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HEAVY METAL LEVELS OF MULBERRY (*MORUS ALBA L.*) GROWN AT DIFFERENT DISTANCES FROM THE ROADSIDES

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ABSTRACT

The heavy metals pollution of mulberry cultivated soil, mulberry leaf and fruit, growing at different distances from the roadside were investigated in the Upper Coruh Valley of Turkey. The rate of heavy metals in soil, leaf and fruit were detected by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). All of the heavy metals (Zn, Mn, Cu, Co, Cr, Pb, Cd and Ni) were found in soil and parts of plant and their highest level at a distance of 20 m from the roadside. At a distance of 20 m from the roadside, the soil was found to be contaminated by Zn (330.7 mg kg^{-1}), Cu (217.5 mg kg^{-1}), Pb (500.2 mg kg^{-1}) and Cd (4.24 mg kg^{-1}) pollutants. The concentration of Pb in mulberry fruit was found to be more than the permissible limits at a 20 and 100 m distances from the roadside with its 0.467 and 0.419 mg kg^{-1} values, respectively. At all of distances, the concentration of Ni in fruit was found higher than the permissible limits. The results reflected a strong relationship between soil and fruits for contamination with some heavy metals.

Key words: *Morus alba* L., heavy metal pollution, the Upper Coruh Valley, Turkey.

INTRODUCTION

Mulberry (*Morus* spp.) is an important fruit specie in Turkey (Ercisli and Orhan, 2007). Although it is extensively grown as food for silkworms in many countries, mulberry fruit production is a main aim in Turkey, which is one of the most important mulberry fruit producers in the world (Ercisli, 2004). Turkey produced 65,140 t of mulberry in 2008 (Anonymous 2009). Its fruit can be eaten fresh or dried. It can also be processed into mulberry juices, paste, jam, pulp and jelly (Maskan and Gogus, 1998) and several traditional products such as “Mulberry Pekmez”, “Mulberry Pestil” or “Mulberry Kome” in the Eastern and Central part of Turkey (Orhan *et al*., 2007).

Although mulberries in the Eastern and Central part of Turkey are grown without using chemical fertilizers and pesticides, these areas are thought to have been affected by heavy metals. Rapid and unorganized urbanization and industrialization has elevated the levels of heavy metals in the environment of developing countries (Sharma *et al*., 2009). Industrial uses of metals and other domestic processes have introduced substantial amounts of potentially toxic heavy metals into the atmosphere and into aquatic and terrestrial environments (O’Connell *et al*., 2008). Heavy metals have a significant toxicity for human, animals, microorganisms and plants (Fotakis and Timbrell, 2006). Thus, the contaminations of fruit with heavy metals pose a serious threat to its quality and jeopardize food safety (Sharma *et al*., 2008). Lead and cadmium are very harmful elements for human body especially in high concentration (Hamurcu *et al*., 2010). Therefore, FAO/WHO established the permissible maximum limit of Cd in fruiting vegetables as 0.05 mg kg^{-1} and Pb in berries and other small fruits as 0.2 mg kg^{-1} (CODEX STAND 1995). Recently, pollution in developing areas has been increasing. Various factors such as exhaust gas, industry waste and waste water have increased the heavy metal contamination in fruit and other edible parts of plants. The most important cause of pollution for the plants on roadsides is probably exhaustion gases. It is expected that heavy metal contents of plants may change according to their distances from the main roads. For example, plants near the roadsides are probably rich in heavy metals as compared to plant grown at distance from the roads (Hamurcu *et al*., 2010). It has been reported that dusts and roadside soils near motorways are polluted with Pb by auto-traffic (Chen *et al*., 1997). Lead-based and gasoline-related emissions from vehicles contribute to the increasing levels of Pb, Cd, Cu, Zn and Ni in soil (Fakayode and Olu-Owolabi, 2003).

The aim of the current study was to determine heavy metal levels in mulberry cultivated soil as well as the leaves and fruits of mulberry grown at different distances from the roadside and elucidate the possible sources of the heavy metal pollution.

