STUDIES ON DEPURATION OF EDIBLE OYSTER *Crassostrea madrasensis* (PRESTON)

by
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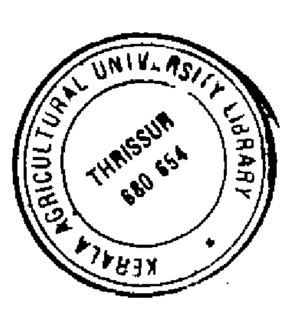

To
My
Daughter

DECLARATION

I hereby declare that this thesis entitled STUDIES ON DEPURATION OF EDIBLE OYSTER *Crassostrea madrasensis* is a bonafide record of research work done by me during the course of reaearch and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society

Place Panangad

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CERTIFICATE

Certified that this thesis, entitled " STUDIES ON DEPURATION OF EDIBLE OYSTER *Crassostrea madrasensis*" is is a record of research work done independently by Mrs Usha P.T under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her

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INTRODUCTION

INTRODUCTION

Molluscan shellfish such as oysters, clams and mussels are soft bodied animals, that are enclosed in a rigid bilaterally symmetrical shell of two parts With the exception of surf clams and ocean quahogs, most bivalve resources of commercial importance grow in shallow, near shore, estuarine waters

The estuarine system is a very dynamic zone in terms of both socioeconomic development and inherent environment factors The quality of estuarine water is complex and influenced by such features as river flows, climatic conditions, tidal stage and flushing, ocean currents, man made pollution sources and configuration of the coastline The sanitary quality of estuarine shellfish is directly related to the quality of the overlying estuarine waters The changes that occur in the quality of the water in which they grow are quickly reflected in shellfish

Shellfish feed by pumping large quantities of water by ciliary action and filtering microscopic particles These particles are passed along the gills and subsequently enter the guts This feeding process concentrate plankton, bacteria, chemical substance and other small sized particles in the digestive tract of the animals Oysters and clams are very effective in concentrating a variety of substances. Concentration

factors for biological and chemical contaminants are dependent upon such conditons as water temperature, levels in overlying waters and physiological characteristics of the species and among individuals eg There may be 1 mg/litre of Zinc in the overlying waters, while the oysters may show upon chemical examination, as high as 150 mg/g of Zn Bacteria are concentrated to a lesser degree exhibiting concentration factors ranging from 1 10 to 1 30 in clear waters (David, 1964).

Particles that do not enter the digestive diverticula are passed out of the stomach into the mid gut and are eventually discharged This process requires about 2 hours in actively feeding adult oyster (Galtsoff, 1964)

It is well established that oysters taken from polluted waters can serve as vehicles for the transmission of diseases to man if consumed raw (Cabelli and Heffernan, 1970 (a), Ayres et al, 1978, Durairaj et al, 1983, Eyles et al, 1985, Cathie et al, 1985)

Food borne diseases arising from oyster consumption range from serious cases of typhoid, cholera, and hepatitis to various forms of mild and severe gastroenteritis (Wood, 1976, Fleet, 1978, Fleet, 1979, Rouse and Fleet, 1982) Between 1900 and 1986 in the United states there were 12,376 documented cases, out of which 26% were typhoid, 11% infectious hepatitis, 11% Norwalk virus, 2% Vibrio, 7% unspecified and 43%

gastroenteritis, food poisoning diarrhoea,etc (Ward and Hackney, 1991)

Bivalves at harvest have SPC 10^3 to 10^5 bacteria/g (Ayres _et al_, 1978, Durairaj _et al_, 1983, Cathie _et al_,1985) The number of heterotrophic bacteria in the bivalve shellfish are greater than that in surrounding water (Cathie _et al_,1985) Oysters accumulate toxic heavy metals even if traces are present in the surrounding waters,and, sites for commercial leasing must be chosen with a full awareness of this possibility (Thrower and Eustace, 1973) Consumption of shellfish so contaminated present an additional public health problem (Cook, 1991)

Microorganisms used as indicators of faecal contamination are necessarily inhabitants of the alimentary tract of warm blooded animals and are important potential sources of faecal contamination in urban and rural areas Anthropogenic sources of faecal organisms to shellfish growing areas include discharges of treated municipal sewage and releases of partially treated or raw sewage

E coli is the dominant faecal coliform in human and animal faeces and is generally considered as indisputable indicator of faecal contamination from warm blooded animals Area that pass a sanitary survey must maintain a median faecal coliform MPN of less than 14/100ml of water to obtain an approved area classification

Following harvest, two microbiological parameters are applied to gauge the acceptability of shellfish meats Wholesale market level meats should have (35 °C) standard plate count of greater than 500,000/g and a faecal coliform level not in excess of 230 MPN/100 g (FDA, 1989)

Procedures for ridding shellfish of microorganisms of public health importance have been under investigation for the past 70 years and have resulted in the wide spread availability of depuration systems that are very effective in removal of microbial contaminants within 36 to 48h (Wood, 1976). Depuration is a dynamic process whereby shellfish are allowed to purge contaminants in tanks of clean water

Attempts to relay shellfish from polluted areas to pristine waters became less feasible as populations expanded and pollution encroached into new areas The Romans, during the first century B C consumed cockles and oysters after keeping them in unpolluted seawaters in tanks which are the earliest known examples of 'Cockle washery' (Younge, 1962)

Many of early depuration studies focussed on the disinfection of shellfish in chlorinated sea water (Wells, 1928) Subsequent work on ultraviolet light and ozone dis infection systems for seawater led to more accepatable technique which are commonly used today

With the exception of vibrios, shellfish moderately contaminated with most bacterial indicators and pathogens can be adequately depurated within 72 hours The presence of trace amounts of bacterial pathogens in depurated shellfish does not generally confer illness to consumers because threshold levels required to cause illness are seldom reached

Toxins, heavy metals, etc, are difficult to be eliminated, they either do not get depurated using current procedures or they are depurated so slowly that commercial steps to purify them would be uneconomical Some of these contaminants might be purged over extended periods, thus making long term relaying the only possible solution for afflicted shell stock In addition, depuration allows sand and grit to be purged from the shellfish gut, thus rendering them more palatable to some consumers. Organoleptic qualities can be enhanced by depurating shellfish in slightly higher salinity sea water which enchances their flavour (Richards, 1990)

The efficiency of depuration process is dependent on the water sterilisation methods and water parameters such as temperature, salinity, dissolved oxygen, turbidity ,flow rate etc, all influence the oyster feeding activity The significance of these factors varies with species and location and tolerable limits of these variables should be determined for each particular case

Although considerable work has been conducted on the depuration of oysters elsewhere, only very little work has been done on the depuration of oyster Crassostrea madrasensis in India, which has got tremendous scope of export

In this context, the present study has been taken up to determine the effect of using various water sterilisation treatments for the depuration of edible oyster C madrasensis The study is also aimed at finding out the effect of higher salinity in the depuration of oyster

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Oysters accumulate human pathogenic bacteria and viruses in their gut when grown in sewage polluted water ways and pose a danger to public health On transfer to tanks of disinfected water, oysters eliminate previously accumulated microbial contaminants with their faeces as part of the normal feeding and digestive activities and become microbiologically cleansed This process termed as depuration, render the polluted oysters safe for human consumption

The sucess of depuration depends upon several factors which include the oyster species , environmental conditions, and the depuration plant Commercial scale oyster depuration has been practised overseas for several decades

2 1 Oysters and Food Poisoning

Oysters have a well documented history of transmitting human diseases. Diseases range from the various infections, bacterial diseases such as typhoid (*Salmonella typhi*), paratyphoid(*Salmonella paratyphi*), cholera (*Vibrio cholerae*) and dysentry (*Shigella dysenteriae*) to varying kinds of gastroenteritis caused by *Salmonella* species, *Vibrio parahaemolyticus* and possibily *Clostridium perfringens* More incidents of infectious hepatitis and other viral based gastrointestinal disturbances have been linked with oyster

consumption Health hazards associated with oyster consumption have been reviewed by Fleet (1971,1979),Wood, (1976).

2 2 Oyster Feeding Mechanism

Oysters obtain their food by feeding Large volumes of water are pumped by ciliary action over the gills which act as a sieve and remove particulate materials including microorganisms The filtered particles become enmeshed in a mucous material and is then directed by ciliary action towards the mouth of the oyster, where depending on the nature of the particle especially the size, entraped material is ingested through mouth and is directed along the rejection part to exterior where it is eliminated as pseudofaeces Where as microorganisms enmeshed in the mucous enter the alimentary tract Waste materials from the alimentary tract is discharged as faeces in the form of fine mucous thread, ingested microorganisms which may still be viable are traped in the discharged faecal mucous and consequently do not recontaminate the surrounding water Thus the uptake and elimination of microorganisms by oysters are natural consequences of their pumping, feeding and excretory activities

Oysters pumping and feeding rates are influenced by the temperature, salinity, dissolved oxygen level and turbidity of the water as well as water movement and physiological state of the oyster (Furfari, 1966) Under optimal conditions,oyster may

filter more than 10 litres per hour so that microorganisms in the surrounding water heavily contaminate the gut region (Wood, 1976) The extend and rates of microbial uptake and elimination are, there fore, ultimately dependend on the number of micro organisms present in the water and the factors that affect pumping and feeding activities When living healthy oysters are transferred to unpolluted or sterilised water, they continue to filter water and the microorganisms previously accumulated within the gut are eventually discharged with the faeces giving microbiologically cleansed oysters (Fleet,1978)

2 3 Accumulation of Microorganisms by Oysters

The microbiological flora of an actively feeding oyster reflects at any one time, the flora of the surrounding water provided there has been no selective uptake, retenion and elimination of microbial species Investigation with Crassostrea gigas and Crassostrea virginica and other species have shown the predominant bacteriological flora to consist of Pseudomonas, Vibrio, Aeromonas, Moraxella, Citrobacter, Chromobacterium, Serratia and Acinetobacter species (Colwell & Liston, 1960). This is consistent with the predominance of these species in the water ways in which these oysters were grown Human pathogenic species represented about 1 to 5 percent of the total microbiological flora within the oyster, but this varied depending upon the extend of sewage pollution of the water ways

It has been suggested that oysters might harbour an indigenous microbial flora which would imply the operation of some selective retension mechanism within the alimentary tract (Colwell & Liston, 1960, Vasconcellos & Lee, 1972), but this concept has not been firmly established

When feeding is disturbed or when an oyster is removed from it's environment,the bivalve shells tightly closed,thus trapping accumulated microbial species An oyster may survive out of water for upto three weeks when stored at temperature between $10^{o}C$ and $25^{o}C$ and provided it is not exposed to' rough, physical treatment During this time, the relative proportion of microbial species may alter as some species die off and others multiply and predominate (Hoff *et al*, 1967)

Some studies in the kinetics of virus uptake and elimination using human enteric viruses as models have shown that,under normal feeding activity oysters and other shellfish rapidly accumulate human enteric viruses and bacteriophages from their growing waters and eliminate these on subsequent depuration (Hedstrom & Lyeke, 1964, Mitchell *et al*, 1966)

The extend of oyster digestion of ingested microbial species is also unknown although it is known that discharged oyster faeces contain large numbers of viable microorganism (Haven *et al*, 1978)

A steady state is known to be rapidly attained for uptake of coliforms beyond which accumulation in the gut does not occur for a given concentration of the bacteria in ambient waters Maximum levels can be attained in the first 6 h by some individuals, but prolonged exposure increase the percentage of the population before reaching a steady state (Perkins *et al*, 1980)

Total viable population in the oyster *Crassostrea madrasensis* and the sea water at Tuticorin were in the range of 10^3 -10^4/g and 10^2 to 10^3 /ml respectively. Pathogenic bacteria were absent, and the study indicated that gram negative asperogenous rod like bacteria such as *Vibrio*, *Flavobacterium*, *Achromobacter* and *Pseudomonas* were the dominant flora (Durairaj *et al*, 1983)

The number of heterotrophic bacteria in bivalve shellfish were always greater than that in surrounding water Over 90% of the coliforms and heterotrophic bacteria in oysters were found in organs associated with digestive tract, coliforms in stomach and heterotrophs in both stomach and lower intestine This suggest that stomach flora of oysters are mainly derived from the external environment and through a process of selection and multiplication it may be gradually replaced by a more indigenous population which dominates lower digestive tract (Kueh *et al*, 1985)

In the U S coastal waters, mean Vibrio parahaemolyticus density was more than 100 times greater in oysters than in water, where as density of faecal coliforms was approximately 10 times higher in oysters Seasonal and geographical distribution of V Parahaemolyticus were related to water temperature, with highest densities in samples collected in the spring and the summer

2 4 Paralytic Shellfish Poisoning

Paralytic shellfish poisoning (PSP) is caused by a neurotoxin produced by certain marine dinoflagellate algae Various mussels, clams, scallops and whelks become toxic if they feed on toxigenic dinoflagellates Incidence of paralytic shellfish poisoning has long been known along the Pacific and Atlantic coast

Sommer and Meyer, (1937) estimated that sickness may result from about 1000-20000 M U and the minimum amount to cause death is about 20,000 M U Bond and Medcof, 1958 on the otherhand, found sickness from about 600 M U and death from 3000-5000 M U Food and Drug Administration of USA promulgated a regulation that shell fish with a toxic level of 400 M U /100 g or more are unsafe for human consumption An outbreak of paralytic shellfish poisoning occured in Kumble near Mangalore following consumption of clams These clams (Meretrix casta) were found to contain paralytic shellfish poison (PSP) at a level of greater than 18,000 M U/100 of Oysters Crassostrea cucullata from the same region also had dangerous levels of PSP Toxin levels were retained in oysters for a longer period than in clams (Karunasagar et al, 1984)

Paralytic shellfish toxin (saxitoxin) was observed in a sample of clams from Tadri estuary by mouse bioassay technique, the

levels were found to be 320 Mouse units/100g (Karunasagar et al, 1986)

Results of a two year (1984-1986) study conducted by Shekar et al, of shell fishes along the coast of Karnataka revealed the presence of PSP in some clams and oysters During April 1985, a sample of oysters from Tadri estuary contained PSP within the permissible limit (less than 400 M U/100 g) while during March April 1986, levels ranging from 370-1200 M U/100 g were recorded Toxin levels declined to safe limits within a week

2 5 Microbiological Indicators of Pollution

Numerous studies have clearly shown that oysters rapidly take up coliforms and E coli from the surrounding waters and concentrate them in their digestive system to very high levels, having the concentration factors as high as 25 to 30 for faecal bacteria, (Mitchell et al, 1976, Railey & Barile, 1987) However, these values fluctuate depending on tidal cycles, local condition and with the organisms

Data from the periodic assays of individual animals suggested that accumulation of the bacteria by the quahogs proceeds to an equilibrium level which is a function of E coli content of water and its overall particulate matter Accumulation takes place in the digestive gland and to a lesser extend in the siphon of the animal (Cabelli & Heffernan, 1970) E coli accumulation factors for quahog clams under optimal conditions were observed by Cabelli & Heffernan (1970a) to be 6 5 to 8 5 while accumulation factor for soft shell clam was 20

When water temperature are lowered below the range of optimum physiological activity there is a decline in pumping and filtration activity and there by an inhibition of accumulation of coliform bacteria (Cabelli & Heffernan, 1971, Haven _et al_,1978)

E coli was found to survive for longer periods of time in unsterile natural seawater when sediment material was present than sea water alone The longer survival of _E coli_ in the sediment is attributed to the greater content of organic matter in the sediment than sea water (Gerba & Johns, 1976)

Gerba _et al_, (1980) analysed statistically three environmental factors such as temperature, salinity, turbidity and bacterial indicators to determine whether these factors would be used to predict enterovirus contamination of oysters There was a moderate correlation between total coliforms in oysters and levels of virus in sea water

It is suggested that _E coli_ reflects faecal contamination more truely and so may be better indicators of human/animal contamination than faecal coliform. (Hackney _et al_, 1983)

Cleansing of _E coli_ under the same depuration conditions, could be used to indicate the cleansing of other pathogenic bacteria like _Bacillus cereus_, _Clostridium perfringens_, _Vibrio parahaemolyticus_ and _Salmonella_ (Son & Fleet,

1980)

Kilgen *et al*, (1988) are of the opinion that the relationship between enteric viruses and bacterial indices in Lousiana oysters and water indicated that viruses do not always correlate with the faecal coliforms indicator system. Louisiana oysters harvested from approved growing waters in summer months contained high levels of non *E coli* faecal coliform which were not of sewage origin

The results obtained by Power & Collins, (1989) suggest that *E coli* is an appropriate indicator of the efficiency of virus elimination during depuration

Higher counts of coliform bacteria were observed in rainy season and cold weather in lower lake of Bhopal *E. coli* formed maximum up to 97 22% of the total coliform (Valecha & Bhatnagar, 1989)

2 6 Depuration Technology

The essential components of a depuration system are tanks to hold sea water and a means of producing sterilised sea water Tank design, size and lay out are determined largely by the number of oysters to be cleansed, handling economics and the amount of space available Facilities for water aeration, heating and cooling may also be required depending upon tank design and

climatic conditions Experience in great Britain and the USA have shown that a depuration time of 36 to 48h is required to obtain safe cleansing, after which the tanks must be cleaned before a new batch of oyster is processed

Seraichekas _et al_, (1968) found that during late phase of depuration, although a great majority of shellfish were free of virus, a few still have found minimal amount of contaminants since the naturally polluted shellfish were shown to contain less virus than those studied in the laboratory It is anticipated that the former type of shellfish may be cleansed more readily by this process within a reasonable period of time

Accumulation and elimination of viral particles by hard clam _Mercenaria mercenaria_ were studied with the coliphage S-13 as a working model by Canzonier, (1971) Upon exposure to virus free running water, clams polluted to relatively low levels (100 plaque forming units /ml) eliminated most of their bacterial contaminants in 24 to 48h Viral contaminants, however persisted for several days to weeks even under ideal conditions for clam activity provided that the temperature remained below the inactivation threshold for the virus

The results of the experiments conducted by Qadri _et al_, (1976) indicate that one week is sufficient to clean polluted Sydney rock oysters by relaying if the water quality,

temperature, water salinity and the season of the year are conducive to the active feeding But the handling and the transport cost of such an operation are rarely economical (Qadri et al, 1976, Fleet, 1978)

The National Health and Medical Research Council of Australia (NHMRC) has introduced a microbiological standard for oysters, stating that oysters for human consumption should not contain more than 2 3 E coli cells/g oysters With levels of E coli greater than 2 3/g oysters will have suffered from unacceptable levels of sewage pollution and therefore represent a potential danger to public health This is the generally accepted international standard for depurated animals (Fleet, 1978)

It was found that contaminated oysters cleansed themselves to NHMRC standard within 24 to 48 h using water continually recirculated through a uv sterliser The trials had been based on tanks containing 1000 and 5000 oysters on the basis of two oysters for each litre of water (Fleet, 1978)

Depuration was carried out by Metcalf et al, (1979) using clams carrying faeces associated natural virus bioaccumulated during a 24 h exposure period The bioaccumulated virus was reduced significantly within the first 24 h of depuration The maximum rate of depletion of bioaccumulated natural virus took place within the first 48 h 80 to 88 % of the virus content of the clam was eliminated during this period

Heavily polluted oyster Crassostrea commercialis were cleansed to acceptable NHMRC recommendations within a 48 h operation Total plate counts of the oysters generally decreased by a factor of ten fold or higher depending upon the initial microbial load Tank water counts were reduced from their initial levels and maintained at about 10^3 cells/ml throughout the 48 h operation (Souness & Fleet, 1969)

If oyster pumping occurs and the rates are in the range of 1 5 to 10 5 litres/h then there is a fairly uniform and optimum rate of elimination If oysters pump, they will produce biodeposits and elimination occurs However in looking at the rates of biodeposits it was found that biodeposition is not required as a prerequisite for depuration to occur Healthy pumping oysters may have the capacity to inactivate and digest significant numbers of coliforms without obvious defaecation (Perkins et al, 1980)

Oysters C commercialis which were unaccepably polluted on the basis of high E coli counts were cleansed to acceptable levels of less than 2 3 E coli cells/g after 2 days, after 6 days no E coli could be detected in any of the relayed oyster samples Laboratory depuration system gave very effective oyster cleansing within 48 h with a reduction E coli numbers from 100 cells/g to undetectable levels (Son & Fleet, 1980)

Both Salmonella charity and E coli were recovered from

faeces produced by contaminated oysters and bacterial numbers found in faeces were higher for the more contaminated oysters Oysters contaminated with S charity at levels of 930 and 43 cells/g produced faeces containing 3 3 x 10^5 and 3 5 x 10^3 cells/g (dry weight basis) Similarly oysters with E.coli levels of 430 and 43 cells/g produced faeces containing 1 8 x 10^5 and 1 5 x10^4 cells/g respectievely The findings of the study reveal another mechanism of recontamination that involves the release of viable bacteria from deposited faeces into the overlying water and the data suggested that bacteria may not be highly entrapped and immobilised within the faecal structure (Rowse & Fleet, 1982)

The recirculating depuration plants studied by Eyles & Davey (1984) in New Southwales can substantially reduce the degree of contamination of oysters with bacteria which are present as a result of pollution They showed that commercially purified oysters are much less likely to contain detectable coliforms or E coli than the oysters C commercialis taken directly from the estuary However, the depuration process may be of limited use in controlling the presence of pathogenic vibrios in oysters

The oysters Crassostrea iredalei with initial faecal coliform MPN of 2 2x10^5 /100g meat depurated to acceptable levels (230 MPN/100g meat) after 48 h except those in the middle of the

tank (490 MPN/100g) This suggests the presence of a dead spot in a depuration system Neverthless the same oysters depurated successfully within 72 h (Gacutan et al,1986)

Acid insoluble ash (sand) content in the muscle could be brought down to an insignificant level by depuration in the water (Surendran & Balachandran, 1988)

The observation recorded by Venkatanarasimha Pillai & Selvan, (1988) indicated that the bacterial count of the oysters, Crassostrea madrasensis and mussel Perna indica could be brought down effectively either by washing them in filtered sea water for 24 h or keeping them in aerated sea water for 48 h The bacterial quality could be further improved by chlorination at the end of depuration

The elimination of sewage effluent associated poliovirus, E coli and 22nm icosahedral coliphage by the common mussel Mytilus edulis was studied by Power and Collins, (1989). In the laboratory system, the logarithms of the poliovirus, E coli and coliphage levels were reduced by 1.86, 2 9, & 2 16 respectively within 52 h of depuration In the commercial scale depuration system, the logarthm of the E coli levels reduced by 3 18 and logarithm of the coliphage levels reduced by 0 87

The initial total and faecal coliform counts of 14000 and 11000/100g oysters were reduced to 300/100g after 24 h and

68 MPN/100g for after 48h for total coliforms and to 78/100 g in 24 h for faecal coliform and total coliform count 45 and 68/100 g respectively (Sangrungruang & Sahavachanin, 1989).

