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The comparison of heavy metal accumulation ratios of some fish species in Enne Dame Lake (Kütahya/Turkey)

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Abstract The metal accumulation levels for muscle, skin, gill, liver and intestine tissues of some Cyprinidae species (*Carassius carassius*, *Condrostoma nasus*, *Leuciscus cephalus* and *Alburnus alburnus*) in Enne Dame Lake (Kütahya/Turkey), which is mostly fed by hot spring waters, were investigated. Analyses were performed for copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), cobalt (Co), magnesium (Mg), nickel (Ni), chrome (Cr) and boron (B) using inductively coupled plasma-optic emission spectroscopy (ICP-OES), and cadmium (Cd) using atomic absorption spectrophotometer (AAS) utilizing microwave digestion techniques. The concentrations of the heavy metals found in the fish varied in the following ranges: Cu: < DL-7.04, Zn: 6.96–357.25, Mn: < DL-20.70, Ni: < DL-6.21, Fe: 9.62–2500.33, Cr: < DL-1.74, Co: < DL-0.54, Cd: 0.01–0.27 and Mg: 197.44–904.90 mg/kg wet weight. While B had the second highest concentration in the water of the lake, it was not encountered in any tissue of the investigated species. In all tissues and the species, While the bioaccumulation factors (BAFs) of Mn, Zn, Fe and Cu were remarkably high, the BAFs of Mg, Cr, Co, and B were also fairly low or none. Although the heavy metal accumulation levels for the muscle were generally lower than other tissues, there were some exceptions. Cd level in the muscle of *C. carassius* was higher than the permissible limit stated by Turkish legislation, FAO and WHO. The mean metal amounts for all the investigated tissues and species are statistically compared and discussed in this study.

Keywords Enne Dame Lake · Freshwater fish · Heavy metal · ICP-OES

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Introduction

Fish is an important source of food for humans. Also it has been advised that fish should be consumed two or three times weekly, because of the pharmaceutical effects of omega 3 polyunsaturated fatty acids, which exist abundantly in fish oil

(Hu et al. 2003). But wide ranges of contaminants are continuously introduced into the aquatic environments and fish from polluted waters seriously threaten human health due to the bioaccumulation of toxic substances in muscle and other tissues (Sekhar et al. 2003). Furthermore, these contaminants also accumulate in the some organs of fish and can cause lethal and a variety of sub lethal effects (Ozmen et al. 2006). Among these toxic substances, heavy metals constitute one of the main dangerous groups, because they are toxic, persistent and not easily biodegradable. The pollution and the contamination of many ecosystems with heavy metals result from both anthropogenic and geologic sources. Geothermal springs may also have a significant role in the contamination of local waters by some heavy metals (Sabadell and Axtmann 1975; Ellis 1997).

In a recent century, it is stated that many priority heavy metals had made their way into the aquatic ecosystem and that their concentrations constantly increase (Topcuoglu et al. 2002; Barlas et al. 2006). Fish have been found to be good indicators of the heavy metal contamination levels in aquatic systems because they occupy different trophic levels (Burger et al. 2002; Karadede-Akin and Ünlü 2007). The amount of the toxic elements in fish is dependent on the concentration levels of these elements in the food and the habitats of the fish, and the detoxification rate of the metals (Urena et al. 2007).

Enne Dam Lake is approximately situated 10 km north of the city of Kütahya and is fed mostly by hot spring waters. Waters discharged to the Lake carry the volcano sedimentary units containing metallic ores, and the boron units in the Tunçbilek formation from the coal mine in the north of area. The dame lake supplies the water necessary for the cooling of the Seyitömer Thermic Station and for the irrigation of Kütahya Plain. The lake also receives agricultural and cattle related wastewater from surrounding villages, and all the wastewater, which is not adequately processed, from the Yoncalı hot spring water resorts. It has been reported that the pollution levels of this lake and its basin are remarkably high (Nalbantçılar et al. 2006; Canbek et al. 2007). Enne Dam Lake and its surrounding area were designated as a picnic area for Kütahya, because of its natural beauty. In the Lake, *C. carassius*, *C. nasus*, *L. cephalus* and *A. alburnus* are the fairly common freshwater fish and are caught in big numbers especially by the local sportive fishermen. The aim of the present study is to compare the bioaccumulation levels of Cd, Cu, Zn, Mn, Fe, Co, Mg, Ni, Cr and B in various tissues of some Cyprinid species (*Carassius carassius*, *Condrostoma nasus*, *Leuciscus cephalus* and *Alburnus alburnus*) in this lake.

