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The Impact of Pollution on the Cuttlefish *Sepia officinalis*

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Abstract: Contaminations of Persistent Organic Pollutants (POPs) such as Dichlorodiphenyl Trichloroethane (DDT), Hexachlorocyclohexane (HCH) and Chlordane (CHLs) were examined in the edible mantle tissues of the commercial cuttlefish *Sepia officinalis* Ehrenberg 1831, collected from the marine water of the Arabian Gulf. The mean concentrations of DDT, CHLs and HCH were in the ranges of 29.4-56, 47.4-100 and 1-4 ng g^{-1}, respectively. Among the POPs analyzed, HCH showed the lowest concentrations ranging between 1-5 ng g^{-1} lipid wt. However, concentrations of DDT, CHLs and HCH detected in this study were generally comparable or lower than those found in studies of similar Cephalopod species from other areas subject to a high anthropogenic impact. Relationships between total body lengths and/or dorsal mantle lengths of the organisms and the concentration values of the studied POPs were also considered. Compared with recommendations of the international organizations there are no potential risks associated with consuming the studied cuttlefish species.

Key words: Cuttlefish, *Sepia officinalis*, organochlorine, DDT, CHLs, HCH, Arabian Gulf

INTRODUCTION

Organochlorinated substances are listed as Persistent Organic Pesticides (POPs) by the United Nations Environment Program (UNEP) in the 1995 Stockholm convention. Twelve POPs were identified of which nine are pesticides (aldrin, endrin, dieldrin, heptachlor, Chlordane (CHL), mirex, toxaphene, Dichlorodiphenyltrichloroethane (DDT) and Hexa Chlorobenzene (HCB) (Wei *et al*., 2007). Although, the usage of technical different types of POPs in several countries as Japan, United States and the former Soviet Union were officially banned >20 years ago, continuing usage of these compounds for agricultural or public health purpose in these countries have been reported (Nakata *et al*., 1995; Depeng *et al*., 1996; McConnell *et al*., 1996; MAFFJ, 2001). In the last 2 decades, POPs have shifted from industrialized countries of Northern Hemisphere to developing countries in tropical and sub-tropical regions including India and China (Wong *et al*., 2005). Many countries have now implemented the Stockholm Convention on POPs (2001) which suggests ending commercial use of 12 POPs and reducing or eliminating their emission into the environment. POPs are highly persistent, they may have a half-life of years or decades in soil/sediment and several days in the atmosphere (Jones and Voogt, 1999). In the aquatic environment, they partition strongly to organic phase and accumulate in the lipid rich tissues in animals and become soluble in fatty tissues rather than entering into the aqueous milieu of cells. Because of this, these chemicals are persistent in biota and biomagnify in food chains causing severe injury to the nervous, endocrine, reproductive and immune systems in birds, fish and mammals (Ratcliffe, 1967; Khan, 1977; Kelce *et al*., 1995; Skaare *et al*., 2000; Toft *et al*., 2003).

The Arabian Gulf is represents a highly stressful environment due to a combination of both prevailing natural conditions and development pressures along its coastline (Tolosa *et al*., 2005). The countries that located along its coast undergone considerable development and consequently urbanization, industrialization. The major sources of POPs in the coastal zone in the Gulf are exports of agricultural chemicals from coastal catchments, heavy manufacturing effluent discharge, municipal and industrial sewage.

Cephalopoda, one of the most important groups of marine invertebrates are consumed throughout the world both as food and as feed supplement and have great commercial value (Navarro and Villanueva, 2001; Koueta and Boucaud-Camou, 2001). Demand of Cephalopods, especially small ones is increasing. Indeed, they are actually much appreciated as a protein source and because of their gustative quality. Very little studies have been established to investigate the distribution and analysis of organic and inorganic substances in Cephalopod tissues (Miramand and Bentley, 1992; Butty and Holdway, 1997; Weisbrod *et al*., 2000, 2001;

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Ueno *et al*., 2003; Storelli *et al*., 2005; Won *et al*., 2009). According to Danis *et al*. (2005) and Won *et al*. (2009), more researches are needed in this field. In addition to the rare studies concerning the use of Cephalopods as bioindicator in the Arabian Gulf water of the Kingdom of the Saudi Arabia, there are no researches to determine the residue levels of POPs in soft tissues of Cephalopods there. The broadly distributed neretic demersal Cephalopod species *Sepia officinalis* Ehenberg, 1831 is one of the most common edible cuttlefish species available in the Arabian Gulf and the Oman sea (Tehranifard and Dastan, 2011). In the light of this concern, the study was carried out to determine some POPs residues in mantle of the cuttlefish *Sepia officinalis* to ascertain whether the concentrations exceed the levels fixed by legislation undertaken worldwide in order to assess human health risk. Besides to find out if there is any relationship between the whole body length and/or the dorsal mantle length and the POPs concentration levels.