Poliovirus and hepatilis A virus were rapidly bioaccumulated by mussels and the maximum concentration of about 10^4 TCID 50/ml was reached within 1 5 h Depuration was carried out upto 24 h, infectivity titre decreased to 10^2 TCID 50/ml and $10^{3\ 2}$ TCID 50ml within 6 h in hepatitis A virus and polio virus contaminated mussels respectively Only a very slight decrease was obtained after 24 h (Franco *et al*, 1990)

Two studies were conducted by (Holliday *et al*, 1991) in the purification of Pacific oyster *C gigas* and Sydney rock oyster *C commercialis* in two typical commercial uv light, pool-type purification plants operated using either a flow through or a recirculation water system At the completion of purification the oysters from both the system had standard plate counts and faecal coliform *E coli* and *V parahaemolyticus* levels within in the recommended limits

A depuration time of 24 h reduced substantially the sand content in the oyster *C madrasensis* (Chellappan, 1991)

The relative pattern and rate of elimination of the microorganisms suggest that they are eliminated from shellfish in two different ways, one is mechanical in nature that result in

microbial elimination during the first 12 h, and other depends upon the microbial species and their accumulated number All microorganisms tested were eliminated completely by the mollusc after 3 days of depuration except MS-2 bacteriophage and the results indicate that the MS-2 bacteriophage may be a more reliable indicator of the microbial depuration efficiency (Eduardo et al, 1991)

2 6 1 Water Sterilisation Treatments

2 6 1 1 Chlorination

Chlorination is the oldest disinfection procedure for depuration of waters Sea water was sterilised by the addition of sodium hypochlorite and residual chlorine inactivated by sodium thiosulphate addition Although giving successful cleansing, the process required large tanks and great volumes of water Control of water chlorine levels was necessary, since insufficient chlorination gave inadequate water sterilisation and overchlorination yielded residuals which interfered with oyster feeding activity and cleansing effectiveness (Kelly, 1961) Chlorine was used in great Britain in the earlier parts of this century for sterilising water for oyster depuration, chlorination was replaced by other methods because it interfered with oyster feeding and hence their rate of cleansing (Fleet, 1978)

Chlorination of the water used in the blower, tanks

which contained artifically contaminated and shucked oyster with *V cholera* did not eliminate the organisms from the oyster meat (Motes, 1982) However, Belmonte *et al*, (1984) obtained a decrease of faecal contamination levels to values significantly lower than the international standards in less than 48h of depuration in chlorinated water No important physical and chemical changes were detected in purified molluscs

It was noticed that when the water is chlorinated, the shells remained tightly closed until such time that the available chlorine dissappeared from the system and no activity leading to depuration took place (Balachandran & Surendran, 1984, 1988) The bacterial qualities of the meat of clam *Villorita cyprinoides*, mussel *Perna indica*, oyster *Crassostrea madrasensis* and clam *Meretrix casta* were considerably improved in the case of treatment with chlorine for 2h after depuration in natural water for 24h (Balachandran & Surendran, 1984, 1988, Vemkatanarasimha pillai & Selvan, 1988 and Mishra & Srikar, 1989)

Eventhough chlorine is the cheapest option for sterilisation of sea water it is quickly bound up by organic material and is difficult to maintain accurately controlled dosage Concentrated hypochlorite s lution can be a difficult, dangerous chemical to handle and the combination of sodium chloride, chlorine and organic material could lead to build up of chloramines in the shellfish (Thrower, 1990)

2 6 1 2 Ultraviolet Light Treatment.

It is an effective means of continuously disinfecting large volumes of water rapidly and cheaply Extensive studies in great Britain by Wood (1961) and the USA by Kelly (1961) have shown that uv light is most effective in disinfecting sea water for oyster depuration system Using either continuous flow or recirculating system, heavily contaminated oysters could be cleansed to acceptable microbiological quality within 48 h with no apparent effect on oyster eating quality (Wood, 1961)

Turbidity had an adverse effect on the effectiveness of uv radiation, however by adjusting the flow rates of sea water through the treatment unit, adequate disinfection was shown to be predictable (Hill et al, 1967)

A uv intensity of 960 micro watt/cm^2 reduced the microbial count of sea water from 263 to 13 per ml. The coliform MPN was reduced from a high of 17 to o 18 per 100 ml With the exception of coliforms, the microbial composition of oysters subjected to uv treated sea water remained at levels comparable to the control oysters held in untreated sea water Another experiment conducted with a uv intensity of 12000micro watt/ min/ cm^2 revealed that the increased uv intensity did not increase the degree of microbial inactivation Coliform and some *Pseudomonas* species appeared to be eliminated easily from oysters, but some potentially hazardous microorganisms such as gram positive cocci

and *Vibrio* species tended to persist for longer periods of time (Vasconcelos & Lee, 1972)

A slight decrease in sterilising efficiency is noticed after 48 h circulation of the depuration water and the phenomenon could be explained by a gradual selection of uv resistant bacteria in the tank water (Souness & Fleet, 1979) The interference of water turbidity with uv sterililsing efficiency is not a problem in circulating depuration system because of the filtering effect of oyster feeding (Souness & Fleet, 1979)

The laboratory depuration conducted by Souness & Fleet (1980) gave a very effective oyster cleansing within 48h ie a reduction of 100 cells/ g to undetectable levels Total plate counts decreased by 10 fold over this period, but rarely went below 10^4, cells/g of oyster The failure of total plate count to decrease below this value is not a reflection of inadequacies in the depuration system itself, but rather is related to the maintanance of an indigenous microbial flora within the oyster Longer depuration time were required for the more heavily contaminated oysters with *Salmonella*

The depuration process may be of limited use in controlling the presence of pathogenic vibrios in oyster *Crassostrea commercialis* (Eyles & Davey, 1984)

A great advantage of uv light is the low cost and the absence of residual taints and odours from chemical residues (Thrower, 1990)

The depuration system for *Crassostrea virginica* conducted at temperature 23 ^{o}C caused *Vibrio vunificus* counts to increase in oysters especially in the haemolymph, adductor muscle and mantle indicating that the disinfectiion properties of uv radiation was less than the rate at which *V vunificus* organisms are released into sea water (Tamplin, 1992)

2 6 1 3 Ozone Treatment

Ozone is a powerful oxidising agent capable of rapidly killing bacteria and viruses (Blogoslawski & Rice, 1975, Thrower, 1990) The main critcism for its use have been capital out lay, maintance and running costs The ozone demand of organic material in the water under treatment determines the antimicrobial effectiveness of ozone and ozone levels around 2mg/litre are required for sea water disinfection (Fleet, 1978) Ozone has been successfully used to disinfect sea water for shellfish depuration and several such plants are in operation in France and Spain (Furfari, 1976)

However, ozone depuration was inadequate due to rapid ozone decay at the very high ambient temperature (Blogoslawski & Monesterio, 1982)

2 6 1 4 Io dophore Disinfection

Iodine has powerful antimicrobial property and is used in the form of iodophores as a disinfectant When the iodophore

was used in a recirculating system at levels between 0 1 to 0 4 mg/l shellfish including oysters cleansed themselves within 10h (Fleet, 1978) This represents about one quarter of the cleansing time recommended for conventional uv or ozone depuration plants Further more, it was claimed that this iodophore treatment had no adverse effect on shellfish feeding activity and did not affect their flavour and other eating qualities The iodine content of shellfish flesh increased by 0 1 to 1 0 mg/kg which is considered not to be nutritionally significant to the consumer

2 6 2 Factors Affecting Oyster Depuration

Any factor which affects oyster feeding activity will influence cleansing capability (Fleet, 1978) The water in the tank should simulate the conditions in the natural habitat to ensure that the animals function vigorously and therefore depurate as efficiently as possible Excessive storage time out of water, rough handling and damage of the oyster prior to depuration must be avoided Oysters which are stressed, moribund or dead will not cleanse and jeopardise the gains of any depuration operation Water parameters such as temperature, salinity, dissolved oxygen and turbidity, all influence the oyster feeding activity The significance of these factors varies with the oyster and location and tolerable limits of these variables should be determined for each particular case Comprehensive studies of these variables have been made for the

European flat oyster (*Ostrea edulis*), the Portugese oyster (*Crassostrea angulata*), American Eastern oyster (*C virginica*) the Pacific oyster (*C gigas*) and the Olympia oyster (*O lurida*) (Wood, 1961, Furfari, 1966, Haven *et al*, 1978)

2 6 2 1 Temperature

The ability of shellfish to feed and defaecate at different temperatures varies with species and the habitat from which they have been harvested

In the USA, the minimum temperature recommended for depuration is 10 °C (Furfari, 1966) and in great Britain depuration is not recommended below 5 °C (Wood, 1969)

Oyster feeding activity is generally optimal around 20 °C but 'involves the risk of oyster spawning (Furfari, 1966, Rowse & Fleet, 1984) Depending on the location, it may be necessary to cool the depuration water in summer and warms it in winter

The efficency of the viral depuration is roughly a function of the sea water temperature within the range of 5-20 °C listed The highest temperature used, 20 °C has given the best results and at 18-20 °C, the viral content of both shell,liquor and meat of quohog reached non dectable levels within 24h The same level was also reached at lower temperature, but at a slower

pace (Liu *et al*, 1967)

There was no appreciable effect of temperature between 10 and 20 $^{\circ}$C on the elimination of *E coli* in the quahog (Hefferan & Cabelli, 1970)

The influence of the temperature on the environmental water examined by Cabelli & Heffernan, (1970) with clams taken from water whose ambient temperature was less than 2 $^{\circ}$C Of the four temperatures examined 2,18,12 and 16 $^{\circ}$C, only at 2 $^{\circ}$C environmental water showed a siginificant inhibitory effect on the removal of microorganisms in clam,*Mya arenaria* At 6 $^{\circ}$C mean bacterial uptake (*E coli*, *S typhimuriium*, and *S flexneri*) and subsequent clearance by the oyster *C virginica* and hard clam *Mercenaria*, *mercenaria* was significantly lower at 20 $^{\circ}$C However, substancial bacterial clearance from the haemolyph occured for both shellfish at each temperature At 20 $^{\circ}$C viable bacteria were no longer detectable after 24h in haemolymph of either clams or oyster after exposure to contaminated water containing $4x10^{3}$ bacteria/ml (Hartland & Timoney, 1978)

Below 10 $^{\circ}$C New England hard clam become physiologicaly incative with accumulation being more strongly inhibited than elimination between 10 and 20 $^{\circ}$C, the rates of elimination are essentially same (Perkins *et al*, 1980) Gulf cost oyster will

depurate faecal coliform to the same levels in 48h over the range of 16 3 to 28 7 ^{o}C Chesepeake Bay oysters will depurate equally as well between about 14 and 29 ^{o}C (Perkins *et al*, 1980)

Temperature and intial level of contamination were the major factors influencing success of depuration of Pacific oysters *C gigas* in New Zealand The closed system using uv disinfection was found to be effective with a flow of 2 5 cycles per hour in 24h at 5 to 24 ^{o}C

Purification of sydney rock oyster was rapid and constant at 18 to 22 ^{o}C with levels of *Salmonella charity* and *Escherichia coli* being reduced to below 1 cell/g within 12h Purification was also effective at the higher temperature range of 24 to 27 ^{o}C for the winter harvested oysters Purification was not effective at temperature below 17 ^{o}C and even after 48h oysters still remained at unacceptable levels (more than one cell/g) of bacteria (Rowse & Fleet, 1984)

Healthy relayed oysters were capable of cleaning in a 7 day period provided the temperature was above 10 ^{o}C Faecal indicator bacteria and enteric pathogenic bacteria were eliminated at similar rates but faecal coliform level did not correlate with viral elimination (Cook & Ellender, 1986) and the authors suggest that faecal coliform may not be useful as endpoint indicators for this methods of oyster purification

A slight increase in temperature from the temperature of habitat water does not affect the pumping of the oysters and there by will not affect the purification process (Rajapandian *et al*, 1988).

Studies were undertaken by Power & Collin, (1990) to determine the effect of temperature on the efficiency of elimination of *E coli* and a 22 nm icosahedral coliphage from experimentaly contaminated mussels, *Mytilus edulis* and they found that initial *E coli* levels were reduced by 99 % within 52h at the test temperaure 5 5, 10 and 16 5 ^{o}C Efficient coliphage elimination occured at 16 5 ^{o}C only

If the water is too warm oysters may become stressed or die due to lack of oxygen, if it is too cold they may go into a state approaching hybernation, and slowed metabolism and feeding rate will impede the depuration process (Thrower, 1990)

Results showed that depuration system conducted at temperatures greater than 23 ^{o}C caused *V vulnificus* counts to increase in oysters In contrast, when depuration sea water was maintained at 15 ^{o}C *V vulnificus* was not detected in seawater and multiplication in oyster tissue was inhibited (Tamplin & Capus, 1992)

2.6.2 2 Salinity

Although the salinity of water is usually about 3.5%

in the open sea, levels can vary considerably in estuaries due to concentration by evaporation and dilution by fresh water run off Eventhough shellfish can adopt to moderate variations in water salinity, feeding activity may be temporarily affected as acclimatisation to changes in salinity takes place

Water for depuration is best taken at the high tide, and salinity values should be carefully observed during periods of heavy rainfall (Wood,1961,Furfari,1966)

Viral depuration of quahogs proceeded rapidly in the water with salinities of 31 ppt and 23 to 28 ppt where as little depuration was obtained with those treated in sea water with salinities of 17 to 21 ppt. A reduction of salinity to 50 to 60 % of the original sea water completely stopped the process (Liu et al, 1967)

Salinity below 16 ppt slows depuration in some Gulf of Mexico oysters and below 7 ppt the rates are highly reduced (Presnell et al,1969)

Quahogs placed in waters having salinities of 31 and 25 ppt rapidly reduced their E coli content within 48h. However, at 20 ppt poor elimination was obtained After a four week adaptation period in 20 ppt water, most of the animals were found to be active at this salinity After these animals were allowed to accumulate E coli from an environmental water level of

4.0×10^3 E coli/100ml, elimination proceeded equally well at 15 ppt (Heffernan & Cabelli,1970).

A marked decrease in elimination activity of soft shell clams was observed when the salinity of the environmental water was decreased from 20 to 10 ppt As 20 ppt is the lower limit for good depuration activity in which the salinity was varied from 15 to 30 ppt (Heffernan & Cabelli,1970)

As excessive variations in water salinity may cause oyster mortality, it is recommended that the salinity of depuration waters should be in the range of 100± 20% of the salinity for the particular growing area (Fleet, 1978)

In Chesapeake Bay oysters, the rates of depuration were unaffected between 14 and 21 4 ppt (Haven et al, 1978)

Purification was clearly ineffective and incomplete at the low salinity, 16 to 20 ppt in the case of Sydney rock oyster, in fact some 20 to 25% of the oysters died during the purification period. In contrast, oyster purification was very effective at the higher salinities (43 to 47ppt) and rates of bacterial cleansing were comparable to those at normal salinties of 33 ppt More over, oysters remained healthy during purification at the higher salinity and exhibited no unusual mortality (Rowse & Fleet, 1984)

Depuration was inconsistant and clearly ineffective in

Crassostrea iredalei when the salinity values were downed to 9 9 to 14 4 ppt Initial MPN levels did not change, and/or even were found to increase until 48 h However, when the oysters were exposed to salinity levels of 17.5 to 31.1 ppt depuration was effective where the coliforms were reduced microbiologically safe levels The minimum salinity for .successfull depuration by C iredalei based on this study is 17 5 ppt (Palpal-Latoc et al, 1986)

Although oysters may be depurated over a wide salinity range (15 to 35 ppt) depuration could be properly effected in salinity ranges in which the oysters have been originally thriving If the oysters are moved from a high to low salinity or vice versa, a period of acclimatisation may be needed for resumption of normal pumping activity of the oyster This will prolong the purification time (Rajapandian et al, 1988)

At 28 6 ppt E coli 4A was eliminated from the mussel Mytilus edulis efficiently and rapidly A final reduction of 3.01 log cycles was achieved within 52 h. At 18 2 ppt E.coli 4A was eliminated efficiently, but less rapidly than during depuration at the higher salinity. A final reduction of 2 18 log cyles was achieved within 48 h (Power & Collins,1990)

Whilst most estuarine molluscs can tolerate a variation in salt levels there will be an optimum preferred salinity which may vary with species and habitat (Thrower,1990)

2 6 2 3 Dissolved Oxygen

The oxygen requirement of oysters during depuration process is to be maintained at a satisfactory level The water oxygen levels depends upon water/oxygen ratio, water temperature and rate of flow of water through the system With recirculating system it may become necessary to aerate the water supply as oxygen is eventually depleted by oyster activity (Wood, 1961, Furfari, 1976).

The dissolved oxygen levels in the water should not fall below 50% saturation during conditions of maximum oyster demand (Fleet 1978, Thrower, 1990).

Appreciable drops in depuration rates occur below 1 8 mg of dissolved oxygen/l in oysters. (Haven et al, 1978).

The normal oxygen level of the sea water is 4 to 7 mg/l During the hosing and jetting of sea water the level is slightly increased by 0 2 to 0 5 mg/l But under static conditions, the oxygen level much reduced and the pumping rate eventually caeses Hence the depuration process is always accomplished by slow flow of running sea water The solubility of oxygen decreases with rise in temperature and with increase in salinity. When supersaturated water warms, it releases excess oxygen and other gases in the form of bubbles and this in turn can cause the death of oyster by embolism (Rajapandian et al,

1988) Therefore supersaturation of water is avoided

The high stocking rates of depuration plants can deplete oxygen levels in a tank Aeration systems which cause turbulance such as injection of compressed air directly into the tank should be avoided as this resuspend faeces containing pathogenic bacteria in the water column (Thrower, 1990)

2 6 2 4 Turbidity

Water turbidity can be an important factor in oyster depuration as excessive turbidity reduces water sterilising effictiveness (Wood, 1961) and may lead to reduced shellfish feeding activity

Turbidity does not affect depuration rates at turbidity levels of 9 4 mg/l in Gulf of Mexico oysters (Presnell et al 1969), 25 mg/l in New England clams (Cabelli & Heffernan, 1970) and as high as 77 mg/l in chesapeake Bay oyster (Haven at al 1978)

It was observed by Heffernan & Cabelli (1976) that a water turbidity of 10 Jackson Turbidity Units (JTU) achievied by the addition of bentonite to the environmental water there was a 24% reduction in the number of animals that accumulated E coli optimally. At 25 JTU only 5% of the animals accumulated the organisms to levels in excess of that in the water.

Elimination however, was not decreased when the water was adjusted to a turbidity of 24 JTU, infact,the increased turbidity appeared to have enhanced the depuration process

Water turbidity may be controlled through preliminary filtration, but this introduces extra capital and maintanance cost (Fleet,1978, Rajapandian et al, 1988). Water turbidity is less of the problem with recirculating systems since the oysters rapidly remove particulate matter through their feeding activity and subsequent trapping in faecal material that accumulate on the tank bottom (Fleet, 1978)

Depuration process will not be effective with turbid waters (Rajapandian et al, 1988) Turbidity caused by the suspended materials in the water can clog the gills of the molluscs, impairing normal feeding and it can reduce penetration of uv light in the sterilising unit. If the material is organic in nature, it can bind the chlorine and ozone when chemical sterilisation is used (Thrower, 1990)

2 6 2 5 Flow Rate

Flow rate through the dupuration tanks and steriliser units should be such to permit effective disinfection and to prevent build up of viable microorganisms in the tank

Rates of sea water flowing through the depuration tanks were found to be unimportant above O 5 l/oyster/h (Presnell et

al, 1969) and 1 0 l/oyster/h (Haven et al, 1978) as long as sediments in the tanks were not stirred in to suspension resulting in recontamination of shellfish

No difference in the elimination of elevated temperature coliforms were observed when the flow rates of environmental water in the depuration system was varied between 3 and 24ml/min/animal. There was a significant increase in the soft shell clam mortality over that in the animals treated at high flow rate At the lower flow rate,the siphons were observed to be marketely distended, however, pumping activity did not appear to be decressed (Cabelli & Heffernan, 1970 b).

Experience in Australia has shown the need for one exchange of depuration water through the tanks and sterilising units every 30 minutes, otherwise build up of viable microorganisms occurs leading to a substancially high oyster contamination than the initial one (Fleet, 1978)

The closed system using uv disinfection was found to be effective with a flow of 2 5 cycles/h in 24h at 5 to 24 ^{o}C using upto 3 C gigas/loading rate provided inital concentration rates are 350 to 35000 as MPN faecal coliform/100g.

