SUB - LETHAL EFFECTS OF HEAVY METALS ON PERNA INDICA (KURIAKOSE & NAIR) AND DONAX INCARNATUS GMELIN

THESIS SUBMITTED TO THE
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FOR THE DEGREE OF

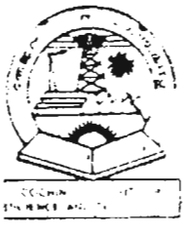
DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY

By

PHILIP MATHEW



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AUGUST 1990

To
My Loving Parents

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CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Shri **PHILIP MATHEW**, under my scientific supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology and no part thereof has been presented before for the award of any other degree, diploma or associateship in any University.

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DECLARATION

I, **PHILIP MATHEW,** do hereby declare that this thesis entitled **"SUB-LETHAL EFFECTS OF HEAVY METALS ON PERNA INDICA (KURIAKOSE& NAIR) AND DONAX INCARNATUS GMELIN,"** is a genuine record of the research work done by me under the scientific supervision of **PROF. DR. N. RAVINDRANATHA MENON**, Head, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, and has not previously formed the basis of the award of any degree, diploma or associate-ship in any University.

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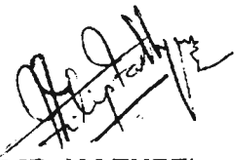

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CONTENTS

PREFACE

Pollution of the aquatic environment by agricultural and industrial chemicals, spilled oil, mine effluents and many other chemical contaminants has been recognised for years. The rapid industrialization and successful green revolution have introduced a large variety of chemicals in our environment. Unfortunately, many of these chemicals due to their chemical structure and properties remain in the environment for prolonged periods and continue to pose health risks and environmental problems. Man has now become aware of the fact that organic and inorganic chemicals can be transported over long distances by water movement, wind and precipitation, accumulating in oceans and coastal waters as the ultimate sink. It is now known that the discharged materials do not undergo degradation or even mineralization as rapidly as expected previously.

Extensive investigations on the effects of hazardous chemicals on estuarine and marine organisms continue to be the interest of most marine scientists. Many studies are confined to the delineation of the cause and effect of pollution due to the most common pollutants among which heavy metals rank a very important position. Marine pollution documented in terms of relative concentration of the contaminants in water alone is no longer acceptable to the scientific world. It is now widely accepted that bioassays form an integral part of a comprehensive approach to pollution assessment. The information so gathered can be of use in management of pollution.

Studies on the lethal and sub-lethal toxicity provide an interesting

plethora of information on the possible consequences of the presence of toxicants on the life and activity of marine organisms. Further, it is also known that chemicals seldom occur singly in nature but in combination.. Thus combined toxicity studies open up another interesting facet of toxicity studies.

The information presented here, gathered during the present investigation underlines the usefulness and importance of such studies especially with reference to heavy metals. However, it should be realised that any study of this sort inevitably require continuation for a more detailed elucidation of the scientific results obtained. Although a wealth of literature is available on the effect of individual heavy metals on marine organisms, information on the combined toxic effects are rather scarce. These aspects were the most important guiding principles for launching the present study. It is hoped that the results obtained would assist in future planning of more elaborate and in depth studies on cytological and molecular biological aspects of toxicology.

1
INTRODUCTION

The apparently limitless marine habitat has long been taken for granted with its supposedly vast capacity to absorb pollutants. However, in recent years it has become clear that the marine environment has limitations to destroy or dilute pollutants to insignificance.

Pollution of the aquatic environment by various types of pollutants have received considerable attention in recent years from among scientists all the world over. Among the common toxicants, heavy metals pose serious hazards to the normal life and activity of marine flora and fauna. Metals from anthropogenic source reach the sea through rivers and outfalls, fall out from atmosphere, direct dumping through ore tailings, marine mining, drilling and also from ships. Several instances have been observed when anthropogenic inputs have exceeded natural inputs, by more than order of magnitude. Heavy metals when present above the threshold concentrations in the marine ecosystem act as pollutants to organisms. Relatively protracted biological half life, inherent toxic nature at sub-lethal concentrations and the capacity to undergo bioaccumulation makes a few heavy metals serious contaminants of the marine ecosystem. Scientific investigations and interpretations in this direction have permitted establishment of maximum "safe" concentration of metals in fish consumed by man.

Scientific investigations have elucidated that free ions are biologically the most available inorganic species of trace metals in sea water and that various factors like total metal concentration, inorganic and organic complexation control their concentration in sea water. Although

diet is the principal source of metals in marine organisms of higher order, the lower group of animals can incorporate considerable quantity of heavy metals from sea water. Uncertainty still exists as to which is the most important source of heavy metals to lower marine invertebrates.

It is well known that metal toxicants seldom occur individually in the aquatic environment. Industrial effluents and agricultural run off into aquatic systems almost certainly burden the ecosystem with mixtures of toxic or potentially toxic metals. Heavy metals in their pure state pose little hazard except those having a high vapour pressure such as mercury and those which may be present in particulate form in the atmosphere such as vanadium. It is the soluble fractions which cause biological alterations (Waldichuk, 1974). Jensen and Jernelov (1969) opined that the danger of discharging some of the metals into the environment in inorganic form lies in their conversion into such highly toxic metallo-organic compounds through biological action. An understanding of the combined toxic effects of heavy metals thus becomes essential, as their effects on marine organisms are not simply due to individual toxicants. The interaction of chemicals may alleviate or aggravate toxicity (Sprague, 1970). Tsai and Mc Kee (1980) suggested that toxicants interact additively or synergistically and that the process appears to depend more on their respective rates of toxic action than on their basic chemical properties. Commonly known as synergism or more than additivity, majority of heavy metals bring about synergistic reaction. Antagonism or less than additivity can also occur as a result of combined action, but at majority of such instances one of the metals will be a non-essential one.

Bryan (1976 a) suggested that the process of heavy metal uptake

from water is a passive one. It has been demonstrated that pinocytosis is the key process involved in the uptake and transfer of metals in molluscs. Endocytosis being a common phenomenon is also believed to play a significant role in the transfer of heavy metals. In marine invertebrates several factors are believed to be involved in the detoxification process. Among these, those that bind to non-specific high molecular weight proteins or to specific low molecular weight metallothionein like proteins seem to be the most important ones.

Winner and Farrel (1976) suggested that although survival is considered as the best index of heavy metal stress, an analysis of sub-lethal toxic stress is quite useful as the sensitivity of this index is relatively higher than that of lethal toxicity indices. The sub-lethal toxic response to a toxicant stems from the long term biological effect on the organism and reflects the environmental changes produced by anthropogenic activities. Reish et al. (1974) opined that such effects may produce alterations in the biological processes which in their turn may influence the normal functioning of the organism or its offspring. Organisms generally can tolerate large doses for short periods of time and progressively lower doses for increasingly longer periods of time. Thus, there seem to exist a time-dose interaction in nature. Arnott and Ahsanullah (1979) are of the view that a knowledge of the acute toxicity studies on individual species, ecosystem studies on laboratory communities or contained "natural" environments, sub-lethal behavioural and physiological tests and toxicant accumulation studies in animal tissues and their transfer through food chain would also give reasonable information on heavy metal toxicities.

The use of sessile marine invertebrates in environmental quality assessment has received considerable thrust from the scientific world since they are unable to escape from environmental deterioration and must either adjust to the change or succumb. Among these, marine mussels, clams and oysters have been shown to exhibit the capacity of concentrating a variety of pollutants within their tissues and they therefore represent good indicators of pollutant bioaccumulation. Thus, they offer convenient indicators of environmental pollution. Perna indica and Donax incarnatus, the two conspicuous and sensitive bivalve species of the intertidal realm have therefore been chosen and put to experimentation in the present scientific investigation. Moreover, information on the combined effects of toxicants on marine bivalves are rather scarce although effects of individual toxicants are very exhaustive.

The present scientific investigation of the effects of copper, mercury and cadmium has focussed on their effects on two commercially important marine bivalve species, Perna indica (brown mussel) and Donax incarnatus (wedge clam), conspicuous representatives of the tropical intertidal areas. The investigation centred around delineating the cause and effects of heavy metal stress, individually and in combination on these species under laboratory conditions. A clear understanding of the cause and effect can be had only if laboratory experiments are conducted employing sub-lethal concentrations of the above toxicants. Therefore, during the course of the investigation, sub-lethal concentrations of copper, mercury and cadmium were employed to assess the concentration dependent effects on survival, ventilation rate, O:N ratio and tissues. The results obtained has been compared with the already available information and partitioned

in sections to make a meaningful presentation. Investigations of this sort would throw more light into the exciting knowledge on marine toxicology and help to understand and delineate the detrimental effects of heavy metal pollution on the flora and fauna of the coastal waters.

2
ACUTE TOXICITY

2.1 INTRODUCTION

The use of bioassays as part of a comprehensive approach to marine pollution assessment is widely accepted nowadays. Toxicity is a biological response, which when quantified in terms of concentration of the toxicant can constitute the basis for a bioassay procedure. Bioassay (= toxicity) tests are defined here as estimation of the amount of biologically active substances by the level of their effect on test organisms (Chapman and Long, 1983). The majority of present bioassays are concerned with determination of survival related to effluent or single aqueous toxicant concentration. Information generated from various toxicity tests can be of use in the management of pollution for different purposes like prediction of environmental damage of a waste, comparison of various toxicants, animals or test conditions and regulations of waste discharge.

2.2 REVIEW OF LITERATURE

Bryan (1984), in his exhaustive treatise on Ocean Management has made an attempt to review the literature available on the various

aspects of heavy metal pollution with reference to marine animals. However, several factors have contributed their might, which has made a comprehensive understanding of the subject difficult. These include, lack of proper concepts from the factors contributing to pollution, diversity in the reporting of literature and the adoption of non-conventional methodologies.

"Stress" as Bayne (1985) puts it can be applied to properties of the environment imposing forces on the organisms. This could be a stimulus, which by exceeding a threshold value, disturbs the normal animal function. On the other hand, a stress response has been defined as "from a strictly biological point of view, it is the population and not the individual that is important and that unless an effect has consequences at the population level, it is insignificant" (Mc Intyre, et al., 1978). A stress response may be primary (mostly of neuro-hormonal character) or secondary, those which are the physiological consequences of the primary response, mostly changes to respiratory and metabolic processess (Pickering, 1981). Such a distinction would not be feasible in the case of marine invertebrates since the categorization has been based on studies in fishes and virtually nothing is known of the invertebrate hormonal aspects of stress response. However, detailed investigations have been carried out in marine invertebrates on the morphological, structural, physiological and behavioural aspects of stress response.

Heavy metals have been defined as those with a specific gravity greater than 4 or 5, located from atomic numbers 22-34 and 40-52 in the periodic table (as well as the lanthanide and actinide series) and having specific biological response (Murphy and Spiegel, 1983).

The Oslo and Paris conventions have classified the contaminants in the marine environment to black and grey lists. The release of the black list substances to the marine environment is completely banned and includes heavy metals such as mercury and cadmium and their compounds. Although heavy metals are one of the most hazardous group of pollutants met with in the marine environment, they pose little hazard to the life and activity of the marine organisms in their pure state. Bryan (1976 a) is of the view that heavy metals when above the optimal values produce serious damage to the ecosystem in general and marine organisms in particular, although many of the metals are highly essential for the normal physiology of marine organisms. Many of the metals do enter into the composition of enzymes, respiratory pigments or structural components. The background concentrations of trace metals are found to be low, although anthropogenic activities tend to increase their concentration several fold. Studies have indicated that marine animals are capable of accumulating trace metals by a concentration factor 10^3 to 10^5. Organisms inhabiting polluted waters therefore tend to exhibit very heavy tissue metal load (Kinne, 1984).

Heavy metal pollution studies of marine animals include lethal and sub-lethal toxicity, accumulation, depuration, histopathology etc. It is believed that the soluble compounds of metals pose the most serious toxicological problem. The toxicity of the same metal varies depending upon the salt form employed. Bryan (1976 a) is of the view that the form of the metal, environmental factors, condition of the animal, acclimation and acclimatization to the metal have a direct bearing on metal toxicity. Thus LC_{50} definitions probably indicate the toxicity as well

as the rate of entry of heavy metals in marine invertebrates (Corner and Sparrow, 1956).

Studies have demonstrated that at toxic levels copper, mercury, cadmium, lead and silver function as enzyme inhibitors (Vallee and Wacker, 1970). Davenport and Redpath (1984) reported that copper is one of the essential metals and that its average natural concentration in coastal waters is around 2-5 ppb. Copper is known to enter into the composition of cytochrome oxidase, lysine oxidase and tyrosinase and is believed to be associated with the enzyme systems involved in removing the toxic side products of aerobic metabolism (Simkiss et al., 1982). Although Perna does not possess any haemolymph oxygen binding pigment, copper is still an essential constituent of Perna haemolymph protein. The known deleterious effects of excess copper are largely associated with interference in enzyme activities and electron transport reactions while membrane permeabilities and cell division may also be affected (Davenport and Redpath, 1984).

Extensive literature is available on the chemistry of copper in sea water (Zirino and Yamamoto, 1972; Ahrland, 1975; Lewis and Cave, 1982). Among the various factors that have been found to influence the chemistry of copper in sea water, the notable ones are solubility, capacity to form complexes, colloids, adsorption and pH. Copper is found to enter the marine ecosystem through terrestrial run off, aeolian import and geothermal addition.

Owing to diverse experimental approach and criteria for toxicity, considerable variations in the LC_{50} values of copper have been observed in Mytilus edulis. While Scott and Major (1972) reported values as high

as 200 ppb, values as low as 15 ppb has also been reported (Manley, 1980). The variations in these values could partly be attributed to the adopted experimental exposure pattern which varied between a total static system and a continuous flow-through system. The effect of copper on the developmental stages of bivalves have been subjected to extensive research. Wisely and Blick (1967) reported a 2 h LC_{50} of 22 ppm copper on pediveliger. However, some stages have been found to be more tolerant to copper (Sheffrin, 1982). In Mytilus plantigrades, 137 ppb of copper was required to kill 50% of the test animals in 52-53 d. MacInnes (1981) working on the response of oyster embryos to copper found that 12.3 ppb of cupric chloride or 14.3 ppb of copper nitrate would produce 10% abnormality of the larvae.

Prabhudeva and Menon (1988) observed that the sulphate forms of copper (individually as well as in combination) were more toxic to Perna indica than the nitrate form; the additive index being more than additive (synergistic) in both the cases. Studies by Lakshmanan and Nambisan (1977) showed that copper was the most toxic metal ion to Perna viridis, Meretrix casta and Villorita cyprinoides var. cochinensis. It was found that variable salinity influenced bioaccumulation of copper by Sunetta scripta (Latha et al., 1982). The rate of uptake of copper was found to have a linear relation to the concentration in water (Nambisan et al., 1977).

Mercury entering the marine environment as divalent inorganic form or as phenyl mercury acetate, is first incorporated into the bottom sediments and thence mobilized by the formation of soluble complexes

such as mercuric chloride or by biological methylation to dimethyl mercury (Jernelov, 1972). Menon N.R. (personal communication) has opined that the 96 h acute toxicity studies employing mercury in inorganic form by direct injection to the test medium cannot involve such a possibility.

Studies on the survival of scallop (Argopecten irradians) exposed to mercury indicated that mercury is less toxic than silver but more toxic than cadmium. Further, the toxicity of mercury is influenced by salinity and temperature of the test medium. Portmann (1970) reporting on the lethal toxicity of mercury on Cardium edule suggested that this species is relatively hardy and compares well with Crangon crangon in its sensitivity to mercury. The lethal toxicity of mercury on the embryos and larvae of Crassostrea virginica has been extensively investigated (Calabrese et al., 1973; MacInnes and Calabrese, 1978; Calabrese et al., 1977). Conner (1972) described the mortality rates in Ostrea edulis larvae exposed to mercury. Calabrese and Nelson (1974) and Calabrese et al. (1977) furnished explanation for the lethal effects of mercury on the larvae of Mercenaria mercenaria. Studies on the mortality rates of juveniles of Argopecten irradians revealed that this animal is relatively less sensitive to mercury (Nelson et al., 1976). Pelletier (1988) reported that Mytilus edulis under laboratory conditions cannot use selenium, even available under its reduced form, to protect itself against inorganic and organic mercury toxicity; the reason of this lack of selenium protection being unclear.

Flatau and Aubert (1979) investigating into the acute toxicity of cadmium in the marine environment reported that although the acute toxicity of cadmium is rather slow, the concentration phenomenon appears

rapidly; the concentration factor of a decreasing order from phytoplankton to fish. Studies by Johnson and Gentle (1979) revealed the acute toxicity of cadmium to the larvae of American lobster, Homarus americanus. Ray and Coffin (1977) are of the view that cadmium is extremely toxic to most aquatic organisms and that its toxicity is cumulative. Thomman et al. (1974) opined that cadmium toxicity to aquatic life has generated great concern since it affects all levels of food chain. Increasing evidence go to show that a short term bioassay may not produce conclusive results on cadmium toxicity since toxicity is generated slowly. However, a better understanding of the precise mechanism of cadmium toxicity can be had by adopting a continuous flow through bioassay or by prolonged chronic exposure as demonstrated by Baby (1987).

Ahsanullah (1976) working on the toxicity of cadmium on Mytilus edulis opined that the species is relatively more sensitive than the common crustaceans. However, the intermoult stages of these crustaceans may be more prone to cadmium toxicity. Further the mortality pattern of invertebrates reflected the cumulative nature of cadmium toxicity. This observation has been confirmed by the fact that at certain concentrations the animals were unaffected initially but thence, in a brief span, very high mortality rate occurred. Briefing on the outcome of acute toxicity tests, Bryan (1984) remarked that the rate of absorption of metals is rather a slow process so that only extremely high concetrations are effective in bioassay tests which utilises death as the end point. Working on Crassostrea virginica, Schuster and Pringle (1969) noted that a concentration of 100 ppb of cadmium could induce mortality in about 15 w although the same species when exposed to 15 ppb of copper for 40 w produced

no mortality inspite of an accumulation of 290 ppb of copper by the tissues (Zaroogian, 1980).

Mohan et al. (1984) delineated the acute toxicity of cadmium to four species of intertidal molluscs following static bioassay technique. The studies revealed that Perna viridis was much more sensitive to cadmium than two species of Modiolus. They further feel that the drastic variations observed in the LC_{50} values reported by various authors owe largely to the non-uniformity of the adopted experimental techniques and in the selection of species from diverse geographical areas. Lack of information on the toxicity of heavy metals on tropical invertebrates often makes comparisons difficult. Studies on the toxicity of cadmium to selected pelecypods indicated that Mytilus edulis, the most distributed temperate representative of this genus is the least sensitive to cadmium (Eisler, 1971; Portmann and Wilson, 1971; and Calabrese et al., 1973). In Mytilus irradians, cadmium was the least toxic compared to the other heavy metals tested. Increasing evidence go to indicate that early life stages of molluscs are more sensitive to mercury and silver than cadmium (Calabrese et al., 1977). Saliba and Ahsanullah (1973) reported that pre-exposure of marine animals to sub-lethal concentrations of heavy metals influence their lethal toxicity. Studies by Krishnakumar et al. (1987) revealed that in Perna viridis, smaller size groups (15-20 mm) are more sensitive than the larger size groups (30-40 mm); the order of toxicities of the metals tested being copper, mercury and zinc.

Despite considerable amount of information available on the effects of individual heavy metals on marine organisms, reports on the adverse effects of heavy metal mixtures to aquatic organisms and their

interaction at sub-lethal levels are rather scarce. Studies on heavy metal interactions might prove a more realistic assessment of their toxicity to coastal organisms since metals usually occur as mixtures rather than singly. Recent investigations have revealed that metals may interact synergistically or antagonistically on the survival and development of various marine organisms (Phillips, 1980; Ahsanullah et al., 1981; MacInnes, 1981; Cooper et al., 1982; Murthy, 1982; Prabhudeva, 1983; Prabhudeva and Menon, 1986 b; Mohan et al., 1986 a and b; Prabhudeva and Menon, 1987 a and b). Commenting on the effects of independent variables on biological responses, Aldrice (1972) stated that the effect of more than one variable and the resultant biological response of an organism may depict plasticity, interaction, tolerance variability and changes in capacity adaptation. Among these, the reaction of the animal experiencing continuous exposure to multiple environmental variables or toxicants can trigger capacity adaptation. Moulder (1980) discussing on the combined toxicity of heavy metals suggested that relative increase in the rate of uptake, formation of toxic metabolites, reduction in excretion, alteration of distribution and inhibition of detoxification could explain more than additive toxicity of metal combinations.

Experiments on the combined toxicity of mercury and copper showed that the combination increased the rate of mortality in Artemia salina (Corner and Sparrow, 1956). A simple additive effect was observed when copper and zinc were supplied to the juveniles of Atlantic salmon (Salmo salar) (Sprague and Ramsey, 1965). Discussing on the effects of chelation on the toxicity of copper, Morris and Russel (1973) opined that the properties of metals are modified greatly when complexed with organic

compounds. A mixture of cadmium, copper and zinc salts were found to be more toxic to estuarine teleosts than individual metals. Eisler and Gardner (1973) stated that concentrations of cadmium not ordinarily lethal, produced a negative effect on the survival of fish pre-exposed to copper or zinc salts or both.

In Ostrea edulis, Coombs (1974) demonstrated that both zinc and copper were weakly complexed to the small molecular weight proteins, taurine, lysine, ATP etc. In developing sea urchin eggs, Murakami et al. (1976) demonstrated an additive effect for mercury and copper in combination. The fact that the interaction of metals was temperature dependent was elucidated by MacInnes and Calabrese (1979). Hrs Brenko et al. (1977) demonstrated synergistic effects for lead, salinity and temperature on the embryonic development of Mytilus galloprovincialis and that at comparatively high temperature the presence of lead had a deliterious effect on the development. Eisler and Gardner (1973) found that mixtures of copper and zinc caused more than expected mortality in mummichogs (Fundulus heteroclitus). In juvenile long fin dace Agosia chrysogaster (Lewis, 1978) and eggs of Cinchlasoma nigrofasciatum (Ozoh and Jacobson, 1979), similar synergistic effects of copper and zinc salts have been demonstrated.

Discussing on the combined toxicity of silver and copper on Perna viridis, Mathew and Menon (1983) found that silver in combination with copper becomes more toxic. MacInnes and Calabrese (1978) in a detailed study on the effect of mercury, silver, zinc and copper, individually and in combination on the embryos of Crassostrea virginica, found

that these two mixtures exerted a less than additive toxicity at low temperatures. Cadmium and zinc increased the toxic resistance of the embryos of Mytilus galloprovincialis (Pavicic, 1977).

Eisler (1977) in an illuminating paper on the evaluation of the toxicity of complex metal mixtures to Mya arenaria, stated that the presence of more than one metal in very low concentration can either result in death or variable rates of metal uptake. Experiments by Roales and Perlmutter (1974) and Moulder (1980) have demonstrated the antagonistic effect of copper on mercury toxicity. Moulder (1980) explaining the effects of chlorides of mercury and copper on Gammarus duebeni, stated that the presence of a sub-lethal level of cupric chloride protected the species against the toxic action of mercuric chloride. He also discussed in detail the nature of mercury-copper interaction. When mussels were exposed to a mixture of zinc and mercury, an antagonistic effect was evident when the concentration of mercury reduced considerably (Breittmayer and Galindo, 1981). Mohan et al. (1986 b) investigating into the combined toxicity of mercury and cadmium observed that mercury and cadmium in mixture interacted more than additively in producing mortality in 96 h in Perna viridis. It seems that concentrations of metals in mixture also affect the toxicity to which component interactions are additive or synergistic. Prabhudeva and Menon (1986 b) obtained comparable results in Perna viridis exposed to a mixture of zinc and copper.

Studies by Baby and Menon (unpublished data) revealed that the presence of cadmium did not have any influence on the uptake of mercury. However, they observed that the presence of mercury can influence

the uptake of cadmium. Therefore, it is possible that the toxicity of a metal could be influenced by the presence of another (Baby and Menon, 1990 (in press)).

2.3 MATERIAL AND METHODS

This part of the investigation has centred mainly around delineating the toxic effects of copper, mercury and cadmium, individually and in combination at lethal levels on two marine bivalve species, Perna indica and Donax incarnatus. Both the selected species are ecologically and economically relevant.

2.3.1 TEST ANIMALS

2.3.1.1 **Perna indica (Kuriakose and Nair)**

This commercially important bivalve species are abundant on the rocky beaches along the south west and south east coasts of India (Kuriakose and Nair, 1976). Major recruitment of this species occurs around September. Animals of 20-25 mm length were dislodged from an unpolluted open shore population on the rocky beaches of Sakthikulangara (76°34'24" Long, 9°56'09" Lat) along the coast of Quilon.

2.3.1.2 **Donax incarnatus Gmelin**

This species is a dominant inhabitant of the exposed sandy beaches of the west coast of India and is commercially important. Pre-monsoon period is the major period of settlement. Animals of 20-25 mm length were sampled sub-tidally from the populations at Ambalapuzha beach (76°21' 22" Long, 9°23'20" Lat).

Both the animal species were transported to the laboratory in polythene trays in sea water collected in situ with no aeration.

2.3.2 WATER

The sea water used during the period of experimentation was collected from an unpolluted area in the Arabian sea, off Cochin. The water was transported to the laboratory in large plastic carbouys of 50 l capacity and kept in total darkness for about one week for aging. The particulate fractions (living and non-living) of the sea water were allowed to settle. Before experiments, the sea water was filtered using fibre glass filter (length 32 cm, breadth 16 cm) containing glass wool and activated charcol. The sea water used for the experiments (salinity : 33-35‰ and pH : 8.2-8.4) were aerated to saturation before use. The addition of toxicants did not cause any appreciable variations in pH and all sets of experiments were performed at room temperature (30 ± 2°C).

2.3.3 LABORATORY CONDITIONING OF TEST ANIMALS

In the laboratory the collected animals were acclimatized in aerated sea water at 30 ± 2°C for 24 to 48 h prior to experimentation. Specimens from the same population was used for a single set of experiments. Contamination by pseudo-faeces and metabolites was checked by daily renewal of water. The test animals were not fed before or during the course of the short term (96 h) studies.

2.3.4 TOXICANTS

2.3.4.1 Copper

Analar grade of copper sulphate (M.W.249.68) was the source of copper. The salt was dissolved in distilled water and added to achieve the required concentration.

2.3.4.2 Mercury

Standard solutions of mercury were prepared using analar grade mercuric chloride (M.W.271.50) in glass distilled water and stored in amber coloured bottles. Since mercury solutions are not stable for long periods, they were prepared afresh for each set of experiments and added to make up the required concentration.

2.3.4.3 Cadmium

Analar grade of cadmium chloride (M.W.201.32) was the source of cadmium. It was dissolved in glass distilled water and added to achieve the desired concentration. Cadmium solutions are relatively stable and hence was prepared fresh only every 15 d.

2.3.5 TOXICITY STUDIES

2.3.5.1 Lethal toxicity studies

Lethal toxicity tests have remained unsurpassed as a screening technique and a comparative tool and is a classic approach to the study of stress response due to pollution. Death is considered as the chief criterion for assessing the lethal effects. The lethal concentrations were obtained by following short term (96 h) static renewal bioassay technique

recommended by Sprague (1969).

2.3.5.1.1 Mortality tests

The laboratory acclimatized animals (size 20-25 mm) were sorted out using vernier callipers. Ten mussels were exposed to 5 l of the toxicant solution in fibreglass tubs of 10 l capacity with perspex lids. The toxicant solution was renewed every 24 h and was not aerated. The animals were not fed during the experiment. In both Perna indica and Donax incarnatus, valve gaping beyond 5 mm and the inability to close the valves under mechanical stimulation were considered indicative of death. The 96 h LC_{50} values and their 95% confidence limits were calculated as described in section 2.3.6.2.

In metal combination studies, the concentrations of the toxicants (ie toxicant A & B and A, B and C) were calculated based on their respective 96 h LC_{50} values. The concentration of one of the toxicant was kept constant while the others varied. Usually 25 (5x5) combinations were tested to complete one set of experiments on combined toxicity of two toxicants and 125 (25x5) for three toxicants. The 96 h LC_{50} levels, their 95% confidence limits and the additive indices were calculated as described in section 2.3.6.

2.3.6 COMPUTATION OF DATA

2.3.6.1 **Combined toxicity analysis**

The concept of prediction of toxicity of pollutant mixtures provide scope for the study of simultaneous effects of several pollutants

in a single set of experiments.

The effect of mixtures of two or more chemicals is commonly referred to as additive, synergistic or antagonistic depending on the relation of the toxicity of the mixture to that of the individual components. Because these terms are ambiguous and non-quantitative(Fingl and Woodbury 1965) a better system of quantification was employed (Marking and Dawson, 1975). The toxicity unit or sum of the biological activity of a solution containing metal mixtures are calculated as follows:

$$S = \frac{Am}{Ai} + \frac{Bm}{Bi}$$

where A & B are metals, subscripts i and m are the toxicities (LC_{50} or EC_{50}) of the individual metal and the metal mixture respectively and S is the toxicity unit or sum of the biological activity. To arrive at an appropriate index, one of the following relationships is employed, depending on whether S is more than or less than 1.

$$\frac{1}{S} - 1 \text{ if } S \leq 1.0$$

$$S(-1) + 1 \text{ if } S \geq 1.0$$

When the additive indices have negative values, it indicates less than additivity, 'O' value, simple additivity and a positive value, more than additivity. The significance of the additive indices close to zero can be assessed by substituting for LC_{50}'s , the lower confidence limit of the individual toxicants (Ai and Bi) and the upper limit of the mixture (Am and Bm), to determine the lower limit of the index. Correspondingly the upper limit of the toxicants (Ai and Bi) and the lower limit of the mixture (Am and Bm) were substituted to calculate the upper limit of

the index. If the 95% confidence limits over lap 'O', it is considered as simple additive.

2.3.6.2 Statistical analysis

Probit analysis (Finney, 1971) was used to determine the 96 h LC_{50} levels and their 95% confidence limits.

2.4 RESULTS

Lethal toxicity studies give information about the relative lethality of a toxicant. This test is designed to determine the highest concentration of a pollutant that is sufficient to kill some percentage, usually 50% of a limited number of test organisms. Though this appears to be a crude method of measurement of toxic response, its importance was highlighted by many (Duke, 1974; Buikema Jr. et al., 1982). The individual and combined lethal toxicity of mercury, copper and cadmium on Perna indica and Donax incarnatus were delineated. The results are outlined in Tables 1-20.

2.4.1 PERNA INDICA

2.4.1.1 Lethal toxic response to individual toxicants

The results obtained on the lethal toxicity of mercury, copper and cadmium are presented in Table 1. The experimental results indicated that of the three metals tested, copper was the most toxic, followed by mercury and cadmium. Long duration of exposure resulted in a reduction in the 96 h LC_{50} values. Perna indica was extremely sensitive to copper.

2.4.1.2 Lethal toxic response to metal mixtures

Table 1. *Perna indica*. LC_{50} (ppb), when exposed to mercury, copper and cadmium, over periods upto 96 h, along with the respective 95% confidence limits.

Toxicant	48 h		96 h	
	LC_{50}(ppb)	95% Confidence limits	LC_{50}(ppb)	95% Confidence limits
Mercury	96.0	68.0 - 135.4	78.0	76.1 - 80.1
Copper	28.2	24.4 - 32.5	21.8	17.9 - 26.4
Cadmium	3319.7	2997.8 - 3676.2	2856.4	2837.9 - 2874.7

Tables 2 to 10 present data on the lethal toxicity of Perna indica to metal mixtures.

The effect of cadmium and mercury in combination on the test animals was found out by a constant cadmium and varying mercury concentrations. The results indicated that mortality occurred only in the highest concentration of cadmium. The additivity was simple (Table 2a).

Test media containing constant concentration of mercury and varying concentrations of cadmium did not bring about mortality of the test population (Table 2b).

The toxicity of Perna indica to copper did not vary appreciably when the animals were exposed to constant concentration of mercury along with varying copper concentrations. The additive index was simple additive (Table 3a).

In the reciprocal experiment (copper constant and mercury varying) it was observed that significant mortality occurred only in the highest copper concentration. The mercury was found to become highly toxic in the presence of 20 ppb of copper. The combined effect in this case was also simple additive (Table 3b).

Comparable results of mortality of Perna indica were obtained when they were exposed to combinations of copper (constant) and cadmium (varying). Significant mortality was found to occur only in higher concentrations. However, cadmium was found to become more toxic in the presence of copper even though the confidence limits recorded showed high flexibility. The additive index was simple additive (Table 4a).

Table 2a. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
1100	29.2	14.9 - 57.1	0.32(SA)
900	*	*	
700	*	*	
500	*	*	
300	*	*	

SA : Simple additive

* : No mortality

Table 2 b. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	*	*	
25	*	*	
20	*	*	
15	*	*	
10	*	*	

* : No mortality

Table 3 a. *Perna indica*. 96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Mercury (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	20.3	10.1 - 40.6	-0.32 (SA)
25	ND	ND	
20	23.2	17.7 - 30.4	-0.32 (SA)
15	20.2	10.2 - 40.0	-0.12 (SA)
10	20.2	18.0 - 22.9	-0.06 (SA)

SA : Simple additive

ND : Not determined

Table 3 b. *Perna indica*. 96 h LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.

