Organochlorine Pesticides and Polychlorinated Hydrocarbons in Seafood

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Pesticides and polychlorinated biphenyls (PCBs) are ubiquitous toxic constituents due to their bioaccumulation and specific physicochemical properties. These compounds accumulate in the biota of all levels and the residues are present in all environmental compartments from all geographical areas. Elevated pollutant concentrations are generally the consequence of effluent discharge and agricultural and industrial runoffs. Although majority of these compounds enter the environment through anthropogenic activities, marine algae and invertebrates and natural processes such as forest fire also contribute their share. The development of global quality assurance criteria and the general awareness of the consumer for quality of the food forced a new approach in the quality aspects of food commodities and seafood is no exception to this. In the light of stringent safety measures with respect to seafood imposed by EU and other developed countries, the understanding on pesticides and PCBs assumes significance. Though present in extremely low concentrations, they definitely pose a chronic health hazard. However, the major problem, associated with these chemicals was their method of detection. The advancement of gas chromatographic and HPLC systems has made it possible to quantify these toxic chemicals for the benefit of the mankind. This paper provides an insight into the general aspects of pesticides and PCBs besides highlighting the health hazards, bioaccumulation in sediments, fish and other aquatic biota and their relevance to the quality assurance system of seafood.

Keywords: Organochlorine pesticides, polychlorinated biphenyls, seafood

The organochlorine pesticides and polychlorinated biphenyls (PCBs) are of great concern due to their toxic effects on wildlife and humans. They are in fact anthropogenic contaminants with ubiquitous distribution (Loganathan & Kannan, 1991; Reddy et al., 1991) and their chemical stability reflects their persistence in the environment and the lipophilic nature contribute to their tendency to be magnified in the lipid fractions in the tissue. The use

of pesticides in agriculture has contributed to increasing crop yield and several persistent and banned pesticides including DDT are still being used in several countries. Intensive agriculture, urbanization and industrial activities have contributed to the contamination of ecosystem with organochlorine compounds, petroleum products and heavy metals, world over (Haynes & Johnson, 2000). Some of these have been considered environmental hormones due to their effect on the reproductive system of humans and other lives (Colborn & Smolen, 1996). The usage of organochlorine pesticides and other related chemicals is higher in Asian countries and is reflected in the produce from these countries. Contamination of water areas in Australia, is associated with intensive agriculture in the coastal high rainfall regions (Haynes *et al.*, 2000)

Pesticides

A pesticide is a substance, or mixture of substances, that is used in the control of pests such as plant parasitic viruses, bacteria, nematodes, fungi, insects, weeds, rodents, and birds. Pesticides include all materials that are used to prevent, destroy, repel, attract, or reduce pest organisms. Therefore, pesticides are used in many different applications such as forestry, landscaping, agriculture and domestic use.

Pesticides have been in use for thousands of years. Some of the early demonstrations include burning sulphur in an attempt to control insects by Romans and the use of arsenic and pyrethrum by the Chinese. By the early 20th century, two classes of pesticides were primarily used. These included botanicals, natural chemicals derived from plant material, and inorganic salts which were widely used as fungicides, herbicides and insecticides. The era of synthetic pesticides started with the discovery of DDT (dichloro-diphenyltrichloroethane) in 1934 and was a major advancement in chemicals, which pocessed insecticidal properties. Since then, innumerable preparations have been in use in the name of insecticides, fungicides, herbicides, etc. Development of resistant pests led to the banning of DDT in the 1960s. Globally, about 2.5 million t of pesticides are applied annually to control pest organisms. Most of this application is targeted on agricultural crops. Even though the use of pesticides had a very positive impact in the overall increase in food production, the risks associated with this include human health effects, livestock animal poisoning, beneficial insect losses, water contamination, wildlife losses and the genetic evolution of pesticide resistance.

