Polycyclic aromatic hydrocarbons contaminants in Black-lip (Pearl) Oyster *Pinctada margaritifera* from Kish Island (Persian Gulf)

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Abstract

Twenty-four Black-lip Oysters (*Pinctada margaritifera*) were collected in summer (July) 2004 from six coastal locations in Kish Island (Persian Gulf) and were analyzed at the analytical laboratory of Great Lakes Institute for Environmental Research at the University of Windsor according to the chemical analysis procedures which has been accredited by the Canadian Association for Environmental Analysis Laboratories (CAEAL). In order to obtain information on bioavailability of sixteen EPA priority polycyclic aromatic hydrocarbons (PAHs), concentration of 2 to 6-ring PAHs was determined for all samples with application of a gas chromatograph with a mass selective detector. Oysters from Big Coral site exhibited a wide range of total PAH concentration (1.07 to 77.66 ng/g wet weight). The lowest value (oysters from Foreigner's Pelage) and highest values were 0.7 and 36.33 ng/g wet weight respectively. Comparison of the PAH concentrations in oysters with sediments collected from the same locations showed that the overall bioaccumulation has been performed through the movement of water-soluble lower molecular weight (LMW) PAHs to the oysters in the studied area.

Keywords: Contamination, PAHs, Bioaccumulation, Black-lip Oyster, Kish Island, PersianGulf, Bioavailability

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Introduction

The Persian Gulf and its marine environmental quality preservation is crucial for several socio-economic reasons. Local people rely on seafood (especially fish and shrimp) for both consumption and export income. The region relies heavily upon the sea water itself as a source of fresh water through desalination (Fine et al., 2003). Nevertheless, this semi-closed ecosystem experiences natural and anthropogenic stresses (Sheppard, 1993). The Persian Gulf has suffered from several environmental dilemmas in recent years including the largest oil spill ever recorded that have contributed to the pollution burdens in the region (Gerges, 1993; Literathy, 1993). As a result of the 1991 war, this marine environment was subjected to an estimated six million barrels of crude oil (Burger and Gochfeld, 2006) and in this way coastal and estuarine sediment of the region are exposed by the associated contaminants (Eghtesadi et al., 2002). Additionally over half of the world oil supply is transported from the Persian Gulf and offshore oil exploitation in the Kish this area is considered the most extensive in the world (Golob R, 1984). 0.2 to 7 percent of oil is consisted of PAHs. Many PAHs are ubiquitous because of their mutagenic and carcinogenic properties (Pashin and Bakhitova, 1979; Tokiwa et al., 1994). Coastal and estuarine hydrophobic contaminants tend to be attached to organic matter in the sediments (Amymarie and Gschwend, 2002). Mussels are abundant in many coastal areas and located mostly on the bottom of the sea due to need for a hard substrate; therefore they are often chosen as tools for exposure assessment studies sediment pollution impact on organisms (Francioni et al., 2007a; Francioni et al., 2007b; Perugini et al., 2007). There are few papers reporting PAH biological occurrence in Persian Gulf (DouAbdul et al., 1987; Fayad et al., 1996; Schoket, 1999; Al-Hassan et al., 2000; Al-Hassan et al., 2001; Nour El-Din and Abdel-Moati, 2001; Al-Saleh and Al-Doush, 2002; Eghtesadi et al., 2002). The present work is focused on samples (sediments and oysters) from Kish island (Persian Gulf) which has been identified as one of the "hot spots" with regard to PAH levels in sediment (Eghtesadi et al, 2002). The sampling stations were chosen as related to population and industrial localization. Sediments close to populated sites (Harireh and Arab Town) are exposed to anthropogenic input while the sediments collected from the tourist sampling locations are subjected to different kind of pollution sources. Sixteen parent PAH compounds among 16 PAHs recommended as priority pollutants by the Environmental Protection Agency (EPA) were analyzed. In order to obtain information on the bioavailability of these pollutants, PAH concentration in sediment and oyster samples were analyzed as their tissues PAH concentration gave information on the contamination burden of organisms living in the vicinity with the sediments.