Copper (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	11.4	10.7 - 12.1	-0.06 (SA)
15	*	*	
10	*	*	
5	*	*	
1	*	*	

SA : Simple additive

* : No mortality

Table 4 a. *Perna indica*. *96 h LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	767.2	460.2 - 1279.1	-0.19 (SA)
15	1677.7	704.2 - 3996.7	-0.28 (SA)
10	*	*	
5	*	*	
1	*	*	

SA : Simple additive

* : No mortality

Table 4 b. *Perna indica*. *96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
1100	18.3	17.0 - 19.7	-0.22 (LA)
900	19.9	18.5 - 21.5	-0.23 (LA)
700	21.3	17.2 - 26.3	-0.22 (SA)
500	21.6	20.6 - 22.6	-0.17 (SA)
300	24.7	23.3 - 26.2	-0.24 (SA)

SA : Simple additive

LA : Less than additive

In the reciprocal combination of cadmium (constant) and copper (varying), it was found that the combination was more toxic with relatively high copper concentration and low cadmium (Table 4b). Thus with 300 ppb of cadmium, the 96 h LC_{50} occurred in a medium which contained 24.7 ppb of copper, while with 1100 ppb of cadmium a copper concentration of 18.3 ppb produced 50% mortality of the test population. Further, the additive index showed a gradation from simple additivity to less than additivity with an increase in cadmium concentration.

In the triad combination experiment (Tables 5-10) involving the three metals, clear cut thresholds were delineated.

In the first series of triad combination exposures, mercury was the variant with constant concentrations of cadmium and copper (Table 5). The toxicity of mercury was found to increase with an increase in the copper levels at constant cadmium concentration. Thus, 24 ppb of mercury along with 10 ppb of copper and 300 ppb of cadmium produced 50% mortality of the test population, while 16.4 ppb of mercury produced the same result in combination with 20 ppb of copper and 300 ppb of cadmium. Experiments employing higher concentrations of cadmium revealed that significant mortality occurred mainly in the medium which contained 15 ppb of copper along with low concentrations of mercury. The additive index varied from simple additivity, more than additivity, to less than additivity.

The results of the triad combination experiments involving cadmium (constant) mercury (constant) and copper (varying) on Perna indica are given in Table 6. Elevation in cadmium concentration from 300 ppb

Table 5. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt and copper salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Copper (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
1100	1	28.2	24.2 - 30.5	0.26 (MA)
1100	5	24.9	20.1 - 30.8	0.07 (SA)
1100	10	22.8	16.2 - 32.0	-0.14 (SA)
1100	15	3.1	0.8 - 12.5	-0.11 (SA)
1100	20	**	**	
900	1	*	*	
900	5	29.8	29.7 - 30.5	0.08 (MA)
900	10	24.7	24.1 - 25.3	-0.09 (SA)
900	15	12.9	9.8 - 17.0	-0.17 (LA)
900	20	**	**	
700	1	*	*	
700	5	32.6	21.5 - 49.5	0.12 (SA)
700	10	29.2	ND	-0.08
700	15	14.3	11.2 - 18.3	-0.12 (SA)
700	20	**	**	
500	1	*	*	
500	5	*	*	
500	10	26.4	ND	0.03
500	15	17.9	13.2 - 24.2	-0.09 (SA)
500	20	13.1	ND	-0.26
300	1	*	*	
300	5	*	*	
300	10	24.0	16.3 - 35.2	0.15 (SA)
300	15	18.0	12.2 - 26.5	-0.02 (SA)
300	20	16.4	15.8 - 17.0	-0.23 (LA)

SA : Simple additive
LA : Less than additive
* : No mortality
** : 100% mortality
ND : Not determined
MA : More than additive

to 500 ppb along with varying mercury concentration was found to increase the toxicity of copper. The 96 h LC_{50} of copper was found to decrease with increasing mercury load. However, toxicity of copper in general, increased in mixtures containing low concentration of mercury. The toxicity of copper was found to increase with increasing cadmium and mercury load. With 900 ppb and 1100 ppb of cadmium along with varying concentrations of mercury and copper, significant mortality was limited to the three lower concentrations of mercury tested. Elevation in the cadmium and mercury level did influence the 96 h LC_{50} of copper; the additive index varying from simple additivity to less than additivity.

The triad combination exposures of Perna indica to mercury (constant) copper (constant) and cadmium (varying) produced results which are given in Table 7. When the medium contained 10 to 20 ppb mercury and 1.0 to 10 ppb of copper and varying concentrations of cadmium, no mortality of the test population was recorded. However, the maximum cadmium concentration employed was 1100 ppb. With 20 ppb of mercury, significant mortality was found to occur in the test population exposed to 15 and 20 ppb of copper along with varying cadmium concentrations. Simple additivity obtained with high concentrations of mercury and copper directly implies that cadmium also had contributed to this. The additive index showed a gradation from simple additivity, more than additivity, to less than additivity.

It is evident from Table 8 that irrespective of the copper concentration present in the triad, total mortality was observed in those combinations which contained 30 ppb of mercury, 300 to 1100 ppb of cadmium and 1.0 to 20 ppb of copper. Therefore, it is not possible

Table 6. *Perna indica*. 96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt and mercury salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Cadmium (ppb)	Mercury (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
1100	10	13.3	ND	-0.12
1100	15	12.9	9.9 - 16.9	-0.17 (SA)
1100	20	12.6	10.9 - 14.5	-0.22 (LA)
1100	25	**	**	
1100	30	**	**	
900	10	15.0	ND	-0.13
900	15	14.4	13.7 - 15.2	-0.17 (LA)
900	20	14.1	13.9 - 14.3	-0.22 (LA)
900	25	**	**	
900	30	**	**	
700	10	16.6	ND	-0.13
700	15	14.8	13.1 - 16.6	-0.12 (SA)
700	20	14.7	13.5 - 16.0	-0.18 (LA)
700	25	11.3	ND	-0.08
700	30	**	**	
500	10	19.9	ND	-0.22
500	15	15.1	12.5 - 18.4	-0.06 (SA)
500	20	15.0	ND	-0.35
500	25	11.2	8.0 - 15.7	-0.009 (SA)
500	30	**	**	
300	10	17.0	15.1 - 19.3	-0.01 (SA)
300	15	18.3	17.4 - 19.2	-0.14 (SA)
300	20	19.5	9.1 - 37.1	-0.26 (SA)
300	25	8.8	ND	0.21
300	30	**	**	

SA : Simple additive

LA : Less than additive

** : 100% mortality

ND : Not determined

Table 7. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt and copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Copper (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	1	1007.9	904.3 - 1121.2	0.37 (MA)
30	5	884.9	638.1 - 1227.2	0.08 (SA)
30	10	549.7	254.1 - 1189.0	-0.04 (SA)
30	15	431.9	215.3 - 866.4	-0.22 (LA)
30	20	**	**	
25	1	*	*	
25	5	1094.2	1055.6 - 1134.2	0.07 (SA)
25	10	825.4	599.1 - 1137.4	-0.07 (SA)
25	15	158.1	60.2 - 415.2	-0.06 (SA)
25	20	**	**	
20	1	*	*	
20	5	*	*	
20	10	*	*	
20	15	710.3	573.9 - 878.9	-0.19 (LA)
20	20	440.8	368.3 - 527.7	-0.33 (LA)
15	1	*	*	
15	5	*	*	
15	10	*	*	
15	15	600.1	332.6 - 1082.4	-0.09 (SA)
15	20	ND	ND	
10	1	*	*	
10	5	*	*	
10	10	*	*	
10	15	548.4	483.5 - 622.0	-0.008 (SA)
10	20	ND	ND	

LA: Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

ND: Not determined MA : More than additive

to say with certainty whether copper added to the toxic effects. The subsequent results probably show that it was the combined effect of mercury and cadmium which was more deterimental than low levels of copper in the triad. This statement could be further qualified by the results obtained in the triad combinations which contained 10 to 20 ppb of mercury and 300 to 1100 ppb of cadmium with varying concentrations of copper. Lowering the concentrations of mercury and cadmium slightly reduced the toxicity of copper and the combined toxicity was in majority of cases, simple additive.

Table 9 represents the toxic effect of copper, cadmium and mercury, the former two being maintained constant and the latter namely mercury made to vary. Reduction in the concentration of copper was apparently found to reduce the toxicity of mercury also. So it has to be assumed that mercury and copper play a major role in controlling the toxicity and hence lethality in the case of Perna indica exposed to a combination of the three metals. Obtaining more than additivity at least in two instances indicated combined toxicity and curiously enough this happened when the copper concentration was 1.0 or 5.0 ppb. Shifting from less than additivity to simple additivity and more than additivity in the case of three metals applied in different concentrations, probably indicates concentration dependent additive reaction.

Table 10 gives information on the combined toxicity of mercury, copper and cadmium; copper and mercury kept constant and cadmium varying. It is certain that relatively low concentration of cadmium will not prove lethal in the presence of relatively higher levels of copper and mercury. Lower concentrations of copper and mercury with very high concentration of cadmium was also found to be relatively less toxic. Simple additivity recorded in many cases indicates that the combined

Table 8. *Perna indica*. 96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt and cadmium salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Mercury (ppb)	Cadmium (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	300	**	**	
30	500	**	**	
30	700	**	**	
30	900	**	**	
30	1100	**	**	
25	300	8.8	ND	0.21
25	500	11.2	8.8 - 15.7	-0.009 (SA)
25	700	11.3	ND	-0.08
25	900	**	**	
25	1100	**	**	
20	300	19.5	9.1 - 37.1	-0.26 (SA)
20	500	15.0	ND	-0.35
20	700	14.7	13.5 - 16.0	-0.18 (LA)
20	900	14.1	13.9 - 14.3	-0.22 (LA)
20	1100	12.6	10.9 -14.5	-0.22 (LA)
15	300	18.3	17.4 - 19.2	-0.14 (SA)
15	500	15.1	12.5 - 18.4	-0.06 (SA)
15	700	14.8	13.1 - 16.6	-0.12 (SA)
15	900	14.4	13.7 - 15.2	-0.17 (LA)
15	1100	12.9	9.9 - 16.9	-0.17 (SA)
10	300	17.0	15.1 - 19.3	-0.01 (SA)
10	500	19.9	ND	-0.22
10	700	16.6	ND	-0.13
10	900	15.0	ND	-0.13
10	1100	13.3	ND	-0.12

LA : Less than additive

SA : Simple additive

** : 100% mortality

ND : Not determined

Table 9. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of copper salt and cadmium salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Cadmium (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	300	16.4	15.8 - 17.0	-0.23 (LA)
20	500	13.1	ND	-0.26
20	700	**	**	
20	900	**	**	
20	1100	**	**	
15	300	18.0	12.2 - 26.5	-0.02 (SA)
15	500	17.9	13.2 - 24.2	-0.09 (SA)
15	700	14.3	11.2 - 18.3	-0.12 (SA)
15	900	12.9	9.8 - 17.0	-0.17 (LA)
15	1100	3.1	0.8 - 12.5	-0.11 (SA)
10	300	24.0	16.3 - 35.2	0.15 (SA)
10	500	26.4	ND	0.03
10	700	29.2	ND	-0.08
10	900	24.7	24.1 - 25.3	-0.09 (SA)
10	1100	22.8	16.2 - 32.0	-0.14 (SA)
5	300	*	*	
5	500	*	*	
5	700	32.6	21.5 - 49.5	0.12 (SA)
5	900	29.8	29.7 - 30.5	0.08 (MA)
5	1100	24.9	20.1 - 30.8	0.07 (SA)
1	300	*	*	
1	500	*	*	
1	700	*	*	
1	900	*	*	
1	1100	28.2	24.2 - 30.5	0.26 (MA)

SA : Simple additive
LA : Less than additive
* : No mortality
** : 100% mortality
ND : Not determined
MA : More than additive

Table 10. *Perna indica. 96 h LC_{50} (ppb), when exposed to constant concentration of copper salt and mercury salt, with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Mercury (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	10	ND	ND	
20	15	ND	ND	
20	20	440.8	368.3 - 527.7	-0.33 (LA)
20	25	**	**	
20	30	**	**	
15	10	548.4	483.5 - 622.0	-0.008 (SA)
15	15	600.1	332.6 -1082.4	-0.09 (SA)
15	20	710.3	573.9 - 878.9	-0.19 (LA)
15	25	158.1	60.2 - 415.2	-0.06 (SA)
15	30	431.9	215.3 - 866.4	-0.22 (LA)
10	10	*	*	
10	15	*	*	
10	20	*	*	
10	25	825.4	599.1 -1137.4	-0.07 (SA)
10	30	549.7	254.1 -1189.0	-0.04 (SA)
5	10	*	*	
5	15	*	*	
5	20	*	*	
5	25	1094.2	1055.6 -1134.2	0.07 (SA)
5	30	884.9	638.1 -1227.2	0.08 (SA)
1	10	*	*	
1	15	*	*	
1	20	*	*	
1	25	*	*	
1	30	1007.2	904.3 -1121.2	0.27 (MA)

LA : Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

ND : Not determined MA : More than additive

action commences only when the cadmium concentrations are above threshold levels. Alternation of less than additivity and simple additivity is a factor which cannot be explained properly (See Table 10).

2.4.2 DONAX INCARNATUS

2.4.2.1 **Lethal toxic response to individual metals**

The lethal toxicity of mercury, copper and cadmium on Donax incarnatus is presented in Table 11. From the results the following is evident. Copper was the most toxic, followed by mercury and cadmium. The 96 h LC_{50} of copper was 25.2 ppb while that of mercury was 59.1 ppb and that of cadmium, 320.6 ppb.

2.4.2.2 **Lethal toxic response to mixtures of metals**

The results of the lethal toxicity of metal mixtures to Donax incarnatus are presented in Tables 12 to 20.

The first in the series looked into the effect of a combination of copper and mercury, the former maintained at a constant concentration and the latter varying. With increasing copper concentration, the toxic effect of mercury was found to decrease (Table 12 a). Thus with 15 ppb of copper, the 96 h LC_{50} occurred in a mixture which contained 51.1 ppb of mercury, while with 20 ppb of copper, a mercury concentration of 90.5 ppb was required to produce 50% mortality of the test population. The additive index was simple additive. Curiously enough, mercury was found to become innocuous in the presence of copper as evidenced from the LC_{50} values.

Table 11. *Donax incarnatus*. LC_{50} (ppb), when exposed to mercury, copper and cadmium over periods upto 96 h, along with the respective 95% confidence.

Toxicant	48 h		96 h	
	LC_{50}(ppb)	95% Confidence limits	LC_{50}(ppb)	95% Confidence limits
Mercury	446.8	384.8 - 518.8	59.1	54.7 - 63.9
Copper	158.2	153.2 - 163.2	25.2	23.3 - 27.1
Cadmium	1111.9	972.8 - 1293.9	320.6	301.0 - 341.4

Table 12 a. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	90.5	11.2 - 728.7	-1.33 (SA)
15	51.1	22.1 - 118.1	-0.46 (SA)
10	*	*	
5	*	*	
1	*	*	

SA : Simple additive

* : No mortality

Table 12 b. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	20.2	15.7 - 26.0	-0.31 (LA)
20	33.6	22.2 - 50.8	-0.67 (LA)
15	47.4	34.2 - 65.6	-1.13 (LA)
10	*	*	
5	*	*	

LA : Less than additive

* : No mortality

A mixture of constant concentration of mercury with varying concentrations of copper produced different results. The toxicity of copper was drastically influenced by an elevation in the mercury concentration. Further, the 96 h LC_{50} of copper decreased with increasing mercury concentration. The additive index was less than additive in all the case (Table 12 b).

Donax incarnatus exposed to a metal mixture containing cadmium (constant) and mercury (varying) produced results (Table 13 a) which indicated enhanced toxicity of mercury at relatively low cadmium concentration. The 96 h LC_{50} of mercury was found to decrease with an increase in cadmium load. Thus with 50 ppb cadmium, a concentration of 27.3 ppb of mercury was required to produce 50% mortality in the test population, whereas an increase in cadmium concentration to 300 ppb resulted in a decrease in mercury concentration to 18.3 ppb to produce the same result. However, the additive indices were simple additivity, more than additivity or less than additivity.

More or less comparable results were obtained with reference to mercury (constant) and cadmium (varying). The 96 h LC_{50} of cadmium was found to decrease with increase in mercury concentration. The fact that 41.8 ppb was the LC_{50} in the presence of 30 ppb mercury shows a clear cut more than additive reaction. The additive index showed a gradation from simple additivity to more than additivity.

Copper (constant) in combination with cadmium (varying) resulted in relatively reduced toxicity (Table 14 a). The 96 h LC_{50} of cadmium was found to increase with increasing copper concentration. The additive

Table 13 a. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
300	18.3	16.9 - 19.8	-0.24 (LA)
250	16.7	12.1 - 23.0	-0.06 (SA)
200	18.2	14.0 - 21.2	-0.05 (SA)
100	19.8	16.3 - 24.0	0.55 (MA)
50	27.3	16.2 - 46.3	-0.62 (SA)

LA : Less than additive

MA : More than additive

SA : Simple additive

Table 13 b. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	41.8	31.1 - 54.7	0.57 (MA)
20	199.2	135.9 - 292.1	0.04 (SA)
15	206.0	153.1 - 311.1	0.12 (SA)
10	251.8	232.6 - 272.5	0.05 (SA)
5	*	*	

MA : More than additive

SA : Simple additive

* : No mortality

index varied from more than additivity to less than additivity

Table 14 b depicts the results obtained with Donax incarnatus and a combination of cadmium (constant) and varying concentrations of copper. Significant mortality occurred only in the medium containing 200 ppb or higher concentrations of cadmium, along with varying copper load. The results indicate that enhanced toxicity occurred only at relatively high cadmium concentration. Further, the 96 h LC_{50} of copper showed significant decrease with an increase in cadmium concentration and the additive index was less than additive.

The combined toxicity of constant concentrations of mercury and cadmium and varying concentrations of copper is detailed in Table 15. One of the conspicuous features was that the 96 h LC_{50} values of copper decreased with an increase in the concentration of the other two metals. Further, with the increase in the concentration of metals, significant mortality becomes restricted to clear cut thresholds. In addition, the toxicity of copper in general was found to increase with an elevation in the concentration of mercury and cadmium. The additive index varied from simple additivity to less than additivity.

Table 16 represent the data obtained on the lethal toxicity of mercury, copper and cadmium on Donax incarnatus. Presence of 30 ppb of mercury and 1.0 to 20 ppb of copper in no way influenced the toxicity of cadmium on this species; the cadmium concentrations ranging from 50 to 300 ppb. Total mortality was observed in all the concentrations. However, reduction in mercury and copper concentration influenced the mortality of Donax incarnatus in the presence of cadmium. Cadmium

Table 14 a. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	246.6	242.9 - 250.4	-0.56 (LA)
15	236.8	203.1 - 299.2	-0.33 (LA
10	160.4	153.8 - 167.4	0.11 (MA)
5	116.5	93.3 - 145.1	0.78 (MA)
1	309.4	217.1 - 353.0	-0.005 (SA)

LA : Less than additive

MA : More than additive

SA : Simple additive

Table 14 b. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
300	ND	ND	
250	26.6	15.7 - 45.3	-0.84 (LA)
200	38.1	21.1 - 68.8	-1.13 (LA)
100	*	*	
50	*	*	

LA : Less than additive ND : Not determined

* : No mortality

Table 15. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt and cadmium salt, with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Cadmium	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	50	**	**	
30	100	**	**	
30	200	**	**	
30	250	**	**	
30	300	**	**	
20	50	32.4	31.4 - 33.3	-0.78 (LA)
20	100	13.5	9.9 - 18.2	-0.18 (SA)
20	200	12.2	5.1 - 38.2	-0.44 (LA)
20	250	**	**	
20	300	**	**	
15	50	ND	ND	
15	100	ND	ND	
15	200	12.4	4.1 - 38.1	-0.37 (SA)
15	250	11.1	5.2 - 23.4	-0.47 (LA)
15	300	**	**	
10	50	24.5	22.0 - 27.4	-0.30 (LA)
10	100	18.9	17.4 - 20.5	-0.23 (LA)
10	200	16.0	8.8 - 29.1	-0.43 (LA)
10	250	9.9	4.7 - 21.2	-0.35 (LA)
10	300	**	**	
5	50	26.3	9.5 - 73.0	-0.28 (SA)
5	100	23.6	21.2 - 26.3	-0.33 (LA)
5	200	19.6	18.9 - 20.2	-0.49 (LA)
5	250	17.1	13.5 - 21.6	-0.54 (LA)
5	300	**	**	

LA : Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

ND : Not determined

Table 16. *Donax incarnatus.* *96 h LC_{50} (ppb), when exposed to constant concentration of mercury salt and copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Mercury (ppb)	Copper (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
30	1	**	**	
30	5	**	**	
30	10	**	**	
30	15	**	**	
30	20	**	**	
20	1	107.7	96.8 - 119.9	0.40 (MA)
20	5	127.0	86.0 - 188.0	0.072 (SA)
20	10	ND	ND	
20	15	102.8	82.0 - 128.8	-0.25 (LA)
20	20	59.3	24.8 - 141.9	-0.32 (LA)
15	1	289.1	214.7 - 389.2	-0.19 (SA)
15	5	268.0	231.7 - 310.1	-0.29 (LA)
15	10	243.7	219.8 - 270.1	-0.41 (LA)
15	15	130.1	102.1 - 198.4	-0.21 (LA)
15	20	96.0	28.1 - 328.3	-0.34 (LA)
10	1	306.0	212.4 - 440.8	-0.16 (SA)
10	5	273.8	ND	-0.22
10	10	ND	ND	
10	15	156.4	150.2 - 162.8	-0.25 (LA)
10	20	173.5	42.9 - 702.6	-0.50 (LA)
5	1	*	*	
5	5	*	*	
5	10	*	*	
5	15	258.1	222.4 - 299.4	-0.49 (LA)
5	20	195.7	131.5 - 291.3	-0.49 (LA)

LA : Less than additive
SA : Simple additive
** : 100% mortality
ND : Not determined
* : No mortality
MA : More than additive

was found to become more toxic even at low levels, when copper and mercury concentration was 20 ppb. Uniformly less than additive reaction, however, questions the assumption whether the death is due to a combined effect. It is likely that lethality was rather a result of the presence of mercury and copper rather than cadmium directly. Less than additivity could indicate independent action of one of the metals, probably taking over the lethal effects of the other.

Table 17 depicts the results obtained based on experiments on the combined toxic effects of cadmium (constant), copper (constant) and mercury (varying) on Donax incarnatus. The 96 h LC_{50} levels of mercury decreased with an increase in the concentration of copper and cadmium. The results indicate clear cut thresholds beyond which 100% mortality occurred. The additive index was less than additive.

The combined toxicity of varying concentrations of copper in the presence of cadmium and mercury on Donax incarnatus is presented in Table 18. Copper concentrations above 10 ppb and below 30 ppb were found to be toxic. However, the exact concentration could vary. Total mortality in the presence of 50 to 250 ppb of cadmium and 30 ppb of mercury, irrespective of the copper concentration, cannot be explained.

More or less comparable results were obtained with reference to copper (constant), mercury (constant) and cadmium (varying) (See Table 19). The toxicity of cadmium was found to increase with an increase in the concentration of the other two metals. The additive index was less than additive in almost all the cases.

Table 17. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt and copper salt, with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Cadmium (ppb)	Copper (ppb)	Mercury 96 h LC_{50}(ppb)	95 % Confidence limits	Additive Index
300	1	10.0	8.1 - 12.4	-0.15 (LA)
300	5	2.7	0.3 - 29.5	-0.18 (LA)
300	10	**	**	
300	15	**	**	
300	20	**	**	
250	1	16.3	10.6 - 25.2	-0.09 (SA)
250	5	15.1	10.1 - 22.6	-0.23 (LA)
250	10	12.5	5.1 - 30.5	-0.39 (LA)
250	15	9.7	6.6 - 14.3	-0.54 (LA)
250	20	5.1	3.8 - 6.9	-0.66 (LA)
200	1	*	*	
200	5	*	*	
200	10	*	*	
200	15	17.4	4.3 - 70.2	-0.51 (LA)
200	20	12.6	10.9 - 14.5	-0.63 (LA)
100	1	*	*	
100	5	*	*	
100	10	*	*	
100	15	9.6	6.3 - 14.7	-0.07 (SA)
100	20	0.6	0.08- 5.1	-0.31 (LA)
50	1	*	*	
50	5	*	*	
50	10	*	*	
50	15	*	*	
50	20	18.4	11.8 - 28.7	-0.26 (LA)

LA : Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

Table 18. *Donax incarnatus*. 96 h LC_{50} (ppb), when exposed to constant concentration of cadmium salt and mercury salt, with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Cadmium (ppb)	Mercury (ppb)	Copper 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
300	5	**	**	
300	10	**	**	
300	15	**	**	
300	20	**	**	
300	30	**	**	
250	5	17.1	13.5 - 21.6	-0.54 (LA)
250	10	9.9	4.7 - 21.2	-0.35 (LA)
250	15	11.1	5.2 - 23.4	-0.47 (LA)
250	20	**	**	
250	30	**	**	
200	5	19.6	18.9 - 20.2	-0.49 (LA)
200	10	16.0	8.8 - 29.1	-0.43 (LA)
200	15	12.4	4.1 - 38.1	-0.37 (SA)
200	20	12.2	5.1 - 38.2	-0.44 (LA)
200	30	**	**	
100	5	23.6	21.2 - 26.3	-0.33 (LA)
100	10	18.9	17.4 - 20.5	-0.23 (LA)
100	15	ND	ND	
100	20	13.5	9.9 - 18.2	-0.18 (SA)
100	30	**	**	
50	5	26.3	9.5 - 73.0	-0.28 (SA)
50	10	24.5	22.0 - 27.4	-0.30 (LA)
50	15	ND	ND	
50	20	32.4	31.4 - 33.3	-0.78 (LA)
50	30	**	**	

LA : Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

ND : Not determined

Table 19. *Donax incarnatus.* *96 h LC_{50} (ppb), when exposed to constant concentration of copper salt and mercury salt, with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Mercury (ppb)	Cadmium 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	5	195.7	131.5 - 291.3	-0.49 (LA)
20	10	173.5	42.9 - 702.6	-0.50 (LA)
20	15	96.0	28.1 - 328.3	-0.34 (LA)
20	20	59.3	24.8 - 141.9	-0.32 (LA)
20	30	**	**	
15	5	258.1	222.4 - 299.4	-0.49 (LA)
15	10	156.4	150.2 - 162.8	-0.25 (LA)
15	15	130.1	102.1 - 198.4	-0.21 (LA)
15	20	102.8	82.0 - 128.8	-0.25 (LA)
15	30	**	**	
10	5	*	*	
10	10	ND	ND	
10	15	243.7	219.8 - 270.1	-0.41 (LA)
10	20	ND	ND	
10	30	**	**	
5	5	*	*	
5	10	273.8	ND	-0.22
5	15	268.0	231.7 - 310.1	-0.29 (LA)
5	20	127.0	86.0 - 188.0	0.072 (SA)
5	30	**	**	
1	5	*	*	
1	10	306.0	212.4 - 440.8	-0.16 (SA)
1	15	289.1	214.7 - 389.2	-0.19 (SA)
1	20	107.7	96.8 - 119.9	0.40 (MA)
1	30	**	**	

LA : Less than additive
SA : Simple additive
* : No mortality
** : 100% mortality
ND : Not determined
MA : More than additive

Table 20. *Donax incarnatus*. *96 h LC_{50} (ppb), when exposed to constant concentration of copper salt and cadmium salt, with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.*

Copper (ppb)	Cadmium (ppb)	Mercury 96 h LC_{50}(ppb)	95% Confidence limits	Additive Index
20	50	18.4	11.8 - 28.7	-0.26 (LA)
20	100	0.6	0.08 - 5.1	-0.31 (LA)
20	200	12.6	10.9 - 14.5	-0.63 (LA)
20	250	5.1	3.8 - 6.9	-0.66 (LA)
20	300	**	**	
15	50	*	*	
15	100	9.6	6.3 - 14.7	-0.07 (SA)
15	200	17.4	4.3 - 70.2	-0.51 (LA)
15	250	9.7	6.6 - 14.3	-0.54 (LA)
15	300	**	**	
10	50	*	*	
10	100	*	*	
10	200	*	*	
10	250	12.5	5.1 - 30.5	-0.39 (LA)
10	300	**	**	
5	50	*	*	
5	100	*	*	
5	200	*	*	
5	250	15.1	10.1 - 22.6	-0.23 (LA)
5	300	2.7	0.3 - 29.5	-0.18 (LA)
1	50	*	*	
1	100	*	*	
1	200	*	*	
1	250	16.3	10.6 - 25.2	-0.09 (SA)
1	300	10.0	8.1 - 12.4	-0.15 (LA)

LA : Less than additive SA : Simple additive

* : No mortality ** : 100% mortality

It is clear from Table 20 that the concentration of mercury in the medium assumes importance in the presence of copper and cadmium. Lack of mortality recorded in lower concentrations of copper and cadmium and increased toxicity in combination with high concentration of cadmium and low concentration of copper indicate that mercury and copper may be functioning in a more than additive manner. 300 ppb of cadmium as such is also found to be highly toxic along with copper or mercury and the picture, however, does not change when all the three metals were present in the combination. Less than additive reaction recorded in almost all the combinations is noteworthy.

2.5 DISCUSSION

The lethal toxicity studies are usually aimed at providing a quantitative picture of the toxicant's effect on the death of the test organisms with reference to concentration and time. It is seen that in majority of cases, both these variables are unrealistic in that the concentrations employed are far too high and the time too short. Usually, the 96 h LC_{50} values are used by experimenters to fix sub-lethal concentrations for subsequent chronic investigations. Therefore, the information gathered on acute toxicity becomes an integral part of any laboratory based study to understand sub-lethal effects with reference to metabolism and activity.

The studies conducted in this laboratory from 1984 to 1989 have helped in understanding individual as well as combined toxicity of the most common and hazardous heavy metals encountered as pollutants in the natural environment. The animals used being mussels, collected from the same locality during different periods, covering both post-monsoon

and pre-monsoon seasons have helped in finding out seasonal fluctuations in toxicities also. Perna indica, inhabiting an unpolluted beach of Shakthikulangara has been subjected to rigorous tests to delineate lethal and sub-lethal effects of heavy metals and petroleum hydrocarbons. As a general rule, variations in toxicity was not conspicuous. However, marginal changes in LC_{50} values had been obtained occasionally. This was found to be very true in the case of Perna indica when exposed to a combination of cadmium and mercury. Perna indica collected during early post-monsoon period was found to be more sensitive to the combined toxic effect of mercury and cadmium than those collected during early pre-monsoon. The test animals of size range 20-25 mm attain this dimension in about 4-6 weeks. Therefore, the early post-monsoon samples would have settled at the fag end of the monsoon season when the hydrological features of the area had undergone changes accompanying monsoon. Unstable conditions in the hydrological features are likely to bring in a stress on the animals which would have added to the toxic effects of the heavy metals in these animals. However, lack of mortality of Perna indica subjected to exposure of high concentration of cadmium and mercury in the present instance remains unexplained. Notwithstanding the above limitation, the experimental organisms were collected when the environment was attaining a stable condition characteristic of the area during pre-monsoon periods.

Although Phillips (1980) has discussed extensively on the trace metal seasonality in the aquatic biota, the effects of season on toxicity of heavy metals on bivalves is not properly treated. It is understood that physiology and particularly sexual cycle and changes of ambient water quality can affect uptake of heavy metals by marine bivalves (Phillips,

1980). Changes in toxicity can also therefore be affected by the above factors. Variations in mercury toxicity on P. viridis has been noticed with reference to the locality of occurrence and the physiological status of the Indian mussels (Eknath and Menon, 1983).

Sprague (1970) remarked that the most exciting and potentially useful development in pollution biology has been the method of predicting toxicity of mixtures of toxicants. The methodology developed helps to measure the simultaneous effects of more than one metal and that this could be expressed numerically. Sprague and Ramsay (1965) used the toxic unit method to predict the toxicity of copper and silver to Salmo salar. Majority of techniques for evaluating the toxicity of mixtures of chemicals follow mathematical models for additive joint toxicity that yields their harmonic mean of the LC_{50}'s of the components (Finney, 1971). This model tests the hypothesis that the toxicity of chemical mixtures is simply additive. Smyth et al. (1969) normalised the values obtained from Finney's equation with a frequency distribution curve and adjusted the values to indicate additive toxicity with zero. The study of mixtures of toxic chemicals in sea water and the resultant benefits and hazards are fairly new to science. The simple additive reaction when Perna indica was exposed to combinations of copper, mercury and cadmium indicates that the comparatively low concentrations of the metals employed react together and that the presence of one did not in any way express or suppress the effect of the other. This brings us to the important question whether the combined toxicity of metal combinations is also mainly concentration dependent and not metal dependent. The present series of experiments were conducted using an essential and a non-essential

metal in combination with the contention that the animals will detoxify the non-essential metal and would handle the essential metal later. However, the results do not support this assumption.

Baby and Menon (in press) recorded that the presence of cadmium in the tissue did not have any influence on the uptake of mercury, on the other hand the presence of mercury in the tissue can increase the uptake of cadmium. Therefore, the combined toxicity of mercury and cadmium may be directly influenced by the concentration of mercury rather than that of cadmium and it is interesting to note in this connection that both are non-essential metals. The shift over from simple additivity to less than additivity in the case of cadmium - copper combination may be attributed to the non-toxic nature of copper whereby the concentration of cadmium becomes ineffective, which in the present instance was declining.

