# Comparative Evaluation of Some Selected Fish Toxicants on Certain Aquatic Environmental Health Parameters with Reference to Nutrient Cycling Microbes




*A Thesis*
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*West Bengal University of Animal and Fishery Sciences,*
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By

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*Dedicated to*
*my priced possession*
*my parents*

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# CERTIFICATE

This is to certify that the work embodied in the thesis entitled *'Comparative Evaluation of Some Selected Fish Toxicants on Certain Aquatic Environmental Health Parameters with Reference to Nutrient Cycling Microbes'* submitted by **Ms. Chandrani Sarkhel** in partial fulfilment of requirement for the Degree of **Master of Fishery Science (Aquaculture)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

**Chairman**
**Advisory Committee**

# APPROVAL SHEET

## APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE (AQUACULTURE)

We, the undersigned, having satisfied with the performance of **Ms. Chandrani Sarkhel** in the viva-voce examination, conducted today, the 30th Oct, 2002, recommend that the thesis be accepted for award of the degree.

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# Contents

# List of Tables

# List of Figures

# 1. Introduction

Success of fish culture practices depends largely upon the scientific management of nursery and rearing ponds by providing the youngs an enemy free congenial environment. Therefore, nursery pond management is the prime need for scientific fish culture practices (Jhingran and Pullin, 1985,1988) and the principal step of nursery pond management is the control of predatory and weed fishes (Jhingran, 1991). Though, there is a number of fish enemies in the nursery pond namely frogs, snakes, and some birds who prey upon the young fishes but the worst are the carnivorous fishes (Chakroff, 1993). Besides predatory fishes, some varieties of uneconomic small fishes that naturally occur or accidentally introduced into the nursery ponds along with riverine carp spawns act as weed fishes (Rath, 1993). In fresh water ponds both these predatory and weed fishes not only compete with the carps for food and space but also directly prey upon them (Chatterjee and Ganguli, 1993). Normally, some of the trash fishes breed in ponds little earlier than the major carps or they breed immediately at the onset of monsoon and their young ones feed vigorously on the plankton available in pond and grow so fast that when the carp spawns are released into nursery, the predators are large enough to take a heavy toll of the desired spawn (Jhingran, 1991).

The removal of predatory and weed fishes has thus become one of the most important steps for successful and scientific management of nursery pond (Chatterjee and Ganguli, 1993) and the most dependable and commonly used method is the poisoning of fish pond either by plant poisons or by chemicals.

Most of the plant poisons are bio-degradable and are converted to manures after their application in nursery pond (Mahapatra and Thosar, 1999), but most of the chemicals exert residual toxicity (Chatterjee and Ganguli, 1993). Plant

poisons are cost effective and locally available, so it is within the reach of small and marginal farmers particularly in the developing countries like ours. Therefore, plant poisons are mostly recommended as piscicide because of their double role both as toxicogenic as well as manurial (Banerjee *et al.*, 1987). On the other hand, chemical piscicides are expensive and often proved to have adverse effects on the nutrient level and microbial composition of soil. Moreover, these piscicides are often in short supply. Therefore, although chemical piscicides exert perceptible results within a short period, they are not generally recommended. Some chemicals such as organochlorine pesticides are even banned in our country (Chakroff, 1993) as they are proved to have a role in the recent epizootics of ulcerative fish disease in South East Asia (Perschbacher, 1989). Therefore, the proper dosage of any piscicide should be evaluated on a scientific basis, because the dead or narcotised fishes in many cases are eaten by human beings. So their suitability for consumption also needs to be properly evaluated (Mahapatra and Thosar, 1999).

Piscicide breakdown in soils is largely mediated by microorganisms. The rate and pathway of piscicide bio-degradation in soils are heavily influenced by the soil environment. Most of the piscicides are toxic to micro-organisms but as the chemicals degradate, microbial population recover (Schaffer *et al,* 1993). Generally a series of complicated process between the application of piscicide and ultimate breakdown product that persist in fish pond culture system is involved via production of food organisms, material cycling and energy flow. The microbial degradation of piscicides depend on several operating variables. Heterotrophic bacteria plays the main role in the decomposition process (Krstulovic, 1989). Literature on the consequence of any pisicicide on the nutrient cycling microbes in pond culture systems is extremely meagre.

The present problem has been envisaged to study the comparative responses of some piscicides of natural and chemical origin upon the bio-geochemical cycling bacteria under simulated pond culture conditions. The possible outcome of

the proposed study will have a direct impact upon the fish farmers of this agro-climatic situation to select the proper piscicide with proper dose. Therefore, the objectives of this study is to determine :

i) The relative impacts of different piscicides in the population composition of mineralizing bacteria like aerobic heterotrophic, ammonifying, denitrifying, nitrogen fixing, ammonia oxidising, cellulose decomposing and phosphate solubilizing bacteria and their temporal variability.

ii) The temporal changes in nutrient status of the system due to mineralization under different piscicide treatment.

iii) The status of aquatic environmental health condition following application of piscicide in respect to certain physico-chemical and biological parameters of water and soil.

Efficient pond fish culture entails small and seasonal ponds as preferable as they facilitate effective control of environmental conditions, and also automatic destruction of predatory and weed fishes by complete dewatering of the pond. Whereas, complete draining of deep perennial water bodies are economicaly intensive, therefore, some particular steps are adopted to remove or control predatory fishes (Jhingran, 1991). The unwanted or weed fish are small uneconomic varieties of fishes that occur either naturally or accidentally introduced primarily along with the carp spawn into the fish ponds (Rath, 1993). The role of predatory and weed fishes in nursery ponds have been discussed earlier by Alikunhi *et al.* (1955), Alikunhi (1957), Ibrahim (1957), as well as Choudhuri (1960). Unwanted and predatory fishes are very harmful for nursery, rearing or stocking ponds (Chakroff, 1993), because they not only compete for food and dissolved oxygen but also compete for space with the culturable variety of fishes (Rath, 1993). Choudhuri (1960) studied the food, feeding habits, fecundity and breeding of common species of weed fishes with special reference to their role in the survival of carp spawn in nurseries. Weed fishes have high fecundity and they ripen sexually very fast (Jhingran, 1991). Tripathy and Sharaf (1974) listed *Puntius sophore* followed by *Chela laubuca, Amblypharyngodon mola* and *Rasbora daniconius* as highly destructive and observed that they took a heavier toll of spawn in 24 hrs. than the froglets, tadpoles or the aquatic insects.

### 2.1 Eradication of predatory fishes :

In freshwater ponds predatory fishes not only compete with the carps for food and space but also directly prey on them, therefore, removal of these fishes has become one of the most important task for successful and scientific management of pisciculture (Chatterjee and Ganguli, 1993). In earlier practices, eradication of unwanted fishes were done mainly through hook and line with baits. The customary

methods of removing unwanted fishes from nursery ponds is by repeated drag netting (Jhingran, 1991). By using filtering devices at the inlet, the entry of weed and predatory wild fishes can be reduced to a large extent (Lee, 1973 ; Pillay, 1995). Methods like dewatering and desilting of ponds, repeated netting operation, hooks and lines with baits are found to be incomplete and uneconomical (Rath, 1993). Therefore, limited poisoning of the pond with selective toxicants become popular for the purpose.

### 2.2 Piscicide or fish toxicants :

A suitable piscicide is one which (i) effectively kills the target organisms at fairly low doses and is not injurious to people and cattle who may use the water, (ii) does not render the affected fish unsuitable for human consumption, (iii) gets quickly nullified in water, (iv) leaves no cumulative adverse effect in pond, (v) easily available and at the same time economical (Jhingran, 1991). There are several chemicals and other herbal poisons which are used extensively for eradication of predatory fishes (Chatterjee and Ganguli, 1993). The most common poison used in fish pond is rotenone inherant in mahua oil cake (*Bassica latifolia)*. Others used in fish ponds are quick lime, tea seed cake, tobacco waste and powdered croton seed (Markling, 1992 ,Chakroff, 1993). Antimycin is also very toxic to carp and selectively kills some undesirable species but is ineffective at high pH (Markling, 1992).

Piscicides or fish toxicants can be grouped as per their source or origin. Accordingly, they can be grouped as (1) Plant derivatives and (2) chemical compounds like (a) organophosphate compounds and (b) chlorinated hydrocarbons (Jhingran, 1991 ; Rath, 1993 ; Mahapatra and Thosar, 1999).

#### 2.2.1 Fish poisons of plant origin :

There are quite good number of plant derivatives which could be used for fish poisoning. Generally, to avoid the hazardous effects of chemicals, some less toxic native plant derivatives are now used in India as fish poisons (Chatterjee and Ganguli, 1993). The plant derivatives are advantageous because they are biodegradable and are converted to manures after their application (Mahapatra and

Thosar, 1999). Various parts of the plant as stem, bark, leaves, roots of some of the commonly used plants as fish toxicants are :

**Table. 1 : Various plant sources of pisicides used for controlling predatory and weed fishes**

| | Plant sources of piscicide | Component used | Reference |
|---|---|---|---|
| 1. | *Bassica latifolia* (Mahua) | Oil cake | Tripathy *et al.* (1980), Lakshman (1983) |
| 2. | *Derris trifoliata* | Root powder | Jhigran (1991), Rath (1993) |
| 3. | *Crotton tiglium* | Powdered seed | Bhuyan (1967) |
| 4. | *Walsura piscida* | Bark powder | Nandi and Chakraborty (1970) |
| 5. | *Barringtonia acutangula* | Seed powder | Chakraborty *et al.* (1972) |
| 6. | *Camellia sasargua* | Seed cake | Pillay (1995) |
| | | Seed powder | Banerjee (1991) |
| 7. | *Milletia piscida* | Powdered root | Bhuyan (1967) |
| 8. | *Tamrindus indica* | Seed husk | Jena (1979) |
| 9. | *Randia dumetorum* | Unripe fruit | Nandi and Chakraborty (1970) |

### 2.2.2 Chemical piscicides :

#### a) Organophosphates

Toxicity of organophosphorus insecticides to fish and aquatic insects has been examined by Srivastava, 1966; Srivastava and Konar, 1965; Konar, 1969. In India, three organophosphate compounds like thiometon (Konar, 1969), DDVP (Srivastava and Konar, 1965 ; Konar, 1969) and phosphamidon (Srivastava and Konar, 1965 ; Konar, 1969) have been found successful for killing fish on experimental basis. Though, Shrestha *et al.* (1987) used organophosphate malathion for preparing nursery pond but the most effective and commonly used organophosphates are DDVP, thiometon and phosphamidon (Rath, 1993). Organophosphate has more detrimental effect than other piscicides (Thosar and Das, 1984), but they are comparatively less toxic than chlorinated hydrocarbons to fish (Mahapatra and Thosar, 1999).

DDVP (0 : 0 dimethyl = 2 : 2 - dichlorovenyl phosphate ) is commercially available in India as a spray or as 'Nuvan 100 EC, manufactured by Ciba Ltd. (Jhingran, 1991). Mortality of disease transmitting snails were established under laboratory conditions by using organophosphate piscicides (Panigrahi, 1998). Acute toxicity of DDVP on fish was studied by Pal (1983) and, Perschbacher and Sarkar (1989). It has been observed that DDVP with a mixture of non-ionic detergent is more effective than individual toxicant (Hossain *et al., 1987).*

**b) Chlorinated hydrocarbons**

Chlorinated hydrocarbons like aldrin ($C_{12}H_8Cl_6$), dieldrin ($C_{12}H_8Cl_6O$), endrin ($C_{12}H_8Cl_6O$) and Taffdrin - 20 are used for the eradication of predatory and weed fishes (Jhingran, 1991; Rath, 1993). Chlorinated hydrocarbons could be used economically in cleaning ponds of miscellaneous fishes and other unwanted organisms (Cottam *et al.*, 1946). Chlorinated hydrocarbons are very toxic to fishes and persist in the medium as stable compounds; also they might be biomagnified and bioaccumulated in the food chain (Mahapatra and Thosar, 1999). Chakroff (1993) suggested not to use chemicals like endirn, dieldrin, DDT etc. in ponds as they can persist in the sediment for years and therefore, fish kill may occur afterwards. Organochlorin compounds have been also suggested as contributing to the recent epizootics of ulcerative fish disease in South-East Asia (Perschbacher, 1989).

**2.2.3 Other type of piscicides :**

There are several other chemicals which are very often used for eradication of unwanted or weed fishes. Endosulfan is a registered fish toxicant used extensively to control undesirable organisms (Paul and Rauth, 1987). Ammonia is successfully employed for eradication of unwanted fishes and also it acts as a fertilizer afterwards (Rath, 1993). The combination of commercial grade bleaching powder and urea having equal proportion of 5 ppm each of chlorine and ammonia has been found to be effective, economical and is also considered advantageous (Ram *et al.*, 1988). Though, singlly anhydrous ammonia (Ramachandran, 1960 ; Ramaprabhu *et al.*, 1985, 1986) and commercial bleaching powder (Tripathy *et al.*, 1980) have been used for eradicating unwanted and predatory fish, combination of urea (as source of ammonia)

and bleaching powder proved to be better. Urea, a component of the treatment, besides acting as a piscicide in such combination also helped in the growth of natural fish food organisms, such as algae, diatoms and rotifers and can be considered beneficial in fish culture system (Mohanty *et al.*, 1993 ). Application of urea (200 kg/ha ) is an established fertilization measure in composite fish culture operations in India (Anon, 1981). When it is used as a piscicide the ammonia liberated would act both as piscicide and weedicide (Tripathy *et al.*, 1991). Again ammonia treatment with super phosphate resulted in significantly higher growth increment in case of carp seed (Tripathy *et al.*,1991). The growth and survival of carp fry in pond treated with bleaching powder was comparable with those in oilcake treated and control ponds. So, bleaching powder like mahua oil cake can be effectively used as piscicide without adversely affecting the growth and production of carp seed (Ram *et al.*, 1988).

**2.3 Fish killing mechanism of the piscicides :**

Different piscicides have different type of mechanisms for killing the fish. Saponin derived from mahua oil cake (4-6%) and tea seed cake is the active ingradient that kill the fishes (Perschbacher *et al.*,1989). This saponin act as a haemotoxic which may dissociate the bonding pattern of haemoglobin molecules and also responsible for breakdown of the bio-membrane of red blood corpuscles (Jhingran, 1991 ; Banerjee and Ganguli, 1993). Such toxicity following mahua oil cake application lasts only for 2 days after which fish may be killed due to oxygen defficiency or due to substances like $CO_2$ liberated in large quantities for the decomposition of mahua oil cake (Nath, 1983). Fishes on being affected by mahua oil cake, are reported to appear in a distress condition at first. Later they become inactive and loss their balance. Then they sink to the bottom and lie on as if in a state of coma and finally die (Bhatia, 1970). Other than that tamarind seed (*Tamrindus indica*) husk (Jena, 1979) and sugarcane jaggery (gur) (Jhingran, 1991) also contains saponin like ingradient that act as haemotoxic to fish. The plant derivative derris root powder has 5% rotenone ($C_{23}H_{22}O_6$) as active ingradient that damages the respiratory system of fishes (Jhingran, 1991).

Organophosphate compounds act as neurotoxins, irreversibly inhibits the activity of cholinesterases as a result of which there is accumulation of acetylcholine which is a neurotransmitter substance at parasynthetic neuroeffector junction, autonomic ganglia, somatic myoneural junction and probably certain CNS (Central Nervous Systems) regions. Therefore, DDVP with 76 % active ingradient acted as a neurotoxin to fish when applied for eradication of unwanted fishes (Ghatak and Konar, 1993).

Chlorinated hydrocarbons also act as neurotoxin to fish but it is highly toxic and adversely affect the other biota (Mahapatra and Thosar, 1999). In shallow ponds it acts quicker during sunny days but may have hardly any action in deeper water exceeding the depth of about 20 ft. It's action takes about 2-3 hrs. to affect Indian major carps and 4 to 8 hours to hardy fishes like Tilapia, Singhi, Magur etc. (Rath, 1993).

Other than these piscicides, mixture of urea [$CO(NH_2)_2$] and bleaching powder [Ca (OCl)Cl] is also applied frequently as a fish toxicant (Tripathy *et al.*, 1980). Urea, after application into the pond is hydrolysed to ammonia ($NH_3$) which is liberated within 24-48 hrs. at a temperature ranging from $23^0$ - $30^0$C, while hypochlorous acid (HOCl) is produced instantaneously from the chlorinated compound, bleaching powder under the prevailing environmental conditions. This hypochlorous acid, being a strong oxidizing agent is readily produced in the presence of reducing substances namely, $NH_3$, $Mn^{+2}$, $Fe^{+2}$ etc. of the environment resulting in 'Chlorine demand' of water. In the pond ecosystem Chloramines usually termed "Combined Residual Chlorine" (CRC) are formed with the operation of oxidative-reduction process in the presence of both $NH_3$ and hypochlorous acid (Mohanty *et al.*, 1993). The rate of chloramine formation largely depends upon ambient pH of the system (Mattice *et al.*, 1981). On the other hand, un-ionized ammonia ($NH_3$) and Free Residual Chlorine (FRC) [HOCl +$OCl^-$] are primarily responsible for fish kill when ammonia and chlorine compounds are employed seperately in fish ponds. But when combinations of ammonia and chlorine compounds are used, resultant Total Residual Chlorine (TRC) becomes the toxic component in which both free residual

chlorine(FRC) and Combined Residual chlorine (CRC) are represented. However, the formation of FRC ($HOCl + OCl^-$) and CRC ($NH_2Cl + NHCl_2 + NCl_3$) depends mainly on the molar ratio of both ammonia and chlorine present in the system. The break point of ammonia in the presence of chlorine occurs within a molar ratio of 1 to 2 with complete disappearnce of all ammonia and chlorine from water (Fair and Geyer, 1954). The piscicidal effect at higher level of chlorine as suggested by Tripathy *et al.*(1980) is due to the toxicity of free residual chlorine (FRC) but according to Mohanty *et al.* (1993), the combined residual chlorine is mainly responsible for fish kill. Individually free chlorine, even at low concentration in natural waters has been reported to be toxic to fish by causing osmotic imbalance (White, 1955 ; Tompkins and Tsai, 1976). Similarly increased ammonia concentration adversely affect enzyme - catalysed reactions, membrane stability and gill function resulting in fish mortality (Colt and Armstrong, 1979).