Classification of pesticides

Pesticides can be classified according to their chemical structure. Inorganic pesticides are poisons made from common natural highly toxic and indestructible chemicals like arsenic, copper, lead and mercury and hence can accumulate in the environment. Natural or organic pesticides are generally compounds extracted from plants. Many plants like tobacco. chrysanthemum, and conifers, have evolved the ability to produce substances that are used for this purpose. Another class of chemicals is used to fumigate (fumigants) the insects but due to the danger associated it has been banned. world over. Chlorinated hydrocarbons (DDT, chlordane, aldrin, dieldrin, toxaphene, paradichlorobenzene and lindane) are synthetic organic compounds that affect the nervous system of the pests. These chemicals are highly resistant to decomposition and can remain in the ecosystems for a long time. Organophosphates are chemicals 10 to 100 times more toxic to animals larger than insects, than chlorinated hydrocarbons, but are not persistent as hydrocarbons. Parathion, malathion, dichlorvos. dimethyldichlorovinylphosphate (DDVP) and tetraethylpyrophosphate (TEPP) are some of the examples of this group. Carbamates are urethanes that affect the nervous system of pests. They are very similar to organophosphates and include such chemicals as carbaryl (Sevin), aldicarb (Temik), aminocarb (Zineb) and carbofuran (Baygon).

Pesticides and the environment

The presence of pesticides or their metabolites in low levels in the rood after application is called "residue". The upper limit for each pesticide in the food items, which do not produce any toxicological effect is called "maximum residual limit" (MRL). With increasing awareness among the consumers, the MRL is decreasing creating an ever-increasing demand for selective and sensitive detection technique. Up to 90 % of the pesticides applied never reach the intended targets. As a result, many other organisms sharing the same environment as the pests are accidentally poisoned. Contamination of groundwater and surface water by pesticides is a very common problem. Pesticides can reach water resulting from direct treatment used to control pests or indirectly. Pesticides can contaminate aquatic systems by fallout from aerial sprays, soil erosion or through the disposal of effluent from pesticide factories. Pesticides can enter water through surface runoff, leaching or erosion.

Once the pesticide reaches the water system its distribution and fate will depend on its persistence and solubility. Most pesticides that persist in a water system usually become adsorbed onto floating particles and settle out as sediment. Generally, the more persistent a pesticide is, the greater the effect it will have on the aquatic ecosystem. Also, the more soluble a pesticide is, the greater will be its potential for aquatic system and groundwater contamination. Water composition, pH, temperature, aquatic life present and amount of suspended organic and inorganic material are some of the factors affecting the persistence of a pesticide in a water system. Compounds including lindane (X-HCH), aldrin, heptachlor, chlordane, DDT and dieldrin have been extensively used in agricultural applications for control of insects and weeds (Hagan & Klumpp, 1995). As a result, these have been identified as contaninants in ground water, seawater, freshwater biota, freshwater and estuarine sediments and marine biota (Haynes et al., 2000).

Habitat alteration

Pesticides can also reduce the availability of plants and insects that serve as habitat and food for fish and other aquatic animals. Insect eating fish can lose a portion of their food supply when pesticides are applied. Inadequate supply of insects can force fish to range farther in search of food, where they may risk greater exposure to predation. Spraying herbicides can also reduce reproductive success of fish and aquatic animals. The shallow, weedy nursery areas for many fish species provide abundant food and shelter for young fish. Aquatic plants provide as much as 80% of the dissolved oxygen necessary for aquatic life in ponds and lakes. Spraying herbicides to kill all aquatic plants can result in severely low oxygen levels and the suffocation of fish.

Ecological effects of pesticides

Pesticides are included in a broad range of organic micro-pollutants that have ecological impacts. Different categories of pesticides have different effects on living organisms and hence generalization is difficult. Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contaminated by pesticide runoff. Fish and aquatic animals are exposed to pesticides in three primary ways (i) direct absorption through the skin by swimming in pesticide contaminated waters, (ii) direct uptake of pesticides through the gills during respiration, and (iii) drinking of pesticide contaminated water or feeding on pesticide

contaminated prey. Exposure of fish and other aquatic animals to a pesticide depends on its biological availability, bioconcentration, biomagnification and persistence in the environment.

The property of chlorinated hydrocarbon for bioccumulation is strongly related to their capacity to bind to lipids. Bioavailability refers to the amount of pesticide in the environment available to fish and wildlife. Some pesticides rapidly breakdown after application while some others bind tightly to soil particles suspended in the water column or to stream bottoms, thereby reducing their availability. Some are quickly diluted in water or rapidly volatize into the air and are less available to aquatic life. The transformation of organic compounds directly from water column to the biota via gills or body surface into the circulatory fluids is called bioaccumulation or transferred via blood across the gastrointestinal tract into the circulating fluid by a process called biomagnification (Connell, 1988). Bioconcentration is the accumulation of pesticides in animal tissue at levels greater than those in the water or soil to which they were applied. Some pesticides such as DDT are lipophilic and accumulate in fatty tissue such as edible fish tissue and human fatty tissue. Other pesticides such as glyphosate are metabolized and excreted. Biomagnification is the accumulation of pesticides at each successive level of the food chain. As larger organisms eat smaller organisms, the concentration of pesticides and other chemicals are increasingly magnified in tissue and other organs. Very high concentrations can be observed in top predators, including man.

