

Accumulation of Pesticide Residues by Shrimp, Fish and Brine Shrimp During Pond Culture at Ghorabari (District Thatta)

RAZIA SULTANA, WAJEEHA ALI*, F. AMEER, ALIA BANO MUNSHI AND M. NASIR
 Center for Environmental Studies and Food and Marine Resources Research Center PCSIR Laboratories
 Complex, Karachi, Shahrah-e-Dr Salimuzzaman Siddiqui, Karachi-75280, Pakistan
 wajeeha_ali@hotmail.com*

(Received on 30th November 2010, accepted in revised form 27th February 2012)

Summary: Residual level of persistent organochlorines (OC) such as Σ HCH (α -HCH, β -HCH, γ -HCH, δ -HCH, Σ DDT (o,p'-DDD, op-DDE, p,p-DDE pp-DDD, pp'-DDT, o,p'-DDT), dieldrin and endrin were measured in a number of water samples from Ambro creek and their accumulation in shrimp (*Penaeus merguensis* and *P. penicillatus*), fish (*Otolithes ruber*) and brine shrimp (*Artemia sp.*) reared in ponds for a period of four months. Samples were extracted with organic solvents, and quantified using gas chromatography-electron capture detection (GC/ECD). It has been found that results of animal tissue and water are not same however OCs, (mainly Σ DDT and Σ HCH 4,4-DDT, Dieldrin+2,4-DDT, and Methoxychlor were detected in all samples). Heptachlor exo-epoxide were found in fish and *Artemia sp.* and absent in all shrimp samples. Heptachlor endo-epoxide was detected only in *Artemia sp.* and average residual concentration of OCs in *Artemia sp.* was 0.004-0.09 ppm. Methoxychlor was found in the highest quantity in all the samples whether it was fish, shrimp or *Artemia*. In fish average residual concentration of all (OCs) in individual sample was 0.03 – 0.180 ppm.

Bio-accumulation of pesticides in shrimps and fish was found to be with in the permissible limit after four months rearing since none of the detected pesticides seem to exceed their ADI. It has been found that all the pesticides present in water were not found in similar concentration in animal tissue. The maximum quantity and number of pesticide residues was found in brine shrimp, *Artemia sp.*, the fish and shrimp 35-37 however a number of pesticides were found below the detectable limits of the instrument (BLD). The accumulation of pesticides in shrimps and fish was found to be with in the permissible limit according to codex convention 2003.

Key words: Pesticide residue, shrimp, fish, brine shrimp, *Artemia*

Introduction

Pesticides are used to control pests, including insects, water weeds, and plant diseases or pesticides are beneficial chemicals to provide protection against insects, unwanted plant growth. Pesticides are also instrumental in controlling many insect-borne human diseases such as malaria, encephalitis, and bubonic plague. On the other hand indiscriminate use of pesticides is one of the many factors contributing to the decline of fish and other aquatic species. Lethal or sub-lethal effects of those pesticides have been observed on the organisms accumulating them and these also transfer their harmful effects to human being via food chain, where they can be biomagnified by accumulation of pesticides at each successive level of the food chain [1]. Exposure of fish and other aquatic animals to a certain pesticide depends upon its bioavailability, bioconcentration, biomagnifications, and its persistence in the environment. Some pesticides rapidly breakdown after their application, while some others bind tightly to soil particles suspended in the water column or to stream bottoms, thereby reducing their availability. Some are diluted in water or rapidly volatilize into the air and are less available to aquatic

life [2]. In addition to direct harmful effects, pesticides are harmful indirectly and may cause the habitat degradation by reducing the availability of plants and insects that serve as habitat and food for fish and other aquatic animals.

The Indus river delta (South-east of Sindh province of Pakistan) has been considered as a potential shrimp farming area along the Sindh coast. During 1980's, land of this area was allotted to the different private and public sector organizations including prospective shrimp farmers for shrimp farming in deltaic area for example Ghorabari, that is located at the right bank of river Indus; the soil at Ghorabari has good water retention capacity and rich soil fertility. The sediment is compact, fine grained and ranged mostly between the fine sand and clay size fraction. The clay fraction is 70 %, silt 25 % and fine sand 5 % approximately, (APN Report, 2004) thus giving the sediment more or less the required characteristics for making ponds.