**Table. 2 : Doses of different piscicides prescribed by different authors**

| Piscicide | Test organism | Dose | Author |
|---|---|---|---|
| **Plant origin** | | | |
| Mahua oil cake | - | 60 ppm | Bhatia (1970) |
| | All predatory and weed fishes | 200-250 ppm | Bhatia (1970)<br>Banerjee (1991) |
| | - | 75 ppm | Nath (1983) |
| Derris root powder | - | 0.5 ppm | Hall (1949) |
| | *Mugil parsia*<br>*Channa punctata* | 11-39 ppm | Das (1969) |
| | All weed and predatory fishes | 150 ppm | Shirgur (1972)<br>Banerjee (1991) |
| | - | 6-10 ppm | Jhingran (1991) |
| *Nicotiana tabacem* (Tobacco) | Snails and aquatic organisms | 12-15 ppm | Tang (1967) |
| | | 100-200 ppm | Banerjee (1991) |
| Tea seed cake | - | 216 kg Tea seed cake +144 kg quicklime | Pillay (1995) |

*Contd.....*

| Piscicide | Test organism | Dose | Author |
|---|---|---|---|
| Tea seed powder | - | 100-200 ppm | Banerjee (1991) |
| *Barringtonia acutangula* (Hijal) | - | 200 ppm | Banerjee (1991) |
| *Tamarindus indica* (Tentul) | - | 250 ppm | Banerjee (1991) |
| *Acacia moniliformes* (Sonajhuri) | - | 250 ppm | Banerjee (1991) |
| **Organophosphates** | | | |
| DDVP | Most unwanted fish | 3-30 ppm | Jhingran (1991) Rath (1993) |
| | Phytoplankton | 0.5 ppm | Konar (1964) |
| | Aquatic insects | 0.003-0.5 ppm | Rath (1993) |
| **Chlorinated hydrocarbons** | | | |
| Aldrin | All predatory and weed fishes | 0.2 ppm | Jhingran (1991) Rath (1993) |
| Eldrin | All weed fish | 0.001 ppm | Chaudhuri (1960) |
| Dieldrin | All weed fish | 0.01 ppm | Jhingran (1991) Rath (1993) |
| Toxaphene | Blue gill, Bass | 0.05 ppm | Chaudhuri (1960) |
| DDT | Blue gill, Bass | 0.15 ppm | Chaudhuri (1960) |
| Methoxychor | Blue gill, Bass | 0.2 ppm | Chaudhuri (1960) |
| Chlordane | Blue gill, Bass | 0.2 ppm | Chaudhuri (1960) |
| B S C | Blue gill, Bass | 0.1 ppm | Chaudhuri (1960) |
| **Others** | | | |
| Urea + Bleach | All weed fish | 3 ppm+ 5 ppm | Mohanty *et al* (1993) |
| | *Channa punctata* (fry) | 5 mg $Cl_2$/ lt +5 mg $NH_3$/lt | Ram *et al.* (1988) |
| Un-ionised $NH_3$ | All weed fishes | 5 ppm | Ramaprabhu *et al.* (1990) |
| Bleaching powder | Unwanted fish, crab, benthos, molluscs | 50 ppm | Tripathy *et al* (1980) |

**2.4 Effects on water quality parameters :**

The effect of piscicides on water quality parameters is dependent upon the nature of the compounds used for the purpose. When mahua oil cake was applied in ponds, the DO content of the pond showed a decreasing trend upto trace within 24 hrs. and the BOD of ponds were high (40-44 ppm) which persist upto 5 days (Nath, 1983). After 8-11 days slow increase of DO was noted. In contrast, free $CO_2$ content showed increasing trend with insignificant change in total alkalinity (Nath, 1983). However, Jana *et al.* (1987) observced that bicarbonate alkalinity, total hardness, chloride concentration were greatly increased in mahua oil cake treated water. On the other hand, DDVP as a piscicide in ponds resulted in insignificant fluctuations of pH, temperature, DO but the colour and odour of water changes. The combined application of bleaching powder and urea did not result in any noticeable changes in water quality paramerters, excepting an increase in the chlorine content (Ghatak and Konar, 1992).

**2.5 Effects on nutrient status :**

When mahua oil cake was applied in ponds, the mean values of phosphate and different forms of nitrogen were greatly increased in concentration (Nath, 1983; Jana *et al.*, 1987). Significant increase in phosphate concentration, after mahua oil cake application is due to its biodegradation (Ghatak and Konar, 1993) and the released nutrients help in increasing the productivity afterwards (Nath, 1983). The phosphate and total nitrogen content were found to be high after mahua oil cake treatment upto 13th day until the nutrients were taken up by phytoplankton and algae (Nath, 1983). The maximum toxicity develops during the period of 3-7 days of cake application and was evident from the sharp rise in the concentration of $NH_3$ and $CO_2$ of water during this period (Jana *et al.*, 1987). This is because, a high rate of $NH_3$ oxidation was observed in the water bodies with high DO levels, while the process of denitrification proceeds more rapidly in water which lacked sufficient DO (Sugiyama and Kawai, 1978).

Application of DDVP as fish toxicant in pond resulted in the release of large amount of phosphate in pond water but no significant fluctuation of nitrite and

nitrate level was observed (Ghatak and Konar, 1992). On the other hand when urea and bleaching powder is applied it may increase the level of free chlorine and large amount of ammonia with subsequent rise in nitrate and nitrite (Ram *et al.*, 1988 ; Mohanty *et al.*, 1993). In case of urea and mahua oil cake application, increase in the organic carbon level of soil as well as water was noticed (Ram *et al.*, 1988).

**2.6 Effects on pond productivity :**

A reduction in gross as well as net primary productivity immediately after application of mahua oil cake was observed and the rate of such reduction was directly dose dependent (Pal and Jabeen, 2000). Toxicity of mahua oil cake was found to be responsible for the sharp decline of most members of phyto and zoo-planktons within 3-5 days of treatment (Jana *et al.*,1987). As mahua oil cake acts as a fertilizer in pond following it's application (Banerjee *et al.*, 1987), it has got the utility in adding fertility to the water for the growth of plankton besides controlling weed fishes (Acharjee and Biswas, 1995). Lakshman (1983) reported, highly significant increase in primary productivity and growth of fish at 3200 kg/ha of mahua oil cake application in combination with 1125 kg/ha of lime. A period of 30-45 days should be allowed after application of mahua oil cake before stocking, for maximum benefits from the cake.

DDVP also significantly reduced both phytoplankton and zooplankton population at sublethal concentration and subsequently it resulted in reduction of GPP and NPP (Pal and Konar, 1985). However, combination of urea and bleaching powder did not cause any adverse effect on productivity. Though initial reduction in planktons was noticed, a subsequent increase in plankton population as well as productivity was observed (Shyam *et al.*, 1993).

**2.7 Comparative persistance of piscicide :**

In general, the plant derivaties are bio-degradable in water medium because they are decomposed and converted to manure once their toxic action is nullified (Mahapatra and Thosar, 1999). The effectiveness of saponin persists for about 2 days in water after application of Mahua oil cake (Nath, 1983). Organophosphate piscicide shows the abnormal behaviour of fish within the first 6

hrs. of exposure while with organochlorine piscicides abnormal activities increased after 6-8 hrs. of exposure (Panwar *et al.*, 1976). Chlorinated hydrocarbons are more toxic to fishes and persist in the medium as stable compound, also these chlorinated hydrocarbons get deposited into the sediment, therefore rendering its poisonous effect for long (Chakroff, 1993). They may be biomagnified and bioaccumulated in the food chain (Mahapatra and Thosar, 1999). Therefore, in recent years organophosphates are more preferred than organochlorins due to it's less residual effect (Mahapatra and Noble, 1993). The combination of urea and bleaching powder have a short residual toxicity in fish culture ponds (Janakiram *et al.*, 1988).

**2.8 Comparative cost effectiveness :**

Cost of piscicide like any other inputs is a major consideration for choosing the right piscicide by the fish culturist. Of all the piscicides, plant derivatives are comparatively cost effective. Among others, rotenone is a registered toxicant but it is thought to be expensive by some fish managers (Markling, 1992), but it is of moderate cost compared to organophosphate compounds (Perschbacher *et al., 1989)* which is too costly to be attractive to the fish culturists (Perschbacher *et al.*, 1989). The combination of commercial grade bleaching powder and urea has been found to be most economical and advantageous among the inorganic piscicides (Ram *et al.*, 1989). Because, dual advantage of piscicidal action and fertilization value is attainable from this combination with benefits of cost effectiveness (Janakiram *et al.*, 1988 ; Mohanty *et al.*, 1993).

**2.9 Detoxification of ponds treated with piscicides :**

Detoxification of piscicide treated pond is an important step before subsequent stocking of fish seed into the pond water. Most of the plant originated poisons degradate and disappears from the water within 7-12 days (Chakroff, 1993), whereas, the chemical poisons needed more time to be detoxified. The use of detoxifyiong materials like charcoal powder, sulphuric acid, potassium permanganate etc. has been recommended in the chemically treated ponds to remove the toxicity within a short period. These detoxifying materials can be used as charcoal powder @ 20-25 ppm, $H_2SO_4$ @ 100 ppm, $KMnO_4$ @ 5 ppm which can detoxify the pond

within 4-5 days. Raw cowdung @ 18,000 kg/ha is also found effective in detoxification of treated water (Rath, 1993). The detoxification period of bleaching powder was lengthy (Perschbacher *et al.*, 1989). But application of urea (200 kg $ha^{-1}$) is an established fertilization measure in composite fish culture operations in india (Anon, 1981), so when urea is used as a component of treatment along with bleaching powder it shorten the detoxification period of the pond as well as act as a fertilizer afterwards (Mohanty *et al.*, 1993).

The toxic period of mahua oil cake can be reduced from 20-25 days to 7-10 days or even less by aerating the water mechanically or by suitable doses of oxidising chemicals (Jhingran, 1991). It was observed that $KMnO_4$ solution @ 2.4 ppm indicate no detoxification of saponin, although $KMnO_4$ solution effectively detoxify rotenone of derris root powder. Similarly, treatment of mahua oil cake applied pond water with lime @ 200 ppm indicate no detoxificaion of the saponin, but it was only possible to reduce the toxic period of saponin by aerating the pond water with suitable aeration device (Nath, 1983). If single superphosphate is used along with mahua oil cake it compensate the deletorious effect of the cake. Use of mahua oil cake at high amount or it's frequent addition should be avoided (Sarkar, 1988).

Review of available literature on piscicides in pond culture system indicated very few informations regarding the consequences of fish toxicants upon the bio-geochemical cycling microbes in aquatic systems which is of prime importance so far the natural productivity of the system is concerned. Therefore, it is pertinent to address the problem of toxicity related changes in the community composition of mineralizing microbial profile of fish culture ponds following application of fish toxicants. With this background the present study has been envisaged.

# 3. Materials and Methods

The present investigation on the comparative account of different piscicides has been carried out in the Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia (23°00' N and 88°34' E). The duration of the study lasted for a period of about three months from March to May, 2002.

Twelve experimental cement cisterns (180 l) were selected for the present investigation. The cisterns were provided with an agricultural soil base (pH 7.4) of 15 cm and then filled with ground water (pH 7.1-7.2). All the cisterns were manured with cowdung at the rate of 10,000 kg/ha, as practised in traditional pond preparation. The requisite amount of cowdung were mixed with water in a bucket and dispensed in the form of slurry into the cisterns. They were then grouped into four batches in triplicate following a completely randomized design.

Predatory fish *Channa punctatus* (32 ± 5 g) fry were stocked as test fish at a rate of 5 nos. /cistern 10 days after the application of manuring when the colour of the water changes to greenish blue indicating development of planktonic organisms. The fishes were procured from local market, acclimatised in cement cisterns for seven days and then released in three of the four batches of cisterns designated for three treatments employed (M, D and B + U). The first three batches were subjected to treatment with three different types of piscicides like Mahua oil cake (M), DDVP (D) and combination of Bleaching powder and Urea (B + U) using the doses as practised by the farmers five days after introduction of predatory fishes, whereas the fourth batch without any piscicide as well as fish introduction served as control (C) (Table 3).

All the dead fishes were removed immediately from the cisterns treated with toxicants. The water was tested for residual toxicity after 20 days of toxicant

treatment by placing fingerling of common carp (*Cyprinus carpio*) in beakers of water from the respective cisterns. When there was no mortality, the cisterns were stocked with healthy fingerling of *Cyprinus carpio* at the rate of 15 nos. in each cistern, including the control. They were reared until the water quality reached a congenial steady state in respect to selected water quality parameters upto day 70. No artificial feed was provided to the fishes.

**Table. 3 : Experimental protocol followed in the investigation**

| Variables | | Conditions | | | |
|---|---|---|---|---|---|
| | | **Mahua oil cake** | **DDVP** | **Bleach+Urea** | **Control** |
| Volume of the experimental cisterns (l) | | 180 | 180 | 180 | 180 |
| Duration (days) | | 90 | 90 | 90 | 90 |
| Piscicide Dosage ($mg\ l^{-1}$) | Mahua oil cake | 200 | – | – | – |
| | DDVP | – | 10 | – | – |
| | Bleach (as $Cl^-$) | – | – | 5 | – |
| | Urea (as $NH_3$) | – | – | 5 | – |
| Predatory fish | | *Channa punctatus* | *Channa punctatus* | *Channa punctatus* | – |
| Stocking density (Nos./cistern) | | 5 | 5 | 5 | – |
| Size | Length (cm) | 9±3.2 | 9±1.2 | 9±1.2 | 9±1.2 |
| | Weight (g) | 32±5 | 32±5 | 32±5 | 32±5 |
| Cultured fish | | *Cyprinus carpio* | *Cyprinus carpio* | *Cyprinus carpio* | *Cyprinus carpio* |
| Stocking density Nos./cistern | | 15 | 15 | 15 | 15 |
| Size | Length (cm) | 3.5±2.2 | 3.5±2.2 | 3.5±2.2 | 3.5±2.2 |
| | Weight (g) | 3.1±1.8 | 3.1±1.8 | 3.1±1.8 | 3.1±1.8 |

**3.1 Water replenishment :**

A fixed level of water was maintained in the experimental cisterns by periodic additon of ground water to compensate the losses due to evaporation as well as sampling.