The results obtained from the triad combination gave the information that the combined action of three metals can either be less than additive or simple additive. It may be noted here that the concentrations used for lethality tests were normally 50% of the individual LC_{50} values of the respective metals. As a rule, in all the triad experiments the concentrations of copper seem to be controlling the death of the experimental animals, thereby providing a simple additive reaction. In this connection it is interesting to note that Plackett and Hewlett (1967) predicted mortalities following exposure to mixtures of metals based on two models for the non-interaction of the toxicants, namely, independent dissimilar action and simple similar action. However, Negilski et al. in 1981 disproved the above contention based on their experiments employing metals in com-

bination, where they used Callianassa australiensis to predict mortalities after 14 d exposure. They concluded that metals when used in pairs acted in an interactive manner where as in triad mixtures, mortalities of the shrimp happened in an independent dissimilar action. This cannot be taken as a general rule. Although more than additive reaction were not manifested in any of the combinations studied, examination of the raw data show that not withstanding simple additive reaction, there could be drastic increase in the toxicity of one of the metal components of the mixture. For example, Table 9 shows that in the presence of 1100 ppb of cadmium and 15 ppb of copper, 3.1 ppb of mercury produced 50% mortality and reduction in cadmium concentration to 300 ppb reduced the toxicity of mercury. Further reduction in copper drastically reduced the toxicity of mercury and the combined action was simple additive. Simple additivity can indicate at least two reactions. Independent action of all the metal components involved, where the most toxic component controls mortality or a joint action of two components in the triad. In the present case, the metals used being mercury, copper and cadmium, it is logical to assume that either copper or mercury controlled the mortality rates; cadmium only complementing this reaction. Absence of mortality in lower concentrations of copper in the presence of higher concentrations of mercury or cadmium show that copper was the most toxic component in the triad. This is further exemplified by the absolute mortality recorded when the triad contained comparatively higher concentrations of copper. Shifting of toxicity in the traid combinations was found to be controlled either by mercury or the copper concentration. However, variations from the observations in the lower concentrations of all the metals can be attributed to only the time factor, which was only 96 h.

Employing Callianassa australiensis, Negilski et al. (1981) first assumed that zinc, cadmium and copper acted on different sites within the shrimp and that they would act in a dissimilar manner; the model being independent action. However, the results of their experiments showed that these pairs of metals have different mechanisms of action and therefore did not act in a simple fashion. The basic question arising out of the results obtained during the present investigation is whether it is possible to ignore the amount administered versus the amount reaching the site of action because when copper and cadmium or copper, mercury and cadmium were applied in concert, the 96 h LC_{50} of the metals showed variations, not only from the individual metal stand point but also in dual or triad combinations. In the presence of copper, the LC_{50} of cadmium varied considerably and the variation was not copper concentration dependent when the metals were administered in dual combination whereas in the triad combination, the 96 h LC_{50} of cadmium was both copper and mercury dependent. Negilski et al. (1981) developed predictive models to explain combined toxicity of heavy metals. They developed an independent dissimilar action model to predict the mortalities of shrimp exposed to a combination of zinc, cadmium and copper and found conformity with their predictions. These models were developed assuming "independent dissimilar action" (Page 308), to induce mortalities. The observed mortalities in the zinc, cadmium, copper mixture were not significantly different from predicted mortalities based on independent dissimilar action. However, the variations noticed in the predicted mortalities and the observed values using the same model in bi-metal combinations forced them to assume that the existing models to predict mortality rates in combined toxicity studies are inadequate. The approved toxicity indices worked out based

on Marking and Dawson (1975) have also been found inadequate to assess the combined toxicity of metal mixtures. It is quite likely that the suggestions by Phillips (1976) and Bryan (1984) that it is necessary to delineate the reasons for variations in the uptake and thereby manifestations of mortality in marine mussels exposed to heavy metal combinations, is worth further exploration. Much of the literature reported for the effects of mixtures of toxicants to marine and fresh water biota has been based on the toxicity unit concept alone. In the light of the present observations, it looks as if this method does not have a sound theoretical frame work although there are emperical examples supporting this concept.

Toxicity unit concept seems to be merely a redefinition of the simple similar action model and that it has been employed to characterise the toxicity of mixtures of pollutants without regard for dose dependent mortality brought about by individual metals. This can be easily seen in some of the two metal combination studies conducted in the present instance. The shifting of simple additivity to less than additivity or more than additivity was found to be controlled by the concentration of one of the metals rather than both. Less than additive reaction recorded in the case of Donax incarnatus probably speaks for independent similar action of the metals.

Discussing the effects of concentrations of metals used on the variability in the toxic reactions like less than additivity, simple additivity or more than additivity, MacInnes (1981) opined that at low concentrations metal ions form complexes with each other, other ions in the sea water and organics and these interactions can possibly account for a merely additive (simple additive) or antagonistic effects observed (less than additive),

whereas at high concentrations the complexing capacities are overcome, thereby increasing the availability of ions in sea water and hence resulting in synergism (more than additive). Further, since the dissolved organic matter in sea water plays a cardinal role on the rate of complexation, it is possible that the tropical coastal waters, the experimental medium containing around 1.2 ppm of dissolved organic matter, usually used for toxicity studies can also influence availability and hence the toxicity of the medium.

Examination of the data obtained on two metal combination, especially with reference to cadmium and copper shows that shifting from less than additive to more than additive and then to simple additivity occurs with respect to Donax incarnatus. Since, the sea water used for the experiments came from the same stock this could not be the effect of complexation alone. It is safe to assume that copper an essential and highly toxic metal, when present in higher concentrations was the main offender and cadmium had no effect, death would have been controlled by the toxic reactions of copper alone, indicating independent dissimilar action. The concentration of copper when brought down to 1.0 ppb, brought up the cadmium concentration in the mixture to 310 ppb so as to bring about 50% mortality, which concentration was incidentally very near to the 96 h LC_{50} value. It may be seen here that the high concentrations of the metals employed were near to the respective 96 h LC_{50} values which would have influenced the mortality rates of the animals and the results prove beyond doubt that reduction in toxicity would occur when cadmium was present in high concentrations producing less than additive reaction.

The triad combination showed in majority of cases less than additive (antagonistic) reactions. In triad combinations, concentrations of the individual components to be employed is a very important factor as this can demonstrably influence the data. This aspect is amply evidenced in the case of Donax incarnatus, which is a very sensitive marine intertidal bivalve. Antagonistic reactions can occur when the concentrations of the combinations are relatively low and this would result in decreasing or increasing the toxicity of a single component very clearly shown by the present investigation. Prabhudeva and Menon (1988) found that when Perna indica was exposed to a combination of copper and silver, the toxicity of the individual metals increased. The reasons of the increase of toxicity of the mixture, according to them are not clearly understood. Among the causative factors which may have influenced toxicity, according to these authors include selective absorption of metals, disruption of detoxyfying mechanisms, impediment of the transport of metal ions after entry into the cells and reaction of the metal ions of both the metals on the same site in the cell. However, the present study shows that they could be concentration dependent also.

3
VENTILATION RATE

3.1 INTRODUCTION

The main objective of this series of experiments was to delineate concentrations which would induce alterations in the basic responses of the animal as well as to segregate toxicity bound hyper or lower activity exemplified by one of the behavioural functions namely ventilation rate, for ventilation gives an idea of the animal's capability to perform normally in the environment. Results obtained and presented in the literature available on the rate of ventilation show that the quantity of water filtered is a dependable measure of toxicity stress and well being of bivalves used for the experiments. It is found that the interferance by the byssal mass is an important factor which influences the rate of ventilation of mussels even when exposed to highly toxic chemicals. On the other hand, clams in general can 'clam up' thereby preventing entry of water into the mantle cavity. Wedging the shells or drilling holes on the shell have been suggested as methods to prevent a complete closure of the shells of clams while employing for experiments. However, laboratory studies have shown that the inert material wedged in between the shells could be easily rejected by the animal thereby bringing in considerable fluctuations in the quantity

of water entering the mantle cavity as a function of experimental duration. Therefore, such methods were not resorted to during the course of the present experiments.

3.2 REVIEW OF LITERATURE

The sub-lethal effects of contaminants on marine organisms are usually viewed as modifications in rate functions, behavioural responses, cell structure etc. and are believed to result from exposure of the organisms to pollutant levels much lower than experimentally delineated lethal concentrations. Therefore, the concentrations to be employed in sub-lethal studies should have a direct bearing on the realistic concentrations met with in estuaries and coastal waters. Bryan (1976 a) observed that the concentrations of heavy metals in coastal and estuarine waters are likely to vary considerably depending on the quantum of pollutants in that area. Subtle changes are likely to occur in the activity of the coastal fauna when subjected to chronic exposure to analytically undetectable levels of toxicants. Bayne (1985) suggested that such changes could be detected only if continuous monitoring is carried out so as to assess the "scope for growth" (Widdows, 1985 a). Since the variations in the rate functions (positive or negative) and related aspects of the animal's biology have to be critically evaluated, such studies have to last for considerable period. It is difficult to maintain marine organisms in the laboratory for long durations and under controlled conditions without interfering with the normal physiology of the animal. Sub-lethal toxicity studies should there-

fore take into account both the concentration and time factor. In practice, prolonged durations are compensated for by enhanced concentrations. The concentrations employed in sub-lethal toxicity studies are usually well above realistic concentrations. Normally the rate functions employed to assess the sub-lethal effects are filtration rate (ventilation rate), oxygen consumption, ammonia excretion etc.

In marine animals, the sub-lethal responses to heavy metals have been exemplified as damage to gill epithelial cells and copious secretion of mucous (Eisler and Gardner, 1973; Labat et al., 1974; Martin et al., 1977; Wong et al., 1977; Engel and Fowler, 1979; D'Silva et al., 1979; Reddy and Menon, 1979). In marine molluscs, filtration rate has been proved to be a reliable parameter to assess the effect of pollutants and being a non-destructive method can be carried out with ease. The effects of toxicants on the filtration rate of Mytilus edulis was dealt with in detail by Abel (loc cit.). Environmental factors such as salinity, temperature, pH etc. are known to influence the filtration rate (Cole and Hepper, 1954). Prabhudeva and Menon (1985) using Perna viridis pre-exposed to heavy metals demonstrated that zinc and copper at low concentrations affected the filtration rate conspicuously. A reduction of 15-25% efficiency in filtration rate was observed in concentrations ranging from 40-80 ppb copper. During the period of experimentation, the animals were placed in clean sea water after being pre-exposed to the toxicants for 24-96 h. They feel that pre-exposure to toxicants influences the rate of filtration. Mercury and cadmium were found to alter the filtration rate in six intertidal

animals (Mohan et al., 1986 a). They feel that unlike many functions of potential value as indicators of sub-lethal stress, the filtration rate is based on a response of ecological significance, since bivalves depend on the water they filter for both food and oxygen (Abel, 1976). Brown and Newell (1972) suggested that the valve closure mechanism and inhibitory effect of ciliary action accompanying stress can significantly affect the amount of water drawn into the body cavity. Mercury and cadmium in mixtures were found to interact additively and more than additively (synergism) in depressing the filtration rates in Modiolus spp. and Donax spiculum (Mohan et al., 1986 a). In an attempt to delineate the behavioural and rhythmic aspects of filtration in Argopecten irradians and Crassostrea virginica, Palmer (1980) observed that filtration behaviour was not influenced by the tidal sequence or laboratory conditions. In the case of Argopecten irradians, the filtration rate remained either constant throughout or stabilized after a steady initial decline. It was also noted that rate of filtration was affected by the concentration of suspended silt. In Villorita cyprinoides var. cochinensis, Abraham et al. (1986) found that the filtration rate decreased exponentially with the increase in concentration of chromium, manganese, nickel, copper, cadmium and mercury. Studies by Widdows et al. (1982) and Stickle et al. (1984) indicated that an analysis of filtration rate under pollutant stress has a direct ecological significance, since alterations in feeding rate cause variation in growth rate in marine molluscs. Howell et al. (1985) observed that exposure of Mytilus edulis to dissolved copper led to a decline in the filtration rate when measured in whole animals and that a concentration of 94 ppb of copper produced a 50% reduction

in filtration rate. Grace and Gainey (1987) commenting on the observed reduction in the filtration rate in Mytilus edulis following exposure to dissolved copper stated that the reduction was caused not by valve or siphon closure.

3.3 MATERIAL AND METHODS

This section of the thesis deals with the determination of ventilation rates of Perna indica and Donax incarnatus exposed to sub-lethal concentrations of mercury, copper and cadmium, individually and in combination. Details of test animals employed, experimental media, toxicants etc. have already been given (Section 2.3).

3.3.1 SUB-LETHAL TOXICITY STUDIES

The concentrations of the different toxicants used, individually and in combination, for the sub-lethal study were derived from the 96h LC_{50} levels of the individual toxicants delineated after the static renewal bioassay. The sub-lethal toxicity studies conducted can be categorised into those using test animals pre-exposed to the toxicants (upto 1/10th of the 96 h LC_{50} level) for a period of 24 h (short term) and the other using test animals exposed to the toxicants for a period of 14 d in case of Donax incarnatus or 21 d in case of Perna indica (long term). The test solutions were never aerated during the periods of pre-exposure or experiment. During long term exposure the animals were fed with Synechocystis salina.

Sub-lethal effects were monitored utilising various accepted

reactions of the animal. Concentrations of mercury, copper and cadmium used to study the effects on P. indica ranged between 1.0 and 10 ppb, 0.5 and 6.0 ppb, 25 and 400 ppb respectively; in the case of D. incarnatus the same were 1.0 and 5.0 ppb, 0.5 and 6.0 ppb and 5.0 and 40 ppb.

3.3.1.1 **Ventilation Rate**

3.3.1.1.1 Pre-exposure to the toxicants for 24 h

Bivalve molluscs feed and respire by means of a water current drawn into the body under the influence of the ctenidium. The dye clearance technique was employed to assess the ventilation rate. The technique involves the addition of a known concentration of neutral dye (neutral red: 2 ppm) to the test solution and leaving the animals to clear the dye. The amount of water ventilated after a specific time lapse was determined after having found out the reduction in the dye concentration with the help of a Spectrophotometer (Hitachi: model 220-20) at 435 nm. Ventilation rate was calculated using Abel's equation (Abel, 1976) viz.

$$m = \frac{M}{n.t} \log_e \frac{Co}{Ct}$$

where 'm' is the ventilation rate, 'M' is the volume of suspension, 'n' is the number of test animals per vessel, 't' is the duration of sampling, Co is the initial concentration of the suspension and Ct is the final concentration. Three animals pre-exposed to the respective toxicant concentration were placed in glass beakers of 1 l capacity containing 750 ml of the test solution, with 2 ppm neutral red. The experiment lasted for 2 h and the frequency of sampling was 30 min. After the experiments, the animals were dissected, soft tissues removed, cleaned in distilled water, dried at 70-80°C for 48 h and dry weights were recorded to constancy and the results expressed as ml $h^{-1}mg^{-1}$(dry wt).

Statistical analysis was done of the data gathered following the procedure explained earlier (Section 2.3.6.2). To assess whether there was any significant variation of the sub-lethal responses registered in respect of the test animals, the students 't' test was employed.

3.4 RESULTS

The present study has centred around delineating the rate of ventilation of Perna indica and Donax incarnatus exposed to different metal stress. The results are presented in Tables 21-44d and Figs. 1-24

3.4.1 PERNA INDICA

3.4.1.1 Ventilation rate of Perna indica pre-exposed for 24 h to individual metals

Table 21 and Fig. 1 represent the rate of ventilation of Perna indica exposed to various concentrations of mercury (1.0 ppb to 10 ppb). The rate of ventilation was inversely proportional to increase in concentration of mercury in the medium. The rate of ventilation showed a steady decline with increasing concentration of mercury from 2.5 ppb upto the highest concentration tested. The effective concentration of mercury that resulted in 50% reduction in performance was 10.01 ppb.

The results of the effect of various sub-lethal concentrations of copper on the ventilation rate of Perna indica is presented in Table 22 and Fig. 2. The presence of 0.5 ppb copper resulted in reduction in ventilation rate by about 30%. Further increase in copper concentration registered an increase in the rate of ventilation and at 4.0 ppb of copper

Table 21. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury along with the respective standard deviations, percentage performance and EC_{50} level.*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
1.0	0.668**	0.048	67.7	
2.5	0.925	0.116	93.8	
5.0	0.817	0.140	82.9	10.01
7.5	0.783*	0.076	79.4	
10.0	0.554 ***	0.085	56.2	
Control	0.986	0.115	100.0	

Table 22. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper, along with the respective standard deviations, percentage performance and EC_{50} level.*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
0.5	0.616*	0.046	68.9	
1.0	0.679	0.085	75.9	
2.0	0.809	0.101	90.5	0.25
4.0	0.986	0.122	110.3	
6.0	0.778	0.135	87.0	
Control	0.894	0.189	100.0	

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 23. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium along with the respective standard deviations, percentage performance and EC_{50} level.*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
25	0.904	0.241	84.9	
50	1.162	0.148	109.2	
100	0.859	0.213	80.7	1623.8
200	0.847	0.181	79.6	
400	0.699 **	0.127	65.7	
Control	1.064	0.075	100.0	

** $P < 0.01$

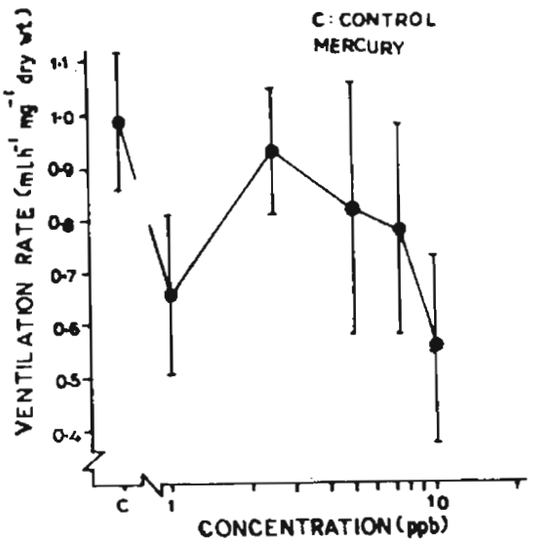


FIG. 1.

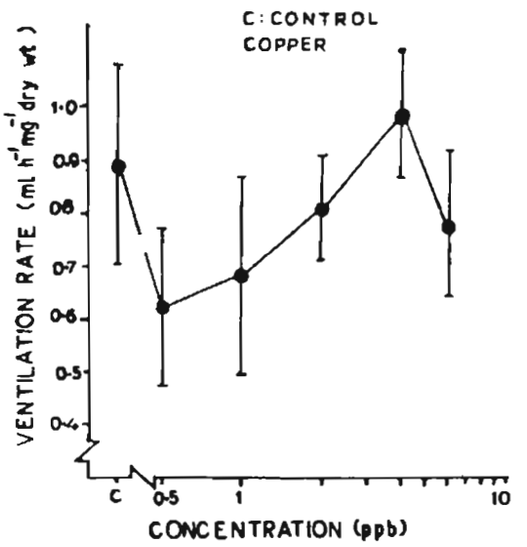


FIG. 2

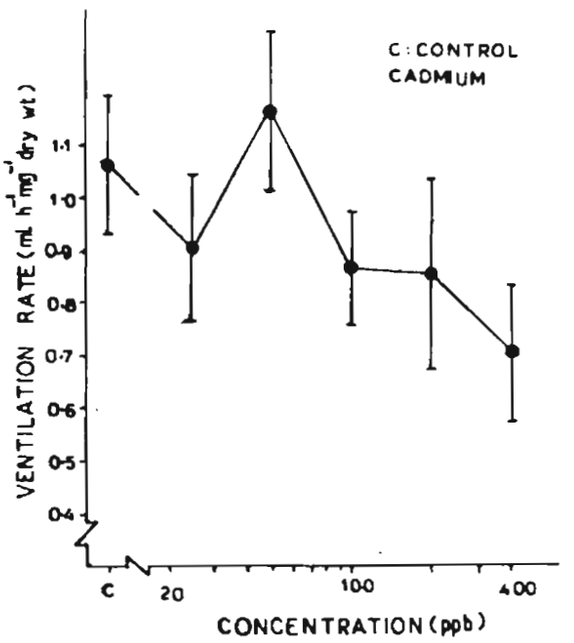


FIG. 3

Figures 1,2&3. Perna indica. Average ventilation, under sub-lethal concentrations of mercury, copper and cadmium (vertical bars indicate standard deviation).

the animals ventilated more water than that of the control. At 6.0 ppb, the rate of ventilation was reduced. The calculated EC_{50} was 0.25 ppb.

Comparable results were obtained on the rate of ventilation in Perna indica exposed to various sub-lethal levels of cadmium (Table 23 and Fig. 3). In general, the rate of ventilation was less than that of the control; the only exception being the rate recorded in the case of test organisms exposed to 50 ppb cadmium. The difference was maximum with those animals exposed to 400 ppb of cadmium. This was the only rate which was statistically different from that of the control. The effective concentration of cadmium to reduce the rate of ventilation by 50% was 1623.8 ppb.

3.3.1.2 Ventilation rate of Perna indica pre-exposed for 24 h to metal mixtures

Tables 24a to 32d and Figs. 4 to 12 give the data on the combined toxicity of mercury, copper and cadmium on the ventilation rate of Perna indica. As indicated before, here one or two of the metals were maintained at a constant level and the other made to vary. All the concentrations used have been employed individually also to assess the sub-lethal stress.

Tables 24a and b and Fig. 4 depict the data obtained on the ventilation rate of Perna indica exposed to a constant concentration of mercury with varying concentrations of copper. The mercury concentration employed was 1.0 ppb and copper varied between 0.5 to 6.0 ppb (Table 24a and Fig. 4). A concentration of 2.0 ppb of copper did not drastically

influence the ventilation rate. However, exposure to higher and lower concentrations did produce statistically significant reduction in the rate of ventilation. The EC_{50} was 8.7 ppb and the combined toxicity was less than additive.

Exposure of Perna indica to 5.0 ppb of mercury (constant) and varying concentrations of copper produced comparable results (Table 24b and Fig. 4). However, the EC_{50} of copper was brought down to 5.68 ppb and the analysis of data showed that the reaction was less than additive.

The presence of copper and mercury at sub-lethal levels resulted only in less than additive reaction with reference to the rate of ventilation. Table 25a and Fig. 5 show the ventilation performance of those animals exposed to 0.5 ppb of copper and 1.0 to 10 ppb of mercury. The presence of 0.5 ppb of copper along with 1.0 or 2.5 ppb of mercury in the experimental medium resulted in statistically significant reduction in the rate of ventilation of Perna indica; the rate being 0.585 and 0.458 ml h^{-1} mg^{-1}(dry wt) respectively. Beyond 2.5 ppb of mercury, however, no significant reduction in the ventilation rate was observed.

Stepping up of copper concentration to 2.0 ppb produced comparable results (Table 25b and Fig. 5). An increase in the mercury concentration from 1.0 ppb to 5.0 ppb produced a steady decline in the rate of ventilation. At 5.0 ppb of mercury, the maximum reduction in ventilation rate which was statistically significant was observed. No significant reduction in the ventilation rate was observed with further increase in the

Table 24 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 24 a. *1.0 ppb Mercury + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.585***	0.065	67.7		
1.0	0.595***	0.031	68.9		
2.0	0.831	0.069	96.2	8.7	-33.9 (LA)
4.0	0.631	0.200	73.0		
6.0	0.623*	0.159	72.1		
Control	0.864	0.034	100.0		

Table 24 b. *5.0 ppb Mercury + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	Sd			
0.5	0.712	0.178	82.4		
1.0	0.849	0.127	98.3		
2.0	0.639*	0.156	73.9	5.68	-22.2 (LA)
4.0	0.577***	0.065	66.8		
6.0	0.474***	0.069	54.9		
Control	0.864	0.034	100.0		

* $P < 0.05$ LA : Less than additive

*** $P < 0.001$

Table 25 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and mercury (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 25 a. 0.5 ppb Copper + Mercury

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.585***	0.065	67.7		
2.5	0.458***	0.051	53.0		
5.0	0.712	0.178	82.4		
7.5	0.729	0.115	84.3		
10.0	0.799	0.172	92.5		
Control	0.864	0.034	100.0		

Table 25 b. 2.0 ppb Copper + Mercury

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.831	0.069	96.2		
2.5	0.732	0.143	84.7		
5.0	0.639*	0.156	73.9	12.12	-8.2 (LA)
7.5	0.758	0.100	87.7		
10.0	0.760	0.092	87.9		
Control	0.864	0.034	100.0		

* $P < 0.05$

** $P < 0.001$

LA : Less than additive

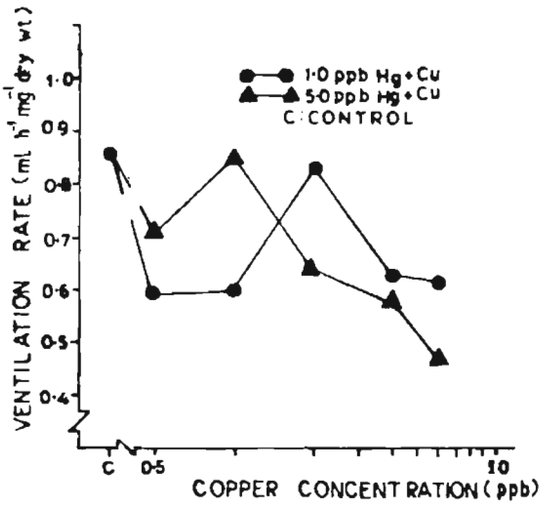


Figure 4. *Perna indica*. *Average ventilation under sub-lethal concentrations of mercury (constant) and copper (varying) (for standard deviations see Tables 24 a&b).*

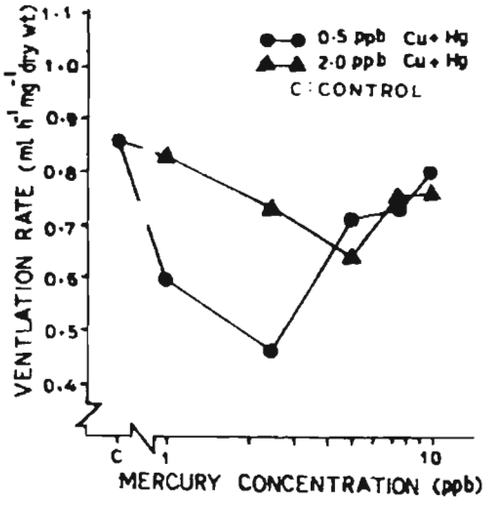


Figure 5. *Perna indica*. *Average ventilation, under sub-lethal concentration of copper (constant) and mercury (varying) (for standard deviations see Tables 25 a&b).*

mercury concentration. The median effective concentration of mercury was calculated and put as 12.12 ppb, which was beyond the concentration employed in the experiments. The combined toxicity worked out showed that the range employed brought about a less than additive reaction.

In the case of mercury along with cadmium, the combined action was simple additive. This was true in the case of both the series of experiments where the mercury concentration was either 1.0 ppb or 5.0 ppb, with cadmium concentrations varying from 25 ppb to 400 ppb. In the series where the mercury concentration was 1.0 ppb, the ventilation rate showed an initial increase with an increase in the cadmium concentration from 25 ppb to 50 ppb. At 50 ppb of cadmium, the animals ventilated 1.047 ml $h^{-1}mg^{-1}$(dry wt) which was more than around 17% of that of the control. However, further increase in the cadmium concentration produced clear cut reduction in the rate of ventilation and at 400 ppb of cadmium along with 1.0 ppb of mercury, the percentage performance was 59.9. The EC_{50} was 518.4 and the combined action was simple additive (Table 26a and Fig. 6).

Table 26b and Fig. 6 out line the data obtained on the ventilation performance when the animals were exposed to 5.0 ppb of mercury and varying concentrations of cadmium. Curiously enough, the ventilation rate was found to decrease with an increase in the cadmium concentration. When the cadmium concentration in the experimental medium was above 50 ppb, the ventilation or the rate of filtration ranged from 0.449 to 0.840 ml $h^{-1}mg^{-1}$(dry wt). In the highest concentration employed (5.0

ppb mercury along with 400 ppb of cadmium), the percentage performance was 93.9. The EC_{50} was 38.9 and the combined toxicity simple additive.

Depicting statistically significant variation, Perna indica exposed to 25 ppb of cadmium and 1.0 to 10 ppb of mercury, drastically reduced the rate of ventilation. In the lowest concentration tested, the animals ventilated around 80.1% of water when compared to those in the control. The decline in the rate of ventilation was steady and when the mercury concentration was 7.5 ppb, the rate of ventilation was as low as 0.416 ml $h^{-1}mg^{-1}$(dry wt). Further increase in the mercury concentration to 10 ppb, however, showed a slight increase in the rate of ventilation. The EC_{50} was 5.52 ppb of mercury along with 25 ppb of cadmium. The combined toxicity was simple additive (Table 27a and Fig. 7).

More or less comparable results were obtained when the experimental medium contained 100 ppb of cadmium along with varying concentrations of mercury. When the test medium contained 1.0 ppb of mercury and 100 ppb of cadmium, the performance of the test animals was more or less comparable with that of the control. An eight fold increase in the mercury concentration reduced ventilation performance considerably. Giving statistically significant results, the rate of ventilation was found to decrease steadily with the increase in mercury concentration. Ventilating 0.282 ml $h^{-1}mg^{-1}$(dry wt), Perna indica reduced their performance to 31.5% in comparison to their counterparts in control when the test medium contained 7.5 ppb of mercury along with 100 ppb of cadmium. An increase in the mercury concentration to 10 ppb showed an elevation in the rate

Tables 26 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and cadmium (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 26 a. *1.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.716	0.169	80.1		
50	1.047	0.088	117.1	518.4	1.39 (SA)
100	0.879	0.054	98.3		
200	0.823	0.103	92.1		
400	0.536*	0.169	59.9		
Control	0.894	0.189	100.0		

Table 26 b. *5.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.489*	0.148	54.7		
50	0.412*	0,240	46.1	38.9	0.91 (SA)
100	0.449*	0.192	50.2		
200	0.808	0.220	90.4		
400	0.840	0.200	93.9		
Control	0.894	0.189	100.0		

* $P < 0.05$ SA : Simple additive

Tables 27 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant) and mercury (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 27 a. *25 ppb Cadmium + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.716	0.169	80.1		
2.5	0.493**	0.048	55.1	5.52	0.76 (SA)
5.0	0.489*	0.148	54.7		
7.5	0.416**	0.140	46.5		
10.0	0.583	0.174	65.2		
Control	0.894	0.189	100.0		

Table 27 b. *100 ppb Cadmium + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.879	0.054	98.3		
2.5	0.564*	0.078	63.1		
5.0	0.449*	0.192	50.2	4.6	0.92 (SA)
7.5	0.282**	0.140	31.5		
10.0	0.543*	0.150	60.7		
Control	0.894	0.189	100.0		

* $P < 0.05$ SA : Simple additive

** $P < 0.01$

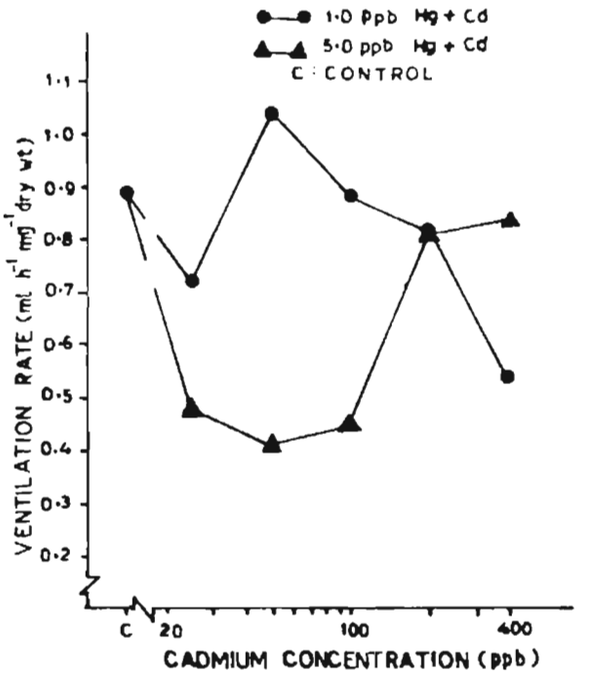


Figure 6. *Perna indica*. *Average ventilation, under sub-lethal concentrations of mercury (constant) and cadmium (varying) (for standard deviations see Tables 26 a&b).*

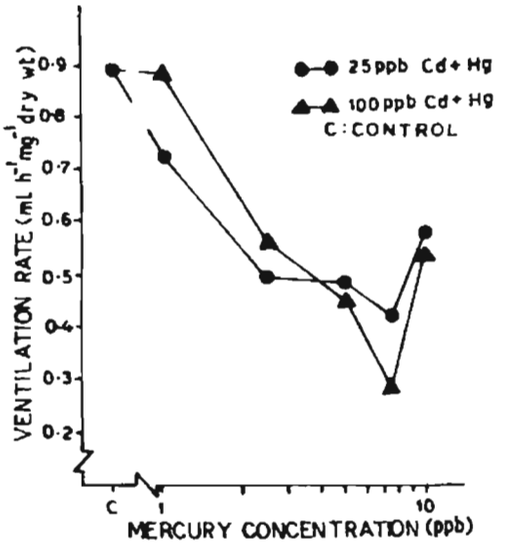


Figure 7. *Perna indica*. *Average ventilation, under sub-lethal concentrations of cadmium (constant) and mercury (varying) (for standard deviations see Tables 27 a&b).*

of ventilation which, however, was only 60.7% of the control. The effective concentration to reduce the rate by 50% was calculated to be 4.6 ppb of mercury. Analysis of the data indicated a simple additive reaction (Table 27 b and Fig. 7).

Tables 28 a and b and Fig. 8 illustrate the average ventilation rate of Perna indica exposed to 0.5 ppb or 2.0 ppb of copper, along with 25 to 400 ppb of cadmium. An increase in the cadmium concentration from 25 ppb to 50 ppb registered an increase in the rate of ventilation which, however, was only 90% of that of the control (Table 28 a and Fig. 8). Further increase in the cadmium concentration to 400 ppb, along with 0.5 ppb of copper did not elicit significant variation in the rate of ventilation. In all cases, the rate of ventilation was lower than that of the control.

A four fold increase in cadmium concentration from 25 ppb reduced ventilation performance considerably, in the combined presence of 2.0 ppb of copper. Thus, 100 ppb of cadmium along with 2.0 ppb of copper produced a statistically significant ventilation rate of 0.374 ml $h^{-1}mg^{-1}$(dry wt), which was 42% of that of the control animals. A further four fold increase in the cadmium concentration was found to increase the ventilation rate. The EC_{50} was 114.7 ppb of cadmium and the combined reaction was less than additive (Table 28 b and Fig. 8).

