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Determination of PCB levels in skin and muscle of northern pike (*Esox lucius*) in Anzali Wetland, Iran

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ABSTRACT

This study was carried out to determine the PCBs levels in skin and muscle tissues of Esox lucius in Abkenar (western area of Anzali wetland) in autumn (December 2009). Water samples, sediment and suspended particles in water and E.lucius caught in Abkenar zone were submitted to four selected stations for analysis. The biometric values including total weight and length were measured for the fish samples. All the samples obtained i.e. water, sediment and suspended particles as well as the fish sample were transferred to the laboratory according to the Moopam standard [8]. The skin and muscle tissues of E.lucius were separated and prepared according to the same standard and the PCBs level was analyzed by using a Gas Chromatography (GC–ECD) equipped with a Ni63 detector. Average concentration of PCBs in E.lucius skin and muscle samples were 24.36 and 14.36 ng/g dry weight, respectively. The average coefficient of variation (C.V) of PCBs concentration in E.lucius skin was more than that of the muscle. A highly significant difference was observed between the muscle and skin tissues of E.lucius ($P<0.000$; $n=9$). Also, a significant difference was observed between PCBs levels in water and sediment samples ($p<0.000$; $n=12$) and particles suspended in filtered water ($p<0.0001$; $n=12$). Moreover, there was a very strong positive correlation found between the PCBs levels in water and the filtered suspended particles in water ($p=0.0001$; $r=0.980$; $r2=96\%$; $n=12$). Level of accumulation of biological in skin texture of E.lucius was about 5835.69 ppb and in its muscle texture was 3440.58 ppb. Maximum level of average PCBs measured in the fish tissues in autumn (maximum 0.03653 ppm. and minimum 0.01025 ppm) was less than those of the FDI standard and Food Standards Australia/NZ MRL and was more than that of USEPA standard (skin texture of E.lucius). The most dangerous isomers of PCB as indicators of PCB in polluting seas and particularly the coastal region, like seaport and estuary are studied (PCB 28,52,101,138,153,180), in skin texture and muscle is being investigated that the maximum density of mentioned dangerous isomers in autumn in skin and muscle of E.lucius was more in isomers with chlorine (related to 153 PCB that its maximum in sample number 9 (3.64ppb) but in measurement environment of water, suspended particles of water and sediment in isomers or less chlorine (PCB 52) had the highest density. The maximum daily consumption limit of E.lucius muscle tissue by a person of 70kg weight in a day averagely is 0.026kg.

Keywords: PCBs, *Esox lucius*, Anzali Wetland, Water, Sediment

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INTRODUCTION

Wetland is a water environment which has considerable scientific, economical, and, social values. Also, they are the habitats of various organisms contributing to the gene cache of microbes, plants and animals on the earth. But today we witness the pollution and degradation of the wetlands. As the wetlands are basically motionless and have a long turnaround time, therefore, adjacency of industries leads to the accumulation of contaminants in wetlands and finally chemical interactions of the pollutants and their sedimentation on wetland's bed [3].

