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Organochlorine pesticides and polychlorinated biphenyls in Blue crabs *Callinectes sapidus* (Rathbun, 1896) from Akyatan lagoon in the Eastern Mediterranean region of Turkey

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Abstract

The aim of this study is to determine the levels of organochlorine-based pesticides and polychlorinated biphenyls in the edible muscle tissue of Blue crabs *Callinectes sapidus* (Rathbun, 1896) that are harvested from Akyatan Lagoon in the Eastern Mediterranean Region of Turkey. The crabs were harvested in October 2010, January 2011 and March 2011. A total of fifty crabs was studied. A quantitative determination of residue levels was carried out through a Gas Chromatography-Electron Capture Detector (GC-ECD), and compared with acceptable contaminant levels. PCBs were the greatest source of contamination. The predominant compounds were α-HCH, o,p'-DDE, PCB 28 (2,2',4,4'-PCB) and PCB 52 (2,2',5,5'-PCB), with mean concentrations of 22.39, 59.45, 347.31 and 362.86 ng/g wet weights, respectively. The present work is highly significant and highlights a chronic exposure to pollutants in Akyatan Lagoon, this ecosystem being protected under the Ramsar Convention (Convention on Wetlands of International Importance, especially as Waterfowl Habitat).

Keywords: Akyatan Lagoon; Blue Crabs; GC-ECD; Organochlorine pesticides; Polychlorinated biphenyls.

Introduction

Organochlorine pesticides (OCs) have been extensively used in agriculture for a long period due to their lipophilic characteristics and resistance to being metabolized. Many organochlorine pesticides were banned in Turkey after 1985 (Daglioglu *et al*., 2010). Pesticides have been reported to cause carcinomas and hormonal disorders in humans (Erkmen, 2010). After entering the ecosystem, pesticides can penetrate to the adipose tissue of living organisms and accumulate in organisms' tissues in higher concentrations than those found in the environment. The accumulated concentration of pollutants increases progressively, from the bottom of the food pyramid to the top (Braune *et al*., 2005). Because crabs are omnivores and eat both fish and plants, they may easily be exposed to more pollutants, thus increasing accumulation in their bodies. Another route of exposure to pollutions for crabs is the water. The residue levels of pollutants in organisms such as fish, crabs, and mussels are indicators of pollution of their habitat. Therefore, predators of Blue crabs are affected even more. Since pollutants are more concentrated near the top of the food chain, humans may also be exposed to toxic doses of pollutants.

Polychlorinated biphenyls (PCBs) are used in hydroelectric power plants, transformers and dyestuffs, but it is prohibited to import and use them in Turkey (Cakirogullari & Secer, 2011). According to the Stockholm Convention that was implemented in 2004, the signatory countries to the agreements are obligated to eliminate persistent contaminants until 2025 (Kilercioglu *et al*., 2015).

PCBs are reported to have adverse effects on human immune, nervous and reproductive systems (Cogliano 1998; Brouwer *et al*., 1999; Darnerud, 2003; Langer *et al*., 2003). Chronic exposure of humans to OCs and PCBs occur through various routes: consumption of contaminated foods, especially those with a high-fat content (fish, meat, chicken etc.) and environmental contaminants (Aytac *et al*., 2010).

The Blue crab is a species native to the western Atlantic coasts but its natural geographical distribution ranges from Canada down to northern Argentina (Wanguemert & Pujol, 2016). However, it was spread to the Atlantic European coasts and in recent years, it was reported in Hawaii, Japan and even in Baltic Sea. The first record of the occurrence of *C. sapidus* in the Mediterranean was in Italy on 4 October 1949. Later, it was reported in the Mediterranean countries including Turkey, Greece, Israel, Albania, and Croatia

(Wanguemert & Pujol, 2016). *Callinectes sapidus* is economically important in the world. Its meat is not as favoured as fish in Turkey, thus it is exported to Asian and European countries (Kucukgulmez, 2005). Due to its high economic value, fishing of the Blue crab is increasing (Bilen *et al*., 2011) and 17 and 77 tons of blue crabs were caught in 2008 and 2009 respectively in Turkey (Ayas & Ozogul, 2011). Crab meat is sold at high prices on international markets, and unused crab meat and shells are used in food production (Kucukgulmez, 2005).