Tables 29 a and b and Fig. 9 show the results obtained on the combined toxic effectsof cadmium and copper on the ventilation performance

of Perna indica. Table 29 a and Fig. 9 show the rate of ventilation by Perna indica exposed to 25 ppb of cadmium and 0.5 to 6.0 ppb of copper. The presence of 25 ppb cadmium and 0.5 ppb of copper resulted in reduced ventilation rate which was around 68% of that of the control and was statistically significant. Increase in the concentration of copper upto 6.0 ppb along with 25 ppb of cadmium did not produce drastic variations in the ventilation rate of the experimental animals.

An increase in the cadmium concentration to 100 ppb along with varying concentrations of copper, however, produced quite different results. Increase in the copper concentration from 0.5 ppb to 2.0 ppb produced statistically significant reduction in the rate of ventilation. Thus, 2.0 ppb of copper with 100 ppb of cadmium produced drastic reduction in the rate of ventilation; the animals ventilating 0.374 ml $h^{-1}mg^{-1}$(dry wt) which was only around 42% in comparison with the performance of the control. Higher concentrations however, resulted in the elevation of ventilation rate, although below that of the control. The EC_{50} was 2.4 ppb of copper and the additive index was less than additive (Table 29 b and Fig. 9).

Tables 30 a to 32 d and Figures 10 to 12 illustrate the average rate of ventilation by Perna indica exposed to triad combinations of mercury, copper and cadmium. Here two of the metals were maintained at a constant level while the third was made to vary. All the concentrations used have been employed individually also to assess sub-lethal stress.

Tables 28 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and cadmium (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 28 a. *0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.603***	0.042	68.6		
50	0.795	0.123	90.4		
100	0.785	0.093	89.3		
200	0.752	0.108	85.6		
400	0.732	0.129	83.3		
Control	0.879	0.051	100.0		

Table 28 b. *2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.837	0.253	95.2		
50	0.795	0.055	90.4		
100	0.374***	0.056	42.5	114.7	-7.1 (LA)
200	0.664*	0.167	75.5		
400	0.769	0.094	87.4		
Control	0.879	0.051	100.0		

* $P < 0.05$ LA : Less than additive

*** $P < 0.001$

Tables 29 a&b. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 29 a. *25 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.603***	0.042	68.6		
1.0	0.816	0.163	92.8		
2.0	0.837	0.253	95.2		
4.0	0.812	0.120	92.3		
6.0	0.789	0.153	89.8		
Control	0.879	0.051	100.0		

Table 29 b. *100 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.785	0.093	89.3		
1.0	0.673	0.186	76.6	2.4	-8.7 (LA)
2.0	0.374***	0.056	42.5		
4.0	0.647**	0.096	73.6		
6.0	0.812	0.134	92.4		
Control	0.879	0.051	100.0		

** $P < 0.01$ LA : Less than additive

*** $P < 0.001$

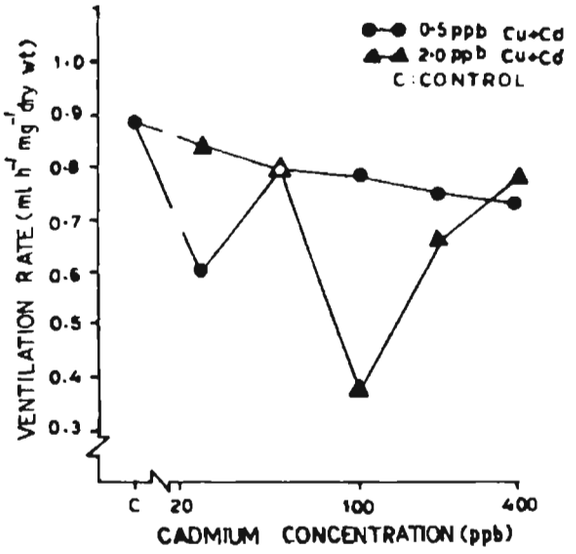


Figure 8. *Perna indica.* *Average ventilation, under sub-lethal concentrations of copper (constant) and cadmium (varying) (for standard deviations see Tables 28 a&b).*

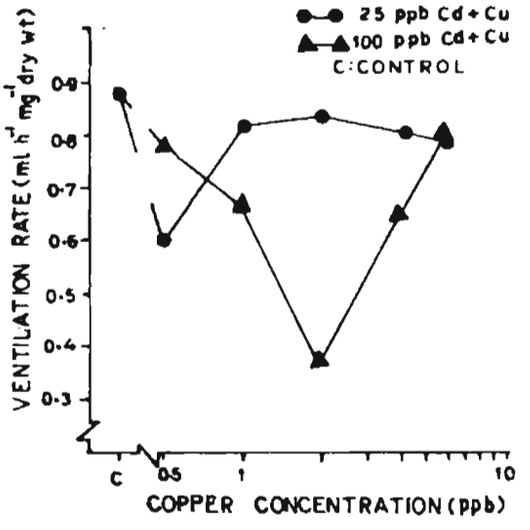


Figure 9. *Perna indica.* *Average ventilation, under sub-lethal concentrations of cadmium (constant) and copper (varying) (for standard deviations see Tables 29 a&b).*

Tables 30 a to d and Fig. 10 outline the combined toxic effects of 1.0 and 2.0 ppb of mercury, 0.5 and 2.0 ppb of copper and 25 to 400 ppb of cadmium on the ventilation rates. Eleciting an EC_{50} of 601.7 ppb of cadmium along with 1.0 ppb of mercury and 0.5 ppb of copper, the combination apparently influenced the ventilation performance of the test animals (Table 30a and Fig. 10). The median concentration of 100 ppb of cadmium along with 1.0 ppb of mercury and 0.5 ppb of copper, did not significantly influence the ventilation rate. However, the lower and higher concentrations of cadmium along with constant concentration of mercury and copper, produced significant reduction in the ventilation rate. Thus, animals exposed to 25 ppb and 400 ppb of cadmium along with 1.0 ppb mercury and 0.5 ppb of copper cleared only 0.741 and 0.729 ml $h^{-1}mg^{-1}$ (dry wt), which was around 65% of that of the control. The combined toxic effect of the three metals was found to be simple additive.

An enhancement in the copper concentration to 2.0 ppb along with 1.0 ppb of mercury and 25 to 400 ppb of cadmium brought about clear cut reduction in the (Table 30 b and Fig. 10) ventilation rate. Here, the rate of ventilation came down to 0.406 ml $h^{-1}mg^{-1}$ (dry wt), when the cadmium concentration was 100 ppb at a constant copper and mercury load. Although higher concentrations of cadmium brought about a slight elevation in the ventilation rates, the performance showed real stress and was statistically significant. The EC_{50} was calculated to be 196.85 and the reaction of the animals with respect to ventilation was less than additive.

Clear cut reduction in the ventilation performance was observed when mercury concentration was stepped up to 5.0 ppb along with 0.5 ppb copper and 25 to 400 ppb of cadmium. The medium containing 50 ppb of cadmium along with constant concentration of copper and mercury, did not produce significant reduction in the ventilation rate. However, lower and higher concentrations of cadmium at constant mercury and copper load did produce real stress and the ventilation rates ranged from 0.662 to 0.742 ml $h^{-1}mg^{-1}$(dry wt). The EC_{50} was 868.1 ppb of cadmium at constant mercury and copper load and the combined toxic action was less than additive (Table 30 c and Fig. 10).

Table 30 d and Fig. 10 depict the data on the rate of ventilation by Perna indica exposed to 2.0 ppb of copper along with 5.0 ppb of mercury and 25 to 400 ppb of cadmium. In this series, clear cut reduction in the ventilation performance was observed only when the cadmium concentration was 200 ppb along with constant mercury and copper load. Other combinations did not elecit any statistically significant reduction. The EC_{50} was brought down to 439.5 ppb and the reaction was calculated to be less than additive.

The data obtained on the combined toxic effect of constant concentration of cadmium and copper and varying concentrations of mercury on the ventilation rate of Perna indica are presented in Tables 31 a to d and Fig. 11. In the case of constant concentration of cadmium and copper along with varying concentrations of mercury, the combined action was simple additive. This was true in the case of all the four series

Tables 30 a,b,c&d. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying), along with the respective standard deviations, percentage performance EC_{50} level and additive index.*

Table 30 a. *1.0 ppb Mercury + 0.5 ppb Copper + Cadmium.*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.741**	0.125	66.4		
50	0.783**	0.053	70.2		
100	1.035	0.096	92.7		
200	0.858*	0.132	76.9	601.7	-1.5 (SA)
400	0.729**	0.133	65.3		
Control	1.116	0.159	100.0		

Table 30 b. *1.0 ppb Mercury + 2.0 ppb Copper + Cadmiun*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.893	0.132	80.0		
50	0.818*	0.152	73.3		
100	0.406***	0.013	36.4	196.85	-7.2 (LA)
200	0.632**	0.094	56.6		
400	0.655**	0.098	58.8		
Control	1.116	0.159	100.0		

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

LA : Less than additive
SA : Simple additive

Table 30c. 5.0 ppb Mercury + 0.5 ppb Copper + Cadmium.

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	0.662**	0.173	58.3		
50	0.880	0.185	78.9		
100	0.757*	0.144	67.8	868.1	-2.0 (LA)
200	0.753**	0.113	67.4		
400	0.742**	0.088	66.5		
Control	1.116	0.159	100.0		

Table 30 d. 5.0 ppb Mercury + 2.0 ppb Copper + Cadmium.

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
25	1.067	0.058	95.6		
50	1.054	0.152	94.5		
100	0.979	0.256	87.7		
200	0.799*	0.154	71.6	439.5	-7.8 (LA)
400	1.022	0.248	91.6		
Control	1.116	0.159	100.0		

* $P < 0.05$

** $P < 0.01$

LA : Less than additive

of experiments where the cadmium concentration was 25 or 100 ppb, copper concentration 0.5 or 5.0 ppb and mercury concentration varying from 1.0 to 10 ppb. In the series where the cadmium concentration was 25 ppb and copper concentration 0.5 ppb, along with varying concentrations of mercury, the rate of ventilation showed significant reduction only when the mercury concentration was 1.0 ppb and 5.0 ppb. The other combinations, however, did not produce any drastic influence on the ventilation rate. The median effective concentration of mercury along with constant concentration of cadmium and copper was found to be 4.1 ppb (Table 31 a and Fig. 11).

Stepping up of copper concentration to 2.0 ppb along with 25 ppb cadmium and 1.0 to 10 ppb of mercury produced a less than additive reaction. No significant reduction in the ventilation rate was observed in any of the mercury concentrations along with constant concentration of cadmium and copper. The EC_{50} was found to be 0.37 ppb of cadmium (Table 31 b and Fig. 11).

The results obtained when the medium contained 100 ppb of cadmium along with 0.5 ppb of copper and 1.0 to 10 ppb of mercury are shown in Table 31 c and Fig. 11. The rate of ventilation in the experimental animals was significantly reduced only when the concentration of mercury was either 2.5 or 5.0 ppb, along with constant cadmium and copper load. The EC_{50} was 1.90 ppb of mercury and the combined toxicity was of a simple additive nature.

Tables 31 a,b,c&d. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying), along with the respective standard deviations, percentage performance EC_{50} level and additive index.*

Table 31 a. *25 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.741**	0.125	66.4		
2.5	1.018	0.245	91.2		
5.0	0.662**	0.173	58.3	4.1	-1.4 (SA)
7.5	1.067	0.129	95.6		
10.0	1.048	0.183	93.9		
Control	1.116	0.159	100.0		

Table 31 b. *25 ppb Cadmium + 2.0 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.893	0.132	80.0		
2.5	0.874	0.219	78.3		
5.0	1.067	0.058	95.6	0.37	-7.1 (LA)
7.5	1.089	0.114	97.6		
10.0	1.159	0.258	103.9		
Control	1.116	0.159	100.0		

** $P < 0.01$ LA : Less than additive

SA : Simple additive

Table 31 c. *100 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	1.035	0.096	92.7		
2.5	0.731*	0.179	65.5		
5.0	0.757*	0.144	67.8	1.90	-1.3 (SA)
7.5	1.040	0.205	93.2		
10.0	1.047	0.161	93.8		
Control	1.116	0.159	100.0		

Table 31 d. *100 ppb Cadmium + 2.0 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.406***	0.013	36.4		
2.5	0.865*	0.122	77.5		
5.0	0.979	0.256	87.7	1.32	-7.2 (LA)
7.5	0.984	0.211	88.1		
10.0	1.071	0.229	95.9		
Control	1.116	0.159	100.0		

** $P < 0.01$ *** $P < 0.001$

SA : Simple additive LA : Less than additive

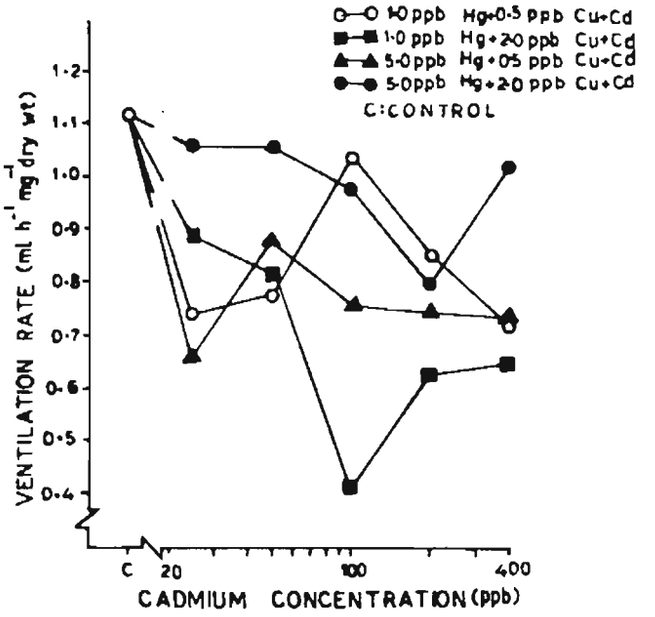


Figure 10. *Penna indica.* *Average ventilation, under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying) (for standard deviations see Tables 30 a, b, c & d).*

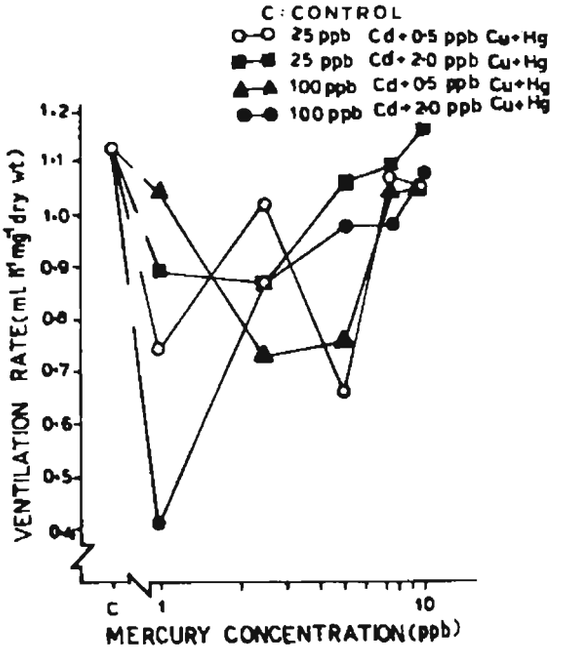


Figure 11. *Penna indica.* *Average ventilation, under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying) (for standard deviations see Tables 31 a, b, c & d).*

Table 31 d and Fig. 11 depicts the data obtained when Perna indica was exposed to 100 ppb of cadmium, 2.0 ppb of copper, along with 1.0 to 10 ppb of mercury. Mercury concentration of 1.0 ppb along with constant concentration of cadmium and copper resulted in animals' reduced rate of ventilation to 36% of that of the control whereas in the highest mercury concentration of 10 ppb, it was almost unaffected; in terms of water cleared it was 1.071 ml $h^{-1}mg^{-1}$(dry wt). The EC_{50} was brought down to 1.32 ppb of mercury along with 100 ppb of cadmium and 2.0 ppb of copper. Analysis of the data indicated a less than additive reaction.

The presence of constant concentration of mercury and cadmium along with varying concentrations of copper elecited both simple and less than additive responses. Table 32 a and Fig. 12 detail out the rate of ventilation by Perna indica exposed to 1.0 ppb of mercury and 25 ppb of cadmium, along with 0.5 to 6.0 ppb of copper. When the medium contained 0.5 ppb of copper along with constant concentration of mercury and cadmium, the animals propelled only 0.741 ml $h^{-1}mg^{-1}$(dry wt), which was only 66% of the overall performance of the control animals.

Registering a simple additive reaction as indicated earlier, the rate of ventilation by Perna indica was reduced significantly when the medium contained 0.5 ppb of copper along with 5.0 ppb of mercury and 25 ppb of cadmium. Higher concentrations of copper along with constant mercury and cadmium load, however, did not produce any drastic influence on the ventilation rate. The quantity of water ventilated by the animals

exposed to 0.5 ppb of copper along with 5.0 ppb of mercury and 25 ppb of cadmium was rather low; being 0.662 ml $h^{-1}mg^{-1}$(dry wt), which was only 58% of that of the control. The EC_{50} was found to be 0.18 ppb of copper (Table 32 b and Fig. 12).

Table 32 c and Fig. 12 depict the ventilation performance by Perna indica exposed to 1.0 ppb of mercury and 100 ppb of cadmium, along with 0.5 to 6.0 ppb of copper. The results indicated that significant reduction in the ventilation performance occurred only in the median copper concentration of 2.0 ppb at constant mercury and cadmium load. Higher and lower concentrations of copper at constant concentration of mercury and cadmium, however, did not produce any serious effect on the rate of ventilation by the test animals. The effective concentration to reduce the ventilation performance by 50% was calculated to be 2.3 ppb of copper.

Enhancement of mercury concentration to 5.0 ppb along with 100 ppb of cadmium and 0.5 to 6.0 ppb of copper (Table 32 d and Fig. 12) produced a simple additive reaction with respect to the ventilation performance by Perna indica. The data showed that significant reduction in the ventilation rate occurred only when the medium contained 0.5 ppb of copper along with constant concentration of mercury and cadmium. Further increase in copper concentration, however, did not register any significant variation in the rate of ventilation. The animals in the lowest test concentration ventilated only 0.757 ml $h^{-1}mg^{-1}$(dry wt), which was around 68% of that of the control. The EC_{50} was found to be reduced to a low value of 0.05 ppb of copper.

Table 32 a,b,c&d. *Perna indica.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 32 a. *1.0 ppb Mercury + 25 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.741***	0.125	66.4		
1.0	0.893	0.234	80.0		
2.0	0.893	0.132	80.0		
4.0	0.894	0.210	80.1		
6.0	0.794*	0.142	71.1		
Control	1.116	0.159	100.0		

Table 32 b. *5.0 ppb Mercury + 25 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.662**	0.173	58.3		
1.0	0.879	0.111	78.8		
2.0	1.067	0.058	95.6	0.18	-2.3 (SA)
4.0	1.076	0.156	96.4		
6.0	0.955	0.187	85.6		
Control	1.116	0.159	100.0		

* $P < 0.05$ SA : Simple additive

** $P < 0.01$

*** $P < 0.001$

Table 32 c. 1.0 ppb Mercury + 100 ppb Cadmium + Copper

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	1.035	0.096	92.7		
1.0	0.936	0.203	83.9		
2.0	0.406***	0.013	36.4	2.3	-8.4 (LA)
4.0	0.999	0.162	89.5		
6.0	1.007	0.194	90.2		
Control	1.116	0.159	100.0		

Table 32 d. 5.0 ppb Mercury + 100 ppb Cadmium + Copper

	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.757*	0.144	67.8		
1.0	0.914	0.151	81.9		
2.0	0.979	0.256	87.7	0.05	-0.3 (SA)
4.0	0.982	0.145	87.9		
6.0	0.977	0.282	87.5		
Control	1.116	0.159	100.0		

* $P < 0.05$ LA : Less than additive

*** $P < 0.001$ SA : Simple additive

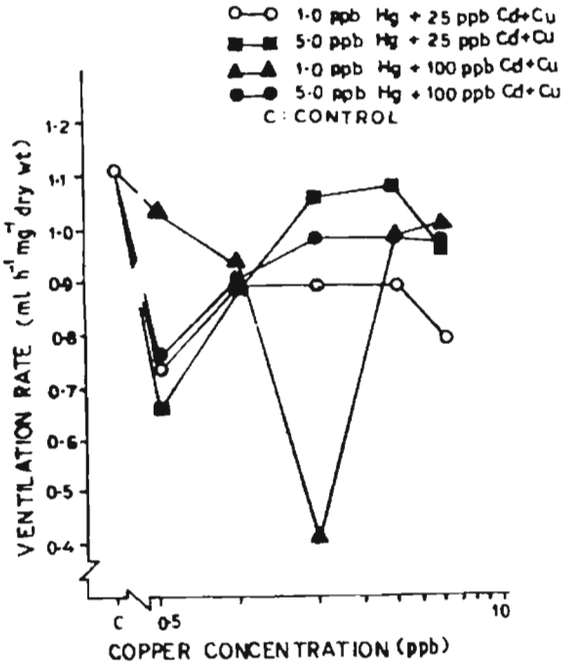


Figure 12. *Perna indica*. *Average ventilation, under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying) (for standard deviations see Tables 32 a, b, c&d).*

3.4.2 DONAX INCARNATUS

The rate of ventilation in marine bivalves is a meaningful index of activity. The amount of water that passes over the gills of lamellibranchs is of considerable interest in the study of the nutritional, respiratory, excretory and the overall performance of these animals. The rate of ventilation of Donax incarnatus, subjected to different metal stress were worked out and the results are presented in Tables 33 - 44 d and Figs 13 - 24.

3.4.2.1 Ventilation rate of Donax incarnatus pre-exposed for 24 h to individual metals.

The rate of ventilation of individuals of Donax incarnatus exposed to various concentrations of mercury, copper and cadmium, singly for a period of 24 h is outlined in Tables 33 to 35 and Figs. 13 to 15.

Table 33 and Fig. 13 give the rate of ventilation by Donax incarnatus after pre-exposure to 1.0 to 5.0 ppb of mercury. The ventilation rate showed a significant elevation when the mercury concentration was increased from 1.0 to 2.0 ppb. Thus, when the medium contained 2.0 ppb of mercury, the individuals ventilated 0.427 ml $h^{-1}mg^{-1}$(dry wt), which was 74% more than that of the control animals. Further increase in mercury concentration to 3.0, 4.0 and 5.0 ppb, however, produced a steady decline in the ventilation rate, but still remained well above the rate maintained by the control animals.

The average ventilation rate by Donax incarnatus exposed to different sub-lethal concentrations of copper is presented in Table 34 and Fig. 14. The animals exposed to 0.5 ppb of copper ventilated 0.405 ml $h^{-1}mg^{-1}$(dry wt) which was around 65% more than those of the control. Increase in concentration from 2.0 ppb to 6.0 ppb of copper had little effect on the ventilation performance of the animals which approached a rate nearly equal to that of the control.

The influence of different concentrations of cadmium on the rate of ventilation of Donax incarnatus is shown in Table 35 and Fig. 15. Even concentrations upto 20 ppb of cadmium was not found to drastically influence the rate of ventilation. However, an increase to 30 ppb of cadmium produced a 22% increase in the rate of ventilation. A further increase to 40 ppb produced a highly significant increase in the rate of ventilation and the animals exposed to 40 ppb of cadmium had a ventilation performance which was around 96% more than that of the control.

3.4.2.2 **Ventilation rate of Donax incarnatus pre-exposed for 24 h to metal mixtures**

Donax incarnatus when exposed to mercury along with copper, the combined toxic action was simple additive. This was true in the case of both the series of experiments where the mercury concentration was either 1.0 ppb or 3.0 ppb with copper concentrations ranging from 0.5 to 6.0 ppb. In the series where the mercury concentration was 1.0 ppb, the

Table 33. *Donax incarnatus*. *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury, along with the respective standard deviations, percentage performance and EC_{50} level.*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
1.0	0.197	0.035	80.1	
2.0	0.427**	0.068	173.6	
3.0	0.382	0.109	155.3	
4.0	0.341	0.148	138.6	
5.0	0.289	0.041	117.5	
Control	0.246	0.053	100.0	

Table 34. *Donax incarnatus*. *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper, along with the respective standard deviations, percentage performance and EC_{50} level.*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
0.5	0.405	0.184	164.6	
1.0	0.329	0.119	133.7	
2.0	0.246	0.073	100.0	
4.0	0.251	0.095	102.0	
6.0	0.253	0.149	102.8	
Control	0.246	0.053	100.0	

** $P < 0.01$

Table 35. *Donax incarnatus*. *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium, along with the respective standard deviations, percentage performance and EC_{50} level.*

Cadmium (ppb)	Ventilation rate (ml h^{-1} mg^{-1} dry wt)		Performance (% of control)	EC_{50} (ppb)
	Mean	SD		
5	0.247	0.093	100.4	
10	0.261	0.052	106.1	
20	0.271	0.073	110.2	
30	0.299	0.097	121.5	
40	0.481**	0.064	195.5	
Control	0.246	0.053	100.0	

** $P < 0.01$

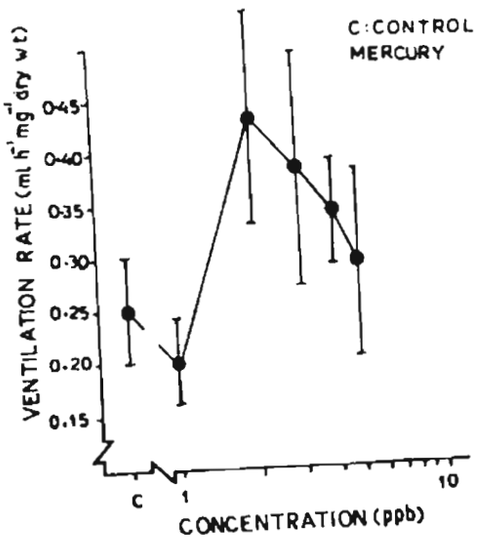


FIG. 13

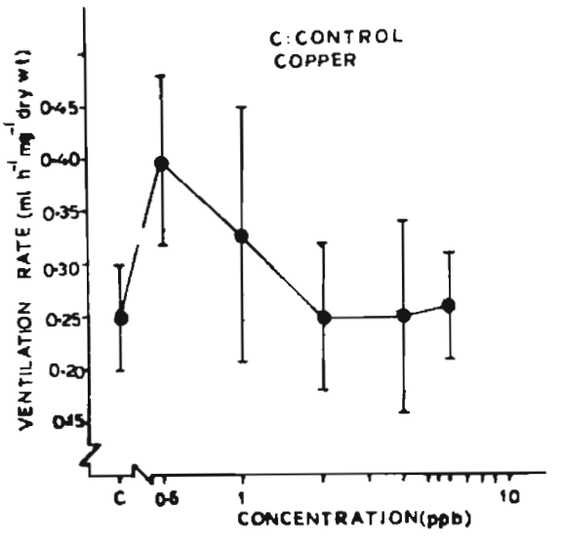


FIG. 14

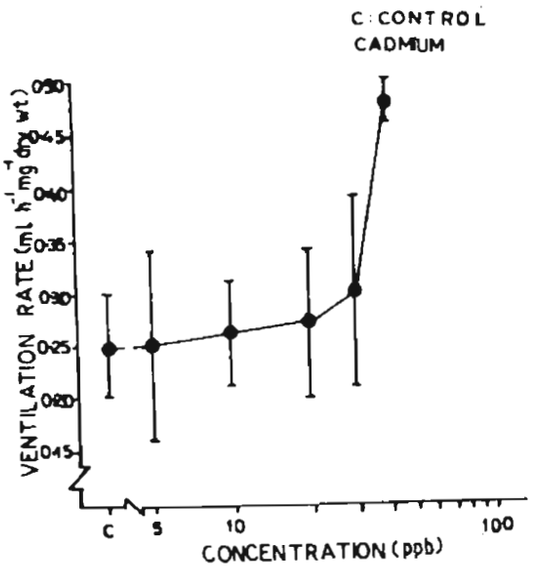


FIG. 15

Figures 13,14 & 15. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of mercury, copper and cadmium (vertical bars indicate standard deviation).*

ventilation rate showed clear cut variation only in two lower copper concentrations. When the medium contained 0.5 ppb of copper along with 1.0 ppb of mercury, the ventilation performance of the animals showed significant increase which was around 76% more than that of the control. However, an increase in copper concentration to 1.0 ppb with constant mercury load depicted a statistically significant reduction in the ventilation rate which was 30% of that of the control. Further increase in the copper concentration upto 6.0 ppb produced no added effect on the performance of the animals. The EC_{50} required to produce a reduction in the ventilation rate by 50% was 1.2 ppb of copper with a constant mercury load (Table 36 a and Fig. 16).

Table 36 b and Fig. 16 outline the data obtained with 3.0 ppb of mercury and varying concentrations of copper on the average rate of ventilation by Donax incarnatus. Comparable results were obtained even though the mercury concentration was increased to 3.0 ppb. Thus when the concentration of copper was 0.5 ppb, the animals ventilated 0.232 ml $h^{-1}mg^{-1}$ (dry wt). A doubling of copper concentration resulted in a sudden reduction in the ventilation rate which was around 53% of that of the control. A further increase in the copper concentration along with 3.0 ppb of mercury, produced a steady increase in the ventilation rate and when the medium contained 4.0 ppb of copper, the performance of the animals was almost equal to that of the control. The EC_{50} was 1.1 ppb of copper with a constant mercury load and the combined toxicity was of a simple additive nature.

Tables 36 a&b. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 36 a. *1.0 ppb Mercury + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.297*	0.074	176.8		
1.0	0.048*	0.032	28.6		
2.0	0.152	0.071	90.1	1.2	-1.09 (SA)
4.0	0.163	0.033	97.0		
6.0	0.181	0.051	107.8		
Control	0.168	0.059	100.0		

Table 36 b. *3.0 ppb Mercury + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.232	0.063	138.1		
1.0	0.089	0.055	52.9		
2.0	0.106	0.029	63.1	1.1	-1.58 (SA)
4.0	0.161	0.073	95.8		
6.0	0.197	0.082	117.3		
Control	0.168	0.059	100.0		

* $P < 0.05$ SA : Simple additive

The ventilation performance of Donax incarnatus exposed to 0.5 ppb of copper and 1.0 to 5.0 ppb of mercury is depicted in Table 37 a and Fig. 17. The presence of 1.0 ppb of mercury along with 0.5 ppb of copper resulted in significant elevation in the ventilation rate of Donax incarnatus. Beyond 1.0 ppb of mercury, the animals ventilated less quantity of water, but at a rate higher than those in the control. A concentration of 2.0 ppb of mercury along with constant concentration of copper in the medium resulted in a ventilation rate of 0.191ml h^{-1} mg^{-1}(dry wt), which was around 14% more than those in the control. Further increase in mercury concentration along with constant copper load resulted in higher ventilation rates.

Table 37 b and Fig. 17 present the results obtained on the rate of ventilation by Donax incarnatus exposed to 2.0 ppb of copper with 1.0 to 5.0 ppb of mercury. The data showed that the presence of even 1.0 ppb of mercury along with 2.0 ppb of copper resulted in a reduction in ventilation rate, which was around 90% of that of the control. Further increase in mercury concentration resulted in a reduction in the ventilation rate. A four fold increase in the mercury concentration along with constant copper load was found to evoke a significant reduction in ventilation rate which was 0.075 ml $h^{-1}mg^{-1}$(dry wt). Further increase in mercury concentration to 5.0 ppb resulted in an increase in the ventilation performance, a rate which almost equalled that of the control. The EC_{50} was calculated to be 4.6 ppb of mercury.

Tables 37 a&b. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and mercury (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 37 a. *0.5 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.297*	0.074	176.8		
2.0	0.191	0.054	113.7		
3.0	0.232	0.063	138.1		
4.0	0.242	0.061	144.0		
5.0	0.242	0.101	144.0		
Control	0.168	0.059	100.0		

Table 37 b. *2.0 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.152	0.071	90.1		
2.0	0.132	0.055	78.5		
3.0	0.106	0.029	63.1	4.6	-3.43
4.0	0.075*	0.029	44.6		
5.0	0.182	0.053	108.3		
Control	0.168	0.059	100.0		

* $\underline{P} < 0.05$

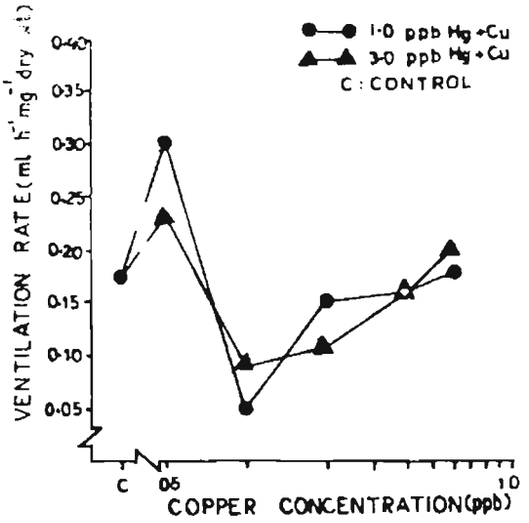


Figure 16. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of mercury (constant) and copper (varying) (for standard deviations see Tables 36 a&b).*

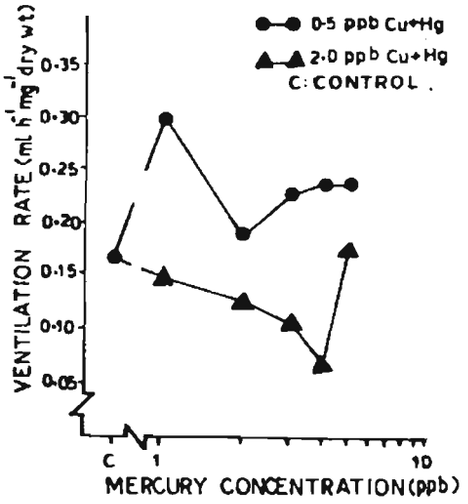


Figure 17. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of copper (constant) and mercury (varying) (for standard deviations see Tables 37 a&b).*

The effect of 1.0 or 3.0 ppb of mercury and 5.0 to 40 ppb of cadmium in the experimental medium on the ventilation performance of Donax incarnatus is explained in Tables 38 a and b and Fig. 18. The experimental animals showed clear cut stress, ventilating only 0.075 ml $h^{-1}mg^{-1}$(dry wt) when exposed to 5.0 ppb of cadmium along with 1.0 ppb of mercury. Exposure to higher concentrations of cadmium with constant mercury load, evoked little impact on the ventilation performance as evidenced by the amount of water ventilated in unit time.