Anzali wetland is an internationally recognized wetland located in Abkenar, Iran. The major pollutions increasingly devastation this wetland are brought in by the Pirbazar River which originates from two minor rivers namely Koohgard and Zarchoob. Also, domestic and industrial wastewaters and pollutants originated from cities such as Bandar Anzali, Fooman, Somesara, Rasht and Masal are poured in the wetland. In addition to domestic wastewater, agricultural pesticides used in the surrounding paddy fields end up directly into the wetland through drainage. One of these pollutants with a very high level of stability is the Poly Chlorinated Biphenyls compounds (PCBs). PCBs are a group of synthetic organic chemicals which are white and crystalline when pure but appear as colorless solution in industrial compounds. Their viscosity and density increase strongly and its limited solubility decreases but easily dissolve in fats [5]. These compounds are often absorbable through digestion, breathing and skin. People are often poisoned by these compounds through consuming fish and shellfish which have been exposed to PCBs [1,12]. Intestinal absorption of PCBs by fish occurs quickly. PCBs enter the fat tissues through blood circulation and remain there or are converted to their various forms of metabolites by liver and excreted in urine and bile. In addition to fat tissues, the maximum level of PCBs accumulation has been reported in kidney, liver and brain and the minimum level in muscles. These compounds are more stable with the increase of chlorine atomic number and the level of excretion of these compounds decreases with the increase of chlorine atomic number [4]. Sagratini et al. [10] conducted a research on the level of PCBs in fish and shellfish in Adriatic Sea and the study showed that the maximum level of PCBs in Zagreb was from 0.02 to 0.05mg/kg. In a similar study, Bosiner et al. [4] measured the levels of six PCB derivatives in freshwater fish of Zagreb region and the highest level was between 0.02 to 0.05mg/kg depending on the type of derivatives although the total level of measured derivatives was lower than the maximum acceptable level of 2mg/kg [4]. Antunes et al. [2] studied the level of PCBs (33 group of compounds) in large fish species, food and environmental compounds in water (suspended particles). Water and its suspended particles were shown to contain higher density of lower PCB derivatives (PCBs with less chlorine) while the fish and food were revealed to contain higher density of higher PCBs derivatives (PCBs with higher chlorine).

As Abkenar is a free fishing zone and *E.lucius* is a native species in the region and is of considerable importance [11], therefore, the aim of the present study was to determine the level of PCBs in the aquatic environment of this zone.

MATERIALS AND METHODS

Anzali wetland is located on southwest of Caspian Sea and south of Bandar Anzali (E=49° 28′; N=37° 25′) with an area of 15000 hectare and is 23 meter lower than the free waters. Anzali wetland is a collection of natural freshwater wetlands fed by 11 rivers. Anzali wetland is also a local habitat for spawning and reproduction of fish as well as, over wintering and incubation of many species of aquatic and near aquatic birds [6]. This wetland is classified in the group of coastal-marine wetland and in the year 1975 was recorded in the international convention for the protection of wetlands [3].

1.1 Sampling stations

After field inspection and the identification of the study zone, aerial photographs were obtained and sampling stations were determined accordingly as follows:

Station 1: N: 37 o 26' 57.33" E: 49o 23' 22.13"
Station 2: N: 37 o 28' 19.78" E: 49 o 20' 52.64"
Station 3: N: 37 o 29' 50.73" E: 49 o 18' 14.37"
Station 4: N: 37 o 26' 4.23" E: 49 o 26' 5.60"

Figure 1: Sampling site in Abkenar (Western part of Anzali wetland)

Sampling

1.1.1 Water sampling

Glass water samplers with their interior parts covered with aluminum foil (to prevent any potential reactions with the pollutants) were used. Before sampling, glasses were thoroughly washed with detergent and rinsed by distilled water several times followed by a finally rinse with hexane. They were then dried in an oven at 180 °C. Water sample (2 L each) was collected in triplicates at the depth of 1 m in December 2008. After filtration, fixing materials including hexane and dichlorophenol methane were added to the samples and transferred to the laboratory.

1.1.2 Sampling from the suspended particles in water

Suspended particles were separated from water by using a 0.45 µ filter made of Teflon or poly tetra ethylene (PTFE) before adding the fixatives. The samples were then placed in aluminum containers, frozen and transferred to the laboratory [8]. Totally 12 samples were collected.

1.1.3 Sediment sampling

5-kg Sediment samplers (gerp sampler, model Hydro Bios) were prepared as previously described for glass water samplers. From the 4 stations shown in figure 1, a total of 12 samples (4 samples from each station in triplicates) were collected in December 2009. Sediment sample were put in a metal container, frozen and transferred to the laboratory [8].

1.1.4 *E.lucius* sampling

None fish were caught by fishing net and the biometric measurements were carried out (total length in cm using a biometry ruler and weight in g using a conventional 5 kg balance with an accuracy of 1g). The samples were then frozen at -20 °C and transferred to the laboratory for separating the muscle and skin tissues in order to analyze the PCBs content. Tissue separation was done according to the Moopam [8] standard.

