water

3.2 Impact of municipal wastewater irrigation on soil properties

Data of Table 2 indicate that application of municipal wastewater were resulted an increase (0-60 cm soil layer; mean of soil layers) in pH, EC, C, organic matter and $CaCO_3$ of wastewater-irrigated soil as compared to well water-irrigated soil. Increase in pH was 1.02 unit and EC 1.68 times in soil of wastewater treatment compared to the soil of well water treatment. The increase in pH and EC of soil in the wastewater-irrigated stand may have been due to alkaline nature of municipal wastewater (Singh & Bhati, 2005). SOC as a basic index of soil playing a variety of roles in nutrient, water, and biological cycles (Rattan et al., 2005) was 1.17%–1.29% in municipal wastewater-irrigated soil, whereas it was 0.88%–1.14%

Soil properties	Clay (%)	Silt (%)	Sand (%)	texture	pH	EC (dS/m)	C (%)	Organic matter (%)	$CaCO_3$ (%)
Wastewater irrigated soil	29.25	36.20	34.55	Clay loam	8.17^{a} (0.03)	1.28^{a} (0.04)	0.718^{a} (0.032)	1.23^{a} (0.05)	20.20^{a} (0.57)
Well water irrigated soil	27.14	37.86	35	Clay loam	7.94^{b} (0.10)	0.763^{b} (0.036)	0.585^{b} (0.062)	1.00^{b} (0.107)	18.55^{b} (0.45)
Values are mean of four replications with ± SD in parentheses; Different superscripts in columns indicate significant ($P < 0.01$) difference									

Table 2. Soil properties of two stands (0-60 cm)

in soil irrigated with well water. Increase in SOC content might be due to municipal wastewater application (Bhati & Singh, 2003). In general, the suitability of soils for receiving wastewater without deterioration varies widely, depending on their infiltration capacity, permeability, cation exchange capacities, phosphorus adsorption capacity, texture, structure, and type of clay mineral (Ivan & Earl, 1972).

The concentration of heavy metals (Zn, Cu, Pb and Ni) was higher in all depths of wastewater irrigated soil compared to those of well water irrigated soil (Fig. 1). As a matter of fact, high concentration of heavy metals in wastewater leads to increase them in soil (Huerta et al., 2002; Nan et al., 2002; Mapanda, et al., 2005). The comparison of soil Zn, Cu, Pb and Ni with critical range of heavy metals in soil (Table 3) showed that only Ni of soil treated with municipal wastewater and Pb of soil treated with the both municipal wastewater and well water were higher than the standard amounts of soil. The effects of wastewater irrigation on accumulation of soil heavy metals depend on various factors such as concentration of wastewater heavy metals, the period of wastewater irrigation, and soil properties (pH, texture, organic matter) (Rattan et al., 2005). And also generally, 10 to 50 years is needed so that the heavy metal levels precede the standard levels (Smith et al., 1996). Because of the high concentration of Pb in all soil and water samples, it can be predicted that besides the municipal wastewater, Pb probably has been added to the water and soil from other sources such as air pollution.

In the present investigation the concentration of heavy metals decreased with soil depth in both stands (Fig. 2). These results are in agreement with the findings obtained later (Yadav