Materials and methods

Sample collection: Sediment and oyster samples were collected at six sites in Kish Island (Table 1, Fig. 1). Twenty four surficial sediments and 24 oysters (Black-lip Oyster**,** *Pinctada margaritifera)*

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samples were collected while SCUBA diving in July 2004 at Simorgh harbor, Harireh pelage, Arab town, Big Corals, Foreigners pelage and water distillation sites (Fig. 1 and Table 1). After collection, sediments and oysters were placed and wrapped in Aluminum container and foil respectively which were previously washed with spectroscopic grade hexane. They were frozen in dry ice and transported to Great Lakes Institute for Environmental Research (GLIER) where they were frozen at -20°C until analysis. **Sediment sample preparation:** All

Table 1: Specifications of sediment and oyster sampling sites

Station Number	Station Code	Station name	Latitude (°N)	Longitude (°E)
1	WS	Water Distillation	26 33 9880	053 59 6220
2	HS	Harireh	26 34 2760	053 58 3380
3	PH	Arab town	26 34 5490	053 57 8350
4	B5M	Big Corals	26 32 0716	054 02 4010
5	B3M	Foreigners Pelage	26 31 4274	054 02 2828
6	CS	Simorgh Harbor	26 34 0250	053 59 6220

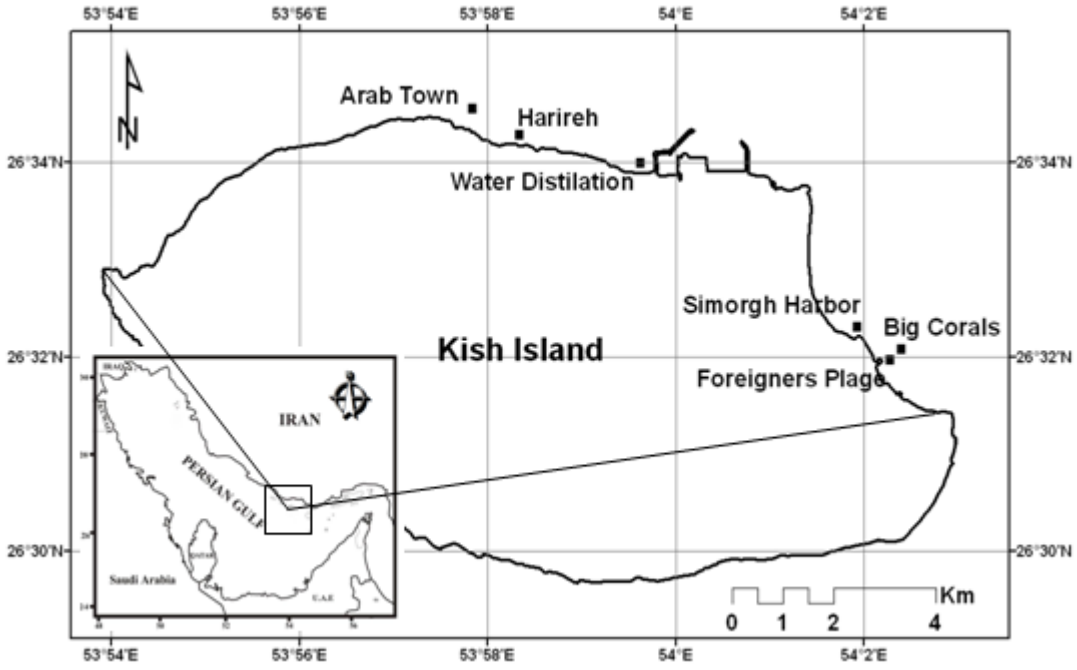


Figure 1: Sampling stations in Kish Island along the coastal areas

chemical analysis procedures was performed at the analytical laboratory of GLIER at the university of Windsor which has been accredited by the (Canadian Association for Environmental Analysis Laboratories: CAEAL). The PAH analysis is approved by CAEAL, and reference CAEAL proficiency samples are routinely approved followed the protocol presented by Drouillard et al. (Lazar, 1992). Briefly, sediment samples were homogenized and weighted for 20gr for chemical analysis. Sediment total organic carbon (TOC) was determined using loss on ignition (LOI). Organic contaminant extraction was performed by mixing 20 g of wet sediment by grinding with 100 g anhydrous Na_2SO_4 (ACS grade, BDH, ON, Canada) using a