1.2 Sample preparation

Preparation process is critical in order to achieve accurate and reproducible analyses. The preparation was conducted following the Moopam [8] and Khatami [7] standard. The preparation stages according to the type of analysis and type of sample were: A) washing utensil B) drying C) Milling D) Sieving and E) Homogenizing.

1.3 Instrumental analysis

Extraction and re-doping were carried out on the prepared samples according to the Moopam [8] standard. The PCB compounds in all samples including water, sediment, suspended particles in water and fish were measured by using a Gas Chromatography (GC–ECD) equipped with a Ni63 detector [8].

RESULTS

The maximum and minimum levels of PCBs contents in various samples obtained from Anzali wetland including water, sediment, particles suspended in water as well as skin and muscle tissues of *E.lucius* are presented in table 1.

Table 1: Measured PCBs content various samples obtained from Anzali wetland in December 2009 (ng/g dry weight)

Row	PCBs Measured environment	level of PCB density with 95% accuracy	Average of sample	Maximum level of PCB density with 95% accuracy
1	*E.lucius* skin	16.23	24.36	36.53
2	*E.lucius* muscle	10.25	14.36	18.46
3	water	0.0012	0.0042	0.014
4	Particles suspended of water	0.90	5.42	32.54
5	Sediment	3.94	0.894	5.83

As shown in table 1 and figure 2, the average level of PCBs in *E.lucius*`s skin was more than that of the muscle. The highest amount of PCB was measured in suspended particles of water. The density of pollutants in water was significantly different from those of the sediment and suspended particles of water. There is no significant difference observed between the pollutants density in sediment and suspended particles of water (Fig. 2).

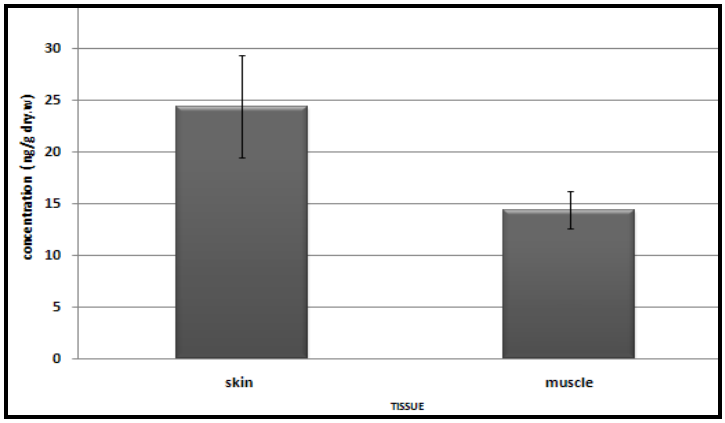

Figure 2: Comparison of the average (±S.E) of actual PCBs content in the skin and muscles tissues of E.lucius in Abkenar (December 2009)

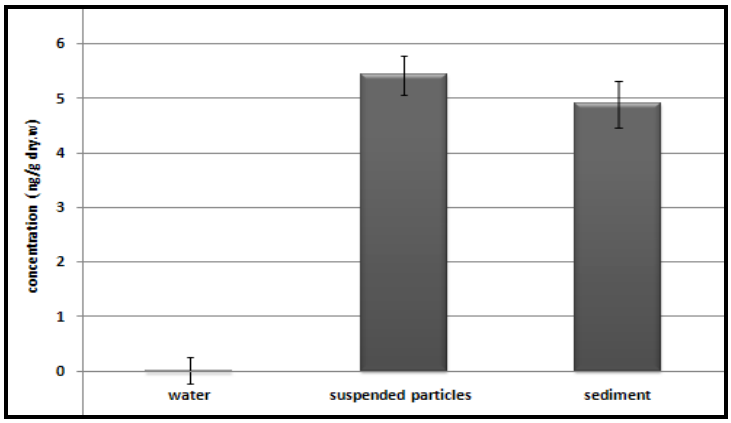

Figure 3: Comparison of the PCB average (±S.E) measured in different environments in Abkenar (December 2009)

The highest coefficient of variation in skin of *E.lucius* and the lowest coefficient of variation are in muscle of *E.lucius* (Table 2).