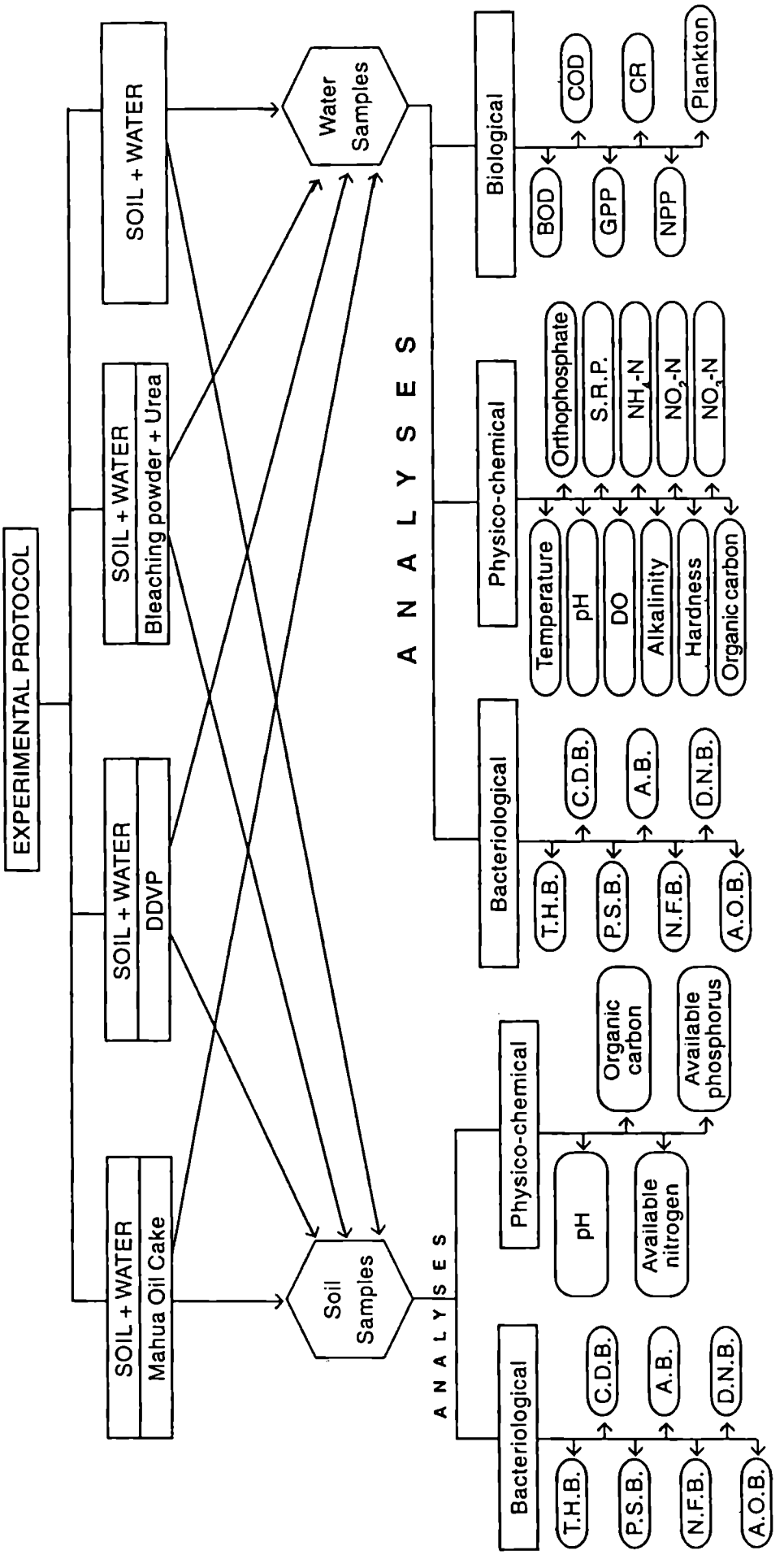


**Fig. 1 : Diagramatic Representation of the Experimental Protocol**

### 3.2 Collection of sample :

Water samples were collected at 10 days intervals from each of the cisterns at a fixed hour of the day (10:00 AM) following all the necessary precautions.

The soil samples from each of the cisterns were collected from two different places of the soil bed using a mini hand grab sampler. They were then mixed, air dried, pulverized with pestle and mortar and sieved through 150 μm mesh size sieve and stored in labelled polythene packets for analyses.

A conical plankton net made of bolting silk cloth (no. 21 with 77 meshes per square centimeter) was used to collect the plankton samples. About 5 l of water from each of the cisterns was collected from randomly selected locations with the help of a 500 ml beaker and pooled together for filtering through plankton in 4% formalin solution and stored in labelled vials for subsequent quantitative analysis.

The water samples for the purpose of microbial plating were collected at ten days intervals. The samples were aseptically collected in sterilized reagent bottles. The plating was carried out on the same day.

### 3.3 Analyses of samples :

#### 3.3.1 Water Quality :

**a) Temperature and Hydrogen-ion concentration :**

The samples of water collected were analysed following the procedures described in APHA (1995). The temperature of water was recorded with the help of a centigrade mercury thermometer. The pH of water was measured using a pH meter (Systronics, MK VI).

**b) Alkalinity :**

Estimation of alkalinity of water samples were done immediately after collection. Carbonate alkalinity of water samples were analysed by titrating the samples against N/50 $H_2SO_4$ using phenolphthalein as indicator. Bicarbonate alkalinity was determined against N/50 $H_2SO_4$ using methyl orange indicator (APHA, 1995).

**c) Dissolved Oxygen :**

For estimation of dissolved oxygen content, water samples were collected in triplicate in narrow mouth glass stoppered bottle, taking necessary precaution to exclude air bubbles. Winkler's method was used to measure the amount of dissolved oxygen of water (APHA, 1995).

**d) Hardness :**

Total hardness of water was determined by using N/50 EDTA as a titrant and Eriochrome Black-T as indicator.

**e) Organic carbon :**

Organic carbon content of the water samples were determined by titrating against 0.05 N Mohr's salt (Golterman, 1978).

**f) Biochemical Oxygen Demand :**

Biochemical oxygen demand (BOD) of water was estimated following the method described by Golterman *et al.* (1978). For estimation of BOD, water samples were collected in duplicate, in narrow mouth glass stoppered bottles, taking necessary precautions to exclude air bubbles. The dissolved oxygen content of water was measured in one of the bottles immediately after collection following modified Winkler's method. The other sample was incubated in dark at 20°C for 1 day, after which the dissolved oxygen content of water in the incubated sample was determined. The difference between the initial dissolved oxygen content and that of the incubated sample was expressed as $BOD_1$ of the sample.

**g) Chemical oxygen demand :**

Chemical oxygen demand of water was determined by digesting the samples with the mixture of potassium dichromate and concentrated $H_2SO_4$ and titrating against ferrous ammonium sulphate taking phenanthroline as indicator (Golterman, 1978).

**h) Soluble reactive phosphate :**

Soluble reactive phosphate content of water was measured at 880 nm following ascorbic acid method (APHA, 1995).

**i) Orthophosphate :**

The orthophosphate content of water was determined ccolorimetrically at 690 nm following the stannous chloride method (APHA, 1995).

**j) Ammonium-N :**

Ammonium nitrogen of water was measured at 630 nm in a spectrophotometer (Systronics, 118) after one hour of colour development following the modified phenate method (Wetzel and Likens, 1991).

**k) Nitrite-N :**

The concentration of nitrite was measured at 543 nm in a spectrophotometer (Systronics, 118) using α-napthylamine and sulphanilic acid (Wetzel and Likens, 1991).

**l) Nitrate-N :**

The amount of nitrate was determined by UV-spectrophotometric method (APHA, 1995), using aluminium hydroxide suspension and 1N HCl at 220 nm and 275 nm in a spectrophotometer (Systronics, 118). Measurement of the ultraviolet absorbtion at 220 nm enable rapid determination of nitrate. Because dissolve organic matter may also absorb at 220 nm and nitrate does not absorb at 275 nm a second measurement was made at 275 nm to correct the nitrate value.

**m) Primary productivity :**

The dark and light bottle technique described by Winberg (1963) was followed for the measurement of primary productivity of phytoplankton.

Water samples were collected in 125 ml Borosil glass bottle in triplicate from each cistern taking all necessary precautions during filling to prevent air bubbles from remaining in the bottle. All the bottles were then exposed at the surface layer of water under normal light conditions for 4 to 5 hours of day light depending upon the photoperiod. The oxygen content of all the dark and light bottles were monitored using modified Winkler's method. The calculation described by Vollenweider (1974) was used to measure the rate of primary production. The results of primary production in terms of $O_2$ was converted into mg carbon by multiplying with a factor 0.375

(Natarajan and Pathak, 1983). The calculation described by Vollenweider (1974) was used to measure the rate of primary productivity.

**3.3.2 Soil Quality :**

**a) Hydrogen-ion concentration :**

The soil pH was measured with a digital pH pen (Systronics, MKVI), using a 1 : 2 suspension of soil and water.

**b) Available phosphorus :**

Available phosphorus was determined using 1 : 20 soil to Olsen's extractant (0.5 N $NaHCO_3$ adjusted to pH 8.5) (Olsen *et al.*, 1954) followed by Dickman and Bray's (1940) chlorostannous reduced molybdophosphoric blue colour method in hypochloric acid system as described by Jackson (1967).

**c) Available nitrogen :**

Available nitrogen was determined by using KCl as extractant and distillating in 4% boric acid solution with Devadray's alloy as catalyst (Jackson, 1967).

**d) Organic Carbon :**

For estimation of organic carbon, air dired powdered sediment (500gm) was digested with 1N $K_2 Cr_2O_7$ (20 ml) and concentrated $H_2SO_4$ (20 ml) and kept for 30 minutes in dark. The digerted sample was then diluted with 150 ml distilled water and 10 ml phosphoric acid and 1ml diphenyl amine indicator were added. It was then titrated against 0.5 N ferrous ammonium sulphate (Mohr's salt) until brilliant green colour appeared (Walkley and Black, 1934).

**3.3.3 Plankton :**

Quantitative determinations of plankton were made in the graduated centrifuge tubes as the volume of plankton in 20 l of water sample and then expressed in units per litre (Jhingran, 1988).

**3.3.4 Enumeration of Bacteria :**

Enumeration of total aerobic heterotrophic bacteria, ammonifying bacteria, denitrifying bacteria, nitrogen fixing bacteria, ammonia oxidising bacteria, phosphate solubilizing bacteria and cellulose decomposing bacteria was done from water and

soil samples at 10 days interval. Collection of samples were performed as per the method of Rodina (1972) and Antipchuk (1979). All the routine procedures for bacterial culture as sterilization of glass wares and media, inoculation of samples and incubation of petridishes were followed.

**a) Total aerobic heterotrophic bacteria :**

Population of heterotrophic bacteria were grown in standard nutrient agar medium having the following composition (APHA, 1995) :

| | |
|---|---|
| Petone | 10 g |
| Beef extract | 1.5 g |
| Sodium Chloride | 2.0 g |
| Bacto agar | 20 g |
| Distilled water | 1000 ml |

The medium was sterilized in the autoclave at 15 lbs. pressure for 15 minutes.

**b) Denitrifying bacteria :**

Asparagine - nitrate - citrate agar medium was used for growth of denitrifying bacteria. Asparagine and nitrate compound acting as a source of nitrogen substrate and citrate as organic source of carbon. Alexander (1978) mentioned that nitrate acts as an exogenous terminal $H^+$ acceptor in the oxidation of organic substrate. The composition of this medium was.

**Solution A :**

| | |
|---|---|
| Asparagine | 1.0 g |
| $KNO_3$ | 1.0 g |
| Distilled water | 250 ml |

**Solution B :**

| | |
|---|---|
| Neutral Na - citrate | 8.5 g |
| $KH_2 PO_4$ | 1.0 g |
| $Mg SO_4, 7H_2 O$ | 1.0 g |
| $CaCl_2, 6 H_2O$ | 0.6 g |
| $FeCl_3, 6 H_2O$ | traces |
| Distilled water | 250 ml |

Solution A and Solution B were mixed and made up to 1 litre by adding of extra distilled water. Bacteriological agar at the rate of 2% was added maintaining pH at 7.5. The medium was then sterilzed in autoclave at 10-12 lbs pressure for 15 minutes.

**c) Ammonifying bacteria (Alexander, 1978) :**

| | |
|---|---|
| Peptone | 5 g |
| Beef extract | 4 g |
| NaCl | 5 g |
| Agar | 20 g |
| Distilled water | 1000 ml |

**d) Phosphate solubilizing bacteria [PSB] :**

PSB are capable of utilizing tri-calcium phosphate. The production of clear zone around the colonies of the organisms was an indication of the presence of PSB (Subba Rao, 1977). The composition of modified Pikovskaya's agar medium was as follows.

| | |
|---|---|
| Glucose | 10 g |
| Tri calcium phosphate | 5.0 g |
| Ammonium sulphate | 0.5 g |
| KCl | 0.2 g |
| $MgSO_4$ | 0.1 g |
| Mn $SO_4$ | Trace |
| Ferrous sulphate | Trace |
| Yeast extract | 0.5 g |
| Agar powder | 20.0 g |
| Distilled water | 1000 ml |

**e) Ammonia oxidizing bacteria (Drews, 1974) :**

| | |
|---|---|
| Ammonium sulphate | 1.0 g |
| $K_2HPO_4$ | 1.0 g |
| Mg $SO_4$, $7H_2O$ | 0.5 g |
| NaCl | 1.0 g |
| Fe $SO_4$, $7H_2O$ | 0.05 g |
| Bacto agar | 15.0 g |
| $CaCO_3$ | 5.0 g |
| Phenol red | 0.01 g |
| Distilled water | 1000 ml |

**f) Nitorgen fixing bacteria :**

**Solution A :**

| | |
|---|---|
| Manitol | 10 g |
| $K_2H$ $PO_4$ | 0.5 g |
| Mg $SO_4$, 7 $H_2O$ | 0.2 g |
| NaCl | 0.2 g |
| Bacto agar | 20.0 g |
| Distilled water | 1000 ml |

**Solution B :**

| | |
|---|---|
| $MnSO_4$ | Trace |
| $FeCl_3$ | Trace |
| $CaCO_3$ | Trace |

Solution A and Solution B were sterilized and then mixed.

**g) Cellulose decomposing bacteria :**

| | |
|---|---|
| Cellulose powder | 2.5 g |
| Peptone | 0.5 g |
| $KH_2$ $PO_4$ | 0.2 g |
| Mg $SO_4$ | 0.2 g |
| $K_2$ $CO_3$ | 0.4 g |
| Ca $Cl_2$ | 0.2 g |
| Agar | 20 g |
| Distilled water | 1000 ml |

Aliquots of water samples and soil samples were prepared in sterile distilled water as per the dilution required for inoculation. The conventional spread plate techniques (Chan and Kueh, 1976) under aerobic condition was used to enumerate viable counts of different groups of bacteria. Each dilution of sample was grown in bacteriological culture plate (10 cm dia) in quadruplicate. The plates were incubated at 37± 2°C for 48 hours. The colonies were then counted and the numbers recorded.

### 3.4. Statistical Analysis :

All the results were subjected to statistical analyses. One way analysis of variance (ANOVA) were applied to test the significance among the treatment differences followed by critical difference test (CD) to find out significance in difference between any pair of treatment combination. Correlation-co-efficient (r) test was applied to establish relationship between selective parameters using appropriate software.

**4.1 Water :**

**4.1.1 Microbial parameters :**

**Total heterotrophic bacteria [THB] :**

Total heterotrophic bacterial population exhibited a sharp peak 10 days after application of either (B + U) or (M) followed by a decline. However, in the other two systems the bacterial population continued to decline all throughout (Fig. 2). The overall mean value was highest (27.133 x $10^3$ nos. $ml^{-1}$) in (B + U) and lowest in control (7.3 x $10^3$ nos. $ml^{-1}$).

One way analysis of variance indicated significant differences among the treatments ($F \geq 3.98$; $P < 0.05$) in most of the dates of observations. However, CD test revealed insignificant differences ($P > 0.05$) between (M) and (D) (Fig.2).

**Cellulose decomposing bacteria [CDB] :**

A sharp decline was encountered in all the treatments following application of toxicants and a more or less steady state condition was achieved after 40 days except in mahua treatment where an increasing trend was observed from 30th to 50th day (Fig. 3). The overall mean value in mahua treatment was 3.05-3.67 times higher than the rest of the treatments.

Highly significant differences were exhibited ($F \geq 21.46$; $P < 0.01$) among the treatments but CD test established insignificant differeces ($P>0.05$) either between (B + U) and (D) or with control.

**Phosphate solubilizing bacteria [PSB] :**

PSB population tendend to increase till day 25 in (D) and (M) then gradually decline similar to that of (B + U) and (C) (Fig. 4). The population density was always higher (57.25 – 84.52%) in (D) than the rest of the systems.

Differences among the treatments were significant ($F \geq 31.18$; $P < 0.01$) till day 40, however, such differences either between (M) and (D) or between (B + U) and control were insignificant (CD = 0.789; $P > 0.05$).

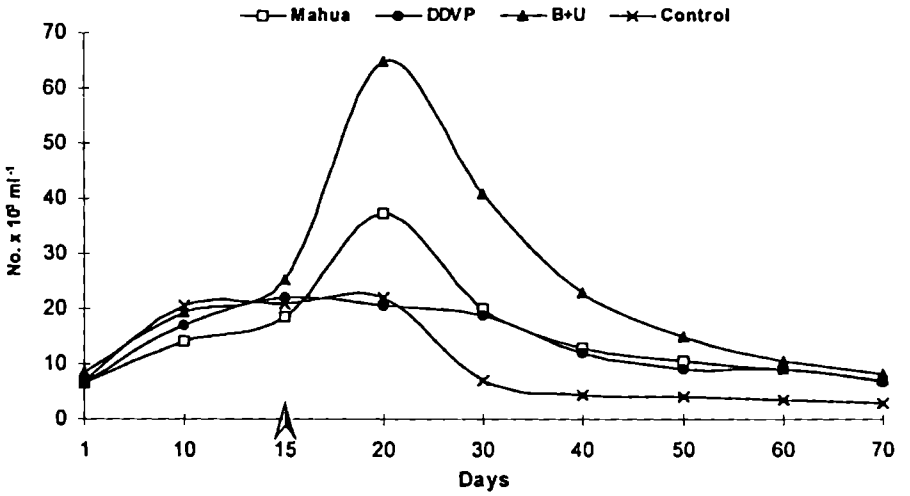


Fig. 2 : Temporal changes in the population of total Heterotrophic bacteria in water under different treatments employed.