2 6 2 6 Initial Concentration of Bacteria

It was shown that purification of lightly polluted shell fish,northern quahong with virus, was achieved sooner than

of the heavily polluted ones The time required to depurate the lightly polluted shellfish (50 PFU/ml of the homogenate) to nondetectable level was 24 h and that for the heavily polluted ones (100 PFU/ml) was 72 h This finding definitely bears out the orginal suspicion that the time of depuration is proportional to the degree of pollution of shellfish (Liu at al, 1967)

Depuration effectiveness of soft shell clam *Mya arenaria* depended upon the number of virus bioaccumulated and whether virus was solid associated All bioaccumulated faeces associated natural virus was deposited either in hepatopancreas or in siphon tissue and eliminated within 24 to 48 h depuration

Level of initial contamination was one of the major factors influencing the success of depuration of the Pacific oyster *C gigas* (Buissan *et al*, 1981, Corre *et al*, 1990)

Results showed that depuration system conducted at temperatures greater than 23 ^{o}C caused *V vulnificus* counts to increase in oysters In contrast, when depuration sea water was maintained at 15 ^{o}C, *V. vulnificus* was not detected in sea water and multiplication in oyster tissue was inhibited. (Tamplin, 1992)

2 6 3 Heavy Metal Accumulation and Elimination from Oysters

Brooks and Rumsby (1967) suggested that some metals in the oyster Ostrea sinuata were bound in the faeces and pseudofaeces and could be eliminated by depuration for 100 h. Their work, which compared only 6 individuals before and after depuration did not indicate a change in Zn or Cd concentration Although a high cadmium concentration did not appear to displace other ions, there was some evidence for loss of ions from the visceral mass

Oysters which abnormally accumulated Zn and Cu were transplanted into the water area of normal oyster and the process of disappearance indicate that (1) the accumulated Cu doesnot begin to disappear until half a month or more after transplantation (2) the accumulated Zn begin to disappear immediately after the transplantation (lkuta, 1968)

Oysters accumulate certain toxic heavy metals if traces of these are present in the surrounding waters and sites of commercial leases must be chosen with a full awareness of this possibility (Thrower & Eustace, 1973, Ratkowsky et al, 1974)

A close association was obtained between the proximity to heavily urbanised areas and concentration of metals found, oysters growing nearest urban areas having the highest concentration of one or more of the metals. Examination of samples of native oysters could be useful in providing an index of measure of environmental pollution. (Ratkowsky et al, 1974)

Concentration levels of Fe, Mn, Cu and Zn in the oyster C.madrasensis were in the order of 120 to 1600mg/Kg 5.8mg/Kg, 70 to 205mg/Kg and 2450 to 12500mg/Kg respectively. High concentration were observed during December to May Low values were confined to June to November when the fresh water discharge through the rivers was maximum(Sankaranarayanan et al, 1978)

Cadmium was not depurated by C virginica within a period of 16 week in flowing ambient sea water and no decrease or change in copper concentration occured with increase or decrease in temperature within a depuration period of 56 week (Zaroogian, 1979)

Cadmium contaminated oysters containing 5 9ppm Cd was depurated for 42 days in flowing estuarine water and the Cadmium content decreased to 3ppm Results showed that lab dozed oysters eliminated Cd in the natural estuarine environment and suggested that the rate of elimination is affected by changing water temperature and salinity The rapid elimination suggested the presence of free or unbound Cd (Mowdy, 1981)

Study conducted by Kumagai and Sacki (1982) indicated that there was no relationship between the heavy metal content of clams, Tapes japonica and the mud in which they inhabited

Oysters were found suitable for monitoring Zn,Cd,Pb and Cu contamination The rate of accumulation and depuration of

metals by oysters and mussels was related to the anatomy of the animals and sequestering of some metals in granulocytes Uneven metal distribution between gonad and other tissues was found to cause seasonal variation of heavy metals in oysters (Cooper et al, 1983)

The results of the accumulation of metals Zn,Cd,Cu,Pb,Ni,Co and Cr by the oysters C gigas and C margarilacea for 3 weeks showed that all seven elements were accumulated to a greater or lesser extend. During the 3 week period of exposure, the oysters accumulated Zn and Cu at the fastest rates, Pb and Cd at intermediate rates and the remaining elements were accumulated more slowly (Watling, 1983)

Mean Zn concentration of oysters, C gigas depurated in the filtered seawater was 1044 mg/Kg compared with 725 mg/Kg in filtered diluted seawater in the 48 h experiment and 1122 mg/Kg and 860 mg/Kg respectively in the 100 h experiment, 1122 mg/Kg and 860 mg/Kg respectively in the 100 h experiment Iron was the only metal that appeared to be lost in faeces and pseudofaeces (Thomson, 1983)

The high atomic ratios of Zn to Cd (1200 to 1400:1) found at certain times in oysters in the Derwent estuary appeared to suppress food intake, growth and faecal conversion in rats fed with diets containing greater than 15 mg/Kg Cd (dry wt) Little

Cd was deposited in the usual target organs, that is, Liver and Kidney (Thrower & Olley, 1983)

Concentration of Fe, Cu, Zn and Pb in the mollusc Villorita cyprinoides var cochinensis, M casta and P viridis studied were influenced by season. Highest concentration of these metals were found during low salinity and low pH of habitat water (monsoon periods) Metal concentration decreased in these species in summer months (Lakshmanan & Nambisan, 1983)

From the experiments conducted by Zaroogian and Johnson (1984) it was found that, after 12 week treatment with 5 and 20×10^{-9} mg Ni/Kg seawater, mean tissue concentration in C viriginica were 9 62±3 56 and 12 96± 5 15 mg/Kg dry wt After holding C virginica in ambient flowing seawater for 28 week, 48 and 68% loss of Ni concentration occurred

Ranges of concentration (mg/Kg wet wt) of Ag(0-0 4) Cd (0-0 8) Cr (0-0 8) Mn (0 4-3 2) Ni (0 2-1 7) and Pb (0-1 7) were determined by Talbot (1985) in S cucullata and Saccostrea spp from several locations in Dampier Archipelago and nearby Cape Lambert Concentrations of Cu and Zn in individual specimen ranged from 1.4 to 555 and from 55 to 1000 mg/Kg wet wt reached maximum value at areas adjacent to township and iron ore importing terminals and correlated significantly with length and wet wt Fe concentration (4 2-1629 mg/Kg wet wt) did not correlate significantly with oyster length or wet wts

The rate and extend of bioconcentration of iron in gill, mantle and adductor muscle of the oyster Crassostrea madrasensis were studied in specimens collected from the different stations of vellar estuary by Sentilnathan et al, (1986) The overall results showed higher concentration in the mantle than in the gill and adductor muscles Organisms collected from freshwater zone contained higher concentration of iron than those collected from tidal and marine zones A clear seasonal trend in distribution of iron was noted with the maximum concentration during postmonsoon season and minimum during summer

The ranges of heavymetal concentration (ppm) in the water were Fe-1 26-7 70, Zn 0 69-4 0 and Cu 0 10-1 09 The concentrations of heavy metals in oysters were higher than the metal concentrations in water and sediment (Rajendran & Kurian, 1986)

The distribution of metal load in the tissue of C madrasensis exhibited seasonality Higher metal load was observed by Unnikrishnan Nair (1986) during the breeding period and also when the estuary maintained high salinities from October to April

Analysis of the tissues of mussels, clams and edible oysters from different areas revealed significant variations in the concentrations of metals Fe, Zn, Cu, Pb, Cd, Ni and Co in their rank order The results indicate the sutability of these species as sentinel organisms in monitoring programmes for heavymetal contamination in bivalves in coastal waters of India

The levels of Fe, Cu, Ni, Mn, Co, Cr, Si, Pb and Cd were determined in tissues of natural and cultured M galloprovincialis

from the Eastern Black Sea The average content of the metals was found to be lower and more stable in cultured animals and it is concluded by Pavlova (1987) that cultured mussels can be used as indicator spp to control metal concentrations in coastal ecosystems

Lakshmanan (1988) found that the average content of Cd in canned mollusc was 0 5552 ppm The data showed that the toxic metals like Mg, Pb and Cd were below the permitted limits The depuration studies showed that the concentration of Cu and Zn in the tissue declined in both clams and oysters In the oyster, the mean Zn concentration (ppm) in filtered sea water was 743 vs 497 in seawater containing EDTA in the 48 h depuration period compared to the back ground level of 892 ppm

The level of total mercury content in the edible oyster C madrasensis varies from 0 0024 ppm to 0 17 ppm The mercury level in the edible oyster was decreasing with increase in size groups of breadth and flesh weight (Jasmine et al, 1988)

The geographic variation of metal content in sediments of coastal lagoon of Ria Formosa reflected anthropogenic sources in the Lagoon Higher contents of Zn, 434 mg/kg were recorded in sediments near major population area (Menden & Vale, 1988)

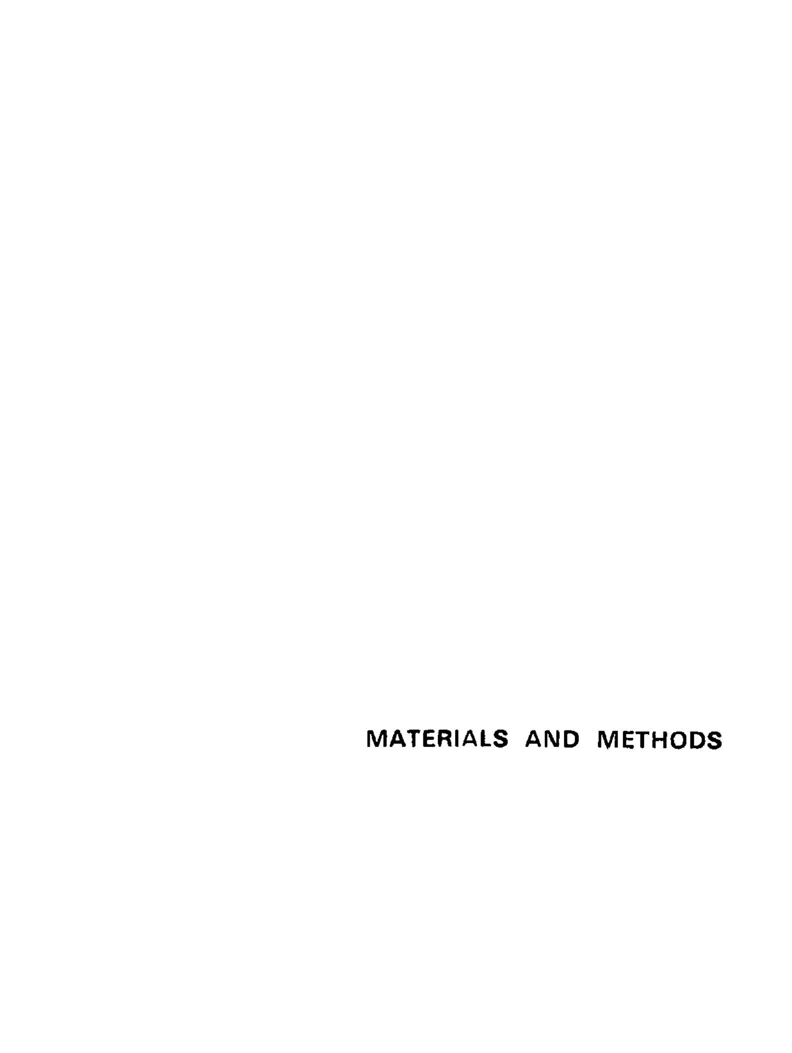
Hutagulung (1989) found that individual M virdis from the same population accumulated different concentration of the metals

35 species of large M viridis out of 105 had Mg and Cd concentration ranges of 11 to 848 ppb and 627 to 2436 ppb respecti

The contents of Fe, Zn, Cd,and Pb in 20 series of samples of mussels collected from the entire life cycle was determined by Serra et al,(1989) and found that in no case did the concentration of these elements exceed the maximum authorised by the Spanish regulation

The concentration As,Cd,Cr,Sn,Hg,Pb and Zn in each of the 38 spp of molluscs (clams, oysters and cockles) varied greatly in the three groups (Lopes et al, 1989).

The conc of Zn,Cd,Cu,Pb,Al,Ni,Mn and Fe in the oysters in Northern Australia were higher than that present in the habitat waters and concentration of Cd in oysters exceeded the NHMRC recommended limit of 2 mg/kg



MATERIALS AND METHODS

3.MATERIALS AND METHODS

3 1 Collection and Transportation of Oyster

Oyster used for the study were harvested from Cochin backwaters near Munambam by hand picking The live oysters were then transported to the laboratory in a container full of habitat water Upon reaching the lab, they were examined for any morbidity and removed from the shell stock. The salinity of the habitat water was noted and the animals were kept in the same water for further study

For the initial microbiological analysis of the shellfish and sea water, the oysters were collected from the natural habitat and transported in insulated container, containing ice Care was taken to avoid direct contact of ice with oyster The sample of seawater for bacteriological examination was collected in a clean, sterile glass bottle with metal closure and was transported to the laboratory in the same container with oyster Date and time of harvesting, salinity and pH of the habitat water etc were noted

3 2 Microbiological Examination of Oyster and Habitat Water

Preparation of the sample for microbiological examination was done according to APHA 1970 The oysters were

washed and scrubbed under running tapwater to remove surface mud and marine life and surface sterilised with 70% alcohol, after which they were dried, shucked asceptically and the flesh (25g) transferred to a sterile blender jar and blended for one minute The oyster homogenate were diluted in sterile 0 1% peptone water to 10^{-6}

Total plate counts were performed by spread plating samples of homogenate on to nutrient agar containing 3% NaCl and by examining for colony development after incubation at 30°C for 48h

Escherichia coli levels were measured with a 3 tube most probable number (MPN) procedure. Tubes of lactose broth were inoculated at 37°C for 24 hours Tubes displaying positive acid and gas production were inoculated into tubes of E C broth and incubated at 44 5°C for 24 h Tubes which have shown gas production and turbidity were streaked on Mac Conkey agar plates and red non mucoid colonies were subjected for IMVC tests for confirming them as E coli Quantitative estimation of Salmonella was carried out by MPN method Tubes of lactose broth were inoculated with homogenate and incubated at 37°C for 24h and they were inoculated into selenite cysteine broth, tetrathionate broth, and nutrient broth followed by streaking on to bismuth sulphide agar Plates were incubated at 37°C for 24h and examined for typical Salmonella colonies. They were streaked on

to nutrient agar plates for checking the purity and subjected for tests such as triple sugar ion agar, production of urease, indol, oxidase etc

Vibrio parahaemolyticus was enumerated by the 3-tube MPN procedure Tubes of seawater yeast extract broth were inoculated with oyster homogenate and incubated at 37 ^{o}C for 24 h, followed by plating on to thiosulphate citrate-bile salt sucrose agar After incubation at 37 ^{o}C for 24 h, typical blue green colonies of *V parahaemolyticus* were counted and the following confirmation tests such as triple sugar ion agar, vogus proskaur, sensitivity to O/129, growth in the absence of NaCl, growth in the presence of 8% and 10% NaCl

Shigella was quantified by inoculating the lactose broth tubes with the diluted homogenate and incubated at 37 ^{o}C for 24h followed by subculturing into tubes of tetrathionate broth and incubated at 37 ^{o}C for 24 h The positive tubes showing growth were then plated on to desoxycholate citrate agar and incubated for 24h at 37 ^{o}C and examined for typical *Shigella* colonies Representative colonies were restreaked onto nutrient agar by performing tests such as motility, reactions on tripple sugar iron agar, utilisation of citrate and production of urease

Water sample was also analysed following the same procedure as described above, but without further dilution

3 3 Determination of Biochemical Composition of Oyster

Biochemical composition of the sample including moisture, total nitrogen, salt soluble nitrogen, non protein nitrogen, ash, acid insoluble ash and crude fat were determined according to standard procedures

Determination of moisture was done by drying the sample at elevated temperature (Boyds, 1979) Percent moisture is derived from the difference in weight of the sample before and after drying

Total Nitrogen in percentage was detrermined by the Microkjeldahl's method (AOAC, 1975)

Ash content was determined by igniting the preweighed sample at 550 ^{o}C in a muffle furnace until free from carbon, allowed to cool and weighed (AOAC, 1975)

Acid insoluble ash is a measure of the sand and other silicious matter in the sample and was determined by boiling the ash in dilute HCl for 5 minutes. The filtered residue was ignited and content of acid insoluble ash was calculated as acid insoluble ash = Total ash % - Acid soluble ash %

Salt soluble nitrogen (extractable nitrogen) as percentage of total nitrogen was determined by extracting the protein in Dyer's buffer (5 % NaCl and O O2 $NaHCO_3$, pH 7 2) at O

-3 ^{o}C and centrifuged,aliquot of the extract is digested and it's nitrogen content was determined by Microkjeldahl's method (Dyer et al, 1950)

Non protein nitrogen in the oyster meat was obtained by determining the nitrogen content of protein free trichloroacetic acid extract by Microkjeldahl's method (AOAC, 1975)

3 4 Determination of Maximum Biological Activity

Excretion of ammonia by the animals will be maximum at the maximum biological activity and the activity of the bivalve will vary depending on the salinity of the water in which they are thriving Eventhough oysters are euryhaline and they can tolerate wide variation in the salinities, there is an optimum preferred salinity at which their activity will be maximum To find out the optimum preferred salinity, the following experiment was conducted

Oysters at a size range of 6 to 9cm length were introduced into troughs of water with varying salinity such as 15ppt, 20ppt, 25ppt and 30 ppt All the trays contained five animals each with 3 litres of water They were observed for the appearance of faecal matter and rate of survival upto four days The ammonia content of the water of each salinity was measured for 24 h and 48h with same salinity water The experiment was repeated 3 times

3 5 Accumulation of *Escherichia coli* by the oyster *C madrasensis*

For effective accumulation of bacteria the salinity of water used for maintanance during accumulation should be compromising for both bacteria and bivalves The pathogens which are seeded into the water should remian alive during the period of accumulation and the salinity had to be optimum for the animals to accumulate the pathogens to the maximum extent possible

To find out the optimum salinity for the accumulation, the following experiment was done

15 oysters each were kept in 4 tubs of ~ 3000 ml brackishwater at salinities 15,20,25 and 30 ppt respectively (in triplicate) and seeded with *E coli* to attain a number of $1x10^9$ cells/ml of water After 6h, 12h, and 18h one sample from each set was drawn and analysed for the extent of accumulation Water from the troughs were replaced with fresh seawater of the same salinities inorder to reduce the level of ammonia to a minimum, so that the toxic effect of ammonia which retard the activity of the oysters could be minimised The practice had an added advantage of keeping the number of the pathogens more or less constant and viable throughout the period of accumulation The experiment was repeated thrice.

3 6 Depuration studies

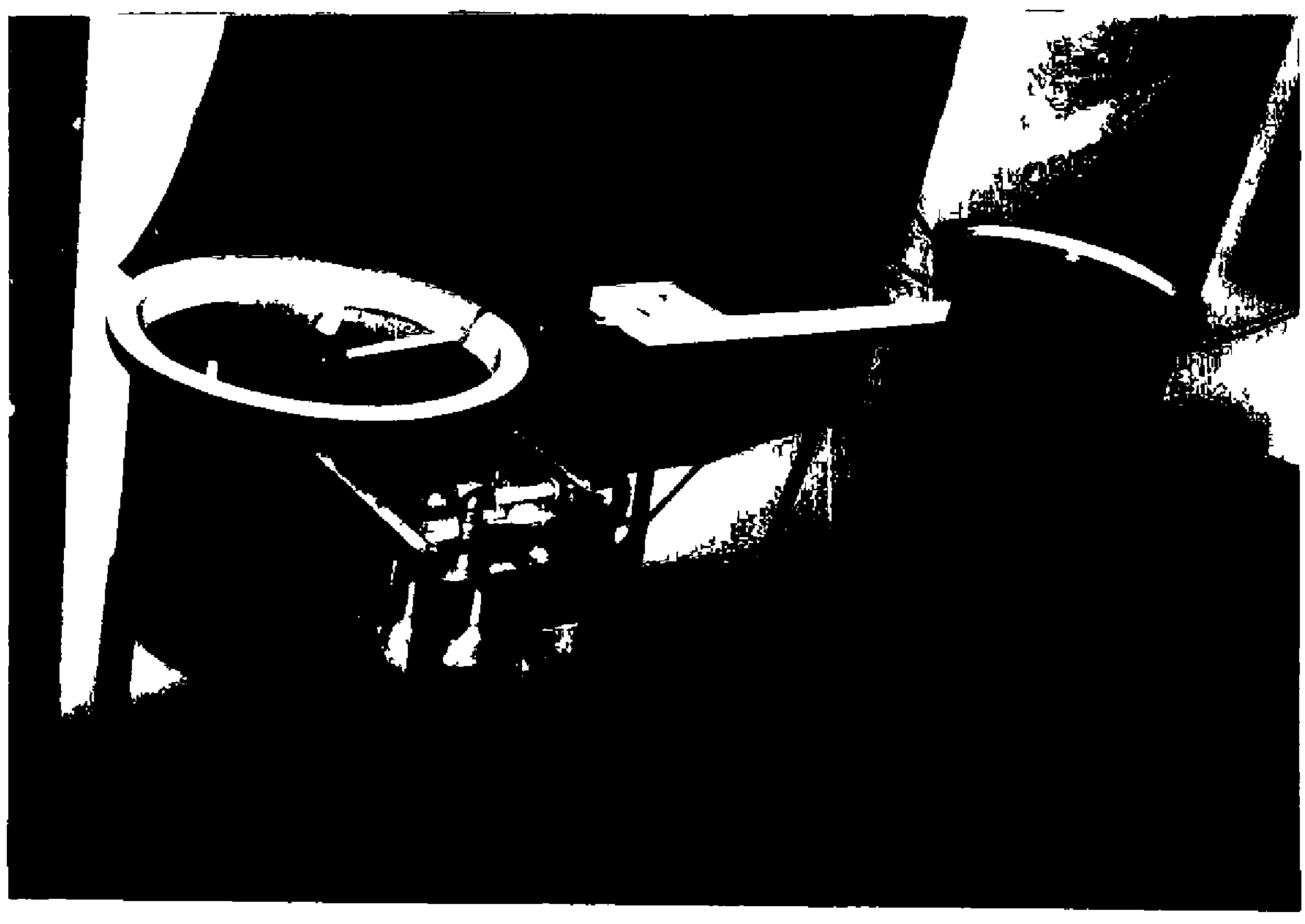
3 6 1 Depuration System

3 6 1 1 Description of the Equipment

The depuration system used for the study was earlier fabricated in the Department of Processing Technology as part of an I C A R project The system consists of three components (Fig 1) They are depuration chamber, biological filter and ultra violet light chamber The depuration chamber is fitted with removable meshed platforms arranged in rows for spreading the oysters This conical shaped chamber is connected to the biological filter, which oxidises ammonia to nitrate thereby reducing the toxic effect of ammonia, through a rubber tubing From the top of the biological filter, an air lift pump drives water to the uv chamber inorder to make the circulating water free from pathogens which takes water back to the depuration chamber