Methoxychlor was found in the highest quantity in all the samples whether it was fish,

*To whom all correspondence should be addressed.

shrimp or *Artemia*. It is most commonly used as an organo-chlorine insecticide against flies, mosquitoes, cockroaches, chiggers, and a wide variety of other insects and also It is used widely on agricultural crops and livestock, and in animal feed, barns, grain storage bins, home garden, and on pets. It does not dissolve easily in water and once in water, it binds to the sediments and settles to the bottom. Methoxychlor breaks down slowly in air, water and soil by sunlight and microscopic organisms, which may take time of several months. Methoxychlor does not usually build up in the food chain and most of the information available from human and animal studies suggests that it is not carcinogenic and the International Agency for Research on Cancer (IARC) Environmental Protection Agency the (EPA) have determined that methoxychlor is not classifiable as to its carcinogenicity to humans.

The area houses three aquaculture projects and the water intake points (N 24.349533 E 67.608433) for these projects are located in the Ambro creek. The present study has, therefore, been planned to determine the amount of pesticide residues in creek water and to monitor their accumulation in shrimp (*Penaeus merguensis* and *P. penicillatus*), fish (*Otolithes ruber*) and brine shrimp (*Artemia*) reared for four months in the ponds.

Results and Discussion

Physical Observations

Artemia sp. And fish sample were in very good appearance having bright red color and water was not turbid.

Pesticide Residues

Besides routine testing and analysis services, PCSIR is also engaged in to give awareness about hazardous chemical residues in all type of sea food [3]. All samples were analyzed at PCSIR Laboratories for pesticides and metabolites including Σ HCH, Σ DDT (p,p-DDD, p,p-DDE, p,p-DDT), dieldrin & endrin and hexachlorobenzene (HCB).

The concentration of pesticide residues was determined in source water (Table-1) as well as in the six samples of shrimp fish and *Artemia* (Table-1).

Table-1 shows the concentration of pesticide residue in Ambro Creek water collected from pumping site. Out of 31 pesticides analyzed eight were absent in the source water. The concentration of remaining 23

pesticides were found to be in the range of 0.002 (2, 2, 4-Trichlorobiphenyl) to 0.264 ppm (alpha-HCH).

The pattern of accumulation of pesticide residues by shrimp, fish and *Artemia* has been shown in Table 2 and it reveals the presence of few pesticides in low concentration ranged between 0.017 ppb to 137.44 ppb in all the investigated samples (Table 2). LOD is the limit of detection up to lowest range which does not deal to higher value. Σ DDT group pesticides were found to be the frequently in all samples. The pesticide residues like 4, 4-DDE and 2, 4-DDT (metabolites of DDT) and Dieldrin were found in the wild caught shrimp samples also.

Six pesticide namely, alpha-HCH, 4, 4-DDE, Dieldrin+2, 4-DDT, 4, 4-DDT, beta endosulfan and Methoxychlor were found in the shrimp samples. Alpha-HCH and beta endosulfan were determined in samples after four month rearing. However, accumulation of 4-4 DDE was found after one month of rearing and 4-4 DDT after two months rearing. The highest concentration of both 4-4 DDE and 4-4 DDT (0.305 ppb and 0.083 ppb respectively) was found in the second month, which again decreased to 0.296 ppb and 0.017 ppb respectively after four months rearing (Table 2). Dieldrin+2, 4-DDT was accumulated in shrimp from the very first month (1.364 ppb) and there after reached to optimum level in second month (2.737 ppb). However, both were not found to be in detectable limit in the sample after four month rearing. These observations may be suggestive of the environmental biodegradation of the pesticides (4-4 DDE, 4-4 DDT, Dieldrin+2, 4-DDT) which may initiated after two to three months. The accumulation of Methoxychlor was found to be increasing progressively during the four months rearing period though the rate of accumulation was fast during the last two months. It may be observed from Table 2 that amount of Methoxychlor after one month rearing was 11.208 ppb, which increased to 16.166 ppb after two months and it further increased to 137.444 ppb after four months time.

Six of pesticides were found in the analyzed fish sample; 4, 4-DDE, Dieldrin+2, 4-DDT, 4, 4-DDT and Methoxychlor were found to be accumulated in fish samples (Fig. 1). The quantity of Methoxychlor was found to be far less in fish samples (5.756 ppb) than that accumulated by shrimp (137.444 ppb), after four month rearing. It may be seen in Table 2 that Heptachlor exo-epoxide was found only in fish samples, whereas, alpha-HCH was found only in shrimp samples.