MATERIALS AND METHODS

Samples collection: The study was carried out in the Upper Coruh Valley in the northeast of Turkey in July, 2007 and 2008. The sampling points vary between 1190 and 1240 m altitudes. Five sampling sites, situated along the D-925 road in the section of Erzurum to Ispir were selected for this study. The road, carrying more than 2.000 motor vehicles per day, is the main road connecting Erzurum to Rize. Mulberry leaves and fruits were sampled at a distance of 20, 100, 400 and 1000 m from the roadside including two trees for each distance with five replicates (five sites) in a completely randomized design. From two trees, about 1 kg samples for fruit and a sufficient amount of samples for leaves were collected at each distance and each sampling site for the study. Soil samples were taken from the canopy areas of sample mulberry trees at a depth of 0-10 cm. All of the samples were analyzed as two parallels.

Analyses of soil physical and chemical characteristics: The soil was air-dried and passed through a 2 mm sieve for chemical and physical analyses. Particle-size analysis was performed by the pipette method after pretreatment with 35% H2O2 according to Gee and Bauder (1986). Cation-exchange capacity (CEC) was determined by using sodium acetate-ammonium acetate buffered at pH 7 according to Sumner and Miller (1996). Available P was determined using the sodium bicarbonate method of Olsen *et al*., (1954). Soil-pH determinations were done in 1:2 (w/v) water extract, and calcium carbonate levels were determined according to McLean (1982). Soil organic matter was determined using the Smith-Weldon method as described by Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 Rhoades (1982) was used to determine exchangeable cations (K, Mg, Na and Ca), using a Perkin-Elmer 360 Atomic Absorption Spectrophotometer (Perkin-Elmer, Waltham, Massachusetts, USA).

Analyses of soil and plant tissue heavy metal levels: Soil samples were air-dried, ground, and passed through a 100 mesh plastic sieve. Plant samples (mulberry fruits and leaves) were oven-dried at 68^{o}C for 48 h and ground to pass through 1 mm sieve. Soil samples were digested with concentrated HCl +concentrated HNO_3 + HF + $HClO_4$ (10:5:5:3, v/v) and plant tissues (fruits and leaves) digested with concentrated HNO_3-H_2O_2 acid mixture (2:3 v/v) in three step (first step; 145ºC, 75%RF, 5 min; second step; 180ºC, 90%RF, 10 min and third step; 100ºC, 40%RF, 10 min) in microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens 2005a). Contents of Co, Cr, Zn, Pb, Cd, Mn, Cu and Ni in soil samples and plant tissue were determined by using an Inductively Couple Plasma, Optical Emission Spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens 2005b).

Data analysis: Descriptive statistics for each trait were expressed. No significant effect of year factor was found. Therefore, the obtained data from two years was pooled and re-analyzed using two-way ANOVA. Significant differences were determined by LSD test.

RESULTS

The main physical and chemical soils properties: Some physical and chemical properties of mulberry cultivated soils are given in Table 1. At different distances from the road, soil sand, silt, clay, pH , cation exchange capacity (CEC), $CaCO_{3,}$ organic matter (OM), P, Na, Ca, K and Mg ranged from 37.65 to 46 g/kg, 18.12 to 25.24 g/kg, 35.67 to 42.19 g/kg, 7.13 to 7.65, 21.40 to 26.48 $cmol_c$/kg, 0.92 to 1.52, 1.07 to 2.00%, 2.23 to 2.4, 0.64 to 0.77, 11.58 to 14.66, 5.94 to 6.33 and 2.21 to 2.68, respectively. The mean pH of the soil samples was 7.28 and CEC was 23.64 meq 100 g^{-1} and soil texture was clay loam.

Heavy metal in soil: Heavy metals (Co, Cr, Zn, Pb, Cd, Mn, Cu and Ni) concentration of soil samples showed a variation depending on the distances from the roadside (from 20 to 1000 m) in the mulberry cultivated area. It was observed that the motor traffic activity of roadsides increased all of the metal content, especially Co, Cr, Pb, Cd and Ni in the soil. There were statistically significant differences between the distances in respect of total element concentration (Table 2). The available and total Mn, Zn, Cu, Co, Cr, Pb, Cd and Ni contents of the soil near to road side (20-100 m) were considerably higher than samples taken farther away from the road side (> 400 m) (Table 2).