The ecological effects of pesticides (and other organic contaminants) are varied as given below and are often interrelated. The types of effects will vary depending on the organism under investigation and the type of pesticide.

- Death of the organism
- · Cancers, tumours and lesions on fish and animals
- · Reproductive inhibition or failure
- Suppression of immune system
- · Disruption of endocrine (hormonal) system
- Cellular and DNA damage
- Teratogenic effects (physical deformities such as hooked beaks on birds)

- Poor fish health marked by low red to white blood cell ratio, excessive slime on fish scales and gills, etc.
- Intergenerational effects (effects that are not apparent until subsequent generations of the organism).
- Other physiological effects such as eggshell thinning

These effects are not necessarily caused solely by exposure to pesticides or other organic contaminants but may be associated with a combination of environmental stresses such as eutrophication and pathogens.

Factors that degrade pesticides

Pesticides are subject to degradation by chemical and photochemical reactions. They are also degraded by two other principal biological mechanisms, viz., (i) microbiological processes in soils and water and (ii) metabolism of pesticides that are ingested by organisms as part of their food supply. While both processes are beneficial in the sense that pesticide toxicity is reduced, metabolic processes do cause adverse effects in, for example, fish. Energy used to metabolize pesticides and other xenobiotics (foreign chemicals) is not available for other body functions and can seriously impair growth and reproduction of the organism.

Many pesticides dissipate rapidly in soils. This process of mineralization results in the conversion of the pesticide into simpler compounds such H₂O, CO₂, and NH₃. While some of this process is a result of chemical reactions such as hydrolysis and photolysis, microbiological catabolism and metabolism are usually the major routes of mineralization. Soil microbiota utilizes the pesticide as a source of carbon or other nutrients. Some chemicals (for example 2,4-D) are quite rapidly broken down in soil while others are less easily attacked (2,4,5-T). Some chemicals are very persistent and are only slowly broken down (atrazine) (Stephenson & Solomon, 1993).

Metabolism of pesticides in animals is an important mechanism by which organisms protect themselves from the toxic effects of xenobiotics in their food supply. In the organism, the chemical is transformed into a less toxic form and either excreted or stored in the organism. Different organs, especially the liver, may be involved, depending on the chemical. Enzymes play an important role in the metabolic process and the presence of certain enzymes, especially "mixed" function oxygenases (MFOs) in liver, is now used as an indicator that the organism has been exposed to foreign chemicals.

Pesticides and fish

The rivers, backwaters and ocean are the ultimate dumping place for all the pollutants, whether it is industrial effluents or agricultural runoffs. The toxicity of the pollutants depends on the physical and chemical parameters of water namely, pH, temperature, solubility and chelation with different metals (Chevreuil et al., 1995). The accumulation of organochlorine compounds in fish is related to its lipid content, habitat, dietary intake, growth rate and metabolism (Pastor et al., 1996). Muscle tissues from a wide range of fish species were found to contain organochlorine pesticides including chlordane, dieldrin and heptachlor epoxide (Roach & Runnie, 1990).

Some mussels have the capacity to take in these chemicals and can act as biological markers. The blue mussels and zebra mussel accumulate most hydrocarbons present in the environment (Murray et al., 1991). The larger organisms accumulate pesticides in the body by bioconcentration and these in turn cause health hazards. Chlorinated pesticides (particularly DDT group of pesticides and lindane and chlorophenols were reported in eight of the fish samples anlaysed from Isipingo estuary, Natal, South Africa but were found to be within the WHO prescribed limits (Grobler et al., 1996). Similarly, about twenty-seven species of marine and freshwater fish from Cambodia were found to accumulate organochlorines with DDT and related pesticides and among them, the freshwater fish had higher accumulation of pesticides suggesting possible contamination from watershed (Monirith et al., 1999). Bioaccumulation of these chemicals were related to the food habits as reported by their accumulation in higher concentrations in carnivorous fish sea mullet and the bottom feeder red mullet than in sea bass of Mediterranean region (Pastor et al., 1996). Seafood for human consumption has been contaminated with low levels of PCBs, DDT and its metabolites, chlordane compounds and lindane isomers (Kannan et al., 1993; Kannan et al., 1994; Kannan et al., 1995). In Indian food fishes and shellfishes, 0.005 to 0.35 ppm level of pesticides have been detected and these are within the prescribed limits (Radhakrishnan et al., 1986; Radhakrishnan, 1994; Radhakrishnan & Antony, 1989).