Table-1: Recovery of pesticides from RKB samples (n= 10)

	Organochlorine	Recovery(%)	R.S.D(%)*	LODmg/kg	LORmg/kg
1.	alpha-HCH	95.4	4.6	0.0310	0.0940
2.	HCB	97.8	5.1	0.0240	0.0729
3.	beta-HCH	92.4	5.0	0.0304	0.0920
4.	gamma-HCH	98.5	4.1	0.0292	0.0886
5.	delta-HCH	97.9	5.2	0.0024	0.0072
6.	epsilon-HCH	95.4	4.5	0.0540	0.1637
7.	2,4,4-Trichlorobiphenyl	97.8	5.2	0.0607	0.1840
8.	Heptachlor	92.4	6.0	0.0152	0.0461
9.	2,2,5,5-Tetrachlorobiphenyl	98.5	4.3	0.1968	0.5963
10.	Aldrin	97.9	5.4	0.1584	0.4800
11.	Isodrin	95.4	4.7	0.0072	0.0219
12.	Heptachlor-exo-epoxide (cis-isomer B) + Oxy-chlordane	97.8	5.2	0.0236	0.0716
13.	Heptachlor-eNDo-epoxide (trans- isomer A)	92.4	6.0	0.0266	0.0806
14.	Trans-chlorodane(gamma)	98.5	4.3	0.0154	0.04
15.	cis-Chlordane(alpha)	95.4	4.7	0.0310	0.0940
16.	Oxy-chlordane	97.8	5.2	0.003	0.0092
17.	trans-Chlordane(gamma)	92.4	6.0	0.002	0.0061
18.	2,4-DDE	98.5	4.3	0.003	0.0092
19.	2,2,4,5,5-Pentachlorobiphenyl	97.9	5.4	0.002	0.0061
20.	cis-Chlordane(alpha) + alpha-Endosulfan	95.4	4.7	0.003	0.0092
21.	4,4-DDE	97.8	5.2	0.002	0.0061
22.	Dieldrin	92.4	6.0	0.003	0.0092
23.	2,4-DDD	95.4	4.7	0.002	0.0061
24.	2,2,4,4,5,5-Hexachlorobiphenyl	97.8	5.2	0.007	0.0219
25.	4,4-DDT + 2,2,3,4,4,5-Hexachlorobiphenyl	92.4	6.0	0.003	0.0092
26.	Methoxychlor	98.5	4.3	0.007	0.0219
27.	2,2,3,4,4,5,5-Heptachlorobiphenyl	97.9	5.4	0.003	0.0092
28.	Methoxychlor	95.4	4.7	0.007	0.0219
29.	chlorpyrifos	97.8	5.2	0.003	0.0092
30.	Mirex	92.4	6.0	0.003	0.0092
31.	Heptachlor-endo-epoxide	98.5	4.3	0.002	0.0061
32.	Heptachlor-exo-epoxide (cis-isomer B) + Oxy-chlordane	97.9	5.4	0.003	0.0092
33.	Endrin+beta-Endosulfan	95.4	4.7	0.002	0.0061
34.	Dichlorvos	97.8	5.2	0.003	0.0092
35.	2,2,3,4,4,5-Hexachlorobiphenyl+4,4-DDT	92.4	6.0	0.002	0.0061
36.	2,2,4,4,5,5-Hexachlorobiphenyl	98.5	4.3	0.007	0.0219