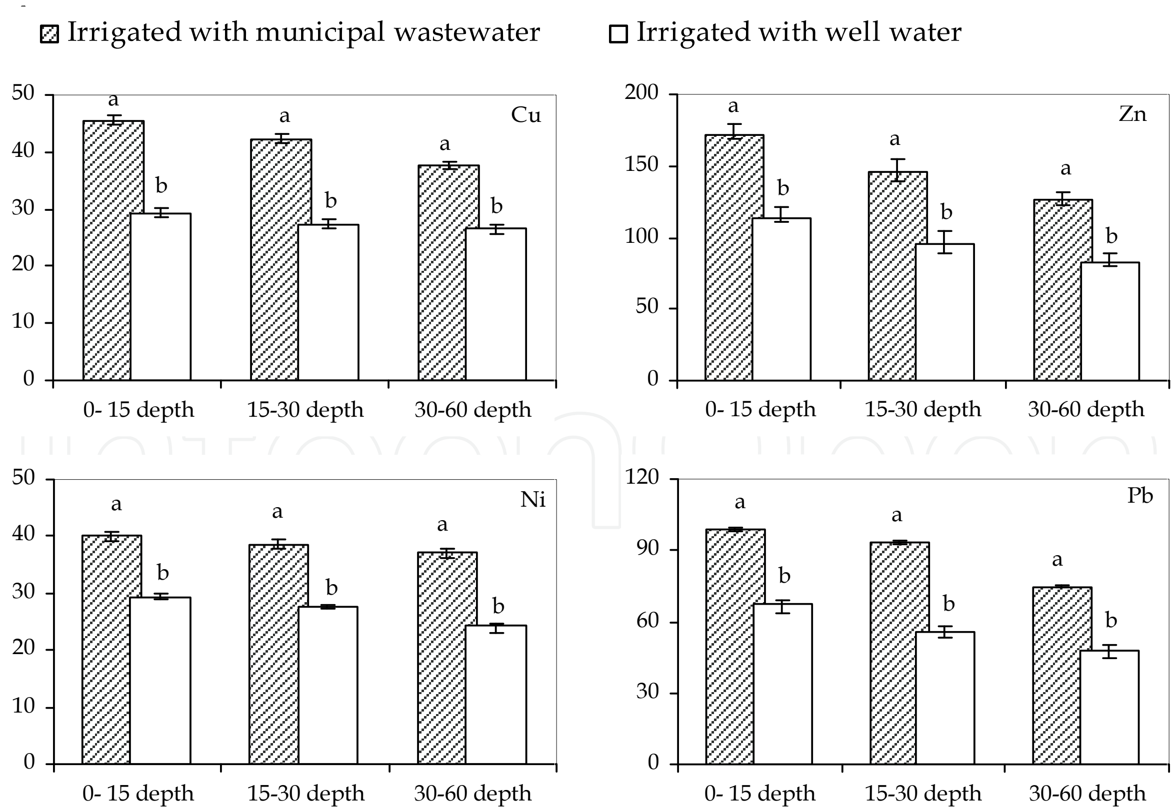

Fig. 1. Comparison of heavy metals in similar depths (0-15, 15-30 and 30-60 cm) between soils irrigated with wastewater and well water (mg/kg); Error bars are ± SE

et al., 2002). Since, the soil surface is richer in heavy metals than the underlying layers, greater accumulation in the topsoil probably is due to soil texture (the soil texture in both stands is clay-loam, as a result penetrability is decreased and accumulation of heavy metals are often observed at upper layers), low mobility of heavy metals in soil (Afyoni et al., 1998), and surface application of municipal wastewater.

Heavy metals	Critical range *
Zn (mg/kg)	10-500
Cu (mg/kg)	5-400
Pb (mg/kg)	40
Ni (mg/kg)	30
* Zn and Cu: Salardiny (1992); Pb and Ni: (EPA)	

