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Fish Bile as Biomarker of Total Petroleum Hydrocarbon Pollution in the Khure Mussa – Persian Gulf

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ABSTRACT: Total Petroleum Hydrocarbon (TPH) are environmental contaminants that are released into the marine environment through oil spills, industrial and domestic activities. TPH are readily transformed into more hydrophilic metabolites, which are accumulated in bile. Thus fish bile can be used as a biomarker of exposure of fish to TPH in the marine environment.
in this study several stations were selected from the Khure Mussa in the northern part of the Persian Gulf. Fish and sediment sampling were collected in the 2009. Preparation and analysis of the samples were performed according to MOOPAM method using Spectroflorophotometer (UVF(. The highest average concentration of TPH in sediment was 364.91 mg/kg (dry weight) and was observed in Jafari station.
The maximum concentration of TPH in bile liquid of fish (Euryglossa orientalis) was 525 mg/l which was found in Zangi station. Statistical analysis revealed a significant differences between TPH concentration in sediment and in bile liquid of fish (Euryglossa orientalis) in the study area ($P<0.05$). High concentration of TPH in fish bile observed in this study suggests that fish bile can be used as a tool for bio-monitoring of TPH pollution.

Keywords: *fish bile; Total Petroleum Hydrocarbon (TPH); Euryglossa Orientalis; Khure Mussa; Persian Gulf*

INTRODUCTION

Total Petroleum Hydrocarbon (TPH) are environmental contaminants that end up in the marine environment from varions sources like oil spills, domestic heating, transport and power plants. TPH are absorbed and bioaccumulated by fish via food and water and from sediments, reaching higher levels in bile than in those mediums (Neff, 1985).

Severity of the effects of chemical contaminents on an organism depends on type,entry value and level of accumulation, which is a dependeat parameter of environment (water or land) and parameters such as distance or proximity to the contaminent source (Karbasi, 1998).

If information about level of contaminents in the environment is available,it is possible to estimate contaminent level by comparing it with international standard values and ultimately find a way to reduce its unfavorable levels. Having this type of information also helps to assess the effects of contaminents on local aquaculture considering aquaculture type and required standards (EPA, 2003).

In general, TPH are readily metabolized in fish. They undergo electrophilic substitution oxidation and reduction reactions. Biotransformation reactions are the major determinants for a hydrophobic molecule to be toxic to fish, to be distributed in tissues and to be excreted. Without these biochemical processes half-lives of hydrocarbon would be much longer in fish and other vertebrates (Neff, 1985).

In fish the major metabolization pathways are conducted by cytochrome P450 monooxygenase, epoxide hydrolase and several conjugating enzymes (Tuvikene, 1995). These enzymes, as the first step (phase I enzymes), add a polar group into a hydrocarbon

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molecule through oxidative, reductive or hydrolytic processes. In the second step (Phase Π) the phase I metabolite of a hydrocarbon molecule is conjugated with polar endogenous substances like glucuronide, sulphate, glutation or amino acide to produce a water- soluble compound that is excreted via bile (Tuvikene, 1995). Khure Mussa in the northwest of Persian Gulf, due to its, geographic location is exposed to, a series of major petrochemical industries and ports such as Razi petrochemichal complex and Imam Khomeini port. High tide in the region, is expanding polluted area greatly and has led to expansion of pollution from the affected region in Khure Mussa to the estuary whole (Kazemi, 2003).

Considering that wastewater effluent and out fall of petrochemical complex located in the Khure Mussa enters into the estuary, the monitoring of parameters affecting the distribution of oil hydrocarbons (TPH) in order to , to manage waste disposal in this water body, appears necessary.

Fig. 1 shows the geographical location of samling stations in Khure Mussa

MATERIALS AND METHODS

The studied area includes Ganam, Jafari, Ghazaleh, Ahmadi, Zangi channel in Khure Mussa. The geographical position of these areas in shown in Table 1 and Fig. 1. Sampling from sediment and fish was performed in November 2009.

In order to colect, the sediment samples, 3 stations in first, middle and end of each channel was considered, and sediments were sampled using Van Veen.

Sampling of each fish from any channel was done along the channel by Toor Tral. Fishes sampled from each channel was wrapped separately inside Aluminum foils. Samples were kept in ice-chest, transformed to laboratory and after dissecting, bile samples were separated without gallbladder and transferred in to small Vials and kept in the frizzer with temperature lower than -20°c.