Available form heavy metals in the soil were determined between 2.48-1.1 mg kg^{-1} Zn, 105.8-38.2 mg kg^{-1} Mn, 4.14-1.72 mg kg^{-1} Cu, 1.48-0.37 mg kg^{-1} Co, 0.05-0.02 mg kg^{-1} Cr, 3.89-1.09 mg kg^{-1} Pb, 0.05-0.02 Cd and 0.66-0.33 mg kg^{-1} Ni, while total heavy metal in the soil varied from 330.7 to 295.4 mg kg^{-1} for Zn, 527.3 to 436.8 mg kg^{-1} for Mn, 217.5 to 184.7 mg kg^{-1} for Cu, 11.4 to 7.95 mg kg^{-1} for Co, 48.3 to 33.69 mg kg^{-1} for Cr, 500.2 to 76.6 mg kg^{-1} for Pb, 4.24 to 1.73 mg kg^{-1} for Cd, and 26.45 to 16.96 mg kg^{-1} for Ni (Table 2).

Heavy metal in mulberry leaves and fruits: The concentrations of heavy metal levels in the mulberry leaves and fruits from different distances from the road are summarized in Table 3. In the leaves, the heavy metals concentrations ranged from 18.55-23.88 mg kg^{-1} for Zn, 234.0-354.4 mg kg^{-1} for Mn, 6.38-8.26 mg kg^{-1} for Cu, 0.24-0.72 mg kg^{-1} for Co, 0.28-0.61 mg kg^{-1} for Cr, 0.86-1.28 mg kg^{-1} for Pb, 0.03-0.06 mg kg^{-1} for Cd and 0.92-2.02 mg kg^{-1} for Ni, while the concentration of the heavy metal in the fruits varied from 5.97 to 8.74 mg kg^{-1} for Zn, 74.1 to 127.2 mg kg^{-1} for Mn, 2.12 to 3.14 mg kg^{-1} for Cu, 0.08 to 0.26 mg kg^{-1} for Co, 0.09 to 0.23 mg kg^{-1} for Cr, 0.28 to 0.47 mg kg^{-1} for Pb, 0.01 to 0.02 mg kg^{-1} for Cd and 0.3 to 0.75 mg kg^{-1} for Ni. The heavy metals in the mulberry leaves and fruits, as in the soil samples, were detected their highest levels at a distance of 20 m from the road in the study.

Relationships between soil heavy metals content and mulberry plants (Fruits): The relationships between the heavy metal contents of soil and mulberry fruits at different distances from the road are illustrated in (Table-4). The correlation analysis showed a strong relationship between heavy metal (available form) concentration in soil and in mulberry fruit, particularly for Co, Cr, Zn, Pb, Mn, Cu and Ni. A significant correlation was found between mulberry cultivated soil and mulberry fruits in terms of Zn, Pb and Cu concentration at a distance of 20 m from the road. However, the correlation between soil and fruit samples for all heavy metals at a distance of 100 m from the road was non-significant. A strong relationship was also detected between soil and fruits in terms of Co, Pb and Mn concentrations at a distance of 400 m from the road and Cr, Pb, Mn, Cu, Ni and B concentrations at a distance of 1000 m from the road. Accordingly, some heavy metal available forms were highly expressed in mulberry trees and their fruits along the road side, except for a distance of 100 m, in soils.

