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BIOACCUMULATION RATIOS OF SEDIMENT-BOUND HEAVY METALS OF PORSUK AND ENNE DAM LAKES (KÜTAHYA/TURKEY) TO DIFFERENT TISSUES OF COMMON CARP (*Cyprinus carpio*)

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ABSTRACT

In this study, sediment-bound heavy metal levels of Enne and Porsuk Dam Lakes and their bioaccumulation factors (BAFs) to various tissues (Muscle, skin, gill, liver and intestine) of common carp (*Cyprinus carpio*) were investigated. After per month acclimation period the fingerlings were subjected to one month assay in eight tanks. The analyses of heavy metals (Hg, Cd, Pb, Cu, Zn, Mn, Ni, Fe, Cr, B, Ag and Se) were performed with Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) in Laboratory of Environmental Issues Research and Training Centre, Anadolu University. Hg and B were not encountered in sediments of both Enne and Porsuk Dam Lakes. There were any statistical differences (Except Zn) between heavy metal concentrations of both dame lakes. According to international criterias and Turkish regulations, heavy metal concentrations (Except Ni) in sediment of both dame lakes were markedly below the maximum permissible levels. BAFs of the elements were significantly different. Metal levels in various tissues of the sediment-treated group were generally higher than those of the control group. Althouhg BAFs (from sediments) of some elements were above 1 in some tissues, BAFs of all elements were also markedly below 1 in the muscle tissue.

Key Words: Bioacumulation, *Cyprinus carpio,* Heavy Metal, Sediment, ICP-OES.

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ÖZET

PORSUK VE ENNE BARAJ GÖLLERİ (KÜTAHYA/TÜRKİYE) SEDİMENTİNE BAĞLI AĞIR METALLERİN PULLU SAZANIN (*Cyprinus carpio*) FARKLI DOKULARINA BİYOAKÜMÜLASYON ORANLARI

Bu çalışmada, Enne ve Porsuk Baraj Göl'leri sedimentine bağlı ağır metal seviyeleri ve *Cyprinus carpio*'nun farklı dokularına (Kas, deri, solungaç, karaciğer ve bağırsak) bioakümülasyon faktörleri (BAF) araştırılmıştır. Bir aylık alıştırma periyodundan sonra, fingerlingler toplam 8 tanklık deney düzeneğinde bir ay süre ile beslenmişlerdir. Ağır metal (Hg, Cd, Pb, Cu, Zn, Mn, Ni, Fe, Cr, B, Ag ve Se) analizleri Anadolu Üniversitesi Çevre Mühendisliği Bölümü'nde bulunan İndüktif Eşleşmiş Plazma-Optik Emisyon Spektroskopi (ICP-OES) ile yapılmıştır. Hem Enne hem de Porsuk Baraj Gölleri sedimentinde Hg ve B elementleri tespit edilememiştir. Her iki gölün ağır metal konsantrasyonları arasında (Zn hariç) istatistiksel fark yoktur. Uluslar arası kriterlere ve Türk standartlarına göre her iki gölün sedimentinde ağır metal konsantrasyonları (Ni hariç) izin verilen maksimum seviyelerden önemli derecede düşük bulunmuştur. Elementlerin bioakümülasyon faktörleri önemli derecede farklıdır. Sediment muamele edilmiş grupların farklı dokularında metal seviyeleri genel olarak kontrol grubundan yüksektir. Bioakümülasyon faktörleri (BAF) bazı dokularda 1'in üzerinde bulunmasına rağmen kas dokusunda bütün elementler için hesaplanan biokümülasyon faktörlerinin 1 den önemli derecede düşük olduğu tespit edilmiştir.

Anahtar Kelimeler: Biyoakümülasyon, *Cyprinus carpio*, Ağır Metal, Sediment, ICP-OES.

