BIOACCUMULATION OF HEAVY METALS AND THEIR EFFECT ON VITAL ORGANS OF *LABEO ROHITA* IN PONDS AND NATURAL WATER BODIES

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ABSTRACT

Labeo rohita was sampled to determine the contamination levels of chromium, lead, nickel, copper and manganese from wild (Trimmu and Balloki Headworks) and controlled fish ponds at Ravi Campus (UVAS) from September to December, 2013. Sampled fish were blotted dry, weighed and liver and intestine were removed to study histology and metals concentration. Chromium seems ubiquitous and was detected in all the water samples as well as liver and intestinal tissues of experimental fish. Its level was lowest in water samples and fish organs in samples collected from UVAS fish ponds while the highest in samples collected from Balloki Headworks, the main recipients of waste from Lahore city. Fish liver depicted Chromium (Cr) only but the intestine revealed the presence of Nickel (Ni), Lead (Pb), Manganese (Mn) and copper (Cu) too. Cadmium was below the detectable limit in all water and tissues (liver, intestine) of sampled fish. Heavy metals load and their range induced pathological alterations, infiltration and necrosis in liver and intestinal tissues. Canonical discriminate analysis of Cr, Cu, Mn, Ni, Pb and Cd and the extent of histopathological changes differed significantly among localities establishing an appropriate link with living media and their vertical flow in fish.

Key words: *Labeo rohita*, heavy metals toxicity, water sources, vital organs, histo-pathological changes.

INTRODUCTION

Heavy metals are naturally found in aquatic environments as positive ions and now they constitute a considerable part of water pollution due to anthropogenic activities like industrialization, urbanization, as well as commercial activities. Although, these are basically required for the normal functioning of aquatic creatures but the excessive amounts may accumulate in the aquatic animals like fish and gradually increase to levels causing toxic effect (Shah *et al.* 2012 & 2013 and Izuchukwu *et al.* 2017). Heavy metals are among the most serious environmental pollutants due to their high toxicity, abundance and ease of accumulation due to their non-biodegradable and non-thermo degradable nature and are extremely persistent in the environment (Guevara-Riba *et al.* 2004).

Heavy metals for example As, Hg, Cd, and Pb are severe toxicant and have the ability to alter the freshwater quality and aquatic life, especially fish which sometimes can metabolize metals to even more harmful derivative compound (Shah *et al.* 2012 and Khan *et al.* 2012). Being the top consumers, fishes can have heavy metals in four routes via food consumption, water uptake (gills), consumption of nonedible particles and through skin absorption. The absorbed heavy metals accumulate in the different body organs especially in the liver, kidney and gills prior to being eliminated or stored (Azaman *et al.* 2015). Accumulation of heavy metals may reduce fish appetite and metabolic activities that leads to hamper growth and development (Javid and Saeed, 2010) and can be serious hazard when transferred to humans by fish consumption (Rahman *et al.* 2014). Thus, the fishes are suggested as environmental biomarkers and are considered as an early warning of environmental degradation (Verlecar *et al.* 2006). The gills (Alazemi *et al.* 1996), liver (Braunbeck, 1998) and kidney (Bernet *et al.* 1999) are the main organs for pollution target as these are the main sites for metabolic activity and hence can be used as bio indicator of metals bioaccumulation (Al-Ghanim *et al.* 2015).

Pakistan has been blessed with number of water resources with World's largest water distribution system but the anthropogenic activities destroy the water quality to such an extent that it became unfit for human and aquatic life (Muhammad *et al.* 2010 & 2011; Khan *et al.* 2013). Industrialization and urbanization play a major roll in dumping of heavy metals in the environment up to the alarming level (Hussain *et al.* 2014) especially in the Indus River and its tributaries. Fatoki and Mathabatha (2001), Qadir *et al.* (2009), Qadir and Malik (2011), Javed and Usmani (2013), Cardwell *et al.* (2013), Khan *et al.* (2018) and many other researchers have worked on heavy metal contamination but the information are limited. The objective of this study was to investigate the concentration of heavy metals in different organ of *Labeo*

rohita selected from Balloki Headworks and Trimmu Headworks and was compared with fish cultured in the pond system at Fish Farm complex at Ravi campus, Pattoki.