It is reported that the gender of a Blue crab is easily recognised by the shape of its abdomen, the abdomen is long in males, while it is wider in females. *Callinectes sapidus* (Rathbun, 1896) is a decapod crab of the swimming crab family, Portunidae. They are mainly omnivores, and consume both animals (small fish, some bivalves, annelids, dead organic matter) and plants. Moreover, eels, trout, and some carnivorous species such as octopus and shark are predators of blue crabs (Davis & Sizemore, 1982). Blue crabs act as opportunistic benthic predators and also some cases of cannibalism have been reported (Wanguemert & Pujol, 2016). Akyatan Lagoon is an important habitat for Blue crabs. As a marine product, blue crab is in high demand in developed countries (Celik *et al*., 2004), but its consumption by Turkish people is unknown, due to lack of data (Atar & Secer, 2003). No current data about consumption of blue crab in Turkey has been found in the literature.

In this study, our aim was to show that Blue crabs, *Callinectes sapidus* (Rathbun, 1896), are exposed to environmental pollutants such as OCs [alpha-Hexachlorocyclohexane (α-HCH); beta-Hexachlorocyclohexane (β-HCH); delta-Hexachlorocyclohexane (δ-HCH); Hexachlorobenzene (HCB); Ortho,para'-dichlorodiphenyldichloroethylene (o,p'-DDE); para,para'-dichlorodiphenyldichloroethylene (p,p'-DDE); ortho,para'-dichlorodiphenyltrichloroethane (o,p'-DDT); para,para'-dichlorodiphenyltrichloroethane (p,p'-DDT)], and Polychlorinated biphenyls (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) in their natural habitat in Akyatan Lagoon.

Study Area: Akyatan Lagoon (Fig. 1) (36° 37' N – 35° 16' E) is located at the southernmost edge of Turkey, and the north-eastern corner of the Mediterranean Sea, approximately 30 km south of the city of Adana. The lagoon is connected to the Mediterranean Sea via a canal (Pelc *et al*., 2014). There is much fertile farmland around the lagoon. At present, the contaminated waste water is discharged into the lagoon through drainage-irrigation canals that are fed by farmlands and industrial zones. The region that surrounds and includes the lagoon is very important due to being on a bird migration route (Sonmez & Aytuk, 2011). It is a typical alluvial lake, and is the largest lagoon in Turkey with an average of 1400 hectares of water surface area. Furthermore, Akyatan Lagoon is protected under the Ramsar Convention (Convention on Wetlands of International Importance, especially as Waterfowl Habitat) (Davutluoglu *et al*., 2010).

Materials and Methods

A total of 50 blue crabs was harvested (15 in October 2010, 15 in January 2011, and 20 in March 2011) from Akyatan Lagoon. Crabs were harvested by fishermen using cages. Dissected muscle samples were homogenized and preserved at -20°C until the time of analysis, and tissue samples were extracted according to previously described methods, with modifications (Yang *et al*., 2006). 1 g of homogenized muscle tissue was extracted with 80 mL (millilitre) of dichloromethane and n-hexane (1:1/v:v) for 16 hours using a magnetic stirrer.

After this period, the combined extract was filtered through 5 g of anhydrous sodium sulfate (Merck, Germany) and evaporated to 5 mL under a stream of nitrogen. To remove the excess lipid, a two-layer florisil column was prepared in the following order: glass wool (bottom layer, 1cm); and 8 g of florisil (activated at 300°C for 8 hours) as the top layer. The column was pre-washed with 70 mL of dichloromethane and n-hexane (1:1/v:v).

After pre-washing, 5 mL of the extract was added in the column and slowly eluted with 80 mL of dichloromethane and n-hexane (1:4/v:v), then the extract was evaporated under a nitrogen atmosphere. 6mL of n-hexane was added and mixed, and then the upper layer was removed. Aldrin and PCB 202 were added as internal standards (25 ng/mL) and mixed. 1 µl was injected to the sampler of Gas Chromatography. The calibration standard solutions of 7.8-1000 ng/g and correlation coefficient (r^2) was higher than 0.99.

Gas Chromatographic analysis was performed using a Perkin Elmer Model Autosystem XL Gas Chromatography (United Kingdom) equipped with a ^{63}Ni-EC detector. Chromatographic determination of OCs and PCBs was carried out using a 30 m x 0.32 mm D.I. fused silica capillary column HP-5 from Hewlett-Packard (Agilent, U.S.). The operating conditions were as follows: injector temperature: 250°C; detector temperature: 300°C; column: 1 min. at 150°C; 3°C/min., from 150 to 200°C; 8°C/min., from 200 to 280°C; 10 min. at 280°C. The carrier gas was helium, flow rate: 1mL/min. Injection volume was 1µl.