* = not calculated

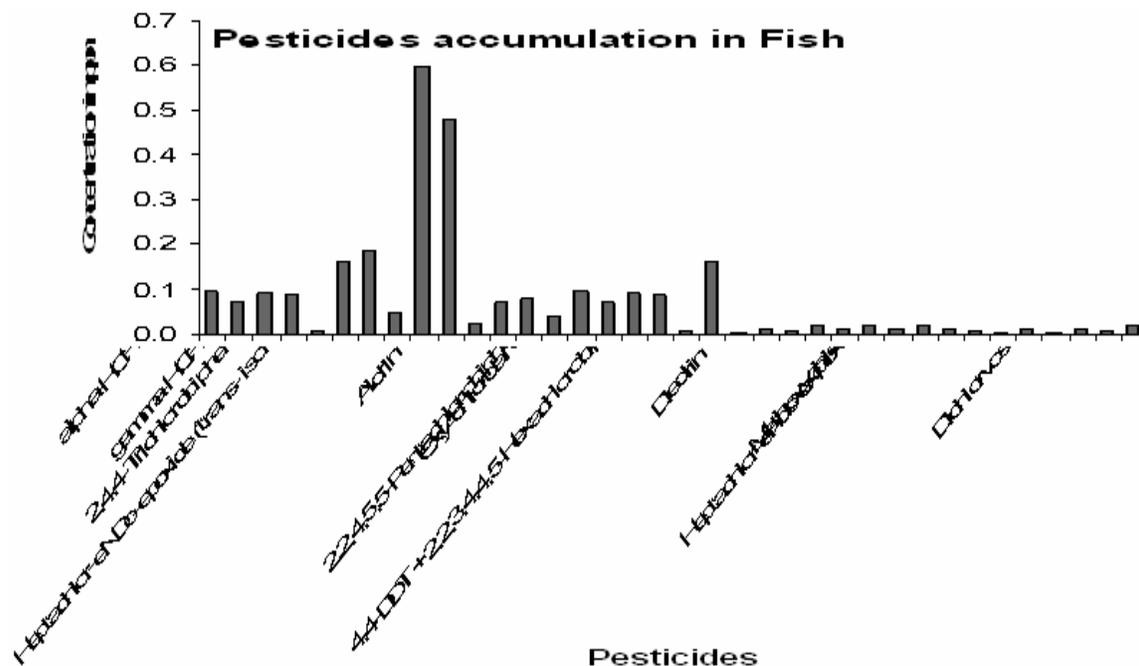


Fig. 1: Accumulation of pesticides in Fish samples.