INTRODUCTION

Heavy metals have discharged into the environment for a long time. Therefore, this types of pollution is now a series problem in all of the word, especially in the industrialized countries. Heavy metals in any environment have eventually collected in aquatic reservoirs, especially in their sediments. Therefore, aquatic animals, especially ones connected sediment, may be exposed to elevated levels of heavy metals (Altındağ & Yiğit, 2005; Sekhar et al. 2003). Metals bound to the sediment might pose a threat to aquatic biota, and consequently might cause detrimental effects to human health through the consumption of aquatic food (Chen & Chen, 1999; Yılmaz et al. 2003). Therefore, heavy metal louts of aquatic environments and their risk potentials must be investigated. Bioaccumulation factor (or transfer factor) is the process which causes an increased chemical concentration in an aquatic organism compared to those in aquatic ecosystem (Water, sediment and food). Bioaccumulation factor (BAF) indicates whether heavy metal biomagnification take place. This factor greater than 1 indicates biomagnification (Rashed, 2001; Mackay et al. 2000).

Porsuk and Enne Dame Lakes are known as a highly polluted freshwater reservoir in Kütahya/Turkey due to the industrialization and urbanization activities. The surroundings of these lakes are also very rich in boron minerals and thermal springs. There are some studies on heavy metal levels of fish in these two dame lakes (Uysal et al. 2009; Canbek et al. 2007). But it has not been investigated the heavy metal lout of

sediment and its bioaccumulation ratios to fish tissues in control conditions. The main aim of this study was to investigate the sediment-bound heavy metal (Hg, Cd, Pb, Cu, Zn, Mn, Ni, Fe, Cr, B, Ag and Se) concentration of Enne and Porsuk dama lakes (in Turkey), and the bioaccumulation ratios of these heavy metals to different tissues (Muscle, gill, skin, intestine and liver) of common carp (*Cyprinus carpio)*.

MATERIALS AND METHODS

Experimental Assay

Sediment collection and preparation

Sediment samples used in the experiments were collected from both Porsuk (39°33'-39°40'N, 30°05'-30°16'E) and Enne (39° 28.483' N, 29° 51.663'E) dame lakes in the Middle West Anatolia of Turkey. Sediment sampling was performed from six stations of each dame lake using Ekman sediment grab. Collected sediment samples were dried first on sunshine and later in an etuv, and then sieved through a 0.5cm mesh to remove coarse fragments. After sieving, sediment samples from six stations of each dame lake were well homogenized in itself.

Origin of Cyprinus carpio and preperation of aquarium tanks

Cyprinus carpio fingerlings (8.18±0.3 g wet weight and 10.15±0.9 cm total lenght) were obtained from *The Mediterranean Fisheries Research, Production and Education Institute* (Antalya/Turkey). Fish fingerlings were acclimated the experimental conditions for one month. After acclimation period the fingerlings were also subjected to one month assay in eight tanks (100x40x30 cm): 3 tanks withought sediment as control, 3 tanks with sediment from the Porsuk dame lake, and 3 tanks with sediment from the Enne dame lake. Each tank approximately contained 120 l of water. The six tanks except control were added 15 g/l sediment. The aquariums were well aerated in order to maintain the oxygen levels above 6 mg/l, but without disturbing the sediment stratum. Each tank held six randomly selected individuals. Fish were fed with a commercial fish food twice a day. The daily feeding amount was approximately 2% of the total fish weight.

Temperature was maintained constant at 25±1 ^{o}C and other some physico-chemical parameters (pH, ammonia, ammonium, nitrite, nitrate and dissolved oxygen) of tanks' water were also regularly measured. Heavy metal concentrations of experimental water, sediment and commercial food were initially also measured. Any fish did not die during experiment.

Heavy Metal Analysis

Fish exposed to sediment throughout one mounth were dissected for muscle, skin, gill, liver and intestine. Due to the small size of fish tissues, pooled tissues of the same type from 3 fish were used for metal analyses. Sediment, food and tissue samples were dried for 24 h at 105°C. After all samples had been passed through a nylon sieve (0.5 mm). 0.5 g of each sample was placed in pyrex reactors of a (CEM Marsxpress) microwave digestion unit. $HClO_4$: HNO_3 acids of 1:3 proportions for fish were inserted in the reactors respectively. Samples were mineralized at 200^{o}C for thirty minutes.