Sediments were dried by Freeze-Dryer ZIRBUS (Model VaCo511). 10 to 20 grams of frizze dried desiment was put in the Hexan-Dicloromethan solvent

Table 1: Geographical location of sampling stations

Sampling locations	Longitude (E)	Latitude (N)
Ghanam	49° 13´ 395″	30° 27´ 591″
Jafari	49º 02´ 798″	30º 23´ 361″
Ghazaleh	49º 07´ 465″	30° 26´ 387″
Ahmadi	49º 08´ 729″	30° 26´ 964″
Zangi	49º 03´ 520″	30º 28´ 520″

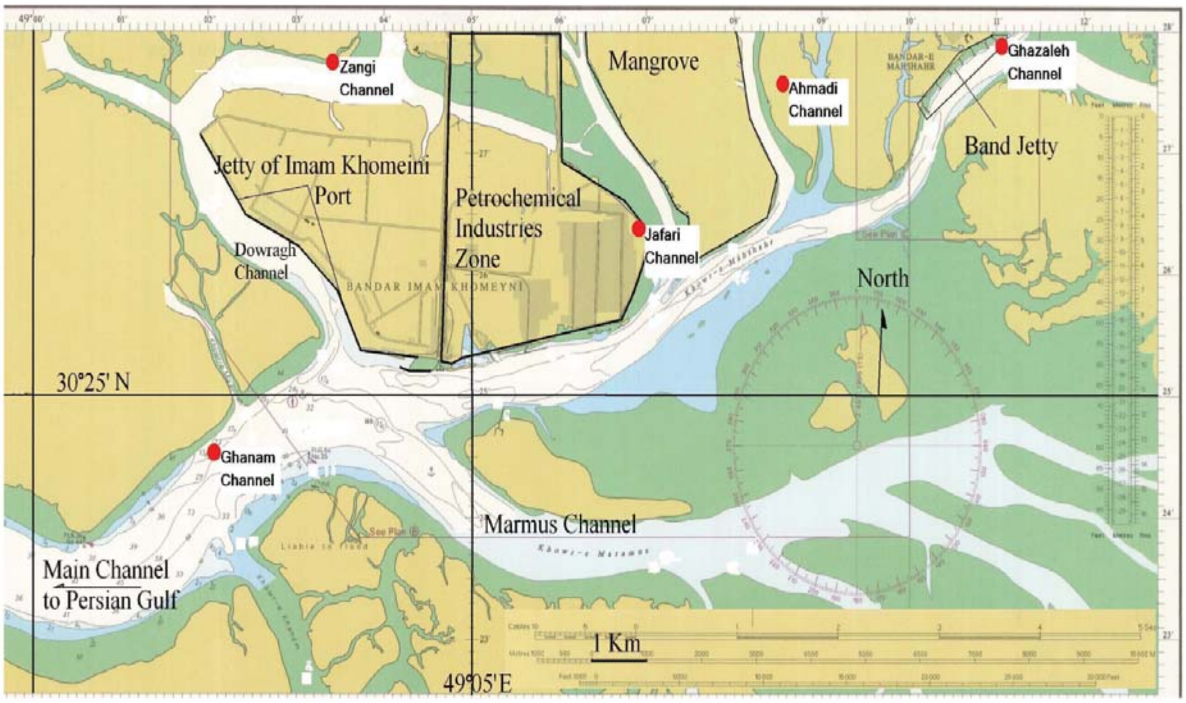

Fig. 1: Sampling stations in Khure Mussa

inside the soxhlet system. Obtained organic extract was passed from Silicagel column (Takada *et al*,. 1991).

After concentrating by rotary evaporate or machine Heidolph (Model TPY4902), the sample was put in 5 ml Vials and it was ready for instrumental analysis (MOOPAM, 1999).

Absorption in the two different wavelengths and simuttaneously raised the wavelength of 310 nm and emission wavelength of 360 nm by device spectroflurophotometer (UVF) were measured. Finally, TPH concentrations in sediments from the standard curve obtained crude oil (MOOPAM, 1999).

1- For analysis of bile samples, liquid bile at 1 : 1000 ratio, was diluted by MeOH solvent mixed with water (50% = 1:1) (Krahn *et al*., 1986).