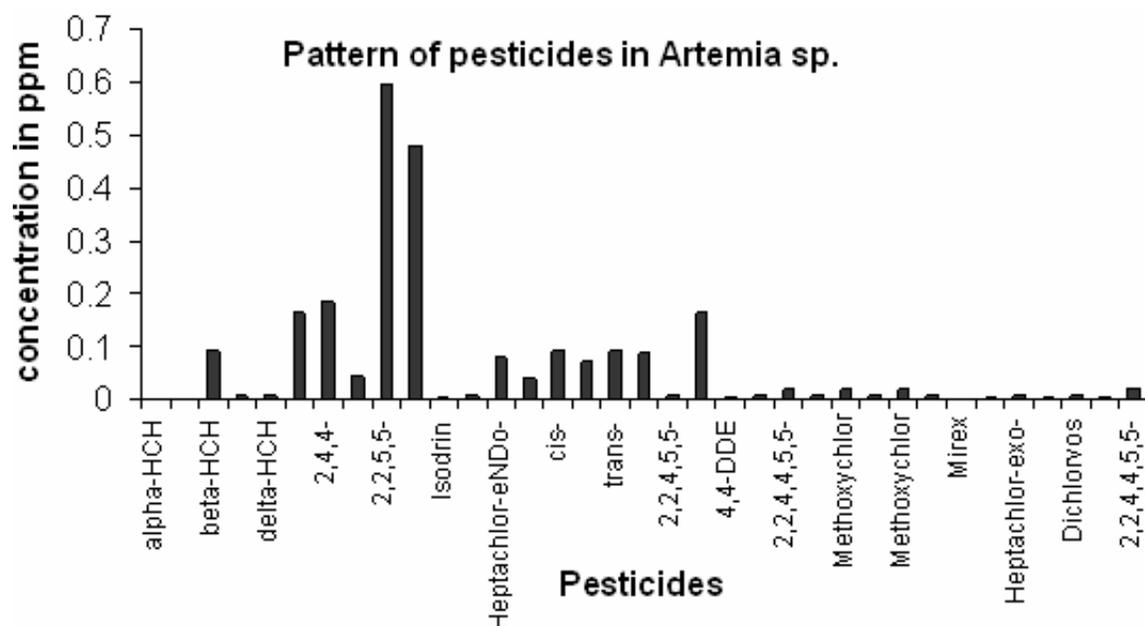


Fig. 2: Pattern of organochlorines in *Artemia sp.*

Maximum number and quantity of pesticides was found in the *Artemia* samples (Table 2). A sum of eight pesticide residues was detected in *Artemia* samples. The *Artemia* have accumulated the highest concentration of alpha HCH (0.703 ppb), and Dieldrin & 2, 4-DDT (9.815 ppb) (Fig. 2). Aldrin was found only in *Artemia*, but not in fish and shrimp. However like shrimps, Methoxychlor was found in the highest concentration (35.323 ppb) also in *Artemia*. The ability of *Artemia* to ingest and accumulate a wide variety of foods in the body has given them the reputation of biocapsules. Bioencapsulation methods of *Artemia* have been developed for oral delivery of vitamins, chemotherapeutics and vaccines to the predator organisms, which feed on *Artemia* [4, 5] The accumulation of pesticides by *Artemia* has no correlation with its nutritional value [6] and have reported that larval survival was not affected in crab *Leptodius* even when they were fed *Artemia* with Dieldrin level of 150 ng, g⁻¹, marine shrimp contains noticeably less pesticide residues than the fish reared in Tambaks in Indonesia [7,8] Heptachlor exo-epoxide was not found in any of the samples of shrimps, whereas, it was found in the fish samples. Fish absorbs pesticides partly with water and mainly with food, and these pesticides are metabolized or accumulated in animal tissue [9-11] The concentration of 4, 4-DDT and 4, 4-DDE was higher in fish than that of shrimps, whereas, Methoxychlor was found in the highest concentration in the shrimp samples and its concentration was also found to be increasing gradually with their rearing period.

From 1950's to 1970, aldrin and dieldrin were the most widely used pesticides for crops like corn and cotton. However due to the concerns about their after effects and damage to the environment and potentially to human health, EPA have banned all uses of aldrin and dieldrin in 1974, except to control termites. In 1987, EPA has strictly banned all their uses. Exposure to aldrin and dieldrin happens mostly from eating contaminated foods, such as root crops, fish, or seafood as these build up in the body after years of exposure and may affect the nervous system. Sunlight and bacteria change aldrin to dieldrin it is because that we mostly find dieldrin in the environment as it they bind tightly to the soil and breaks down very slowly and evaporate in the air. People who intentionally or accidentally ingest large amounts of aldrin or dieldrin suffer convulsions which may also cause death. Health effects may also occur even with their smaller amount but after a long period of exposure as these chemicals build up in the body. The Food and Drug Administration of USA (FDA) [12] regulates the residues of aldrin and dieldrin in raw foods and their permissible range is between 0 to 0.1 ppm, depending on the type of food product.

Substantial Information is available in literature for various aquatic organisms on accumulation of pesticide residues in the water [8]. The lethal dosage of pesticides to human being is normally related to its lethal dosage by oral intake for rats or mice and the data as regards these dosages are supplied by the pesticide producing company or

health organizations. These values may be used as a measure for the evaluation of the hazards to humans by consumption of fish which contains a certain concentration level of pesticide residue.

The main group of pesticides found in fish samples collected from culture ponds, cages and sea are the pesticides related to DDT that is DDT and its metabolites TDE, DDE, and DDMU. The levels of their concentration were rarely above 1 ppm /wet weight, however some exceptions were found with concentrations up to about 1.5 ppm for total DDT (DDT plus its metabolites). The hazards for the consumption of sea fish/ shrimp by human beings with such levels of DDT are negligible, since the oral toxicity of DDT has been found to basis at the level of 200 mg/kg body weight. The limit per kg body weight, calculated on this would require a consumption of 100 kg fish containing 2 ppm of total DDT [13]. For a man of 50 kg body weight it means that the limit of consumption (in a short period) is 5000 kg of fish. Hence forth even with a safety factor of 10, there is no danger in consuming fish [8]. During the present study pesticides mainly belonging to the DDT group have been found their concentration was under the permissible limit. This observation has been substantiated further by the absence of changes in behavior, weight loss, impaired reproduction, inability to avoid predators, and lowered tolerance to extreme temperatures, which are reported in the cases of the intake of sub lethal doses of such pesticides [2]. The hydrocarbons and chlorinated hydrocarbons though found in considerable quantity in water (unpublished manuscript), their accumulation in shrimp were not up to the toxic level. It may be due to the fact that the pumping of water has been done at high tide and fresh water containing the pesticide residues is drained at low tide, therefore the water entering into the pond may have somewhat lower level of pesticides.