Afterwards, the samples were filtered (Whatman No.41, pore size 20-25 µm or Macherey-Nagel MN 640 de) in such a way as to make their volumes to 100 ml with ultra-pure distilled water. A blank digest was carried out in the same way. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/l of each element supplied from SCP SCIENCE. Heavy metals concentrations were measured by Varian 720 ES model ICP-OES (ASTM, 1985; APHA, 1992; Bialonska & Dayan, 2005; EPA 3051, 1994). All element calibration solutions were arranged in accordance with the concentrations in the sample. Hg and Se were determined by hydride generation-ICP OES. Data presented are the averages of at least three independent experiments each of them in three replications. The analytical process quality was also controlled by certified reference material of NCS DC73350 (leaves of poplar). The analysis of these standard reference materials showed good accuracy, with the recovery rates of the metals, between 90.3 % and 108.9 % (Table 1). Recoveries were compared with the American Organization of Analytical Chemists criteria (AOAC, 1993). In ICP-OES analyses, the method detection limits (MDL) of the measured elements were defined as the concentration of an element which will give a signal three times higher than the standard deviation of seven to ten replicate measurements of five reagent blanks belonging to five different sets of the experiments. The wave lengths and detection limits were given Table 2.

Table1. Element levels results of reference materials (mg kg^{-1})
Tablo 1. Referans metaryallerin element seviye sonuçları (mg kg^{-1})

Elements	NCS DC73350 (leaves of poplar)		Recovery %	AOAC[a] %
	Certified	Analytical value		
Ag	(0.013)	0.015±0.004	115.3	85-110
Cd	0.32±0.07	0.32±0.01	100.0	80-110
Cr	0.55±0.07	0.56±0.005	101.8	80-110
Cu	9.3±1.0	8.4±0.45	90.3	80-110
Fe	274±17	287.66±11.06	104.9	90-107
Mn	45±4	43.6±6.41	96.8	80-110
Hg	0.026±0.03	0.024±0.004	92.3	75-120
Pb	1.5±0.3	1.63±0.37	108.6	80-110
Ni	1.9±0.3	2.00±0.28	105.2	80-110
Zn	37±3	35.93±3.23	97.1	80-110
B	53±5	54.5±2.98	102.8	80-110
Se	0.14±0.02	0.15±0.01	107.1	80-115

[a] AOAC American Organization of Analytical Chemists.

Table 2. The wave lengths and detection limits for ICP-OES of elements.
Tablo 2. Elementlerin ICP-OES için dalga boyları ve dedeksiyon limitleri

Elements	Wave Lengths	Detection Limits (μg L^{-1})
Ag	328.068	0.5
B	249.773	0.07
Cd	228.802	0.4
Cr	267.716	0.5
Cu	324.753	0.3
Fe	259.940	0.4
Hg	194.164	2
Mn	257.610	0.05
Ni	231.604	1.3
Pb	220.353	3
Zn	213.856	0.3
Se	196.026	5

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 & Fraser, 2000).

Condition Factor (Kf)

Condition factor (Kf) was calculated according to the equation of [Kf=total weigh/(total lenght)^3 x 100] (Martinez, 2001; Korkut, 2007).

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

Total weights, total lenghts and condition factors (Cf) of experimental fish were given in Table 3. None of the experimental fish died along the one month test period At the end of experiment, although total weight and lenth of the fish increased, their condition factors (Cf) insignificantly decreased. Nevertheless Cf of the fish were withing the normal range. Because it has been indicated that growth performance of fish is well if Cf is about 1 or above 1 (Korkut et al., 2007).