Table 2: Coefficient of variation of skin and muscle texture of E.lucius in absorbing PCB

Fishes texture	C.V. (%)
E.lucius skin	61
E.lucius muscle	37

Biological accumulation of PCBs in skin and muscle tissues of *E.lucius* showed the accumulation occurred in skin was higher that that of muscle (Table 3).

Table 3: Biological accumulation of PCBs in fish tissues

Fishes tissues	BCF=C tissue ppb /Cw ppb
E.lucius skin	5835.69
E.lucius muscle	3440.58

Table 4 shows the correlation between the PCBs density in the *E.lucius*`s muscle and skin tissues and those of the other environments. The following conclusions could be drawn:

- There is a very strong positive correlation between the length and weight of *E.lucius.*
- There is a very strong positive correlation between PCBs density in water and in suspended particles in water.

Table 4: Correlation between the PCBs density in the E.lucius`s muscle and skin tissues and those of the other environments

Sample	Sediment	Particles suspended in water	Water	*E.lucius* skin	*E.lucius* muscle	*E.lucius* weight
Particles suspended in water	R=-0.323 P=0.305 N=12					
Water	R=-0.356 P=0.257 N=12	R=0.980 P=0.0001 R^2=%96 N=12				
E.lucius skin	R=0.169 P=0.663 N=9	R=-0.162 P=0.677 N=9	R=-0.365 P=0.334 N=9			
E.lucius muscle	R=0.442 P=0.233 N=9	R=-0.228 P=0.555 N=9	R=-0.395 P=0.293 N=9	R=0.127 P=0.745 N=9		
E.lucius weight	R=-0.468 P=0.204 N=9	R=0.030 P=0.938 N=9	R=-0.032 P=0.934 N=9	R= -0.662 P=0.052 N=9	R=0.100 p=0.799 N=9	
E.lucius length	R=-0.313 P=0.413 N=9	R=-0.005 P=0.989 N=9	R=-0.330 P=0.386 N=9	R=-0.294 P=0.442 N=9	R=0.402 P=0.284 N=9	R=0.871 P=0.002 R^2=%76 N=9

Table 5 shows the calculation of maximum daily consumption limit of *E.lucius*`s skin and muscle tissues by a 70 kg person based on PCBs density. In muscle texture the minimum limit of daily consuming is in fish number 5 about 0.013g and the maximum amount is fish number 8 by 0.0385g.

Table 5: The maximum daily consumption limit of E.lucius`s skin and muscle tissues by a 70kg person based on the PCBs density

No. of fish	Average of PCBs in muscle	Average of PCBs in skin	Maximum of acceptable risk of cancer-causing	Terracing factor of cancer-causing (mg/g day)		Maximum daily limit of consuming	Maximum daily limit of consuming
1	0.01656	0.01494	0.00001	0.04	2	0.021	0.023
2	0.01917	0.014118	0.00001	0.04	2	0.018	0.0247
3	0.01226	0.017080	0.00001	0.04	2	0.028	0.020
4	0.013206	0.021228	0.00001	0.04	2	0.026	0.016
5	0.025836	0.035304	0.00001	0.04	2	0.0135	0.0099
6	0.01228	0.05877	0.00001	0.04	2	0.0285	0.0059
7	0.010566	0.023046	0.00001	0.04	2	0.033	0.015
8	0.009192	0.010044	0.00001	0.04	2	0.038	0.0348
9	0.010188	0.024708	0.00001	0.04	2	0.034	0.0141

***The standard ∑PCB according to FDI standard is about 2 ppm, according to USEPA standard is about 0.02 ppm and according to Food Standards Australia/NZ MRL is 0.5 ppm.*

DISCUSSION AND CONCLUSION

As shown in Table 1, the average density level of PCBs in *E.lucius*`s skin (24.36 ng/g dry weight) was more than the that of the muscle tissue (14.36ng/g dry weight). This could be ascribed to fat accumulation under skin and the relatively high dissolution of PCBs in fat [5]. The high amount of PCBs accumulation in *E.lucius* in general is due to its position in the food chain where it feeds on smaller fish and shellfish. These creatures feed on Phytoplankton, suspended particles in water and the benthos which are high in PCBs.