2- 5 ml of diluted sample was transferred to centrifuged (3000 rpm at 4ºC) for five minutes, to remove debris that would interfere with analysis (Krahn et al., 1986).

Fluorescence of the TPH metabolites was measured at the excitation/ emission wavelength pair (310.360 nm) (MOOPAM, 1999).

RESULTS AND DISCUSSION

Results of mean concentration of TPH in sediments in studied station is illustrated in Fig. 2.

Highest average concentration of TPH was observed in Jafari station with the value of 364.91 mg/kg, while lowest value was observed in Zangi station with 234.66 mg/kg.

The results of analysis of Variance (ANOVA) and Tukey test showed that between the average concentration of TPH in the sediments in studied stations there was a significant difference($P<0.05$).

According to Fig. 3, concentration of TPH in fish bile, the highest concentration levels were observed in fish from Zangi 525 mg/l and lowest concentrations Ghazaleh f 313.33 mg/l.

The results of analysis of Variance (ANOVA) and Tukey test showed that between the average concentration of TPH in fish bile in studied stations

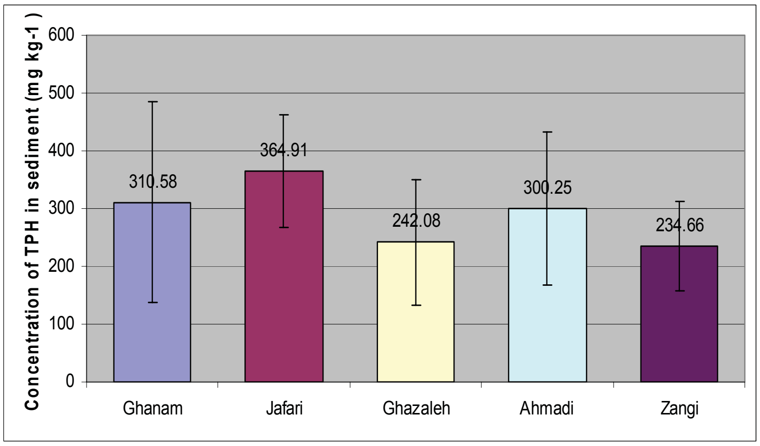

Fig.2: Mean concentration of TPH in sampled sediments

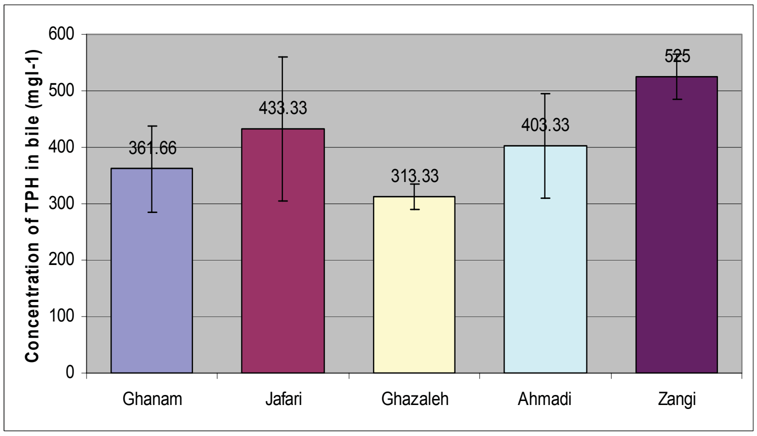

Fig.3: Mean concentration of TPH in fish bile

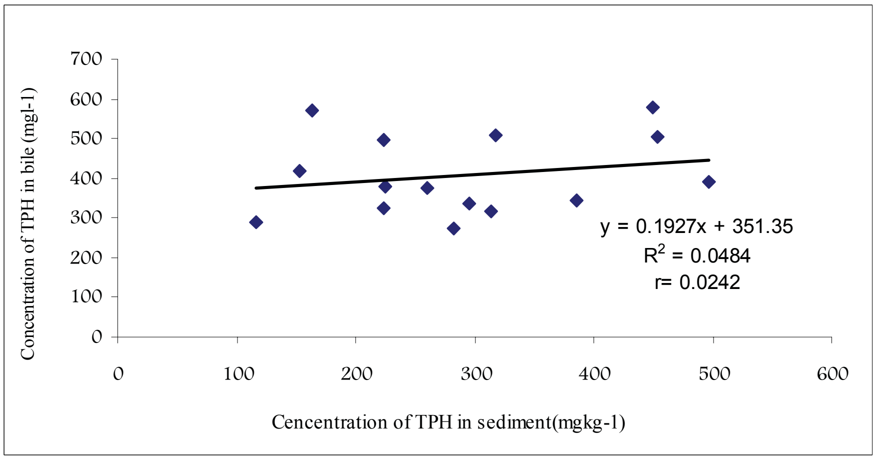

Fig. 4: Relationship between concentration of TPH in sediment and fish bile

there was no significant difference (P>0.05).
Fig. 4 shows the correlation between the concentration of TPH in sediments and fish bile in the studied stations.

CONCLUSION

The highest value of TPH in sediments was observed in Jafari station. It can be noted that petroleum material which entered the water was settled after some changes, by different factors such as waves and solar radiation. Some parts of them became floated in water, some evaporated and heavier parts sank in sediments of sea floor. These petroleum materials have disadvantages for benthical and other benthic feeding organisms(Guzzella, and De Paulis, 1994).
The main canals of effluent of petrochemical companies from Special Economic Zone as well as the main canal of effluent from Imam Khomeini petrochemical company go through Khure Jafari. Therefore it is not unexpected that this station has the highest value of contaminants. On the other hand, because Khure Ghannam is the connection point between Khure Mussa and the Persian Gulf, contaminents move to this station and that is why this station has a high level of contamination.
Khure Ghazaleh and khure Ahmadi are both located in downstream of Mahshahr petrochemical company where a variety of hydrocarbon compounds are kept. There is a possibility of leaking and pouring of these compounds in these channels and this can be the reason why the level of contamination by hydrocarbon compounds is high in these channels.