Materials and methods

The study area and the fish samples

Enne Dam Lake (39° 28.483′ N, 29° 51.663′ E) is located in the north of Kütahya, a city in the district of the Middle West Anatolia of Turkey, at an altitude of 1008 m above the sea level. The fish samples (*C. carassius*, *C. nasus*, *L. cephalus* and *A. alburnus*) used in the experiment were caught using gill nets, cast nets and fishing lines. Sampling was performed in July 2005. The fish were first wrapped in polyethylene plastic and placed in an isolated container and transported to the Biology Laboratory of Dumlupınar University. Biometric measurements were performed, and the fish were immediately frozen and stored at −80°C until dissection. The sampled fish were of the mostly consumed sizes (Table 1).

Sample preparation

To minimize contamination, all the materials used in the experiments were previously washed in ultra pure water, and a stainless steel knife was used to cut the tissues. Before analysis the fish were thawed and a 0.5 g sample was taken from each tissue (Muscle, gill, skin, intestine and liver).

Table 1 Mean lengths and weights (grams) of the investigated fish

Species	Total length (cm)	Weight (g)
Carassius carassius	15.3 ± 0.83	72.75 ± 0.76
Condrostoma nasus	24.43 ± 0.35	160.36 ± 0.72
Leuciscus cephalus	35.90 ± 0.87	503.84 ± 0.57
Alburnus alburnus	12.06 ± 0.55	12.82 ± 0.65

Microwave method was applied for the digestion procedure of samples. Three thawed 0.5 g homogenates from each tissue were placed in a Teflon digestion vessel with 7 ml of concentrated nitric acid (HNO_3) and 1 ml of Hydrogen peroxide (H_2O_2). The samples in the vessels were then digested using an optimized microwave method. Then, they were let to cool at room temperature. The residues were then dissolved and diluted to 50 ml. The chemicals used for the sample dissolution were of analytical grade. Ultra pure water was used throughout the study (Moeller et al. 2001; Sandroni et al. 2003; Tuzen 2003).

Chemical analysis

Cu, Zn, Mn, Fe, Co, Mg, Ni, Cr and B were determined by a Perkin-Elmer DV 4300 inductively coupled plasma-optical emission spectrometry (ICP-OES). In the ICP-OES analysis, the following wavelength lines were used; Cu 324.8 nm, Zn 213.9 nm, Mn 232 nm, Ni 324.7 nm, Fe 248.3 nm, Cr 357.9 nm, Co 240.7 nm, Mg 285.2 nm, and B 249.7 nm. The quality of the analytical process was also controlled by the analysis of NIST-CE278 certified standard reference materials of mussel tissue. Analysis of Cd was also performed by Hitachi 180-70, polarized Zeeman Atomic Absorption Spectrophotometer (AAS) equipped with a graphite furnace. A cadmium hollow cathode lamp was used. Calibration was performed by analyzing three standard solutions (10, 20, 40 μg/l).

Bioaccumulation factor (BAF)

The bioaccumulation factor (BAF) was calculated according to the equation of BAF = CB/CWT; CB: the concentration of the heavy metals in the fish, CWT: the concentration of the heavy metals in the water (Mackay and Fraser 2000).

Statistical analyses

Each reported result was the average value of the three analyses. The results were offered as means ± SEM. The statistical differences of mean metal levels among tissues and species were analyzed using multiple comparison tests (SPSS package program). One-way ANOVA was utilized to compare the data by species and by tissue. Results were considered significant at $p < 0.05$.