Table 3. Critical range of heavy metals in soil

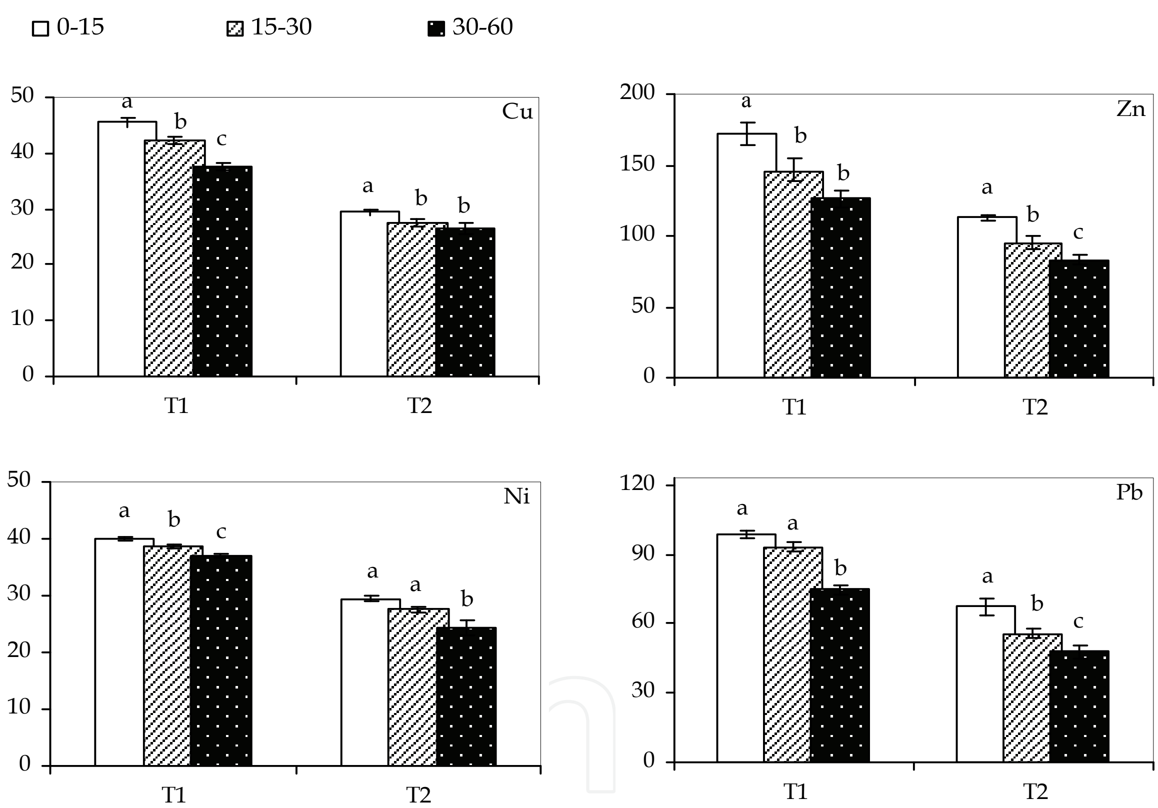

Fig. 2. Comparison of heavy metals among different depths (0-15, 15-30 and 30-60 cm) of soil in each irrigated stand (mg/kg); T_1: Soil irrigated with wastewater, T_2: Soil irrigated with well water; Error bars are ± SE

3.3 Changes in concentration of leaf heavy metals

The concentration of Zn and Cu elements in the leaves of black locust trees differed significantly under impact of two irrigation treatments. These concentrations in the leaves of wastewater irrigated trees were about 1.5 times higher than those of well water irrigation. However, irrigation with municipal wastewater did not result in toxicity to Zn and Cu of leaves (Table 4). Marked difference in Zn and Cu of tree leaves may be due to the increase of them through municipal wastewater (Meli et al., 2002). This result is in agreement with Singh & Bhati (2005) and Aghabarati et al. (2008), where substantially greater concentration of these elements were observed in leaf of *Dalbergia sissoo* seedlings and *O. europaea* trees irrigated with municipal wastewater compared to control. Ni and Pb were not detected in leaf samples which may be due to the low dynamic of heavy toxic metals, whereas it was likely accumulated in lower parts of the plant, such as root and stem. Nevertheless, Madejo´on et al. (2006) reported the presence of some heavy toxic metals in leaf of olive and holm oak trees. In fact, the quantity of element absorption using plant depends upon many factors including the total quantity of the elements applied through wastewater application, soil properties, and type of plant (Bozkurt & Yarilga, 2003; Kalavrouziotis and Arslan-Alaton, 2008).

Heavy metals	Wastewater	Well water	*P*-value	Range*
Zn (mg/kg)	30.62 ± 6.00^{a}	20.63 ± 2.60^{b}	<0.05	10-100
Cu (mg/kg)	4.87 ± 0.77^{a}	2.81 ± 0.23^{b}	<0.01	2-20
Ni (mg/kg)	nd	nd	------	-------
Pb (mg/kg)	nd	nd	------	-------
Values are mean of four replications with ± SD; different superscripts in rows indicate significant difference; nd: not detected; * Salardiny (1992)				

Table 4. Concentration of heavy metals in leaf of black locust trees irrigated with wastewater and well water

4. Conclusion

Today, the reuse of municipal wastewater for land irrigation constitutes a practical method of disposal which is expected to contribute decisively to the handling and minimization of environmental problems arising from the disposal of wastewater effluents on land and into aquatic systems. The application of wastewaters onto appropriate forest species will enable long term environmental protection, creating a new water source in significant quantities for the irrigation of forested areas at the same time. Again, the use of wastewaters for irrigating maybe increases heavy metals and pathogens in soil and plant. Hence, the control of all of parameters associated with the disposal of wastewaters on land should be done for safe reuse of them. Furthermore, the method and extent of use of wastewaters, however, vary according to the infrastructure and the local socio-economic conditions prevalent from country to country.

According to the results of the present paper from the area under study where municipal wastewater is being used for about 15 years, high level of some heavy metals in irrigation water and soil treated with municipal wastewater and possibility of accumulation of heavy toxic metals in lower parts of the plant, it is said that regulations about the utilization of municipal wastewater in irrigation should consider in order to minimize the risk of negative effects to ecosystem health. This can be controlled by avoiding toxic elements from entering the municipal wastewater and continued monitoring or treatment of wastewaters before it is put into disposal channel for irrigation.

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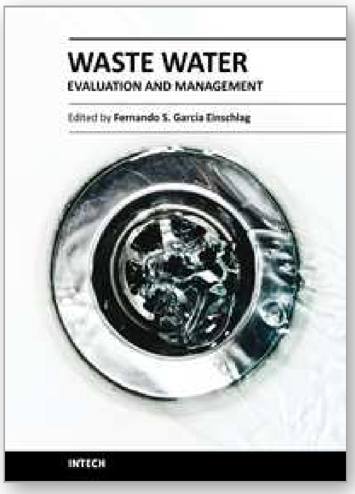

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Fresh water resources are under serious stress throughout the globe. Water supply and water quality degradation are global concerns. Many natural water bodies receive a varied range of waste water from point and/or non point sources. Hence, there is an increasing need for better tools to asses the effects of pollution sources and prevent the contamination of aquatic ecosystems. The book covers a wide spectrum of issues related to waste water monitoring, the evaluation of waste water effect on different natural environments and the management of water resources.

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