Marine plants

Algae and sea grass tend to concentrate organochlorines to some extent and the major effect noticed is the decreased photosynthesis, respiratory inhibition or enhancement and growth reduction (Haynes & Johnson, 2000)

through inhibition of cyclic phosphorylation and suppression of electron transport chain and ATP turn over (Ramachandran *et al.*, 1984). Endrin, dieldrin and aldrin cause most toxic effects to aquatic plants even at concentrations lower than 1 g.l⁻¹ while DDT and DDE require slightly higher concentrations (Menzel *et al.*, 1970; Powers *et al.*, 1975). Besides causing harmful effects on the metabolism in these plants, they indirectly enter the fish and other forms of life and ultimately enter human beings through fish and other aquatic organisms.

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of fat-soluble organic compounds with a biphenyl molecule, basically two benzene molecules attached together, with chlorine atoms attached at varying positions. They are oily liquids or solids, clear to light yellow in color and with no smell or taste. PCBs are also known as arochlors and chlorodiphenyls. The PCBs are generally represented by a four-digit number. The first two digits represent the type of molecule and the second two digits the percentage makeup of chlorine in the molecule. For example, Arochlor-1242 and Arochor-1254 contain 42 and 54 percent chlorine by weight, respectively (Moore & Ramamoorthy, 1984). PCBs are composed of 209 closely related compounds that differ from each other by the number and position of chlorine on the phenyl ring (Lee *et al.*, 2001).

PCBs have widespread industrial applications. At one time PCBs were common components of hydraulic fluids, lubricants, heat transfer fluids, and insecticides. PCBs were primarily manufactured as dielectric fluid for transformers and capacitors because of their ability to absorb heat, low flammability, low electrical conductivity, and favorable dielectric constant (Kennish, 1997). Currently, heat transfer fluids residing in old transformers and capacitors used in power distribution systems are the main sources of PCBs. Non-liquid PCBs (NLPCBs) can be found in various items such as fluorescent light ballast potting material, ceiling tile coatings, and certain painted surfaces. However, due to its varied effects on health, U.S. has stopped producing PCBs. As levels in the environment increased, the potential for harmful effects increased. PCBs are generally liquid, viscous or viscous sticky resins. They are sparingly soluble in water and the solubility decreases with increasing chlorine content. They are stable compounds that break down very slowly in the environment.

There are extensive reports available indicating the concentration of PCBs in the water resources throughout the world (Moore & Ramamoorthy, 1984; Armstrong et al, 1993; Kamman et al., 1993; Sugiura et al., 1986; Grobler et al., 1996; Everaarts et al., 1991; Everaarts et al., 1998; Pastor et al., 1996; Corsolini, 2000). PCBS enter the environment through many ways. They enter air as solid or liquid aerosol and remain there for longer time. In the process they get moved to other places. Through rain or snow they reach the water bodies and soil where they get adsorbed to soil particle and remain there for many years. Being sticky in nature they get adsorbed to particles in water and either move down to the sediments or remain suspended. Inshore marine areas closer to industrial zones are often the highly polluted areas as far as PCBs are concerned. In the process, PCBs enter water plants and other living forms in water. As the proportion of dissolved portion of PCB is relatively low, their residual level in the plants and animals are also low. Zooplanktons are often associated with low residual levels than benthic species with exceptions being the association of high concentrations of PCBs in planktons with high lipid content.

PCBs can bioaccumulate in the fatty tissue of fish, birds, and mammals. The bioconcentration depends not only on the concentration of these chernicals in the environment but also on the physiological and biochemical processes within the organism concerned and these processes are species dependent (Pastor et al., 1996). From different experiments it was found out that mussels and benthic fish are the best indicators of coastal pollution in water and sediments (Porte & Albaiges, 1993) Higher concentrations of PCBs in the range of 2.0 to 6.1 mg.kg⁻¹ were noticed in migratory shad (Pastel et al., 1980; Skea et al., 1979) compared to the non-migratory species from the same area (Kilikidis et al., 1981). In most cases, the PCB concentrations are related to the high fat seasons (Moore & Ramamoorthy, 1984). As far as concentration in the body is concerned, maximum concentration is associated with fat deposits followed by liver and gonads (Loizeau, 1992). The accumulation is by and large lowest in muscle and is related to the fat content.

