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Translocation and enrichment of heavy metals in *Brassica juncea* grown in Paper mill effluent irrigated soil

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Abstract: The present study observed the accumulation of heavy metals in *Brassica juncea* irrigated with paper mill effluent (PME) and control Bore well water (BWW). The soil was treated to five rates of effluents viz. 10, 25, 50, 75 and 100 ml/Kg soil. It was revealed 100% PME irrigation of soil increased Pb (+46.44%), Cr (+83.21%), Ni (+38.43%) and Cd (+78.92%). The enrichment factor (Ef) for Cr and Cd showed moderate enrichment with 10% to 75% PME irrigated soil, except Cr (5.96) which showed significant enrichment with 100% PME irrigated soil. Ef value for Pb and Ni showed deficiency to mineral enrichment with different concentrations of PME irrigated soil. The maximum accumulation of Pb (42.66±2.05 mg/kg), Cr (39.80±5.95 mg/kg), Ni (88.64±11.29 mg/kg) and Cd (5.85±0.29 mg/kg) were recorded in leaves of *B. juncea*, while that of Pb (43.85±3.46 mg/kg), Cr (48.59±3.81 mg/kg), Cd (6.74±1.22 mg/kg) with 100% and Ni (74.93±2.54 mg/kg) were recorded with 75% PME after 60 days in roots of the *B. juncea*. Ef value was found maximum for Cr (5.08) in leaves and for Pb (6.64) in roots, while the Translocation factor (Tf) was found maximum for Pb (2.45) in root of the crop irrigated with PME. The use of PME with proper dilution and with the metallic concentrations in permissible limit can be used as biofertigant for irrigation of *B. juncea*.

Keywords: *Brassica juncea*, Heavymetals, Enrichment factor, Translocation factor

INTRODUCTION

Waste water irrigation, solid waste disposal, sludge application, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals (Gupta *et al.*, 2010, Harmanescu *et al.* 2011, Chopra and Pathak, 2012). Long term irrigation with such effluents increases organic carbon content, heavy metal accumulation in soil and the chances of their entrance in food chain and that may ultimately cause significant bioaccumulation (Chopra *et al.*, 2009). On the other hand, wastewater is also a resource that can be applied for productive uses as it contains nutrients that can be used for the cultivation of agricultural crops (Hati *et al.*, 2007; Chandra *et al.*, 2009; Rath *et al.*, 2011). The excessive accumulation of heavy metals in agricultural soils through wastewater irrigation may not only result in soil contamination, but also affect food quality and safety (Hati *et al.*, 2007; Bharagava *et al.*, 2008; Chopra *et al.*, 2009).

Pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulogic materials and water during the manufacturing process, and release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbon in the effluent (Liss *et al.*, 1997 and Singh, 2007). In India, there are 666 pulp and paper mills, out of which 632 mills are agro- based residue mills (Malla and Mohanty, 2005; Kumar and Chopra, 2012). They generate a huge amount of wastewater (black liquor) having high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Mapanda *et al.*, 2005; Kumar, 2010). These mills are highly water intensive, consuming 100-250 m^3 freshwater/ ton paper and also generate the corresponding wastewater 75-225 m^3 wastewater/ton paper (Thompson, 2001 and Tewari *et al.*, 2009).

Irrigation of crops with effluents is a very common practice in India due to scarcity of water for irrigation (Sharma *et al.*, 2007, Arora *et al.*, 2008). The effect of effluents irrigation on various crops/vegetables has been studied to observe the concentration of accumulated metals to which human beings are exposed (Ismail *et al.*, 2005, Singh and Kumar, 2006). Keeping this in view, the present study was carried out to investigate the translocation and enrichment of heavy metals in leafy vegetable, *B. juncea* after irrigation of soil with Paper mill effluent (PME).