Because Khure Zangi is located upstream of the company, the level of petroleum contaminants is low at this site which is probably not affected by the effluents.
Ghadamgahi (2009) studied PAHs (anthracene, pyrene, benzo-α pyrene) in (Johnius belangerii) and sediments in Jafari, Zangi and Ghanam stations. He concluded that the concentration of PAHs in sediments was higher than FAO and WHO international standards, but this value was lower in muscle tissue.
Moreover, anthracene and benzo-α pyrene had higher values in khure Jafari because this Khure located near the out fall of effluents of petrochemical companies. Our study suggests that the same reason may have caused contamination in Khure Jafari.
A study for evaluation of TPH level in southeast Nigeria coasts by Eja and Ogri (2003) revealed that average TPH in water (149± 81.11) mg/l, in sediments (339.2± 245.7 mg/kg) and in animal tissue was(198.9 ± 50.08 mg/kg). In the present study, the average TPH in sediment was lower than the reported value of Eja and Ogri (290.49 mg/kg).
Veerasingam *et al*. (2010), analysed the distribution of TPH in surface sediments in six stations between Pondicherry and Nagapattinam in Bengal Gulf in India. They found that concentration of petroleum hydrocarbons lied between 1.48 and 4.23 ppm. Petroleum hydrocarbons level in Bengal Gulf was lower than level in our study. Petroleum hydrocarbon concentration in muddy and clay sediments was higher than in sandy sediments. The concentration of hydrocarbon petroleum in present study is much higher than that of Veerasingam report, probably due to higher contamination level of oil industries in Persian Gulf.
The present study shows that bile liquid bioaccumulated petroleum hydrocarbons and their metabolit and can be used to analyse this type of the contaminant concentration in the aquatic environment.
In our study TPH level in bile liquid of fish was highest in Khure Zangi. Establishment of Special Economic

Zone, entering of refined and unrefined industrial effluents into the Khure, with sever tides in Khure Mussa have all together caused, different contaminants enter this Khure.

Larger fish live in this Khure and they absorb much of these water through their gill which results in absorbing contaminants.

Vuorinen *et al*. (2003) studied on PAH metabolite concentration in Salmon of Baltic Sea and fish bile Perch (Perca fluviatilis) in Finland Gulf which are subject to oil spills. They reported that contaminants were not found in fish muscles, but they immediately go through many hydrophilic metabolites that leave the bile. They also showed that the contamination level was higher in fish which live closer to the oil spill, and 1 hydroxy pyren concentration in Perch near refinery was higher than level at contamination source. Therefore bile liquid can be studied to show the contamination level (Vuorinen *et al*., 2003).