OC and PCB standards that were used in the analysis were suitable for chromatographic studies (Dr. Ehrenstorfer, Augsburg) and all solvents that were used were analytical grade reagents (Merck, Germany). Excellent linearity was observed in the concentration range studied, and the determined correlation coefficients were r^2>0.99 for OCs and r^2>0.99 for PCBs. The limit of detection (LOD) was 0.20 ng/g for α-HCH and β-HCH, 0.16 ng/g for HCB, 0.30 ng/g for δ-HCH, 0.34 ng/g for o,p'-DDE, 0.26 ng/g for p,p'-DDE and p,p'-DDT, 0.21 ng/g for o,p'-DDT;0.85 ng/g for PCB 28, 0.80 ng/g for PCB 52, 0.47 ng/g for PCB 101, 0.25 ng/g for PCB 138, 0.28 ng/g for PCB 153, 0.184 ng/g for PCB 180. Control experiments with the reagents were carried out before the analysis. To assess the quality of the method, a recovery study was performed on overspiked replicates of blank fish muscle samples. Recovery rate of OCs and PCBs were from 70 to 80% and from 60 to 70%, respectively.

Fig. 1: Location of the sampling area of Akyatan Lagoon and its drainage channel to Mediterranean (Google Earth Inc. 2017).

All analysis results are presented in ng/g. Statistical analysis was performed using SPSS 15.0 software package. The Spearman Correlation coefficient was employed to investigate the correlation between pesticides. Linear Regression was employed to model ∑HCH and ∑DDT values. A $p< 0.05$ value was considered statically significant. All results were calculated based on wet weight.

Results and Discussion

The mean values of the persistent pollutant concentrations that were detected in muscle tissue of blue crabs are shown in Table 1. Samples below the detection limits were not included in the statistical analysis. The numbers of samples not included in the analysis were 14, 15, and 22 respectively for PCB 28, PCB 52 and PCB 101. The numbers of samples not included in the analysis were 22, 33, and 17 respectively for o, p'-DDE, p, p'-DDT and ∑DDT. The numbers of samples not included in the analysis were 32, 45, and 44 respectively for α- HCH, δ- HCH and HCB. The percentage column on the table gives the percentage of the samples which were contaminated. Three samples were females, the remainder were males; however, our study was conducted without regard to gender. The sampling months were compared with the statistical analysis method of one-way analysis of variance. Statistical difference was not found ($P>0.05$) between months but residue levels were found to be higher than designated limits for each month. A comparison of months is given in Table 2.

At least one PCB compound was found in all of the samples. DDTs and β- HCH had, respectively, the highest and lowest contamination levels. α-HCH, o,p'-DDE, PCB 28, and PCB 52 were the predominant compounds with concentrations of 22.39, 59.45, 347.31, and 362.86 ng/g, respectively.

β-HCH was detected in only one sample. Contamination levels of HCB were found to be lower than ∑HCH, but higher than β-HCH. European Union Reg. (EC) No:149/2008 Maximum Residue Limits (MRL) List (EU, 2008), Republic of Turkey's Agriculture Ministry's Food Codex MRL List (Official Gazette of the Republic of Turkey 2011), and Turkey's National Residue Monitoring Plan (Turkey's Food Control Directorate, 2016) were taken into consideration in order to evaluate the data. The maximum residue limit for PCBs is 75 ng/g wet weight. The use of OCs was banned in Turkey in 1983 (Erdoğrul *et al*., 2005), but o,p'-DDE and p,p'DDT were found at higher levels than other DDT compounds.

α-HCH was observed in 18 samples, and the contamination levels that were found were higher than the limits in all the samples. β-HCH was not found except in one sample (3.5 ng/g). δ-HCH was found in five samples, four of which exceeded the limits. The highest level was 113.8 ng/g for δ-HCH. HCB was identified in seven samples, five of which exceeded acceptable limits.

The lagoon receives fresh water from drainage-irrigation canals. A drainage canal carries waste water to Akyatan Lagoon from Incirlik Industrial Zone. The farmland around Akyatan Lagoon is fertile and cotton, watermelons, melons, oranges, and sugar cane are cultivated in the area. Irrigation canals pass through the farmlands, thus pollution affects the plants and animals in the lagoon.

It is reported by Cakirogullari & Secer (2011) that PCBs are used in the production of oil-based paints, cable insulation, carbonless copy paper, adhesives and tapes, transformers, hydraulic systems, and plastics. In our study, the levels of PCB 28 and PCB 52 were found to be higher than other PCBs. Moreover, in one sample the concentration of PCB 138 was 154 ng/g.

Table 1. Residue levels of studied pollutants in muscle tissue of Blue crabs (n = 50) given in ng/g.