MATERIALS AND METHODS

Location: *Labeo rohita* was collected from Trimmu Headworks located at the junction of River Chenab and Jhelum (District Jhang), Balloki Headworks at River Ravi (District Kasur) and from UVAS-Fish ponds, C-block Ravi Campus Pattoki, district Kasur in the month of September to December 2013. Fish were harvested, weighted and transported in the ice boxes to the histology lab of Anatomy and Histology department, UVAS, city campus, Lahore.

Histopathological studies of liver and intestine: Samples collected on monthly basis from the selected stations were subjected to histological studies according to Tao *et al.* (1999). Fish were dissected for intestine and liver, sliced and fixed in fixative solution containing ethanol, formaldehyde and glacial acetic acid (1:3:7). Tissues were dehydrated in ethanol and embedded in Paraffin wax. Histological sections of 2 μm were then cut at a microtome and stained with hematoxylin-eosin. Light microscopy performed and stained section was photographed under photoscope.

Determination of heavy metals in fish: Metal analysis was done in the chemistry lab of Government College, University, Lahore. Samples of liver and intestine were subjected to the wet digestion in the anatomy lab for the metal analysis viz. chromium, copper, cadmium, lead, nickel and manganese following S.M.E.W.W. (1989). Tissues (50g each) were washed with deionized water, dried on blotting paper and digested in 5ml solution of perchloric and nitric acid at 200 to 250°C to obtain a transparent solution. 100 ml double distilled water was added and heavy metals were analyzed through atomic absorption spectrophotometer.

Water Quality Analysis: Water samples (1L) from all studied sites were collected in water sampler bottles. The samples were collected below the surface about 2-3 feet away from the banks in such a way that no bubbles were allowed. Temperature, dissolved Oxygen, total dissolved solids (TDS), conductivity and pH were determined on site with the help of YSI Digital meters to establish correlation between type of water quality and response of fish. Further, samples were brought to the chemistry lab of Government College, University, Lahore for metal analysis (S.M.E.W.W, 1989)**.** Water samples were filtered and preserved in 5 ml of 55% HNO3/L of water to prevent metal adsorption on the inner surface of the container and stored at 4∘C before metal analyses

Statistical Analysis: Data collected were subjected to ANOVA for identification of statistical significance (Steel *et al.*, 1996). Variable means among three different sites were compared using Duncan's Multiple Range Test to ascertain their level of superiority or inferiority. Probability level was set at P<0.05.

RESULTS AND DISCUSSION

Statistical analysis of metal contamination indicate that water and fish organs contain less accumulation of heavy metals or even below detectable limits (BDL) in control culture system at Ravi campus, Pattoki except one water sample in which Cr, Pb and Cu were detected. Pb and Cr were present in the intestine and Pb and Cu were present in the liver of pond fish. Cr was the only metal present in all the samples from the pond whereas, Ni, Mn and Cd were below the detectable limits in water, intestine and liver of fish.

Statistical analysis of water samples from Trimmu Headworks indicates significantly higher concentrations of Cr whereas Pb concentration was highest at Balloki Head works. Other heavy metals were below detectable limits in both headworks. Intestine of fish samples from the Balloki head works showed higher concentrations for Cr, Pb, Cu, Ni, and Mn except Cd that was BDL. The intestine of Trimmu headworks fish showed higher concentrations of metals then the pond fish. The liver analysis indicates only Cr with higher concentration in Balloki Headwork followed by Trimmu headworks and Ravi campus pond complex, respectively. All the other metals were BDL in the liver. Thus the statistical analysis indicate that Balloki headworks had highest metal contamination as compared to the Trimmu headworks and ponds culture system. Lowest accumulation level in the pond system at Ravi campus, indicated that fish grown in the controlled system are more suitable for consumption. The values for the metals concentrations were quite higher from the maximum allowable concentration in the trimmu and Balloki headworks. The maximum allowable concentrations ((μg/ml) for the metals are given in table 1 according to EPA (Mills, 1995).