DISCUSSION

Heavy metal in soil: The acceptable limits of some available form of heavy metals in the soil are 1 mg kg^{-1} for Cd, 8 mg kg^{-1} for Cu, 18 mg kg^{-1} for Pb and 43 mg/kg for Zn (Davidescu *et al*., 1988).. In the present study, Cd, Cu, Pb and Zn did not exceed the acceptable limits reported by Davidescu *et al*., (1988) and Özkutlu *et al.*, (2009). The maximum permissible levels of total heavy metals in the soil were defined by Turkish Ministry of Environment and Forest (TMEF) with national norms (Anonymous 2005). It must be noted here that these norms do not vary much with the norms of European countries. According to these norms (25831-2005, for soil pH>6: Cr ≤ 100 mg kg^{-1}: Zn ≤ 300 mg kg^{-1}: Pb ≤ 300 mg kg^{-1}: Cd ≤ 3 mg kg^{-1}: Cu ≤ 140 mg kg^{-1}: Ni ≤ 75 mg kg^{-1}), the concentrations of Cr and Ni levels in the mulberry cultivated soil did not exceed permissible upper limits at any of distances from the road. By contrast, the concentration of Zn, Pb and Cd at 20 m distance from the road while Cu at all distances from the road exceeded the permissible limits compared to the national norms (Table 2). There is no information about Mn and Co levels in soil samples in the national norms. Excess amount of total heavy metal levels in the soil may have toxic effects on the plants (Li *et al*., 2007). Kuno (1984) reported that an excess of heavy metals such as Cd, Cu, Zn and Ni in the soil causes inhibition of mulberry growth and visible symptoms such as chlorosis and malformation of roots and leaves. Generally, phytotoxicities of soil have been associated with 60-125 mg kg^{-1} total Cu (Ross 1994), 70-150 mg.kg^{-1} total Cd (Jiang *et al*., 2001), 500-1000 mg kg^{-1} total Pb (Foy *et al*., 1978), and 41.528-120.028 mg kg^{-1} total Mn (Paschke *et al.*, 2005). In the present study, total heavy metal concentration in the soil such as Cu (at all distances from the road), Pb (at 20 m distance from the road) and Mn (at all distances from the road) were at phytotoxicity levels in comparison with Ross (1994); Foy *et al*. (1978); Paschke *et al*., (2005).

Heavy metal in mulberry leaves and fruits: The current data showed that the heavy metal levels of the mulberry leaves were higher than the mulberry fruits (Table 3). Previously, Hamurcu *et al*., (2010) reported that the leaves of vegetables and fruit plants contained more trace elements and heavy metals than the edible parts. Nookabkaew *et al*., (2006) reported that the average concentrations of heavy metals in the mulberry leaves were 28.5 mg kg^{-1} for Zn, 155.6 mg kg^{-1} for Mn, 8.0 mg kg^{-1} for Cu, 0.09 mg kg^{-1} for Co, 0.79 mg kg^{-1} for Cr, 0.40 mg kg^{-1} for Pb, 0.01 mg kg^{-1} for Cd and 0.96 mg kg^{-1} for Ni. In the another study, it was reported that the concentrations of Zn, Cu and Pb in the mulberry leaves was 0.42, 0.60 and 0.77 mg kg^{-1} in polluted areas and 0.32, 0.46 and 0.49 mg kg^{-1} in unpolluted areas, respectively (Arnaudova and Grekov, 2003). The present study shows that the concentrations of Mn, Co, Pb, Cd, Ni in the mulberry leaves was observed higher than those detected in Thailand (Nookabkaew *et al*., 2006) and the concentration of Zn, Cu and Pb was higher than those detected in polluted and unpolluted areas in Bulgaria (Arnaudova and Grekov, 2003).

The maximum permissible level of Cu, Ni, Pb and Cd concentration for the foods are determined as 5 mg kg^{-1}, 0.2 mg kg^{-1}, 0.4 mg kg^{-1} and 0.05 mg kg^{-1}, respectively according to Turkish Food Codex (Anonymous 2002). There is no information about Zn, Mn, Co or Cr levels in fruit samples in Turkish standards. In the current study, the concentration of Pb (at 20 and 100 m distance from the road) and Ni (at all of distances) levels in mulberry fruits exceeded maximum permissible limits compared to Turkish Food Codex . Trichopoulos (1997) has reported the Pb has a toxic effect for human metabolism even in low amounts and may have carcinogenic effects.

Duran *et al*., (2008) have reported 1.5-10 fold higher concentration of heavy metals (Ni: 2.12, Pb: 5.50, Co: 1.78, Cr: 0.80 and Cd: 0.63 µ/g) in dried mulberry fruits compared to results obtained in the present study. Also, our results were comparable with those reported by other authors (Hamurcu *et al*., 2010; Kumar *et al*., 2007; Ozkutlu *et al*., 2009).