Barbour *et al.* (2009) compared the level of organic contaminants in bile and muscle of Mugil spp. Following a major oil spill in the eastern Mediterranean Sea, they concluded showed their that correlation between TPAH level and carcinogenic aromatic hydrocarbon (cPAH) in bile was positive and they suggested to reduce analysis time for measuring organic contaminants in muscle, bile can be used for measurement of these contaminents. Present study has tried to follow up this suggestion (Barbour *et al*., 2009).

Kazemi *et al.* (1999) studied on accumulation of Aromatic hydrocarbons in Euryglossa orientalis and in sediments of Khure mussa. They measured three compounds of PAH, called pyrene,anthracene, benzo-α pyrene in three Khures (Ghanam,Jafari and Ahmadi). Their results showed higher values in fish. This emphasizes on the idea that fish which have PAH metabolism enzymes have lower concentration of these compound in their tissues. Like the present study, they also concluded that level of aromatic hydrocarbons in Khure Mussa is higher than standards. Highest value of PAHs was observed, in Jafari station, but the difference of TPH in the selected stations was not significant.

In addition, fish biomarkers have been used to assess the contaminant exposure and effects in Lake Erie tributaries by Xuan Yang (2004). This study concluded that in sampled stations there was a positive relationship between PAH metabolite concentration in fish and in sediments.

In the present study a strong relation between TPH in sediments and bile was not observed. Positive Correlation between TPH in bile and sediments was not statistically significant, showing that the relationship was direct ($P> 0.05$), but according to correlation factor ($r = 0.024$) the linear relation is weak.

While TPH were elevated in samples from fish bile in the present study, TPH concentration was not related to concentration of TPH in sediments. This is not unexpected for fish that are relatively mobile in areas that have a high variability of sediment contaminant concentrations (Collier *et al.*, 1993; Arcand- Hoy and Metcalfe, 1999). Because fish move around, within each location, the exposure they receive is likely to be variable,and not necessarily correlated with contaminant levels at sampling sites they were collected. Sediment data from seven sites within Marina del Rey demonstrated that variable PAH concentrations may happen over small distances (Noblet *et al*., 2002). Previous studies that measured the levels of Fluorocence Absorbing Compounds (FACs) in fish from contaminated sites, found inconsistent relationship between FACs and sediment PAHs, depending on the mobility of the species of fish studied. Stein *et al.* (1992), and Arcand-Hoy and Metcalfe (1999) found a poor agreement between FACs and PAHs used by relatively mobile species of benthic fish English sole (Pleuronectes vetulus) in Puget Sound, WA, and brown bullhead (Ameiurus nebulosus) in the lower Great Lakes, USA, respectively. A high correlation between FACs and sediment PAH shas been observed, however, using a species with limited migratory movement (Oyester toadfish Opsanis tau) (Collier *et al*., 1993)

In conclusion, this study revealed that hydrocarbons and their metabolites are present in high levels in fish bile samples, therefore fish bile can be used as a simple tool with simple preparation procedure for moniboring the level of hydrocarbon contamination in the marine environment.

REFERENCES

Arcand- Hoy, L.D.; Metcalfe, C.D., (1999). Biomarkers of exposure of brown bullheads (Ameiurus nebulosus) to contaminants in the lower Great Lakes, North America. Environ. Toxicol. Chem., 18, 740- 749.

U.S. Environment Protection Agency (EPA), (2003) Ground water & Drinking water contaminents and MCLs.

Barbour, E.K.; Mastori, F.A.; Shib, H.A.; Abdel Nour, A.; Jaber, L.S., (2009). Comparsion of the level of organic contaminants in bile and muscle of Mugil spp. Following a major oil spill in the eastern

Mediterranean Sea. Veterinaria Italiana., 45(3), 405- 412.

Collier, T.K.; Stein, J.E.; Goksoyr, A.; Myers, M.S.; Gooch, J.W.; Huggett, R.J.; Varanasi, U., (1993). Biomarkers of PAH exposure in oyster toadfish (Opsanis tau) from the Elizabeth River, Virginia. Environ. Sci., 2, 161- 177.