Results and discussion

The heavy metal contents and bioaccumulation factors (BAFs) of the tissues of the investigated species were presented in Table 2. The comparisons of the heavy metal contents of the tissues and the species were also shown in Tables 3 and 4. The heavy metal levels were as follows for the water of Enne Dam Lake: Mg>B>Fe>Zn>Ni>Co>Cu>Mn. While B was determined to be in the highest concentration, following Mg, in the lake water, it was found to be below the detection limit with ICP-OES in all the tissues of the species. This implicates that boron can be detoxified by fish or does not accumulate as much as the detectable amount in fish tissues. It was also informed in the literature that B was not biomagnified in the aquatic food chain and its concentration in fish tissues was generally lower than biota (Saiki et al. 1993; Allen et al. 2001; Dashti et al. 2004). Our result concerning B accumulation to fish tissues was best fit to that result. The relatively high concentration of B in Enne Dam water can also be related to geological structure of the lake basin. Because it was reported that approximately 2/3 of the World boron reserve was in the Turkey and that a large part of Turkey's reserve was in the district of Kütayta (Güyagüler 2001).

In general, magnesium, zinc and iron had the highest concentrations in all tissues of the investigated species while cadmium was the element that had the lowest detected concentration. For example, the accumulation levels of heavy metals in tissues of *C. carssius* were: Mg>Zn>Fe>Cu>Mn>Cr>Cd for muscle; Mg>Zn>Fe>Mn>Cu>Co>Cd for gill; Mg>Zn>Fe>Mn>Cu>Cr>Cd for skin, Zn>Fe>Mg>Mn>Cu>Ni>Cd for intestine and Fe>Mg>Zn>Cu>Mn>Cr>Cd for liver. Approximately, the same orderings were also seen in the other investigated fish. These results show that the ordering of abundances of the metals does not considerably change among fish tissues. This may be the result of the fact that the investigated species are of the same family (*Cyprinidae*) and

Table 2 Heavy metal contents (milligram per kilogram wet weight) and BAFs of different tissues of the species

Water (mg/l)		Cu	Zn	Mn	Ni	Fe	Cr	Co	Cd	Mg	B
		0.003 ± 0.0	0.022 ± 0.0	0.001 ± 0.0	0.007 ± 0.0	0.076 ± 0.0	<DL	0.005 ± 0.0	0.003 ± 0.0	290.516 ± 3.5	0.129 ± 0.0
C. carassius	Muscle	1.51–503.3	30.06–1366.3	0.48–480.0	<DL-0	18.44–242.6	0.39	<DL-0	0.16–43.2	324.10–1.1	<DL-0
	Gill	1.51–503.3	166.75–7579.5	20.70–20700.0	<DL-0	130.60–1718.4	<DL	0.54–108.0	0.17–45.9	717.00–2.6	<DL-0
	Skin	0.99–330.0	126.45–5747.7	1.52–1520.0	<DL-0	44.53–585.9	0.08	<DL-0	0.01–2.7	224.85–0.7	<DL-0
	Intestine	1.02–340.0	357.25–16238.6	8.18–8180.0	0.80–114.2	291.70–3838.1	<DL	<DL-0	0.17–45.9	282.65–0.9	<DL-0
	Liver	7.04–2346.6	76.78–3490.0	3.18–3180.0	<DL-0	2500.33–32899.0	1.32	<DL-0	0.02–5.4	230.60–0.7	<DL-0
C. nasus	Muscle	0.27–90.0	6.96–316.36	<DL-0	6.21–887.1	49.86–656.0	0.54	<DL-0	0.07–18.9	364.46–1.2	<DL-0
	Gill	1.08–360.0	32.90–1495.45	14.57–14570.0	<DL-0	103.92–1367.3	<DL	<DL-0	0.22–59.4	699.10–2.4	<DL-0
	Skin	0.30–100.0	28.44–1292.72	0.43–430.0	<DL-0	24.66–324.4	0.33	<DL-0	0.08–21.6	197.44–0.6	<DL-0
	Intestine	1.00–333.3	18.43–837.72	19.28–19280.0	<DL-0	498.45–6558.5	1.74	<DL-0	0.10–27.0	359.00–1.2	<DL-0
	Liver	2.59–863.3	19.25–875.0	3.54–3540.0	1.78–254.2	489.66–6442.8	0.63	<DL-0	0.01–2.7	236.36–0.8	<DL-0
L. cephalus	Muscle	0.87–290.0	16.31–741.36	0.38–380.0	1.11–158.5	11.32–148.9	<DL	<DL-0	0.07–18.9	292.75–1.0	<DL-0
	Gill	<DL-0	51.45–2338.63	4.14–4140.0	0.64–91.4	43.76–575.7	<DL	<DL-0	0.02–5.4	503.95–1.7	<DL-0
	Skin	<DL-0	28.49–1295.0	<DL-0	<DL-0	9.62–126.5	<DL	<DL-0	0.01–2.7	254.66–0.8	<DL-0
	Intestine	<DL-0	29.29–1331.36	<DL-0	<DL-0	64.80–852.6	<DL	<DL-0	0.01–2.7	220.40–0.7	<DL-0
	Liver	<DL-0	29.81–1355.0	<DL-0	<DL-0	64.84–853.1	<DL	<DL-0	0.02–5.4	211.75–0.7	<DL-0
A. alburnus	Muscle	<DL-0	21.10–959.09	<DL-0	<DL-0	24.88–327.3	<DL	<DL-0	0.05–13.5	243.93–0.8	<DL-0
	Gill	<DL-0	74.99–959.09	10.98–10980.0	<DL-0	102.76–1352.1	<DL	<DL-0	0.06–16.2	833.56–2.8	<DL-0
	Skin	<DL-0	96.05–4365.90	10.64–10640.0	<DL-0	38.67–508.8	<DL	<DL-0	0.07–18.9	904.90–3.1	<DL-0