MATERIALS AND METHODS

Sampling: *Sepia officinalis*, a common edible cuttlefish species were caught by local professional fishermen from the Arabian Gulf water of Saudi Arabia coast (Fig. 1). After capture, care was taken to avoid any contamination of the specimens that were immediately packed in ice and sent to the laboratory. The collected specimens were identified accordingly (Silas, 1985; Aoyama and Nguyan, 1989; Pierce *et al*., 1994).

The Total Length (TL) and Dorsal Mantle Length (DML) were measured (Table 1). After that specimens were divided into 8 groups (each contains 5 animals) according to the dorsal mantle length. The collected animals were dissected and the edible mantle, the contaminant storage tissues were obtained and homogenized. All the homogenized samples were stored at -20°C until chemical analysis was performed.

Chemichal analysis: Organochlorine insecticides, Chlordane compounds (CHLs) and Hexachlorobenzene (HCB) were analyzed using a Hewlett Pachard HP5890-GC with split/splitless injector and a 25 m×0.3 mm fused silica capillary with a chemically bonded gum phase SE54 sodium chloride and sodium sulfate that were kiln fired at 450°C overnight and cooled in a greaseless desiccator. Silica gel used for column chromatography was solvent extracted with n-hexane in a flass cartridge inserted into an extraction apparatus as described by Ehrhardt (1987). After extraction, the silica gel was first dried in the same cartridge by passing ultra-pure nitrogen through and was then activated by heating the cartridge in an electric tube oven to 200°C for 6 h with nitrogen stream reduced to a few mL min^{-1}.

The extraction method was based upon that Wade *et al*. (1988). A total 5 g of dried tissues was Soxhlet-extracted with methyl chloride and concentrated in Kuderna-Danish tubes. The extracts were fractionated by alumina; silic gel (80-100 mesh) chromatography.

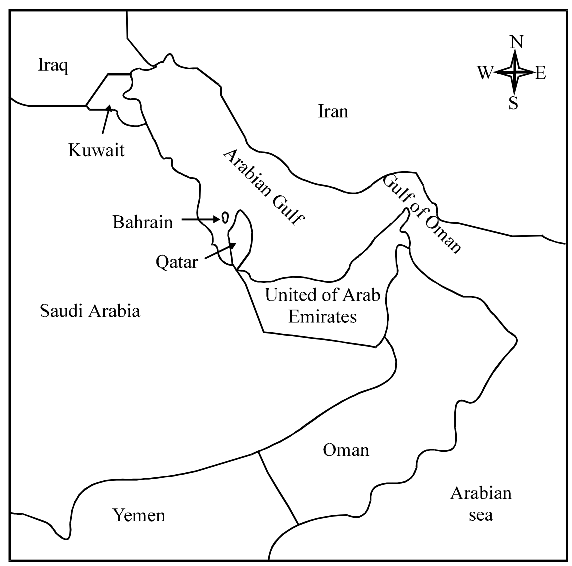

Fig. 1: Map of the Arabian Gulf, locality of the studied cuttlefish

Table 1: Total Length (TL), Dorsal Mantle Length (DML), mean concentrations, Standard Deviation (SD) and Standard Error (SE) for the 8 groups of the edible cuttlefish *Sepia officinalis* collected from the Arabian Gulf of Saudi Arabia

			CHL		HCH		DDI	
Groups	TL	DMI	Mean±SD	SE	Mean±SD	SE	Mean±SD	SE
1	10.0	6	80.0±24.40	10.95	1.81±0.83	0.37	55.0±13.22	5.91
2	11.0	7	75.0±17.16	2.67	3.00±1.58	0.70	40.0±11.10	4.96
3	13.0	8	70.6±19.04	8.51	1.00±1.00	0.44	29.4±9.520	4.26
4	14.5	9	89.2±14.25	6.37	5.00±1.58	0.70	56.0±15.57	6.96
5	16.0	10	47.4±10.85	4.85	1.00±1.00	0.44	40.4±11.10	4.96
6	17.0	11	100.0±12.24	5.47	2.00±1.22	0.54	52.0±10.81	4.83
7	17.5	12	71.6±18.78	8.40	4.00±1.58	0.70	11.0±12.10	7.64
8	19.0	13	55.0±13.22	5.91	3.00±1.58	0.70	48.0±15.65	7.00

The extracts were sequentially eluted from the column with 50 mL of pentane (aliphatic fraction) and 200 mL of 1:1 Pentane-dichloromethane (POPs fraction) and concentrated for GC analysis. POPs were romatography in the split/splitless mode using an Electron Capture Detector (ECD). A 30 m× 0.32 mm i.d., fused-silica capillary column was used for this purpose with a chemically bonded gum phase SE54 (J&W Scientific, Inc.).