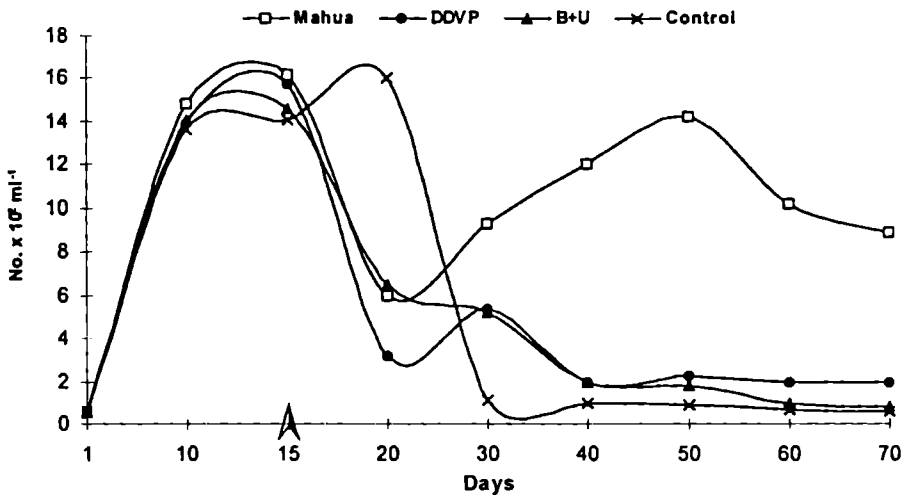


Fig. 3 : Temporal changes in the population of Cellulose decomposing bacteria in water under different treatments employed.

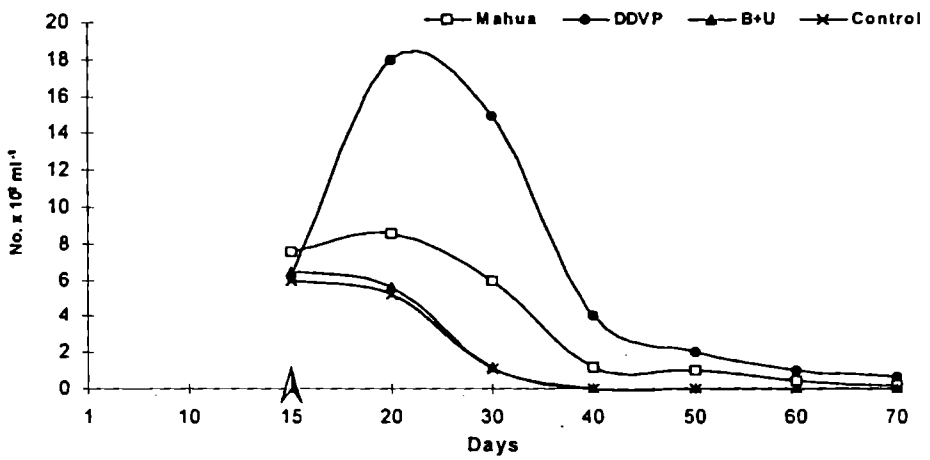


Fig. 4 : Temporal changes in the population of Phosphate solubilizing bacteria in water under different treatments employed.

⍋ – indicates application of fish toxicants

**Nitrogen fixing bacteria [NFB] :**

Temporal pattern of changes in the NFB population showed an immediate decline in any of the treatments followed by a sharp increase till day 40 to 50. The values then declined again (Fig. 5). Remarkably higher (17.21 – 35.59%) population density were encountered in (D) and (M) whereas the minimum value was encountered in control (15 x 10 nos. $ml^{-1}$).

Treatment differences become more prominent ($F \geq 2.467$; $P < 0.05$) after day 30 and continued till day 60 after which it became insignificant ($P > 0.05$). CD test indicated lack of significance between the treatments with (D) and (B + U) during most of the periods of observation.

**Ammonifying bacteria (AB) :**

Reduction of AB by 31 – 96.5% following application of any of the fish toxicants was observed. A uniform peak in all the treatments was observed on day 30 followed by a steep decline again. The mean value was maximum (6.882 x $10^4$ nos. $ml^{-1}$) in control followed by mahua (4.047 x $10^4$ nos. $ml^{-1}$), (B + U) (3.621 x $10^4$ nos. $ml^{-1}$) and (D) (1.673 x $10^4$ nos. $ml^{-1}$) (Fig. 6). Treatment differences were insignificant ($F \geq 2.148$; $P < 0.05$) only in 28% of the total observations.

**Denitrifying bacteria [DNB] :**

Similar to AB the population of DNB in water declined in any of the treatments following application of fish toxicants, however, the values remained more or less stationary thereafter (Fig. 7). Treatment differences were not prominent (ANOVA, $P > 0.05$) almost during the entire period of observation.

**Ammonia oxidising bacteria [AOB] :**

The AOB population gradually increased in any of the treatments by 57.42 – 83.57% and attained peak on day 30 and then gradually declined (Fig.8). The maximum value (15.5 x 10-92x10 nos. $ml^{-1}$) was always observed in (B + U) which was 23.52 and 76.73% higher then (M) and (D) respectively.

Treatment differences became conspicuously significant ($F \geq 124.36$; $P < 0.001$) after day 25. Again differences between any of the treatment pairs remained significant ($CD = 2.676$; $P < 0.05$) during the said period.

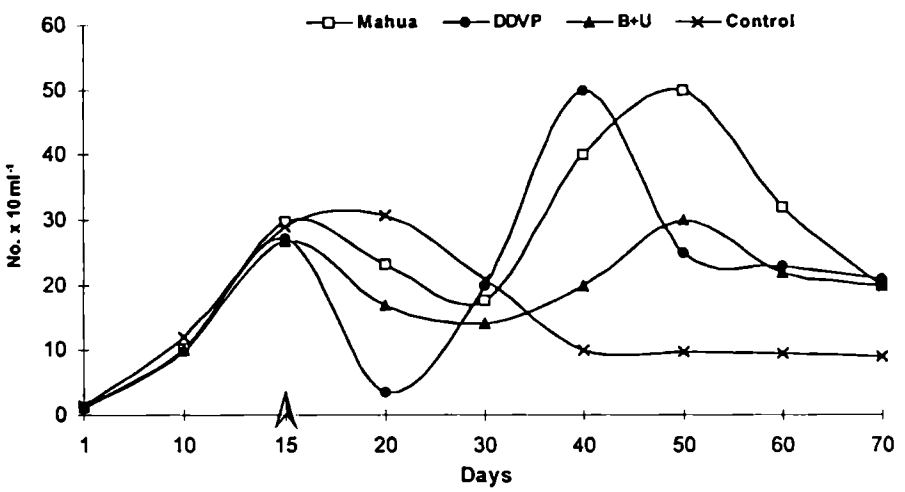


Fig. 5 : Temporal changes in the population of Nitrogen fixing bacteria in water under different treatments employed.

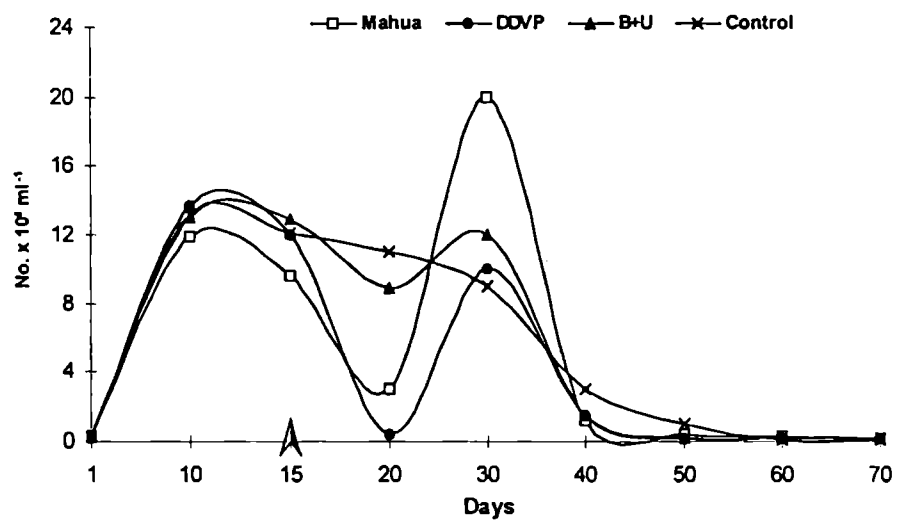


Fig. 6 : Temporal changes in the population of Ammonifying bacteria in water under different treatments employed.

⍋ – indicates application of fish toxicants

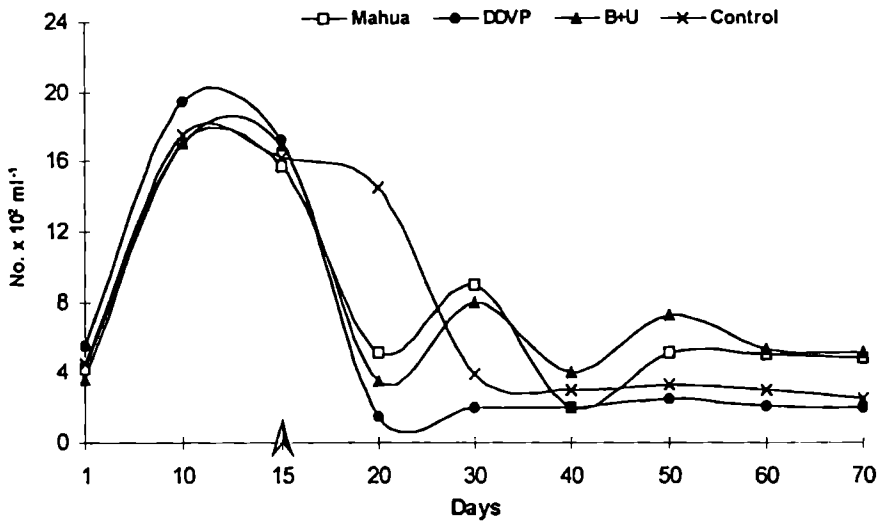


**Fig. 7 : Temporal changes in the population of Denitrifying bacteria in water under different treatments employed.**

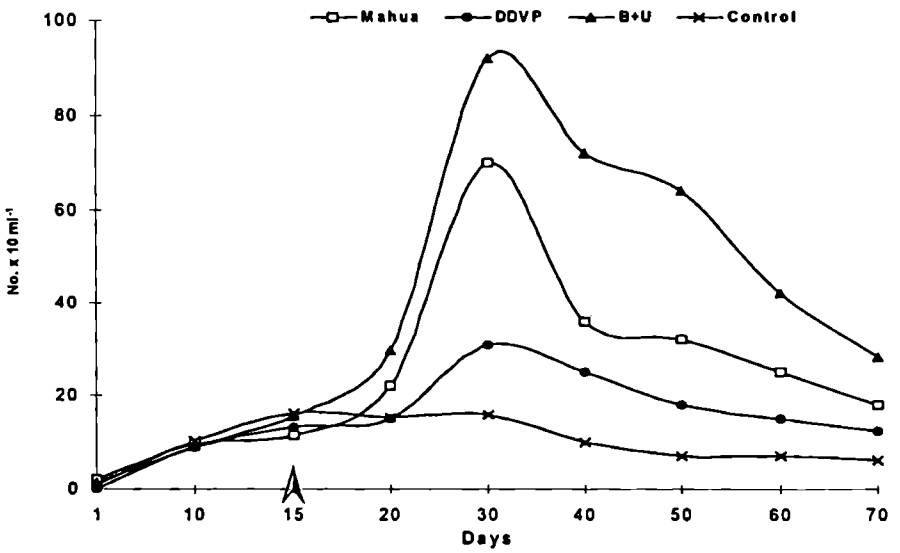


**Fig. 8 : Temporal changes in the population of Ammonia Oxidizing bacteria in water under different treatments employed.**

⍋ – indicates application of fish toxicants

### 4.1.2 Nutrient parameters :

**Ammonium Nitrogen :**

A sharp increase was observed in the values the $NH_4$–N in the treatments with (B + U) and (M) followed by a gradual decline after day 30. In contrast, the values continued to declined after application of toxicant either in (D) or in (C). The peak value attained in (B + U) was 21.8 – 58.5% higher during day 30 but during the later half, $NH_4$–N value was consistently remained maximum in the (M) treatment (Fig. 9).

Treatment differences were significant (ANOVA, $P < 0.05$) during the first half after application of toxicant.

**Nitrite Nitrogen :**

Temporal changes in the values of $NO_2$–N in any of the treatments were identical to that of $NH_4$–N. However, the highest value was observed in (M) instead of (B + U) (0.267 mg $l^{-1}$). Also treatment differences were prominantly significant (ANOVA, $P < 0.05$) during most of the study period (Fig. 10).

**Nitrate Nitrogen :**

Similar to that of $NO_2$–N the values of $NO_3$–N increased till day 25 to 30 followed by a decline after which a stead state achieved in (M) and (B+ U) (Fig 11). Whereas, in the other two systems the decline continued from day 20 onwards. The highest values were always observed in (M) which was 10 – 80% higher compared to that of (B + U) and (D) respectively.

Treatment differences were significant ($P < 0.05$) all throughout, however, differences between (B + U) and (D) became insignificant ($P > 0.05$) during the final phase of investigation.

**Soluble reactive phosphate [SRP] :**

The overall mean value of SRP was highest in (D) (0.0386 mg $l^{-1}$) followed by (M) (0.02 mg), Control (0.0163 mg $l^{-1}$) and (B + U) (0.0152 mg $l^{-1}$). The SRP increased substancially in the treatments with (D) and (M) followed by a gradual decline. In contrast, the other two treatment [(B + U) and (C)] it continued to decline after application of test material (Fig. 12).

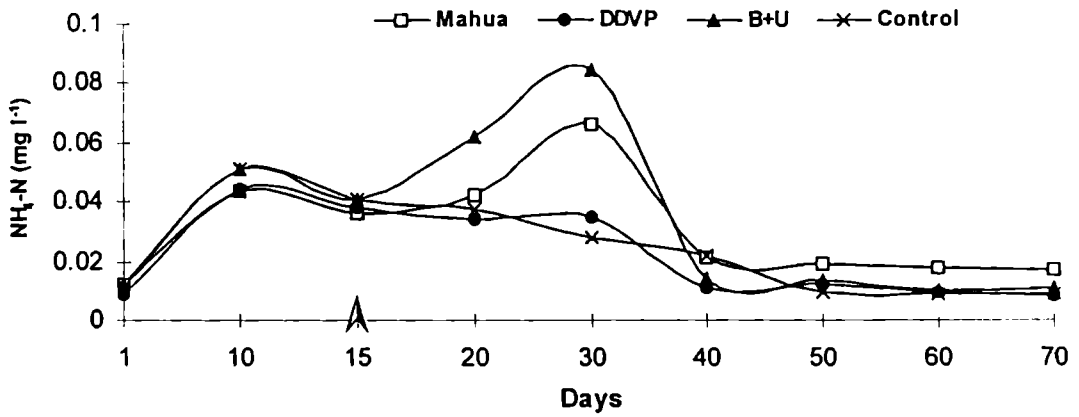


Fig. 9 : Temporal changes in the $NH_4$-N concentration of water under different treatments employed.

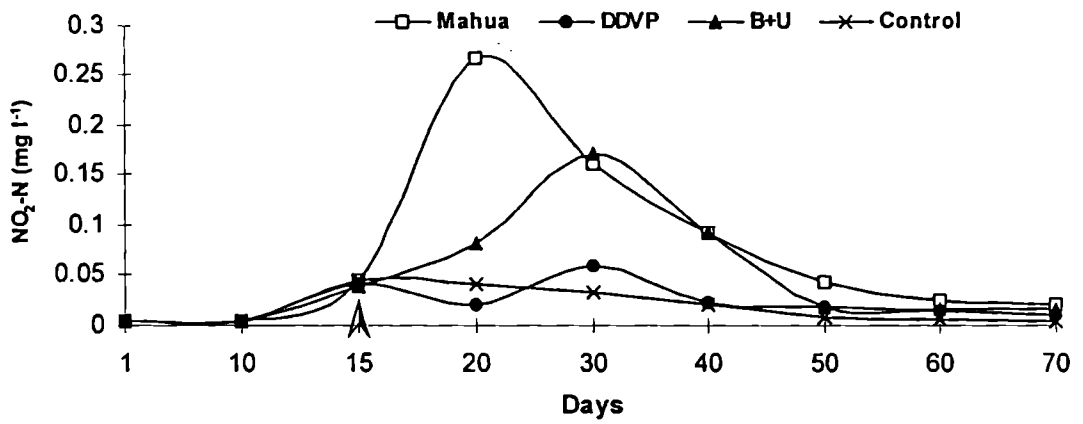


Fig. 10 : Temporal changes in the $NO_2$-N concentration of water under different treatments employed.