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mortar and pestle. The surrogate spiking standard was added to each thimble. Then the samples were Soxhlet-extracted with 300 ml of acetone: hexane (1:1) for 16 hours. Each sample was concentrated to 50 ml back extracted in a separatory funnel containing Millipore grade water with hexane and concentrated again to 50ml and passed through an anhydrous Na_2SO_4 column, then further concentrated to 5ml. The samples were then cleaned up further with Florisil®. The column were then eluted with %100 hexane and then dichloromethane (DCM) : hexane (1:1) for 16 hours. Each sample was concentrated to 50 ml, back extracted in a separatory funnel containing Millipore® grade water with hexane and concentrated again to 50 ml; back extracted three times with hexane Omnisolv ® grade and rotaevaproated to 50 ml and dehydrated by passing through a chromatographic column of anhydrous sodium sulphate. The elutant was rotaevaporated and cleaned up and purified with a Florisil® column and rotaevaporated to 2 ml, cleaned up with activated copper and analyzed with 5890/5979 gas chromatograph with a mass selective detector (GC-MSD) equipped with a Hewlett-Packard 7973A autosampler and a 30 m x 0.25 mm DB-5 column. A method blank (clean Na_2SO_4) and certified reference sediment (NRC-IMB of Canada, SRM 1944 New Jersey Sediments) was extracted with each batch of 5 samples.

Biological sample preparation: The extraction of organic contaminants from biological tissue followed the protocol presented by Lazar et al. (Lazar, 1992). Briefly, each oyster was thawed and tissue homogenized with anhydrous sodium sulfate. The samples were added to 2 X 35 cm glass columns previously filled with 2 cm height of Na_2SO_4 (ACS grade, BDH, ON, Canada) and 30 ml hexane: dichloromethane (1:1, v/v) (Omnisolv grade, VWR Scientific). The sample was spiked with a reference standard (Accustandard, Conneticut, U.S.A.) to determine extraction efficiency. After 1 hour samples were eluted with 250 ml 1:1 v/v hexane: dichloromethane and rotaevaporated to approximately 5 ml. The extracts were made up to 25 ml with hexane and 2 ml aliquots of the extracts were taken for determination of lipid content by weighting. The remaining extracts were concentrated to 2 ml and transferred to 35 cm x 1 cm glass columns packed with 6 g activated Florisil in hexane. PAHs were eluted with 100 ml 3:2 v/v dichloromethane: hexane. Extracts were concentrated and made to a final volume of 1 ml with isooctane. Analysis by gas chromatography was performed on a Hewlett-Packard (Avondale, PA) 5890/5979 gas chromatograph with a mass selective detector equipped with a Hewlett-Packard 7973A autosampler and a 30 m x 0.25 mm DB-5 column. Injection was 1 µ splitless at 250°C and the oven temperature was programmed from 100-270°C at 3°C/min. Carrier gas was ultra-high purity helium at 30 cm/s flow rate. The detection level was 0.48 ng/g dry weight. An analytical blank was analyzed for every five samples with the same weight of sodium sulfate used for drying the samples. The concentration of the

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PAHs in the blank was deduced from those in the extract of the samples. Sediment and biological sample extracts were analyzed using a Hewlett-Packard (Avondale, PA) 5890/5979 gas chromatograph with a mass selective detector equipped with a Hewlett-Packard 7973A autosampler and a 30 m x 0.25 mm DB-5 column. Injection was 1 µ splitless at 250°C and the oven temperature was programmed from 100-270°C at 3°C/min. Carrier gas was ultra-high purity helium at 30 cm/s flow rate. The detection level ranged from 0.48-xx ng/g dry weight for individual PAHs. Quality assurance was performed by determination of range of recoveries of the surrogate spiking solution for sediments and for mussel samples. The average % recoveries for PAHs in the NIST standards ranged from 70-130% and the results for individual PAHs were corrected accordingly.