Recovery and quality assurance: Recovery standards were added at the start of the procedure and carried through the extraction, cleanup and instrumental analysis steps to determine the recovery of the analysis. The recoveries of DDT, CHLs and HCH in this analytical procedure were 94.5-7.6% for the studied pesticides. The specific quality assurance steps were used to ensure measurement accuracy and precision; one procedural blank, one matrix spike, one duplicate sample and one standard reference material were run with each batch. Quality assurance for the measurement of POCs was confirmed by analyzing standard reference materials and the results agreed well with the certified values. Concentrations of POCs were not corrected for the recoveries and were given as nanograms per gram on a lipid weight basis.

Statistical analysis: An independent t-test analysis was applied to the observed data to check the level of significance site-wise. Pearson's correlation coefficient statistical functions on Microsoft Excel were used to test the relation between the body length and/or the dorsal mantle length and the POPs concentration levels.

RESULTS AND DISCUSSION

The persistent organochlorine pesticide residues in the mantle tissues of the common cuttlefish, *Sepia officinalis* from the Arabian Gulf water of the Kingdom of the Saudi Arabia are shown in Table 1 and Fig. 2. The accumulation order of the different contaminants was CHLs>DDT>HCH.

DDT: In the present study, DDT mean values ranged between 29.4-55 ng g^{-1} lipid weight in the mantle tissues of *S. pharaonis*. These values are remarkably higher than those detected in the same species collected from the Red sea of Yemen (1.8±0.02 ng g^{-1}) and the Gulf of Aden (1.5±0.02 ng g^{-1}) (Al-Shwafi *et al*., 2009). Moreover, the mean values were higher than that recorded in the edible parts of *Sepia officinalis* (9.1 ng g^{-1}) from the Gulf of Naples, Southern Italy (Naso *et al*., 2005). However, the values were lower than the Maximum Residue Limits

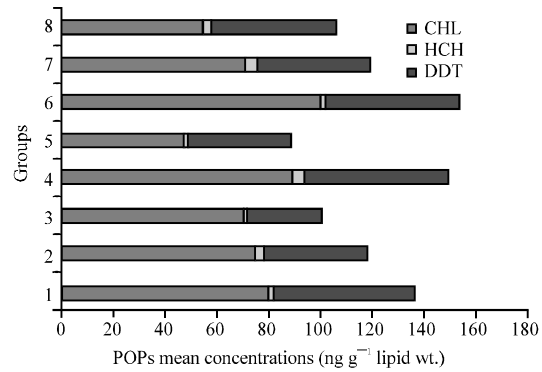

Fig. 2: Concentrations (ng g^{-1} lipid wt.) of organochlorine compounds in squid samples from Arabian Gulf of Saudi Arabia

(MRL) (1 mg kg^{-1}) for some foods of animal origin. They were also below the Maximum Acceptable Limit (MAL) (0.3 mg g^{-1}) proposed by FAO (1983). On the other hand, the highest mean concentration (118-698 ng g^{-1}) of DDTs, so far recorded in squid collected from offshore Korean waters (Yellow sea) (Won *et al*., 2009).

A preliminary survey of marine samples revealed the presence of DDT derivatives in the oysters (*Pinctada margartifera*) collected from Kuwait waters (Anderlini *et al*., 1981).

Douabul *et al.* (1987) proved the presence of DDT residues in 13 commercially important fish species collected from the North West of the Arabian Gulf although, DTT has been officially banned in Iraq country. Organochlorine pesticides such as DDT are generally higher in the sediments of Northwestern Gulf and Shatt Al-Arab than in the more Southern sediments in Saudi Arabia, Qatar and AUE, suggesting the influence of Shat Al-Arab discharge into the Gulf (Fowler, 2002). There are 31 countries requesting public health exemptions for DDT under the Stockholm convention, the Kingdom of the Saudi Arabia is one of them, those lists these additional countries as current users of DDT for vector control (WHO, 1995).