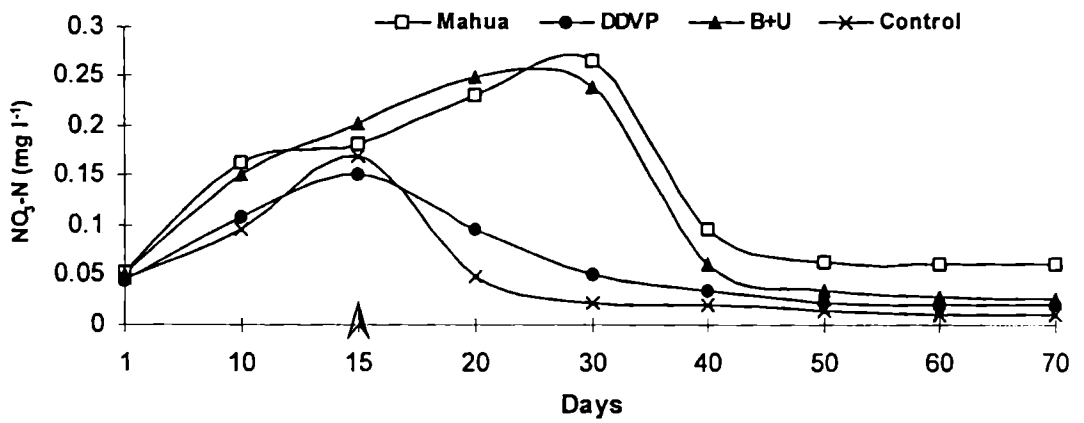


Fig. 11 : Temporal changes in the $NO_3$-N concentration of water under different treatments employed.

⅄ – indicates application of fish toxicants

ANOVA indicated a high level of significance ($P < 0.001$) among the treatments allthroughout, though differences between (B + U) and (C) were insignificant during most of the periods of observations.

**Orthophosphate :**

Similar to SRP the orthophosphate value was highest by 17.35 – 58.77% in (D) compared to the rest of the systems and the temporal pattern was also identical (Fig. 13).

Stastical differences among treatments were highly significant ($P < 0.01$). Likewise SRP differences between (B + U) and (C) were insignificant ($P > 0.05$) in most cases.

**Dissolved Oxygen (DO) :**

The values of dissolved oxygen showed an overall increasing trend following the application of fish toxicants except in (M) where a steep fall was observed till day 5 after its application. The values of dissolved oxygen in (M) then sharply increase (Fig. 14). Treatment differences were significant ($P< 0.05$) only upto day 15 after the application of fish toxicants.

**Biochemical Oxygen Demand (BOD) :**

Followed by an initial increase, the BOD values declined in any of the systems (Fig. 15). Distinctly higher values (0.7-5.2 mg $l^{-1}$) were observed in (M) whereas, the values in other two treatments along with the control did not differ much and ranged from 0.9-4 mg $l^{-1}$ during the investigation period. The overall treatment differences were significance ($P< 0.05$).

**Chemical Oxygen Demand (COD) :**

The COD values increase gradually in all the treatments after application of fish poison, then gradually declined. The values in the control were always much lower (18.1-27.16 mg $l^{-1}$) than any of the treatments employed (Fig. 16). Treatment differences were insignificant during most of the periods of observations.

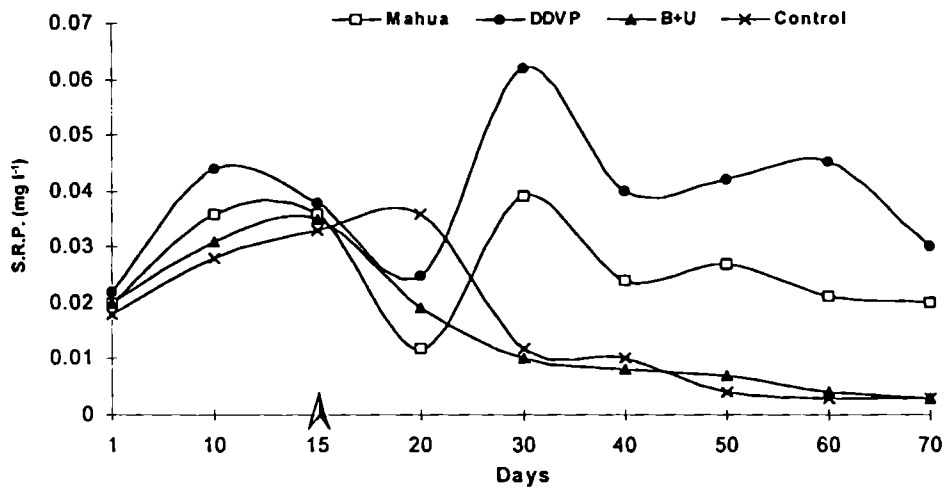


**Fig. 12 : Temporal changes in the Soluble Reactive Phosphate concentration of water under different treatments employed.**

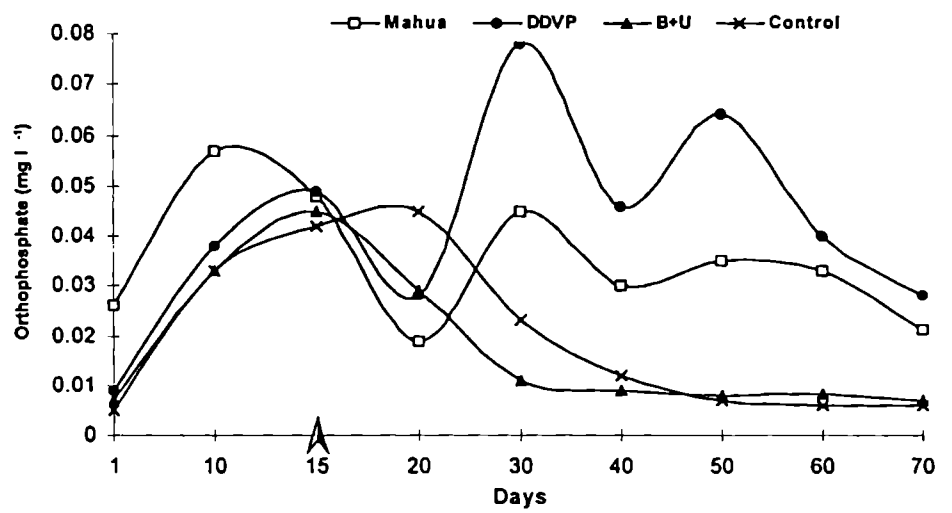


**Fig. 13 : Temporal changes in the Orthophosphate concentration of water under different treatments employed.**

↑ – indicates application of fish toxicants

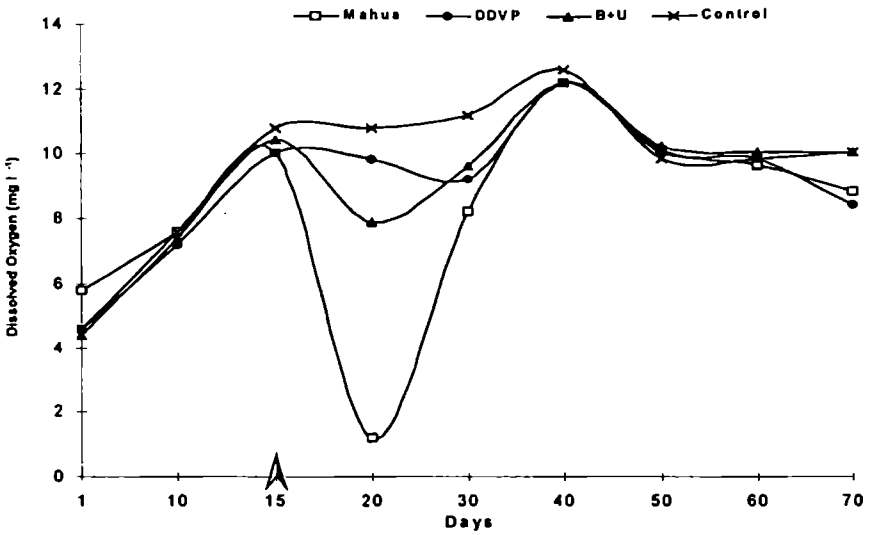


Fig. 14 : Temporal changes in the Dissolved oxygen concentration of water under different treatments employed.

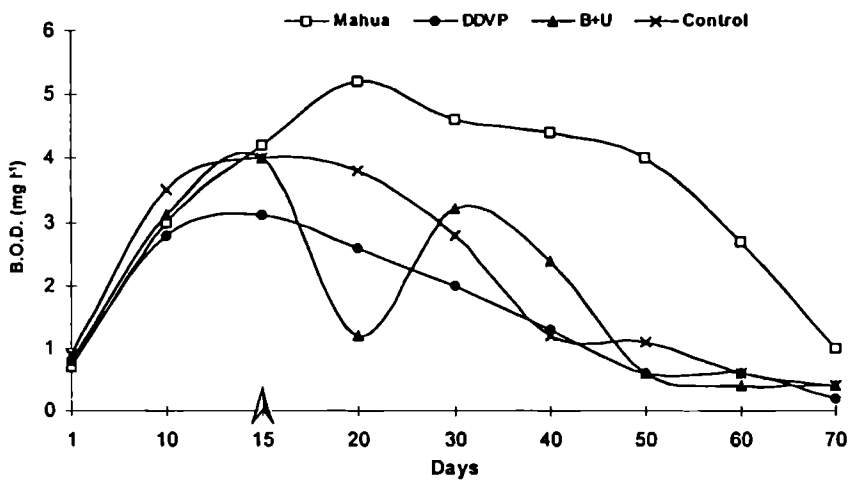


Fig. 15 : Temporal changes in the Biochemical Oxygen Demand of water under different treatments employed.

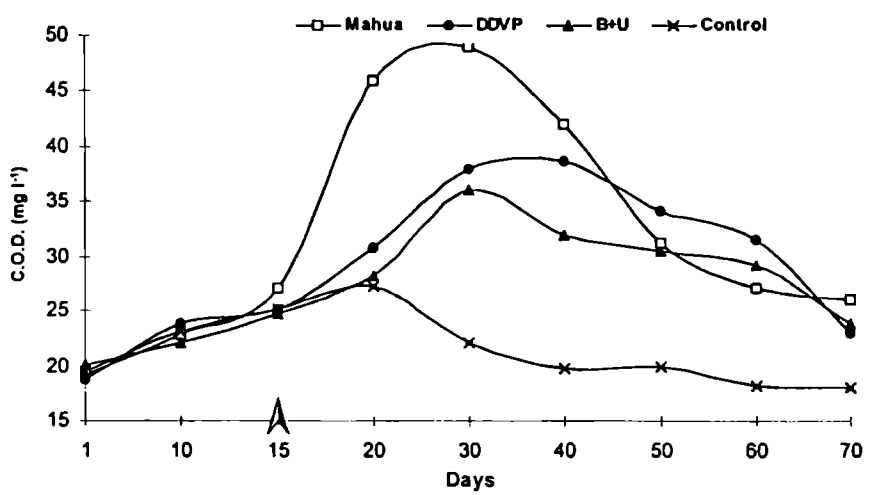


Fig. 16 : Temporal changes in the Chemical Oxygen Demand of water under different treatments employed.

⅄ – indicates application of fish toxicants

**4.1.3 Physico-chemical parameters :**

Water temperature ranged from 28°-34°C during the period of investigation with little variations among the treatments (Table 4).

The minimum pH (7.1) was encountered in (M) whereas, the maximum pH (8.4) was encountered either in (D) or (B + U).

The overall mean value of dissolved free carbon di-oxide was highest (2.88 mg $l^{-1}$)in (M) followed by 0.38 mg $l^{-1}$ either in (D) or (B + U) and lowest in control (0.22 mg $l^{-1}$). However, the maximum value (16.9 mg $l^{-1}$) of carbonate alkalinity was encountered in (D) and minimum (10.55 mg $l^{-1}$) in (M). The values are not significantly different in (B + U) (13.88 mg $l^{-1}$) and control (13.33 mg $l^{-1}$). Similar to dissolved free carbon di-oxide highest mean value of bicarbonate alkalinity (139.7 mg $l^{-1}$) was observed in (M). The values in other treatments were not prominantly different from each other (121.38-122.5 mg $l^{-1}$) (Table 4). The variation between the minimum and maximum values of hardness ranged from 0.17-1.26 mg $l^{-1}$ times in the treatments and the overall mean value was highest in (M) (180.5 mg $l^{-1}$) followed by (C) (160 mg $l^{-1}$), (B + U) (154 mg $l^{-1}$), (D) (142 mg $l^{-1}$) respectively. The overall mean value of dissolved organic carbon in (M) (3.3 mg $l^{-1}$) was 15.1-31.9% higher than (D) and (B + U) respectively.

The magnitude of variation between the minimum and maximum values of plankton volume was distinctly higher (> 300 times) in (D) followed by (M) (> 150 times) and (B + U) (> 100 times) respectively. Such variation was however very less (< 4 times) in the control. The highest volume of total plankton was achieved in (M) (0.49 ml/20 l), and lowest in (B + U) (0.37 ml/20 l).

Exhibiting 16 times variations between the minimum and maximum value of NPP, the treatment with DDVP showed highest mean value (168.4 mg C/$m^3$/hr) which was only 22.47 and 17.69 % higher than (B + U) and (M) respectively. Similar to plankton volume the flactuation of NPP in control was 4 times. Community respiration in (M) (135.4 mg C/$m^3$/hr) was distinctly higher than the lowest value (69.79 mg C/$m^3$/hr) encountered in (D). The values were not distinctly different in (B + U) (99.65 mg C/$m^3$/hr) and control (98.18 mg C/$m^3$/hr). The gross

Table 4 : Range value for pH and mean values (±SE) of different physico-chemical parameters of water :

| Parameters | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mahua Oil Cake | | DDVP | | Urea + Bleach | | Control | |
| | Range | Mean±SE | Range | Mean±SE | Range | Mean±SE | Range | Mean±SE |
| Temperature ($^0$C) | 28-34 | 31±3.18 | 28-34 | 31±3.18 | 28-34 | 31±3.18 | 28-34 | 31±3.18 |
| Hydrogen-ion concentration | 7.1-8.3 | - | 7.9-8.5 | - | 7.6-8.5 | - | 7.6-8.4 | - |
| Dissolved Oxygen (mg $l^{-1}$) | 1.2-12.2 | 7.9±1.01 | 4.6-12.2 | 8.4±0.87 | 4.4-12.2 | 8.62±0.84 | 4.6-12.6 | 8.82±0.82 |
| Dissolved free carbon di-oxide (mg $l^{-1}$) | 0-15 | 2.88±1.91 | 0-1.5 | 0.38±1.39 | 0-1.5 | 0.38±1.39 | 0-1.0 | 0.22±1.15 |
| Carbonate alkalinity (mg $l^{-1}$) | 0-20 | 10.55±2.75 | 0-35 | 16.9±3.63 | 0-25 | 13.88±2.5 | 0-20 | 13.33±2.33 |
| Bicarbonate alkalinity (mg $l^{-1}$) | 95-120 | 139.7±15.98 | 70-187.5 | 121.38±17 | 80-180 | 122.5±15.24 | 85-192.5 | 121.38±14 |
| Hardness (mg $l^{-1}$) | 100-280 | 180.5±29.3 | 80-210 | 142.22±17.44 | 90-250 | 154±21.77 | 100-230 | 160±17.41 |
| Dissolved organic carbon (mg $l^{-1}$) | 2-4.5 | 3.3±0.28 | 1.9-3.9 | 2.35±0.25 | 1.7-4.1 | 2.8±0.3 | 1.9-4.2 | 2.99±0.36 |
| Plankton volume (ml/20 lt) | 0.006-1.0 | 0.49±0.14 | 0.004-1.2 | 0.47±0.19 | 0.008-0.9 | 0.37±0.11 | 0.24-0.89 | 0.45±0.09 |
| Net primary productivity (mg C/$m^3$/h) | 31.25-312.5 | 138.61±31.5 | 31.25-500 | 168.4±52 | 75-250 | 130.55±25.1 | 62.5-250 | 141.5±19.8 |
| Community respiration (mg C/$m^3$/hr) | 56.25-225 | 135.4±24.6 | 37.5-112.5 | 69.79±9.03 | 75-150 | 99.65±8.53 | 50-150 | 98.18±10.9 |
| Gross primary productivity (mg d/$m^3$/hr) | 106.25-537.5 | 274.03±28.1 | 68.75-550 | 238.19±37.2 | 150-400 | 230.20±22.3 | 112.5-329.875 | 239.69±12.2 |

primary productivity value was maximum in (M) (274.03 mg C/$m^3$/hr) followed by control (239.69 mg C/$m^3$ /hr.), (D) (238.19 mg C/$m^3$/hr.) and (B +U) (230.20 mg C/ $m^3$/hr).

**4.2 Soil**

**4.2.1 Microbial parameters :**

**Total heterotrophic bacteria (THB) :**

The THB population of soil, as expected, exhibited in higher population densities as encountered in the water medium. The maximum mean value (30.83 x $10^5$ nos. $g^{-1}$) was observed in (B + U) and minimum value (7.44 x $10^5$ nos.$g^{-1}$) was observed in (D) (Fig. 17 ).

Treatment differences were significant ($F \geq 7.667$; $P < 0.01$) in most of the dates of observations.

**Cellulose decomposing bacteria (CDB) :**

Likewise CDB population in water, the values in the soil sharply declined after application of the toxicants. However, unlike the water system no peak was observed in the treatment with mahua during the second half of observation in the soil system (Fig. 18).

Overall treatment differences were significant ($F \geq 3.384$; $P < 0.05$) althroughout, although differences among (D), (B + U) and control became insignificant ($P > 0.05$) on day 40 onwards.

**Phosphate solubilizing bacteria (PSB) :**

The responses of PSB in soil was almost indentical to that of water and distinctly higher (9.64 x $10^4$ nos. $g^{-1}$ – 11.31 x $10^4$ nos. $g^{-1}$) values were observed in the treatment with (D), compared to the other systems (Fig. 19).