As PCBS are present in the atmosphere one get exposed to PCB by breathing particularly around electrical installation and dumping yards. It can also enter the system through contaminated drinking water or eating fish and other seafood, dairy products. If the mother is exposed to PCB it enters the offspring through the breast milk. Exposures to PCBs over a long time may cause harmful effects to the skin (acne, rashes, and coloring of the nails and

skin) and eyes (redness, burning, irritation, and discharge) and irritation in the nose. PCBs in the diet of animals produced similar effects. Repeated skin contact to PCBs in rabbits caused liver, kidney and skin damage. Rats and other animals that breathed very high levels of PCBs over several months had liver and kidney damage. A single, large exposure of PCB to skin caused death in rabbits. Based on animal studies, Health and Human Services of USA has classed PCBs as potentially carcinogenic, even though their carcinogenic effect on humans is not fully confirmed.

PCB concentration generally causes growth retardation at 10-100 ppm levels while reduction in photosynthesis and carbon uptake are shown to occur at a still lower concentration of 0.1-1 ppb (Moore & Ramamoorthy, 1984). The toxicity varies with species of plant and invertebrates and also with the type of the arachlor. The fish are found to be sensitive to PCB. The normal reproduction was reported to be affected by exposure to PCB. The birds like waterfowl, effects similar to that produced by organochlorine pesticides, like reduction in eggshell thickness have been detected (Moore & Ramamoorthy, 1984).

The levels and residue profiles of concentration of poly chloro biphenyls (PCB), polychloronaphthalenes (PCN), and chlorobenzenes (CBZ) in fish, moss, algae, sediment and water from the polluted river Krupa, Bela krajina, Slovenia, have been reported (Jan et al., 1994). They also reported that longer duration of exposure, favoured bioconcentration of PCBs. Accumulation of PCB in the Zebra mussel was several folds higher than that of other organochlorines (Chevreuil et al., 1996). PCBs were reported in eight of the fish samples anlaysed from Isipingo estuary, Natal, South Africa but were found to be with in the WHO prescribed limits (Grobler et al., 1996). PCBs constitute the second highest pollutant after organochlorine pesticides in Cambodian fishes and were predominating in marine fish than in freshwater fish (In et al., 1999). Mediterranean red mullet were found to accumulate large concentrations of PCBs primarily due to their high lipid content (Pastor et al., 1996). As far studies with mammals are concerned the effects are almost similar to that produced by organochlorine pesticides. However, the severity is somewhat less compared to the latter. PCBs are also known for their mutagenic properties.

Pollutants like PCBs reach fat tissues through blood stream and get accumulated. As long as no metabolism of fat take place, the accumulation continues. When fat metabolism takes place as in the case of starvation, they

re-enter the blood stream and reach different tissues (Corsolini et al., 2000). Recurrent cycles of accumulation and mobilization cause the organism high risk of toxic contamination. When a xenobiotic enters the system the hydrosoluble ones gets degraded by the mixed function oxygenase system and is eliminated or converted into less toxic materials while the fat-soluble portions accumulate in the adipose tissues (Corsolini, 2000). Regulations Environmental Protection Agency (EPA) of USA, prescribed PCB levels of 0.001 ppb in lakes and streams to prevent cancer. In drinking water, the PCB level should not be more than 4 mg.l-1 for adults and 1 mg.l-1 for children to prevent non-cancer harmful effects. The Food and Drug Administration (FDA) requires milk, eggs, other dairy products, poultry fat, fish, shellfish, and infant foods to contain no more than 0.2-3 ppm to prevent non-caner harmful effects. The National Institute for Occupational Safety and Health (NIOSH) recommends workers not breathe air with more than 0.001 mg.m⁻³, for a 10 hour workday, 40 hour work week. The Occupational Safety and Health Administration (OSHA) requires workplace exposure limits of 0.5 mg.m⁻³ (54% chlorine) or 1 mg.m⁻³ (42% chlorine) for an 8 hour workday to protect workers from non-cancer harmful health effects.