Experimental

Description of the Study Area

The area receives marine water from Ambro Creek and fresh water from drain canal (Fig. 3) The drain canal collects waste water from upland agriculture lands and hence contaminated with pesticide residues. The fresh water is drained through regulatory gates of drain canal during low tide. At high tides the regulatory gates are kept closed to prevent entry of the sea water to upland agricultural areas and their the Ambro creek is replenished and flushed by seawater twice daily during high and low tides. The tidal amplitude is over three meters and the

salinity of seawater varies at different locations with high and low tides.

1) Sampling

Shrimps were checked for molts and mortalities; molts and dead individuals were removed immediately to avoid spoilage (Figure 4B). *Artemia* were reared in a separate one acre pond and everytime 10-20 shrimp samples were collected from the cages with monthly interval till four months (Figure 4A) for biometry measurement, similarly wild caught shrimp control. Fish and *Artemia* samples (Figure 5 B, C) were analyzed only once at the end of experiment. Samples were kept in deep freezer and transported in ice box from Ghorabari to PCSIR Labs Complex, Karachi. Samples were kept at - 80 °C. All samples for four months were analyzed simultaneously to avoid any discrepancy in results and the fresh sample collected from Creek was taken as control.

2) Determination of Pesticide

The analytical method was validated and based on [14].

Chemicals

High purity pesticide grade solvents (hexane, dichloromethane) and certified ACS reagents were used, but reagent grade acetone and hexane were distilled and evaluated by GC. Florisil was activated at 675°C for 4h, and deactivated with few drops of water with gentle shaking before use.

Pesticide Standards

A mixture of 37 pesticide standards was purchased from Dr. Ehresnstorfer's laboratory, Germany and certified reference material (IAEA - 406) from International Atomic Energy Agency. Stock solutions of pesticides (Table 1) were prepared using pesticide grade solvents. Spiking solutions for measuring method efficacy (percent recovery) were prepared from stock solutions. Calibration standards solutions at least three concentrations were also prepared from stock solutions in hexane. All stock, spiking and calibration standards were stored at 4°C.

Water samples were screened for a total of 37 pesticides. Calibration curves of working standards were used to evaluate the linearity of the gas chromatograph response each day of analysis and pesticide residues were quantified based on these external standards.



Fig. 3: The map of study area, indicating the drain canal, regulatory gates, pumping site and *Artemia* ponds.

To study the accumulation of pesticide residues, about 400 juvenile shrimps (*Penaeus merguensis* and *P. monodon*) and 40-50 fishes (*Otolithes ruber*) collected from the Ambro creek were reared in pond (1-acre). Shrimps were kept in a cage (Fig. 4A), whereas, fishes were kept free in the pond. Fish/shrimp were fed on the natural food organisms (like mysids, copepods, larvae of other crustaceans and small fishes etc) collected from creek and transferred daily to the pond and cage for shrimps.

Sample Preparation

For determination of pesticide residues in source water, samples were collected from Ambro creek at pumping station as shown in Fig. 5. The shrimp reared in cages were collected at monthly intervals for four months to study the progressive pattern of accumulation of pesticides residues; whereas fish and *Artemia* samples were analyzed only after four months of rearing. Samples (fish shrimps and water) were collected from rearing ponds for pesticide residues analysis. A sub-sample about 5g was homogenized tissue of fish and *Artemia* sp. used for analysis.

The description of the six samples is as under.

1. Sample 1: Control, the wild caught shrimps
2. Sample 2: Shrimps after one month rearing
3. Sample 3: Shrimps after two month rearing
4. Sample 4: Shrimps after 4 month rearing
5. Sample 5: Fish reared in ponds for four months
6. Sample 6: *Artemia* reared in ponds for four months



Fig. 4: A. Shrimps reared in cage placed in the same pond to avoid predation by carnivorous fishes; the fishes were left free in the same pond; (B) Shrimps were checked daily for molts, general health condition and mortalities