Table 1. Some physical and chemical characteristics of mulberry cultivated soil at different distances from the road

Soil characteristics		Distances from the road (m)				*Mean*	*LSD*	*Probability*
		20	100	400	1000			
Physical characteristics	Sand%	37.65^{d}	40.98^{b}	46.00^{a}	38.56^{c}	*40.80*	*0.815*	*0.0001*
	Silt%	20.16^{b}	18.12^{c}	18.33^{c}	25.24^{a}	*20.46*	*1.456*	*0.0001*
	Clay%	42.19^{a}	40.90^{b}	35.67^{d}	36.20^{c}	*38.74*	*0.412*	*0.0001*
	pH	7.13^{b}	7.65^{a}	7.15^{b}	7.20^{b}	*7.28*	*0.329*	*0.0314*
	CEC	26.48^{a}	22.23^{c}	21.40^{d}	24.46^{b}	*23.64*	*0.512*	*0.0001*
Chemical characteristics	$CaCO_3$	1.52 a	1.03^{a}	0.92^{a}	0.96^{a}	*1.11*	*1.144*	*0.5124*
	OM	1.41^{bc}	1.80^{ab}	2.00^{a}	1.07^{c}	*1.57*	*0.430*	*0.0130*
	P	2.40^{a}	2.23^{a}	2.38^{a}	2.23^{a}	*2.31*	*0.255*	*0.2416*
	Na	0.75^{a}	0.64^{a}	0.64^{a}	0.77^{a}	*0.7*	*0.333*	*0.6095*
	Ca	14.66^{a}	12.70^{b}	14.39^{a}	11.58^{b}	*13.33*	*1.477*	*0.0123*
	K	6.33^{a}	5.94^{a}	5.80^{a}	6.20^{a}	*6.07*	*1.313*	*0.6913*
	Mg	2.21^{a}	2.33^{a}	2.68^{a}	2.56^{a}	*2.44*	*1.048*	*0.6304*

Values with different letters in a row are significantly different at the 0.05 level according to LSD test

Table 2. Some heavy metal available and total form concentrations of mulberry cultivated soil at different distances from the road (mg kg^{-1})

Distances from road (m)	Zn		Mn		Cu		Co		Cr		Pb		Cd		Ni	
	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total
20	2.48^{a}	330.7^{a}	105.8^{a}	527.3^{a}	4.14^{a}	217.5^{a}	1.48^{a}	11.40^{a}	0.05^{a}	48.3^{a}	3.89^{a}	500.2^{a}	0.05^{a}	4.24^{a}	0.66^{a}	26.45^{a}
100	1.43^{a}	298.5^{b}	86.1^{b}	468.9^{b}	2.76^{b}	186.7^{b}	0.90^{b}	8.78^{b}	0.03^{b}	36.41^{b}	2.33^{b}	390.6^{a}	0.04^{b}	2.81^{b}	0.59^{a}	19.02^{b}
400	1.31^{a}	296.3^{b}	65.1^{c}	438.7^{b}	2.61^{b}	185.2^{b}	0.55^{c}	8.33^{b}	0.03^{b}	34.3^{b}	1.66^{bc}	178.3^{b}	0.03^{c}	2.12^{bc}	0.55^{a}	17.28^{b}
1000	1.1^{a}	295.4^{b}	38.2^{d}	436.8^{b}	1.72^{c}	184.7^{b}	0.37^{d}	7.95^{b}	0.02^{c}	33.69^{b}	1.09^{c}	76.6^{b}	0.02^{d}	1.73^{c}	0.33^{b}	16.96^{b}
Mean	*1.58*	*305.2*	*73.8*	*467.9*	*2.81*	*193.5*	*0.83*	*9.12*	*0.03*	*38.18*	*2.24*	*286.40*	*0.033*	*2.73*	*0.53*	*19.93*
LSD	*1.59*	*12.15*	*19.16*	*41.94*	*0.891*	*11.63*	*0.12*	*1.144*	*0.01*	*9.73*	*0.742*	*116*	*0.005*	*0.776*	*0.188*	*2.5*
*P**	*0.265*	*0.0004*	*0.0002*	*0.0035*	*0.0017*	*0.0004*	*0.0001*	*0.0005*	*0.0007*	*0.0034*	*0.0001*	*0.0001*	*0.0001*	*0.0003*	*0.019*	*0.0001*