MATERIALS AND METHODS

Experimental design: A field study experiment was conducted in the Experimental garden of the Department of Zoology and Environmental Sciences, Faculty of Life

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Sciences, Gurukula Kangri University Haridwar (29°55'10.81''N and 78°07'08.12''E) during the period of November, 2010 to January, 2011. The poly bags (dia-30cm) were used for growing *B. juncea*. The pots were arranged in a completely randomized design with four replicates. Twenty four poly bags were filled with soil and used for the cultivation of *B. juncea*. The proper distance was maintained between each replicate (30 cm) between all treatments (60 cm) and plant to plant (5cm) for the maximum performance of the crop. Each poly bag was made porous for aeration. The poly bags with *B. juncea* were given various treatments viz. 10, 25, 50, 75 and 100% of the Paper mill effluent (PME) and with Bore well water (BWW) taken as control, separately.

Analysis of soil, leaves and roots of the crop: The soil samples were analyzed before and after irrigation of soil with PME and BWW, while *B.juncea* samples of leaves and roots were analyzed after irrigation of the soil and harvesting of the crop for heavy metals (Pb, Cr, Cd and Ni) as per standard methods of APHA (2005) and Chaturvedi and Sankar (2006).

The samples of soil as well as the leaves and roots were dried separately in the air at room temperature and sieved through a 2 mm sieve. Samples were digested in nitric acid (HNO_3) and perchloric acid ($HClO_4$) acid as per the method described in AOAC (Association of Official Analytical Chemists, 1990). After digestion, all samples were filtered through Whatmann No. 42 filter paper and in each case volume was made with 50 ml. The heavy metals Pb, Cr, Cd and Ni were determined in the digested aliquot by Atomic Absorption Spectrophotometer (AAS) (Make-ECIL, Model No. 4129) using a specific lamp of particular metal using appropriate drift blank.

Data analysis: The heavy metal contents in both the *B. juncea* and adjoining soil were expressed as means and standard deviation of six replicates calculated in Microsoft Office Excel (2007) and Axum 50.

RESULTS AND DISCUSSION

Heavy metals of soil irrigated with PME: The mean±SD values of heavy metals (Cd, Ni, Pb and Cr) of the soil before and after irrigation with different concentrations of PME viz. 10%, 25%, 50%, 75% and 100% are given in the Table 1.

In the present study, maximum concentrations of the heavy metals were recorded for Pb (17.40±0.78 mg/kg), Cr (32.02±1.18 mg/kg), Ni (67.02±1.46 mg/kg) and Cd (6.35±0.49 mg/kg) with 100% PME irrigated soil and minimum concentrations were recorded for Pb (10.89±0.45 mg/kg), Cr (13.76±0.57 mg/kg), Ni (45.28±1.14 mg/kg) and Cd (2.77±0.43 mg/kg) with 10% PME irrigated soil in comparison to control.

There was a remarkable increase of 60.94% Cr with 10%, 68.52% with 25%, 75.55% with 50%, 80.27% with 75%, 83.21% with 100% and of 51.71% Cd with 10%, 64.45% with 25%, 69.57% with 50%, 74.40% with 75% and 78.92% with 100% concentration of PME irrigated soil in comparison to control. It may likely occur due to the presence of significant quantity of heavy metals at higher concentrations of PME. During the present study heavy metals contents were found minimum at lower concentrations (10 to 50%) and maximum at higher concentrations (75 to 100%) of PME irrigated soil. There was much variation in the metal contents of soil irrigated different concentration of PME.This was likely due to dilution of the PME, which minimizes the quantity of heavy metals in the effluent irrigated soil. The present data revealed that the level of heavy metals in soil increased as per dilution quantity of PME. In the present study heavy metal content such as Pb (17.40±0.78 mg/kg), Cr (32.02±1.18 mg/kg), Ni (67.02±1.46 mg/kg) and Cd (6.35±0.49 mg/kg) with 100% PME irrigated soil was higher than those reported by Pathak *et al.* (2013) for Cr (29.53±3.33 mg/kg) and Cd (6.11±1.74 mg/kg) in 100% PME irrigated soil, except Ni (206.18±13.44 mg/kg) in PME irrigated soil at Haridwar, whereas Sinha *et al.* (2008) found Fe (14,285 ± 1244 mg/kg), Cr (197.76 ± 12.83 mg/kg) and Zn (104.91 ± 0.97 mg/kg) in soils treated with different tannery sludge applications.

