Seafood Safety - Proceedings of the Symposium on Seafood Safety - Status and Strategies, 28-30 May 2002, Cochin, India

Accumulation and Release of Petroleum Hydrocarbons by *Mytilopsis sallei* from the Harbour Waters of Visakhapatnam

R. RAGHU PRAKASH

Visakhapatnam Research Centre of Central Institute of Fisheries Technology Visakhapatnam - 530 003, India

Marine organisms are known to accumulate petroleum hydrocarbons in their tissues from the surrounding environment or through the food chain. Bivalves being sedentary filter feeders are extremely vulnerable to both chronic and acute exposures to petroleum hydrocarbons. In the recent years, due to increasing activity of bulk carriers, oil tankers, industrial activities and other anthropological activities, considerable amount of oil is finding its way into the coastal waters around Visakhapatnam. *Mytilopsis sallei*, a marine bivalve occurs in the vicinity of the harbour waters where petroleum hydrocarbons are present in high concentrations. The initial levels of petroleum hydrocarbons in the tissue of *M. sallei* reflected on the amount of petroleum hydrocarbons in the medium. *M. sallei* accumulated PHC in the tissue to a level greater than that found in the surrounding medium. A direct relationship exists between the initial concentration and that of the amounts retained after depuration. Total elimination of petroleum hydrocarbons could not be achieved even after 30 days of depuration.

Key words : Petroleum hydrocarbons, bioaccumulation, *Mytilopsis sallei*

The immediate effect of petroleum hydrocarbon discharge on the health of human population is the possible risk from the consumption of seafood contaminated by oil derived carcinogens such as polycyclic aromatic hydrocarbons (PHC). In addition to the possible effects on human health, crustaceans fishes and mollluscs exposed to oil contamination can acquire an obnoxious oily taste associated with the presence of volatile compounds derived from oil (Samiullah, 1985). Extensive data is available on the accumulation of petroleum hydrocarbons in natural laboratory conditions (Farrington *et al.*, 1983; Stegeman & Teal, 1973; Anderson, 1973).

Bivalves being sedentary filter feeders are extremely vulnerable to both chronic and acute exposures to pollutants such as petroleum hydrocarbons.

The potential for elimination of contaminants by marine organisms, when they are transferred to clean water, plays an important factor for survival and adaptive behavior in polluted waters and also from the point of view of food safety.

In the recent years due to increasing activity of bulk carriers, oil tankers, industrial activities and other anthropological activities, considerable amount of oil is finding its way into the coastal waters around Visakhapatnam. *Mytilopsis sallei*, a marine bivalve occurs in the vicinity of the harbour waters where PHCs are present in high concentrations. This study attempts to throw some light on the accumulation of petroleum hydrocarbons into the tissue and its release from the tissues of marine bivalve, *M. sallei.*

Materials and Methods

Specimens of *M. sallei* were collected from three stations from the harbour area. Water samples were also collected from the area. The initial concentration of petroleum hydrocarbons from the tissues of *M. sallei* was estimated from the three stations. The petroleum hydrocarbons from the medium was also estimated.

Petroleum hydrocarbons were extracted from seawater with CCl_4 and transferred to n-hexane phase and passed through a silica gel column (UNESCO, 1967). PHC concentrations in seawater were measured following the method of Levy (1972) and NAS (1975) using scanning mode Beckman's ACTA II double beam Spectrophotometer in the region 210-350 nm. The PHCs from the tissues were extracted from the tissues with acetone and methylene chloride and then transformed into n- hexane phase (Bromann & Ganning, 1985) and analysed by UV fluorescence (UNESCO, 1976).

Animals of the three groups were maintained in clean flowing sea water to facilitate gradual release of PHC. The concentration of PHC was measured in the tissues of the animals for a period of 30 days continuously at regular intervals of 1, 2, 5, 10, 15 and 20 days.

Results and Discussion

The PHC concentrations from the medium was found to be 80 $\mu g.g^{-1}$, 58 $\mu g.g^{-1}$ and 21$\mu g.\ g^{-1}$ in stations I, II, and III, respectively. The initial levels of PHC concentration in the tissues of *M. sallei* was estimated to be 109 $\mu g.g^{-1}$, 62 $\mu g.g^{-1}$ and 15 $\mu g.g^{-1}$ from station I, II, and III, respectively.