Each value is an average of three representative samples

<DL below detection limit

Table 3 The comparison of different tissues of the species with respect to the metal accumulation levels

	C. carassius	*C. nasus*	*L. cephalus*	*A. alburnus*
Cu	$L^2 > M^1 = G^1 > I^1 > S^1$	$L^3 > G^2 > I^2 > S^1 > M^1$	M	
Zn	$I^4 > G^3 > S^3 > L^2 > M^1$	$G^2 > S^2 > L^{1,2} > I^{1-2} > M^1$	$G^2 > L^{1-2} > I^{1-2} > S^{1-2} > M^1$	$S^3 > G^2 > M^1$
Mn	$G^3 > I^2 > L^{1-2} > S^1 > M^1$	$I^2 > G^2 > L^1 > S^1$	$I^2 > M^1$	$G^1 > S^1$
Ni	I	$M^2 > L^1$	$M^1 > G^1$	–
Fe	$L^5 > I^4 > G^3 > S^2 > M^1$	$I^3 > L^3 > G^2 > M^1 > S^1$	$L^2 > I^2 > G^2 > M^1 > S^1$	$G^2 > S^1 > M^1$
Cr	$L^3 > M^2 > S^1$	$I^2 > L^1 > M^1 > S^1$	–	–
Co	G	–	–	–
Cd	$G^2 = I^2 > M^2 > L^1 > S^1$	$G^3 > I^2 > S^2 > M^2 > L^1$	$M^3 > L^2 = G^2 > I^1 = S^1$	$S^3 > G^2 > M^1$
Mg	$G^2 > M^1 > I^1 > L^1 > S^1$	$G^3 > M^2 > I^2 > L^1 > S^1$	$G^2 > M^1 > S^1 > I^1 > L^1$	$S^2 > G^2 > M^1$

The difference in the metal accumulation levels of the tissue with a different number in the same row relating to any species is significant ($p < 0.05$)

M muscle, *G* gill, *S* skin, *I* intestine, *L* liver

share the same habitat. But there were significant differences in the heavy metal amounts among tissues (Table 3). This result also shows that accumulation ratio and biological demand of any metal are different for each fish tissue. A lot of researchers have reported that metals accumulate in high concentrations in the liver, the gill and the intestine, because these organs have relatively higher potential for metal accumulation than muscle (Demirak et al. 2006; Yang et al. 2007). For example, it was found that the higher accumulation ratios of metals in the liver could be due to the greater tendency of the elements to react with the oxygen carboxylate, the amino group, the nitrogen and/or the sulphur of the mercapto group in the metallothionein protein (Al-Yousuf et al. 2000; Usero et al. 2004). Although these findings are generally consistent with our results, there are also exceptions. For instance, the muscle Cd contents of *C. carssius*, *C. nasus* and *L. cephalus* were remarkably higher than some metabolite organs such as the liver (Table 3). As indicated in the literature, this finding may be the result of specific bioaccumulation characteristics, special to the species and the elements (Henry et al. 2004; Papagiannis et al. 2004).