PME irrigation increased Pb, Cr, Cd and Ni of the soil. As per Indian Standards (Awashthi, 2000), Pb, Cr and Ni were below the permissible limits while Cd was above the permissible limits except in case of 10% PME irrigated soil. The concentration of Pb and Cr were below the permissible limits, while Cd and Ni in 100% PME irrigated soil were above the permissible limits reported by Kabata-Pendias and Pendias (1992).

Ef for soil irrigated with PME: In the present study the EF value of the Pb, Cr, Cd and Ni were found maximum for Cr (5.96) with 100% PME irrigation and minimum for Ni (1.10) in 10% PME irrigated soil. The Ef was found in the order of Cr (2.56) > Cd (2.07) > Pb (1.17) > Ni(1.10) for 10%, Cr (3.18) > Cd (2.81) > Pb (1.33) > Ni(1.14) for 25%, Cr (4.09) > Cd (3.29) > Pb (1.49) > Ni (1.24) for 50%, Cr (5.07) > Cd (3.91) > Pb (1.69) > Ni(1.40) for 75% and Cr (5.96) > Cd (4.74) > Pb (1.87) > Ni (1.62) for 100% PME irrigated soil. The Ef value for Cr and Cd showed moderate enrichment with 10%, 25%, 50% and 75% PME irrigated soil, except Cr (5.96) which showed significant enrichment in 100% PME irrigated soil. Ef value for Pb and Ni showed deficiency to mineral enrichment with 10%, 25%, 50%, 75% and 100% PME irrigation. The Ef value of Ni for soil was lower than Ni (3.27) as reported by Pathak *et al.* (2013) for PME irrigated soil. Kumar and Chopra (2010) also reported more Ef for Cr (11.24), Cd (5.04) in soil irrigated with Sugar mill effluent.

Heavy metals in leaves and root of *B. juncea* irrigated with PME: The metal concentrations in *B. juncea* roots/

Table 1. Heavy metals contents before and after irrigation with PME and BWW irrigated soil.

Metals	Before effluent irrigation	After BWW Irrigation	After effluent irrigation					Limit(a)	Limit(b)
			10%	25%	50%	75%	100%		
Pb	8.57	9.32±0.73	10.89±0.45 (+14.42%)	12.37±0.85 (+24.67%)	13.92±0.48 (+33.06%)	15.71±0.45 (+40.68%)	17.40±0.78 (+46.44%)	250-500	50
Cr	12.34	12.38±0.72	13.76±0.57 (+60.94%)	17.07±1.44 (+68.52%)	21.99±1.74 (+75.55%)	27.24±2.43 (+80.27%)	32.02±1.18 (+83.21%)	n/a	100
Ni	43.26	41.27±3.58	45.28±1.14 (+8.87%)	47.10±2.49 (+12.39%)	51.12±1.65 (+19.27%)	57.65±1.02 (+28.41%)	67.02±1.46 (+38.43%)	75–150	30
Cd	3.24	2.09±0.46	2.77±0.43 (+51.71%)	3.76±0.98 (+64.45%)	4.40±0.17 (+69.57%)	5.23±0.42 (+74.40%)	6.35±0.49 (+78.92%)	3–6	1

(a) Source: Awashthi (2000); (b)Source: Kabata-Pendias and Pendias (1992), n/a - not avialable

shoots, Ef value and Tf for *B. juncea* are given in Fig 1and 2.

The maximum heavy metals contents was found for Pb (42.66±2.05 mg/kg), Cr (39.80±5.95 mg/kg), Ni (88.64±11.29 mg/kg) and Cd (5.85±0.29 mg/kg) in leaves, while that of Pb (43.85±3.46 mg/kg), Cr (48.59±3.81 mg/kg), Cd (6.74±1.22 mg/kg) in root of *B. juncea* irrigated with 100% PME concentrations. Ni was recorded maximum (74.93±2.54 mg/kg) in roots of *B. juncea* irrigated with 75% PME concentration.