3 6 1 2 Working Principle

Sea water at salinities required by the animal is circulated through the biological filter for a week inorder to deplete the ammonia to the maximum possible extent. UV light is switched on for sufficient length of time to eliminate the pathogens and to make it ideal for the operation. Oysters after seeding are introduced into the tank and the valve leading to the



biological filter is closed and the one to the uv chamber is opened This will lead to the destruction of the organisms as soon as they are expelled from the animals without contaminating the biological filter

After completing depuration the water can again be circulated through the biofilter to reuse for the next batch

3 6 2 Determination of Biochemical and Heavy Metal Changes during Depuration

100 healthy animals (not seeded) were selected from the shell stock and arranged on the removable meshed platforms without touching each other and arranged in rows in the depuration chamber The water in the depuration tank was maintained at 30 ppt as it was the salinity level found to be optimum A sample of the animals were taken from the shellstock before introducing into the chamber to analyse the initial biochemical composition The samples were taken from the system at every 12h, 18h,24h,36h and 48h and analysed for the biochemical changes such as total nitrogen, salt soluble nitrogen, non-protein nitrogen, ash and acid insoluble ash following the same procedure as described earlier

For the heavy metal anlaysis samples were drawn at 0h, 24h and 48h About 5g sample (meat) was taken into a 250 ml round bottom flask of Bethge apparatus (Analytical methods

committee, 1965) 25 ml conc HNO_3 and 6 ml conc H_2SO4 were added to it along with 2 to 3 glass beads, connected the flask to the condensate receiver and reflex condenser and kept for overnight The sample was heated till the solution becomes clear The sample was cooled and filtered through a filter paper and made the volume to known amount and analysed for Cd,Pb,Sn and Zn in an Atomic Absorption Spectro Photometer using respective cathodes and standards Mercury was analysed by Mercury Analyser (MA 5800A) using mercuric chloride standard solutions

3 6 3 Bacterial Depuration of Oysters

After seeding with the indicator organisms, *E coli*, the oysters were introduced into the depuration tank and allowed to remain there for 48h The salinity of the water in the depuration tank was maintained at 30 ppt except for the depuration at 35 ppt

Efficiency of depuration of oyster at various treatments such as depuration with uv light, without uv light, chlorination of water at different levels such as 10 ppm, 20 ppm and 30 ppm and depuration at 35 ppt salinity was evaluated by drawing samples at different intervals and determining residual organisms

Before and after each depuration, water samples for determining the environmental parameters such as salinity, pH,

dissolved oxygen samples were drawn at 0h, 12h, 18h, 24h, 36h, 48h and analysed for the residual level of E coli in the sample

The water used for the depuration system was collected from the backwater, allowed to settle and fed into the tank The salinity was adjusted either by common salt or by potable tap water

The environmental parameters were determined by the following methods,

1 Temperature - Using a graduated mercury thermometer with an accuracy of 0 1 °C

2 Dissolved Oxygen - Winkler's method (Stickland & Parson 1968)

3 Salinity - Using a salinorefractometer

4 pH -By using universal indicator solution checked by digital pH meter

3 6 4 Sensory Evaluation of Depurated Animals

In sensory analysis studies, the samples were assessed on the basis of aroma, and flavour characteristics using hedonic scale The aroma and flavour characteristics were assessed using a

10 point hedonic scale for raw and cooked oysters before depuration, after 24h depuration and after 48h depuration, ranging from extremely fresh (10) to extremely stale (1) Grittiness characteristics was assessed using a five point hedonic scale for cooked oyster before depuration, after 24h depuration and after 48h depuration ranging from no sand content (5) to very high sand content (1) In all instances 10 panelists were presented with samples

3 7 Statistical Analysis of the Data

The data obtained from the studies were analysed using analysis of variance technique (Snedecor & Cochran, 1968).

The data on bacterial population were subjected to logarithmic transformation before analysis, to contain the high variations present

Pair wise comparisons using critical difference values were made for those treatments which were found statitically significant

RESULTS

4 RESULTS

4 1 Proximate Analysis

The results of the experiments to determine proximate analysis of oyster are shown in the Table 1

4 2 Microbiological Examination of Oyster

Oysters (C madrasensis) and water collected from backwater near the cochin bar mouth were examined for total bacterial count and pathogens such as Escherichia coli, Salmonella, Vibrio parahaemolyticus, Vibrio cholerae and Shigella The viable bacterial count of oyster was $3\ 1750 \times 10^{4}$ /g The pathogenic bacteria were found to be absent except E.coli in oysters and seawater samples E coli count was very less and within the permissible limits. E.coli was found to be 2/g in oyster and 14/100ml in seawater The salinity of water during the collection of water and oyster samples was 30 ppt

4 3 Determination of Maximum Biological Activity

The maximum biological activity of oysters were determined on the basis of three factors, viz,presence of faecal matter, survival rate and production of ammonia. All sample produced faecal matter irrespective of the salinity of the water in which they were kept.

Table 2 shows the average rate of survival with respect

Table 1 Proximate composition of oyster , C madrasensis

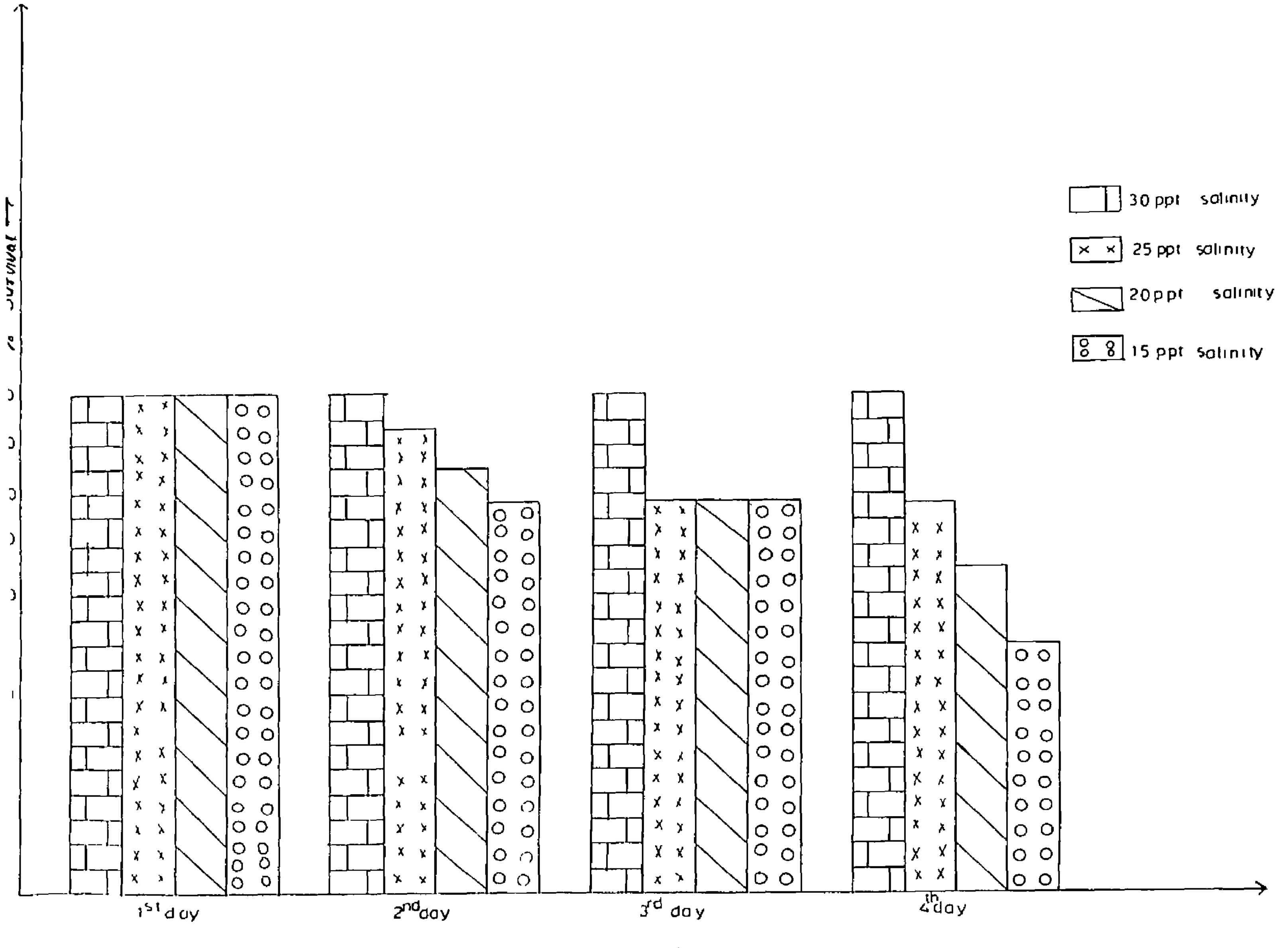
Parameters	Content %
Moisture	87.825
Total Protein	7 37
Total Nitrogen	1 1795
Non Protein Nitrogen	0 3875
Salt soluble Nitrogen	0.4814
Ash	1 654
* Crude fat	12 27

* On a dry weight basis

Table 2 Rate of survival[a] (%) of oyster C madrasensis at different salinities

Salinity ppt Days	15	20	25	30
1	100	100	100	100
2	78	85	93 3	100
3	78	78 3	78 3	100
4	50	65	78 3	100

a Average of three experiments



30 ppt salinity
25 ppt salinity
20 ppt salinity
15 ppt salinity
1st day
2nd day
3rd day
4th day
Period

to different salinities and periods Sarvival rate of the oysters kept at 30 ppt salinity was 100% even after four days Average survival rate of oysters kept in 25 ppt, 20 ppt and 15 ppt showed a decline as shown in Fig 2.

Ammonia production which is an indication of biological activity, was found to be maximum for oysters kept at 30 ppt salinity as shown in Table 3 and Fig 3

Analysis of variance (Tables 4 & 5) indicates that, activity at 15 ppt and 20 ppt salinities differ from 25 ppt and 30 ppt salinities significantly at 5% level However, the activity at 30 ppt salinity and 25 ppt salinity showed no significant difference at 5% level

The results of the above three experiments show that 30 ppt salinity is best for maximum survival and biological activity for oysters compared to 15 ppt, 20 ppt and 25 ppt salinities Hence the depurations were carried out in seawater of 30 ppt salinity

4 4 Seeding Studies

Results of the seeding studies showed that 20ppt salinity is ideal for seeding oysters with *E coli* After periods of 6h, 12h and 18h, oysters accumulated 1,323 x 10^5 *E coli*/g, 2 232 x 10^6 *E.coli*/g and 1.888 x 10^7 *E.coli*/g respectively (Table6)

Table 3 Ammonia excretion[a] by oyster at different salinities[b]

Period (h)	Salinity ppt			
6	15	20	25	30
24	1 25	7 26	10 75	19 6
48	below 1	4 92	10 67	19 5

a - Values in microgram ammonia

b - Each value is average of three experiments

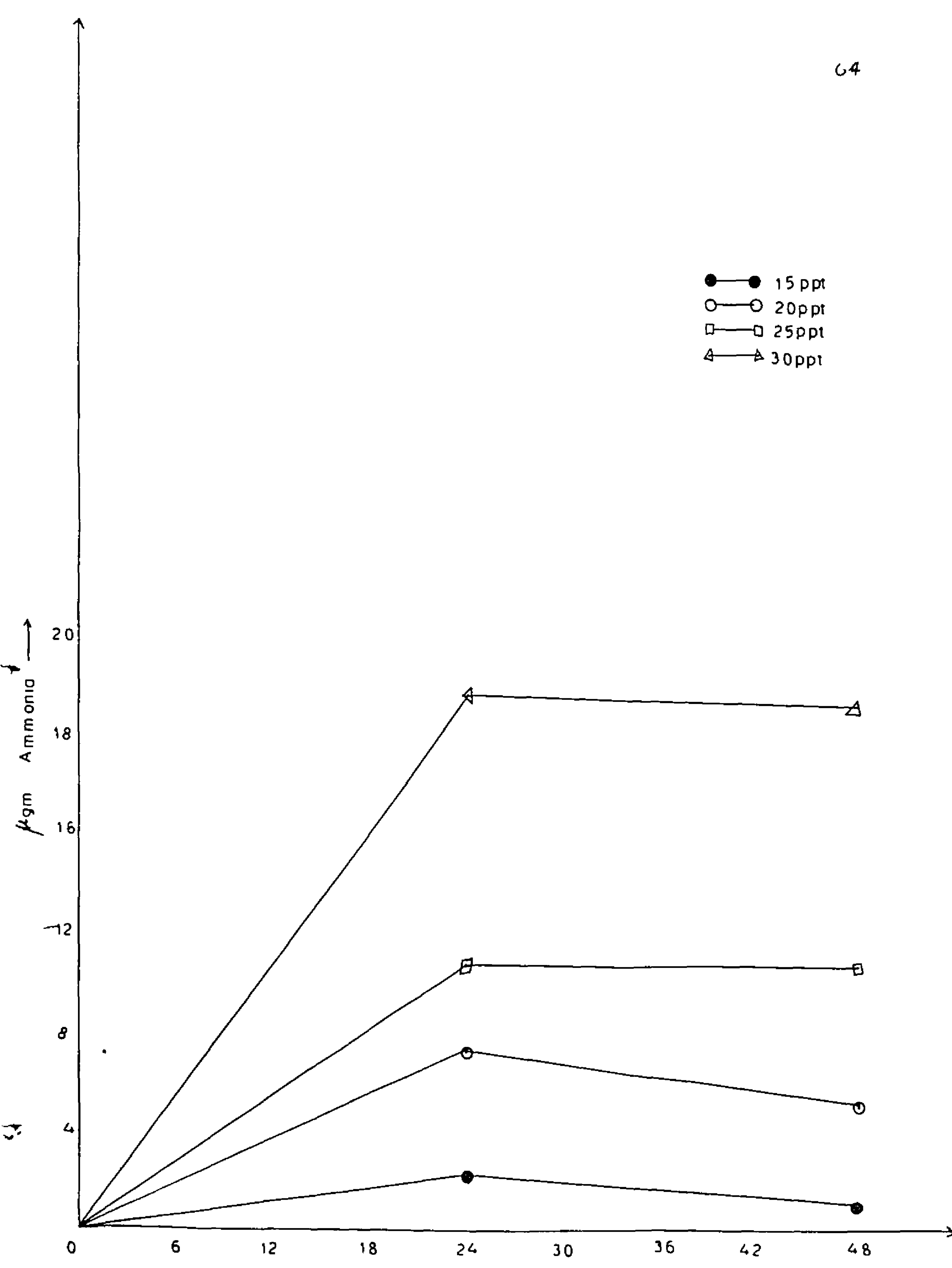
Table 4 ANOVA table for excretion of ammonia by oyster at various salinities at 24 h

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table 5 %
Treatment	3	533 416	177 805	11 47*	4 07
Error	8	124 007	15 50		
Total	11	657 493			

t value = 2 306

Critical difference = 7.41

Treatment means -1 25 ,7 26 ,10 75, 19 6



15 ppt
20 ppt
25 ppt
30 ppt
μgm Ammonia
20
18
16
12
8
4
0
6
12
18
24
30
36
42
48
Period h

Oysters kept at salinity 15 ppt accumulated 2 64 x 10^{2} *E coli*/g 5 09 x 10^{2} *E coli*/g and 5 115 x 10^{3} *E coli*/g after a period of 6h,12h and 18h and accumulation after the same periods were 1 41 x 10^{2} *E coli*/g, 1 58 x 10^{3} *E coli*/g and 1 33 X 10^{4} *E coli*/g respectively for oysters kept at salinity 25ppt for seeding

Oysters which were kept in 30 ppt salinity water accumulated less *E coli* compared to others Average accumulation of *E coli* by oysters were 2 5 x 10^{1} ,1 89 x 10^{2} and 5 8 x 10^{2} , after 6h, 12h and 18h respectively

Analysis of variance also showed that there is significant difference among different salinities and periods (Table 7).

From the graph (Fig 4) also it can be seen that average maximum accumulation of *E coli* by oysters at 20ppt is higher and 6h of exposure is effective in achieving high counts of organisms in the oyster bodies Hence in this experiment, 20 ppt salinity was taken as optimum for accumulation of the pathogenic organism, *E coli* by the oyster and an exposure period of 6h was adopted

4 5 Depuration Studies

4 5 1 Biochemical Changes during Depuration

Oysters were kept in depuration tank containing

Table 5 ANOVA table for the excretion of ammonia by oyster at various salinities at 48h

Source	Degrees of freedom	sum of squares	Mean sum of squares	F value Computed	F value Table
Treatment	3	575 5175	191 839	7 682*	4 07
Error	8	199 7917	24 974		
Total	11	775 3074			

t value =2 306

Critical difference = 9 409

Treatment means - 1 , 4 92 , 10 67,19 50

Table 6 Accumulation of *E coli** by oyster *C madrasensis* at different salinities

Salinity (ppt)	15	20	25	30
Period (h)				
6	2 640x10^{2}	1 323x10^{5}	1 41x10^{2}	2 50x10^{1}
12	5 090x10^{2}	2 232x10^{6}	1 58x10^{3}	1 89x10^{2}
18	5 115x10^{3}	1 888x10^{7}	1 33x10^{4}	5 80x10^{2}

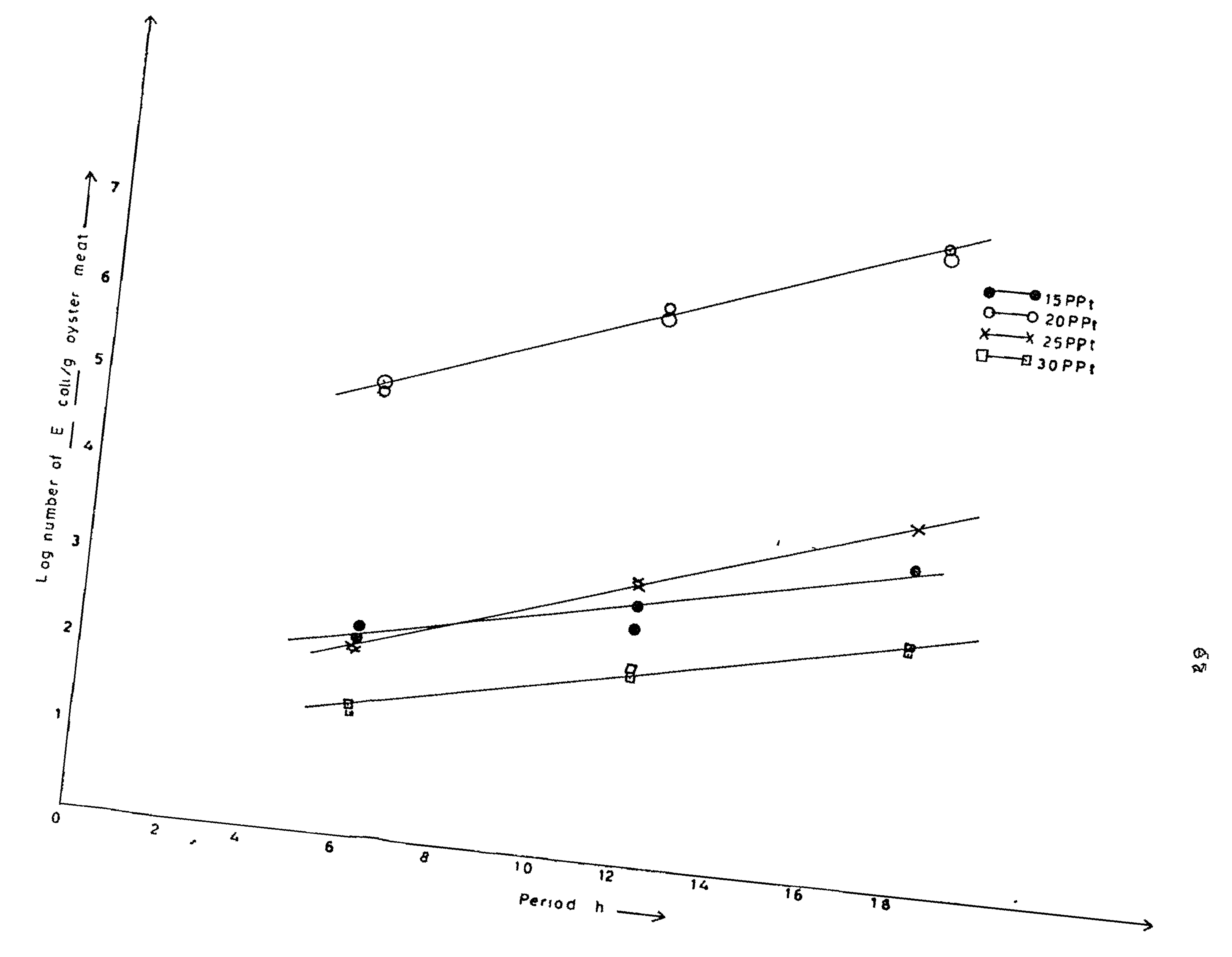

Log number of E coli/g oyster meat
Period h
15PPt
20PPt
25PPt
30PPt
0
1
2
3
4
5
6
7
2
4
6
8
10
12
14
16
18

Table 7 ANOVA Table for the Accumulation of <u>E coli</u> by oyster at differe
salinities

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	
				Computed	Table
Treatment	3	118 27808	39 42603	26 4138*	3 01
Period	2	9 92698	4 96349	3 3253	3 40
Interaction	6	1 05179	0 17530		
Treatment					
x period	11	129 25685	11 7506		
Error	24	35 82305	1 49263		
Total	35				

t = 2 064

Critical differance = 2 0589

Treatment means - 2 9457, 6 2488, 3 1574, 2 1369

seawater of 30 ppt salinity and depurated for 48h. Samples of oysters were taken out from the tank at frequent intervals viz 12h,18h,24h,36h and 48h and analysed for it's changes in biochemical composition such as total nitrogen (TN), salt soluble nitrogen (SSN), non protein nitrogen (NPN) ash and acid insoluble ash Before keeping the oysters in depuration tank, a sample was taken from the lot and analysed for the above biochemical components