On the basis of the initial concentration, the animals can be grouped into three groups. Animals from station I (group I) as having high initial levels of PHC in the tissue, animals from station II (group II) as having medium levels of PHC concentration and animals from station III (group III) as having low initial levels of PHC in the tissue.

The elimination of PHC in Group I was rapid during the first 48 h, where the concentration came down to almost 54 μg.g^{-1} After 96 h, the PHC concentration came down to 32 μg.g^{-1}, which is about 70 % of the initial level (Fig. 1). After 96 h the elimination was rather slow with only slight reduction, the tissue concentration reaching 25 μg.g^{-1}, 14 μg.g^{-1} 12 μg.g^{-1}, 14 μg. g^{-1} and 10μg.g^{-1} during the 10th, 15th, 20th and 30th days, respectively (Fig. 1).

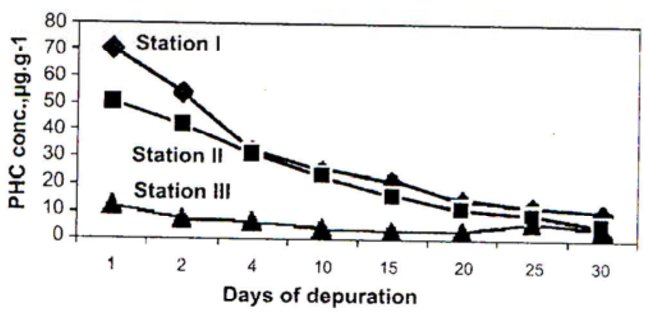

Fig. 1. Release of petroleum hydrocarbon by *Mytilopsis sallei* as a function of time

In the II group having initial concentration of 60 μg.g^{-1}, the PHC level came down to almost 50 % of the initial concentration after 96 h, after which the elimination was rather slow. Total elimination of petroleum hydrocarbons from the tissues could not be achieved after 30 days.

In the third group having a initial concentration of 15 μg.g^{-1} in the tissues, the elimination was rather slow when compared to the other two groups. Though there was a slight fall in the PHC levels for the first 2 days, there was no apparent decrease later. After the 30th day the petroleum hydrocarbons in the tissues remained unaltered with the concentration of 4μg.g^{-1}.

Petroleum hydrocarbon concentrations introduced into natural marine systems can be expected to occur in the water column, in solution and colloidal suspension and droplets in addition to being adsorbed to phytoplankton, suspended particles, detritus and sediments. The entrance of hydrocarbons into shellfish presumably occur by various routes. Possible uptake across outer membranes, particularly the gills should be significant when hydrocarbons concentrations are high and as hydrocarbons move out of the water onto the organics, ingestion of contaminants might become a major path (Fossato, 1975).

The initial levels of PHC in the tissue of *M. sallei* reflect on the amount of PHC in the medium. *M. sallai* accumulated PHC in the tissue to a level greater than that found in the surrounding medium. Similar accumulation patterns were shown by *M. edulis* (Ehrhdart & Heineman, 1975). Bivalves are capable of concentrating and retaining in their tissues at least some hydrocarbons fractions at levels considerably higher than the concentration in which they reside (Fosatto & Conzoiner, 1976). The temporal trends in the accumulation of PHCs in the tissues was observed in *M. edulis* and *M. baltica.*

The elimination process in the group I, with a high initial petroleum hydrocarbon concentration in the tissue may be divided into an initial phase with a rapid reduction within the first 2 days and subsequent phase of slight or slow reduction or plateau phase. Similar elimination pattern was observed in *Mytilus galloprovincialis* (Fosatto, 1975). Animals having higher initial concentrations of PHC relatively retained higher levels even after depuration. A direct relationship exists between the initial concentration and that of the amounts retained after depuration. *Crassostrea virginica* exposed to petroleum hydrocarbons when transferred to clean sea water showed an apparent linear rate of decrease in hydrocarbon concentration, resulting in a loss of 90% of the accumulated burden of 334 $\mu g.g^{-1}$ in 2 weeks period after which the rate of loss slowed down. At the end of the 4 weeks discharge of accumulated hydrocarbons, leveled up to 34 $\mu g.g^{-1}$. Lower molecular weight and more water-soluble compounds are discharged more rapidly (Steagman & Teal, 1983).