Compound	%	October			January			March			Maximum residue limits in Turkey (wet weight) ng/g[b]
		X±S.S[a]	Min.	Max.	X±S.S[a]	Min.	Max.	X±S.S[a]	Min.	Max.	
HCB	14	27.22±28.14	2.97	65.75	5.8	5.8	5.8	30.55±23.97	13.6	47.5	≤ 10
α- HCH	36	44.34±15.01	28.77	68.18	99.79±160.86	22.03	387.4	50.7±34.3	16.82	101.7	≤ 10
β- HCH	2	-	-	-	3.5	3.5	3.5	-	-	-	≤ 10
δ- HCH	10	54.50±52.46	14.12	113.80	11.71±4.12	8.80	14.63	-	-	-	≤ 10
∑ HCH	40	59.24±54.46	14	182	87.65±147.43	9	387	51.7±34.3	17	102	
o, p'-DDE	56	95.60±141.17	9.40	558.51	153.96±241.5	13.84	642	89.55±87.58	14.03	325.84	
p, p'-DDE	12	-	-	-	-	-	-	74.15±15.4	63.25	85.04	
o, p'-DDT	24	17.59±14.37	3.82	43.51	7.6	7.6	7.6	15.79±0.37	15.53	16.06	
p, p'-DDT	34	49.39±72.60	7.20	220.80	94.49±87.9	14.76	243.09	269.6±141.65	114.87	436.51	≤ Total 40
∑ DDT	66	145.89±209.08	17	820	175.49±199.83	29	642	172.04±238.46	14	847	
PCB28	72	634.88±306.74	250	1377.98	417.06±499.37	85.64	1741	325.47±157.32	100.8	598.8	
PCB52	70	371.51±222.68	80.15	771.16	424.07±732.15	36.99	2700	693.6±438.2	72.90	1478.11	
PCB101	54	175.40±226.29	25.05	968.85	211.68±387.58	39.5	1309	71.92±47.05	28.21	158.08	
PCB138	2	154	154	154	-	-	-	-	-	-	
PCB153	28	386.76±734.70	12.49	1488.8	19.7±9.79	10.3	34.27	109.64±114.84	12.25	266.16	≤ Total 75
PCB180	56	26.60±26.48	7.87	45.33	149.2±236.02	10.57	716.58	436.4±465.79	48.14	1436	

a: Arithmetic mean ± standard deviation.
b: Turkey's Food Control Directorate, 2016.

Table 2. Statistical comparison of result by months.

Pollutant	Months	X±S.S[a] (ng/g)
∑HCH	October	46,3167±23,05705
	January	31,5500±12,54701
	March	47,9225±26,52784
HCB	October	27,2225±28,14156
	January	*
	March	30,5500±23,97092
∑DDT	October	89,7475±54,66502
	January	108,8414±71,65974
	March	81,0120±59,06200
∑PCB	October	1133,6500±917,49960
	January	907,3813±1040,80677
	March	1069,5815±849,15600

a: Arithmetic mean ± standard deviation.

*: The number of sample that contaminated is low for statistical analysis.

The region has many hydroelectric power plants (Seyhan 1, Seyhan 2, Catalan, and Yuregir) that generate electricity. The use of PCBs were reported in the power plants and in electrical equipment, and it is thought that these PCBs are leaking into the water; these pollutants were not usually subject to emission limits (Dyke *et al*., 2003). Seyhan River flows into the Mediterranean Sea in an area close to Akyatan Lagoon. There is a single passage between Akyatan Lagoon and the Mediterranean Sea, and fish and crustacean species pass into the lagoon using this passage. The several drainage canals drain to Akyatan constitute the main sources of pollution. In addition to waste waters that are transported by drainage canals, the hydroelectric power plants on Seyhan River are thought to cause PCB contamination in the surrounding area, including Akyatan Lagoon. Moreover, there is an organized industrial zone (Haci Sabanci Organized Industrial Zone) near Ceyhan River, in which electrical equipment, motor vehicles, and chemical products are manufactured. (Kilercioglu *et al*., 2015). Ceyhan River also flows into the Mediterranean Sea in an area near Akyatan Lagoon.

In 1996, Turkish authorities found that an area of 5080 m^2 in Incirlik Air Base, at Incirlik village, was contaminated with PCBs. After the research period that ended in February 1997, a decision was made to send the contaminated soil abroad to be burned, and to improve the water treatment systems (Buyuker, 2009). The distance between Incirlik Air Base (Incirlik AB) and Seyhan River is 6 km, and the distance from Incirlik AB to Akyatan Lagoon is approximately 39 km. PCBs are very persistent and have a potential for long-range atmospheric transport to remote areas. Atmospheric transport of PCBs may occur through an exchange process commonly known as "grass-hopping" (Eckhardt *et al*., 2007). However, we could not found any information about atmospheric transport of PCBs in Incirlik AB in the literature. However, this cannot be ruled out as one of the possible causes of contamination.