Probably, this elucidates the compositions and the elevated residue concentrations of DDT found in cuttlefish samples in this study. This is confirmed by the research of Al-Saleh *et al.* (2009) who mentioned that although DDT is banned for agricultural purpose in Saudi Arabia, it is occasionally used to control vector-borne diseases in certain regions of the country. Naso *et al.* (2005) explain that the presence of the highest levels of DDT in (benthic and neritic) marine species clearly points to local sources of contamination mainly located along the marine coasts.

CHL: The current study showed that the mean concentration values of CHL compounds ranged from 47.4-100 ng g^{-1} lipid wt. These values represent the maximum attainable concentration among the POPs analyzed. The peak concentration values of CHL in the present study may reflect the recent continuing illegal input of technical CHL from the countries located along the Arabian Gulf. Naso *et al.* (2005) found that the strong interaction with sea bed and its sediments may have an important influence on the degree of contamination detected in the benthic and neritic Cephalopods. To safeguard public health, CHL concentration standards in food has been established by various organizations. In 1970, the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluated chlordane and established tolerances for residues in food of 0.02-0.5 mg kg^{-1} (FAO/WHO, 1970). In 1986, an Acceptable Daily Intake (ADI) in food of 0-0.0005 mg kg^{-1} b.w. was established (FAO/WHO, 1987). In 1994, the ADI was changed to a Provisional Tolerable Daily Intake (PTDI) value at the same level, 0.0005 mg kg^{-1} b.w. (WHO, 1999).

CHL concentrations in the mantle of squid from the Korean offshore waters were ranging from 4-22 ng g^{-1} lipid wt. for animals collected from the Yellow sea and from 9-12 ng g^{-1} lipid wt. for squids collected from the East sea (Won *et al.*, 2009).

HCH: Among the POPs analyzed, HCH showed the lowest concentrations (Table 1, Fig. 2). The mean values ranged 1-5 ng g^{-1} lipid wt. These low values are most probably due to the fact that HCHs have high vapor pressure and volatilize easily from the contamination source for wider atmospheric distribution (Loganathan and Kannan, 1994). HCHs were determined in the mantle of the Japanese common squid collected from the offshore waters of Korea.

The concentrations were ranged between 3-11 ng g^{-1} in samples having mantle lengths ranged from 22.6-25.4 cm (Won *et al.*, 2009). Obviously, these values are remarkably higher than those detected in the present study. Several studies have discussed the relationship between HCH and DDT. Beyer *et al.* (2000) assessed long-range transport potential of OCs via air and water and found that a-HCH is 4-5 times more transportable than DDT breakdown product of DDE.

Hong declared that HCHs show an even distribution in the environment with little spatial variation in comparison to DDTs. Won *et al.* (2009) mentioned that the overall level of HCH compounds in squid were clearly lower than that of DDTs which has been frequently observed in various marine organisms (Lee *et al.*, 1997; Kajiwara *et al.*, 2002, 2004; Monirith *et al.*, 2003). Compared with other POPs, the low bioaccumulation of HCHs is due to their physicochemical properties such as high biodegradability and low lipophilicity (Loganathan and Kannan, 1994). Tanabe *et al.* (1984) reported that the bioconcentration factor of HCHs between squid and seawater in the Western North Pacific ecosystem was 300-400 times lower than those of DDTs.

Relationship between POPs concentrations and body-length and/or mantle length: Results from the statistical analysis showed no significant relationship between body length and/or mantle length and the concentrations of the studied POPs (Fig. 3). These findings are in agreement with the results obtained by Al-Shwafi *et al.* (2009). They concluded that there is no relationship between body length and DDTs concentration in the muscle of the *S. pharaonis* collected from both the Red sea of Yemen and the Gulf of Aden. Similarly, this agrees with the results of Ueno *et al.* (2003)