References

Armstrong, D.E., Shafet, M.M. and Dean, K.E. (1993) PCBs in Lake Michigan: Linkages to the Cycles of Biogenic Particles and Organic Carbon, ASLO and SWS, Annual Meeting, USA (abstract)

Chvreuil, M., Granier, L. and Carro, A.M. (1995) Water Air Soil Poll. 81, 107

Chvreuil, M., Blanchard, M., Teil, M.J., Carru, A.M., Testard, P. and Chesterikoff, A. (1996) Water Air Soil Poll. 88, 371

Colborn, T. and Smolen, M.J. (1996) Review of Environ. Contamin. Taxicol., 146, 91

Connell, D.W. (1988) Review Environ. Contamin. Toxicol. 101, 117

Corsolini, S., Aurigra, S. and Focardi, S. (2000) Marine Pollution Bull. 40, 952

Everaatts, J.M., Weerlee, E.M.V., Fischerm, C.V. and Hillebrand, M.Th.J. (1998) Marine Pollution Bull. 36, 492

Everaats, J.M., Bano, M., Swennen, C. and Hillebrand, M.Th.J. (1991 J. Sci. Soc. Thailand 17, 31

Grobler, D.F., Badanhorst, J.E. and Kempster, P.L. (1996) Marine Pollution Bull. 32, 572

Hagan, V.W. and Klumpp, D.W. (1995) Marine Pollution Bull. 30, 166

Haynes, D. and Johnson, J.E. (2000) Marine Pollution Bull. 41, 267

Haynes, D., Muller, J. and Carter, S. (2000) Marine Pollution Bull. 41, 279

Kammann, U., Landgraff, O. and Stainhart, H. (1993) Marine Pollution Bull. 26, 629

- Kannan, K., Borrell, R., Auguilar, A., Focardi, S. and Tatsukaw, R. (1993) Arch. Environ. Contam. Toxicol. 25, 227
- Kannan, K., Tanabe, S., Tatsukawa, R. (1994) Science Total Environ. 153, 29
- Kannan, K., Tanabe, S., Tatsukawa, R. (1995) Environ. Sci. Tech. 25, 227
- Kennish, M.J. (1997) in Practical Handbook of Estuarine and Marine Pollution. CRC Press, Boca Raton, Fl.
- Lee, K., Tanabe, S., Koh, C. (2001) Marine Pollution Bull. 42, 273
- Loganathan, B.G. and Kannan, K. (1991) Marine Pollution Bull. 22, 582
- Loizean, V. (1992) J. Rech. Occeanogr. 17, 61
- Menzel, D.W., Anderson, J. and Randtke, A. (1970) Science 167, 1724
- Monirith, I., Nakata, H., Tanabe, S. and Tana, T.S. (1999) Marine Pollution Bull. 38, 604
- Moore, J.W. and Ramamoorhty, S. (1984) in Organic Chemicals in Natural Waters, Applied Monitoring and Impact Assessment, Spring-Verlaug, New York: 289 p.
- Murray, A.P., Richarson, B.J. and Gibbs, C.F. (1991) Marine Pollution Bull. 22, 595
- Pastor, D., Beix, J., Fernandez, V. and Albaiges, J. (1996) Mar. Poll. Bull. 32, 257
- Porte, C. and Albaiges, I. (1993) J. Arch. Environ. Contam. Toxicol. 26, 273
- Powers, C.D., Rowland, R.G., Michels, R.R., Fisher, N.S. and Wurster, C.F. (1975) Environ. Poll. 9, 253
- Radhakrishnan, A.G., Antony, P.D. Mukundan, M.K. and Jose Stephan, (1986) On the use of mussels as a pollution indicator, p. 13, National Seminar on Mussel Watch, Cochin
- Radhakrishnan, A.G. (1994) in *Nutrients and Bioactive Substances in Aquatic Organisms* (Devadasan, K., Ed.), Society of Fisheries Technologists (India), Cochin
- Radhakrishnan, A.G. and Antony, P.D. (1989) Fish. Technol. 26, 60
- Ramachandran, S., Rajendran, N., Nandakumar, R. and Venugopal, V.K. (1984) Aqu. Botany 19, 395
- Reddy, M., Echols, S., Finklea. B., Busbee, D., Reif, J. and Ridgway, S. (1991) Marine Pollution Bull, 36, 892
- Roach, A.C. and Runnie, J. (1990) Marine Pollution Bull. 36, 323
- Sugiura. K., Kitamura, M., Matsumoto, E. and Ooto, M. (1986) Arch. Environ. Contam. Toxicol. 15, 69