The results of the above experiments are shown in Table 8

Average initial content of total nitrogen was 1 218% From the graph, (Fig 5) it can be seen that total nitrogen content does not vary much till 36 h of depuration, but after that it shows a decline But analysis of variance showed that there is no significant change in the content of total nitrogen with different periods of depuration at 5% level of significance (Table 9)

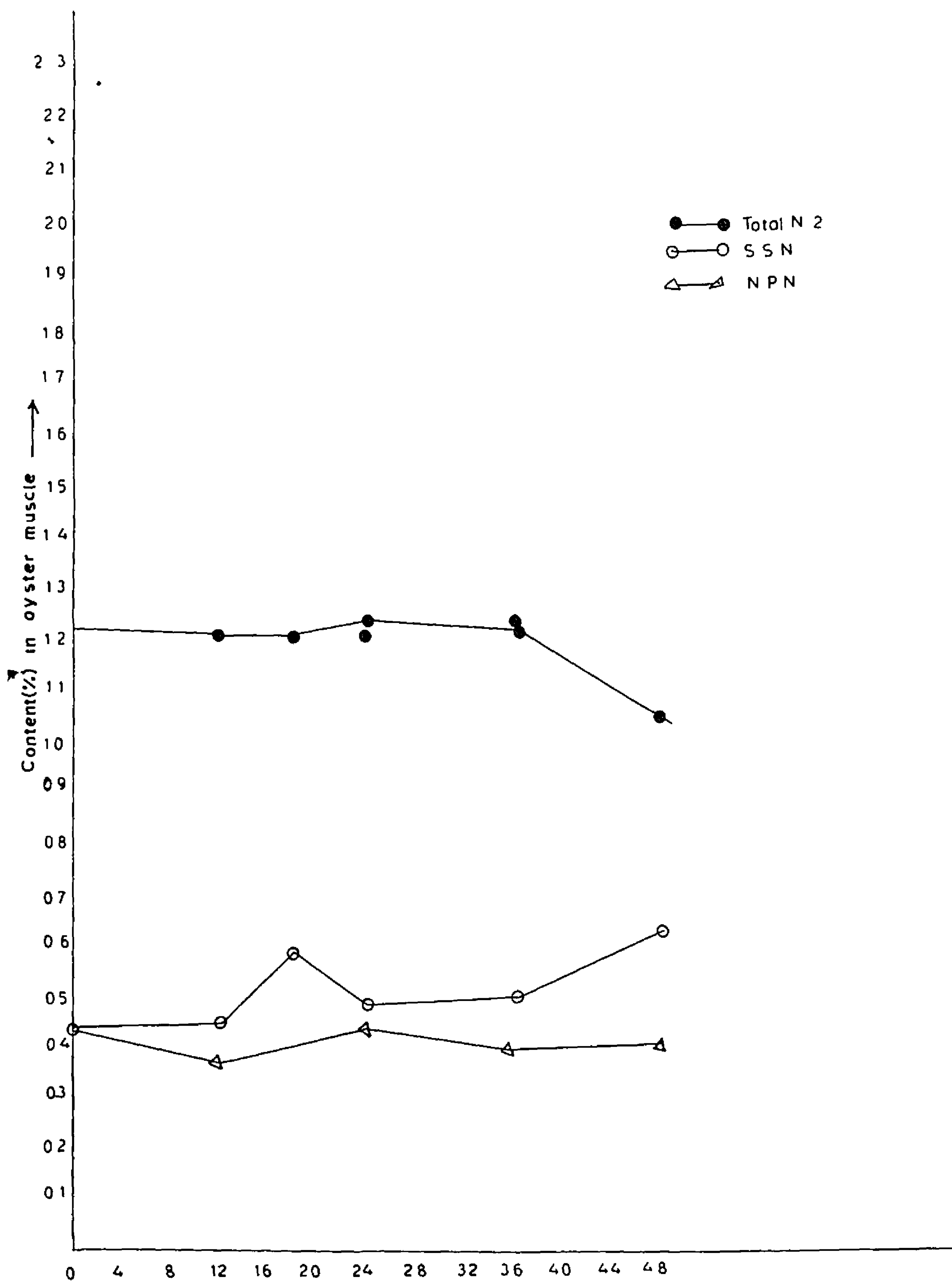
Average content of salt soluable nitrogen before and after depuration was 0 4% and 0 515% respectively showing an increasee in SSN during depuation (Fig 5) However, analysis of variance showed that there is no significant differance in the content of SSN with periods of depuration at 5% level of significance (Table 10)

From the Table 8 and Fig. 5 it can be seen that, the

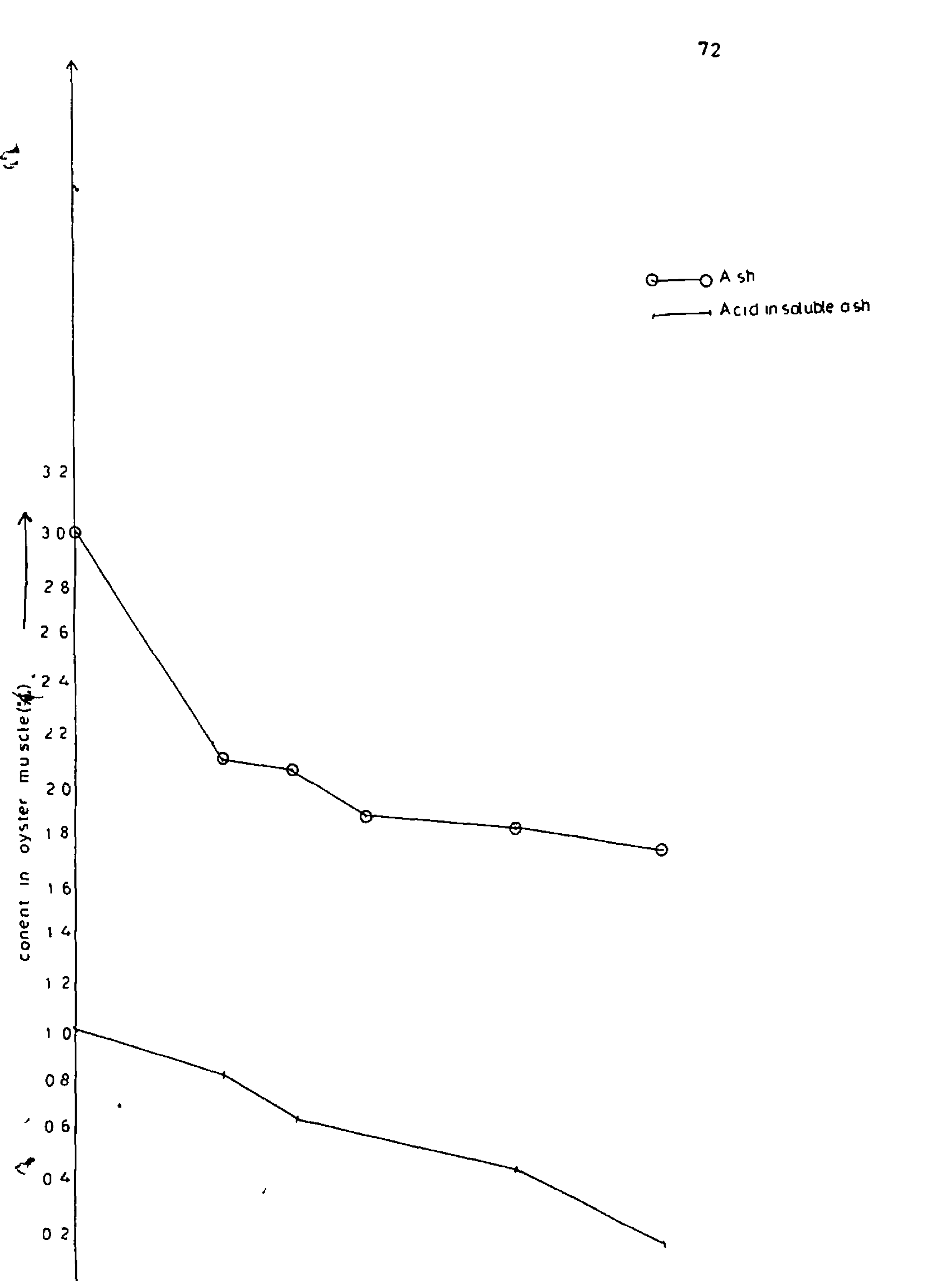
Table 8 Biochemical changes during depuration of oyster*

Parameters %	Period, h 0	12	18	24	36	48
Total N2	1 218	1 207	1 205	1 238	1 22	1 049
Salt Soluble N2	0 437	0 453	0 585	0 492	0 496	0 632
Non Protein N2	0 433	0 367	0 395	0 435	0 401	0 412
Ash	3 03	2 14	2 053	1 8799	1 84	1 745
Acid Insol-uble Ash	1 0441	0 869	0.683	0.619	0 487	0 187

* - Each value is the average of three experiments



Total N 2
S S N
N P N
Content(%) in oyster muscle
2 3
2 2
2 1
2 0
1 9
1 8
1 7
1 6
1 5
1 4
1 3
1 2
1 1
1 0
0 9
0 8
0 7
0 6
0 5
0 4
0.3
0 2
0 1
0
4
8
12
16
20
24
28
32
36
40
44
48



A sh
Acid insoluble ash
conent in oyster muscle (%)
3 2
3 0
2 8
2 6
2 4
2 2
2 0
1 8
1 6
1 4
1 2
1 0
0 8
0 6
0 4
0 2
0
4
8
12
16
20
24
28
32
36
40
44
48

NPN content reduced to 0 367% at 12h depuration from the initial content of 0 435% and NPN content increased again to 0 435% after 24h of depuration However, statistical analysis shows that there is no significant difference in the NPN content with periods of depuration at 5% level of significance (Table 11)

From the Fig 6 and Table 8 it is evident that the samples showed a significant reduction in ash content and acid insoluble ash which represent the sand content in the gut of oysters Ash content reduced from the initial 3 03% to 1 745% after 48h of depuration And acid insolu ble ash content showed a reduction from the initial 1 0441% to 0 187% after 48h depuration The analysis of variance of data showed that there is significant difference (Table 13) in acid insoluble ash content

4 5 2 Bacterial Depuration of Oysters

4 5 2 1 Depuration of Oyster in Seawater with Ultra Violet light Treatment

Since the natural levels of *E coli* in local oysters are variable, it became necessary to seed the oysters with this organism in the laboratory allowing oysters to accumulate on an average 1 7183 $x10^{5}$ *E coli*/g within 6 h of exposure The seeded oysters were depurated for 48 h in sea water sterilised by uv light

Table 14 shows the effect of uv light in the

Table 9 ANOVA table for comparing Total Nitrogen in oyster at different periods of depuration

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	
				Computed	Table at 5%
Treatment	5	0 07353	0 01471	0 9751	4 74
Block	2	0 13615	0 0681	4 5152	
Error	10	0 15082	0 01508		
Total	17	0 3605			

Table 10 ANOVA table for comparing Salt Soluble Nitrogen in oyster at different periods of depuration

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	
				Computed	Table at 5%
Treatment	5	0 0881	0 01762	1 4485	3 33
Block	2	0 03825	0 019125	1 5722	
Error	10	0 121644	0 0121644		
Total	17				

Table 11 ANOVA table for comparing Non Protein Nitrogen at different periods of depuration

Source	Degrees of freedom	Sum of squares	Mean sum squares	F value	
				Computed	Table at 5%
Treatment	5	0 00977	0 001954	0 9637	3 33
Block	2	0 00039	0 000196	0 0966	
Error	10	0 02028	0 002028		
Total	17				

Table 12 ANOVA table for comparing Ash content in oysters during depuration

Source	Degrees of freeedom	Sum of squares	Mean sum of squares	F value	
				Computed	Table at 5%
Treatment	5	3 31569	0 663138	3 2294	3 33
Block	2	2. 1476	1 073799	5 2293	
Error	10	2 05344	0 205344		
Total	17				

Table 13 ANOVA table for Acid Insoluble Ash content in oyster during depuration

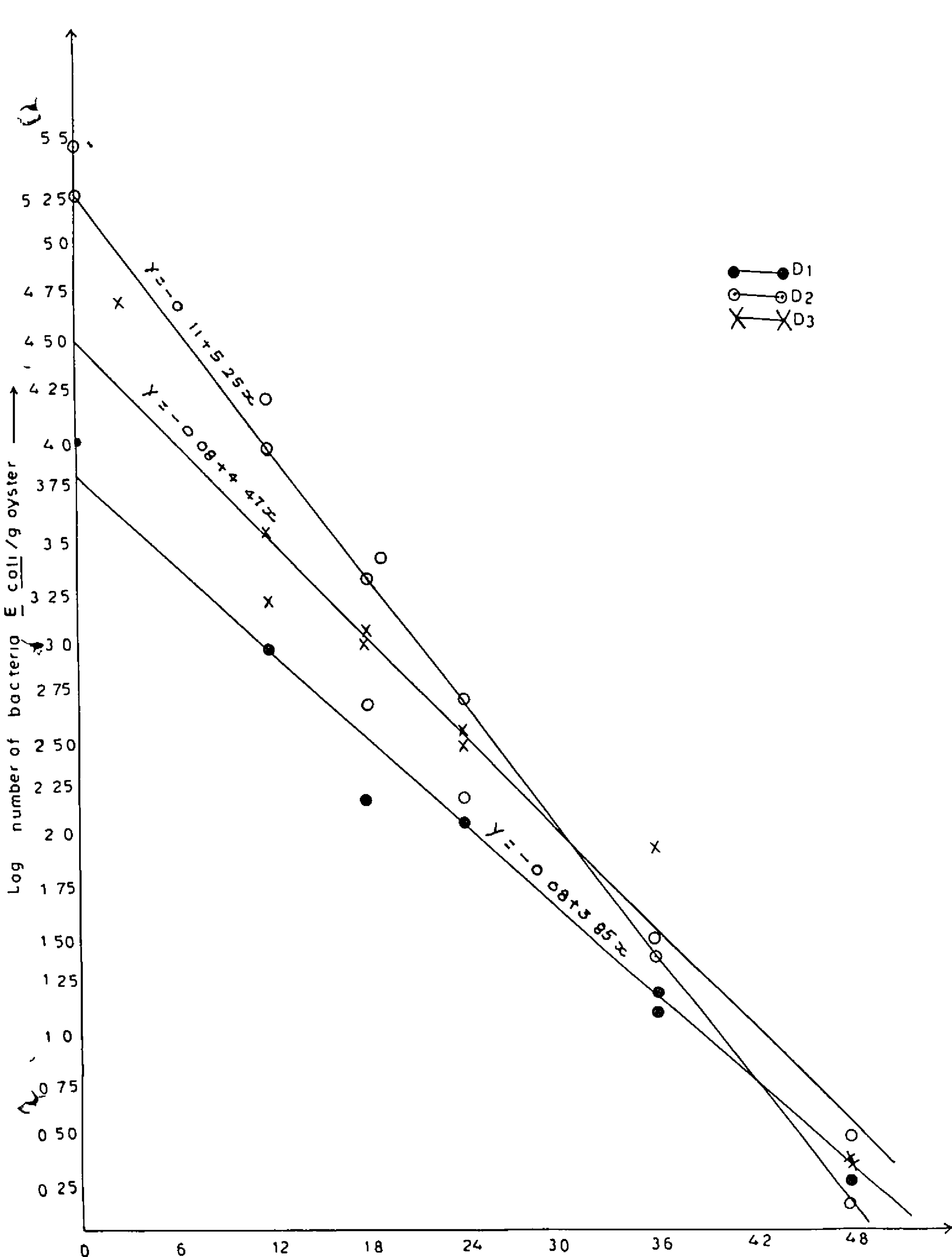
Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	
				Computed	Table at 5%
Treatment	5	1 33826	0 26765	4 12189 *	3 33
Block	2	1 54175	0 77087	11 87159	
Error	10	0 64934	0 06493		
Total	17				

Critical difference = 0 4634

Treatment means = 1.044 ,0 869 ,0.683 , 0.619 , 0.487
0 187

Table 14 Effect of depuration with ultra violet light on the numbers of organisms

Period h	*E coli*/g oyster meat			Average number o organisms per g oys
	Depuration I	Depuration II	Depuration III	
0	1 1x 10^{4}	4 6x10^{5}	4 45x10^{5}	1 7183x10[illegible]
12	1 5x10^{3}	1 5x10^{4}	1 498x10^{3}	5 999x10^{3}
18	1 5x10^{2}	4 4x10^{2}	9 36x10^{2}	5 08x10^{2}
24	7x10^{1}	1 5x10^{2}	3 03$10^{2}$	1 74x10^{2}
36	7	3 0x10^{1}	9 15x10^{1}	4 28x10^{1}
48	2	2 0	2 2	2 03


Log number of bacteria E coli /g oyster
5 5
5 25
5 0
4 75
4 50
4 25
4 0
3 75
3 5
3 25
3 0
2 75
2 50
2 25
2 0
1 75
1 50
1 25
1 0
0 75
0 50
0 25
0
6
12
18
24
30
36
42
48
D1
D2
D3
Y = -0 11 + 5 25 X
Y = -0 08 + 4 47 X
Y = -0 08 + 3 85 X

purification of oysters kept at 30 ppt salinity

E coli elimination was efficient at all the three replications with greater than 99 9 % reduction always occuring within 48 h Average initial levels of 1 7183 $x10^5$ were reduced to 1 $03x10^1$ after 48 h of depuration.

A final reduction of 2 68 log cycles in depuration I, 2 75 log cycles in depuration II and 3 48 log cycles in depuration III were achieved within 48h (Fig 7)

By 24h after axposure,a reduction of 2 175 log cycles in depuration I , 3 25 log cycles in depuration II and 2 log cycles in depuration III were achieved But after 24 h,the rate of elimination was not as rapid as before, and the rate of elimination was 2 13, 1 7 and 1 08 log cycles reductions in depuration I, II and III respectievely

Average residual organisms after 48h depuration was in the order of 2/g, 2/g and 2 2/g respectively in depurations I,II and III which is less than the NHMRC standard of 2 3 *E coli*/g

4 5 2 2 Depuration of Oyster in Sea Water without Ultra Violet light Treatment

From table 15 it is evident that samples showed a significant reduction in faecal colifirm MPN at the end of 48 h depuration even without uv light But the rate of reduction is less compared to that of previous experiment, even though the elimination was efficient with greater than 99 8% reduction always occuring within 48 h

The initial E coli levels of 4 6 x 10^4/g were reduced to 3 0 x10^1/g after 48 h of depuration

A final reduction of 2 68 log cycles in depuration I, 2.75 log cycles in depuration II, 3.48 log cycles reduction in depuration III were achieved after 48h of depuration

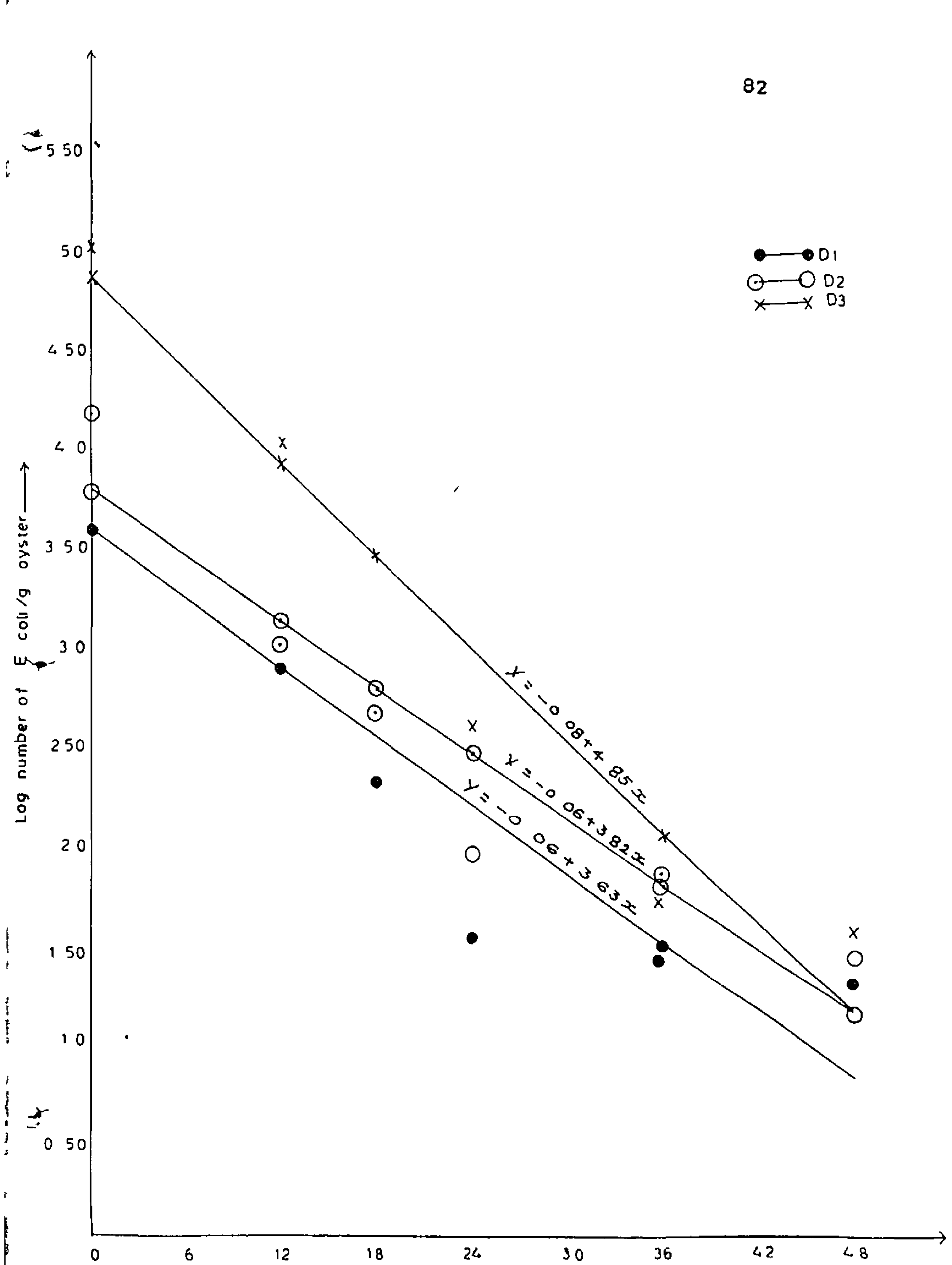
By 24h after depuration, a reduction of more than 2 log cycles was achieved in all the three replications But after 24h exposure the elimination was not rapid and a reduction of only 0 25 log cycle in depuration I, 0 5 log cycle in depuration II and 1 5 log cycles in depuration III were achieved (Fig 8)

The average residual organisms after 48 h of depuration was in the order of 30/g which is higher than the standard stipulated

The efficiency of depuration of oyster in seawater

Table 15 Effeect of depuration without ultra violet light on the number of organisms

Period h	*E. coli* /g oyster meat			Average No of organis
	Depuration I	Depuration II	Depuration III	
0	$1\ 1\text{x}10^{4}$	$1\ 6\text{x}10^{4}$	$1\ 1\text{x}10^{5}$	4 5666x
12	$1\ 1\text{x}10^{3}$	$1\ 050\text{x}10^{3}$	$1\ 2\text{x}10^{4}$	4 716x1
18	$2\ 1\text{x}\ 10^{2}$	$4\ 6\text{x}\ 10^{2}$	$2\ 9\text{x}10^{3}$	1 19x10
24	$3\ 5\text{x}10^{1}$	$9\ 3\text{x}10^{1}$	$4\ 3\text{x}10^{2}$	1 86x10
36	$2\ 1\text{x}10^{1}$	$7\ 5\text{x}10^{1}$	$5\ 3\text{x}10^{1}$	5 23x10
48	$2\ 1\text{x}10^{1}$	$2\ 9\text{x}10^{1}$	$3\ 9\text{x}10^{1}$	2 97x10



D1
D2
D3
Log number of E coli/g oyster
5 50
50
4 50
4 0
3 50
3 0
2 50
2 0
1 50
1 0
0 50
0
6
12
18
24
30
36
42
48
Y = -0 08 + 4 85 X
Y = -0 06 + 3 82 X
Y = -0 06 + 3 63 X

sterilised with uv light is more than depuration in sea water not exposed to uv light

4 5 2 3 Depuration of Oyster in 10 ppm Chlorinated Water .