Results

The polycyclic aromatic hydrocarbon concentration and composition (2- to 6-ring parent and branched) were determined for all sediment and oyster samples and were normalized to the organic carbon (OC) content of sediment as well as tissue lipid concentration correspondingly. A typical concentration data for oyster and sediment samples on Simorgh site are presented in figures 2 and 3 respectively. The concentrations of PAHs in oysters are orders of magnitude higher in comparison to the sediment samples. These figures also demonstrates the overall tendency of lower molecular weight PAHs (LMW PAHS) for bioaccumulation in oysters while there has been a decrease in tissue concentration of higher molecular weight PAHs (HMW PAHs) in comparison to the sediment samples. Similar pattern was observed for the lipid normalized average PAHs in oysters in all sampling sites (Fig. 5).

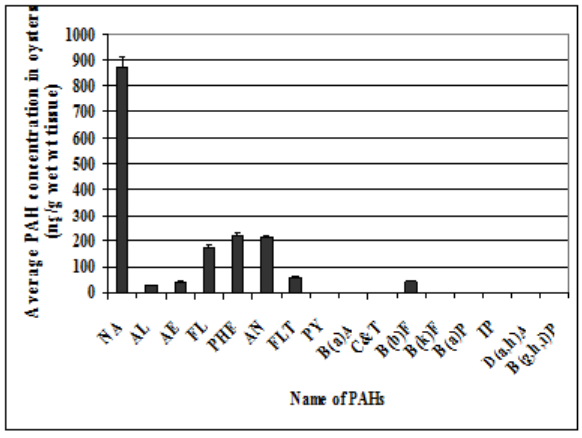


Figure 2: A typical example of lipid normalized individual PAH concentration in oysters of Simorgh site*(For abbreviations refer to fig. 4)

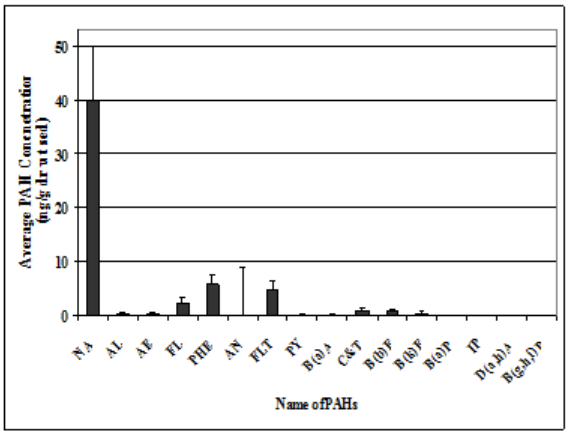


Figure 3: A typical example of organic carbon normalized individual PAH concentration in sediments of Simorgh site*(For abbreviations refer to fig. 4)

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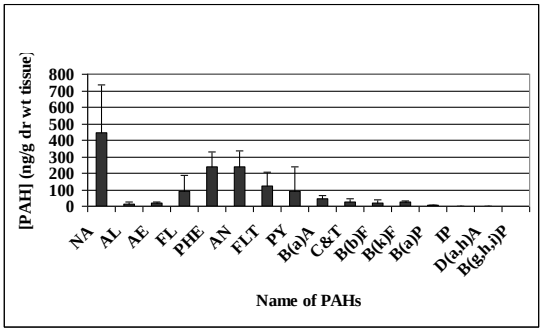


Figure 4: Lipid Normalized Average PAHs in Oysters*

* NA: Naphthalene; AL: Acenaphthylene; AE: Acenaphthene; FL: Fluorene; PHE: Phenanthrene; AN: Anthracene; FLT: Fluoranthene; PY:Pyrene; B(a)A: Benzo(a)Anthracene; C&T: Chrysene; B(b)F: Benzo(b)fluoranthene; B(k)F: Benzo(k)fluoranthene; B(a)P: Bezo(a)pyrene IP: Indeno(1,2,3)pyrene; D(a,h)A: Dibenzo(a,h)anthracene; B(g,h,i)P: Benzo(g,h,i)pyrene