Although, treatment differences were significant ($F \geq 29.367$; $P < 0.001$) during the major part, CD test (CD = 1.5933) revealed that differeces among (M), (B + U) and control were insignificant ($P > 0.05$).

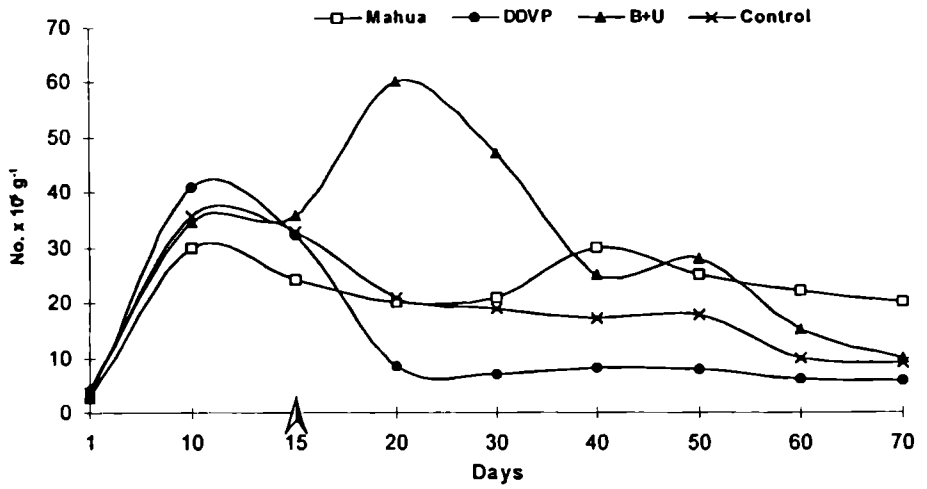


**Fig. 17 : Temporal changes in the population of total Heterotrophic bacteria in soil under different treatments employed.**

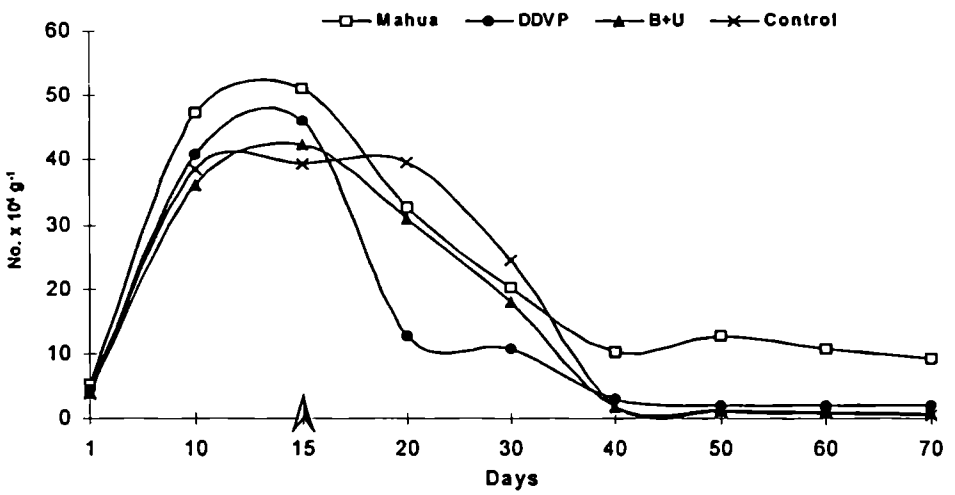


**Fig. 18 : Temporal changes in the population of Cellulose decomposing bacteria in soil under different treatments employed.**

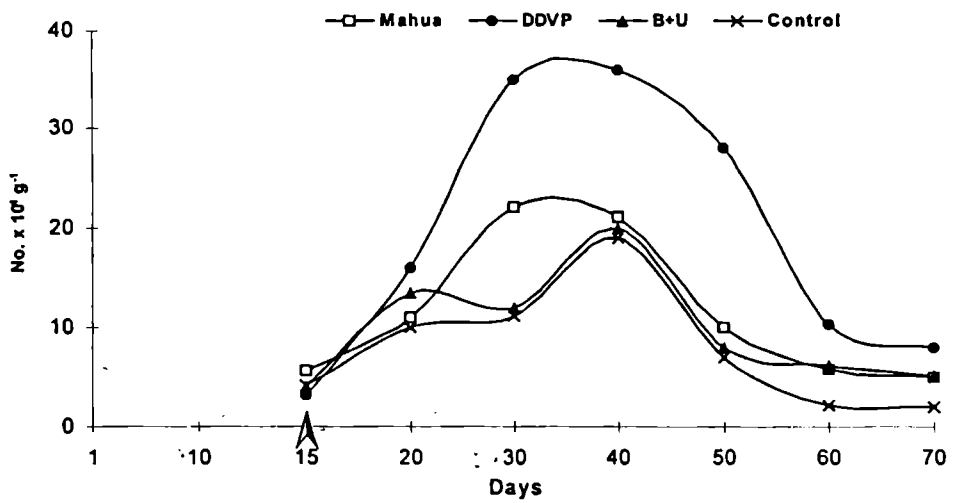


**Fig. 19 : Temporal changes in the population of Phosphate solubilizing bacteria in soil under different treatments employed.**

⍋ – indicates application of fish toxicants

**Nitrogen fixing bacteria (NFB) :**

The values of NFB attained peak during 40 to 50 days in any of the treatments employed (Fig. 20). The overall mean value was highest (35.167 x $10^2$ nos. $g^{-1}$) in (D) followed by (B + U) (32.244 x $10^2$ nos. $g^{-1}$), control (28.422 x $10^2$ nos. $g^{-1}$) and (M) (25.133 x $10^2$ nos. $g^{-1}$).

Statistical analysis (ANOVA) exhibited a clearcut significance ($F \geq 13.524$; $P < 0.001$) among the treatment means throughout the period of observations excepting during the last date when the difference became narrowed down.

**Amonifying bacteria (AB) :**

After a sharp decline, the values of AB increased substantially in all the treatments (Fig. 21). The maximum value (25.683 x $10^5$ nos. $g^{-1}$) was attained in (M) followed by (B +U) (17.283 x $10^5$ nos. $g^{-1}$), control (15.95 x $10^5$ nos. $g^{-1}$) and (D) (9.144 x $10^5$ nos. $g^{-1}$).

Highly significant ($F \geq 4.421$, $P < 0.05$) differences were observed among the treatment means during the period of investigation.

**Denitrifying bacteria (DNB) :**

The values of DNB population gets stabilized after a sharp fall till day 25 in all the treatments. (Fig. 22) The mean values were comparatively higher in (B + U) ( 2.933 x $10^5$ nos. $g^{-1}$) and (M) (2.336 x $10^5$ nos. $g^{-1}$) than (C) (2.058 x $10^5$ nos. $g^{-1}$) and (D) (1.116 x $10^5$ nos. $g^{-1}$).

Differences were significant ($F \geq 5.513$; $P < 0.05$) only in about 50% of the total observations. However, insignificant ($P > 0.05$) differences were observed between (M) and (B + U).

**Ammonia oxidising bacteria (AOB) :**

The temporal pattern of changes of AOB in soil was identical to that of its' water counter part (Fig. 23). The maximum value (43.5 x $10^2$nos. $g^{-1}$) was observed in (B + U) followed by (M) (32.833 x $10^2$ nos. $g^{-1}$), (D) (19.717 x $10^2$ nos. $g^{-1}$) and control (12.167x $10^2$ nos. $g^{-1}$).

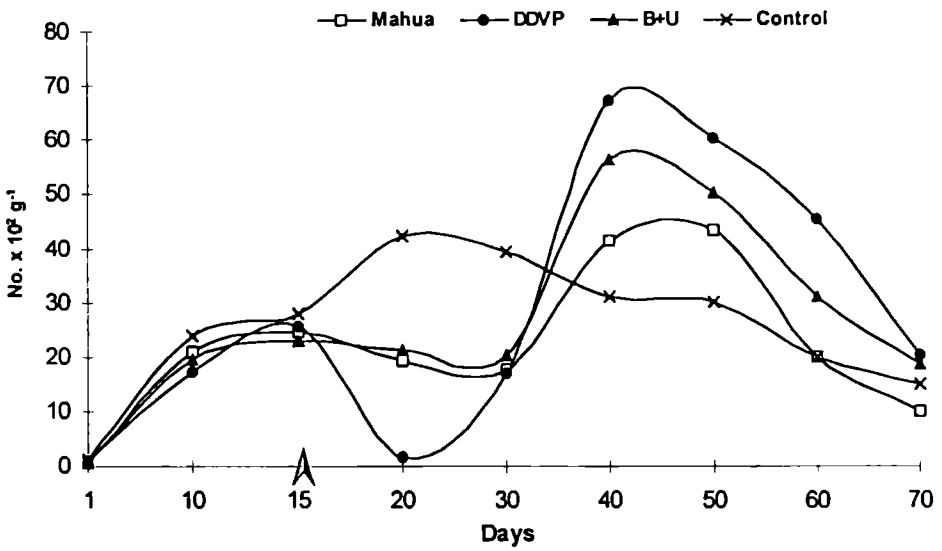


Fig. 20 : Temporal changes in the population of Nitrogen fixing bacteria in soil under different treatments employed.

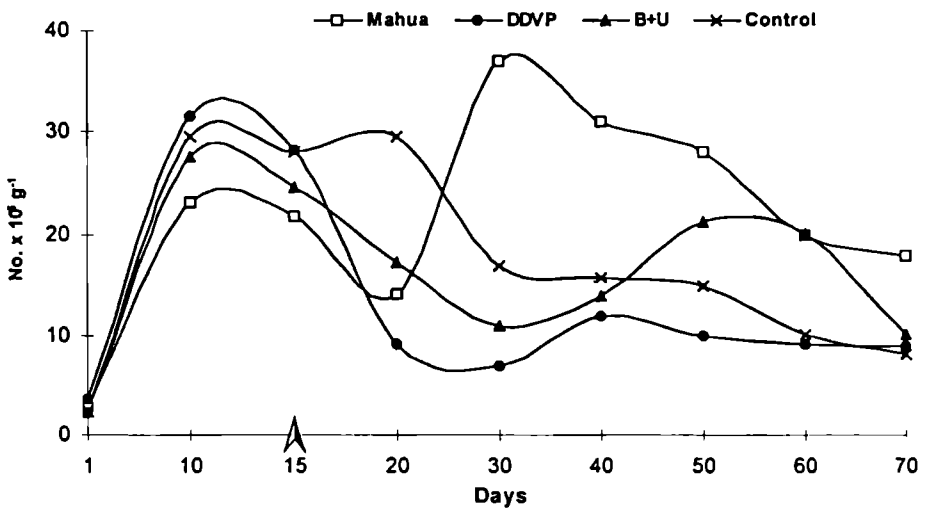


Fig. 21 : Temporal changes in the population of Ammonifying bacteria in soil under different treatments employed.

⍋ – indicates application of fish toxicants

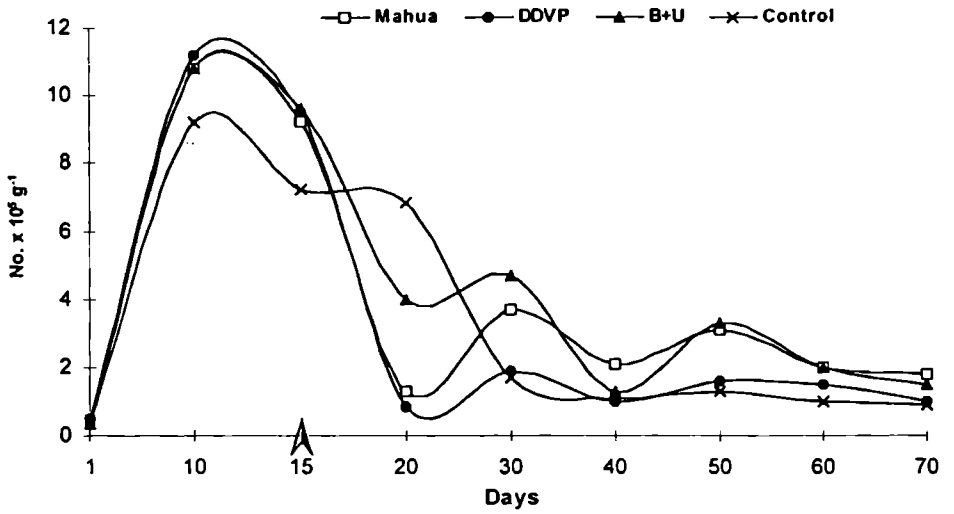


Fig. 22 : Temporal changes in the population of Denitrifying bacteria in soil under different treatments employed.

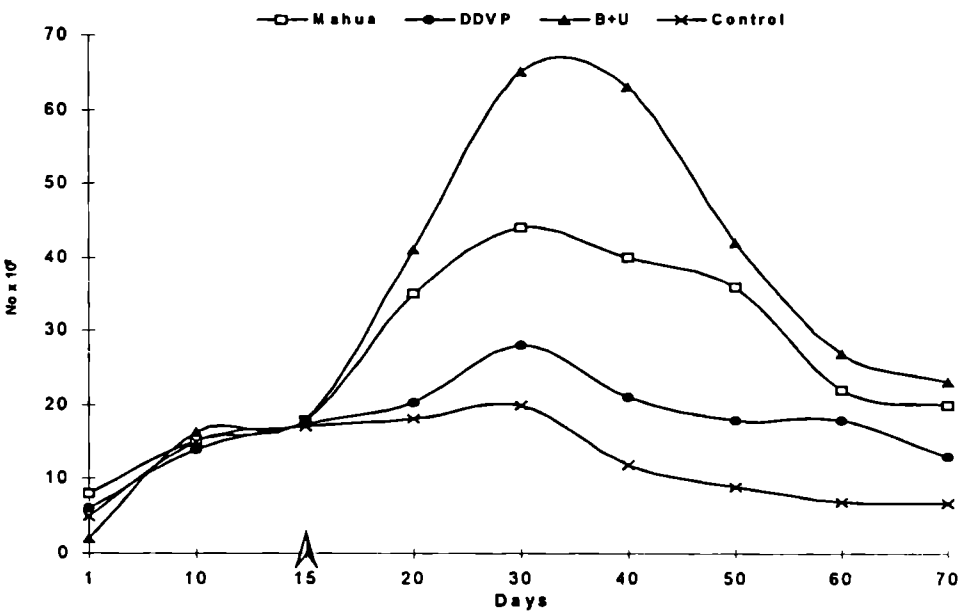


Fig. 23 : Temporal changes in the population of Ammonia oxidizing bacteria in sediment under different treatments employed.

⅄ – indicates application of fish toxicants

Treatment differences either among the systems or between any possible pair of treatments were significant ($F \geq 22.12$; $P < 0.001$) throughout the period of observations.

**4.2.2 Physico-chemical parameters of soil :**

Soil pH did not differ much among the treatments and it ranged from 7.6-8.2 during the study period. The maximum mean value (1.21 mg $100g^{-1}$) of available nitrogen in (M) was 29.75-36.36% higher than (D) (0.77 mg $100g^{-1}$) and (B + U) (0.85 mg 100 $g^{-1}$). On the contrary the highest mean value of available phosphate was achieved in (D) (1.16 mg $100g^{-1}$) followed by (M) (0.95 mg 100 $g^{-1}$), (C) (0.72 mg $100g^{-1}$) and (B +U) (0.61 mg 100 $g^{-1}$). The soil organic carbon was maximum (0.685%) in (M) and minimum in (D) (0.466%) (Table 6).

**4.3 Fish mortality and growth :**

Fish mortality calculated from the recovered fishes at the time of harvest indicated that the maximum mortality (46%) was encountered in (M) and minimum in (26%) in control. Such mortality (26%) did not differ between (D) and (B + U).

The absolute growth achieved in (M) (2.32 g) was maximum and closely followed by (B + U) (2.10 g). The growth in (D) was much lower (0.54 g). However, the production of fish in (B + U) (58 g) was 22.8% and 27.2% higher compared to (M) and (D) respectively. The production was lowest in control (40.7 g) (Table 5).

**Table 5 : Mortality and growth performances of *Cyprinus carpio* in different treatments**

| | Treatments | | | |
|---|---|---|---|---|
| | (M) | (D) | (B + U) | (C) |
| Mortality (%) | 46 | 30 | 30 | 26 |
| Net growth (g/fish) | 2.32 | 0.54 | 2.10 | 0.30 |
| Yield (g/cistern) | 44.76 | 42.2 | 58.0 | 40.7 |

Table 6 : Range value for pH and mean values (±S.E.) of different physico-chemical parameters of soil :

| Parameters | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mahua Oil Cake | | DDVP | | Urea + Bleach | | Control | |
| | Range | Mean±SE | Range | Mean±SE | Range | Mean±SE | Range | Mean±SE |
| Hydrogen-ion concentration | 7.6-8.1 | - | 7.7-8.2 | - | 7.8-8.1 | - | 7.6-8.1 | - |
| Available-N (mg 100 $g^{-1}$) | 0.55-3.1 | 1.21±0.28 | 0.55-1.42 | 0.77±0.09 | 0.55-1.42 | 0.85±0.12 | 0.55-1.45 | 0.82±0.11 |
| Available P (mg 100 $g^{-1}$) | 0.4-2.0 | 0.95±0.24 | 0.4-2.5 | 1.16±0.32 | 0.4-1.75 | 0.61±0.2 | 0.4-1.5 | 0.72±0.16 |
| Organic carbon (%) | 0.26-1.2 | 0.685±0.11 | 0.28-0.62 | 0.466±0.04 | 0.2-0.78 | 0.497±0.06 | 0.2-1.4 | 0.6±0.12 |

A negetive impact of any of the fish toxicant immediately after it's application was pronounced in the poplulation of CDB, AB, DNB and NFB of both water and soil phase of the environment. However, CDB population of water in mahua treatment recovered quickly and attained pre-treatment level within 25-30 days after application (Fig. 3). This is identical also in case of NFB population both in (M) as well as in (B+U) treatment (Fig. 5).