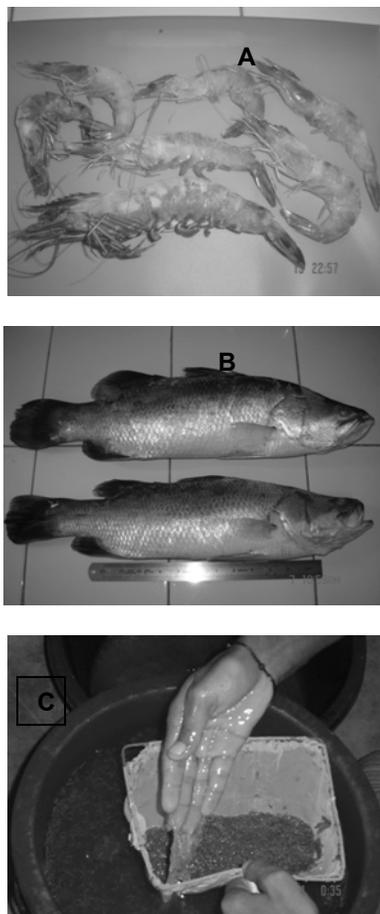


Fig. 5: Samples of (A) shrimp, (B) fish and (C) Artemia, analyzed for pesticide accumulation after four months rearing.

The shrimp reared in cages were collected at monthly intervals for four months to study the progressive pattern of accumulation of pesticides residues; whereas fish and *Artemia sp.* Samples were analyzed collectively only after four months of rearing.

Chemical Analysis

Determination of Pesticide

Pesticides were determined in the samples by lab validated method based on Association of official Analytical chemists [14] method 18th Ed. 2005 using a Gas Chromatograph coupled with an ECD detector. Samples storage, preparation for extraction, concentration and chromatographic separation were conducted in the Center for Environmental Studies at PCSIR Labs Complex, Karachi.

Sample Preparation

Extraction

Fish samples were thawed cut into small pieces with a knife and finely chopped samples were weighed (0.5g). Finely chopped fish tissue (0.5 g) was weighed in a small plastic weigh boat. The weighed tissue was transferred to a glass mortar and pestle along with 2g Bondesil® C-18 sorbent (Varian Inc. CA).Using the pestle, the tissue and C-18 sorbent were thoroughly ground in the mortar to a homogenous consistency with the appearance of small grains, A 20 ml bond Elute® Florisil solid phase extraction cartridge (Varian Inc. CA) was affixed to a vacuum manifold (Supelco, PA) and the sample/C-18 mixture was transferred to the cartridge using a metal spatula . A polyethylene frit (Varian Inc. CA) was firmly tamped in to the place above the sample /C-18 mixture using a disposable syringe plunger. The sample was eluted from the cartridge under vacuum with successive washes (3x 5 ml) of dichloromethane. The elute was collected in a glass, 25ml test tube, evaporated to about 1 ml using a nitrogen evaporator, and quantitatively transferred to a centrifuge tube using about 10 ml Hexane than evaporated a second time diluted to final volume, and transferred to an auto sampler vial to gas chromatographic analysis.

Chromatographic System:

Determination of chlorinated pesticides is performed on Perkin Elmer Gas Chromatograph Clarus 500 equipped with Electron Capture Detector (ECD) using N₂ as carrier gas. Hydrogen at 2 ml min⁻¹ and nitrogen gas at 30 ml min⁻¹ were used as the carrier and makeup gases, respectively. Analysis is performed on cross bond 5% diphenyl , 95% dimethyl polysiloxane capillary column having 30 meter length , 0.35 mm ID and 0.50 μm df.

Initial oven temperature was 100°C which is increased with the rate of 4 °C/ min upto 240°C. Holding time at 100°C was 5 min and at 240°C was 10 min respectively. Injector temperature was 200 °C and detector temperature was 270 °C and attention -3.

Performance and Quality Control

All the glassware was washed. No rubber or plastic items, other than Polytetrafluoro ethylene (PTFE) were “Blanks” periodically analyzed to ascertain absence of contamination. Internal spiking and reagent blanks were used to determine recovery values. Quality assurance and Quality control

measures included the use of reagent blanks, surrogate and matrix spike recovery. Calibration and calibration verification were routinely checked at the beginning and end of each batch of 6 samples. All laboratories batches of samples contained one procedural blank, one laboratory control sample, a duplicate sample. The procedural blank was spiked with the solvent and a surrogate internal standard pesticides mixture 5 and 11 from Dr. Ehrnestoffer laboratory, Germany. All samples were spiked with the surrogate compound to determine efficiency.