The highest Cd, Cu, Zn and Mn accumulations were in the muscles of *C. carassius*. The levels of Cu and Zn in all tissues and organs of *C. carassius* were also higher than the other species studied (Tables 3 and 4). The high heavy metal, especially Cu, Zn and Cd, bioaccumulation tendency of *C. carassius* tissues may be due to their ecological demands. Because this species dependent on sediment and burrows in mud in the dry season or during winter.

The bioaccumulation factor (BAF) is a number that describes the bioaccumulation as the ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment (Mackay and Fraser 2000). In all investigated tissues of the species, while the BAFs of Mn, Zn, Fe and Cu were remarkably high, the BAFs of Mg, Cr, Co, and B were also fairly low or none. The highest BAFs were determined in liver of *C*. nasus for Cu and Ni; in the intestine of *C. carassius* for Zn; in the gill of *C. carassius* for Mn and Co; in the liver of *C. carassius* for Fe; in the gill of *C. nasus* for Cd and in the skin of *A. alburnus* for Mg (Table 2). It has been indicated that BAFs from environment to fish tissue changes according to the species of the chemical, the metabolite properties of the tissues and the pollution degree of the environment (Ayas et al. 2007; Ayaş 2007; Ozmen et al. 2008). The BAFs of Cd, the most dangerous element, also ranged from 2.7 to 49.9 in all tissues of the species. While Mg was of the most abundant elements in all tissues of the species, its BAFs were quite low, ranged from 0.6 to 2.8.

The heavy metal concentrations of fish species in different freshwater reservoirs of Turkey have been widely studied (Karadede and Unlu 2000; Altındag and Yigit 2005; Mendil et al. 2005; Canbek et al. 2007; Dural et al. 2007; Karadede-Akin and Ünlü 2007; Mendil and Uluözlü 2007; Yilmaz et al. 2007). There are similarities, as well as differences, between the results from our study

Table 4 The comparison of the species with respect to the metal accumulation levels of the tissues