In this experiment, sterilisation of sea water was effected by chlorination with sodium hypochlorite solution at a concentration of 10 ppm The seeded oysters were depurated for 48 h and at regular intervals samples were drawn and examined for residual organisms

An average initial level of 5 5 x 10^4 *E coli*/g was achieved after seeding and the level reduced to 6 2 x 10^1/g at the end of 48h of depuration (Table 16)

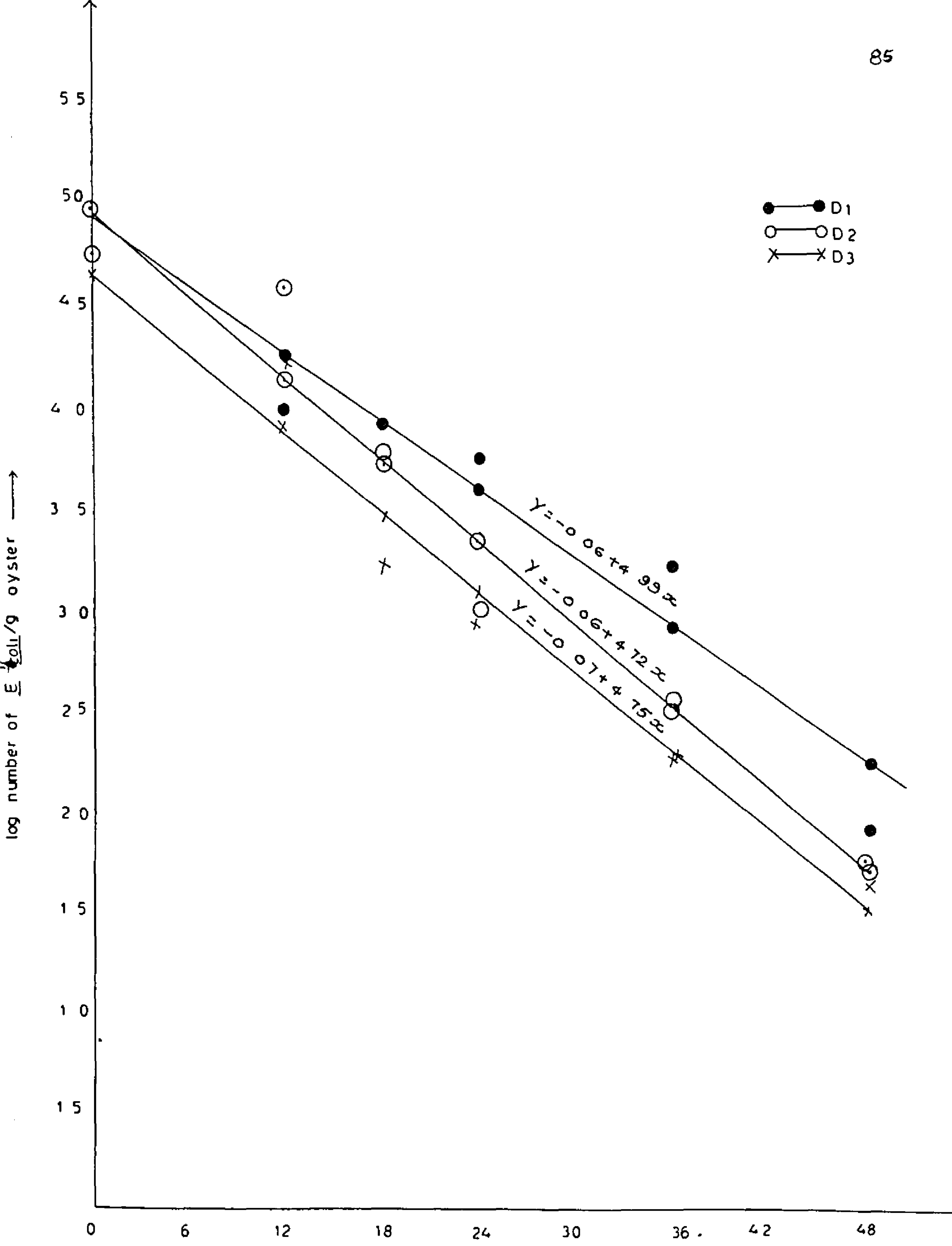
By 24h exposure , a reduction of 1 log cycle in depuration I, 1.78 log cycles in depuration II, and 1 78 log cycles in depuration III were achieved But after 24h exposure, except in depuration I, where a reduction of 1 8 log cycles was achieved, the rate of eliminations were not as rapid as before, being 1 25 and 1 3 log cycles in second and third depuration respectively after 48h depuration (Fig 9)

A final reduction of 2 8 log cycles in depuration I, 3 01 log cycles in depuration II and 3 08 log cycles in depuration III were achieved after 48h

The residual organisms after 48h depuration were in the

Table 16 Effect of depuration of oyster in 10 ppm chlorinated water on the number of organism

Period h	Number of *E coli* /g oyster meat			Average No of organisms
	Depuration I per g oyster	Depuration II	Depuration III	
0	$5\ 7585 \times 10^{4}$	$6\ 0332 \times 10^{4}$	$4\ 7764 \times 10^{4}$	$5\ 5227 \times 10^{4}$
12	$1\ 8616 \times 10^{4}$	$3\ 92 \times 10^{4}$	$1\ 6642 \times 10^{4}$	$2\ 4819 \times 10^{4}$
18	$9\ 5035 \times 10^{3}$	$6\ 488 \times 10^{3}$	$1\ 745 \times 10^{3}$	$5\ 912 \times 10^{2}$
24	$5\ 94 \times 10^{3}$	$1\ 086 \times 10^{3}$	$9\ 0 \times 10^{2}$	$2\ 644 \times 10^{3}$
36	$1\ 786 \times 10^{3}$	$3\ 72 \times 10^{2}$	$1\ 85 \times 10^{2}$	$7\ 8 \times 10^{2}$
48	$8\ 5 \times 10^{1}$	$5\ 5 \times 10^{1}$	$4\ 5 \times 10^{1}$	$6\ 16 \times 10^{1}$


D1
D2
D3
Y=-0 06+4 99x
Y=-0 06+4 72x
Y=-0 07+4 75x
log number of E coli /g oyster
Period h
5 5
5 0
4 5
4 0
3 5
3 0
2 5
2 0
1 5
1 0
1 5
0
6
12
18
24
30
36
42
48

order of 45/g, 85/g and 55/g respectively in the three replications as seen from table 16 These values are higher than the standards stipulated

4 5 2.4 Depuration of Oysters in 20 ppm Chlorinated Water

The seeded oysters were depurated for 48h in seawater sterilised at 20 ppm residual chlorine using sodium hypochlorite.

It can be seen from the Table 17 that an average initial level of $6\ 6 \times 10^{4}$ E coli/g was achieved after seeding which reduced to $1\ 0 \times 10^{1}$/g after 48h depuration

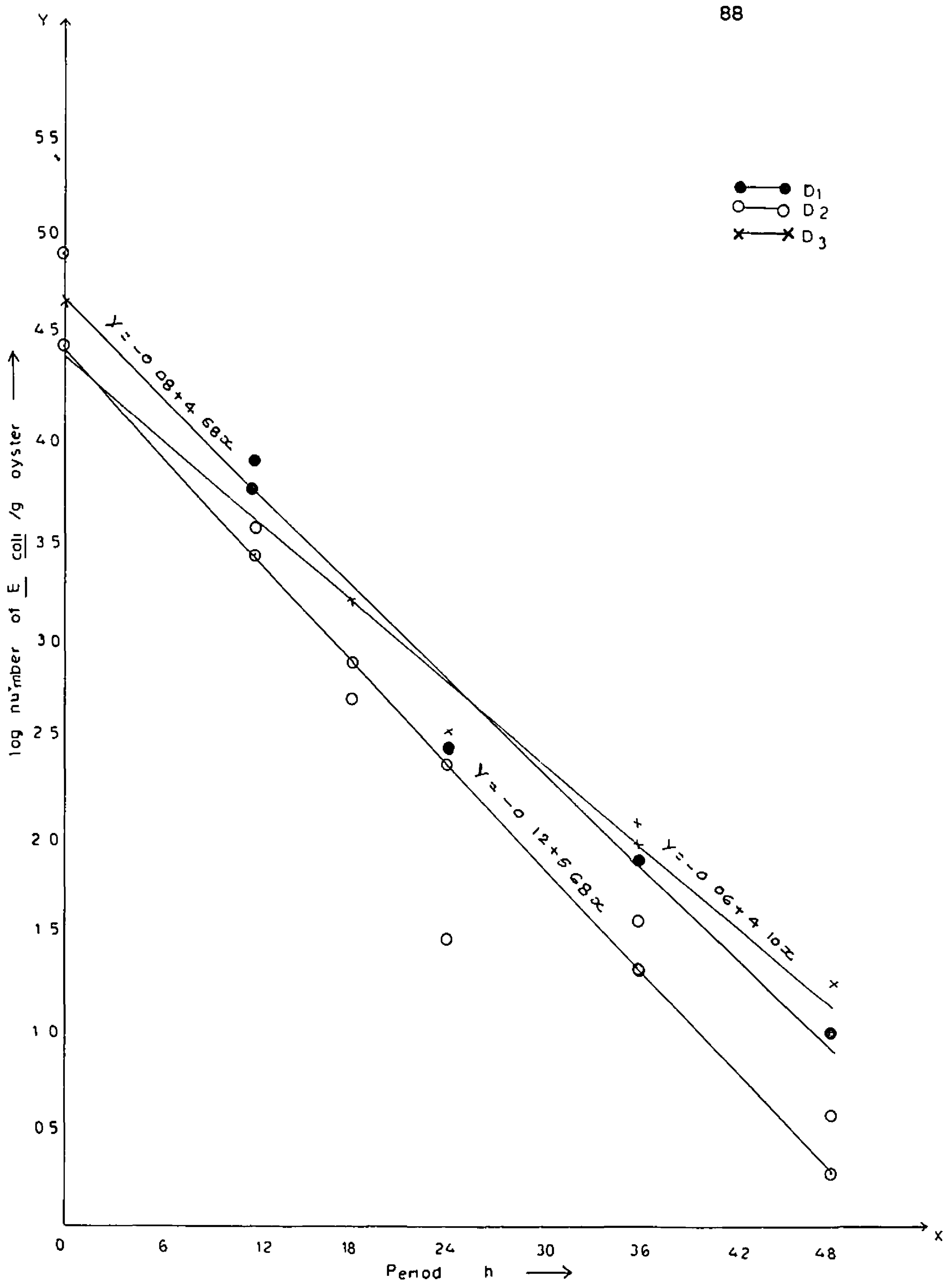
From the Fig 10 it can be seen that by 24h exposure, a reduction of 2 45 log cycles in depuration I, 3 38 log cycles in depuration II and 2 18 log cycles in depuration III were achieved But after 24h, the elimination was not so rapid and only less than 1 5 log cycle reduction was achieved in depuration I and III, where as in depuration II only 0 93 log cycle reduction in bacterial numbers was achieved

A final reduction of 3 38 log cycles in depuration I, 4 31 log cycles in depuration II and 3 43 log cycles reduction in depuration III were achieved after 48h of depuration

The residual organisms after 48h depuration were in the order of 10/gm, 3/g and 18/g in depurations I,II and III which are higher than the NHMRC standard of 2 3/g

Table 17 Effect of depuration on oyster purification in 20ppm chlorinated water

Period h	*E coli*/g oyster			Average No. of organisms
	Depuration I	Depuration II	Depuration III	
0	7 6901x10^{4}	7 2897x10^{4}	4 8228x10^{4}	6 6000x10^{4}
12	7 0170x10^{3}	3 2810x10^{3}	2.8030x10^{3}	4.3670x10^{3}
18	1 3440x10^{3}	4.8400x10^{2}	1 3680x10^{3}	1 0650x10^{3}
24	2 5200x10^{2}	3 0000x10^{1}	2 8000x10^{2}	1 8800x10^{2}
36	6.9000x10^{1}	3 8000x10^{1}	1.1500x10^{2}	6 2800x10^{1}
48	1.0000x10^{1}	3 100x10^{1}	1 8000x10^{1}	1 0000x10^{1}


Y
55
50
45
40
35
30
25
20
15
10
05
0
6
12
18
24
30
36
42
48
X
Period h
log number of E coli /g oyster
D1
D2
D3
Y = -0 08+4 68x
Y = -0 12+5 68x
Y = -0 06+4 10x

4 5 2 5 Depuration of Oysters in 30 ppm Chlorinated Water

In this experiment, the seeded oysters were depurated in sea water sterilised with sodium hypochlorite solution at 30 ppm residual chlorine level

As evident from the Table 18 the average level of 3 7197 x 10^4 E coli/g after seeding was reduced to 3 5 E coli/g after 48h of depuration which is a little higher than the NHMRC standard stipulated

By 24h exposure, a reduction of 2 60 log cycles in depuration I, 2 88 log cycles in depuration II and 2 73 log cycles in depuration III were achieved (Fig 11) But after 24h exposure, the rates of elimination decreased as can be seen from the Fig 11, which indicates a reduction of 1 38 log cycles, 1 15 log cycles and 1 1 log cycles in depurations I,II and III respectively

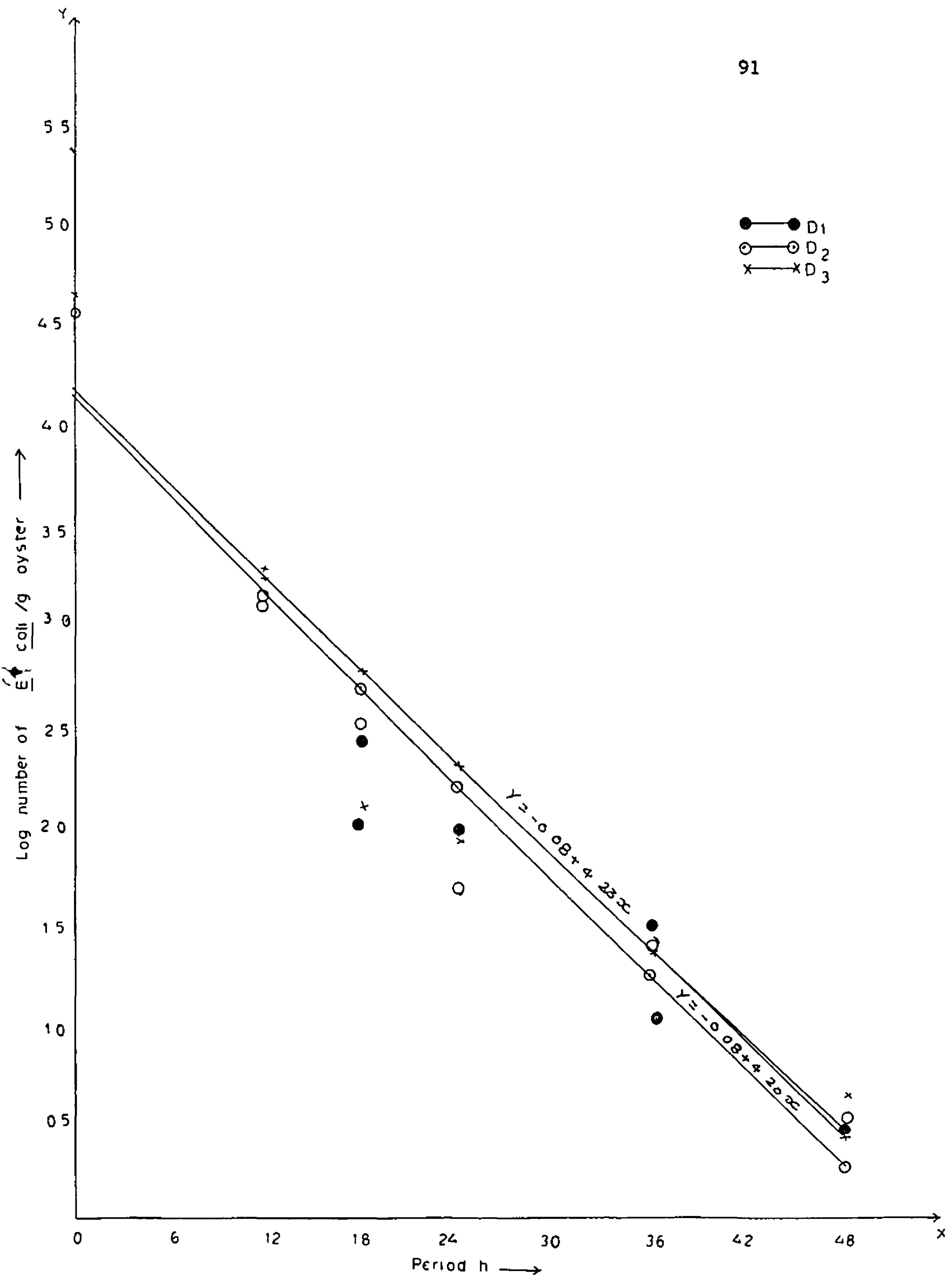
However, a final reduction of 3 98 log cycles, 4 03 log cycle and 3 73 log cycles were achieved within 48h in depurations I,II and III respectively (Fig 11)

4 5 2 6 Depuration of Oyster in 35 ppt Salinity

From the earlier experiment (3.3) it was found that 30 ppt salinity is optimum for the survival and biological activity of oysters compared to 15,20 and 25 ppt salinities So inorder to

Table 18 Effect of depuration on oysters in 30 ppm chlorinated water on the numbers of organisms.