The values for the Pd, Cr were lower as compared to the values evaluated by the Chatha *et al.* (2016) who reported higher values for these metals and indicate the increasing concentration level of metals in the farmed fishes in Head Qadirabad area along the Chenab River and declare them not safe for human consumption due to the presence of Pb, beyond acceptable limits. Similarly the values of Pd in the trimmu and Balloki headworks were higher than that of allowable ranges thus, the fish in that water bodies were not fit for human consumption. Presences of Pb beyond acceptable limits may cause headaches, inflammation, and abdominal pains. Children can experience loss of

concentration and behavioral disturbances and in case of long exposures intellectual ability may decline (Jabeen and Javed, 2013). The consistent consumption of contaminated fish can also cause renal impairment (WHO, 2008).

Similar findings were reported by the Qadir *et al.* (2009) and Qadir and Malik (2011) from the Nullah Aik and Nullah Palkhu area supplied by untreated metal contaminants from tanneries and other industrial and city wastes from Sialkot and Wazirabad area. Similarly, Rauf *et al.* (2009) reported Cd and Cr in *Labeo rohita* and *Cirrhinus mrigala* with higher concentrations. Shivakumar *et al.* (2014) from india and Ahmad *et al.* (2015) from Karachi fish harbor reported higher metal concentrations due to the anthropogenic activities and industrial waste dumping into the natural water bodies. Our findings were also in accordance to the findings of Javed and Usmani (2013), Izuchukwu *et al.* (2017) and Khan *et al.* (2018) who reported that heavy metals bioaccumulate in the fish bodies that may be used as bio-indicator of environmental pollution.

The maximum dissolved oxygen level was observed in Ravi campus fish ponds while the minimum level was in Balloki Headworks experimental site. The water temperatures in all three sites were in the range of 23.46 ± 1.309 to 26.0 ± 0.816 °C. Total dissolved solids were highest in Pattoki campus fish ponds followed by Trim Headworks and Balloki Headworks. Maximum electrical conductivity was in campus ponds while the minimum in Balloki Headworks. pH value was highest in campus ponds while minimum was recorded in Trimu Headworks (Table 2). Water quality parametrs like temperature and pH were within the acceptable ranges for fish culture in all three experimental sites however, values for DO and EC were lower in the trimmu and Balloki headworks that may retatrd the growth of fish and other aquatic animals. The value for TDS were within acceptable range in the Balloki and Trimmu headworks but was significantly higher in the pond culture system at Ravi campus Pattoki.

Histological studies of intestine: In Pakistan there are few studies on the effect of metals pollution on the aquatic organism and ecosystem (Waseem *et al.* 2014). Metal concentration in the fish intestine were studied by the Rajeshkumar and Li (2018) indicating higher values for the Pb followed by the chromium and copper however the data on the histopathology studies are scare especially on the intestine and related glands. In present study, pathological changes of fish intestine indicate the gut mucosa had lifted off (Fig. 3) and narcotized (Fig. 1-9). Individual epithelial cells appeared rounded up with dense chromatin and most of them were with eosinophilic cytoplasm. In some cases strips of epithelial cells appeared sloughed into the lumen (Fig. 3) giving appearance of copious catarrhal exudates (Fig. 9). There

is an acute inflammatory response; particularly it was in the posterior part of the intestinal mucosa, which sloughs into the lumen. Apart from the degranulation of eosinophilic granular cells, there was also hyperaemia, infiltration of macrophages with massive necrosis of gut (Fig. 6). Khan *et al.* (2018) reported histopathological alterations in digestive gland i.e atrophy, hydropic vacuolation, necrosis, and lipofuscin which was in accordance of our results. Damage to intestinal tissue, particularly to enterocytes and villi structures, was detected histologically in tilapia by Younis *et al.* (2013). They reported degenerated nuclei and apoptosis in the crypts of Lieberkuhn and abnormally dilated lamina propria with inflammatory leukocytes at 10% of LC50 concentration of metals. Disturbed longitudinal muscularis and proliferation of mucous-secreting goblet cells indicated the defense mechanism against the pathological changes induced by $CdCl_2$ contamination.

Histological studies of Liver: The parenchymatous tissue had many important physiological functions like the intermediate metabolism of protein, carbohydrate and lipid, the synthesis of plasma protein such as albumen, ovalbumen (reproductive protein) and also the formation and secretion of bile. It also cause the detoxification of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides. Anatomically it differs from that of higher animals in lacking fixed Kupffer cells lining the sinusoids, which, in mammals, are among the most significant components of the reticuloendothelial system (Hinton and Lauren, 1990, Latif *et al.* 2012).