**probability* *Values with different letters in a column are significantly different at the 0.05 level according to LSD test*

Table 3. Some heavy metal contents of mulberry leaf and fruits at different distances from the road (mg kg^{-1})

Distances from road (m)	Zn		Mn		Cu		Co		Cr		Pb		Cd		Ni	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
20	23.88^{a}	8.74^{a}	354.4^{a}	127.2^{a}	8.26^{a}	3.14^{a}	0.72^{a}	0.26^{a}	0.61^{a}	0.23^{a}	1.28^{a}	0.47^{a}	0.06^{a}	0.020^{a}	2.02^{a}	0.75^{a}
100	21.96^{b}	8.21^{a}	315.8^{b}	116.3^{a}	7.46^{ab}	2.89^{ab}	0.59^{b}	0.22^{a}	0.42^{b}	0.16^{b}	1.13^{b}	0.42^{ab}	0.04^{b}	0.015^{ab}	1.54^{ab}	0.57^{ab}
400	20.62^{b}	7.73^{ab}	294.5^{c}	108.8^{a}	7.07^{bc}	2.74^{ab}	0.39^{c}	0.14^{b}	0.35^{c}	0.14^{bc}	0.95^{c}	0.35^{bc}	0.04^{b}	0.014^{b}	1.27^{bc}	0.48^{ab}
1000	18.55^{c}	5.97^{b}	234.0^{d}	74.1^{b}	6.38^{c}	2.12^{b}	0.24^{d}	0.08^{c}	0.28^{d}	0.09^{c}	0.86^{c}	0.28^{c}	0.03^{b}	0.010^{b}	0.92^{c}	0.30^{b}
Mean	*21.25*	*7.66*	*299.70*	*106.60*	*7.29*	*2.72*	*0.48*	*0.18*	*0.41*	*0.16*	*1.06*	*0.37*	*0.04*	*0.015*	*1.44*	*0.52*
LSD	*1.87*	*1.92*	*17.51*	*24.87*	*0.93*	*0.79*	*0.11*	*0.06*	*0.06*	*0.05*	*0.116*	*0.09*	*0.01*	*0.006*	*0.502*	*0.29*
*P**	*0.0011*	*0.0473*	*0.0001*	*0.006*	*0.0096*	*0.0844*	*0.0001*	*0.0005*	*0.0001*	*0.002*	*0.0001*	*0.0088*	*0.0039*	*0.0329*	*0.0058*	*0.0325*

**probability* *Values with different letters in a column are significantly different at the 0.05 level according to LSD test]*

Table 4. Pearson correlation coefficients between heavy metals contents in mulberry cultivated soil and in mulberry fruits

Distances (m)	Zn	Mn	Cu	Co	Cr	Pb	Cd	Ni
20	0.994**	0.781	0.987**	0.536	0.876	0.942*	0.639	0.713
100	-0.510	0.496	0.406	0.510	-0.404	-0.343	-0.619	-0.079
400	-0.588	0.997**	0.739	0.954*	0.620	0.969*	0.620	0.528
1000	-0.289	0.979*	0.975*	0.748	0.999**	0.975*	0.506	0.984**

*P<0.05 **P<0.01

The results obtained in the current study had differences with literature values. These differences depend on multiple factors. In different environments, many factors are known to affect the concentration of heavy metals in both soil and plants, including industrialization, traffic density and unknown atmospheric deposits.

The current study showed that mulberry cultivated soil was polluted by Pb, Cd, Cu, Mn and Zn heavy metals at 20 and 100 m distances from the road. The study also indicated that the Pb (at 20 and 100 m distances from the road) and Ni (at all distances from the road) contents of mulberry fruits were higher than permissible limits. There are some factors for heavy metal contamination of soil and plants like growth media, nutrients, agro inputs, soil, pesticides and fertilizers. However, the main source of heavy metal contamination in the current study is likely emissions from vehicles. The levels of Ni, Co and Cr do not appear to reach pollution levels in the soil.

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