Period h	*E coli*/g oyster Depuration I	Depuration II	Depuration III	Average No of organisms
0	3 6150x10^{4}	3 3212x10^{4}	4 2228x10^{4}	3 7197x10^{4}
12	1 4120x10^{3}	1 2232x10^{3}	1 9147x10^{3}	1.5160x10^{3}
18	2 7700x10^{2}	3 0750x10^{2}	1 1910x10^{2}	2 3500x10^{2}
24	9 5500x10^{1}	4 5860x10^{1}	8 1300x10^{1}	7.4000x10^{1}
36	2 9000x10^{1}	2 3500x10^{1}	5.2300x10^{1}	3.5000x10^{1}
48	4	3	3 7	3 5



Y
5 5
5 0
4 5
4 0
3 5
3 0
2 5
2 0
1 5
1 0
0 5
Log number of E. coli /g oyster
D1
D2
D3
Y = -0 08 + 4 23 x
Y = -0 08 + 4 20 x
0
6
12
18
24
30
36
42
48
X
Period h

find out the effect of higher salinity on depuration, the oysters were depurated in 35 ppt salinity seawater sterilised by exposure to uv light

Table 19 shows the bacterial cleansing results for oysters depurated at 35 ppt salinity seawater

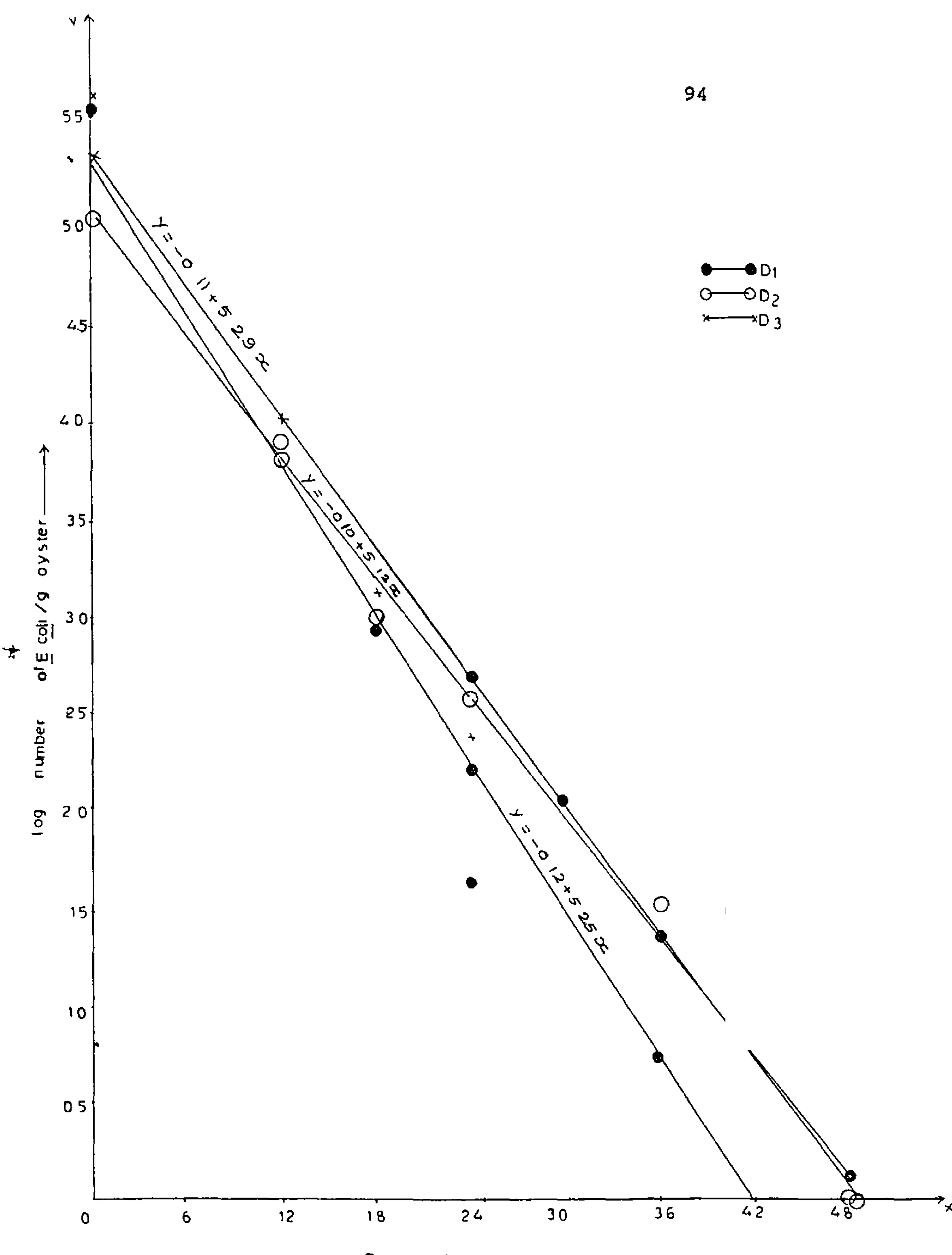
Oyster purification was very effective at this salinity and rates of bacterial cleansing were higher than that of previous experiments

It can be seen from the Table 19 that an average initial level of 3 006 x 10^{5} organisms /g oyster was achieved after seeding, and within 48h of depuration all organisms were purged out of oysters In depuratuion I, 100% purging achieved even after 36h of depuration

The slope of the Fig 12 itself indicates the efficiency of depuration By 24h exposure, it can be seen from the figure that a reduction in E coli of 3 93, 2 33 and 3 25 log cycles were achieved in depuration I, II and III respectively. In depuration I a final reduction of 5 58 log cycles was achieved within 36h In depuration II and III a final reduction of 5.01 and 5 13 log cycles respectievly were achieved within 48h The results indicate the effectiveness of oyster purification at 35 ppt salinity No residual organisms were found after 48h depuration in any of the three experiments

Table 19 Effect of depuration of oyster in 35 ppt salinity seawater on the number of organisms

Period	*E coli*/g oyster			Average No of organism
h	Depuration I	Depuration II	Depuration III	
0	3 7843x10^{5}	1 0416x10^{5}	4 1928x10^{5}	3 0060x10^{5}
12	8 8850x10^{3}	7 5230x10^{3}	6 3250x10^{3}	7 5780x10^{3}
18	8 5400x10^{2}	9 6500x10^{2}	1 2760x10^{3}	1 0320x10^{3}
24	4 5000x10^{1}	4 6900x10^{2}	2 3400x10^{2}	2 4900x10^{2}
36	0	3 3000x10^{1}	7 1000x10^{1}	3 5000x10^{1}
48	0	0	0	0



Y
55
50
45
40
35
30
25
20
15
10
05
0
6
12
18
24
30
36
42
48
X
log number of E coli /g oyster
Period h
D1
D2
D3
Y = −0 11 + 5 29 x
y = −0 10 + 5 12 x
y = −0 12 + 5 25 x

4 5 2 7 Statistical Analysis

Statistical analysis of the data (Table 21) shows that all the six treatments applied are significantly different to each other at 5% level of significance

For better and easy comparison of the results, the data were plotted together with the linear equations obtained which are given in Fig 7,8,9,10,11,12 and 13 Data on bacterial population were transformed into logarithmic values before analysis

Figure 13 gives comparison of effectiveness of various treatments Treatment I and VI were most effective and those also agrees with the standard residual organisms of 2 3/g

Depuration at 10 ppm chlorinated water was less effective followed by depuration in unsterilised water The various physical parameters of the water during depuration are shown in Table 22

Table 20 Effect of depuration on oyster purification, (bacterial cleansing) under different treatments.

Period	Treatments					
	I	II	III	IV	V	VI
	with uv	with out uv	10 ppm Chlori nation	20 ppm chlori nation	30 ppm chlori nation	35p sal nity
0	1 7183x10^{5}	4 5666x10^{4}	5 2270x10^{4}	6 6000x10^{4}	3 7197x10^{4}	3 Ø
12	5 9990x10^{3}	4 7160x10^{3}	2 4819x10^{4}	4 3670x10^{3}	1 5160x10^{3}	7 5
18	5 0800x10^{2}	1 1900x10^{3}	5 1920x10^{3}	1 0650x10^{3}	2 3500x10^{2}	1 0
24	1 7400x10^{2}	1 8600x10^{2}	2 6440x10^{3}	1 8800x10^{2}	7 400x10^{1}	2 4
36	4 2800x10^{1}	5 2300x10^{1}	7 8100x10^{2}	6 2800x10^{1}	3 500x10^{1}	3 5
48	2 0300	3 0000x10^{1}	6 1600x10^{1}	1 0000x10^{1}	3 500x10^{1}	0

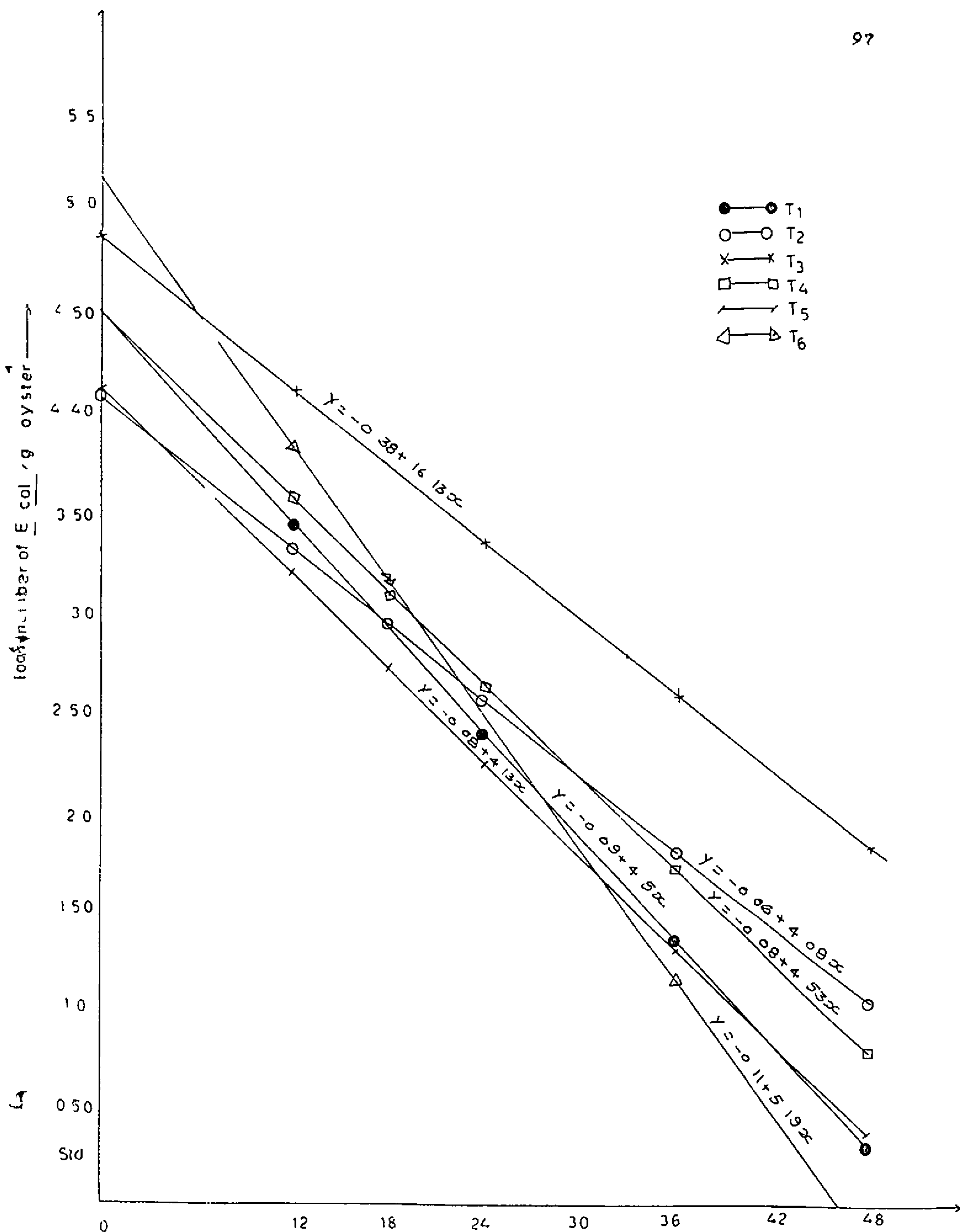

T1
T2
T3
T4
T5
T6
Y = -0 38+ 16 13x
Y = -0 08+4 13x
Y = -0 09+4 5x
Y = -0 06+4 08x
Y = -0 08+4 53x
Y = -0 11+5 19x
5 5
5 0
4 50
4 40
3 50
3 0
2 50
2 0
1 50
1 0
0 50
Std
0
12
18
24
30
36
42
48
oyster

Table 21 ANOVA table for depuration done under various conditions

Source	Degree of freedom	Sum of square	Mean sum of square	F value Computed	F value Table at 5%
Treatment	5	12 5699	2 51397	6 74*	2 60
Period	5	176 2678	35 25357	94 56*	2 60
Interaction	25	9 3204	0.37282		
Treatment x Period	35	198 1581	5 6617		
Error	72	11 5706	0 16070		
Total	107	209 7287			

t value = 2 060

Critical difference = Ø.4985

Treatment means

Treatment I - 2 4522

Treatment II - 3 9371

xTreatment III - 5 0428

Treatment IV - 4 0231

Treatment V - 3 4694

Treatment VI - 3 6264

Table 22 Physical parameters observed during bacterial depurations under different treatments

Parameters	Salinity (ppt)		Dissolved Oxygen mg/litre		Temperature oC		pH		Flow rate ml/sec	
	Before Depu	After Depu	Before Depu	After Depu	Before Depu	After Depu	Before Depu	After Depu	Before Depu	After Depu
I	30	30	6 1	4 24	32	32	7 5	7 5	20 5	20 5
II	30	30	10 21	5 118	29	29	7 6	7 3	20 5	20 5
III	30	30	5 23	4 53	30 5	30 5	7 0	7 0	20 5	20 5
IV	30	30	5 24	4 23	30 5	30 5	6.8	6 8	20 5	20 5
V	30	30	6 12	4 78	32 0	33 0	6 9	6 9	20 5	20 5
VI	35	35	9 66	4 92	32 0	32 5	7 3	7 2	20 5	20 5

4 5 3 Sensory Analysis

Immediately after depuration the oysters were compared with oyster samples before depuration and after 24h depuration for aroma (fresh and cooked) taste and grittiness (cooked) and the mean scores are shown in the Table 23

There were slight improvements in the organoelectric quqlities of oyster after depurations as can be seen from the table 23 Analysis of variance indicated that no significant differences exist at 5% levels between samples of different treatments with respect to aroma of fresh and cooked meat (Table 24 & 25) and significant difference existed in the taste characteristics of cooked oyster (Table 26)

Analyses of variance for grittiness characteristics indicates that a significant difference exist at 5% levels Depuration at 10 ppm chlorination and without uv radiation depuration differ from the rest of treatments (Table 27)

Similarly analysis of variance indicates that significant difference exist between o h and 48h depuration However there was no significant difference between 24h and 48h depuration

4 5 4 Changes in Heavy Metal Concentration during Depuration

Concentrations of Cadmium, Zinc, Lead, Mercury and Tin in oysters during different periods of depuration viz, oh, 24h and 48h are shown in Table 28

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Table 23 Sensory evaluation of depurated oysters

Sensory Scores	Aroma (Fresh)			Aroma (Cooked)			Taste (Cooked)			Grittiness (Coo ed,		
Treatments	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h
I	8 75	8 82	9 00	8 89	9 13	9 17	8 90	9 20	9 36	4 52	4 75	4 7
II	8 55	8 80	9 02	8.67	9 20	9 40	8 50	9 20	9 20	4 25	4 30	4 2
III	8 50	8 80	9 00	8 80	8 60	8 90	8 60	8 75	8 80	4 20	4 30	4 3
IV	8 80	9 00	8 90	9 10	9 20	9 10	9 10	9 30	9 10	4 60	4.80	4 7
V	8 90	8 60	9 10	8 90	9 00	9 00	9 10	9 00	9 20	4 50	4 60	4 7
VI	8 80	8 87	9 03	8 99	9 13	9 20	9 10	9 30	9 30	4 50	4 80	4 9

Table 24 ANOVA table for aroma of fresh depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	5	0 0474	0 00948	0 5243	3.33
Period	2	0 2642	0 05284	2 9226	
Error	10	0 1808	0 01808		
Total	17	0 4924			

Table 25 ANOVA Table for aroma of cooked depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Table at 5%
Treatement	5	0 3876	0 07752	3 1823	3 33
Period	2	0 1735	0 08675	3 5612	
Error	10	0 2436	0 02436		
Total	17	0 8047			

Table 26 ANOVA Table for taste of cooked depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	Table at 5%
Treatement	5	0 58167	0 11633	4 6070*	3 33
Period	2	0 20243	0 101211	4 0084	
Error	10	0 2525	0 02525		
Total	17	1 0366			

t value = 2 228

Critical difference = 0 2991

Treatment means - 9 0633 , 9 09, 8 7676 , 9 133 , 8 967, 9 107

Table 27 ANOVA Table for grittiness of cooked depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	5	0 7242	0 14484	23 990 *	3 33
Period	2	0 12003	0 060017	9 9419 *	4 10
Error	10	0 060367	0 006037		
Total	17	0 9046			

(NHMRC) recommended maximum concentration of these metals in seafoods

The NRMRC recommendation for Zinc in sea foods is 1000 ppm None of the homogenates showed values in excess of the NHMRC standard The initial concentration of Zinc in oysters was 9 036 mg/Kg The Zinc concentration showed reduction during the 48 h depuration as shown in table 28

Concentration of Cadmium in oysters were higher than that of the NHMRC standard of 2 0 ppm The mean initial concentration was 5 9197 mg/Kg which reduced to 5 3406 mg/Kg after 48h

The initial concentration of Lead was 26 6282 mg/Kg which was increased to 34 38 mg/Kg after 48 h of depuration and this exceeds the NHMRC recommendation of 2 0 ppm

Initial concentration of Mercury in oysters was 0 048 mg/Kg which reduced to 0 0355 mg/Kg with an increase to 0 1163 during 24 h depuration (BIS specification of Hg in sea foods is 0 5 ppm)

Tin concentration was higher compared to other metals in oysters with an initial concentration of 103 74 mg/Kg The tin concentration increased to 110 2345 during the first 24 h exposure, there after the concentration decreased to 101 7074 mg/Kg after 48 hr exposure (BIS recommended for Tin = 250 ppm)

Table 28 Change in heavy metal concentration during depuration @

Period h	Heavy metal content(ug/g) Cadmium	Zinc	Lead	Mercury	Tin
0	5 9197	9 036	26 6282	0 0488	103 74
24	6 6197	8 847	36.7489	0 1163	116 2345
48	5 3406	5 614	34 3809	0 0355	101 7074

@ Average values of three depuration

Table 29 ANOVA Table for Lead in depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	2	476 2622	238 1311	0 1782	5 14
Error	6	8018 5294	1336 42		
Total	8	8494 5294			

However, the analyses of data showed that there is no significant difference in metal concentration with periods of depuration within 48 h of depuration under the conditions adopted (Table 29,30,31,32,33)

Table 30 ANOVA Table for Cadmium in depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	2	8.67997	4 339986	0 2036	4.26
Error	9	191 8038	21 3115		
Total	11	200 4837			

Table 31 ANOVA Table for Tin indepurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	2	7551 7207	3775.8604	0 1934	5 14
Perıod	6	117118 299	19519 7165	3 5612	
Total	8	124670 0194			

Table 32 ANOVA Table for Zinc in depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatement	2	26336 236	131168 118	0 0107	4 26
Error	9	11039690 65	1226632 29		
Total	11	11066026 89			

Table 33 ANOVA Table for Mercury in depurated oysters

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value Computed	F value Table at 5%
Treatment	2	0 0182778	0 009139	0 7826	4.46
Error	8	0 0934253	0 11678		
Total	10	0 11170308			

DISCUSSION

5 DISCUSSION

5 1 Microbiological Examination of Oyster

The total bacterial count of oyster was 3 1750x10^{7}/g and the pathogenic bacteria were found to be absent except *E coli* in oysters and seawater These findings are in agreement with the findings of Durairaj *et al*, (1983) and Pillai and Selvan, (1988) The values of *E coli* were found to be in the order of 2/g in oyster and 14/100 ml in seawater According to NHMRC standard, the permissible limits of *E coli* is 2 3/g in depurated oyster Coliform counts of water were reported to be maximum under low salinity conditions (Presnell & Kelly, 1981) At higher salinities accumulation of coliforms will be less The salinity recorded was 30 ppt during the collection of oyster and water samples The low TBC load of oyster and complete absence of pathogens except faecal coliforms on few occasions showed that the oysters were free from microbial pollution in Cochin waters

5 2 Determination of Maximum Biological Activity

In order to find out the optimum salinity level for the maximum biological activity for purification, activities of oysters viz, presence of faecal matter, survival rate and production of ammonia at different salinities of 15,20,25 and 30 ppt were determined

Oysters kept in all the salinities produced the faecal matter indicating that they are active at all the four salinities However, the results of the survival rate and ammonia production show that, 30 ppt is the optimum salinity for the maximum biological activity Even after 4 days, the survival rate of oyster at 30 ppt was 100 % compared to other salinities where as the survival rate was less than 100 % as shown in Fig 2 As seen from the Fig 3 the ammonia production, which is an indication of biological activity, was maximum at 30 ppt with gradually decreasing upto 15 ppt

The effectiveness of purification is related to the rates of oyster pumping and feeding In turn, water salinity affect these activities, so that control of such parameters during purification is an important aspect of public health protecction (Rowse & Fleet,1984) While most estuarine mollusc can tolerate a wide variation in salt levels, there will be an optimum preferred salinity which may vary with species and habitat (Thrower,1990) and from this study, it was found out that 30 ppt, out of the four salinities tested was the preferred salinity Since the salinity of the habitat water was 30 ppt, it was taken as the upper limit of the salinities tested

5 3 Accumulation of *Escherichia coli* by Oysters

Results of the accumulation studies of *E coli* by the oysters showed that 20 ppt salinity was ideal for seeding since

out of the four salinities tested, maximum accumulation occured t 20 ppt salinity And within a period of 6h, the oysters accumulated 1 3228 x 10^5 *E coli*/g oyster meat The results obtained by Timmoney and Abston (1984) showed that each clam accumulated 5x10^6 to 1x10^7 CFU of the test organism during the 15 min exposure This represented a contamination rate of about 1x10^5 to 2x10^5 CFU per gram of clam tissue and was approximately a three fold increase over the bacterial counts per ml of water in the exposure tank The results of the present study is in agreement with the findings of Presnell & Kelly (1981,) that is, coliform counts were maximum under low salinity conditions

In the present study, the bacterial counts per ml of water in the exposure tank was 1x10^9 and the oysters accumulated only upto 1 888x10^7 *E coli*/g during 18 h exposure period at 20 ppt. Reily and Barile (1987) was of the opinion that the accumulation factor (median level in animals/level in water) can be as high as 25 to 30 for faecal bacteria, however these values fluctuates depending on tidal cycles and local conditions A significant feature in all accumulation studies in which levels above environmental titers have been achieved is the use of flow through system Accumulation of both bacteria and viral particles is generally poor in standing water system (Hedstrom & Lycke, 1964, Hoff & Becker, 1968) The results obtained in the present study is in agreement with the above statement Anyhow, an accumulation level of 1 3228x10^5 *E coli*/g oyster meat is

sufficient for depuration studies and hence 6h exposure period was taken for the seeding The accumulation studies of quahog reported by Cabelli and Heffernan(1970) showed that maximum accumulation of *E coli* in active animals occured within 6h of contamination Accummulation of *E coli* by soft shell clam, *Mercenaria mercenaria*, followed the same pattern observed with the Northern quahog, that is the level achieved was determined by the *E coli* density in the environmental water and not by the accumulation interval after the first 6h (Heffernan & Cabelli, 1970, Perkins *et al*, 1980)

The rates of accumulation were less in other salinities tested The low salinity, 15 ppt, is occassionally encountered in natural environment during periods of heavy rainfall Sudden exposure of oyster to lowered salinity is stressfull, leading to weakened physiological activity (Rowse & Fleet, 1984) The results obtained for the seeding of oysters at 15 ppt is in agreement with the above statement The low salinity may have an influence on the physiological activity and hence the low rate of accumulation At higher salinities of 25 and 30 ppt, the accumulation rates were comparatively less Thus the results confirm with the findings of Presnell and Kelly(1981)