Liver cell necrosis is a common finding in both primary liver disease and in reactive hepatitis in response to systemic infection. Small clusters of necrotic hepatocytes (focal necrosis) may be isolated within normal hepatic tissue. Focal necrosis and the various prior changes may be associated with a variety of toxic conditions, including virus infections, fungal infection and other toxic bacteremias, pesticide poisoning and heavy metal toxicity.

There were focal lesions which indicated confluent necrosis because it involved a significant area of liver (Fig. B). There were some areas which do not have inflammatory infiltrate but lot of dead cells or apparently dying were observed which are indicative of infarctive necrosis (Fig. C). Malignant hepatoma appeared in some cases, it was darkly stained and apparently appeared normal (Fig. A)

Fatty infiltration of liver cells is common in farmed as well as in wild fishes. It can be due to feeding on rancid fats, vitamin deficient diets or in toxic conditions. Presence of heavy metals have damaged cell membranes causing widespread necrosis and ceroid build-up in remaining hepatocytes with severe congestion and generalized intracellular oedema which is generally

observed in fish exposed to algal toxin but heavy metal toxicity gave the same results (Fig. A-F). Hepatocytes swelled and contained large accumulation of ceroid within cytoplasm giving it granular appearance (Fig. E). Ceroid has aggregated to form intracellular nodules. Studies on *Clarias gariepinus* treated with sewage/domestic wastewater containing Cu, Fe, Pb, Cd, Mn and Zn (Authman *et al.* 2013) was also in accordance with present study.

There is a diffused increase in the fibrous tissue of the liver, usually associated with chronic damage and destruction of hepatocytes. This damage can result from a standing biliary obstruction, heavy metal or pesticide poisoning. Cirrhotic livers are occasionally seen in wild fish with little evidence for the cause. Cells of the nodule have become enlarged and bizarre in shape, enlarging and showing a high level of mitosis (Fig. D). Similar findings have been reported in liver of different fishes for example exposure of *Oreochromis mossambicus* (Van Dyk *et al.* 2007), *Clarias gariepinus* (Gabriel, 2007 a & b), *Channa punctatus* (Mishra and Mohanty 2008 a & b) and *Channa striatus* and *Heteropneustes fossilis* (Fatima and Usmani, 2013) to cadmimum and zinc compounds. Parvathi *et al.* (2011) exposed *Cyprinus carpio* to lethal concentrations of Cr whereas *Tilapia zilli* was exposed to Al by Hadi and Alwan (2012) reported degeneration of liver tissues and necrosis due to infiltration of leukocytes.

Table 1. Level of heavy metals present in water and tissues of liver and intestine of fish sampled from experimental sites

Source		Chromium (Cr)	Nickel (Ni)	Lead (Pb)	Manganese (Mn)	Copper (Cu)	Cadmium (Cd)
EPA (1995) Max. allowable concentration (µg/ml)		0.1	0.2	0.5	0.05	0.6	0.04
Ravi Campus	Water Sample	0.288[ac]	BDL	0.047 [b]	BDL	0.0046	BDL
	Intestine	0.107±0.03[bc]	BDL	0.090±0.02 [a]	BDL	BDL	BDL
	Liver	0.125±0.16 [bc]	BDL	BDL	BDL	0.0051±0.01 [b]	BDL
Balloki Headworks	Water Sample	0.494±0.14[bb]	BDL	0.095±0.05[b]	BDL	BDL	BDL
	Intestine	0.548±0.37[aa]	0.17±0.047 [b]	0.698±0.24 [a]	15.30±2.93 [a]	0.553±0.28 [a]	BDL
	Liver	0.515±0.27[ab]	BDL	BDL	BDL	BDL	BDL
Trimmu Headworks	Water Sample	0.517±0.10 [aa]	BDL	0.025±0.01 [b]	BDL	BDL	BDL
	Intestine	0.420±0.21 [bb]	0.18±0.107 [a]	0.66±0.15 [a]	BDL	BDL	BDL
	Liver	0.406±0.13 [cb]	BDL	BDL	BDL	BDL	BDL

Table 2. Physiochemical parameters of three experimental sites with acceptable ranges.