The concentrations of Pb, Cr, Cd and Ni in edible parts of *B. juncea* were above the permissible limits of FAO/WHO standard (Codex Alimentarious Commission, 1984) and Indian Standard (Awashthi, 2000) except Cr with 10% and 25% PME irrigation. The metal accumulation in the PME irrigated *B. juncea* showed that the concentration of Pb, Cr, Ni and Cd increase in the soil as per dilution, because the maximum contents of heavy metals increased up to 100% PME concentration. This might be due to increase in concentration of organic and inorganic materials, which are mainly responsible for the increase in the contents of metals in PME. From the above scenario based on 10%, 25%, 50%, 75% and 100% effluent irrigation, it was observed that the metal status increased to their higher concentrations in soil irrigated with PME as also reported by Kumar *et al.* (2010). Similar observations of higher metal accumulation have been reported by Kumar and Chopra (2012) for *Trigonella foenum-graecum* L. (Fenugreek) plants irrigated with Distillery effluent (DE).

Ef for *B. juncea* irrigated with PME:The Ef values of different metals indicated that root to shoot translocation of metals in *B. juncea* was quite higher after 60 days of growth period, showing their ability to translocate metals from the root to the shoot, or to compartmentalize it in order to continue the absorption of metals from the growth media. The EF values for leaves of *B. juncea* were in the order of Cd (2.37) > Cr (1.56) > Ni (1.45) > Pb (1.20) in 10%, Cd (2.58) > Pb (2.09) > Cr (2.12) > Ni (1.68) in 25%, Cr (3.10) > Cd (2.75) > Pb (2.74) > Ni (2.00) in 50%, Pb (3.46) > Cr (3.46) > Cd (2.68) > Ni (2.35) in 75%, Pb (5.56) > Cr (5.08) > Cd (4.37) > Ni (2.95) in 100% PME irrigated soil. In case of 10% PME irrigated *B. juncea* leaves, Ef values for Pb, Cr and Ni showed the minimal enrichment, while Cd showed moderate enrichment in 10% PME irrigated soil. In case of 25% PME irrigated *B. juncea* leaves Pb, Cr and Cd showed moderate enrichment and Ni showed the minimal enrichment. The Ef values with 50%, 75% and 100% PME irrigated *B. juncea* leaves for Cr, Cd and Ni showed moderate enrichment except Pb, which showed moderate enrichment with 50%/75% and significant enrichment with 100% PME irrigation.

According to enrichment categories in *B. juncea* leaves, the Ef values for Cd showed moderate enrichment with

Table 2. Heavy metals contents in leaves and roots of *B. juncea* grown in PME and BWW irrigated soil.

Metals	Plant parts	After BWW irrigation	After effluent irrigation					Limit(a)	Limit(b)
			10%	25%	50%	75%	100%		
Pb	Leaves	7.67±0.69	9.21±0.55	16.06±0.88	20.99±0.52	26.56±1.53	42.66±2.05	5	2.5
	Root	6.60±0.72	9.76±0.51	19.51±1.04	15.27±1.08	23.04±6.34	43.85±3.46		
Cr	Leaves	13.35±1.12	12.21±0.88	16.64±0.45	24.27±0.60	27.13±3.23	39.80±5.95	5	20
	Root	11.01±1.33	13.49±0.91	17.49±1.09	29.90±2.96	31.16±1.55	48.59±3.81		
Cd	Leaves	1.34±0.13	3.17±0.55	3.45±0.31	3.68±0.41	3.59±0.25	5.85±0.29	0.3	1.5
	Root	1.35±0.17	4.02±0.64	4.34±0.86	4.59±0.15	6.01±0.72	6.74±1.22		
Ni	Leaves	30.06±1.39	43.61±3.94	50.46±1.52	60.19±3.87	70.67±6.01	88.64±11.29	20	1.5
	Root	25.38±2.11	45.88±2.32	52.93±1.07	62.69±3.08	74.93±2.54	51.78±4.10		

(a) FAO/WHO standard (Codex Alimentarious Commission 1984) (b) Indian standard (Awashthi 2000).