The greater abundance of CDB population in mahua treatment might have contributed to the dissolved organic carbon content of water, as a direct relationship was exhibited between them, also the highest concetration of organic carbon (3.3 mg $l^{-1}$) was acheived in the above treatment. The higher level of organic carbon of water in (M) helps in sustaining greater abundance of nearly all the mineralizing microbes tested. Several literatures emphasize the role of carbon as energy source for the microbial population operating in the mineralizing process (Olah, 1986 ; Avnimelech, 1999). Also, the favourable N : P ratio in (M) (4.5-5.5) contributed to the higher population of most of the mineralizing bacterial populations. Gaudy and Gaudy (1980) explained that N : P ratio normally required for microbial growth is around 4.

The population of AOB increased in any of the treatments employed. Such increase was maximum in (B+U) treatment because urea component of this treatment hydrolysed and the resultant ammonia (Mattice *et al.*, 1981 ; Mohanty *et al.*, 1993) perhaps acted as a substrate for the AOB population. This is evident because of the direct relationship ($r=0.744$ ; $P< 0.001$) between them. Sugiyama and Kawai (1978) observed that a high rate of ammonia oxidation proceeds in the water bodies with high DO level.

As expected, a direct relationship between AOB and $NO_3$ - N concentration of water ($r \geq 0.78$ ; $P < 0.001$) was exhibited in any of the treatments. This might be explained as the AOB population under a congenial oxidizing environment acted upon the ambient $NH_4$-N of water resulted in increased amount of $NO_3$ -N. Several authors (Brix, 1994 ; Reddy and D' Angelo, 1994) confirmed nitrifying-denitrifying process as the dominant nitrogen removal mechanism in aquatic systems and nitrification is the limiting step (Willadsen *et al.*, 1990) which is primarily an aerobic process regulated by availability of dissolved oxygen (Reddy *et al.*, 1980).

NFB population of both water and soil irrespective of treatments decreased exponentially with increasing values of total inorganic nitrogen ($NH_4$ +$NO_2$+$NO_3$) of water as depicted in Fig. 24. Such a modular relationship has been explained by 68% and 52% in case of NFB population of water and soil respectively. Therefore, it might be concluded that nitrogen fixation mediated by NFB in aquatic system is more intense in a nitrogen defficient environment. It implies that fixation becomes advantageous only when $NO_3$ and $NH_4$ are no longer available (Welch, 1996) and nitrogen fixation decreases with higher concentration of nitrate and ammonia and in the presence of oxygen (Wetzel, 1975; Petterson and Bostrom, 1989). Therefore, one would expect an inverse relationship between the rate of nitrogen fixation and the concentration of combined nitrogen in water bodies (Wetzel, 1983).

A sharp fall in the DO values of water in mahua treatment was expected because of high organic loading following it's decomposition. This is evident from the fact that the BOD values increased simultenously (Fig. 15) indicating oxygen demand due to the decomposition process involved. Such decreasing trend of DO with simultaneous increase in BOD following mahua oil cake application upto 5 days was also observed by Nath (1983).

Both SRP and orthophosphate concentration of water consistantly exhibited higher values following application of DDVP (Fig. 12, 13). This is because DDVP instantly supplied inorganic phosphorus to the medium. Ghatak and Konar (1992) observed that application of DDVP as fish toxicant resulted in the release of

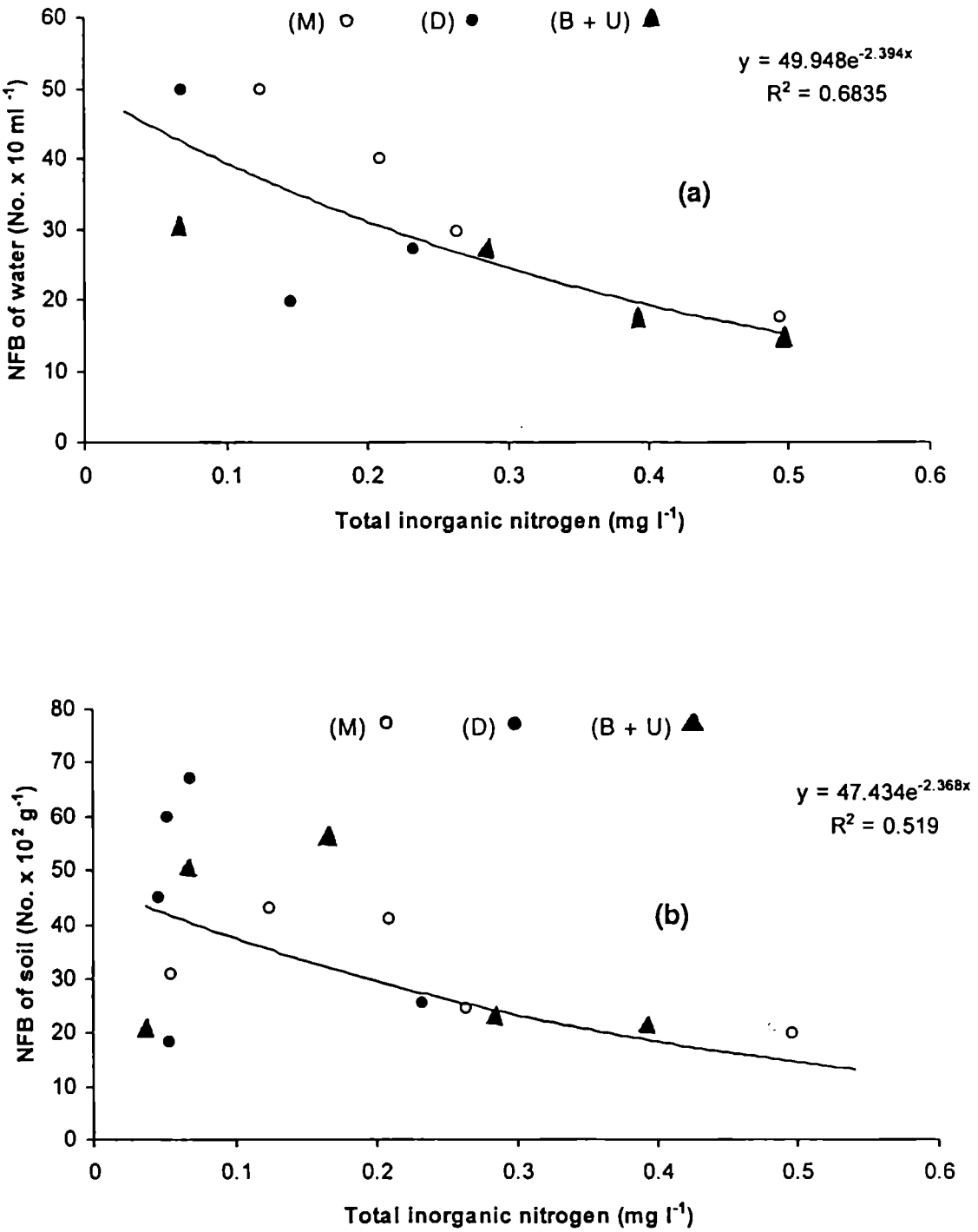


**Fig. 24 : Relationship between NFB population of water (a) and soil (b) with total inorganic nitrogen of water.**

large amount of phosphate in pond water but no significant fluctuation of nitrite and nitrate level. But such high concentration in this treatment (0.039-0.042 mg $l^{-1}$) seemed to be eutrophic as per the classification adopted by Reckhow and Simpson (1980), and Welch (1996).

The net primary productivity in (M) (138.61 mg C/$m^3$/hr) did not differ much with (B+U) (130.55 mg C/$m^3$/hr). The DO values among the treatments attained maximum in (B+U) (8.62 mg $l^{-1}$), also the dissolved organic carbon in (B+U) (0.37 mg $l^{-1}$) is satisfactory.

Application of (B+U) shifted the environment to a P-limitted condition with high N :P ratio (10-45) during a considerable period. Contrary to this DDVP application resulted the environment to somewhat N-limited as the N:P ratio remained consistantly lower (2-3) (Fig. 25).

The high mortality rate (46%) encountered in (M) is perhaps due to the high organic load under a very shallow culture environment. However, the absolute growth achieved in this treatment was maximum (2.32 g) because of the congenial N:P ratio (4.5-5.5) during most of the culture period (Fig. 25). A congenial N : P ratio of 4 : 1 to 8 : 1 has been advocated in aquatic production systems (Winberg and Liakhnovich, 1965). However, net yield was maximum in (B+U) as because urea, a component of the treatment besides acting as a piscicide influenced favourable growth of fish food organisms. Mohanty *et al.* (1993) opined that the combination treatment of bleaching and urea helped in the growth of natural fish food organisms such as algae, diatoms and rotifers and can be consiered beneficial in fish culture system.

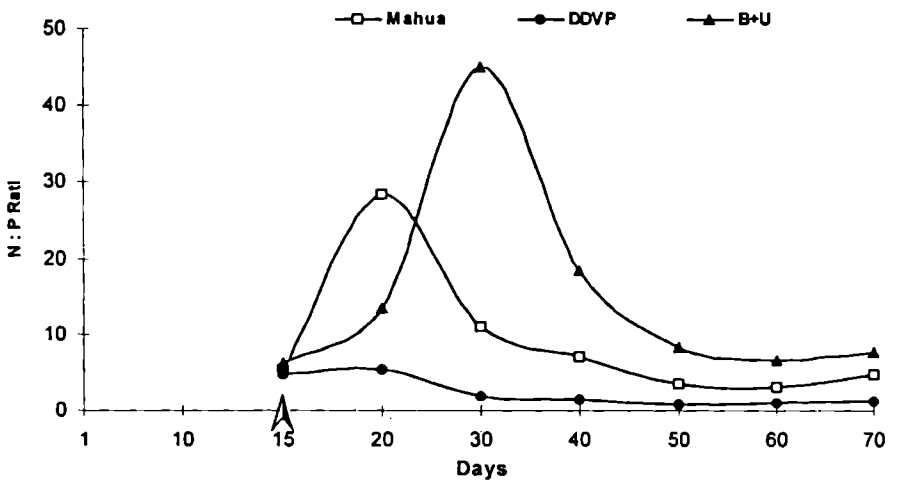


**Fig. 25 : Changes in N : P ratio of waters in different treatments employed.**

⍋ – indicates application of fish toxicants

# 6. Summary

Comparative responses of three selective pisicides of chemical and plant origin upon some of the bio-geochemical cycling bacteria under simulated pond culture condition in the outdoor have been studied. The whole work embodied in this thesis consisting of seven chapters. viz. Introduction, Review of Literature, Materials and Methods, Results, Discussion, Summary and References. Some interesting observations has been made on the subject and valuable informations have come out during the course of a short investigation period. The main outcome of this work is tabulated herein :

i) All the three piscicides are equally effective in eradicating the test predatory fish *Channa punctatus*.

ii) Toxicity of any of the piscicides deminished within three weeks.

iii) Immediately after application, each toxicant exerted a negetive impact upon the population of nutrient mineralizing bacteria like CDB, DNB, NFB and AB both in water as well as soil phase of the environment.

iv) CDB and NFB population recovered within 25-30 days in mahua and bleaching - urea combination treatments.

v) CDB population in mahua treatment and AOB population in bleaching-urea combination treatment was favoured because of high concentration of organic carbon (3.3 mg $l^{-1}$) and ammonia respectively.

vi) A direct relationship between AOB population and $NO_3$-N of water ($r \geq 0.78$ ; $P < 0.001$) as well as between AOB population and $NH_4$-N ($r = 0.744$ ; $P < 0.001$) was achieved.

vii) DDVP treatment instantly caused eutrophication because of high concentration of inorganic phosphate (0.039 - 0.042 mg $l^{-1}$) in the water phase.

viii) An exponentially decreasing pattern of relationship has been modelled between total inorganic nitrogen of water and NFB population in the water phase ($y = 49.948e^{-2.39x}$) and soil phase ($y = 47.434e^{-2.368x}$) which is explained by 58% and 62% respectively.

ix) The favourable N:P ratio (4.5-5.5) during most of the culture period has been attained in mahua application.

x) Combination treatment of bleaching and urea shifted the environment to a P limited condition with high N:P ratio (10-45), whereas, DDVP application resulted the environment to N limited as the N:P ratio remained consistantly lower (2-3).

# 7. References


Acharjee, S. K. and Biswas, A. 1995. Some contributory factors on the level of production in composite fish culture. Environment and Ecology, 13 (1) : 22-25.

Alexander, M. 1978. Introduction to soil microbiology. Willey Eastern Limited, New Delhi : 467 pp.

Alikunhi, K. H., Chaudhuri, H. and Ramachandran, V. 1955. On the mortality of carp fry in nursery ponds and the role of plankton in their survival and growth. Indian J. Fish., 2 (2) : 257-313.

Alikunhi, K. H. 1957. Fish culture in India. Fm. Bull. Indian Coun. Agri. Res., 20:144 pp.

Anon. 1981. Proceedings of the 5th Workshop of AICRP on composite fish culture and fish seed production. CIFRI, Dhauli, Orissa, India, 19-20 Jan.

Antipchuk, A. F. 1979. Microbiological control in the fish farming ponds ( in Russia) Pishevaya Promishlenolv, Moscow : 145 pp.

APHA. 1995. Standard Methods for the examination of water and waste water. 19th Edition, American Public Health Association, Washington, New York.

Avnimelech, Y. 1999. Carbon / Nitrogen ratio as a control element in aquaculture systems. Aquaculture, 176 : 227-235.

Ayyappan, S. 1992. Microbial communities in aquatic production and deomposition process. Training mannual on aquaculture biotechnology, Central Institute of Freshwater Aquaculture, ICAR : 19-27.

Ayyappan, S. 1992. $N_2$ fixation and biofertilization in fish pond ecosystem. Training Mannual on Aquaculture Biotechnology, Central Institute of Freshwater Aquaculture, ICAR : 31-36.

Banerjee, S. C. 1970. Detoxification of pond water treatment with endrin. Indian J. Appl. Chem., 33 : 285 - 290.

Banerjee, S., Das, A. and Chakraborty, P. S. 1987. Evaluation of efficiency of mahua oil cake as fish poison in relation to hardness of water. Environment and Ecology, 5 : 165-167.

Banerjee, S. 1991. Control of weed and predatory fish. Training manual on 'Intensive Fish Farming'. Ramkrishna Ashram Krishi Vigyan Kendra, Nimpith, West Bengal : 1-6.

Bhatia, H. L. 1970. Use of mahua oilcake in fishery management. Indian Fmg., 20 (4) : 39-40.

Bhuyan, B. R. 1967. Eradication of unwanted fish from ponds by using indigenous plant fish poison. Sci. Cult., 33 (2) : 82 -83.

Boyd, C. E. 1982. Water quality management for pond fish culture. Elsevier Scientific Publishing Company, New York : 318 pp.

Boyd, C. E. 1995. Bottom soils, sediment and pond aquaculture. Chapman and Hall, New York : 258-264.

Brix, H. 1994. Consctructed wetland for municipal wastewater treatment in Europe. In : Global Wetlands : Old World and New (Ed. W. J. Mitsch), Elsevier Science BV, Amsterdam, The Netherlands : 325-333.

Chakraborty, D. P., Nandy, A. C. and Philipose, M. T. 1972. *Barning acutangula* (Linn.) Gaertn as a fish poison. Ind. J. Expt. Biol., 10 (1) : 78-80.

Chan, K., and Kueh, C. S. W. 1976. Distribution of heterotrophic bacteria related to some environmental factors in Tolo herbour. Int. J. Ecol. Environ. Sci., 1 : 47-57.

Chatterjee, Subimal and Ganguli, Swapna 1993. Effect of mahua oil cake on the blood of the fish *clarias batrachus*. Environment and Ecology, 11 (4) : 888-891.

Choudhuri, H. 1960. Contribution to the techniques of pond fish culture in India. Unpublished D. phill. Thesis. University of Calcutta.

Coffin, C. C. Hayes, F. R., Godfrey, L. H. and Whiteway, S. G. 1949. Exchange of materials in a lake as studied by the addition of radio active poshphorus. Can. J. Res., 27 : 207-222.

Colt, J. and Armstrong, D. 1979. $N_2$ toxicity to fish, crustaceans and mollucs. Dept. Civil Eng. California : 30 pp.