According to the results obtained by using ANOVA test, there were significant differences observed between the PCBs density in water, PCB level in sediment ($p<0.0001$; n=12) and the filtered suspended particles in water ($p<0.0001$; n=12). This difference could be due to the PCB absorption by the suspended materials and remnants of wetland creatures that had led to PCBs accumulation in sediments.

A very strong positive correlation was found between the PCBs density in water and that of the filtered suspended particles (p=0.0001; r=0.980; r2=96%; n=12). This could be explained by the fact that by increasing the pollutants level in water, their density in suspended particles in water also increases, as the pollutants are absorbed by the suspended particles as well as the suspended phytoplankton and zooplankton creatures.

The most dangerous isomers of PCB were also investigated as indicators of PCB contamination (PCB 28,52,101,138,153,180), in investigated muscle and skin texture that the maximum density of the dangerous isomers

in fall was in the skin and muscle of *E.lucius* in isomers with more chlorine (related to PCB153 that the maximum was in number 9 (3.64ppb). But in the measuring environment of water, suspended particles in water and sediment was exactly opposite from obtained findings, means in isomer with lower chlorine (PCB52) had the highest density [2].

Considering calculation of maximum daily consumption limit of skin and muscle tissues of *E.lucius* by a 70 kg person, one can only consume the maximum amount of 0.026 kg of *E.lucius* as a food source.

The measured level of PCBs in *E.lucius* tissues (maximum 0.03653 and minimum 0.01025 ppm) was less than those of the FDI Guidelines standard (2 ppm) and the Food Standards Australia/NZ MRL standard (0.5 mg per kg). Moreover, the maximum real average level (95% certainty) of the measured PCB in Abkenar - Anzali wetland (water and sediment samples) in autumn was less than the suggested values by the USA and Canada standards. (Recommended Water Quality Criteria E.P.A and sediment quality guidelines Interim Canadian).

REFERENCES

[1] Adeyemi D, Ukpo G, Anyakora C, Uyimadu JP., *Afr. J. Bio*. **2009**. 8(12): 2811-2815.
[2] Antunes P, Gil O, Armanda Reis-henriques M., *Sci. Tot. Env*. **2007**. 377:36-44.
[3] Behroozi Raad, B., *Pub. Mil. Geo*. Org. **2008**.
[4] Abdoli A, Nadery M., *Abzian publication (in Persian)*, **2008**.
[5] Bosnir J, Puntari D, Klari M, Šmit Z., *Arh. Hig. Rada. Toksikol*, **2004**. 56:303-309.
[6] Esmaeeli Sari A., *Naghshe Mehr pub. Tehran, Iran (in Persian)*. **2002**.
[7] Karimi Bagherzadeh, Ruhani Rankoohi., *Roozeno Publication (in Persian)*. **2007**.
[8] Khatami Seyed Hadi., *Env. Prot. Agency (in Persian)*. **2003**.
[9] Moopam A., 2nd Edn, Regional Organization for the Protection of the Marine Environment, ROPME, **2005**. Safat, Kuwait, p 337-355.
[10] National Recommended Water Quality Criteria US EPA mht.
[11] Sagratini G, Buccioni M, Ciccarelli C, Conti P, Cristalli G, Giardina D, Lambertucci C, Marucci G, Volpini R, Vittori S., Food Add. Cont. **2008**. part B, 1:1, 69-77.
[12] Vosooghi G, Mostajir B., *Tehran Univ. pub. (In Persian)*. **2002**.
[13] Zahed Mohammad A., *Univ. Sci. Mar. tech. Azad Univ. North Tehran Branch*. **1997**.
[14] http://www.caspianenvironment.org/Newsite/Activities.2August2010. RiverPol.htm.
[15] http://water.epa.gov/scitech/swguidance/waterquality/standards/criteria/index.m.**2010**
[16] www.Bing.**2010**/07/24.YARRAAND MARIBYRNONG Estuaries: Investigation of Contamination in Fish.