Ventilating less quantity of water even in the lowest cadmium concentration of 5.0 ppb (Table 38 b and Fig. 18) along with 3.0 ppb of mercury, the animals tended to ventilate less and less as the cadmium concentration increased to 20 ppb. Curiously enough, a further increase in cadmium concentration to 30 and 40 ppb with constant mercury load produced significant increase in the ventilation rate of the exposed animals when compared to that of the control. Here, less than 50% reduction occurred when the medium contained around 20 ppb of cadmium. The EC_{50} worked out was 17.1 ppb. However, the combined action was of a simple additive nature.

Tables 39 a and b and Fig. 19 illustrate the average ventilation rate of Donax incarnatus exposed to 5.0 or 20 ppb of cadmium and 1.0 to 5.0 ppb of mercury. Depicting statistically significant reduction in the ventilation rate, the performance was found to increase with increasing mercury load with 5.0 ppb of cadmium. Thus, when the medium contained 5.0 ppb of mercury along with 5.0 ppb of cadmium, the animals

Tables 38 a&b. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and cadmium (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 38 a. *1.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.075*	0.028	44.6		
10	0.148	0.102	88.1		
20	0.185	0.102	110.1	3.0	1.36 (SA)
30	0.146	0.058	86.9		
40	0.143	0.068	85.1		
Control	0.168	0.059	100.0		

Table 38 b. *3.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.166	0.057	98.8		
10	0.105	0.056	62.5		
20	0.085*	0.012	50.6	17.1	-0.54 (SA)
30	0.334*	0.120	198.8		
40	0.381**	0.071	226.8		
Control	0.168	0.059	100.0		

* $P < 0.05$ SA : Simple additive

** $P < 0.01$

ventilated 0.209 ml $h^{-1}mg^{-1}$(dry wt), which was 25% more than the performance of the control animals. (Table 39 a and Fig. 19).

A four fold increase in the cadmium concentration in the test medium along with 1.0 to 5.0 ppb of mercury produced reduced rates of ventilation in two combinations. Thus, a three fold increase in the mercury concentration from 1.0 ppb produced a statistically significant reduction in the ventilation rate by about 51%. Further increase in mercury concentration with constant cadmium load produced increased rates of ventilation which, however, was lower than that of the control. A 50% reduction in the ventilation performance occurred around 3.0 ppb of mercury along with 20 ppb of cadmium and the EC_{50} was 3.1 ppb of mercury. The combined toxic action in this case was also of a simple additive nature (Table 39 b and Fig. 19).

Tables 40 a and b and Fig. 20 show the results obtained on the average rate of ventilation of Donax incarnatus subjected to exposure to 5.0 or 20 ppb of cadmium, along with 0.5 to 6.0 ppb of copper. Although ventilating less quantity of water in the presence of 0.5 ppb of copper along with 5.0 ppb of cadmium, the rate increased with increasing copper concentration upto 4.0 ppb along with a constant cadmium load. The test animals exposed to 0.5 ppb of copper ventilated only 0.244 ml $h^{-1}mg^{-1}$(dry wt) while those in the medium containing 4.0 ppb of copper ventilated 0.389 ml $h^{-1}mg^{-1}$(dry wt). However, an increase in the copper concentration to 6.0 ppb produced a sudden declension in the ventilation

Tables 39 a&b. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant) and mercury (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 39 a. *5.0 ppb Cadmium + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.075*	0.028	44.6		
2.0	0.166	0.076	98.8		
3.0	0.166	0.057	98.8		
4.0	0.168	0.031	100.0		
5.0	0.209	0.085	124.4		
Control	0.168	0.059	100.0		

Table 39 b. *20 ppb Cadmium + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.185	0.102	110.1		
2.0	0.175	0.054	104.2		
3.0	0.085*	0.012	50.6	3.1	-0.67 (LA)
4.0	0.130	0.028	77.4		
5.0	0.167	0.073	99.4		
Control	0.168	0.059	100.0		

* $P < 0.05$ LA : Less than additive

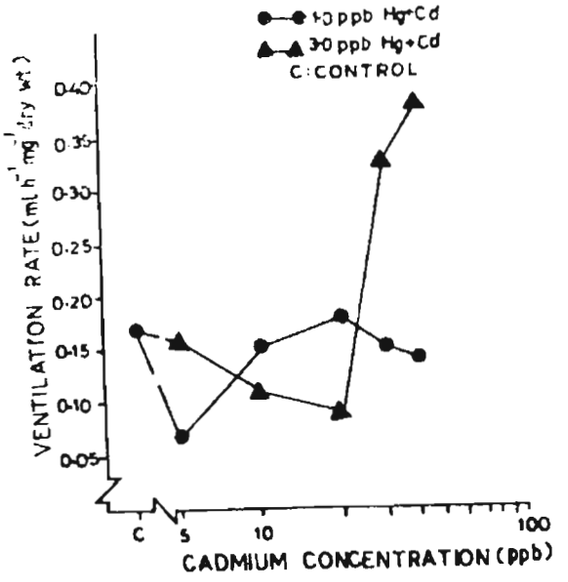


Figure 18. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of mercury (constant) and cadmium (varying) (for standard deviations see Tables 38 a&b).*

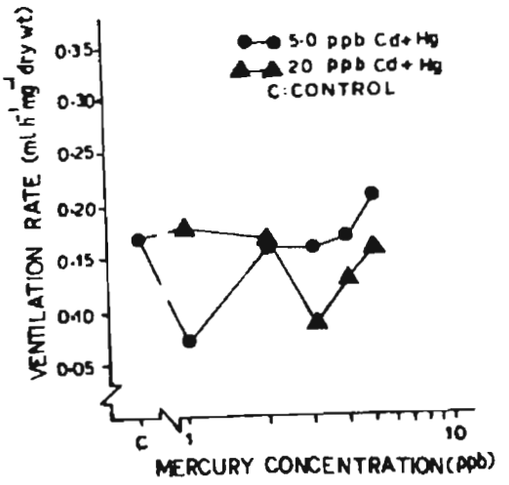


Figure 19. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of cadmium (constant) and mercury (varying) (for standard deviations see Tables 39 a&b).*

rate which was only 77% of the overall performance of the control animals (Table 40 a and Fig. 20).

A four fold increase in the concentration of cadmium (20 ppb) in the test medium along with 0.5 to 6.0 ppb of copper produced variable responses with respect to the rate of ventilation by Donax incarnatus. The rate of ventilation by animals exposed to 0.5 ppb of copper along with 20 ppb of cadmium was 0.198 ml $h^{-1}mg^{-1}$(dry wt). A doubling of the copper concentration produced an increase in the ventilation rate to 0.310 ml $h^{-1}mg^{-1}$(dry wt). A further doubling of the copper concentration tended to reduce the ventilation rate to 0.295 ml $h^{-1}mg^{-1}$ (dry wt). An increase in the copper concentration to 6.0 ppb with a constant cadmium load was found to produce a steady reduction in the ventilation rate.

The results obtained on the combined toxicity of 0.5 or 2.0 ppb of copper along with 5.0 to 40 ppb of cadmium on the ventilation rate of Donax incarnatus are presented in Tables 41 a and b and Fig. 21. Here also the ventilation performance showed a dual nature. Increase in the cadmium concentration from 5.0 to 10 ppb produced a reduction in the ventilation rate of the exposed animals. However, further increase in the cadmium concentration to 20, 30 and 40 ppb with constant copper concentration produced a steady increase in the rate of ventilation by Donax incarnatus. Thus, animals in the medium containing 0.5 ppb of copper along with 40 ppb of cadmium ventilated 0.419 ml $h^{-1}mg^{-1}$(dry wt), which was about 70% more than that by the control animals. This

Tables 40 a&b. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 40 a. *5.0 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.244	0.063	99.2		
1.0	0.319	0.069	129.7		
2.0	0.322	0.122	130.9		
4.0	0.389	0.123	158.1		
6.0	0.189	0.103	76.8		
Control	0.246	0.053	100.0		

Table 40 b. *20 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.198	0.069	80.4		
1.0	0.310	0.114	126.0		
2.0	0.295	0.099	119.9		
4.0	0.289	0.043	117.5		
6.0	0.191	0.053	77.6		
Control	0.246	0.053	100.0		

Tables 41 a&b. *Donax incarnatus*. *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and cadmium (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 41 a. *0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.244	0.063	99.2		
10	0.139*	0.040	56.5		
20	0.198	0.069	80.4	28.1	-0.68 (SA)
30	0.362	0.109	147.2		
40	0.419*	0.080	170.3		
Control	0.246	0.053	100.0		

Table 41 b. *2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.322	0.122	130.9		
10	0.294	0.032	119.5		
20	0.295	0.099	119.9		
30	0.368*	0.061	149.6		
40	0.428*	0.101	173.9		
Control	0.246	0.053	100.0		

* $P < 0.05$ SA : Simple additive

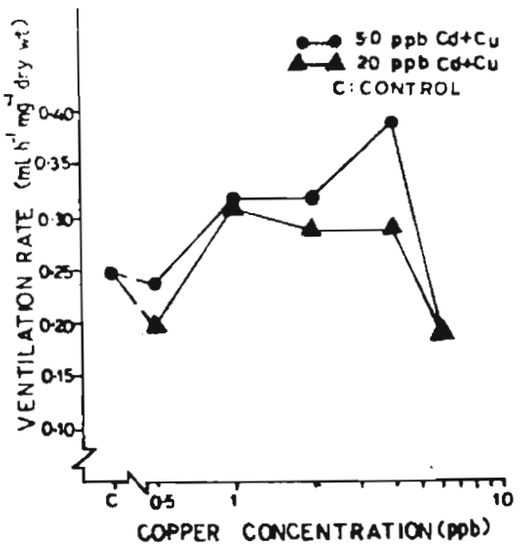


Figure 20. <u>*Donax incarnatus*</u>. *Average ventilation, under sub-lethal concentrations of copper (constant) and cadmium (varying) (for standard deviations see Tables 40 a&b).*

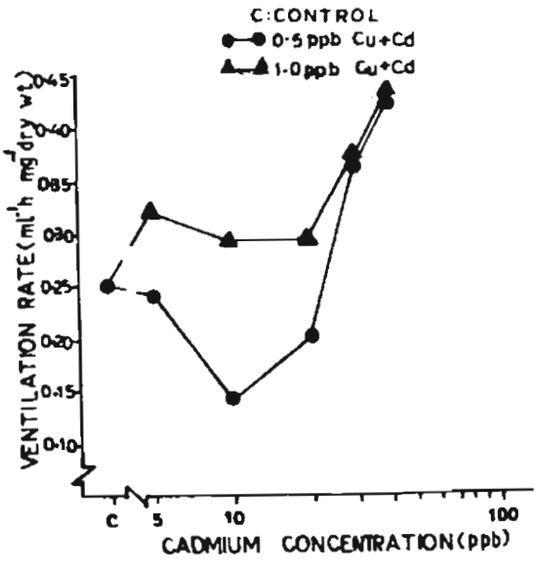


Figure 21. <u>*Donax incarnatus*</u>. *Average ventilation, under sub-lethal concentrations of cadmium (constant) and copper (varying) (for standard deviations see Tables 41 a&b).*

rate was significantly different. The EC_{50} was 28.1 ppb of cadmium and the additivity was found to be of a simple additive nature (Table 41 a and Fig. 21).

Table 41 b and Fig. 21 detail out the results obtained on the ventilation pattern of Donax incarnatus exposed to 2.0 ppb of copper along with 5.0 to 40 ppb of cadmium. The results obtained were of a comparable nature; the rate of ventilation showing a decrease with increase in cadmium concentration at constant copper concentration. The rate of ventilation by animals in the medium containing either 30 or 40 ppb of cadmium at constant concentration of 2.0 ppb of copper were found to be statistically significant at 0.05 level. The animals exposed to the highest concentration tested ventilated 0.428 ml $h^{-1}mg^{-1}$(dry wt) which was around 74% more than that of the control.

Tables 42 a, b, c and d and Fig. 22 illustrate the average ventilation rate by Donax incarnatus exposed to triad combinations of mercury, copper and cadmium. As mentioned earlier, here the concentration of two of the metals was maintained at a constant level and the third was made to vary. Table 42 a and Fig. 22 depict the ventilation performance by animals exposed to 1.0 ppb of mercury (constant), 0.5 ppb copper (constant) and 5.0 to 40 ppb of cadmium (varying). Depicting statistically significant increase in the ventilation rate when exposed to the two highest concentrations of cadmium along with constant mercury and copper load, none of the other combinations was found to produce real stress.

The trend in the rate of ventilation of Donax incarnatus subjected to an exposure to 2.0 ppb of copper along with 1.0 ppb of mercury and 5.0 to 40 ppb of cadmium was assessed and the results presented in Table 42 b and Fig. 22.

The animals' performance was decidedly more in all the concentrations tested and was statistically significant. It is clear from the results that the combination of the three metals at realistic concentrations resulted in increased activity of the animals (Table 42 b and Fig. 22).

Registering the impact of stress only at one instance (20 ppb of cadmium along with 3.0 ppb of mercury and 0.5 ppb of copper), a dual response with reference to ventilation performance by Donax incarnatus was indicated. Here also, the animals were decidedly more active in majority of the concentration ranges employed (Table 42c and Fig. 22).

The effect of a four fold increase in the copper concentration to 2.0 ppb along with 3.0 ppb of mercury and 5.0 to 40 ppb of cadmium on the ventilation performance is presented in Table 42 d and Fig. 22. As observed in the previous cases, here also the animals were hyperactive; the activity increasing with enhanced cadmium load.

Tables 43a,b,c and d and Fig. 23 show the average rate of ventilation by Donax incarnatus exposed to various triad combinations of mercury, cadmium and copper. Table 43 a and Fig. 23 illustrate the effect of 1.0 ppb of mercury, 5.0 ppb of cadmium and 0.5 to 6.0 ppb of copper

Tables 42 a,b,c&d. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 42 a. *1.0 ppb Mercury + 0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.323	0.104	186.7		
10	0.315	0.161	182.1		
20	0.186	0.041	107.5		
30	0.346**	0.067	200.0		
40	0.346*	0.077	200.0		
Control	0.173	0.073	100.0		

Table 42 b. *1.0 ppb Mercury + 2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.313	0.102	180.9		
10	0.359**	0.049	207.5		
20	0.499***	0.071	288.4		
30	0.486***	0.066	280.9		
40	0.381**	0.103	220.2		
Control	0.173	0.073	100.0		

* $P < 0.05$ *** $P < 0.001$

** $P < 0.01$

Table 42 c. 3.0 ppb Mercury + 0.5 ppb Copper + Cadmium

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.395**	0.104	228.3		
10	0.175	0.043	101.1		
20	0.149	0.057	86.1		
30	0.193	0.066	111.6		
40	0.379*	0.102	219.1		
Control	0.173	0.073	100.0		

Table 42 d. 3.0 ppb Mercury + 2.0 ppb Copper + Cadmium

Cadmium (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
5	0.192	0.074	110.9		
10	0.283	0.170	163.6		
20	0.283	0.143	163.6		
30	0.310	0.103	179.2		
40	0.419**	0.070	242.2		
Control	0.173	0.073	100.0		

* $P < 0.05$ ** $P < 0.01$

on the ventilation performance of the test animals. Registering no significant increase in the rate of ventilation, the animals ventilated more or less equal quantity of water when the copper concentration was increased from 0.5 through 4.0 ppb along with constant concentration of mercury and cadmium. However, increase in the copper concentration to 6.0 ppb produced a drastic reduction in the ventilation rate.

The effect of 1.0 ppb of mercury, 20 ppb of cadmium and 0.5 to 6.0 ppb of copper on the ventilation rate of Donax incarnatus is outlined in Table 43 b and Fig. 23. The rate of ventilation was higher in all the animals maintained in the different concentrations.

An increase in the mercury concentration to 3.0 ppb along with 5.0 ppb of cadmium and 0.5 to 6.0 ppb of copper produced quite contrary results with respect to the ventilation performance of Donax incarnatus. Registering statistically significant increased rate of ventilation only in the lowest copper concentration tested, the animals were found to ventilate more water than that by the control animals. (Table 43 c and Fig. 23).

Table 43 d and Fig. 23 illustrate the combined toxic effect of 3.0 ppb of mercury, 20 ppb of cadmium and 0.5 to 6.0 ppb of copper on the rate of ventilation by Donax incarnatus. An increase in the copper concentration from 0.5 to 1.0 ppb elicited an increase in the rate of ventilation by the test animals. However, a further increase in the copper concentration upto 6.0 ppb produced a drastic reduction in the average ventilation

Tables 43 a, b, c&d. *Donax incarnatus. Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 43 a. *1.0 ppb Mercury + 5.0 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.323	0.104	186.7		
1.0	0.323	0.136	186.7		
2.0	0.313	0.102	180.9		
4.0	0.310	0.140	179.1		
6.0	0.138	0.028	79.8		
Control	0.173	0.073	100.0		

Table 43 b. *1.0 ppb Mercury + 20 ppb Cadmium + Copper*

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.186	0.041	107.5		
1.0	0.227	0.068	131.2		
2.0	0.499***	0.071	288.4		
4.0	0.288	0.134	166.5		
6.0	0.217	0.080	125.4		
Control	0.173	0.073	100.0		

*** $P < 0.001$

Table 43 c. 3.0 ppb Mercury + 5.0 ppb Cadmium + Copper

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.395**	0.104	228.3		
1.0	0.307	0.119	228.3		
2.0	0.192	0.074	110.9		
4.0	0.198	0.062	114.5		
6.0	0.217	0.057	125.4		
Control	0.173	0.073	100.0		

Table 43 d. 3.0 ppb Mercury + 20 ppb Cadmium + Copper

Copper (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
0.5	0.149	0.057	86.1		
1.0	0.352	0.131	203.5		
2.0	0.283	0.143	163.6		
4.0	0.138	0.056	79.8		
6.0	0.138	0.049	79.8		
Control	0.173	0.073	100.0		

** $P < 0.01$

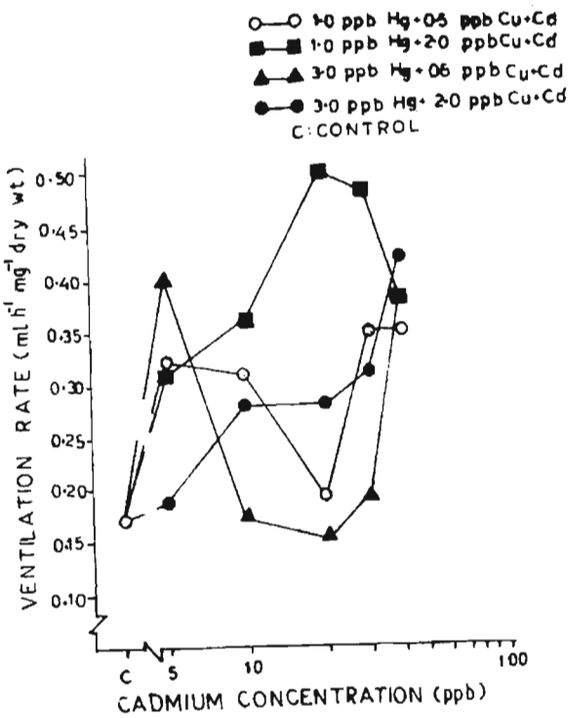


Figure 22. <u>*Donax incarnatus*</u>. *Average ventilation, under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying) (for standard deviations see Tables 42 a, b, c&d).*

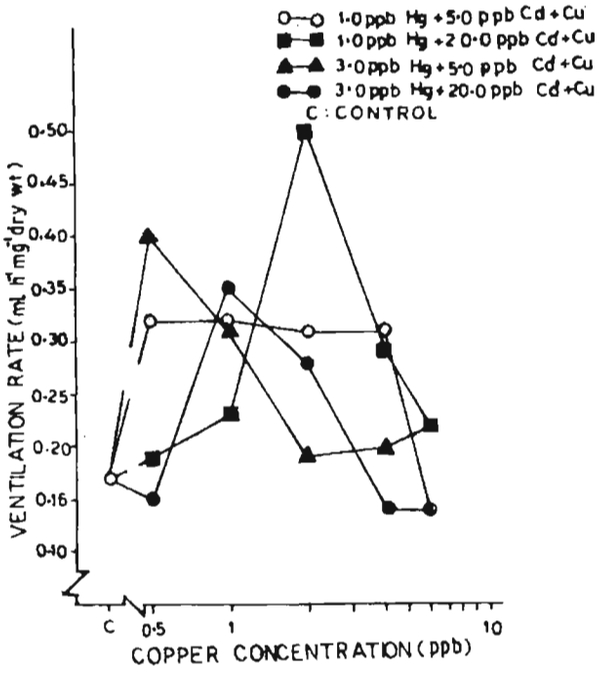


Figure 23. <u>*Donax incarnatus*</u>. *Average ventilation, under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying) (for standard deviations see Tables 43 a, b, c&d).*

performance. The animals exposed to the highest copper concentration (6.0 ppb) along with 3.0 ppb of mercury and 20 ppb of cadmium ventilated only 0.138 ml $h^{-1}mg^{-1}$(dry wt), which amounted to 80% of that of the control animals.

The effect of 5.0 or 20 ppb of cadmium, 0.5 or 2.0 ppb of copper and 1.0 to 5.0 ppb of mercury on the ventilation efficiency is explained in Tables 44a,b,c and d and Fig. 24. Ventilating more quantity of water [0.323 ml $h^{-1}mg^{-1}$ (dry wt)]even in the lowest mercury concentration of 1.0 ppb, the animals tended to ventilate more water as the mercury concentration in the medium increased to 2.0 ppb at constant concentration of cadmium (5.0 ppb) and copper (0.5 ppb). Although slight reduction in the rate was noticed when the media contained 4.0 or 5.0 ppb of mercury, the animals were hyperactive in all the concentrations (Table 44 a and Fig. 24).

Exposure of Donax incarnatus to 5.0 ppb of cadmium along with 2.0 ppb of copper and 1.0 to 5.0 ppb of mercury yielded the results presented in Table 44 b and Fig. 24. Here also, the animals exposed to 4.0 or 5.0 ppb of mercury with 5.0 ppb of cadmium and 2.0 ppb of copper were hyperactive.

Comparable results were obtained when Donax incarnatus was exposed to 20 ppb of cadmium, 0.5 ppb of copper and 1.0 to 5.0 ppb of mercury (Table 44 c and Fig. 24).

Tables 44 a, b, c&d. *Donax incarnatus.* *Average ventilation rate (ml h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying), along with the respective standard deviations, percentage performance, EC_{50} level and additive index.*

Table 44 a. *5.0 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.323	0.104	186.7		
2.0	0.431**	0.102	249.1		
3.0	0.395**	0.104	228.3		
4.0	0.284	0.093	164.1		
5.0	0.248	0.101	143.3		
Control	0.173	0.073	100.0		

Table 44 b. *5.0 ppb Cadmium + 2.0 ppb Copper + Mercury*

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.313	0.102	180.9		
2.0	0.138	0.055	79.8		
3.0	0.192	0.074	110.9		
4.0	0.206	0.081	119.1		
5.0	0.207	0.091	119.7		
Control	0.173	0.073	100.0		

** $P < 0.01$

Table 44 c. 20 ppb Cadmium + 0.5 ppb Copper + Mercury

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.192	0.074	110.9		
2.0	0.161	0.090	93.1		
3.0	0.149	0.057	86.1		
4.0	0.389**	0.088	224.9		
5.0	0.389**	0.062	224.9		
Control	0.173	0.073	100.0		

Table 44 d. 20 ppb Cadmium + 2.0 ppb Copper + Mercury

Mercury (ppb)	Ventilation rate (ml $h^{-1}mg^{-1}$ dry wt)		Performance (% of control)	EC_{50} (ppb)	Additive Index
	Mean	SD			
1.0	0.499***	0.071	288.4		
2.0	0.285	0.069	164.7		
3.0	0.283	0.143	163.6		
4.0	0.230	0.101	132.9		
5.0	0.133	0.094	76.9		
Control	0.173	0.073	100.0		

*** $P < 0.001$ ** $P < 0.01$

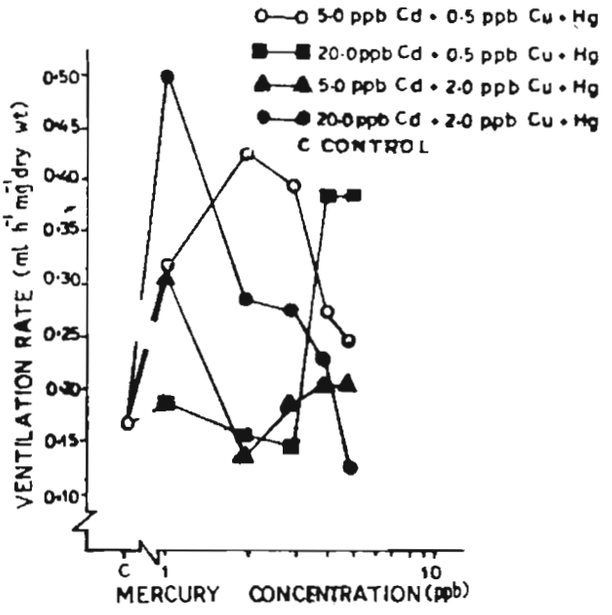


Figure 24. *Donax incarnatus*. *Average ventilation, under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying) (for standard deviations see Tables 44 a, b, c & d).*

Table 44 d and Fig. 24 present the results obtained on the rate of ventilation by Donax incarnatus exposed to 20 ppb of cadmium, 2.0 ppb of copper and 1.0 to 5.0 ppb of mercury. Except in the highest mercury concentration of 5.0 ppb, in all the other combinations employed, the animals ventilated more water. When the mercury load was enhanced to 5.0 ppb a reduction was recorded. It is quite clear that the animals were over active when the media contained less of mercury with 2.0 ppb of copper and 20 ppb of cadmium.

3.5 DISCUSSION

The reasons for the study of filtration/ventilation rate to assess pollutional impacts are many. The concept of assessing ventilation rate to explain stress basically arose out of the knowledge that metabolism and activity are interrelated. Variations in metabolic rate modify the scope for activity and the degree of activity affects metabolic rate. The rates of metabolism and activity may not necessarily attain comparable intensities synchronously. The sudden burst of activity will result in a metabolic debt and this is only slowly repaid. A temporary decline in activity may not be accompanied by comparable decline in metabolic rate. Lot of information is available on the effects of variable environmental parameters on the metabolism and activity of marine intertidal and subtidal bivalves. When a bivalve is exposed to a toxicant, reduction of contact with the medium can be brought about by the secretion of mucous or/and closure of shells. However, the period of contact reduction has to be limited since the animal must feed, exchange gases and defecate.

Closure of shells to enable reduction of contact is probably a very important behavioural response of a bivalve exposed to a toxicant. Notwithstanding these limitations, the ventilation rate has been accepted as a confirmed technique to assess pollutional effects in bivalves.

During the last fifty years, lot of data concerning filtration rates of filter feeding bivalves have been documented. The results were obtained using different experimental conditions and a great variety of suspended materials such as colloidal graphite, clay, chalk, starch, milk, blood, unicellular algae, bacteria and sea water with its natural seston. However, comparison of such data by Winter (1973) showed that most of the determinations represented true values and that particle concentration seems to be the most important factor affecting filtration rate. Mytilus edulis is known to modify the rate of filtration which can result in high rate of filtration at reduced particle number and low rate of filtration at increased particle number. Therefore, the concentration of neutral red used at 2 ppb would not necessarily be an impediment in making comparisons in the rate under varying stressed conditions.

It was demonstrated that feeding in mussels was impaired in the presence of pollutants (Abel, 1976). The presence of copper in the whole tissue was found to influence filtration rate and the rate of filtration was indirectly proportional to the body burden. Mohan et al. (1986a) opined that unlike many systems of potential value as indicators of sub-lethal stress, the filtration rate is an important one since this activity gives a direct idea on the quantity of water propelled through the gills. A

few papers which have dealt with filtration under stress are of Cole and Hepper (1954), Eknath and Menon (1979), Palmer (1980), Reddy and Menon (1980), Widdows et al. (1982), Mathew and Menon (1984), Stickle et al. (1984), Prabhudeva and Menon (1985), Mohan et al. (1986a),Grace and Gainey (1987) and Prabhudeva and Menon (1988, 1989). The rate of filtration can be affected by various factors. To understand this, it is essential to delineate the various processes involved. The water enters the pallial cavity of a bivalve through the inhalent siphon before passing through the gill ostia or into the supra branchial chamber. Such circulated water is expelled through the exhalent siphon, which is narrower than the inhalent siphon. Both the siphons posses the velum which can regulate the current flow. Usually, the bivalves reduce the effective area of lamellar contact with the water by means of mucous. Mytilus edulis does not filter in very dilute suspensions (Widdows et al., 1989). Davids (1964) found that the pumping rate of Mytilus edulis was reduced when the cell concentration increased beyond a particular level. Bayne et al. (1973) found a close agreement between scope for activity and filtration rate. Commenting on the filtering efficiency of Perna perna, Bayne (1985) opined that physiological flexibility accompanying food availability is reflected in the clearance rate, oxygen consumption and hence scope for growth. The flexibility in feeding response allows these animals to control the net energy balance in response to changes brought about in its physiology or metabolism.

A conspicuous feature of the results obtained was variability in the EC_{50} values. It may be pointed out here that differences in the EC_{50} were noticed in respect of ventilation rate in the case of Perna

indica, collected during two years from the same locality. In the case of mercury it was 7.6 ppb by animals collected during 1986 (Baby, 1987) whereas it was 10.1 when the animals were collected and tested in 1988. It may be assumed that these variations are within the range of possible flexibility. However, the variations noticed in the case of experiments where cadmium was used was around five times. No rational explanation can be given for this observation. It may be pointed out that Perna indica is a very sensitive marine mollusc and normally reacts sharply to elevated stress. The possibility of influence by body size, physiological status and experimental procedure have to be ruled out here, since the animals employed were of the same size and experimental design unchanged. In this connection it is interesting to note that the animals' capacity to get adapted to changing environmental conditions and stress factors are not properly understood. Normally adaptation is visualized as an ecological phenomenon comprising adjustments of organisms to alterations in the intensity pattern of variables in their environment and will ultimately result in a relative increase in their capacity to survive. Adaptation involves practically all levels of organic organisation. The capacity of the animals to tolerate environmentally induced changes can be non-genetic. However, it is possible that capacity adaptation may consist of genetic and non-genetic components. Wide variations in tolerance limit with reference to cadmium poses a very important question to pollution scientists as to the reliability of the information gathered on sub-lethal responses.

Another point brought about by the results on the effect of combined toxicity of cadmium and mercury on the ventilation rate of

Perna indica was that there were differences in the EC_{50} values when Perna indica was exposed to this combination. Further, the combined action was found to be more than additive earlier (Baby 1987) and simple additive in the present case. The reason for such a result was owing to reduction in the EC_{50} values in the case of animals used in 1986. Fluctuations from more than additivity to simple additivity is a serious change since this indirectly says that analysis of combined toxicity with additive indices could be very variable. Such evidences were also noticed in the studies conducted with mercury and cadmium. Here the EC_{50} value obtained in 1986 was 139 ppb, recording a more than additive index whereas the experiments in 1988 shows 518 ppb of cadmium, recording a simple additive response. Whereas increase in the mercury concentration resulted in simple additivity in both sets of experiments, the point to ponder is whether additivity is controlled by concentration. If this is so, the results indicate that depending on the concentration, the effect of the metals on the animal could be the result of either more than or simple additivity. In such a contention, the characteristics of the metal has no role to play which is quite unlikely. The present findings did not indicate any variation in the nature of additivity irrespective of concentrations. Variation was only in the reduction of concentration, when one of the components was supplied in higher concentration. If it is assumed that the combined action of two metals is influenced by the characteristics of the metals, it is quite unlikely for a fluctuation from more than additive to simple additivity. It can be reiterated with certainty that all the concentrations employed were far below the lethal concentrations and

duration of the experiment was less than 24 hours. Mohan et al. (1986a) studied the combined action of mercury and cadmium on the rate of filtration in Perna viridis and found that 14 ppb of mercury with 350 ppb of cadmium reduced the filtration rate by half.

Information on the effect of triad combination on the ventilation performance of Perna indica or any tropical mollusc is uniformly lacking. From the data it is clear that when the concentration of the constant component was less, usually the reaction was simple additive indicating independent action. On the other hand, an increase in the concentration of the constant component resulted in less than additivity. This indicates that at higher concentrations in triad combination, one of the three components exert antagonistic reaction with reference to ventilation. However, the reason for this is not known although it can be assumed that it is a function of increased concentration and not of metal reaction. Further, it is not clear whether this indicates an instance of ion antagonism as suggested by Mohan et al. (1986a). Copper in combination with higher dose of mercury and cadmium was found to exert drastic toxicity reaction on the animals. Curiously enough, even the presence of 0.5 ppb of copper was found to reduce ventilation rate by 50%.