Cu	Muscle	*C. carassius*^{2} > *L. cephalus*$^{1-2}$ > *C. nasus*^{1}
	Gill	*C. carassius*^{2} > *C. nasus*^{1}
	Skin	*C. carassius*^{2} > *C. nasus*^{1}
	Intestine	*C. carassius*^{2} > *C. nasus*^{1}
	Liver	*C. carassius*^{2} > *C. nasus*^{1}
Zn	Muscle	*C. carassius*^{2} > *A. alburnus*$^{1-2}$ > *L. cephalus*$^{1-2}$ > *C. nasus*^{1}
	Gill	*C. carassius*^{4} > *A. alburnus*$^{2-3}$ > *L. cephalus*$^{1-2}$ > *C. nasus*^{1}
	Skin	*C. carassius*^{2} > *A. alburnus*^{2} > *L. cephalus*^{1} > *C. nasus*^{1}
	Intestine	*C. carassius*^{2} > *L. cephalus*^{1} > *C. nasus*^{1}
	Liver	*C. carassius*^{2} > *L. cephalus*^{1} > *C. nasus*^{1}
Mn	Muscle	*C. carassius*^{2} > *L. cephalus*^{1}
	Gill	*C. carassius*^{3} > *C. nasus*$^{2-3}$ > *A. alburnus*$^{1-2}$ > *L. cephalus*^{1}
	Skin	*A. alburnus*^{2} > *C. carassius*^{1} > *C. nasus*^{1}
	Intestine	*C. nasus*^{2} > *C. carassius*^{1}
	Liver	*C. nasus*^{1} > *C. carassius*^{1}
Ni	Muscle	*C. nasus*^{2} > *L. cephalus*^{1}
	Gill	*L. cephalus*
	Skin	–
	Intestine	*C. carassius*
	Liver	*C. nasus*
Fe	Muscle	*C. nasus*^{2} > *A. alburnus*^{1} > *C. carassius*^{1} > *L. cephalus*^{1}
	Gill	*C. carassius*^{3} > *C. nasus*$^{2-3}$ > *A. alburnus*$^{2-3}$ > *L. cephalus*^{1}
	Skin	*C. carassius*^{3} > *A. alburnus*$^{2-3}$ > *C. nasus*$^{1-2}$ > *L. cephalus*^{1}
	Intestine	*C. nasus*^{3} > *C. carassius*^{2} > *L. cephalus*^{1}
	Liver	*C. carassius*^{3} > *C. nasus*^{2} > *L. cephalus*^{1}
Cr	Muscle	*C. nasus*^{1} > *C. carassius*^{1}
	Gill	–
	Skin	*C. nasus*^{1} > *C. carassius*^{1}
	Intestine	*C. nasus*
	Liver	*C. carassius*^{1} > *C. nasus*^{1}
Co	Muscle	–
	Gill	*C. carassius*
	Skin	–
	Intestine	–
	Liver	–
Cd	Muscle	*C. carassius*^{2} > *L. cephalus*^{1} = *C. nasus*^{1} > *A. alburnus*^{1}
	Gill	*C. nasus*^{4} > *C. carassius*^{3} > *A. alburnus*^{2} > *L. cephalus*^{1}
	Skin	*C. nasus*^{2} > *A. alburnus*^{2} > *C. carassius*^{1} = *L. cephalus*^{1}
	Intestine	*C. carassius*^{3} > *C. nasus*^{2} > *L. cephalus*^{1}
	Liver	*L. cephalus*^{2} = *C. carassius*^{2} > *C. nasus*^{1}
Mg	Muscle	*C. nasus*^{2} > *C. carassius*^{2} > *L. cephalus*^{1} > *A. alburnus*^{1}
	Gill	*A. alburnus*^{2} > *C. carassius*^{2} > *C. nasus*$^{1-2}$ > *L. cephalus*^{1}
	Skin	*A. alburnus*^{2} > *L. cephalus*^{1} > *C. carassius*^{1} > *C. nasus*^{1}
	Intestine	*C. nasus*^{2} > *C. carassius*$^{1-2}$ > *L. cephalus*^{1}
	Liver	*C. carassius*^{1} > *C. nasus*^{1} > *L. cephalus*^{1}

The difference in the metal accumulation levels of the species with a different number in the same row is significant ($p < 0.05$)

and the findings from other researches. It has been indicated that the levels of heavy metals in fish depend on habitats (Canli and Atli 2003), the durations of exposure of the fish to contaminants, their feeding habits (Canli and Kalay 1998), and the age and the size of the species (Rashed 2001; Fernandes et al. 2007). Therefore, it is very difficult to compare the metal concentrations, even between the same tissues, for two different species.

In the literature, the permissible levels (milligram per kilogram wet weight), above which human consumption is not permitted for Turkish legislation, FAO and WHO are: 0.1 for Cd, 20 for Cu and 50 for Zn. The edible parts of the investigated tissues are muscle, skin, and, rarely, liver. Cu concentrations of all edible tissues of the species were considerably lower than the permissible levels set by Turkish legislation, FAO and WHO. But the permissible limits for Cd and Zn were exceeded in some of the edible tissues of the species analyzed in this study. Zn contents of both the skin and the liver of *C. carassius* and the skin of *A. alburnus* exceeded the permissible tolerable limits. In all species, Zn concentrations of the muscle, the main edible portion of fish, were lower than the maximum limit. Cd content of the muscle was higher than the legal limit only in *C. carassius*. Therefore, we can conclude that the investigated species except *C. carassius* and *A. alburnus* present no hazard for the consumption of humans. But we can say that all edible parts of *C. carassius*, and the skin of *A. alburnus* must not be consumed for the present.

Conclusions

The accumulation ratios of heavy metals in four fish species from Enne Dam Lake were studied. Although boron had the second highest concentration among the investigated elements in the lake water, it was not accumulated in the fish tissues in range of detectable limits. While the heavy metal levels in the tissues of different species showed significant difference, when ranked by the magnitude of their corresponding accumulation levels, the orderings were generally similar each other. The muscle accumulates some metals more compared to the active metabolite tissues or organs like the liver. Among the fish in Enne Dame Lake, especially the *C. carassius* must not be consumed by humans, because the Cd concentration levels of the muscle are higher than the permissible limits.

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