5 4 Depuration Studies

5 4 1 Biochemical Changes during Depuration

These studies were conducted to find out whether any changes in the proximate composition,viz,total nitrogen, salt soluble nitrogen, non protein nitrogen, ash and acid insoluble ash of oyster during depuration since it involves direct feeding and pumping activities Ash and acid insoluble ash are the direct indices of grittiness and there by the accumulated sand and mud content, since the oysters accumulate large quantities of sand and mud by their filter feeding activity

The results of the experiments show that there is no significant changes in the content of total nitrogen, salt soluble nitrogen and non protein nitrogen. The data concerning the changes of biochemical components of oyster during depuration are lacking

The initial ash content of oyster meat was 3 03% which reduced to 1 745% after 48h depuration Similarly, the initial acid insoluble ash content of 1 0441% was reduced to 0 187% after 48h of depuration The results of the depuration conducted by Balachandran and Surendran (1988) on clams found that the acid insoluble ash (sand) in the muscle could be brought down to an insignificant level by depuration in water for 18h. Eventhough the present study could not bring down the sand content to

insignificant levels in oyster, more than 80% reduction in sand content could achieve within 48h of depuration But in the case of ash, only 42% reduction could achieve during the 48h depuration However, the analysis of variance data shows that there is significant change in sand content of oyster with respect to different periods of depuration tested Hence, the present study reveals that the process is sufficient for the removal of sandy matter from the oyster, C madrasensis

5 4 2 Bacterial Depuration of Oyster

5 4 2 1 Depuration of Oyster in seawater, with and without Ultraviolet Sterilisation

Ultraviolet lights are now widely used to sterilise seawater They have been operating in a number of overseas countries for at least 30 years Ultraviolet light is very effective in killing both bacteria and viruses and is cheap to produce and maintain

From the results shown in Table 14, it was found that the depuration of oyster in seawater at 30 ppt salinity sterilised with uv light was effective in killing greater than 99% of the bacteria and it was also found that the contaminated oysters cleansed themselves to NHMRC standards within 48h using water continually recirculated through the uv steriliser The residual oraganisms after 48h in the oyster were in the order of

2 0/g, 2 0/g and 2 2/g oyster meat This ıs ın agreement wıth the findıngs of Wood, (1961), Fleet,(1978), Souness and Fleet, (1980) and Mitchell *et al*, (1966)

Table 15 shows the effect of depuration of oyster in seawater wıthout sterılısation Eventhough the elımınation was efficient with greater than 99% reduction always occuring within 48h of depuration, the residual organisms after 48h were in the order of 21/g, 29/g and 30/g oyster meat which were higher than the NHMRC standard of 2 3 *E colı*/g oyster meat

Figure 7 represents the depuration of oyster in seawater sterılısed with uv lıght and Fıg 8 represent the depuration in seawater without sterilisation A steep slope of the graph of the former represent more efficientt depuratıon compared to the latter even when the initial *E coli* levels were higher ın the former case From these results it is concluded that ultraviolet light is efficient in depurating oyster to acceptable levels

On the basis of inıtıal *E coli*level of MPN 1 7183x10^5/g oyster meat, elimination rates were calculated as more than 99 99% after 48h, 99 89% observed after 48h This finding is in agreement with the findıngs of Haven *et al*, (1978) and Gacutan *et al*, (1986) Findings of Haven *et al*, (1978) showed elimination rates between 93 and 98% in the first 24h

Experience in Great Britain and in the USA on the *Crassostrea virginica* showed that a depuration time of 36 to 48h is enough to cleanse oysters to acceptable levels (Haven *et al*, 1978) In Australia with *C commercialis*, the depuration process is for 36h as legislated (Souness *et al*, 1979, Rowse & Fleet, 1984)

In this depuration, cleansing to acceptable levels of *E coli* was achieved with a very conservative flow rate of 1 23 l/min which is much less than the required flow rate of 11 5 l/100 oysters/min In recirculating system in Australia, flow rates are such that atleast two complete water changes are effected in an hour (Souness *et al*, 1979, Rowse & Fleet, 1984) In the present study, it took about 2 hours for one complete water exchange

The success of the experiments conducted by Liu *et al*, (1967) was partially attributed to the use of purdy type of ultraviolet system, by which the seawater was completely sterilised before being used to treat the shell fish

A uv intensity of 960 micro watt/cm^2 reduced the microbial content of seawater from 263 to 13 per ml and the coliform MPN from 17 to less than 0 18 per 100ml However, an increase in the uv intensity to 12000 micro watt/cm^2/min did not increase the degree of microbial contamination (Vasconcelos &

Lee,1972)

But one of the problem with uv light as explained by Souness and Fleet, (1979) is that there could be a gradual selection of uv resistant bacteria in the tank water there by decreasing the sterilising efficiency after 48h However, in the present study, the oysters cleansed themselves to NHMRC standard within 48h in the uv light sterilised depuration system Hence further research is required to refine and optimise the variables of operations and it may be possible to reduce the time of cleansing operation from 48 to 36h there by avoiding this problem and also making it a more ecnomic and convenient operation

However the depuration process may be of limited use in controlling the presence of pathogenic vibrios in *Crassostrea commercialis*, (Eyles & Davey, 1984) and *V vulnificus* in *C virginica* (Tamplin, 1992) Longer depuration time was required for the more heavily contaminated oysters with *Salmonella* (Souness & Fleet, 1980)

Coliforms and some *Pseudomonas* spp. appeared to be eliminated easily from oysters, but some potentially hazardous microorganisms such as gram positive cocci and *Vibrio* species tended to persist for longer period of time (Vasconcelos & Lee 1972) In view of these, there is a pressing need for a more detailed examination of kinetics of uptake and elimination of

specific food poisoning bacteria by oysters

A great advantage of uv light is the low cost and the absence of residual taints, and odours from chemical residues (Thrower, 1990) The sensory evaluation of the oyster after depuration in the present study also reveals that there was no residual taints or odour in depurated animals

From the present study, the author is of the opinion that, had the flow rate been higher than the one tested in the present study, that is, within the normal range of operation, it would have been possible to depurate the oyster, C madrasensis to the NHMRC standard at 30ppt salinity even without sterilising the seawater with ultraviolet light The studies conducted by Pillai and Selvan, (1988) revealed that, the bacterial count of oyster C madrasensis could be brought down effectively either by washing them in filtered seawater for 24h or keeping them in aerated seawater for 48h

Since the naturally polluted shellfish were shown to contain less E coli than those studied in the laboratory, it is anticipated that the former type of shell fish may be cleansed more readily by this process within a reasonable period of time

5 4 2 2 Depuration of Oyster in Seawater Sterilised with Chlorination

The present study was aimed at finding out the

efficiency of Sodium hypochlorite in sterilising seawater for depuration and chlorination at 3 levels, viz, 10ppm, 20 ppm, 30 ppm were tested in order to find out comparative efficiency of different levels of depuration

Of the three levels tested depuration in 10 ppm chlorination was found to be less effective and in 30 ppm was found to be more effective

Eventhough, the depurations of oyster in water sterilised with chlorination were effective in killing more than 99 5% E coli within 48h The residual E coli levels after depurations were in the order of 45/g, 85/g and 55/g of oyster meat respectively in three replications of 10 ppm chlorination, 10/g, 3/g and 18/g of oyster meat respectively in three replications of 20 ppm chlorination and 4/g, 3/g and 3 7/g of oyster meat respectively in the three replications of 30 ppm chlorination as can be seen from the tables 16, 17 and 18

From the Fig 9,10 and 11 it is evident that depuration in 30 ppm chlorinated water is most effective and the same in 10 ppm chlorinated water is least effective since the slope of the graph, representing depuration in 30 ppm chlorination , is steep and that of 10 ppm chlorination is the least steep

The average residual organisms after 48h depuration of

oyster in 30 ppm chlorinated water is 3 5 which is only little higher than the NHMRC standard of 2 3 E coli/g oyster meat This study reveals that by improving the conditions of depuration, the elimination rates can be increased and chlorination at 30 ppm level could be adopted for sterilisation of seawater for depuration As mentioned earlier the flow rate during these depurations were very less ie, 1 231/min Other environmental parametrs noted were as follows,salinity 30 ppt, Dissolved Oxygen 4 23 to 6 12 mg/I Temperature 30 5 to 32 ^{o}C and P^{H} 6 8 to 7 0

chlorination was replaced by other methods because it interfered with oyster feeding mechanism and hence their rate of cleansing (Fleet, 1978)

Kelly, (1961) was of the opinion that insufficicent chlorination gave inadequate water sterilisation and this statement is in agreement with the present study especially in 10 ppm and 20 ppm chlorination level

However, Belmonte etal ,(1984)obtained a decrease of faecal contamination levels to values significantly lower than the international standards in less than 48h of depuration in chlorinated water

Though the depuration in chlorinated water was found to provide no significant improvement, bacterial qualities of the meats of clam,Villoria cyprinoids ,mussel,Perna viridis and

oyster , Crassostrea madrasensis were considerably improved in the case of treatement with chlorine for 2h after depuration in natural water for 24h (Balachandran & Surendran , 1984,1988), Pillai and Selvan, (1988)

5 4 2 3 Depuration of Oyster in 35 ppt Salinity Seawater

It has been reported by several authors that an increase in salinity from the natural habitat could improve the depuration effectiveness Hence the present study was undertaken to find out any increased effect in the depuration of oyster, Crassostrea madrasensis under conditions of high salinity

It is evident from the results presented in Table 19 and Fig 12 that depuration of oyster, C madrasensis in 35 ppt salinity was very effective when compared to 30 ppt salinity Out of the three trials, complete removal of E.coli from the oysters was achieved within 36h of depuration in the first trial and in the other two trials, complete elimination of E coli from oyster was achieved within 48h of depuration

The environmental parameters recorded during the process were as follows, Dissolved Oxygen 4 92 to 9 66 mg/l, Temperature 32 to 2 5 ^{o}C, P^{H} 7 2 to 7 3 and flow rate 1 231/min

The findings of the present study is in agreement with the findings of Liu et al, (1967), Heffernan and Cabelli, (1970a,b)

Rowse and Fleet, (1984), Palpal-Latoc et al, (1986) and Power and Collins, (1990)

A reduction of salinity to 50 to 60% of the original seawater completely stopped the process of depuration in quahog (Liu et al, 1967) where as the depuration proceeded rapidly at 31 ppt (Liu et al, Heffernan and Cabelli, 1970)

Salinity below 16 ppt slows depuration in some Gulf of Mexico oysters (Presnell et al, 1969)

20 ppt salinity is the lower limit for good depuration activity of soft shell calm in which the salinity was varied from 15 to 20 ppt (Heffernan and Cabelli, 1970 b)

Purification was clearly ineffective and incomplete at low salinity, 16 to 20 ppt in Sydney rock oyster In contrast higher salinities of 43 to 47 ppt, the depuration was effective (Rowse and Fleet, 1984) Where as in the case of C iredalei, depuration was ineffective at salinity values of 9 9 to 14 4 ppt Minimum salinity for successful depuration by C iredalei is 17 5 ppt

In the case of Mytilus edulis salinity of 28 6 ppt was better compared to 18 2 ppt for eliminating E coli 4A (Power and Collins, 1990)

As stated earlier, in these depuration runs also the

flow rate was very low and it is evident from the results that bacterial cleansing to acceptable levels can be achieved even within 24 to 36 h of depuration at 35 ppt salinity, provided the flow rate increased to the required level

5 4 2 4 Comparative Effectiveness of Different Treatments in Eliminating E.coli from the Oyster C madrasensis

From the results shown in Table 20 and Fig 13 it is seen that depuration of oyster, C madrasensis in seawater sterilised with ultraviolet light at salinities 30 ppt and 35 ppt can effectively cleanse the oyster of the pathogenic indicator organism E coli within 48h of depuration to the acceptable levels of less than 2 3 E coli/g oyster meat Depuration of oyster at 35 ppt salinity is most effective and after 48h depuration no residual E coli was present

As it can be seen from the table 20, the initial bacterial counts of oysters were higher in treatment I (seawater sterilised with uv light at 30 ppt salinity and treatment VI (seawater at 35 ppt salinity sterilised with uv light) than the other treatments Initial average bacterial count in treatment I was $1\ 7183 \times 10^{5}$ and of treatment VI was $3\ 006 \times 10^{5}$ Even then the rates of eliminations were higher when compared to the other treatments, thus indicating the relative efficiency in

depurating E coli from the oyster, C madrasensis

Depuration at 35 ppt salinity seawater was the most efficient process as it is evident from the graph (Fig 13). The steepness of the slope of the graph itself indicate its comparative efficiency of depuration

Treatment III (depuration in 10 ppm chlorinated water) and Treatment II (depuration in unsterile water) were the least efffective treatments as evident from the Fig 13 and Table 20

Due to limitations of design of the depuration system a flow rate of only 1 23 l/min or 12 3 ml/min/animal had been maintain during the present study At flow rates below 13 ml/min/animal, elimination of E coli from quahog was significantly reduced and at flow rate of 3 ml/min/animal there was a significant increase in the soft shell calm mortality (Heffernan and Cabelli,1970) However, rates of seawater flowing through the depuration tanks were found to be unimportant above 0 5 l/oyster/h (Presnell et al, 1969) or 1 l/oyster/h (Haven et al 1978) as long as sediments in the tank were not stirred into suspension resulting in recontamination of shell fish

Experience in Australia has shown that there is need for one exchange of depuration water every 30 minutes (Fleet, 1978)

The closed system using uv disinfection was found to be

effective with a flow of 2 5 cycles/h in 24h at 5 to 24 °C using upto three *C gigas*/loading rate provided the initial contamination rates are 350 to 35000 MPN coliform/100 g (Fleet, 1978)

However, data concerning the rates of elimination of *E coli* during depuration of *C.madrasensis* in relation to flow rates are lacking

From the present study it is clear that depuration in 30 ppt and 35 ppt salinity seawater sterilised with uv light is effective in reducing the indicator *E coli* to acceptable levels

Depuration in 35 ppt salinity water is more economical and convenient since it takes comparatively less time in cleansing the indicator organism *E coli*

5 5 Sensory Evaluation of Depurated Oysters

Sensory evaluation was included in the present study in order to find out whether there is any change in the sensory characteristics such as aroma, taste and grittiness characteristics of the depurated samples compared to the samples not depurated

As it can be seen from the table 23, the results of the evaluation show that there was a slight improvement in the sensory characteristics such as aroma, and taste after 48h of depuration

It was found that there was no residual chlorine smell or taste to the meat of the oyster samples depurated in chlorinated waters .

However, the analyses of variances show that there were no significant changes in the aroma ' characteristics between the depurated and undepurated animals So it is concluded that the aroma, of the oysters could not be improved by depuration

From the table 23, it was found that there was a marked improvement in the grittiness characteristics The sand content reduced considerably during the 48h depuration Analysis of variance also showed that there was significant difference between the different treatments used in removing grittiness from oyster and the analysis showed that depuration carried out in waters sterilised with 10 ppm chlorination and without sterilisation differ significantly from the rest of the treatments And also there was a marked change in the sand content from the initial level (level at 0h) to the final level (level at 48h), but sand content at 24h and 48h period did not differ significantly

The results of the earlier experiment also (Table 13) showed that there was significant difference in the sand content with periods of depuration at 30 ppt salinity Sensory

evaluationof depuration of oyster shows that there was a significant change in the initial sand content after 48h depuration Depuration of oyster in unsterilised water and in 10 ppm chlorinated water were less effective in removing sand content from the meat This result is in agreement with the earlier findings, that is, depuration of oyster in unsterilised water and 10 ppm chlorinated water were less effective in removing E coli So it is assumed that the above two depurations are not at all effective for the removal of bacteria and sand content from the oyster, C madrasensis

5 6 Changes in Heavy Metal Concentration during Depuration

The data expressed in Table 28 indicated that Cadmium, Zinc, Lead, Mercury and Tin were not depurated by C madrasensis under the conditions of this experiment

Only a slight decrease from the initial content of 5 9197mg/Kg Cadmium was noticed after 48 hr of depuration The statistical analysis of the result show that the change is not significant The result is in agreement with the findings of Brooks and Rumsby, (1967), Zaroogian, (1981)

No significant decrease in Zinc concentration occured in the oyster within 48h of depuration as can be seen from Table 32 Although not significant, Zinc concentration showed gradual decrease from the content of 9 036 mg/Kg to 5 614mg/Kg

This is in agreement with the findings of Lakshmanan(1988) However, Thomson, (1983) found that there is no significant difference in the content of Zinc after 48 or 100h depuration Ikuta, (1968) is of the opinion that accumulated Zinc begin to disappear immediately after the transplantation

Sankaranarayanan *et al*, (1978) obtained a Zinc content of 2450 to 12500 mg/Kg in *C madrasensis* However , in the present study, Zinc concentration was only 9 036mg/Kg

The initial Lead concentration was 26 63 mg/Kg which increased to 34 38mg/Kg after 48h depuration

Lakshmanan and Nambisan, (1983) found that concentration of Fe, Cu, Zn, and Pb in mollusc were found to be highest during low salinity and low pH and lowest in summer months However, Sankaranarayanan *et al*, (1978) were of the opinian that low values of heavy metals in *C madrasensis* were confined to monsoon months when the freshwater discharge through the river was maximum.

The initial Mercury content in *Crassostrea madrasensis* was 0 0488mg/Kg which was reduced to 0 0355mg/Kg after 48h of depuration Jasmine *et al*, (1988) found out a Mercury concentration of 0 0024 to 0 17 mg/Kg in *C .madrasensis*

Individual *Mytilus edulis* from the same population accumulated different concentrations of the metal 35 out of 105

specimens had Hg and Cd content concentrations ranges of 11 to 848 ppt and 627 to 2436 ppt respectively

Tin concentration did not vary too much from the initial concentration of 103 74mg/Kg

Ratkowsky *et al*, (1974) is of the opinion that examination of oysters could be useful in providing index of measure of environmental pollution

The rate of contamination and depuration of metals by oysters and mussels was related to the anatomy of the animals and sequestering of some of the metals in granulocytes (Cooper *et al*, 1983)

The results of the present study indicate that concentrations of Cadmium and lead exceeded the NHMRC recommendation Hence there is a need to improve the depuration process to eliminate the toxic heavy metals to acceptable levels within reasonable period Various authors have found that oysters depurate heavy metals slowly with time The normal depuration time for oyster is 2 days, the cost would increase and oysters would loss conditions if kept longer in depuration tanks

SUMMARY

SUMMARY

1 The objective of the study was to find out the effectiveness of different water sterilisation methods and higher salinity in the depuration of the edible oyster _Crassostrea madrasensis_ Study included (1) Microbiological examination of oyster _C madrasensis_ and habitat water (2) Investigation on artificial accumulation of _E coil_ by oyster (3) Bacterial depuration of oyster using different treatments viz depurations of oyster in seawater sterilised with ultra violet light, in unsterile seawater, in seawater sterilised with chlorination at 10,20 and 30 ppm levels and in seawater at 35 ppt salinity sterilised with ultraviolet light (4)Investigations on biochemical changes during depuration (5)Sensory evaluation of depurated oysters (6) Investigations on changes in heavy metal concentration during depuration

2 Studies on microbiological examination of oyster and habitat water indicate that pathogenic bacteria were found to be absent in Cochin back waters except _E coli_ The levels of _E coli_ in water and oyster were within the permissible limits

3 The present study reveals that accumulation of _E coli_ by oyster was maximum at 20 ppt salinity and 6h exposure period was sufficient for seeding oyster with _E coli_

4 The results of the depuration studies indicate that there were no significant change in the content of Nitrogen, Salt soluble Nitrogen, Non Protein Nitrogen, and content with

periods of depuration However, the depuration system evolved could achieve more than 80% reduction in sand content and the analysis of variance also indicated that significant difference existed in the content of Acid Insoluble Ash with periods

The results of the bacterial depuration of the oyster indicate that out of the six treatments, depurations in seawater at salinities 35 ppt and 30 ppt sterilised using uv light were effective in cleansing the oyster of the pathogenic indicator organism within 48h of depuration to the recommended limit of less than 2 3 E coli /g oyster meat Depuration of oyster at 35 ppt salinity was found to be most effective

Sensory evaluations of the depurated oyster showed that there were no differences in the sensory characteristics,viz, aroma and taste between the depurated and non depurated samples However, there was significant difference in the grittiness characteristics between the depurated and non depurated samples and also significant difference existed between different treatments used in removing grittiness from oyster Depurations carried out in waters sterilised with 10 ppm chlorination and in unsterile water differ significantly from the rest of the treatments

There was no significant change in heavy metal concentration during 48h depuration

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STUDIES ON DEPURATION OF EDIBLE OYSTER *Crassostrea madrasensis* (PRESTON)

by
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ABSTRACT OF A THESIS

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ABSTRACT

Oysters Crassostrea madrasensis harvested from Cochin back waters are commonly contaminated with low levels of food poisoning organisms such as E coli, Salmonella, Shigella, Vibrio cholerae, V.parahaemolyticus etc, heavy metals like Cadmium, Lead, Copper, Zinc, Mercury etc and sand content. Depuration studies were conducted to find out any changes in the biochemical constituents such as Total Nitrogen, Salt Soluble Nitrogen, Non Protein Nitrogen, Ash and sand content The studies revealed that there were no significant changes in the biochemical constituents during the 48h depuration period

Oysters were laboratory contaminated to levels in excess of 10000 cells/g with E coli and it was cleansed from such oysters during purification in a laboratory depuration unit that used ultraviolet light and chlorination for sterilising the depuration water Depuration in sterilised water using ultra violet light treatment was found to be more efficient in cleansing the oyster of pathogen, E coli than using chlorination Of the two salinity tested depuration of oyster in seawater at 35 ppt salinity gave better results compared to that in 30 ppt salinity sea water both sterilised with uv light Depuration of oyster in unsterile seawater and in seawater sterilised 10 ppm

chlorination was found to be least effective in cleansing the oyster to the acceptable international standard of less than 2.3 E coli/g oyster meat within 48h depuration

There was no appreciated change in the sensory characteristics of oysters such as aroma, taste, and flavour However there was significant change in the grittiness characteristics of oyster after 48h depuration

Depuration was not effective in removing heavy metals such as Cadmium, Lead, Zinc, Tin and Mercury from the oyster within the depuration period of 48h