Table 3. Total weights, total lengths and condition factors (Cf) of experimental fish.
Tablo 3. Deneyde kullanılan balıkların toplam ağırlık,toplam boy ve kondüsyon faktörleri

	Control (Aquariums withought any sediment)		Fish in aquariums with Enne Dame Lake's sediment		Fish in aquariums with Porsuk Dame Lake' sediment	
	BE	AE	BE	AE	BE	AE
Total weight (cm)	9.9±1.2	13.8 ±3.6	10.2 ±0.6	15.5 ±3.4	10.1 ±0.9	12.3 ±2.4
Total length (g)	8.1±0.3	9.2 ±0.8	8.4 ±0.2	9.8 ±0.6	7.9 ±0.2	9.0 ±0.6
Cf	1.8±0.02	1.6 ±0.01	1.6±0.03	1.57 ±0.02	1.9±0.01	1.5 ±0.04

BE: Before experiment; AE: after experiment

Heavy metal concentrations of experimental water, sediment and commercial food were also given in Table 4. The orders of heavy metal concentrations were Fe> Mn> Zn> Ni> Pb> Cr> Cu> Cd> Se in sediment of Enne Dama Lake, Fe> Zn> Mn> Ni> Pb> Cr> Cu> Cd> Se> Ag in sediment of Porsuk Dama Lake, Fe> B> Cd> Pb> Se> Cu> Cr> Ni> Ag in experimental water and Fe> Mn> Zn> Cu> Cr> Ag> Pb> Ni> Se> Cd in the fish diet. There was not substantially significant difference between the heavy metal concentrations of the sediments of both dame lakes.

Table 4. Heavy metal concentrations of Enne and Porsuk Dam Lakes' sediment, water and diet used in experiment.
Tablo 4. Enne ve Porsuk Baraj Gölü suyunun, sedimentinin ve deneyde kullanılan yemin ağır metal konsantrasyonları.

	Enne Dam Lakes' sediment (mg kg^{-1})	Porsuk Dam Lakes' sediment (mg kg^{-1})	Water (mg L^{-1})	Diet (mg kg^{-1})
Hg	BDL	BDL	BDL	BDL
Cd	5.04 ±0.15	3.36 ±0.13	0.0297 ±0.0003	0.80 ±0.12
Pb	88.96 ±3.22	90.00 ±40.66	0.02 ±0.006	3.33 ±0.64
Cu	27.84 ±0.68	26.08 ±1.20	0.008 ±0.0006	31.53 ±5.13
Zn	272.00 ±11.47	656.40 ±32.39	BDL	222.13 ±21.81
Mn	626.40 ±15.32	642.80 ±23.90	BDL	320.00 ±10.26
Ni	136.82 ±5.10	159.12 ±5.65	0.004 ±0.002	3.30 ±2.37
Fe	34030.00 ±1194.32	36550.00 ±1688.96	0.15 ±0.001	1082.20 ±295.77
Cr	59.08 ±1.79	78.40 ±4.57	0.0067 ±0.0007	13.93 ±1.07
B	BDL	BDL	0.052 ±0.014	BDL
Ag	BDL	0.73 ±0.10	0.0015 ±0.0004	7.80 ±2.60
Se	2.70 ±0.32	2.40 ±0.54	0.01 ±0.003	1.93 ±0.27

BDL: Below detection limit

While B was determined the highest concentration in the experiment water, it was found to be below the detection limit with ICP-OES in sediment of both Dam Lakes.

Because B was a soluble element in water, it was expected result that B was higher concentration in water than in sediment. Nevertheless, B was not detected in any tissue of experimental fish tissue as well. This result indicates that B can be detoxified by fish or does not accumulate as much as the detectable amount in fish tissues. It was informed that B was not biomagnified in the aquatic food chain and its concentration in fish tissues was generally lower than biota (Allen et al, 2001; Saiki et al.,1993). Our result concerning B accumulation to fish tissues was fit to that result.