Sites/ Parameters	Ravi Campus Ponds	Balloki Headworks	Trimu Headworks	Acceptable Ranges
DO (mg/L)	3.40±0.216[a]	2.36±0.097[b]	2.50±0.326[b]	3-5 mg/L
Temp. (°C)	25.73±0.543[a]	23.46±1.309[a]	26.00±0.816[a]	15-35 ºC
TDS (mg/L)	947.00±45.760[a]	132.66±17.745[c]	251.33±10.964[b]	400mg/L
EC (mS/cm)	1.23±0.047[a]	0.30±0.081[b]	0.50±0.163[b]	15-50 mS/cm
pH	6.50±0.24[a]	5.47±0.026[b]	5.30±0.294[b]	6-9
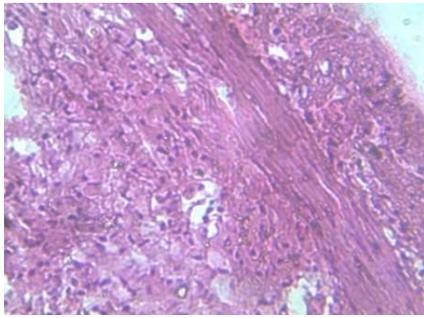
Fig.1
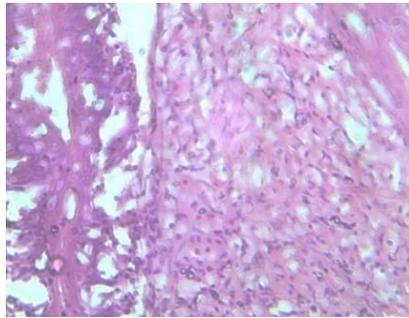
Fig.2
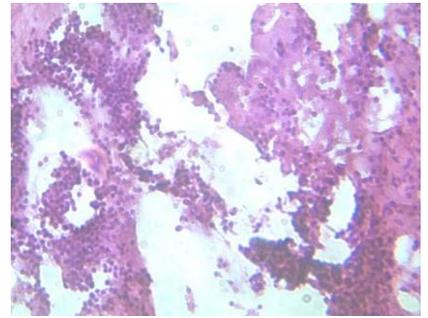
Fig. 3
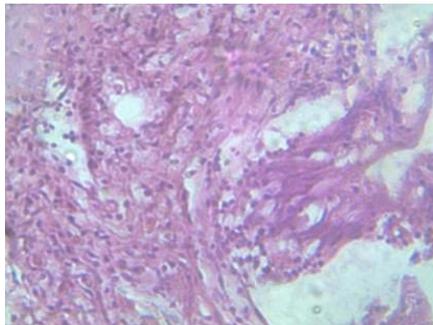
Fig.4
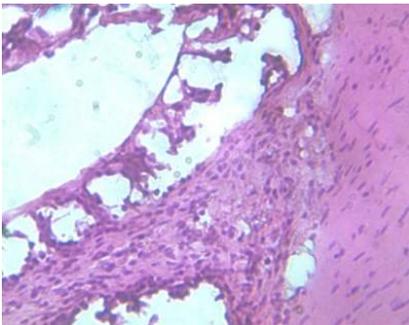
Fig. 5
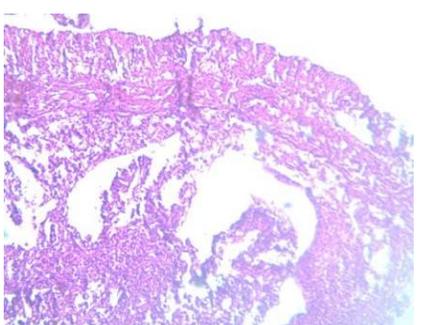
Fig. 6

Fig.7

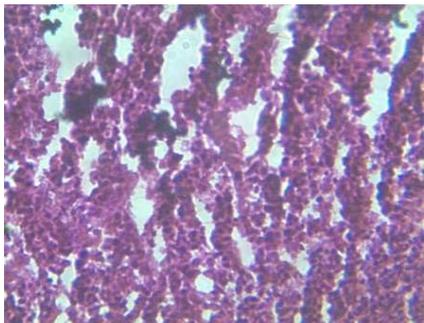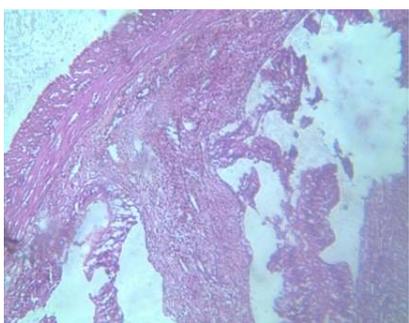**Fig.8**