3) Method Performance

Applied method performance based on analysis of control (fish and shrimp tissue) and spiked samples, were extracted with each analytical set (about every six samples) using the same method.

4) Recovery Studies

A recovery study was carried out with each pesticide. A control (with known level of pesticides as checked for residue- no pesticide residues was detected) fish and *Artemia sp.*) was spiked with appropriate standard solution to a final concentration of 0.05 -0.50ppm, vigorously vortexes to distribute the spiked pesticide and extracted as detailed above. Data of average recovery and R.S.D. (n = 10) for organochlorine in the samples. The recovery was determined by using a mixture of pesticide, values were in the range 95–120% and 90–114% for OC and PCBs pesticides, respectively. Precision was estimated from the multiple analyses of spiked samples and was in the range 0.1 -1.00ng /g for the different compounds. The reporting limits of the analyzed organochlorine compounds were 1.00 ng/g for OC pesticides.

Quality Assurance and Quality Control

Following the published procedures for quality assurance and quality control ICH Q2B (1996) [15, 16] the limit of detection (LOD) was established at a signal to noise ratio equal to three; whereas the limit of quantification (LOQ) was ten times that of LOD.

Results are calculated on the basis of the mean value of specimens. % recovery, %RSD, LOD and LOR calculated during the analysis and values are given in Table 1.

Statistical Analysis

All calculations were carried out by using Excel for Windows and level of significant was measured based on average values.

Conclusion

The accumulation rate of different pesticides varied with their type and also found to have varied with type of organism (shrimp, fish and brine shrimp). The concentration of pesticide residues found accumulated in *Artemia* was more than in the shrimp. The accumulation of pesticides by shrimps was found to be within the permissible limit after four months of rearing and should not pose any risk to human beings on their consumption, since none of the detected pesticides seem to exceed their ADI.

In shrimp culture, grow-out cycle should be completed within 4-5 months time. Since the experiment was run for a period of four months. The present study has generated reliable data as regards accumulation of pesticides in shrimps, fish and brine shrimp at experimental ponds at Ghorabari thatta, Pakistan. The data generated is quite important and useful for investors and researchers for fish, shrimp and *Artemia* farming activities since the shrimp culture project of the Sindh Fisheries Department and two private farms are also located besides Ambro Creek, from where sea water is being pumped.

References

1. J. Waynon and M. T. Finley, U. S. Fish and Wildlife Service Publication 137. Washington, D.C. (1980).
2. L. A. Helfrich, A Guide to Reducing Impacts on Aquatic Systems. Virginia Cooperative Extension, Publication 420, 20 (1996).
3. A. Bano, 1991, Weekly Magazine issue on Saturday Nov. 05 (1999).
4. M. Touraki, S. Mourelatos, G. Karamanlidou, S. Kalaitzopoulou and C. Kastritsis, *Aquacultural Engineering*, **15**, 133 (1996).
5. F. G. Rolando, *Aquaculture*, **216**, 1 (2003).
6. P. Leger and P. Sorgeloos, *International study on Artemia*. **XXV**. (1985)
7. S. McLean, M. Sc. Thesis, University of Rhode Island, USA., 72 (1980).
8. E. K. Duursma and FAO Report (FI:INS/72/003/4) (1976).
9. C. E. Epifanio, *Marine Biology*, **13**, 292 (1972).
10. Hites, R. Foran, A. Jeffery, O. David Carpenter, M. Coreen Hamilton, Barbara A. Knuth, Steven J. Schwager, "Global Assessment of Organic Contaminants in Farmed Salmon." *Science*. **303**, 226 (2004).

11. D. M. Jones, W. J. Berry and McLean, International study on Artemia. VII. *J. World Mariculture Society*, **12**, 303 (1981).
12. FDA. *FDA Veterinarian*. **16**, 5 (2001).
13. B. Kiziewicz and B. Czeczuga, *Polish Journal of Environmental Studies* **1**, 123 (2003).
14. AOAC AOAC International: Gaithersburg, MD, Section 10.1.01 (2005).
15. Eurachem. A Laboratory guide to method validation and related topics, LGC, Queens Rd, Teddington, Middlesex, TW11 0LY, United Kingdom (1998).
16. M. Zahoor, *Journal of the Chemical Society of Pakistan*, **33**, 305 (2011).