A single study exists on the accumulation of persistent pollutants in the muscle tissues of two fish species (Sea bream and Grey mullet) and Blue crabs living in Akyatan Lagoon (Tekin & Pazi, 2017). According to that study, ten Blue crabs (*C. sapidus*) were collected in 2013 and quantitative analysis was performed with GC-MS. The average concentrations of p,p'-DDE and p,p'-DDT were found to be 0.48±0.06 and 0.28±0.03 ng/g respectively, but HCB and p,p'-DDD were not determined. These concentrations are much lower compared with the data obtained in our study.

In a study carried out by Clarke *et al*. (2013), it was reported that some pollutants were found in crabs (*C. pallidus*) that were caught in Ologe Lagoon in Nigeria, and especially α-HCH and γ-HCH were found to be less than 100 μg/kg. In another study in the northern Persian Gulf, PCBs were investigated in muscle tissues and hepatopancreas of *Portunussegnis* (Ghaeni *et al*., 2015). It was reported that accumulation levels were found to be higher in males. Compared to our work, the PCB derivatives are different and the detected contamination levels are lower.

It was reported that king crabs [*Paralithodes camtschaticus*, (Tilesius, 1815)] were caught in different areas of the Barents Sea and the presence of some pollutants (dioxins, furans, non-ortho and mono-ortho PCBs) in claw and leg meats was investigated. The average contamination was found to be in the range of 0.08 – 0.61 μg/kg. (Julshamn *et al*., 2015). The results showed that contamination was below the limits set by European Union (EU, 2008). Ashley *et al.* (2000) reported that PCB concentrations in Striped bass have been monitored in the Hudson River and total PCB levels were found to be between 500 ± 400 and 4000 ± 1000 ng/g wet weight. These results are quite high when compared to the results we obtained. Howell *et al*. (2008) reported that in channel-crab samples, the average total concentration of PCBs was found to be 29 ± 21 in summer 2002 and 21 ± 18 ng/g in fall 2002. Furthermore, total PCB concentration in cat fish was determined to be between 16.5 and 1596 ng/g. Total concentrations of PCBs in crabs were lower than those found in our study.

The effects of OCs on human health are not adequately understood. It was reported by Dorgan *et al*. (1999) that exposure to DDT and PCBs does not increase the risk of breast cancer; nevertheless, some studies have associated exposure with diseases such as non-Hodgkin's lymphoma, leukaemia, brain and uterus cancers, and low sperm concentrations (Daglioglu *et al*. 2010). In the Cukurova Region, levels of HCH, DDT and PCB were investigated in the adipose tissue of 82 autopsy cases. It was reported that average residue concentration levels of ∑ DDT, ∑ HHC and HCB were 55.87, 6.05 and 5.34 ng/g, respectively (Daglioglu *et al*., 2010). This study showed that all humans living in this region are exposed to environmental pollution in various ways (eating, breathing, touching).

According to the Stockholm Convention that was implemented in 2004, the signatory countries to the agreements are obligated to eliminate persistent contaminants until 2025 (Kilercioglu *et al*., 2015).

However, the data provided in our study shows that the contamination of pollutants (especially PCBs) was higher than legal limits (≤ 75 ng/g). We should evaluate OCs and PCBs separately due to their separate contamination routes. The use of pesticides is a source of contamination of OCs, but PCBs are released to the environment by machines and equipment. The Eastern Mediterranean Region of Turkey contains lagoons, National Parks (Karatepe - Aslantas), fertile farmland, and villages, towns, and cities in which millions of people live. Pollutants can spread in nature. Organochlorine pesticides have been implicated in the aetiology of various diseases (Daglioglu *et al*., 2010). It was reported that the consumption of oyster meat polluted with DDTs increases the risk of cancer (Han *et al*., 2000). Other organisms that live in same area and are consumed by people puts the people at risk of pollution. Smith & Gangolli (2002) reported that some cancers and low birth weights in babies are associated with intake of PCB containing fish.

Pollution is a huge worldwide problem and different results were reached in studies that were carried out in different regions of the world. This study is an important asset to the scientific literature since it is one of the first studies in this region with a detailed description of its methodology. The data obtained will constitute the basis for future studies. We expect that further similar studies on this subject in Akyatan Lagoon and other regions of Turkey will confirm and add to our findings. In any case, similar studies should be regularly conducted in order to monitor pollution levels.

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