The process of elimination or the metabolism involved in the process are yet to be elucidated. In fishes the path of hydrocarbon included entrance through the gills, metabolism by the liver, transfer of hydrocarbons and their metabolites to the bile and finally excretion (Lee *et al.,* 1972). Blumer (1967) reported that naturally occurring hydrocarbons in the Basking shark's major food source, zooplankton, passed through the sharks digestive tract without fractionation or structural modification. Canzoiner (1971) suggested that the plateau phase might represent that residual portion of the contaminant that is more intimately sequestered in some tissue or tissues of the bivalve, the unloading of which involves a completely different pathway than that followed by major fraction of the contaminant. Such biphasic mechanism had been postulated for virus elimination in bivalve shell fish (Canzoiner, 1971).

Retention of some accumulated hydrocarbons after depuration reflect the combined effects of many factors One of the factors might be that the transfer of organisms contaminated to clean water might result in the establishment of a new equilibrium concentration of hydrocarbon in the organism. The other possibility is that at least some of the petroleum hydrocarbons retained might have entered a stable compartment characterized by low molecular hydrocarbon turn over rate, thereby becoming less available for removal. It would be of obvious advantage for marine organisms to possess a means to avoid loss by equilibrium of important biogenic hydrocarbons and one would expect a certain amount of hydrocarbons to be retained by such means, Such a possibility is consistent with the observation that biogenic hydrocarbons are persistent in marine organisms (Blumer *et al.*, 1970) and fresh water algae (Kauss *et al.*, 1973).

The release of petroleum hydrocarbons by *M. sallei* after transfer to clean water show that depuration can be effective in lowering hydrocarbons concentrations and plays an important factor in the survival and adaptive behavior of this bivalve polluted waters.

References

Anderson, J.W. (1973) *Uptake and depuration of specific hydrocarbons from fuel oil by bivalve Rangia cuneata and Crassostrea virginica*, Background paper prepared for the National Academy of Sciences, Ocean Affairs Board Workshop on Inputs, Fate and Effect of Petroleum Pollution in Marine Environment Arlie Virginica: 21-25

Blumer, M. (1967) *Science* **156**, 390

Blumer M.M., Mullin, M. and Guillard, R.L. (1970) *Mar. Biol.* **6,** 226

Bromann and Ganning (1985) *Ophilia* 25(1), 49

Canzoiner W.J. (1971a) *Appl. Microbiol.* **21**, 1024

Canzoiner W.J. (1971b) *Microbiology of estuarine pollution and its relation to shell fish contamination*, Proc. 13th Week of Science Conf. Aleppo: 17-37

Ehrhardt, M., (1972) *Environ. Pollut.* **3**, 257

Ehrhardt, M. and Heinemann, J. (1975) *Environ Pollut.* **9**, 263

Farrington, J.W., Risebrough, R.W. Parker, P.L. Davis, A.C., Delappe, B., Winters, J., Boatwright D. and Frew, N. (1983) *Hydrocarbons and polychlorinated biphenyls and DDE in Mussel and oysters from US coast, 1976-1978*. The Mussel Watch Tech. Rep. WHOI – 82-42 Woodshole Oceanographic Institute

Fosatto and Conzoiner (1976) *Mar. Biol.* **36**, 243

Fosatto, V.U. (1975) *Mar. Pollut. Bull.* **7**, 10

Fossato V.U. and E. Siviero (1974) *Mar. Biol.* **25**, 1

Kauss, P. Hutchinson, T.C., Soto, C., Hellebust, J. and Griffiths, M. (1973) *The toxicity of crude oil and its components to fresh water algae*, Prevention and Control of Oil Spills, Proc. Am. Petol. Inst. Envir. Prot. Ag. Conf. : 703-714

Lee, R.F. and Sauerlebev, R. and Dobhs, G.H. (1972) *Mar. Biol.* **17**, 201

Levy, E.M. (1972) *Water Res.* **6**, 57

National Academy of Science (1981) *International Mussel Watch*, National Academy of science Washington DC: 11449-57

Neff J.N., Dixit, M., Cox, B.A. and Anderson, J.W. (1976) *Accumulation and release of petroleum derived aromatic hydrocarbon by marine animals*, Effect of Petroleum Hydrocarbons in Arctic and Sub-Arctic Environments and Organisms II, Biological Effects

Saimiullah, Y. (1985) *Oil Petrol. Pollution* **2**, 235

Steageman J.J. and Teal, J.M. (1983) *Mar. Biol.* **22**, 37

UNESCO (1976) Guide to Operational Procedures for IGOSS Pilot Project on Marine Pollution Manual, UNESCO: 47 p.