Cope, O. B., Gjullin, C. M. and Storm, A. 1947. Effects of some insecticides on trout and salmon in Alaska with reference to blackfly control. Trans. Am. Fish. Soc., 77 : 160-177.

Cottam, C. and Higgins, E. 1946. DDT : Its' effect on fish and wildlife services. Circular, 11 : 1-14.

Das, P. R. 1969. A preliminary note on the toxicity of the plant *Derris trifoliata* lour on fishes. J. Indian Pharmac. Mfr., 7 (4) : 197-200.

Dickman, S. R. and Bray, R. H. 1940. Colorimetrie determination of phosphorus. Ind. Eng. Chem. A. E., 12 : 665-668.

Doudoroff, P. Katz, M. and Tarzwell, C. M. 1954. Toxicity of some organic insecticides to fish, sewage and industrial wastes, 25 (7) : 840-844.

Drews, G. 1974. Microbiologisches Praktikum. Springer-Verlag, Berlin, Heidelberg, New York : 230 pp.

Eren, Y., Tsur, T. and Avnimelech, Y. 1977. Phosphorus fertilization of fish ponds in the upper Galilee. Bamidgeh, 29 : 87-92.

Fair, G. M. and Geyer, J. C. 1954. Water supply and waste water disposal. John Wiley and Sons. Inc. New York, London : 973 pp.

Gaudy, A. F. and E. T. Jr. Gaudy. 1980. Microbiology for environmental scientist and engineers. McGraw-Hill, New York : 736 pp.

Ghatak, D. B. and Konar, S. K. 1992. Chronic effects of mixture of heavy metal, pesticide, detergent and petroleum hydrocarbon in combination on plankton, benthic organisms and water quality. Environment and Ecology, 10 (3) : 524-531.

Ghatak, D. B. and Konar, S. K. 1993. Impact of combinations of cadmium, pesticide DDVP, detergent phenol-J and petroleum hydrocarbon n-heptane on aquatic ecosystem. Environment and Ecology, 11 (3) : 553-559.

Golterman, H. L. Chgmo, R. S. and Ohnstad, M. A. M. 1978. Methods of physical and chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford : 213 pp.

Hall, C. B. 1949 : Ponds and fish culture. Faber and Faber, London.

Hargreaves, J. A. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture, 166 (3, 4) : 181-212..

Harrington, R. W. and Bidlingmayer, W. I. 1958. Effects of dieldrin on fishes and invertebrates of a salt marsh. J. Wildlife. Mgmt., 22 (1) : 78-82.

Hoffman, C. H. and Surber, E. W. 1949. Effects of serial application of DDT on fish and fish food organisms in two Pennsylvania water sheds. Prog. Fish. Cult., 11 (4) : 203-211.

Hogan, J. and Gray, L. 1950. The toxicity of aldrin and dieldrin to goldfish and bluegill sunfish. Ark., Game and Fish Com. : 10 pp.

Hossain, Md. M., Ghatak, D. B., and Konar, S. K. 1987. Acute toxicity of mixture of a nonionic detergent ekaline FI and an organic perticide DDVP to fish, plankton and worm. Environment and Ecology, 5 (4) : 778-781.

Ibrahim, K. H. 1957. Bionomics of forage fishes : observations on the fecundity of three common species of minor barbels. J. Bombay Nat. Hist. Soc., 54 (4) : 826-834.

Jackson, M. L. 1967. Soil Chemical Analysis, Prentice Hall of India Prt. Ltd. New Delhi : 498 pp.

Jana, B. B., Manna, A. K. and Kundu, G. 1987. Effect of an oilcake (*Brassica latifolia*) piscicide on the physico - chemical and biological spectrum of waterbodies. Limnologica, 18 (2) : 431-439.

Janakiram, K., Rao, G. R. M., Ayyappan, S., Purushothaman, C. S., Saha, P. K., Pani, K. C. and Muduli, H. K. 1988. A combination of commercial bleaching powder and urea as a potential piscicide. J. Aquaculture, 72 : 287-293.

Jena, S. 1979. Use of harbal piscicides. In : Proceedings of symposium on inland aquaculture, Feb 12-14. CIFRI, Barrackpore.

Jhingran, V. G. and Pullin, R. S. V. 1985. (Revised 1988). A hatchery mannual for the common chinese and Indian major carps. Asian Development Bank and International Centre for Living Aquatic Resource Management. Manila : 191 pp.

Jhingran, V. G., Natarajan, A. V., Banerjee. S. M. and David, A. 1988. Methodology on reservoir fisheries investigations in India. Bull -12, CICFRI, Barrackpore, W. B. India : 102 pp.

Jhingran, V. G. 1991. Fish and Fisheries of India. Hindustan Publishing Co-operation, Delhi, India : 395-397.

Khan, A. 1952. Manure and its source from oil cakes. J. Sci. Club, 5 : 14 - 17.

Konar, S. K. 1964. Field experiments on the eradication of predaceous insects by the insecticide DDVP., Indian J. Fishery, 11 : 689-698.

Konar, S. K. 1969. Laboratory studies on two organophosphate insecticides DDVP and phosphamidon as selective toxicants. Trans. Am. Fish. Soc., 98 : 430-437.

Krstulovic, N. 1989. Bacteriological evaluation of water quality of Kartela Bay. In: Proceedings of International Seminar on combat of pollution and the conservation of manine wealth in Mediterranean sea, 5-8 June, 9 : 117-139.

Lakshman, A. K. 1983. Mahua oilcake in fish culture. Environment and Ecology, 1 (3) : 163-167.

Lawrence, J. M. 1950. Toxicity of some new insectieides to several species of pond fish. Prog. Fish Cult., 12 (3) : 141-146.

Lee, J. S. 1973. Commerical catfish farming : The Interstate Printer and Publishers, Danville.

Mahapatra, B. C., Noble, A. 1993. Effect of 'Nuvan' on some biochemical and phsiological parameters of *Liza parsia.* Mariculture research, under the post graduate programme in mariculture, 54, part - 3, CMFRI : 53-57.

Mahapatra, B. C. and Thosar, V. R. 1999. Toxicity of plants and their derivatives on fishes. In : Aquaculture : 360-371.

Mathur, D. S. 1975. Toxicity of Nuvacron to selected fishes. Proc. Nat., Acad., Sci. Sec., B 45 : 130-132.

Marilyn Chakroff 1993. Fresh water fish pond culture and management. Scientific Publishers, Jodhpur : 171-172.

Markling, L. L. 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. Fisheries, 17 (6) : 6-11.

Mattice, J. S., Tsai, S. C. and Burch, M. B. 1981. Comparative toxicity of hypochlorus acid and hypochlorite ions to mosquito fish. Trans. Am. Fish. Soc., 110 (4) : 519-525.

Mohanty, A. N., Chatterjee, D. K. and Giri, B. S. 1993. Effective combination of urea and bleaching powder as a pesticide in aquaculture operations. J. Aquacult. Tropics, 8 (2) : 249-254.

Nandeesha, M. C., Kerhavanath, P., Dinesh, K. P. 1984. Effect of three organic manures on plankton production in fish ponds. Environment and Ecology, 2(4) : 311-318.

Nandy, A. C. and Chakraborty, D. P. 1976. A note on the use of unripe fruits of *Randia dumetorum* (Lam.) as a fish poison. J. Inland Fish. Soc. Ind., 8 : 134-136.

Natarajan, A. V. and Pathak, V. 1983. Pattern of energy flow in freshwater tropical and subtropical impoundments. CIFRI, ICAR, Bull No - 36, Barrackpore, West Bengal : 1-27.

Nath, D. 1979. Toxicity of mahua oilcake under Laboratory and Field conditions. In : Proceedings of symposium on inland aquaculture, Feb. 12-14. CIFRI. Barrackpore : 50 pp.

Nath, D. 1983. Nature and duration of toxicity and hydrological changes after application of mahua oilcake in fish ponds. J. Inland Fish. Soc. India, 15 (1, 2) : 69-72.

Olah, J. 1986. Carp production in manure ponds. In : Aquaculture of cyprinids (Eds. R. Billard and J. Marcel ). INRA, Paris, France : 293-303.

Olsen, S. R. , Cola, C. V., Wetanabe, F. S. and Dean, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept., Agr. Cir. : 939 pp.

Pal, A. K. 1983. Acute toxicity of DDVP to fish, plankton and worm. Environment and Ecology, 1 : 25 pp.

Pal, A. K. and Konar, S. K. 1985. Influence of organophosphorus insecticide DDVP on aquatic ecosystem. Environment and Ecology, 3 : 489-492.

Panigrahi, A. 1998. Evaluation of molluscicidal effect of the pesticide Nuvan on disease trenrmitting snail *Lymnaea acuminata* and *Bellamya bengalensis.* Environment and Ecologly, 16 (2) : 257-259.

Panwan, R. S., Kapoor, D., Joshi, H. C., Gupta, R. A. 1976. Toxicity of some insecticides to the weed fish, *Trichogaster fasciatus.* J. Inland Fish. Soc. India, 8, dec : 129-130.

Paul, D., Rauth, S. K. 1987. Comparative studies on the toxicity of endosulphan in some fresh water fishes under different pH and hardness of water. Curr. Sci., 56 (7) : 378-379.

Paul, R. and Jabeen, Z. 2000. Influence of three pesticides on the primary productivity of a fresh water ecosystem. Environment and Ecology, 18 (1) : 81-84.

Penschbacher, P. W. and Sarkar, J. 1989. Toxicity of selected pesticides to the snakehead, *Channa punctata.* Asian Fish. Sci., 2 (2) : 249-254.

Petterson, K. and Bostrom, B. 1989. Kvavcomsattining i limniska ekosystem - En literaturoversikt. Naturvardsverket Rapport 3822, Solna, 67 pp. (In Swedish, Summary in English).

Pillay, T. V. R. 1995. Aquaculture-Principles and Practices. Fishing New Books, Combridge, England : 575 pp.

Prochazkova, L., Blazka, P. 1984. Relationship between organic carbon and chemical oxygen demand. ACTA. Hydrochem. Hydrobiol., 12 (4) : 383-393.

Ram, K. J., Rao, G.R.M, Ayyappan, S., Purushothaman, C. S., Saha, P. K., Pani, K. C, Muduli, H. K. 1988. A combination of commercial bleaching powder and urea as a potential piscicide. J. Aquaculture, 72 (3, 4) : 287-293.

Ramachandran, V. 1960. Observations on the use of ammonia for eradication of aquatic vegetation. J. Sci. Ind. Res. Sect. C., 19 (11) : 284-285.

Ramaprabhu, T., Ghosh, S. R., Das, K. M. and Mohanty, A. N. 1986. Some observations on the use of ammonia as a fish toxicant. J. Comp. Anim. Physiol., 4 (1) : 39-48.

Ramaprabhu, T., Tripathy, S. D., Chatterjee, D. K., Jana, S., Das, K.M. 1990. Use of ammonia as a fish toxicant in ponds. Aquacult. Hung., 6 : 97-103.

Ramaprabhu, T., Tripathy, S. D., Chatterjee, D. K., Jena, S. and Das K. M. 1985. Use of anhydrous $NH_3$ as a fish toxicant in pond ecosystem. In : Proceedings of symposium aquaculture of carp, 2-5 Sept. INRA, Evry, France.

Rath, R. K. 1993. Fresh water Aquaculture. Scientific Publishers, Jodhpur, India : 493 pp.

Reckow, K. and J. Simpson. 1980. A procedure using modeling and error analysis for the prediction of lake phosphorus concentration from land use information. Can. J. Fish. Aqua. Sci., 37 : 1439-1448.

Reddy, K. R., Angelo, E. M. D. 1994. Soil processes regulating water quality in wetlands. In : Global Wetlands : Old World and new (Ed. W. J. Mitsch), Elsevier Science, B. V., Amsterdam, Netherland : 309-324.

Reddy, K. R., W. H. Jr. Patrick, and R. E. Phillips. 1980. Evaluation of selected processes controlling nitrogen loss in a flooded soil. Soil. Sci. Soc. Am. J. 44 : 1241-1246.

Rodina, A. G. 1972. Methods in aquatic microbiology. Baltimore University Press : 461 pp.

Saha, L. C., Choudhury, S. K. 1985. Phytoplankton density in relation to abiotic factors of a pond at Bhagalpur. Comp. Physiol. Ecol., 10 (2) : 98-100.

Sarkar, S. R. 1988. Experimental study on addition of super-phosphate and mahua oil cake for increased yield from fish ponds. Proc. Indian Acad. Sci. Anim. Sct., 97 (1) : 89-96.

Schaffer, A. 1993. Soil Biochemistry. (Eds. J. M. Bollag and G. Stotzkyed). Marcel Dekker, New York : 273 pp.

Sharma, B. K. 1974. On the survival and growth of major carp spawn in nurseries manured with organic fertilizers. J. Zool. Soc. India, 26 (1, 2) : 157-158.

Shirgur, G. A. 1972. Development of indigenous derris root powder. Indian Fish. Assoc., 2 (142) : 55-59.

Shrestha, S. B., Jha, S., Wagle, S. K. 1987. The effects of organophosphate insecticides in nursery ponds. NACA Newsletter, NACA, Bangkok, Thailand : 7 pp.

Shyam, R., Tripatly, N. K., Khan, H. A. 1993. Effect of the piscicide mahua oilcake and bleaching powder on benthic population in carp nursery ponds. J. Aquacult. Tropics, 8 (1) : 25-32.

Srivastava, U. S. and Konar, S. K. 1965. Use of phosphamidon for eradication of fresh water fish predators. Experentia., 21 : 390-391.

Srivastava, V. K. 1966. DDVP as a selective toxicant for the control of fishes and insects. Prog. Fish. Cult., 28 (4) : 235-238.

SubbaRao, N. S. 1977. Soil micro-organisms and plant growth. Oxford and IBH Publishing Co., Calcutta.

Sugiyama, M., Kawai, A. 1978. Microbiological studies on the $N_2$ cycle in aquatic environments. Bull. Jap. Soc. Sci. Fish., 44 (4) : 351-355.

Surber, E. W. 1948. Chemical control agents and their effects on fish. Prog. Fish. Cult., 10 (3) : 125-131.

Tang, Y. 1967. Improvement of milkfish culture in the Philippines. Curr. Aff. Bull. Indo - Pacif. Fish. Coun., 49 : 14-22.

Thosar, M. R. and Das, T. K. 1984. A study on the effect of some insecticides on the primary productivity. Indian. J. Environ. Hlth., 26 : 335-357.

Tomkins, J. A. and Tsai, C. 1976. Survival time and lethal exposure time for the blacknose dace exposed to free chlorine and chloramines. Trans. Am. Fish. Soc., 105 : 315-321.

Tripathy, N. K., Radheyshyam, Satpathy, B. B. and Khan H. A. 1980. *Preliminary* observations on the use of bleaching powder as fish toxicant for preparation of nursery ponds. In : Proceedings of symposium on utilization of animal resource of Orissa, Utkal University, Bhubaneswar, India, 22-23 March.

Tripathy, S. D. and Sharaf, R. K. 1974. Predatory role of weed fishes vis-a-vis aquatic insects, tadpole and froglets. JNKVV, Res. J., 8 (2) : 123-127.

Tripatly, S. D., Aravindakshan, P.K., Singh, Jana, S., Ayyappan, S., Muduli, H.K., Mohanty, A. N. 1991. Carp seed rearing in ammonia treated nurseries. Proceedings of National Symposium On New Horizons In Freshwater Aquaculture, 23-25 Jan. : 13-15.

Volenweiden, R. A. 1974. A manual on the methods of measuring primary production in aquatic environment. IBP handbook No.12, 2nd Ed. Blackwell Scientific Publications, Oxford : 225 pp.

Walkley, A. and Black, I. A. 1934. An examination of the Degrjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37 : 29-38.

Welch, E. B. 1996. Eutrophication. In : Ecological effects of wastewater. Applied Limnology and Pollutant Effects. Chapman and Hall, London : 425 pp.

Wetzel, R. G. 1975. Limnology. W. B. Saunders, Philadelphia, PA : 743 pp.

Wetzel, R. G. 1983. Limnology. 2nd Edition. W. B. Saunders Co., Philadelphia : 860 pp.

Wetzel, R. G. and Likens, G. E. 1991. Limnological Analyses. Springer - Verlag, New York : 391 pp.

White, C. R., Jr. 1955. Chlorine : its effect to goldfish, fathead minnows, golden shiners and blue gills and its removal from water. M. S. Thesis, Alabama Polytechnic Institute, Auburn, AL : 58 pp.

Willadsen, C. T., O. Riger-Kush, and B. Qvist. 1990. Removal of nutritive salts from two Danish root zone systems. In : Constructed Wetlands in Water Pollution Control (Eds. P. F. Cooper and B. C. Findlater), Pergamon Press, Oxford : 115-126.

Winberg, G. G. 1963. Primary production of bodies of water, U.S. Atom. Energy Comm. Div. Tech. Info. ABC - Tr : 5692 pp.

Winberg, G. G. and Liakhnovich, W. P. 1965. Udobrenie Prudov. Moskova : 268pp.

Young, L. A. and Nicholson, H. D. 1951. Stream pollution resulting from the use of organic insectieides. Prog. Fish. Cult., 13 (4) : 193-198.