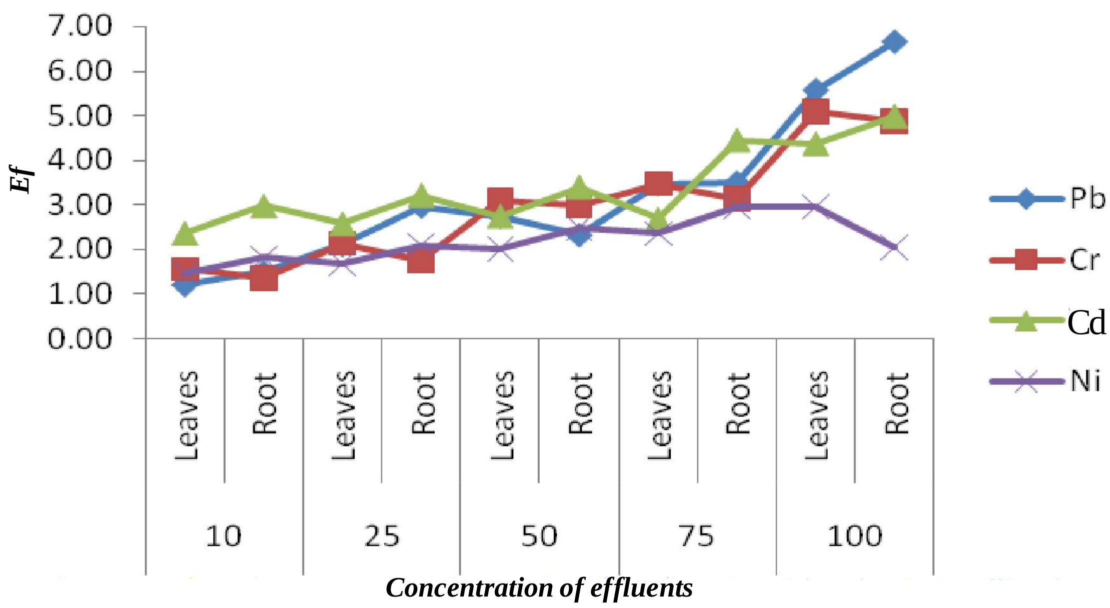

Fig. 1. *Ef for leaves and root of B. juncea irrigated with PME.*

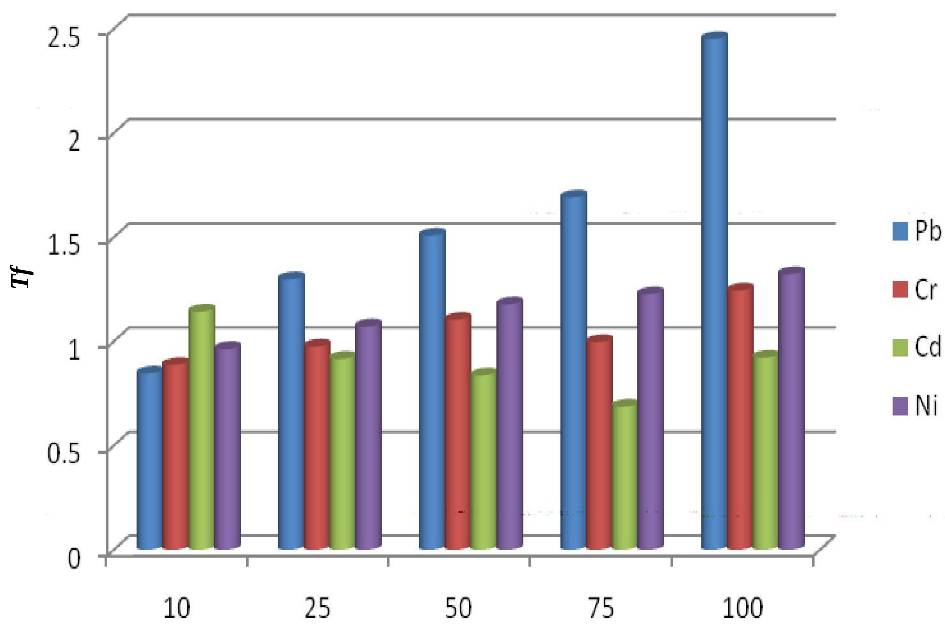

Fig. 2. *Tf for B. juncea irrigated with PME.*

all treatments and for Pb and Cr, it showed moderate enrichment with 25%, 50% and 75% and significant enrichment with 100% PME irrigation, while Ni showed moderate enrichment with 50%, 75% and 100% PME irrigation.