Donax incarnatus is a species which has received least attention from a toxicological stand point although this is an abundant species distributed extensively along the sandy beaches of Kerala. Appearance and disappearance of this species in large numbers along the coast have been reported. During the present investigation also at some instances,

this species was found to be lacking in beaches which otherwise were highly populated by this species. Seasonal variations in the depth of occurance of Donax incarnatus have also been reported (Ansell et al., 1972). The reason for this change in distribution were assigned mainly to variations in hydrographic parameters. This species is known to be very sensitive to environmental alterations and therefore has not been used for stress analysis. In this respect, the information presented in this section of the thesis is absolutely new to toxicologists. Under sub-lethal concentrations of mercury, copper and cadmium, these animals became hyperactive. Increased activity under sub-lethal concentrations of heavy metals has been recorded in the case of Perna viridis exposed to silver (Mathew and Menon, 1984). Curiouly enough, the lowest concentration of copper nearly doubled the ventilation rate of Donax incarnatus whereas in the case of mercury this happened when the concentration of mercury was 2.0 ppb. On the contrary, in the case of cadmium the presence of 40 ppb nearly doubled the ventilation rate.

A combination of mercury - copper or mercury - cadmium or cadmium - mercury or cadmium and copper marginally modified the pattern observed in ventilation rate. The instances where the variations were conspicuous seem to have occurred in the median concentrations employed. For example, the presence of mercury and copper reduced ventilation rate when the copper concentration was either 1.0 or 2.0 ppb with 1.0 or 3.0 ppb of mercury. This indicates that increased ventilation rate, subsequent decrease and then increase, cannot necessarily be controlled by the concentration of heavy metal ions present. If it is to be assumed that the

presence of heavy metal ions result in declension or increase in the ventilation rate, the pattern should have been either an inversly proportional or directly proportional reaction. Notwithstanding the increase in ventilation rate at the lowest concentration in the mercury - copper mixture, the presence of more of mercury or copper in the combination also resulted in the ultimate increase in the ventilation rate. To explain the ventilation rate with reference to concentration and metal, no known theories of metal-animal reaction can be cited. Therefore, it may be assumed that the results obtained shows a totally hitherto unexplained reaction by bivalves to the presence of heavy metals. This may be an indication of positive and negative feed backs, both ultimately leading to breakdown. Further, it may be assumed that Donax incarnatus is subjected to wider environmental fluctuations which would have resulted in variable physiological behaviour. The animal which abound the tropical intertidal and sub-tidal belts is subjected to wide fluctuations in the common environmental parameters such as salinity, temperature and dissolved gases. Therefore, survival of the animal to a great extend depends on its capacity to oscillate in short durations. Compared to Perna indica, the behaviour pattern of Donax incarnatus was found to be more variable. The basic difference between the two bivalves which occupy the intertidal areas is that one is a sedentary form and the other a free living one. A free living animal has the capacity to migrate (in limited sense) so as to achieve the most congenial environmental conditions. In the case of an intertidal animal, restricted to this realm, can also be subjected to wider fluctuations beyond their capacity of migration, so as to achieve congeniality. Therefore

the behavioural pattern of Donax incarnatus must be an admixture of both its physical and physiological ability to compensate. However, the variations noticed in the ventilation performance between concentrations and between metal combinations should be the resultant effect of the above factors and the metal concentration. The presence of cadmium and copper in the test media was found to enhance the animals' capacity to ventilate more water , in majority of cases. Variations in this behaviour can possibly be explained based on assumptions. Increased ventilation rate shows that the animals did not experience any drastic reaction on its physiology that would result in reduction in shell closure ability or siphonal expansion. It is known that bivalves which have got such well developed siphons as in the case of species of Donax incarnatus, can regulate ventilation rate by manipulating the internal lumen of the siphon. Probably, the concentrations were not sufficient to result in either a conspicuous impairment of the ventilation mechanism or increase in the capacity of the animal to ventilate more water. This is evident from the results where the activity of the animal was found to oscillate between hyperactivity and less activity.

Less than additivity or simple additivity was the reaction of the bivalves when they were exposed to a triad combination. This evidently shows that the activity of the animal can depict drastic variations leading ultimately to death. It is reasonable to assume that sub-lethal reactions can also show patterns totally different from the normal trend expected of marine bivalves subjected to stress. Analysis of data available on

combined toxicity as well as publications show that reduced activity is an important reaction of stress. However, it has not been shown hitherto that increased activity also could lead to deleterious effects to marine bivalves when exposed to combinations of heavy metals. The fact that simple or less than additive reaction led to death and that sub-lethal concentrations of heavy metals led to increased rate of performance clearly demonstrates that hyperactivity can also lead to death. As a rule, the ventilation performance of Donax incarnatus was found to shoot up when exposed to concentrations of mercury, copper and cadmium. Reduced rates of performance were only exceptions. It may be recalled here that in the case of Perna indica, triple combination led to reduced activity and that hyperactivity was an exception. Among the combinations employed, constant concentrations of mercury and copper with varying concentrations of cadmium recorded the maximum rate of hyperactivity with reference to ventilation rate.

Trying to explain antagonism, addition and synergism with reference to response of oyster embryos to heavy metal mixtures, MacInnes (1981) suggested that this should reflect on the rate of growth of the embryo. However, the higher concentrations employed by this author depicted synergism. The three metal mixtures according to him showed considerable synergism. Although it is not possible to explain hyperactivity as an index of synergistic reactions, it has to be assumed that Donax incarnatus was trying to propel more water which would have in some fashion, reduced the toxicity of the mixture, probably even before their

entry. Donax incarnatus which is a burrowing bivalve can safely avoid contaminated waters by burrowing deeper into the sediment. It is evident that propulsion of water and the role played by the siphons, help in active burrowing. It is not clear whether the increased propulsion was a reaction of the animal to burrowing deeper and hence may be indicating a toxicity increased behaviour leading to avoidance.

The present evidence further supports the findings of Olla et al. (1983) in the case of Mercenaria mercenaria, wherein they have demonstrated that the behavioural response of the bivalve was affected by oil contaminated sediments. The authors suggested that such effects indicate avoidance behaviour. Mc Greer (1979) studied the burrowing behaviour of the estuarine clam Macoma balthica in response to sub-lethal levels of mercury and cadmium. The correlation between concentration and burrowing rate and speed was attributed to a behavioural avoidance mechanism. Observations made during the course of these experiments showed that when exposed to sub-lethal concentrations of the heavy metals, the animals had kept the siphons protracted and showed evidences of exploration. In the case of Donax trunculus, (Moueza and Frenkiel, 1974 ; Frenkiel 1980) it has been demonstrated that the cruciform muscle functions not as a chemoreceptor but as a vibration receptor. Tactile and chemical sensitivity are usually assigned to the mantle edges in the case of bivalves. Further, the response to heavy metals by the mantle is relatively less than that of the adductor muscle. The fact that when subjected to combined toxicity of heavy metals, Donax incarnatus was groping at the bottom of the experimental vessels, shows that even valve closure

was not effected. In the case of sedentary bivalves like Mytilus edulis, avoidance of lethal levels of copper and zinc solutions was effected by behavioural mechanisms (Black, 1983). However, the above information was based on higher concentrations. In the case of sub-lethal concentrations as evidenced by the experiments conducted on Donax incarnatus, it is quite likely that the animals can perform the avoidance reaction by burrowing deeper into the sediments whereas higher concentrations resulted in total withdrawal of the siphons and valve closure. The presence of higher concentrations of copper along with mercury and cadmium was found to result in reduction in the ventilation performance. Probably this result reflects a reaction totally different from that triggered in the presence of lower concentrations, possibly indicating that the animals can reduce the ventilation rate by reducing the siphonal lumen and closure of the valves. However, a correct understanding of the effects of combined toxicity of more than two metals in the case of this animal requires further investigations, especially with reference to the response of chemoreception areas of the mantle and the siphon. This is an area which promises interesting results.

4
OXYGEN : NITROGEN RATIO

4.1 INTRODUCTION

To understand and to delineate stress effects on bivalves exposed to realistic concentrations of anthropogenic substances especially heavy metals and petroleum hydrocarbons, measurements of various biological rate functions that could be quantified under controlled conditions are employed. Among these, oxygen consumption and nitrogen excretion are the most important physiological parameters usually employed. It is understood that any recognised alteration of the above physiological responses could be modified by behavioural mechanisms of the animal, especially valve closure in sedentary bivalves. The purpose of analysis of the basic physiological functions is to understand subtle variations that would otherwise be unnoticed under chronically polluted conditions. Maintenance of the sedentary animals under laboratory conditions normally would result in a declension in the maintenance metabolism, as the animals are in a totally different environment, subjected to limited physical activity. Notwithstanding these limitations, the O:N ratio is normally used to assess the animals' reaction to a stress.

The utility of O:N ratio to understand "the level of activity

of the oxidative and protein metabolism" (Mayzaud, 1973) is a recognised concept. Bayne (1973 a) described changes in the O:N ratio accompanying changes in season as an outcome of starvation and exposure to increased temperatures. He further assumed that O:N ratio is a sensitive index of nutritive stress in Mytilus. Further, the applicability of this method to temperature stress was also demonstrated by him (1973 a).

4.2 REVIEW OF LITERATURE

Rate of oxygen consumption has been used as a valuable tool by many workers to study the sub-lethal effects of pollutants (Kinne, 1970; Thompson and Bayne, 1972; Mackey and Shumway, 1980; Prabhudeva and Menon, 1986 a; Mohan et al., 1986 a and b). It offers a useful method to assess stress since it is an index of energy expenditure to meet the demands of environmental alterations. The rate of oxygen consumption by marine organisms is influenced by several factors including the type of feeding mechanism, respiratory efficiencies and experimental conditions. Particulate feeding marine animals remove as much as 13% oxygen from the sea water passing over the respiratory surfaces although many others remove around 53%. Compared to indices like growth rate, development and reproduction, a study of respiratory rate under toxicant stress is less sensitive although it is easy and quick.

In Argopecten irradians, Nelson et al. (1976) found toxicant dependent reduction in oxygen consumption. Scott and Major (1972) found that oxygen consumption was reduced in Mytilus edulis by about 12% on exposure

to 300 ppb copper. In Nassarius absoletus, Cheng and Rodrick (1974) reported that 1·0 ppm of copper decreased oxygen consumption by 67-75%. Brown and Newell (1972) concluded that the reduction in respiration in Mytilus edulis in the presence of 500 ppb copper was due to suspension of ciliary activity rather than direct inhibition of respiratory activity. However, the work of Davenport and Fletcher (1978) showed that 0.5 ppm copper does not reduce oxygen consumption. Prabhudeva and Menon (1986a) suggested that oxygen consumption is a product of two important factors, namely ventilation volume and the quantity of gas withdrawn from each litre of water. Therefore, changes in oxygen uptake from the waters by the animal and variations in the amount of water propelled through the gills result in fluctuations in oxygen consumption. Studies on the rate of oxygen consumption by Perna viridis exposed to heavy metals are plentiful (Murthy, 1982; Mathew and Menon, 1983; Prabhudeva and Menon, 1986 a; Mohan et al., 1986 a and b). Mathew and Menon (1982) observed that copper functions as a respiratory depressent in Meretrix casta and Modiolus modiolus. Shapiro (1964) explained that the respiratory depression in Mytilus galloprovincialis could either be due to valve closure or direct impairment of metabolic activity. Akberali and Black (1980) reported aerobic respiration in Scrobicularia plana when exposed to 500 ppb copper for short duration. Prabhudeva and Menon (1986 b) noticed that long duration of exposure to copper and zinc produced cellular damage of gills and cilia bearing cells. Among the three metals mercury, cadmium and zinc, cadmium was found to be the least toxic and mercury the most, bringing about a declension in oxygen consumption (Baby and Menon, 1986).

They further observed that reduced oxygen consumption obtained from whole body analysis indicated the animals' performance. Investigating into the combined toxic effects of heavy metals, Mohan et al. (1986b) found that a combination of cadmium and mercury depressed the oxygen uptake by animals. Studies by Salanki (1965, 1968) revealed that in Perna viridis the animals closed their shells tightly under unfavourable condition resulting in a reduction in oxygen uptake.

Rates of ammonia excretion tend to vary as a function of age (life cycle stage), temperature, salinity (Emerson, 1969), ambient ammonia levels and season. Seasonal variations have been reported in Meganyctiphanes norvegica (Mayzaud, 1973; Conover and Corner, 1968) and comparable fluctuations have also been found to occur in other aquatic invertebrates. Most molluscs excrete ammonia, but of the total nitrogen excreted the ammonia proportion may sometimes be small (Potts, 1967). The average NH_3 excretion rates in marine invertebrates have been described by several authors. A number of amphipods and isopods have been shown to excrete 80-90% of their nitrogenous end products as ammonia. Starved copepods were observed to catabolise more protein carbon than would be accounted for by the respiratory oxygen utilised. In rainbow trout Salmo gairdneri exposed to solutions of ammonium chloride, a direct correlation was observed between blood ammonia (both total ammonia and NH_3) and total ambient ammonia after 24 h (Fromm and Gillette, 1968). The daily excretion rates of ammonia showed an inverse relationship with the starting ambient ammonia levels. They suggested that since blood ammonia levels always

exceeded ambient ammonia levels, accumulation of ammonia in blood must have resulted from excretory inhibition rather than from ammonia uptake against the concentration gradient. The ratio by atomic equivalents of oxygen consumed to nitrogen excreted can provide an index of the balance in the animal's tissue between the rates of catabolism of proteins, carbohydrate and lipid substrates. This ratio can therefore provide a useful integration for understanding "the level of activity of the oxidative and protein metabolism" (Mayzaud, 1973) of the animal.

Among varieties of bivalve species, ammonia comprises about 60 to 90% of total measure of nitrogen excretion (Bayne et al., 1976; Bayne and Newell, 1983). Therefore, the rate of ammonia excretion may be regarded as reflecting the rate of protein catabolism (Widdows, 1978b). The O:N ratio is an index of protein utilization in energy metabolism and low value of O:N ratio is generally indicative of a stressed condition (Bayne et al., 1976; Bayne and Newell, 1983; Widdows,1985a,b and c). It was suggested that for Mytilus edulis O:N ratio values above 50 is representative of a healthy mussel and below 30 of a stressed condition (Widdows, 1985a). Krishnakumar (1987) reported a comparatively low O:N ratio of 23.09 for Perna viridis, which indicates a heavy dependence on protein for energy production. Hawkins et al. (1987) reported that at salinities greater than 20‰, Perna viridis was better able than Perna indica to regulate oxygen consumption independent of PO_2 and that normoxic oxygen consumption remained statistically unchanged in Perna viridis conditioned to salinities between 30 and 15‰ with no obvious signs of distress. Although equally unaffected at salinities between 32 and 20‰, Perna indica showed signifi-

cantly reduced oxygen uptake following transfer from 32 to 15‰. The proportional contribution of protein to total catabolic substrates under natural environmental conditions was as much as 96% in Perna viridis relative to only 19% in Perna indica. Studies have indicated that the O:N ratio may vary depending upon the trophic level, gametogenic cycle, the nature of food and nutrient reserves (Bayne ,1975b; Widdows, 1978b: Worral et al., 1983). Therefore, the interpretation of the O:N ratio should be based on relative change rather than on an absolute value (Shirely and Stickle, 1982; Widdows, 1985 a). The seasonal variation in O:N ratio is marked by a decline during and immediately after the spawning period (Widdows, 1985 a).

Bayne (1973 a) described the seasonal changes in the O:N ratio for Mytilus edulis. For greater part of the year, the O:N ratio for the animals remained relatively constant at about 100, signifying a considerable predominance of carbohydrate and/or lipid catabolism over the utilisation of protein in energy metabolism. However, following spawning the ratio was elevated to higher values indicative of a marked lipid utilisation at a time when glycogen was scarce and protein synthesis was active (Gabbot and Bayne, 1973; Thompson, Ratcliff and Bayne, 1974).

Bayne (1973 a) also recorded changes in O:N ratio following starvation and exposure to increased temperatures. Bayne (1975b) illustrated short term changes in O:N ratio following an increase in temperature. Changes in the O:N ratio due to starvation was further documented for Mytilus edulis (Bayne, 1973 b) and for Mytilus californianus (Bayne et

al., 1975).

Other studies on the O:N ratio in bivalve molluscs are rather scarce and heavy metal stress in particular. Ansell and Sivadas (1973) calculated O:N ratios for the clam Donax vittatus at increased temperatures and expressed the hope that the ratio may be useful in describing the physiological condition of the animal, a point made earlier by Bayne and Thompson (1970).

Hawkins et al. (1986) reported a very low O:N ratio in Perna viridis. Qasim et al. (1977) and Ajithkumar (1984) reported that in Perna viridis the lipid and carbohydrate reserves were low from August to December coinciding with spawning. Studies by Krishnakumar and Damodaran (1987) were carried out during this period and the low O:N ratio reported may be due to the spawning stress. However, further substantiation is required for the observed low O:N ratio obtained for small, immature (10-15 mm size) animals during the investigation. Bayne(1975 a)suggested that utilization of ammonia nitrogen in synthetic pathways or failure to oxidize the carbon skeleton of amino acids will result in deviation from the theoretical explanations of O:N ratio. Therefore, to obtain a base value which can be used as a stress index, it necessitates the collection of information of this index under different ecological and physiological conditions.

4.3 MATERIAL AND METHODS

This part of the investigation looked into the sub-lethal effects

of mercury, copper and cadmium singly and in combination on the oxygen consumption, ammonia - nitrogen excretion and the resultant O:N ratios of Perna indica and Donax incarnatus. The details of animals employed, water used, laboratory conditioning of animals and toxicants are described earlier (Section 2.3 and 3.3.1).

4.3.1 OXYGEN CONSUMPTION

4.3.1.1 **Animals pre-exposed to the toxicants for 24 h**

To find out the sub-lethal toxic effects of mercury, copper and cadmium, individually and in combination, the same concentrations employed to delineate ventilation rate were used to analyse the rate of oxygen consumption. Five test animals pre-exposed to the selected concentrations of metals for 24 h were kept in conical flasks of 1 l capacity containing 1000 ml millipore membrane filtered (0.45 m whatman filter) seawater. Gas exchange from the atmosphere was prevented by sealing the containers with inert liquid paraffin. The duration of the experiment was 4 h and the frequency of sampling 1 h. The water sample used for the determination of dissolved oxygen was siphoned out using a flexible polythene tube. The dissolved oxygen in the samples together with controls was estimated in duplicate following Winkler's method. After the experiments, soft tissues of the mussels were scooped out, cleaned in distilled water, dried at 70°-80° for 48 h and then weighed to constancy. The oxygen consumed is expressed as mg O_2 $h^{-1}mg^{-1}$ (dry wt).

4.3.2 AMMONIA EXCRETION

4.3.2.1 **Animals pre-exposed to the toxicants for 24 h**

Determination of the amount of ammonia excreted by the test

animals pre-exposed to the toxicants for 24 h was performed along side oxygen consumption. Water samples for ammonia determination were siphoned out along side for oxygen estimation. Samples together with controls were analysed for ammonia in duplicate using phenol hypochlorite method of Solorzano (1969).

For Donax incarnatus during the determination of oxygen consumption and ammonia excretion rate, a layer of acid washed sand was placed at the bottom of the test container. No appreciable variation in the result was observed by the presence of sand.

4.3.3 O:N RATIO

The rate of oxygen consumed to nitrogen excreted was calculated in atomic equivalents as follows:

1. Divided the rate of oxygen consumption in $mgO_2h^{-1}mg^{-1}$ by the atomic weight 16.

2. Dividedthe rate of nitrogen excretion in mg NH_4-N $h^{-1}mg^{-1}$ by the atomic weight 14.

Then O:N ratio $= \frac{mgO_2h^{-1}mg^{-1}}{16} : \frac{mg\ NH_4\text{-}N\ h^{-1}mg^{-1}}{14}$

4.3.4 STATISTICAL ANALYSIS

The student's 't' test was employed to assess whether there was any significant variation of the sub-lethal responses registered in respect of the test animals.

4.4 RESULTS

The O:N ratios of Perna indica and Donax incarnatus were worked out after exposing the animals to various concentrations of mercury, copper and cadmium, singly and in combination. The results are presented in Tables 45 to 68.

4.4.1 PERNA INDICA

4.4.1.1 Ratios under individual metal stress

To understand the relation between stress induced by concentrations of heavy metal salts on individuals, mercury, copper and cadmium were utilized employing Perna indica as the target organism.

4.4.1.1.1 Mercury

Five concentrations of mercury ranging between 1.0 and 10 ppb were utilized as stressors. The results obtained are presented in Table 45. It is evident from the table that significant alterations in oxygen consumption were reflected in the O:N ratios.

Ammonia excretion was found to increase significantly in those animals retained in 7.5 and 10 ppb of mercury. However, the presence of 10 ppb of mercury in the surrounding medium resulted in an O:N ratio less than that observed with the control animals. If the O:N ratio is to be taken as an index of stress, only those animals exposed to 5.0 and 7.5 ppb showed distinct evidence of stress, indicated by elevated O:N ratio. The percentage variation in the O:N ratio between the control

and the test organisms was found to vary in a fashion that those animals exposed to 5.0 and 7.5 ppb recorded the maximum increase.

4.4.1.1.2 Copper

Table 46 gives information on the oxygen consumption, ammonia-nitrogen excretion and the O:N ratio when the animals were exposed to copper. It is evident that 2.0 to 6.0 ppb of copper exerted more stress, resulting in increased oxygen consumption. On the other hand, the ammonia excretion was increased only by those animals exposed to 2.0 and 4.0 ppb of copper. Notwithstanding these discrepencies in oxygen and ammonia values, the O:N ratio was high in all the experiments ranging from 1.0 to 6.0 ppb. Differences in O:N ratio with reference to percentage was more or less uniform from 1.0 to 6.0 ppb copper containing media; the differences being always more than that of control.

4.4.1.1.3 Cadmium

This metal on the other hand showed stress of a different nature (Table 47). The O:N ratio was found to go down from that of the control animals in all but one case (50 ppb). Exposure to 400 ppb of cadmium brought down the O:N ratio to 5.9 with the concomittant reduction in oxygen uptake. However, the rate of ammonia excretion was similar to those of the control animals indicating that the reduced oxygen consumption had affected the O:N ratio. The reaction to maximum stress was found in those group of animals where the percentage performance was less than that of the control, but for those organisms exposed in 50 ppb

Table 45. *Perna indica.* *Rates of oxygen consumption ($mgO_2 h^{-1}$ mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.0x10^{-3}$	$1.6x10^{-4}$	$9.9x10^{-5}$	$1.7x10^{-5}$	$6.3x10^{-5}$	$7.1x10^{-6}$	8.8	86.3
2.5	$6.2x10^{-4}$*	$1.8x10^{-4}$	$5.8x10^{-5}$*	$1.5x10^{-5}$	$3.9x10^{-5}$	$4.0x10^{-6}$	9.4	92.2
5.0	$1.6x10^{-3}$**	$1.6x10^{-4}$	$8.5x10^{-5}$	$9.2x10^{-6}$	$1.0x10^{-4}$	$6.1x10^{-6}$	16.5	161.8
7.5	$2.3x10^{-3}$***	$2.5x10^{-4}$	$1.2x10^{-4}$*	$2.4x10^{-5}$	$1.4x10^{-4}$	$8.6x10^{-6}$	16.8	164.7
10.0	$1.1x10^{-3}$	$1.4x10^{-4}$	$1.1x10^{-4}$*	$5.6x10^{-6}$	$6.9x10^{-5}$	$7.9x10^{-6}$	8.8	86.3
Control	$1.0x10^{-3}$	$1.3x10^{-4}$	$8.6x10^{-5}$	$1.2x10^{-5}$	$6.3x10^{-5}$	$6.1x10^{-6}$	10.2	100.0

Table 46. *Perna indica.* *Rates of oxygen consumption ($mgO_2 h^{-1}$ mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper, along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	$9.3x10^{-4}$	$2.1x10^{-4}$	$8.6x10^{-5}$	$1.4x10^{-5}$	$5.8x10^{-5}$	$6.1x10^{-6}$	9.5	93.2
1.0	$1.3x10^{-3}$	$2.6x10^{-4}$	$7.2x10^{-5}$	$1.2x10^{-5}$	$8.1x10^{-5}$	$5.1x10^{-6}$	15.8	154.9
2.0	$2.1x10^{-3}$***	$1.7x10^{-4}$	$1.2x10^{-4}$*	$1.7x10^{-5}$	$1.3x10^{-4}$	$8.6x10^{-6}$	15.3	150.0
4.0	$1.9x10^{-3}$**	$3.4x10^{-4}$	$1.1x10^{-4}$*	$1.4x10^{-5}$	$1.2x10^{-4}$	$7.9x10^{-6}$	15.1	148.1
6.0	$1.3x10^{-3}$**	$8.2x10^{-5}$	$7.6x10^{-5}$	$1.0x10^{-5}$	$8.1x10^{-5}$	$5.4x10^{-6}$	15.0	147.1
Control	$1.0x10^{-3}$	$1.3x10^{-4}$	$8.6x10^{-5}$	$1.2x10^{-5}$	$6.3x10^{-5}$	$6.1x10^{-6}$	10.2	100.0

* $P < 0.05$ *** $P < 0.001$

** $P < 0.01$

Table 47. *Perna indica.* *Rates of oxygen consumption ($mgO_2 h^{-1} mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg NH_4$-N $h^{-1} mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium, along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	1.0×10^{-3}	2.4×10^{-4}	1.0×10^{-4}	2.1×10^{-5}	6.3×10^{-5}	7.1×10^{-6}	8.8	86.3
50	1.7×10^{-3}**	2.6×10^{-4}	1.2×10^{-4}*	1.9×10^{-5}	1.1×10^{-4}	8.6×10^{-6}	12.4	121.6
100	1.1×10^{-3}	1.7×10^{-4}	1.0×10^{-4}	1.4×10^{-5}	6.9×10^{-5}	7.1×10^{-6}	9.6	94.2
200	6.6×10^{-4}**	1.1×10^{-4}	8.1×10^{-5}	1.6×10^{-5}	4.1×10^{-5}	5.8×10^{-6}	7.1	69.6
400	6.2×10^{-4}**	9.6×10^{-5}	9.2×10^{-5}	1.5×10^{-5}	3.9×10^{-5}	6.6×10^{-6}	5.9	57.8
Control	1.0×10^{-3}	1.3×10^{-4}	8.6×10^{-5}	1.2×10^{-5}	6.3×10^{-5}	6.1×10^{-6}	10.2	100.0

* $P < 0.05$

** $P < 0.01$

of cadmium.

4.4.1.2 **Ratios under metal mixture stress**

A series of experiments were conducted to find out the combined effects of mercury and copper, copper and mercury, mercury and cadmium, cadmium and mercury, copper and cadmium and cadmium and copper on the oxygen consumption, ammonia - nitrogen excretion and the resulting O:N ratios. It can be seen from the table that the concentrations of the various metals employed were those which have already been used to assess single metal effects.

Tables 48 a and b give the effect of two concentrations of mercury; 1.0 and 10 ppb, along with 0.5 to 6.0 ppb of copper when present in the culture media. The presence of 1.0 ppb of mercury with varying concentrations of copper gave mixed results. The O:N ratios were either elevated or depressed. Curiously enough, the presence of 4.0 ppb of copper produced the minimum O:N; at the same time when applied with 6.0 ppb the O:N ratio was the maximum (See Table 48 a).

Table 48 b which represents the data obtained from experiments conducted with 5.0 ppb of mercury and varying concentrations of copper, however, showed a reduction in the O:N ratio; the maximum reduction being in that medium which contained 5.0 ppb of mercury and 4.0 ppb of copper. Ammonia- nitrogen excretion was found to be significantly different from that of the control animals in those medium which had 5.0 ppb of mercury with 0.5 or 4.0 ppb of copper. In these combinations,

Tables 48 a&b. *Perna indica.* *Rates of oxygen consumption ($mgO_2 h^{-1} mg^{-1}$ dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 48 a. *1.0 ppb Mercury + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N
	Mean	SD	Mean	SD	O	N	
0.5	$1.1x10^{-3}$	$1.9x10^{-4}$	$9.0x10^{-5}$	$2.1x10^{-5}$	$6.9x10^{-5}$	$6.4x10^{-6}$	10.6
1.0	$1.1x10^{-3}$	$2.1x10^{-4}$	$1.0x10^{-4}$	$1.7x10^{-5}$	$6.9x10^{-5}$	$7.1x10^{-6}$	9.7
2.0	$9.5x10^{-4}$	$1.5x10^{-4}$	$8.6x10^{-5}$	$7.0x10^{-6}$	$5.9x10^{-5}$	$6.1x10^{-6}$	9.6
4.0	$9.9x10^{-4}$	$2.4x10^{-4}$	$1.2x10^{-4}$	$2.6x10^{-5}$	$6.2x10^{-5}$	$8.6x10^{-6}$	7.2
6.0	$1.5x10^{-3}$	$1.7x10^{-4}$	$1.0x10^{-4}$	$1.3x10^{-5}$	$9.4x10^{-5}$	$7.1x10^{-6}$	13.1
Control	$1.3x10^{-3}$	$2.9x10^{-4}$	$1.0x10^{-4}$	$1.8x10^{-5}$	$8.1x10^{-5}$	$7.1x10^{-6}$	11.4

Table 48 b. *5.0 ppb Mercury + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N
	Mean	SD	Mean	SD	O	N	
0.5	$1.7x10^{-3}$	$2.4x10^{-4}$	$1.6x10^{-4}$**	$1.4x10^{-5}$	$1.1x10^{-4}$	$1.1x10^{-5}$	10.3
1.0	$1.3x10^{-3}$	$2.2x10^{-4}$	$1.1x10^{-4}$	$2.7x10^{-5}$	$8.1x10^{-5}$	$7.9x10^{-6}$	10.3
2.0	$1.6x10^{-3}$	$1.3x10^{-4}$	$1.2x10^{-4}$	$8.2x10^{-6}$	$1.0x10^{-4}$	$8.6x10^{-6}$	11.7
4.0	$1.8x10^{-3}$*	$1.7x10^{-4}$	$1.7x10^{-4}$**	$1.9x10^{-5}$	$1.1x10^{-4}$ *	$1.2x10^{-5}$	9.3
6.0	$1.7x10^{-3}$*	$1.2x10^{-4}$	$1.5x10^{-4}$*	$2.5x10^{-5}$	$1.1x10^{-4}$	$1.1x10^{-5}$	9.9
Control	$1.3x10^{-3}$	$2.9x10^{-4}$	$1.0x10^{-4}$	$1.8x10^{-5}$	$8.1x10^{-5}$	$7.1x10^{-6}$	11.4

only at one instance (5.0 ppb Hg + 2.0 ppb Cu) the percentage performance in the O:N ratio was more than that of the control.

The results obtained on the oxygen consumption, ammonia - nitrogen excretion and O:N ratios of Perna indica exposed to constant concentration of copper and varying concentrations of mercury are depicted in Tables 49 a and b. The presence of 0.5 ppb of copper with 10 ppb of mercury resulted in the maximum reduction in the O:N ratio. A similar trend was found in that medium containing 7.5 ppb of mercury. Apart from the medium which contained 1.0 ppb of mercury, the animals exposed under concentrations ranging from 2.5 to 10 ppb of mercury excreted ammonia - nitrogen which was significantly different from the rate of excretion of the control animals (Table 49 a).

On the other hand, significant increase in the ammonia - nitrogen excretion occurred only in those media which contained 7.5 and 10 ppb of mercury in the presence of 2.0 ppb of copper (See Table 49 b). The reaction of the animals which were exposed to 0.5 or 2.0 ppb of copper along with 7.5 or 10 ppb of mercury was directly comparable. In this series of experiments, the presence of 2.5 ppb of mercury with 0.5 ppb of copper resulted in increased O:N percentage. The higher concentrations namely 7.5 and 10 ppb with 0.5 or 2.0 ppb of copper resulted in reduced O:N percentage.

Tables 50a and b explains the consumption of oxygen, excretion of ammonia - nitrogen and the connected O:N ratios of Perna indica exposed to constant concentration of mercury and varying concentrations of cadmium.

Tables 49 a&b. *Perna indica*. *Rates of oxygen consumption ($mgO_2 h^{-1} mg^{-1}$ dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 49 a. 0.5 ppb Copper + Mercury

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percent differe
	Mean	SD	Mean	SD	O	N		
1.0	$1.1x10^{-3}$	$1.9x10^{-4}$	$9.0x10^{-5}$	$2.1x10^{-5}$	$6.9x10^{-5}$	$6.4x10^{-6}$	10.6	92.9
2.5	$5.8x10^{-4}$**	$2.1x10^{-4}$	$4.0x10^{-5}$**	$1.2x10^{-5}$	$3.6x10^{-5}$	$2.9x10^{-6}$	12.6	110.5
5.0	$1.7x10^{-3}$	$2.4x10^{-4}$	$1.6x10^{-4}$**	$1.4x10^{-5}$	$1.1x10^{-4}$	$1.1x10^{-5}$	10.3	90.4
7.5	$1.8x10^{-3}$*	$2.5x10^{-4}$	$1.9x10^{-4}$***	$1.3x10^{-5}$	$1.1x10^{-4}$	$1.3x10^{-5}$	8.3	72.8
10.0	$1.2x10^{-3}$	$2.7x10^{-4}$	$1.4x10^{-4}$*	$1.5x10^{-5}$	$7.5x10^{-5}$	$1.0x10^{-5}$	7.5	65.8
Control	$1.3x10^{-3}$	$2.9x10^{-4}$	$1.0x10^{-4}$	$1.8x10^{-5}$	$8.1x10^{-5}$	$7.1x10^{-6}$	11.4	100.0

Table 49 b. 2.0 ppb Copper + Mercury

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percent differe
	Mean	SD	Mean	SD	O	N		
1.0	$9.5x10^{-4}$	$1.5x10^{-4}$	$8.6x10^{-5}$	$7.0x10^{-6}$	$5.9x10^{-5}$	$6.1x10^{-6}$	9.6	84.2
2.5	$1.3x10^{-3}$	$1.7x10^{-4}$	$1.1x10^{-4}$	$1.9x10^{-5}$	$8.1x10^{-5}$	$7.9x10^{-6}$	10.3	90.4
5.0	$1.6x10^{-3}$	$1.3x10^{-4}$	$1.2x10^{-4}$	$8.2x10^{-6}$	$1.0x10^{-4}$	$8.6x10^{-6}$	11.7	102.6
7.5	$1.4x10^{-3}$	$3.6x10^{-4}$	$1.5x10^{-4}$**	$9.5x10^{-6}$	$8.8x10^{-5}$	$1.1x10^{-5}$	8.2	71.9
10.0	$1.2x10^{-3}$	$2.1x10^{-4}$	$1.4x10^{-4}$*	$1.6x10^{-5}$	$7.5x10^{-5}$	$1.0x10^{-5}$	7.5	65.8
Control	$1.3x10^{-3}$	$2.9x10^{-4}$	$1.0x10^{-4}$	$1.8x10^{-5}$	$8.1x10^{-5}$	$7.1x10^{-6}$	11.4	100.0

Relatively, the animals exposed to 1.0 ppb of mercury consumed less quantities of oxygen than the control with one exception, whereas in the case of ammonia excretion, variations between animals in the lower concentrations of cadmium with 1.0 ppb of mercury and the control were conspicuous. In majority of cases, the O:N ratio was more than that recorded for the control. Lower concentrations of cadmium with either 1.0 or 5.0 ppb of mercury caused elevation in the O:N percentage from that of the control. This trend was found to get reversed at higher concentrations of cadmium.