Fig. 9

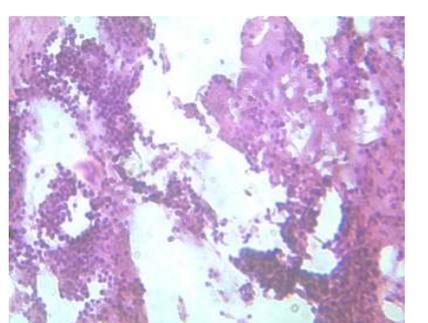**Fig. 1-9. Fish Intestine: Indicating liffted off and narcotized gut mucosa, rounded and individual epithelial with dense chromatin. Acute inflamtory response of mucosa sloughed into the lumen, hyperaemia and infiltration of macrophages.**
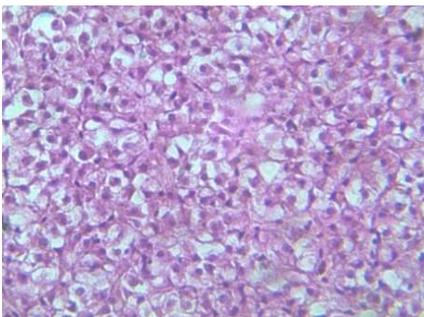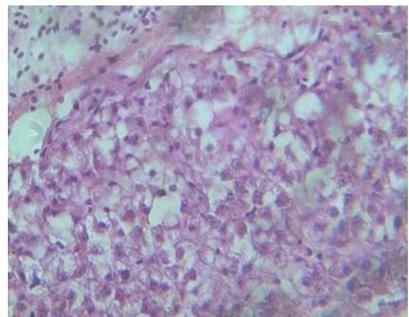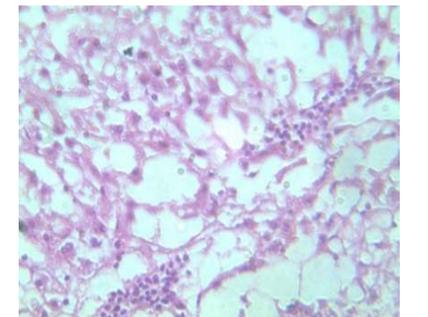

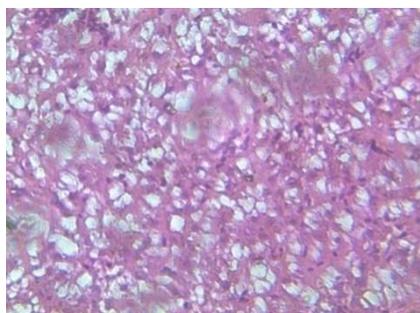**Fig. A**
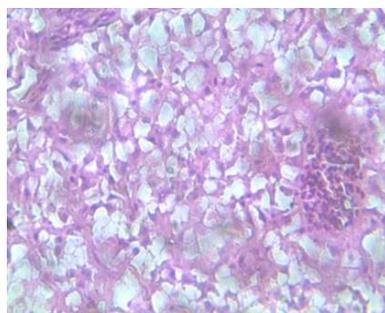

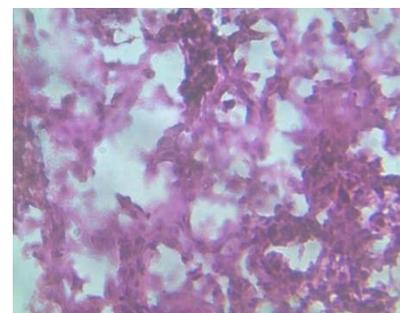**Fig. B**

Fig C

| **Fig. D** | **Fig. E** | **Fig F** |

Fig. A-F Fish Liver: Indicating the inflamation, confluent necrosis, infractive necrosis, malignant hepatoma, fatty infiltration, intracellular oedema and intracellular nodules in the liver of wild fish samples.

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