Dehghan, M.; Eskandari, S.G.; Al Mokhtar, M.; Sabzalizadeh, S., (1998). Determination of Ichthyoplankton density in Creeks of Khuzestan (in Iran). Research and Education Center, Iranian Fisheries Organization.

Eja, M.E.; and Ogri, O.R., (2003). Evaluation of total hydrocarbon (THC) levels in oil polluted coastal areas of South Eastern Nigeria.- Global J.Environ. Sci., 2(1), 8-10.

Ghadamgahi, A., (2009). Polycyclic aromatic hydrocarbons in Johnius belangerii and sediment of Creek Mousa (in Persian Gulf), MS thesis in Biology-Sea contamination, Khoramshahr Marine Science and Technology, Iran.

Guzzella, L. and De Paulis, A.,1994. Polycyclic Aromatic Hydrocarbons in Sediments of the Adriatic Sea, Mar. Pollut. Bull, 28; 159.

Karbasi, A., (1998). Sea contamination caused by fuel transfer in Persian Gulf.

Kazemi, S.M.; Nikpour, I.; Safahie, A.; Mahmoodian, A.; Ghanemi, K., (1999). Study biological accumulation PAHs (anthracene, pyrene, benzo-α pyrene) in water,sediment and Euryglossa Orientalis in Creek Mousa. Ms thesis in biology- Sea Pollution. Khoramshahr Marine Science and Technology, Iran.

Kazemi, J., (2002). Ecological effects of sewage Mercury of Bandare Imam Petrochemical Company on the environment of Creek Mousa. Ms Thesis, Science and Research center of Ahvaz Islamic Azad University, Iran.

Krahn, M.M.; Moore, L.K.; Macleod, W.D., (1986). Standard analytical procedures of the NOAA national analytical facility, 1986: metabolites of aromatic compounds in fish bile. NOAA Technical Memorandum NMFS F/NWC-102.

MOOPAM, Standard methods for Chemical Analysis of Petroleum Hydrocarbons, (1999).

Neff, J. M., (1985). Polycyclic aromatic hydrocarbons. In: Rand, G. M., Petrocelli, S .R. (Eds.). Fundamentals of aquatic toxicology, methods and applications.Hemisphere Publishing Corporation (McGraw-Hill International Book Company). Washington, New York, London, pp.416- 454.

Noblet, J.A.; Zeng,E.Y.; Baird, R.; Gossett, R.W.; Ozretich, R.J.; Phillips, C.R., (2002). Southern California Bight 1998 Regional Monitoring Program: VI. Sediment Chemistry. Southern California Coastal Water Research Project, Westminster, CA.

Stein, J.E.; Collier, T.K.; Reichert, W.L.; Casillas, E.; Hom, T.; Varanasi, U., (1992). Bioindicators of contaminant exposure and sub lethal effects: studies with benthic fish in Puget Sound, Washington. Environ. Toxical. Chem., 11, 701-714.

Takada, H.; Onda, T.; Harada, M.; Ogura, N., (1991). Distribution and sources of polycyclic aromatic hydrocarbons (PAHs) in street dust from the Tokyo Metropolition area. The Science of the Total Environment 107, 45- 69.

Tuvikene, A., (1995). Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Ann. Zool. Fennici 32, 295- 309.

Veerasingam, S.; Raja, P.; Venkatachalapathy, R.; Mohan, R.; Sutharsan, P., (2010). Distribution of Petroleum Hydrocarbon concentrations in coastal sediment Along Tamilnadu Coast, India. Carpathian Journal of Earth and Environment Sciences., 5 (2),5-8.

Vuontisjarvi, H.; Keinanen, M.; Peltonen, K.; Vuorinen, P., (2008). A comparison of HPLC with flurescence detection and fixed wavelength flurescence method for the determination of PAH metabolites in fish bile.17p.

Vuorinen, P.j.; Keinanen, M.; Vuontisjarvi, H., (2003). Bile PAH- metabolite concentration in perch (Perca fluviatilis) exposed to crude oil and sample in the Gulf of Finland near an oil refinery in Baltic salmon (Salmo salar). Finnish Game and Fisheries Research Institute,P.O.Box6, FIN-00721.

Xuan Yang, M.S., (2004). Useof fish biomarkers to assess the contaminant exposure and effects in lake Erie tributaries. Environmental Science Graduate Program.

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