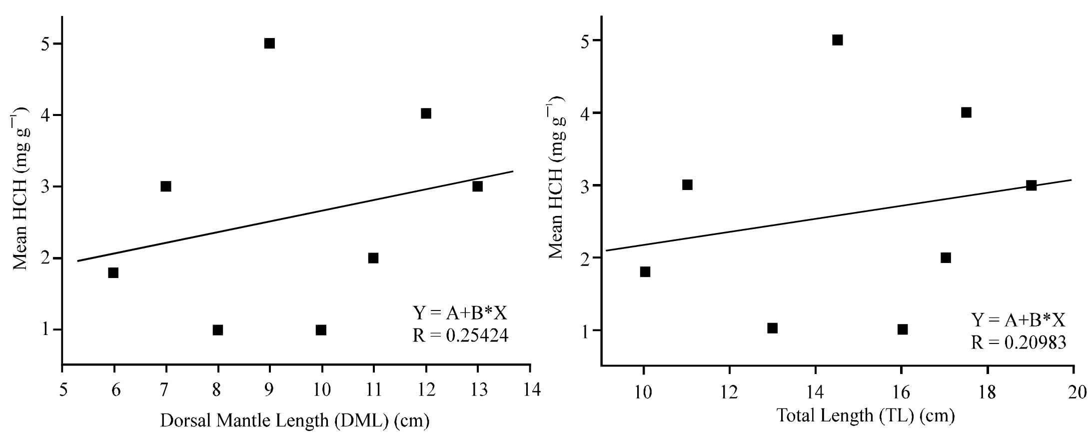

Fig. 3: Continue

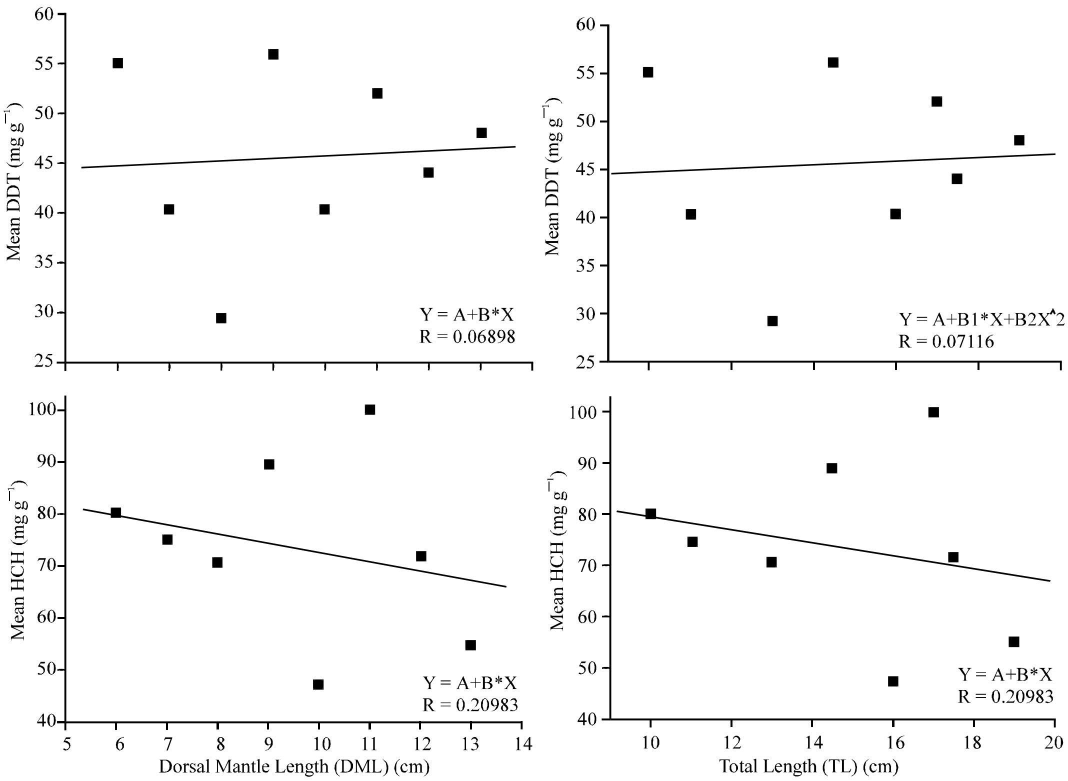

Fig. 3: Variations of metal concentrations (mg kg^{-1} wet weight) in the cuttlefish *Sepia officinalis* in relation to the mantle length and the dorsal mantle length

on the liver Japanese common squid *Todarodes pacificus* and that of Sato *et al*. (2000) who reported no significant correlation between body-length and a-HCH and PCBs concentrations in the Japanese common squid on dry weight basis.

CONCLUSION

POPs such as DDTs, CHLs and HCHs were determined in the mantle of the common cuttlefish, *Sepia officinalis*, collected from the Arabian Gulf water of the Kingdom of Saudi Arabia. Peak levels of DDT and CHL were detected but CHC was low. The present data were compared to the international permissible and/or acceptable levels recommended by the organizations concerned with human health.

From the human health point of view, the concentrations of POPs in the *S. pharaonis* show situation of no hazard effect for the consumers. In the study, no significant relationship was detected between the total body length and/or dorsal mantle length and POPs concentrations. Finally, it is obligatory to enact repressive laws and activate them to control industrial waste discharge and to avoid dispersal of these persistent toxic contaminants into the Arab counties environment. Additional studies should be carried out to monitor the levels of organochlorine pollutants in edible fish and shellfish inhabiting the marine water of the Arabian Gulf.

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