Animals exposed to 25 or 100 ppb of cadmium with varying concentrations of mercury gave interesting results. In the case of those animals exposed to 25 ppb of cadmium and 1.0 to 10 ppb of mercury, a clear cut increase in O:N ratio as a function of concentration was recorded. Animals uniformly used more oxygen and excreted lesser ammonia -nitrogen. On the other hand, in the case of Perna indica exposed to 100 ppb of cadmium, such an increase was not noticed although the O:N ratio was higher than that recorded for control animals. The lack of variations was owing to a rather uniform quantity of ammonia excretion with one exception (See Tables 51 a and b). Relatively low concentration of cadmium along with high concentrations of mercury produced higher percentage of O:N ratio from that of the control in the case of Perna indica. The variations were found to be more explicit when the cadmium concentration was 25 ppb.

In a series of experiments in which either copper or cadmium remained constant, the results obtained were totally different. The presence

Tables 50 a&b. *Perna indica.* Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of mercury (constant) and cadmium (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)

Table 50 a. 1.0 ppb Mercury + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	2.2×10^{-3}	2.9×10^{-4}	1.5×10^{-4}	2.2×10^{-5}	1.4×10^{-4}	1.1×10^{-5}	12.8	130.6
50	1.3×10^{-3}*	2.2×10^{-4}	1.2×10^{-4}**	1.6×10^{-5}	9.4×10^{-5}	8.6×10^{-6}	10.9	111.2
100	1.7×10^{-3}	2.2×10^{-4}	1.4×10^{-4}	1.9×10^{-5}	1.1×10^{-4}	1.0×10^{-5}	10.6	108.2
200	1.5×10^{-3}*	1.9×10^{-4}	1.9×10^{-4}	8.2×10^{-6}	9.4×10^{-5}	1.4×10^{-5}	6.9	70.4
400	1.7×10^{-3}	1.6×10^{-4}	1.7×10^{-4}	1.7×10^{-5}	1.1×10^{-4}	1.2×10^{-5}	8.8	89.8
Control	1.9×10^{-3}	2.5×10^{-4}	1.7×10^{-4}	1.7×10^{-5}	1.2×10^{-4}	1.2×10^{-5}	9.8	100.0

Table 50 b. 5.0 ppb Mercury + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	2.2×10^{-3}	1.8×10^{-4}	1.6×10^{-4}	2.4×10^{-5}	1.4×10^{-4}	1.1×10^{-5}	12.0	122.5
50	2.0×10^{-3}	1.8×10^{-4}	1.2×10^{-4}*	2.1×10^{-5}	1.3×10^{-4}	8.6×10^{-6}	14.6	148.9
100	2.3×10^{-3}	2.2×10^{-4}	1.8×10^{-4}	2.9×10^{-5}	1.4×10^{-4}	1.3×10^{-5}	11.2	114.3
200	3.7×10^{-3}***	4.5×10^{-4}	2.9×10^{-4}***	3.1×10^{-5}	2.3×10^{-4}	2.0×10^{-5}	11.1	113.3
400	1.7×10^{-3}	2.2×10^{-4}	1.6×10^{-4}	1.3×10^{-5}	1.1×10^{-4}	1.1×10^{-5}	9.3	94.9
Control	1.9×10^{-3}	2.5×10^{-4}	1.7×10^{-4}	1.7×10^{-5}	1.2×10^{-4}	1.2×10^{-5}	9.8	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Tables 51 a&b. *Perna indica.* *Rates of oxygen consumption ($mgO_2 h^{-1} mg^{-1}$ dry wt) and ammonia-nitrogen excretion ($mg NH_4\text{-}N\ h^{-1} mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 51 a. *25 ppb Cadmium + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$2.2x10^{-3}$	$2.9x10^{-4}$	$1.5x10^{-4}$	$2.2x10^{-5}$	$1.4x10^{-4}$	$1.1x10^{-5}$	12.8	130.6
2.5	$2.0x10^{-3}$	$2.2x10^{-4}$	$1.3x10^{-4}$*	$1.7x10^{-5}$	$1.3x10^{-4}$	$9.3x10^{-6}$	13.5	137.8
5.0	$2.2x10^{-3}$	$1.8x10^{-4}$	$1.6x10^{-4}$	$2.4x10^{-5}$	$1.4x10^{-4}$	$1.1x10^{-5}$	12.0	122.5
7.5	$1.9x10^{-3}$	$2.2x10^{-4}$	$1.3x10^{-4}$*	$2.2x10^{-5}$	$1.2x10^{-4}$	$9.2x10^{-6}$	12.8	130.6
10.0	$2.1x10^{-3}$	$2.9x10^{-4}$	$1.0x10^{-4}$**	$2.7x10^{-5}$	$1.3x10^{-4}$	$7.1x10^{-6}$	18.4	187.8
Control	$1.9x10^{-3}$	$2.5x10^{-4}$	$1.7x10^{-4}$	$1.7x10^{-5}$	$1.2x10^{-4}$	$1.2x10^{-5}$	9.8	100.0

Table 51 b. *100 ppb Cadmium + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.7x10^{-3}$	$2.2x10^{-4}$	$1.4x10^{-4}$	$1.8x10^{-5}$	$1.1x10^{-4}$	$1.0x10^{-5}$	10.6	108.2
2.5	$1.8x10^{-3}$	$3.1x10^{-4}$	$1.4x10^{-4}$	$2.6x10^{-5}$	$1.1x10^{-4}$	$1.0x10^{-5}$	11.2	114.3
5.0	$2.3x10^{-3}$	$2.2x10^{-4}$	$1.8x10^{-4}$	$2.9x10^{-5}$	$1.4x10^{-4}$	$1.3x10^{-5}$	11.2	114.3
7.5	$1.6x10^{-3}$	$1.7x10^{-4}$	$1.0x10^{-4}$**	$1.9x10^{-5}$	$1.0x10^{-4}$	$7.1x10^{-6}$	14.2	142.9
10.0	$1.9x10^{-3}$	$2.2x10^{-4}$	$1.4x10^{-4}$	$2.9x10^{-5}$	$1.2x10^{-4}$	$1.0x10^{-5}$	11.9	121.4
Control	$1.9x10^{-3}$	$2.5x10^{-4}$	$1.7x10^{-4}$	$1.7x10^{-5}$	$1.2x10^{-4}$	$1.2x10^{-5}$	9.8	100.0

of 0.5 ppb of copper with varying concentrations of cadmium, did not induce any drastic change in the O:N ratio. On the other hand, when the copper concentration was increased to 2.0 ppb, it was noticed that in animals which encountered this copper concentration along with 200 or 400 ppb of cadmium, the O:N ratio was reduced drastically; the most conspicuous difference being shown by those animals exposed to 400 ppb of cadmium (See Tables 52 a and b). The percentage variation in the O:N ratio was rather erratic especially when the copper concentration was raised to 2.0 ppb. It may be noted that with 0.5 ppb of copper, the difference was minimal in all the cadmium concentrations.

In a series of reciprocal experiments, the copper concentration was allowed to vary and the cadmium concentration retained constant. In the case of those animals which were exposed to 25 ppb of cadmium with 0.5 to 6.0 ppb of copper, the O:N ratio was always less than that recorded for the control animals, whereas an enhancement of cadmium concentration to 100 ppb resulted in marginal variation in O:N ratio in the case of those animals which encountered cadmium with 2.0 or 4.0 ppb of copper. Copper seems to exert limited influence only in the presence of lower concentrations of cadmium with reference to the percentage variation in the O:N ratio. This is evident from the results presented in Tables 53 a and b.

Knowledge on the combined effects of more than two heavy metals on the life and activity of bivalve molluscs is rather unique. It is assumed that the presence of more metals might result in increased stress. However, howfar this stress is metal concentration dependent

Tables 52 a&b. *Penna indica.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of copper (constant) and cadmium (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 52 a. *0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	$1.5x10^{-3}$	$2.5x10^{-4}$	$1.8x10^{-4}$	$1.8x10^{-5}$	$9.4x10^{-5}$	$1.3x10^{-5}$	7.3	83.9
50	$1.4x10^{-3}$	$1.3x10^{-4}$	$1.6x10^{-4}$	$1.7x10^{-5}$	$8.7x10^{-5}$	$1.1x10^{-5}$	7.7	88.5
100	$1.2x10^{-3}$	$2.2x10^{-4}$	$1.3x10^{-4}$	$2.2x10^{-5}$	$7.5x10^{-5}$	$9.3x10^{-6}$	8.1	93.1
200	$9.9x10^{-4}$	$1.3x10^{-4}$	$1.0x10^{-4}$	$2.2x10^{-5}$	$6.2x10^{-5}$	$7.1x10^{-6}$	8.7	100.0
400	$1.7x10^{-3}$	$2.1x10^{-4}$	$1.5x10^{-4}$	$1.5x10^{-5}$	$1.1x10^{-4}$	$1.1x10^{-5}$	9.9	113.8
Control	$1.5x10^{-3}$	$2.2x10^{-4}$	$1.5x10^{-4}$	$2.9x10^{-5}$	$9.4x10^{-5}$	$1.1x10^{-5}$	8.7	100.0

Table 52 b. *2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	$1.2x10^{-3}$	$1.8x10^{-4}$	$1.3x10^{-4}$	$2.7x10^{-5}$	$7.5x10^{-5}$	$9.3x10^{-6}$	8.1	93.1
50	$9.9x10^{-4}$*	$1.8x10^{-4}$	$1.0x10^{-4}$*	$1.7x10^{-5}$	$6.2x10^{-5}$	$7.1x10^{-6}$	8.7	100.0
100	$1.2x10^{-3}$	$1.5x10^{-4}$	$8.2x10^{-5}$**	$2.1x10^{-5}$	$7.5x10^{-5}$	$5.9x10^{-6}$	12.8	147.1
200	$1.2x10^{-3}$	$1.3x10^{-4}$	$1.5x10^{-4}$	$1.7x10^{-5}$	$7.5x10^{-5}$	$1.1x10^{-5}$	7.0	80.5
400	$1.3x10^{-3}$	$2.7x10^{-4}$	$2.5x10^{-4}$**	$2.9x10^{-5}$	$8.1x10^{-5}$	$1.8x10^{-5}$	4.6	52.9
Control	$1.5x10^{-3}$	$2.2x10^{-4}$	$1.5x10^{-4}$	$2.9x10^{-5}$	$9.4x10^{-5}$	$1.1x10^{-5}$	8.7	100.0

* $P < 0.05$ ** $P < 0.01$

Tables 53 a&b. *Perna indica*. *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 53 a. *25 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percent differe
	Mean	SD	Mean	SD	O	N		
0.5	$1.5x10^{-3}$	$2.5x10^{-4}$	$1.8x10^{-4}$	$1.8x10^{-5}$	$9.4x10^{-5}$	$1.3x10^{-5}$	7.3	82.9
1.0	$8.5x10^{-4}$**	$1.5x10^{-4}$	$1.0x10^{-4}$	$1.3x10^{-5}$	$5.3x10^{-5}$	$7.1x10^{-6}$	7.4	85.1
2.0	$1.2x10^{-3}$	$1.8x10^{-4}$	$1.3x10^{-4}$	$2.7x10^{-5}$	$7.5x10^{-5}$	$9.3x10^{-6}$	8.1	93.1
4.0	$7.7x10^{-4}$**	$1.4x10^{-4}$	$8.9x10^{-5}$*	$1.6x10^{-5}$	$4.8x10^{-5}$	$6.4x10^{-6}$	7.6	87.4
6.0	$1.2x10^{-3}$	$2.0x10^{-4}$	$1.5x10^{-4}$	$1.4x10^{-5}$	$7.5x10^{-5}$	$1.1x10^{-5}$	7.0	80.5
Control	$1.5x10^{-3}$	$2.2x10^{-4}$	$1.5x10^{-4}$	$2.9x10^{-5}$	$9.4x10^{-5}$	$1.1x10^{-5}$	8.7	100.0

Table 53 b. *100 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percent differe
	Mean	SD	Mean	SD	O	N		
0.5	$1.2x10^{-3}$	$2.2x10^{-4}$	$1.3x10^{-4}$	$2.2x10^{-5}$	$7.5x10^{-5}$	$9.3x10^{-6}$	8.1	93.1
1.0	$1.1x10^{-3}$*	$2.0x10^{-4}$	$1.1x10^{-4}$	$2.8x10^{-5}$	$6.9x10^{-5}$	$7.9x10^{-6}$	8.8	101.1
2.0	$1.2x10^{-3}$	$1.5x10^{-4}$	$8.2x10^{-5}$**	$2.1x10^{-5}$	$7.5x10^{-5}$	$5.9x10^{-6}$	12.8	147.1
4.0	$2.1x10^{-3}$**	$2.1x10^{-4}$	$1.9x10^{-4}$	$2.8x10^{-5}$	$1.3x10^{-4}$	$1.4x10^{-5}$	9.7	111.5
6.0	$1.3x10^{-3}$	$1.7x10^{-4}$	$1.6x10^{-4}$	$1.9x10^{-5}$	$8.1x10^{-5}$	$1.1x10^{-5}$	7.1	81.6
Control	$1.5x10^{-3}$	$2.2x10^{-4}$	$1.5x10^{-4}$	$2.9x10^{-5}$	$9.4x10^{-5}$	$1.1x10^{-5}$	8.7	100.0

* $P < 0.05$ ** $P < 0.01$

is not properly known or understood. The presence of toxic metals in concert could even lead to changes in the animals' capacity adaptation process. Since, majority of the metals present in the sea water are essential to animals and are normally present within the tolerable limits, the animals must have developed methods of segregation and absorption of the metals. Therefore, it was felt that experiments utilizing three metals, of which one is essential and another two non-essential could throw some light on the interaction. A series of experiments utilizing mercury, copper and cadmium in combination with one of the metal components maintained as a variant were conducted. The results obtained from the experiments are presented in Tables 54 a to 56 d.

Tables 54 a,b,c and d present results obtained from experiments conducted on Perna indica employing various combinations of mercury, copper and cadmium. Table 54 a gives information on the combined effect of 1.0 ppb of mercury, 0.5 ppb of copper and 25 to 400 ppb of cadmium. The variations noticed in the oxygen consumption and ammonia- nitrogen excretion were significantly different from that of the control animals. The rate of oxygen consumption was found to increase in all the cases. Similarly, the excretion of ammonia- nitrogen was also found to increase. This resulted in the reduction in the O:N ratio compared to that of the control animals.

Table 54 b gives information on the oxygen consumption, excretion of ammonia-nitrogen and the O:N ratio when the copper concentration was raised to 2.0 ppb, the other metals and their concentrations remaining

the same as that shown in Table 54a. Curiously enough, both oxygen consumption and ammoniacal nitrogen excretion in the media which contained less of cadmium were significantly different from that of the control. On the other hand, only oxygen consumption registered significant difference in higher concentrations of cadmium, resulting in high O:N ratio in those concentrations. When the O:N ratio of control animals is taken as the base line, the major deviations occurred in those animals exposed to 100 and 400 ppb of cadmium with 1.0 ppb mercury and 2.0 ppb of copper.

Tables 54 c and d indicate the results when the animals were exposed to increased concentrations of mercury also. Here the O:N ratio was drastically different in those animals exposed to lower concentrations of cadmium from that of the control. Both ammonia- nitrogen excretion and oxygen consumption recorded significant deviations from the control animals. This trend, however, was not marked when the copper concentration was increased to 2.0 ppb. The O:N ratios were inconclusive. The combined effects of the three metals on the O:N ratio percentage seems to be influenced mainly by mercury variations. Increase in the mercury concentration resulted in elevation in the O:N percentage.

Tables 55a,b,c and d give evidences of the three above mentioned factors when cadmium and copper concentrations were retained constant and mercury subjected to variation. As a rule, oxygen consumption was significantly different in all the animals compared to that of the control. The variations in the O:N ratio was found to be mainly controlled by the fluctuations in the ammonia - nitrogen excretion. The presence of 0.5 or 2.0 ppb of copper with 25 ppb of cadmium did not record any

Tables 54 a,b,c&d. *Perna indica.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying) along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N).*

Table 54 a. *1.0 ppb Mercury + 0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	1.0×10^{-3}***	8.5×10^{-5}	1.1×10^{-4}**	1.9×10^{-5}	6.3×10^{-5}	7.9×10^{-6}	7.9	94.0
50	1.3×10^{-3}***	1.2×10^{-4}	1.3×10^{-4}***	1.5×10^{-5}	8.1×10^{-5}	9.3×10^{-6}	8.8	104.8
100	8.8×10^{-4}***	9.9×10^{-5}	1.0×10^{-4}**	9.6×10^{-6}	5.5×10^{-5}	7.1×10^{-6}	7.7	91.7
200	1.2×10^{-3}***	9.6×10^{-5}	1.4×10^{-4}***	9.6×10^{-6}	7.5×10^{-5}	1.0×10^{-5}	7.5	89.3
400	6.6×10^{-4}	7.0×10^{-5}	8.5×10^{-5}**	6.1×10^{-6}	4.1×10^{-5}	6.1×10^{-6}	6.8	80.9
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 54 b. *1.0 ppb Mercury + 2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
25	1.3×10^{-3}***	1.0×10^{-4}	1.2×10^{-4}***	1.2×10^{-5}	8.1×10^{-5}	8.6×10^{-6}	9.5	113.1
50	9.2×10^{-4}***	8.4×10^{-5}	1.1×10^{-4}***	1.2×10^{-5}	5.8×10^{-5}	7.9×10^{-6}	7.3	86.9
100	6.8×10^{-4}*	5.7×10^{-5}	1.0×10^{-4}**	1.3×10^{-5}	4.3×10^{-5}	7.1×10^{-6}	5.9	70.2
200	1.1×10^{-3}***	1.2×10^{-4}	9.4×10^{-5}*	1.7×10^{-5}	6.9×10^{-5}	6.7×10^{-6}	10.2	121.4
400	1.8×10^{-3}***	5.8×10^{-5}	8.9×10^{-5}*	1.6×10^{-5}	1.1×10^{-4}	6.4×10^{-6}	17.7	210.7
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 54 c. 5.0 ppb Mercury + 0.5 ppb Copper + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percenta differen
	Mean	SD	Mean	SD	O	N		
25	$1.6x10^{-3}$***	$1.0x10^{-4}$	$1.0x10^{-4}$**	$1.1x10^{-5}$	$1.0x10^{-4}$	$7.1x10^{-6}$	14.0	166.7
50	$1.9x10^{-3}$***	$1.0x10^{-4}$	$9.4x10^{-5}$**	$1.1x10^{-5}$	$1.2x10^{-4}$	$6.7x10^{-6}$	17.7	210.7
100	$1.2x10^{-3}$***	$8.2x10^{-5}$	$1.0x10^{-4}$**	$1.6x10^{-5}$	$7.5x10^{-5}$	$7.1x10^{-6}$	10.5	125.0
200	$1.2x10^{-3}$***	$9.6x10^{-5}$	$1.1x10^{-4}$***	$1.1x10^{-5}$	$7.5x10^{-5}$	$7.9x10^{-5}$	9.5	113.1
400	$1.1x10^{-3}$***	$8.2x10^{-5}$	$9.7x10^{-5}$**	$1.1x10^{-5}$	$6.9x10^{-5}$	$6.9x10^{-6}$	9.9	117.9
Control	$5.5x10^{-4}$	$6.6x10^{-5}$	$5.7x10^{-5}$	$1.3x10^{-5}$	$3.4x10^{-5}$	$4.1x10^{-6}$	8.4	100.0

Table 54 d. 5.0 ppb Mercury + 2.0 ppb Copper + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percen differ
	Mean	SD	Mean	SD	O	N		
25	$8.1x10^{-4}$**	$9.8x10^{-5}$	$6.0x10^{-5}$	$1.0x10^{-5}$	$5.1x10^{-5}$	$4.3x10^{-6}$	11.8	140.5
50	$8.8x10^{-4}$***	$8.6x10^{-5}$	$6.7x10^{-5}$	$1.3x10^{-5}$	$5.5x10^{-5}$	$4.8x10^{-6}$	11.5	136.9
100	$8.5x10^{-4}$**	$8.1x10^{-5}$	$9.9x10^{-5}$**	$1.5x10^{-5}$	$5.3x10^{-5}$	$7.1x10^{-6}$	7.5	89.3
200	$6.9x10^{-4}$*	$8.2x10^{-5}$	$7.7x10^{-5}$	$1.4x10^{-5}$	$4.3x10^{-5}$	$5.5x10^{-6}$	7.8	92.5
400	$1.1x10^{-3}$***	$1.0x10^{-4}$	$7.1x10^{-5}$	$7.6x10^{-6}$	$6.9x10^{-5}$	$5.1x10^{-6}$	13.6	161.1
Control	$5.5x10^{-4}$	$6.6x10^{-5}$	$5.7x10^{-5}$	$1.3x10^{-5}$	$3.4x10^{-5}$	$4.1x10^{-6}$	8.4	100.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

drastic variation in the O:N ratio. On the other hand, high concentrations of mercury brought about conspicuous variation in the O:N ratio when the copper concentration was either 0.5 or 2.0 ppb along with 100 ppb of cadmium (See Tables 55a,b,c and d). In the presence of constant concentration of copper and cadmium, the presence of varying concentrations of mercury resulted in increased O:N ratio percentage either at the median level of mercury or at the highest level. Varying levels of mercury with 0.5 ppb of copper and 25 ppb of cadmium produced drastic variation in the O:N ratio when the mercury concentration was only 5.0 ppb.

In another series of experiments, the concentration of copper was allowed to vary with two concentrations of either mercury or cadmium. In those experiments where the mercury concentration was 5.0 ppb and that of copper ranging between 0.5 to 2.0 ppb, the O:N ratio was drastically different. Here also, variations noticed in the oxygen consumption were highly significant in all the experiments (See Tables 56 a to d).

It seems that lower concentrations of copper in these combinations exerted a greater stress on the animals. Variation in the copper concentration with constant concentrations of mercury and cadmium produced changes in the percentage O:N ratio either at the low or median concentration of copper: the shift being controlled by the changes in mercury or cadmium.

4.4.2 DONAX INCARNATUS

Donax incarnatus is a common occupant of the lower intertidal and sub-tidal region along the coasts of Kerala. This species is abundantly

Tables 55 a, b, c & d. *Perna indica.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 55 a. *25 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	1.0×10^{-3}***	8.5×10^{-5}	1.1×10^{-4}**	1.9×10^{-5}	6.3×10^{-5}	7.9×10^{-6}	7.9	94.0
2.5	6.7×10^{-4}	7.3×10^{-5}	6.0×10^{-5}	7.1×10^{-6}	4.2×10^{-5}	4.3×10^{-6}	9.8	116.7
5.0	1.6×10^{-3}***	1.0×10^{-4}	1.0×10^{-4}**	1.1×10^{-5}	1.0×10^{-4}	7.1×10^{-6}	14.0	166.7
7.5	9.4×10^{-4}***	5.9×10^{-5}	1.0×10^{-4}**	1.3×10^{-5}	5.9×10^{-5}	7.1×10^{-6}	8.2	97.6
10.0	1.1×10^{-3}***	8.1×10^{-5}	1.2×10^{-4}***	1.3×10^{-5}	6.9×10^{-5}	8.6×10^{-6}	8.0	95.2
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 55 b. *25 ppb Cadmium + 2.0 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	1.3×10^{-3}***	1.0×10^{-4}	1.2×10^{-4}***	1.2×10^{-5}	8.1×10^{-5}	8.6×10^{-6}	9.5	113.1
2.5	8.4×10^{-4}**	9.0×10^{-5}	7.6×10^{-5}	1.6×10^{-5}	5.3×10^{-5}	5.4×10^{-6}	9.7	115.5
5.0	8.1×10^{-4}**	9.8×10^{-5}	6.0×10^{-5}	1.0×10^{-5}	5.1×10^{-5}	4.3×10^{-6}	11.8	140.5
7.5	9.2×10^{-4}***	3.3×10^{-5}	8.1×10^{-5}	1.7×10^{-5}	5.8×10^{-5}	5.8×10^{-6}	9.9	117.9
10.0	8.9×10^{-4}***	4.9×10^{-5}	8.4×10^{-5}*	8.1×10^{-6}	5.6×10^{-5}	6.0×10^{-6}	9.3	110.7
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

* $P < 0.05$ *** $P < 0.001$

** $P < 0.01$

Table 55 c. *100 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	8.8×10^{-4}***	9.9×10^{-5}	1.0×10^{-4}**	9.6×10^{-6}	5.5×10^{-5}	7.1×10^{-6}	7.7	91.7
2.5	1.2×10^{-3}***	1.0×10^{-4}	1.1×10^{-4}*	3.8×10^{-5}	7.5×10^{-5}	7.9×10^{-6}	9.5	113.1
5.0	1.2×10^{-3}***	8.2×10^{-5}	1.0×10^{-4}**	1.6×10^{-5}	7.5×10^{-5}	7.1×10^{-6}	10.5	125.0
7.5	8.0×10^{-4}**	5.7×10^{-5}	3.9×10^{-5}*	4.3×10^{-6}	5.0×10^{-5}	2.8×10^{-6}	17.9	213.1
10.0	1.1×10^{-3}***	8.6×10^{-5}	7.6×10^{-5}	1.1×10^{-5}	6.9×10^{-5}	5.4×10^{-6}	12.7	151.2
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 55 d. *100 ppb Cadmium + 2.0 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	6.8×10^{-4}*	5.7×10^{-5}	1.0×10^{-4}**	1.3×10^{-5}	4.3×10^{-5}	7.1×10^{-6}	5.9	70.2
2.5	6.8×10^{-4}*	7.1×10^{-5}	9.5×10^{-5}**	1.4×10^{-5}	4.3×10^{-5}	6.8×10^{-6}	6.3	75.0
5.0	8.5×10^{-4}**	8.1×10^{-5}	9.9×10^{-5}**	1.5×10^{-5}	5.3×10^{-5}	7.1×10^{-6}	7.5	89.3
7.5	9.7×10^{-4}***	3.9×10^{-5}	6.5×10^{-5}	1.3×10^{-5}	6.1×10^{-5}	4.6×10^{-6}	13.1	155.9
10.0	1.2×10^{-3}***	1.3×10^{-4}	6.5×10^{-5}	1.0×10^{-5}	7.5×10^{-5}	4.6×10^{-6}	16.2	192.9
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Tables 56 a,b,c&d. *Perna indica.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 56 a. *1.0 ppb Mercury + 25 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	1.0×10^{-3}***	8.5×10^{-5}	1.1×10^{-4}**	1.9×10^{-5}	6.3×10^{-5}	7.9×10^{-6}	7.9	94.0
1.0	9.4×10^{-4}***	6.2×10^{-5}	8.8×10^{-5}**	6.1×10^{-6}	5.9×10^{-5}	6.3×10^{-6}	9.3	110.7
2.0	1.3×10^{-3}***	1.0×10^{-4}	1.2×10^{-4}***	1.2×10^{-5}	8.1×10^{-5}	8.6×10^{-6}	9.5	113.1
4.0	7.3×10^{-4}**	6.2×10^{-5}	5.9×10^{-5}	5.6×10^{-6}	4.6×10^{-5}	4.2×10^{-6}	10.8	128.6
6.0	5.5×10^{-4}	6.6×10^{-5}	7.8×10^{-5}	1.9×10^{-5}	3.4×10^{-5}	5.6×10^{-6}	6.2	73.8
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 56 b. *5.0 ppb Mercury + 25 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	1.6×10^{-3}***	1.0×10^{-4}	1.0×10^{-4}**	1.1×10^{-5}	1.0×10^{-4}	7.1×10^{-6}	14.0	166.7
1.0	1.0×10^{-3}***	5.3×10^{-5}	4.7×10^{-5}	6.7×10^{-6}	6.3×10^{-5}	3.4×10^{-6}	18.6	221.4
2.0	8.1×10^{-4}**	9.8×10^{-5}	6.0×10^{-5}	1.0×10^{-5}	5.1×10^{-5}	4.3×10^{-6}	11.8	140.5
4.0	7.6×10^{-4}**	5.9×10^{-5}	6.9×10^{-5}	1.4×10^{-5}	4.8×10^{-5}	4.9×10^{-6}	9.6	114.3
6.0	1.1×10^{-3}***	1.0×10^{-4}	1.3×10^{-4}***	1.3×10^{-5}	6.9×10^{-5}	9.3×10^{-6}	7.4	88.1
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

** $P < 0.01$ *** $P < 0.001$

Table 56 c. *1.0 ppb Mercury + 100 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	8.8×10^{-4}***	9.9×10^{-5}	1.0×10^{-4}**	9.6×10^{-6}	5.5×10^{-5}	7.1×10^{-6}	7.7	91.7
1.0	1.4×10^{-3}***	1.3×10^{-4}	9.4×10^{-5}**	1.3×10^{-5}	8.8×10^{-5}	6.7×10^{-6}	13.0	154.8
2.0	6.8×10^{-4}*	5.7×10^{-5}	1.0×10^{-4}**	1.3×10^{-5}	4.3×10^{-5}	7.1×10^{-6}	5.9	70.2
4.0	5.9×10^{-4}	8.1×10^{-5}	9.0×10^{-5}*	1.7×10^{-5}	3.6×10^{-5}	6.4×10^{-6}	5.7	67.9
6.0	7.9×10^{-4}**	5.3×10^{-5}	1.2×10^{-4}***	1.5×10^{-5}	4.9×10^{-5}	8.6×10^{-6}	5.7	67.9
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

Table 56 d. *5.0 ppb Mercury + 100 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	1.2×10^{-3}***	8.2×10^{-5}	1.0×10^{-4}**	1.6×10^{-5}	7.5×10^{-5}	7.1×10^{-6}	10.5	125.0
1.0	8.6×10^{-4}***	4.9×10^{-5}	7.2×10^{-5}	1.1×10^{-5}	5.4×10^{-5}	5.1×10^{-6}	10.4	123.8
2.0	8.5×10^{-4}**	8.1×10^{-5}	9.9×10^{-5}**	1.5×10^{-5}	5.3×10^{-5}	7.1×10^{-6}	7.5	89.3
4.0	6.2×10^{-4}	8.7×10^{-5}	7.1×10^{-5}	1.0×10^{-5}	3.9×10^{-5}	5.1×10^{-6}	7.6	90.5
6.0	6.3×10^{-4}	6.0×10^{-5}	6.2×10^{-5}	1.1×10^{-5}	3.9×10^{-5}	4.4×10^{-6}	8.9	105.9
Control	5.5×10^{-4}	6.6×10^{-5}	5.7×10^{-5}	1.3×10^{-5}	3.4×10^{-5}	4.1×10^{-6}	8.4	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

present in the sandy beaches stretching from Shertallai in the north to Ambalapuzha in the south. They have been recorded as tidal migrants and present in large numbers making them a useful candidate for stress assessment. As in the case of Perna indica, this species was also subjected to metal induced stress in the laboratory, involving exposure of the animals to mercury, copper and cadmium, singly or in combination. The stress was quantified employing rates of oxygen consumption, ammonia - nitrogen excretion and the resultant O:N ratios.

4.4.2.1 **Ratios under individual metal stress**

The trend in oxygen consumption, ammonia - nitrogen excretion and the O:N ratios, when Donax incarnatus was exposed to varying concentrations of mercury is presented in Table 57. Concentration bound declension in O:N ratio was a conspicuous feature. Thus, the ratio dipped to 3.0 when the animals were exposed to 5.0 ppb of mercury. 1.0, 2.0 and 3.0 ppb of mercury, resulted in an increase in the O:N ratio from that of the control. When the percentage O:N ratio was compared with that of control animals, it was noticed that this was negatively proportional to increasing concentration of mercury.