Hg concentration in sediment of both dame lake was below the detection limit. Cd and Pb concentration was determined 5.04, 88.96 mg/kg in the sediment of Enne Dam Lake and 3.36, 90.00 mg kg^{-1} in the sediment of Porsuk Dame Lake in this study, respectively. Highest concentrations (as mg kg^{-1}) of these two toxic elements Cd and Pb in sediment of Turkish water were determined 14.25 and 33.45 in Lake Beyşehir (Altındağ & Yiğit, 2005). 0.80 and 83.60 in Dipsiz stream (Demirak et al., 2006), 0.93 and 31.1 in Türkish Coast of the Black Sea (Topçuoğlu et al., 2002), 0.82 and 113 in İzmir Bay (Küçüksezgin, 2006), 0.69 and 20.2 in Southeastern Aegean Sea (Dalman et al., 2006) below dedected limits in Atatürk Dam Lake (Karadede & Ünlü, 2000), 0.69 and 20.2 in Turkish Coast of Southeastern Aegean Sea (Dalman et. al., 2006). In the present study, Cd concentration in the sediment of investigated both dame lakes were remarcably higher than the data reported that reseachers except Altındag & Yigit (2005) for Lake Beyşehir. Fairly higher Cd concentration in the sediment of the both dame lakes may be possibly due to direct contamination of the water by Cd or the geochemical structure of the region. Pb concentrations of the investigated sediment were also higher than the values reported in that aquatic habitats except İzmir Bay. Nevertheless, according to Provincial sediment Quality Guidelines for Metals, Cd and Pb concentrations in sediment of the investigated dame lakes were markedly below the severe effect levels 10 g kg^{-1} for Cd and 250 g kg^{-1} for Pb (Anonim, 1993).

Cd concentration in muscle, gill and skin of the fish in aquariums with sediment was remarcably higher than those of the control groub fish withought sediment. But there were not significantly differences among Cd concentration in intestine and liver of the experimental fish. This result may arise from the relatively high Cd content of the fish diet (Table 4). While and although Pb concentrations in all tissues of the control groub fish was below detection limit, some tissues of fish in aquariums with sediment accumulate relatively high level of Pb. But bioaccumulation factors (BAFs) of both Cd and Pb in all tissues of experimental fish were markedly lower than 1. This finding indicates that Cd and Pb does not bioaccumulate to fish tissues in short time like one month.

Cu concentrations in livers, reverse to other tissues were higher than that of water, diet and sediment of both lakes. BAFs of Cu in liver were also higher than 1. Based on this results, it may be said that liver of fish are very important Cu accumulator organ or that these metal were regulated in the liver (Chen & Chen, 1999). BAFs of Zn and Se in some tissues except muscle were also higher than 1 (Table 5). These findings show that accumulation patterns of metals in fish were also metal and target organ dependent. Cu, Zn and Se are biologically required and essential for all animal. Their concentrations higher than certain limits are only harmful to fish. Maximum levels (mg kg^{-1}) of Hg, Cd, Pb, Cu and Zn above which human consumption is not permitted: were informed in Turkey Legislation 0.5 for Hg, 0.1 for Cd, 1 for Pb, 20 for Cu, 50 for Zn. Our study indicate that the investigated sediment-bound toxic or nontoxic elements in the

Porsuk and Enne dame lakes can not accumulate at the unacceptable levels in edible muscle tissue of common carp in a short time like one month.

Table 5. Heavy metal amounts (mg kg^{-1}dry weight) and bioaccumulation factors (BAF)* of experimental fish

Tablo 5. Deneyde kullanılan balıkların ağır metal miktarları (mg kg^{-1} kuru ağırlık) ve biyoakümülasyon (BAF) faktörleri