In root part of *B. juncea*, the Ef values were found in the order of Cd (2.97) > Ni (1.81) > Pb (1.48) > Cr (1.35) in 10%, Cd (3.22) > Pb (2.96) > Ni (2.09) > Cr (1.75) in 25%, Cd (3.40) > Cr (2.99) > Ni (2.47) > Pb (2.31) in 50%, Cd (4.45) > Pb (3.49) > Cr (3.11) > Ni (2.95) in 75%, Pb (6.64) > Cd (4.99) > Cr (4.86) > Ni (2.04) for *B. juncea* in 100% PME irrigated soil. The Ef values with 10% PME irrigated *B. juncea* root showed minimal enrichment for Ni, Pb, Cr and moderate enrichment for Cd. In case of 25% PME irrigated *B. juncea* root, Cd, Pb, and Ni showed moderate and Cr showed the minimal enrichment. The Ef values with 50%, 75% and 100% PME irrigated *B. juncea* root for Cr, Cd and Ni showed moderate enrichment except Pb, which showed moderate enrichment with 50%/75% and significant enrichment category with 100% PME irrigation. Among the various Ef values in *B. juncea* root, Cd showed moderate enrichment category in all treatments, Cr was found in moderate enrichment category with 50% 75% and 100% PME irrigation, while Ni was in moderate enrichment category in 25%, 50%, 75% and 100% PME irrigation. Pb was in moderate enrichment with 25%, 50%, 75% and significant enrichment with 100% irrigation after irrigation with PME. The present EF values of Cr (4.86) was lower than the values reported by Gupta *et al.* (2008) for Cr (7.58) in the tomato plants grown in the contaminated soil irrigated with sponge iron effluent and also lower than the values reported by Kumar and Chopra (2012) for Cr (21.4), Cd (16.5) in *Trigonella foenum* graecum plants irrigated with DE irrigated soil.

Tf for *B. juncea* irrigated with PME: Soil-to-plant transfer of heavy metals is one of the key components of human exposure to metals through food chain. Variations in Tf among different vegetables may be attributed to differences in the concentration of metals in the soil and to the differences in element-uptake by different vegetables (Cui *et al*., 2004; Zheng *et al*., 2007, Singh *et al.*, 2010). The translocation process of metals from root to shoot includes long distance in xylem and storage in vacuoles of leaf cells and it is affected by several factors (Yang *et al*., 1997).

Among different PME concentration, maximum Tf value off 1.14 were observed for Cd with 10%, 1.30 for Pb and 1.07 for Ni with 25%, 1.51 for Pb, 1.10 for Cr and 1.18 for Ni with 50%, 1.69 for Pb and 1.23 for Ni with 75%, 2.45 for Pb, 1.24 for Cr and 1.32 for Ni was recorded with 100% in *B. juncea* grown in PME irrigated soil.

The present Tf values of Cr (1.24) was higher than that reported by Smical *et al.* (2008) for Cr (0.194), while the values of Ni and Cd were lower than that observed for Ni (0.827), Cd (0.996) in Spinach grown at wastewater irrigated soil (Singh *et al*., 2010). The higher translocation values for these metals from soil to plants indicated a strong accumulation of these metals by *B. juncea*.

Conclusion

It was concluded that PME irrigation increased Pb, Cr, Cd and Ni of the soil. As per Indian Standards, Pb, Cr and Ni were below the permissible limit while Cd was above the permissible limit except in case of 10% PME irrigation. The concentration of Pb and Cr was below the permissible limit while Cd and Ni were above the permissible limit. The level of metals except Cr in *B. juncea* exceeded many folds as per FAO/WHO standard and Indian standards with 10% and 25% PME irrigation The maximum Ef values were found for Cr (5.96), which showed significant enrichment in 100% PME effluent irrigated soil. The maximum Ef value for Pb (5.56 in leaves and 6.64 in root) showed significant enrichment in *B. juncea*. Among the various Tf values, maximum Tf value was recorded for Pb (2.45) in *B. juncea* with 100% PME concentration, which showed high mobility affinity of Pb being translocated from roots to leaves resulting in their greater accumulation and had the capability to tolerate higher levels of these metals. Thus, the use of PME with proper dilution (10%) and metallic concentration in permissible limit can be used as biofertigant for the purpose of this crop.

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