Table 2: Total PAH concentrations in sediment and oyster samples

Name of PAH	in sediment	in oysters
Naphthalene	233.80	3993.54
Acenaphthylene	10.06	127.39
Acenaphthene	9.69	133.95
Fluorene	118.85	819.80
Phenanthrene	163.06	2125.85
Anthracene	161.05	2142.45
Fluoranthene	140.06	1101.30
Pyrene	80.22	442.82
Benzo(a)anthracene	42.71	88.78
Chrysene	120.17	113.16
Benzo(b)fluoranthene	82.50	71.79
Benzo(k)fluoranthene	62.92	143.45
Bezo(a)pyrene	75.58	5.78
Indeno(1,2,3)pyrene	94.92	0
Dibenzo(a,h) anthracene	31.86	0
Benzo(g,h,i)Pyrene	270.55	3598.23

The concentrations of PAHs in oysters are orders of magnitude higher in comparison to the sediment samples. These figures also demonstrates the overall tendency of lower molecular weight PAHs (LMW PAHS) for bioaccumulation in oysters while there has been a decrease in tissue concentration of higher molecular weight PAHs (HMW PAHs) in comparison to the sediment samples. Similar pattern was observed for the lipid normalized average PAHs in oysters in all sampling sites (Fig 5). The total PAH concentration (ΣPAH) in the sediment samples of study area which were normalized to the organic carbon (OC) content of sediment, varied from 9.7 to 270 µg/g dry weight sediment (Table 2). Comparison of the average PAHs content in sediment samples shows that highest contamination was observed in Harireh and lowest in Foreigners Pelage stations (161.69 and 8.95 ng/g dry weight of sediment respectively) (Fig 6). Oysters from Big corals site exhibited a wide range of total PAH concentration (1.07 to 77.66 ng/g dry weight). The lowest value (oysters from Foreigner's Plage) was 0.7 ng/g wet weight while the maximum concentration was 36.33 ng/g wet weight (Fig. 5).

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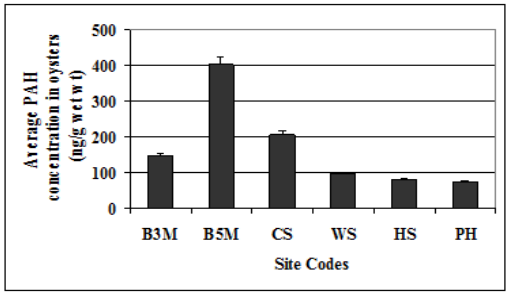


Figure 5: Total average PAH concentration in oysters of sampling Sites

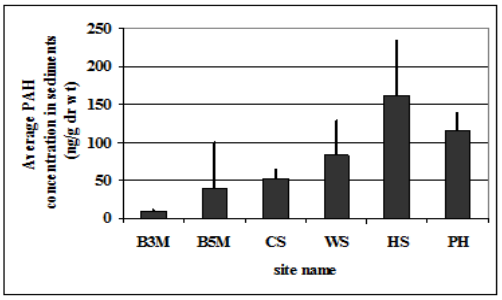


Figure 6: Total average PAH concentration in sediments of sampling sites

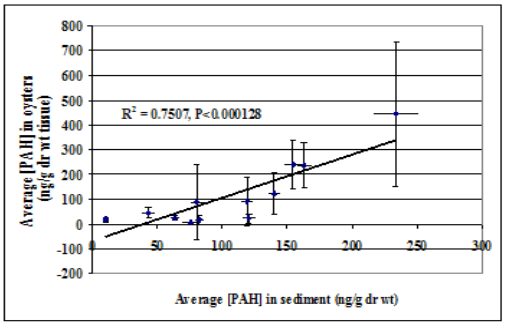


Figure 7: Significant positive correlation between average PAH concentration in oysters and sediment samples

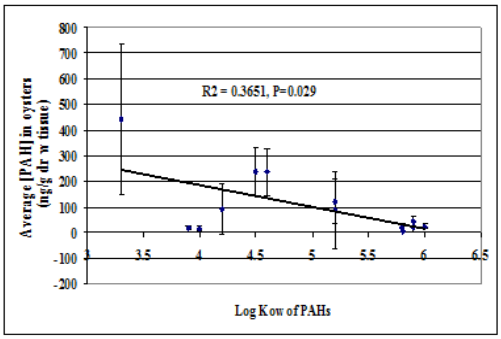


Figure 8: Significant negative correlation between average PAH concentration in oysters and their Octnaol-Water partition coefficient (Kow)