Hg	FC	Muscle	Gill	Skin	Intestine	Liver
		BDL	BDL	BDL	BDL	BDL
	FE	BDL	BDL	BDL	BDL	BDL
	FP	BDL	BDL	BDL	BDL	BDL
Cd	FC	BDL	BDL	0.2±0.0	1.3 ±0.2	0.8 ±0.0
	FE	0.2±0.0 (0.04)	0.6±0.1(0.12)	1.0 ± 0.1(0.20)	1.0±0.0 (0.20)	1.1±0.1 (0.22)
	FP	0.3±0.0-(0.09)	0.5±0.0 (0.15)	2.2±0.2 (0.66)	1.5±0.3 (0.45)	0.9±0.1 (0.27)
Pb	FC	BDL	BDL	BDL	BDL	BDL
	FE	0.7±0.4 (0.007)	BDL	BDL	BDL	1.6±0.4 (0.01)
	FP	1.7±0.7 (0.01)	0.5±0.0 (0.005)	3.7±1.05 (0.04)	BDL	4.5±1.8 (0.05)
Cu	FC	BDL	BDL	BDL	BDL	46.7±14.8
	FE	BDL	BDL	BDL	BDL	43.1±10.4 (1.55)
	FP	BDL	BDL	4.15±0.0 (0.15)	BDL	36.8±2.3-(1.41)
Zn	FC	26.0 ±5.9	944.2±59.0	226.9±19.0	797.2 ±36.6	150.8 ±16.7
	FE	51.2±12.3 (0.18)	865.6±61.6 (3.18)	329.9±32.9 (1.21)	802.4±41.2 (2.95)	231.2±65.6 (0.85)
	FP	56.1±0.7 (0.08)	898.5±47.8 (1.36)	292.8±13.6 (0.44)	778.4±64.2 (1.18)	243.4 ±69.6 (0.37)
Mn	FC	BDL	1.7 ±0.4	BDL	BDL	BDL
	FE	BDL	10.9 ±0.7-(0.01)	BDL	3.8 ±0.2 (0.006)	5.7 ±1.7 (0.009)
	FP	12.6±0.4 (0.01)	6.2±0.7 (0.009)	BDL	BDL	32.9±6.7 (0.05)
Ni	FC	BDL	BDL	BDL	BDL	BDL
	FE	BDL	BDL	BDL	BDL	BDL
	FP	1.1±0.4-(0.006)	BDL	BDL	BDL	1.2±0.2 (0.007)
Fe	FC	60.7 ±15.3	194.7±48.3	69.7±14.2	109.5±38.0	431.0±50.7
	FE	115.0±32.0 (0.003	329.2±38.7 (0.009)	85.0±0.9 (0.002)	405.4±77.5 (0.01)	903.4±140.6 (0.02)
	FP	114.2±25.1 (0.003)	304.3±31.4 (0.008)	115.9±22.0 (0.003)	533.4±96.2 (0.01)	1224.7±242.3 (0.03)
Cr	FC	BDL	3.1±0,1	BDL	BDL	BDL
	FE	BDL	4.7±2.3 (0.07)	BDL	BDL	BDL
	FP	BDL	7.6±0.1 (0.09)	BDL	BDL	1.1±0.1 (0.01)
B	FC	BDL	BDL	BDL	BDL	BDL
	FE	BDL	BDL	BDL	BDL	BDL
	FP	BDL	BDL	BDL	BDL	BDL
Ag	FC	BDL	BDL	BDL	BDL	BDL
	FE	BDL	BDL	BDL	BDL	BDL
	FP	BDL	BDL	BDL	BDL	BDL
Se	FC	BDL	BDL	BDL	BDL	3.2±1.0
	FE	1.2±0.4 (0.44)	5.1±1.4 (1.88)	BDL	BDL	6.3±0.5 (2.33)
	FP	0.7±0.4 (0.29)	2.5±0.1 (1.04)	BDL	3.5±1.0 (1.45)	5.9±2.1 (2.45)

* BAF values are shown in parantheses at the table. BDL: Below detection limit; FC: Fish rared without sediment (Control group); FE: Fish rared in sediment of Enne Dame Lake; FP: Fish rared in sediment of Porsuk Dame Lake

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