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Discussion

The available data for PAHs in sediments from many regions in the Persian Gulf (e.g. Kish Island) are relatively sparse and generally suggest medium levels of PAH contamination relative to other marine areas. Fowler et al. (1993) surveyed surficial sediments from coastal areas around Persian Gulf and reported that the levels of ΣPAHs never exceeded 1–450 ng/g dry weight in industrialized ports of Bahrain and Oman (Fowler et al., 1993). In this study the concentrations of PAHs in sediment are highest at Harireh and Arab Town stations which is in vicinity of the most populated and industrialized places in the island (Figs 6, 7) . Relatively higher concentration of naphthalene might be originated from application of this reagent for household and industrial applications (Figs 2 , 3 and Table2). We believe that this increment should be assessed seriously by fisheries and environmental authorities as in our previous study this event had not been observed in the Island (Eghtesadi et al., 2002).

Correlation between oyster and sediment total parent PAH concentrations is rather high and significant (r^2=0.21, n=25, P=0.000128, F=0.25) and is illustrated in figure 8 which shows the effect of concentration gradient in internalization of PAHs in oysters. This might be due to tendency for accumulation of lipophile PAHs in biological tissues rather than the hydrophilic environment of seawater. This gives rise to the biomagnification in oysters. Similar results were observed in previous studies by Baumard (Baumard et al., 1998).

Compounds characterized by comparable bioavailability are not accumulated to the same extent by oysters. Benzo[e]pyrene has been observed to be preferentially accumulated relative to benzo[a]pyrene by mussels (*Mytilus galloprovincialis*) sampled in the Mediterranean Sea (Baumard et al., 1998). Moreover, while oyster and sediment concentrations of benzo[e]pyrene were correlated to a moderate level, benzo[a]pyrene concentrations were correlated to a much lower level. Different accumulation behavior from the sediment is observed for the different PAHs. Oysters are enriched in low molecular weight PAHs (Fig. 2 and Table2) compared to the high molecular weight relative PAHs in the sediment (Fig. 3 and Table2). This pattern of accumulation shows that more water-soluble compounds are preferentially accumulated in the water-dissolved form through the gills while the heavier molecular weight compounds are favorably passing through the digestive system absorbed from filtered particles (and therefore show higher bioavailability); Sverdrup et al. (2002) tested some PAHs with log $K_{ow} \leq 5.2$ and showed that toxicity significantly goes up together with increasing lipophilicity (increasing log K_{ow}) of the substances. Djomo et al. (2004) showed a direct relation between PAHs toxicity and their K_{ow}. Our results were in agreement with the mentioned researchers showing that LMW PAHs are more bioavalable and less degraded (i.e. more bioaccumulated in the mussels). Different isomeric compound accumulations (based on values of various ratios of isomeric compound concentrations) were observed in oysters and related to pref erential biotransformation capacities of Pinctada margaritifera according to the compounds. The different biotransformation efficiencies of oysters for the different compounds could be related to specific

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properties of the molecules such as different reactivities of the molecules in relation to different molecular structures and especially to the three dimensional geometric descriptors of each compound which determines their bioavailibity and has been noticed in our previous research (Karami-Varnamkhasti). It has been reported by our team that the bioavailability of PAHs in aquatic environments depends mostly to their octanol water partition coefficient (Kow) when Kow≤5.2 (Sverdrup et al. 2002) which is consistent with the results of this study when higher oyster internalization is considered for more water-soluble and sediment concentrated PAHs (e.g. Naphthalene) (Fig 2 and 3). This is consistent with the significant negative correlation between average PAH concentration in oysters and their Octnaol-Water partition coefficient (Fig. 8). According to these and also from the higher concentration of LMW PAHs and lower concentration of HMW PAHs in the oysters (Fig 2) compared to the sediment (Fig 3), we believe that the overall bioavailibity, internalization and bioaccumulation is controlled by their Kow to be within the range of ≤5.2 the movement of more water-soluble LMW PAHs compounds which is consistent with our previous *in vitro* studies (Karami-Varnamkhasti).

Acknowledgments

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