The effect of different concentrations of copper on the oxygen consumption, excretion of ammonia and the resultant O:N ratios are presented in Table 58. Considering the reaction of the control animals based on O:N ratio, copper concentrations of 0.5 to 2.0 ppb, reduced the O:N ratios whereas maximum O:N ratio was recorded in the case of those animals exposed to 4.0 ppb of copper. The rate of ammonia excretion

Table 57. *Donax incarnatus.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt') under sub-lethal concentrations of mercury, along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N')*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	9.4×10^{-4}*	2.2×10^{-4}	3.2×10^{-5}	8.5×10^{-6}	5.9×10^{-5}	2.3×10^{-6}	25.7	115.2
2.0	7.1×10^{-4}	2.8×10^{-4}	2.5×10^{-5}	3.6×10^{-6}	4.4×10^{-5}	1.8×10^{-6}	24.9	111.7
3.0	9.6×10^{-4}**	1.6×10^{-4}	3.6×10^{-5}	2.1×10^{-5}	6.0×10^{-5}	2.6×10^{-6}	23.3	104.7
4.0	2.8×10^{-4}*	1.7×10^{-4}	2.2×10^{-5}	1.9×10^{-5}	1.8×10^{-5}	1.6×10^{-6}	11.1	49.8
5.0	2.3×10^{-4}**	6.6×10^{-5}	6.6×10^{-5}**	1.2×10^{-5}	1.4×10^{-5}	4.7×10^{-6}	3.0	13.5
Control	5.6×10^{-4}	1.3×10^{-4}	2.2×10^{-5}	1.1×10^{-5}	3.5×10^{-5}	1.6×10^{-6}	22.3	100.0

Table 58. *Donax incarnatus.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt') and ammonic - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt') under sub-lethal concentrations of copper, along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N')*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	7.0×10^{-4}	8.1×10^{-5}	5.9×10^{-5}**	1.2×10^{-5}	4.4×10^{-5}	4.2×10^{-6}	10.4	46.6
1.0	7.5×10^{-4}	1.2×10^{-4}	5.2×10^{-5}	3.1×10^{-5}	4.7×10^{-5}	3.7×10^{-6}	12.6	56.5
2.0	5.3×10^{-4}	8.0×10^{-5}	2.9×10^{-5}	3.1×10^{-6}	3.3×10^{-5}	2.1×10^{-6}	16.0	71.7
4.0	1.0×10^{-3}*	2.0×10^{-4}	2.0×10^{-5}	6.1×10^{-6}	6.3×10^{-5}	1.4×10^{-6}	43.7	193.9
6.0	7.1×10^{-4}	2.4×10^{-4}	2.4×10^{-5}	8.7×10^{-6}	4.4×10^{-5}	1.7×10^{-6}	25.9	116.1
Control	5.6×10^{-4}	1.3×10^{-4}	2.2×10^{-5}	1.1×10^{-5}	3.5×10^{-5}	1.6×10^{-6}	22.3	100.0

* $P < 0.05$ ** $P < 0.01$

was found to be significantly different only in the case of those animals exposed to 0.5 ppb of copper. In this case higher concentrations of copper increased the O:N ratio percentage.

The presence of cadmium in concentrations ranging from 5.0 to 40 ppb was also found to have conspicuous effects on the O:N ratios of Donax incarnatus. The presence of 5.0 ppb of cadmium increased the oxygen consumption of the animals drastically, thereby affecting the O:N ratio. On the contrary, the excretion of ammonia-nitrogen was comparable to that of the control in this concentration. The presence of 20 ppb or 30 ppb of cadmium resulted in the reduction of the O:N ratios. Ammonia excretion was found to be significantly different only in the case of those animals exposed to 20 ppb of cadmium (See Table 59). In the case of cadmium exposed animals, curiously enough, both at very low and high concentrations,the percentage O:N ratio was found to be much higher than that of the control.

4.4.2.2 **Ratios under metal mixture stress**

In another series of experiments, mercury, copper and cadmium were used in double combination pattern to study the metal to metal interaction on the oxygen consumption, ammonia excretion and the O:N ratios of Donax incarnatus. In this series, Tables 60 a and b present results on the above factors when Donax incarnatus was exposed to varying concentrations of copper with two constant concentrations of mercury. Drastic differences in oxygen consumption was noticed in the case of those animals kept under a concentration of 1.0 ppb of mercury and copper. On the

Table 59. *Donax incarnatus.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion (mg NH_4-N $h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium, along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios(O : N)*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	$1.5x10^{-3}$***	$2.5x10^{-4}$	$2.1x10^{-5}$	$1.1x10^{-5}$	$2.4x10^{-5}$	$1.5x10^{-6}$	62.5	280.2
10	$8.1x10^{-4}$*	$1.5x10^{-4}$	$2.3x10^{-5}$	$9.1x10^{-6}$	$5.1x10^{-5}$	$1.6x10^{-6}$	30.8	138.1
20	$5.1x10^{-4}$	$1.5x10^{-4}$	$4.8x10^{-5}$*	$1.2x10^{-5}$	$3.2x10^{-5}$	$3.4x10^{-6}$	9.3	41.7
30	$3.5x10^{-4}$*	$9.1x10^{-5}$	$2.4x10^{-5}$	$3.5x10^{-6}$	$2.2x10^{-5}$	$1.7x10^{-6}$	12.8	57.4
40	$5.2x10^{-4}$	$1.7x10^{-4}$	$1.3x10^{-5}$	$6.2x10^{-6}$	$3.3x10^{-5}$	$9.3x10^{-7}$	35.0	157.0
Control	$5.6x10^{-4}$	$1.3x10^{-4}$	$2.2x10^{-5}$	$1.1x10^{-5}$	$3.5x10^{-5}$	$1.6x10^{-6}$	22.3	100.0

* $P < 0.05$ *** $P < 0.001$

other hand, significant variations in the excretion of ammonia - nitrogen was noticed when the concentrations of copper were increased to 2.0, 4.0 and 6.0 ppb. However, the O:N ratio picture showed varying trends in the sense that the reduction occurred only in higher concentrations of copper. The percentage difference with reference to O:N ratio when compared to the control showed the following trend; reduction in both high and low concentrations and increase in one median concentration (Table 60 a).

Table 60 b presents results of similar experiments employing higher constant concentration of mercury. Oxygen consumption was significantly different in three of the five concentrations employed and ammonia -nitrogen excretion rate was significantly different in the three lower concentrations of copper. O:N ratio was reduced in all the animals when compared to that of the control, although the percentage difference was maximum when the medium contained 2.0 ppb of copper and 3.0 ppb of mercury and minimum with 6.0 and 3.0 ppb of copper and mercury respectively.

Tables 61 a and b present data on the oxygen consumption and ammonia -nitrogen excretion with the respective O:N ratios, when Donax incarnatus was exposed to a mixture containing two constant concentrations of copper and 1.0 to 5.0 ppb of mercury. In two concentrations, oxygen consumption and ammonia - nitrogen was significantly different from that of the control. Maximum deviation in O:N ratio occurred in that concentration which contained 0.5 ppb of copper and 3.0 ppb of mercury. Percentage difference in the O:N ratio from that of the control indicated maximum deviation in the median concentration and decreased towards low and

Tables 60 a&b. *Donax incarnatus*. *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 60 a. *1.0 ppb Mercury + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N
	Mean	SD	Mean	SD	O	N	
0.5	$1.3x10^{-3}$	$1.7x10^{-4}$	$1.9x10^{-5}$	$4.9x10^{-6}$	$8.1x10^{-5}$	$1.4x10^{-6}$	59.8
1.0	$1.7x10^{-3}$*	$1.4x10^{-4}$	$2.0x10^{-5}$	$1.7x10^{-6}$	$1.1x10^{-4}$	$1.4x10^{-6}$	74.4
2.0	$1.3x10^{-3}$	$1.3x10^{-4}$	$1.2x10^{-5}$*	$5.8x10^{-6}$	$8.1x10^{-5}$	$8.6x10^{-7}$	94.8
4.0	$1.0x10^{-3}$	$2.5x10^{-4}$	$4.3x10^{-5}$*	$1.0x10^{-5}$	$6.3x10^{-5}$	$3.1x10^{-6}$	20.3
6.0	$1.4x10^{-3}$	$1.7x10^{-4}$	$1.0x10^{-4}$***	$1.3x10^{-5}$	$8.8x10^{-5}$	$7.1x10^{-6}$	12.3
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8

Table 60 b. *3.0 ppb Mercury + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N
	Mean	SD	Mean	SD	O	N	
0.5	$1.5x10^{-3}$	$1.4x10^{-4}$	$7.6x10^{-5}$***	$7.1x10^{-6}$	$9.4x10^{-5}$	$5.4x10^{-6}$	17.3
1.0	$2.5x10^{-3}$***	$2.8x10^{-4}$	$1.3x10^{-4}$***	$2.6x10^{-5}$	$1.6x10^{-4}$	$9.3x10^{-6}$	16.8
2.0	$1.3x10^{-3}$	$2.8x10^{-4}$	$7.0x10^{-5}$**	$2.1x10^{-5}$	$8.1x10^{-5}$	$5.0x10^{-6}$	16.3
4.0	$9.6x10^{-4}$*	$1.6x10^{-4}$	$3.6x10^{-5}$	$5.2x10^{-6}$	$6.0x10^{-5}$	$2.6x10^{-6}$	23.3
6.0	$9.5x10^{-4}$*	$1.3x10^{-4}$	$2.9x10^{-5}$	$1.1x10^{-5}$	$5.9x10^{-5}$	$2.1x10^{-6}$	28.7
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8

* $P < 0.05$ *** $P < 0.001$

** $P < 0.01$

Tables 61 a&b. *Donax incarnatus.* *Rates of oxygen consumption (mgO_2 h^{-1} mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of copper (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 61 a. *0.5 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.3x10^{-3}$	$1.7x10^{-4}$	$1.9x10^{-5}$	$4.9x10^{-6}$	$8.1x10^{-5}$	$1.4x10^{-6}$	59.8	136.5
2.0	$1.3x10^{-3}$	$2.1x10^{-4}$	$1.9x10^{-5}$	$2.1x10^{-6}$	$8.1x10^{-5}$	$1.4x10^{-6}$	59.8	136.5
3.0	$1.5x10^{-3}$	$1.4x10^{-4}$	$7.6x10^{-5}$***	$7.1x10^{-6}$	$9.4x10^{-5}$	$5.4x10^{-6}$	17.3	39.5
4.0	$6.6x10^{-4}$**	$1.3x10^{-4}$	$1.6x10^{-5}$	$7.8x10^{-6}$	$4.1x10^{-5}$	$1.1x10^{-6}$	36.1	82.4
5.0	$1.0x10^{-3}$*	$1.5x10^{-4}$	$1.4x10^{-5}$*	$1.0x10^{-6}$	$6.3x10^{-5}$	$1.0x10^{-6}$	62.5	142.7
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

Table 61 b. *2.0 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.3x10^{-3}$	$1.3x10^{-4}$	$1.2x10^{-5}$*	$5.8x10^{-6}$	$8.1x10^{-5}$	$8.6x10^{-7}$	94.8	216.4
2.0	$3.6x10^{-4}$***	$6.9x10^{-5}$	$2.2x10^{-5}$	$9.3x10^{-6}$	$2.3x10^{-5}$	$1.6x10^{-6}$	14.3	32.7
3.0	$1.3x10^{-3}$	$2.8x10^{-4}$	$7.0x10^{-5}$**	$2.1x10^{-5}$	$8.1x10^{-5}$	$5.0x10^{-6}$	16.3	37.2
4.0	$1.4x10^{-3}$	$2.1x10^{-4}$	$6.6x10^{-5}$*	$2.6x10^{-5}$	$8.8x10^{-5}$	$4.7x10^{-6}$	18.6	42.5
5.0	$1.4x10^{-3}$	$1.5x10^{-4}$	$4.2x10^{-5}$	$1.1x10^{-5}$	$8.8x10^{-5}$	$3.0x10^{-6}$	29.2	66.7
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

high (See Table 61 a). Table 61 b gives information of a similar nature, when the copper concentration was increased to 2.0 ppb. The variation in oxygen consumption and ammonia -nitrogen excretion was confined to only a few concentrations. The O:N ratio was found to get reduced in the case of animals exposed to all the concentrations but with 1.0 ppb of mercury and 2.0 ppb of copper. The percentage was maximum in the case of those animals exposed to the minimum concentration (1.0 ppb) of mercury.

Donax incarnatus was exposed to two constant concentrations of mercury and varying concentrations of cadmium in combination. The results obtained on their rate of oxygen consumption, excretion of ammonia - nitrogen and the respective O:N ratio are presented in Tables 62 a and b. The presence of low concentration of mercury in combination with 20 or 30 ppb of cadmium resulted in significant difference in oxygen consumption and ammonia excretion, resulting in reduced O:N ratios in those combinations. The percentage difference was maximum in the median concentration (20 ppb Cd + 1.0 ppb Hg). The trend was more or less the same even when the mercury concentration was raised to 3.0 ppb. The only difference being the maximum variation in the O:N ratio, being recorded by those test animals retained in 30 ppb of cadmium.

The overall performance of Donax incarnatus exposed to two constant concentrations of cadmium and varying concentrations of mercury is presented in Tables 63 a and b. The rate performance was significantly different only with reference to ammonia excretion in the case of those animals subjected to a stress of 20 ppb of cadmium and 1.0 to 5.0 ppb

Tables 62 a&b. *Donax incarnatus*. *Rates of oxygen consumption (mgO_2 h^{-1} mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant) and cadmium (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 62 a. *1.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	$1.1x10^{-3}$	$1.5x10^{-4}$	$1.8x10^{-5}$	$5.5x10^{-6}$	$6.9x10^{-5}$	$1.3x10^{-6}$	53.5	122.2
10	$1.4x10^{-3}$	$2.4x10^{-4}$	$2.5x10^{-5}$	$4.2x10^{-6}$	$8.7x10^{-5}$	$1.8x10^{-6}$	49.0	111.9
20	$1.9x10^{-3}$**	$2.2x10^{-4}$	$1.2x10^{-4}$***	$2.9x10^{-5}$	$1.2x10^{-4}$	$8.6x10^{-6}$	13.9	31.7
30	$1.8x10^{-3}$*	$2.1x10^{-4}$	$6.3x10^{-5}$***	$8.6x10^{-6}$	$1.1x10^{-4}$	$4.5x10^{-6}$	25.0	57.1
40	$1.2x10^{-3}$	$2.5x10^{-4}$	$2.5x10^{-5}$	$6.4x10^{-6}$	$7.5x10^{-5}$	$1.8x10^{-6}$	42.0	95.9
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

Table 62 b. *3.0 ppb Mercury + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	$9.3x10^{-4}$*	$2.1x10^{-4}$	$1.6x10^{-5}$*	$2.8x10^{-6}$	$5.8x10^{-5}$	$1.1x10^{-6}$	50.6	116.2
10	$8.5x10^{-4}$*	$1.9x10^{-4}$	$2.1x10^{-5}$	$7.1x10^{-7}$	$5.3x10^{-5}$	$1.5x10^{-6}$	35.4	80.8
20	$1.3x10^{-3}$	$9.6x10^{-5}$	$3.9x10^{-5}$*	$2.5x10^{-6}$	$8.1x10^{-5}$	$2.8x10^{-6}$	29.2	66.7
30	$1.6x10^{-3}$*	$1.3x10^{-4}$	$9.4x10^{-5}$***	$9.2x10^{-6}$	$1.0x10^{-4}$	$6.7x10^{-6}$	14.9	34.0
40	$1.3x10^{-3}$	$1.7x10^{-4}$	$4.3x10^{-5}$*	$1.1x10^{-5}$	$8.1x10^{-5}$	$3.1x10^{-6}$	26.5	60.5
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Tables 63 a&b. *Donax incarnatus*. *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 63 a. *5.0 ppb Cadmium + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.1x10^{-3}$	$1.5x10^{-4}$	$1.8x10^{-5}$	$5.5x10^{-6}$	$6.9x10^{-5}$	$1.3x10^{-6}$	53.5	122.2
2.0	$1.7x10^{-3}$*	$2.2x10^{-4}$	$1.6x10^{-5}$	$6.3x10^{-6}$	$1.1x10^{-4}$	$1.1x10^{-6}$	93.0	212.3
3.0	$9.3x10^{-4}$*	$2.1x10^{-4}$	$1.6x10^{-5}$*	$2.8x10^{-6}$	$5.8x10^{-5}$	$1.1x10^{-6}$	50.9	116.2
4.0	$1.6x10^{-3}$	$2.1x10^{-4}$	$3.4x10^{-5}$	$1.6x10^{-5}$	$1.0x10^{-4}$	$2.4x10^{-6}$	41.2	94.1
5.0	$1.3x10^{-3}$	$1.3x10^{-4}$	$3.1x10^{-5}$	$9.8x10^{-6}$	$8.1x10^{-5}$	$2.2x10^{-6}$	36.7	83.8
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

Table 63 b. *20 ppb Cadmium + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.9x10^{-3}$**	$2.2x10^{-4}$	$1.2x10^{-4}$***	$2.9x10^{-5}$	$1.2x10^{-4}$	$8.6x10^{-6}$	13.9	31.7
2.0	$1.2x10^{-3}$	$1.3x10^{-4}$	$4.1x10^{-5}$*	$8.5x10^{-6}$	$7.5x10^{-5}$	$2.9x10^{-6}$	25.6	58.5
3.0	$1.3x10^{-3}$	$9.6x10^{-5}$	$3.9x10^{-5}$*	$2.5x10^{-6}$	$8.1x10^{-5}$	$2.8x10^{-6}$	29.2	66.7
4.0	$1.5x10^{-3}$	$1.7x10^{-4}$	$6.9x10^{-5}$	$3.6x10^{-5}$	$9.4x10^{-5}$	$4.9x10^{-6}$	19.0	43.4
5.0	$2.3x10^{-3}$**	$3.1x10^{-4}$	$1.1x10^{-4}$***	$2.1x10^{-5}$	$1.4x10^{-4}$	$7.8x10^{-6}$	18.3	41.8
Control	$1.3x10^{-3}$	$1.8x10^{-4}$	$2.6x10^{-5}$	$7.6x10^{-6}$	$8.1x10^{-5}$	$1.9x10^{-6}$	43.8	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

of mercury. In general, the O:N ratio was more than that of the control animals when the cadmium concentration was low whereas this was found to get reduced than that of the control, when the cadmium concentration was increased. The maximum percentage difference occurred when the medium contained 2.0 ppb of mercury with 5.0 ppb of cadmium or 1.0 ppb of mercury with 20 ppb of cadmium.

Varying concentrations of copper in combination with 5.0 or 20 ppb of cadmium depicted reversing trends. Although minimal variation in oxygen consumption and ammonia excretion occurred in the case of those animals exposed to 5.0 ppb of cadmium and varying concentrations of copper, the O:N ratio was found to fluctuate more in the case of those test organisms exposed to higher concentrations of cadmium with copper. The percentage difference was more conspicuous in the medium which contained more of cadmium (See Tables 64 a and b).

No significant variation in oxygen consumption or ammonia excretion by Donax incarnatus was noticed when they were subjected to exposure to 0.5 or 2.0 ppb of copper and 5.0 to 40 ppb of cadmium. However, the trend in the O:N ratio was totally dissimilar in these concentrations. In the case of those animals maintained in 0.5 ppb of copper and 5.0 to 40 ppb of cadmium, there was an increase in the O:N ratio in majority of the concentrations employed. On the other hand, conspicuous reduction in the O:N ratio was noticed when the concentrations of the media were 2.0 ppb of copper with 20 or 30 ppb of cadmium. The main difference noticed between these two combinations was an increased O:N ratio in

Tables 64 a&b. *Donax incarnatus.* *Rates of oxygen consumption ($mgO_2\ h^{-1}\ mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}\ mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 64 a. *5.0 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percente differen
	Mean	SD	Mean	SD	O	N		
0.5	6.8×10^{-4}	1.7×10^{-4}	2.6×10^{-5}	1.3×10^{-5}	4.2×10^{-5}	1.9×10^{-6}	22.9	102.7
1.0	6.8×10^{-4}	8.3×10^{-5}	2.5×10^{-5}	1.8×10^{-5}	4.2×10^{-5}	1.8×10^{-6}	23.8	106.7
2.0	6.5×10^{-4}	2.1×10^{-4}	2.3×10^{-5}	2.1×10^{-6}	4.1×10^{-5}	1.6×10^{-6}	24.7	110.7
4.0	9.9×10^{-4}*	2.4×10^{-4}	3.2×10^{-5}	7.7×10^{-6}	6.2×10^{-5}	2.3×10^{-6}	27.1	121.5
6.0	5.2×10^{-4}	7.5×10^{-5}	2.4×10^{-5}	1.3×10^{-5}	3.3×10^{-5}	1.7×10^{-6}	18.9	84.8
Control	5.6×10^{-4}	1.3×10^{-4}	2.2×10^{-5}	1.1×10^{-5}	3.5×10^{-5}	1.6×10^{-6}	22.3	100.0

Table 64 b. *20 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percent differer
	Mean	SD	Mean	SD	O	N		
0.5	6.0×10^{-4}	1.6×10^{-4}	1.5×10^{-5}	8.3×10^{-6}	3.8×10^{-5}	1.1×10^{-6}	35.0	157.0
1.0	8.0×10^{-4}	1.9×10^{-4}	2.6×10^{-5}	2.1×10^{-6}	5.0×10^{-5}	1.9×10^{-6}	26.9	120.6
2.0	2.7×10^{-4}**	4.0×10^{-5}	3.4×10^{-5}	1.5×10^{-5}	1.7×10^{-5}	2.4×10^{-6}	6.9	30.9
4.0	4.9×10^{-4}	1.3×10^{-4}	3.9×10^{-5}	2.1×10^{-5}	3.1×10^{-5}	2.7×10^{-6}	10.9	48.9
6.0	6.6×10^{-4}	6.5×10^{-5}	4.6×10^{-5}*	1.2×10^{-5}	4.1×10^{-5}	3.3×10^{-6}	12.6	56.5
Control	5.6×10^{-4}	1.3×10^{-4}	2.2×10^{-5}	1.1×10^{-5}	3.5×10^{-5}	1.6×10^{-6}	22.3	100.0

* $P < 0.05$ ** $P < 0.01$

Tables 65 a&b. *Donax incarnatus.* *Rates of oxygen consumption (mgO_2 h^{-1} mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt), under sub-lethal concentrations of copper (constant) and cadmium (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 65 a. *0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	$6.8x10^{-4}$	$1.7x10^{-4}$	$2.6x10^{-5}$	$1.3x10^{-5}$	$4.2x10^{-5}$	$1.9x10^{-6}$	22.9	102.7
10	$6.8x10^{-4}$	$2.3x10^{-4}$	$1.7x10^{-5}$	$3.2x10^{-6}$	$4.3x10^{-5}$	$1.2x10^{-6}$	35.0	157.0
20	$6.0x10^{-4}$	$1.6x10^{-4}$	$1.5x10^{-5}$	$8.3x10^{-6}$	$3.8x10^{-5}$	$1.1x10^{-6}$	35.0	157.0
30	$6.8x10^{-4}$	$2.1x10^{-4}$	$1.1x10^{-5}$	$3.7x10^{-6}$	$4.3x10^{-5}$	$7.9x10^{-7}$	54.0	242.2
40	$5.6x10^{-4}$	$1.2x10^{-4}$	$1.8x10^{-5}$	$6.6x10^{-6}$	$3.5x10^{-5}$	$1.3x10^{-6}$	27.2	122.0
Control	$5.6x10^{-4}$	$1.3x10^{-4}$	$2.2x10^{-5}$	$1.1x10^{-5}$	$3.5x10^{-5}$	$1.6x10^{-6}$	22.3	100.0

Table 65 b. *2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	$6.5x10^{-4}$	$2.1x10^{-4}$	$2.3x10^{-5}$	$2.1x10^{-6}$	$4.1x10^{-5}$	$1.6x10^{-6}$	24.7	110.8
10	$6.4x10^{-4}$	$1.3x10^{-4}$	$2.8x10^{-5}$	$8.8x10^{-6}$	$4.0x10^{-5}$	$2.0x10^{-6}$	20.0	89.7
20	$2.7x10^{-4}$**	$4.0x10^{-5}$	$3.4x10^{-5}$	$1.5x10^{-5}$	$1.7x10^{-5}$	$2.4x10^{-6}$	6.9	30.9
30	$3.6x10^{-4}$	$1.4x10^{-4}$	$3.2x10^{-5}$	$1.9x10^{-5}$	$2.3x10^{-5}$	$2.3x10^{-6}$	9.8	43.9
40	$5.5x10^{-4}$	$1.6x10^{-4}$	$1.9x10^{-5}$	$7.5x10^{-6}$	$3.4x10^{-5}$	$1.4x10^{-6}$	25.3	113.5
Control	$5.6x10^{-4}$	$1.3x10^{-4}$	$2.2x10^{-5}$	$1.1x10^{-5}$	$3.5x10^{-5}$	$1.6x10^{-6}$	22.3	100.0

** $P < 0.01$

the median cadmium concentration with 0.5 ppb of copper and reduced O:N ratio in the same concentration of cadmium with 2.0 ppb of copper (See Tables 65 a and b).

Triad combination of mercury, copper and cadmium was utilized to study the effects of varying concentrations of the three metals on the oxygen consumption, ammonia excretion and the O:N ratio. The concentrations of the metals used were mercury : 1.0 to 5.0 ppb, copper : 0.5 to 6.0 ppb and cadmium: 5.0 to 40 ppb. The non-varying component of the metals was 1.0 and 3.0 ppb in the case of mercury, 0.5 and 2.0 ppb of copper and 5.0 and 20 ppb of cadmium. These concentrations were used in varying combinations which resulted in twelve different sets of experiments. The results obtained are presented in Tables 66 a to 68d.

Tables 66 a to d give information on the above aspects when Donax incarnatus was subjected to an exposure of constant concentration of mercury and copper and varying concentrations of cadmium in each set. Here the variants were mercury, copper and cadmium, with cadmium varying to a greater extend than the other two. However, in any single set of experiment only cadmium was the variant. Significant variations in the oxygen consumption rate was observed mainly in those experiments where the mercury concentration was 1.0 ppb, copper 0.5 ppb and cadmium 5.0 to 40 ppb. No significant variations were observed in the oxygen consumption when the mercury concentration was enhanced to 3.0 ppb and copper to 2.0 ppb. The rate of ammonia excretion was found to vary significantly in three sets of experiments where the concentrations

of mercury and copper were 1.0 and 3.0 ppb and 0.5 and 2.0 ppb respectively. However, the presence of 3.0 ppb of mercury and 0.5 ppb of copper with varying concentrations of cadmium did not evince any significant variation in the ammonia-nitrogen excretion, except in the highest cadmium concentration. The O:N ratios were found to be less than that of the control in three sets of experiments (Tables 66 a, b and d). It increased above the control in one set of experiment (Table 66 c). The percentage difference in the O:N ratio was found to alternate in such a fashion that in the presence of 1.0 ppb of mercury with 0.5 ppb of copper and 3.0 ppb of mercury with 2.0 ppb of copper, the median cadmium concentration recorded more difference from the control.

Tables 67 a to d explain the data obtained on the oxygen consumption, ammonia - nitrogen excretion and the O:N ratios of Donax incarnatus, exposed to 1.0 and 3.0 ppb of mercury, 5.0 and 20 ppb of cadmium and varying concentrations of copper. Among these sets of experiments, significant variations in oxygen consumption were noticed in those combinations which contained less quantity of mercury (1.0 ppb). Similarly, the rate of ammonia - nitrogen excretion was significantly different from the control when the animals were exposed to 1.0 ppb mercury, 5.0 ppb cadmium and 0.5 to 6.0 ppb of copper. Apart from these variations, those recorded in the other sets of experiments were not very significant. In general, the O:N ratio was always found to be less than that recorded for the control animals. Very low O:N ratios were recorded in those combinations which contained a minimum quantity of mercury and cadmium. In the median concentration of copper with 1.0 or 3.0 ppb of mercury and 5.0

Tables 66 a, b, c&d. *Donax incarnatus.* *Rates of oxygen consumption (mgO_2 h^{-1} mg^{-1} dry wt) and ammonia - nitrogen excretion (mg NH_4-N h^{-1} mg^{-1} dry wt) under sub-lethal concentrations of mercury (constant), copper (constant) and cadmium (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 66 a. *1.0 ppb Mercury + 0.5 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	1.5×10^{-3}**	2.1×10^{-4}	6.9×10^{-5}**	2.2×10^{-5}	9.4×10^{-5}	4.9×10^{-6}	19.0	40.4
10	1.4×10^{-3}***	1.0×10^{-4}	6.7×10^{-5}**	1.3×10^{-5}	8.8×10^{-5}	4.8×10^{-6}	18.3	38.9
20	3.5×10^{-4}***	8.7×10^{-5}	6.9×10^{-5}**	1.7×10^{-5}	2.2×10^{-5}	4.9×10^{-6}	4.4	9.4
30	6.2×10^{-4}*	1.1×10^{-4}	3.9×10^{-5}*	1.0×10^{-5}	3.9×10^{-5}	2.8×10^{-6}	13.9	29.6
40	5.3×10^{-4}**	9.6×10^{-5}	1.6×10^{-5}	2.1×10^{-6}	3.3×10^{-5}	1.1×10^{-6}	28.9	61.5
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

Table 66 b. *1.0 ppb Mercury + 2.0 ppb Copper + Cadmium*

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	3.2×10^{-4}***	1.1×10^{-4}	6.5×10^{-5}***	5.6×10^{-6}	2.0×10^{-5}	4.6×10^{-6}	4.3	9.1
10	6.1×10^{-4}	1.8×10^{-4}	2.5×10^{-5}	7.6×10^{-6}	3.8×10^{-5}	1.8×10^{-6}	21.4	45.5
20	1.3×10^{-3}**	1.5×10^{-4}	4.8×10^{-5}*	1.6×10^{-5}	8.1×10^{-5}	3.4×10^{-6}	23.7	50.4
30	8.7×10^{-4}	2.1×10^{-4}	3.1×10^{-5}	9.2×10^{-6}	5.4×10^{-5}	2.2×10^{-6}	24.6	52.3
40	9.4×10^{-4}	1.4×10^{-4}	3.4×10^{-5}*	4.9×10^{-6}	5.9×10^{-5}	2.4×10^{-6}	24.2	51.5
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 66 c. 3.0 ppb Mercury + 0.5 ppb Copper + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	8.9×10^{-4}	1.7×10^{-4}	3.0×10^{-5}	9.8×10^{-6}	5.6×10^{-5}	2.1×10^{-6}	26.0	55.3
10	8.5×10^{-4}	1.5×10^{-4}	1.5×10^{-5}	4.7×10^{-6}	5.3×10^{-5}	1.1×10^{-6}	49.6	105.5
20	8.3×10^{-4}	7.6×10^{-5}	1.2×10^{-5}	3.6×10^{-6}	5.2×10^{-5}	8.6×10^{-7}	60.5	128.7
30	7.9×10^{-4}	1.8×10^{-4}	3.1×10^{-5}	7.0×10^{-6}	4.9×10^{-5}	2.2×10^{-6}	22.3	47.5
40	1.1×10^{-3}	2.4×10^{-4}	6.8×10^{-5}***	4.9×10^{-6}	6.9×10^{-5}	4.9×10^{-6}	14.2	30.2
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

Table 66 d. 3.0 ppb Mercury + 2.0 ppb Copper + Cadmium

Cadmium (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
5	1.0×10^{-3}	2.0×10^{-4}	4.5×10^{-5}**	7.6×10^{-6}	6.3×10^{-5}	3.2×10^{-6}	19.4	41.3
10	1.0×10^{-3}	1.7×10^{-4}	4.6×10^{-5}	1.4×10^{-5}	6.3×10^{-5}	3.3×10^{-6}	19.0	40.4
20	8.9×10^{-4}	1.8×10^{-4}	8.8×10^{-5}***	1.4×10^{-5}	5.6×10^{-5}	6.3×10^{-6}	8.8	18.7
30	7.6×10^{-4}	1.2×10^{-4}	7.3×10^{-5}***	6.4×10^{-6}	4.8×10^{-5}	5.2×10^{-6}	9.1	19.4
40	8.4×10^{-4}	2.1×10^{-4}	5.7×10^{-5}**	8.4×10^{-6}	5.3×10^{-5}	4.1×10^{-6}	12.9	27.5
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

** $P < 0.01$ *** $P < 0.001$

Tables 67 a,b,c&d. *Donax incarnatus.* *Rates of oxygen consumption ($mgO_2\ h^{-1}mg^{-1}$ dry wt) and ammonia - nitrogen excretion ($mg\ NH_4\text{-}N\ h^{-1}mg^{-1}$ dry wt) under sub-lethal concentrations of mercury (constant), cadmium (constant) and copper (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 67 a. *1.0 ppb Mercury + 5.0 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	$1.5x10^{-3}$**	$2.1x10^{-4}$	$6.9x10^{-5}$**	$2.2x10^{-5}$	$9.4x10^{-5}$	$4.9x10^{-6}$	19.0	40.4
1.0	$8.9x10^{-4}$	$9.1x10^{-5}$	$7.8x10^{-5}$***	$1.5x10^{-5}$	$5.6x10^{-5}$	$5.6x10^{-6}$	9.9	21.1
2.0	$3.2x10^{-4}$***	$1.1x10^{-4}$	$6.5x10^{-5}$***	$5.6x10^{-6}$	$2.0x10^{-5}$	$4.6x10^{-6}$	4.3	9.1
4.0	$1.3x10^{-3}$*	$2.0x10^{-4}$	$9.6x10^{-5}$***	$8.4x10^{-6}$	$8.1x10^{-5}$	$6.9x10^{-6}$	11.8	25.1
6.0	$1.2x10^{-3}$	$2.5x10^{-4}$	$7.5x10^{-5}$***	$1.4x10^{-6}$	$7.5x10^{-5}$	$5.4x10^{-6}$	14.0	29.8
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100

Table 67 b. *1.0 ppb Mercury + 20 ppb Cadmium + Copper*

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	$3.5x10^{-4}$***	$8.7x10^{-5}$	$6.9x10^{-5}$**	$1.7x10^{-5}$	$2.2x10^{-5}$	$4.9x10^{-6}$	4.4	9.4
1.0	$1.2x10^{-3}$	$2.9x10^{-4}$	$2.9x10^{-5}$	$3.1x10^{-6}$	$7.5x10^{-5}$	$2.1x10^{-6}$	36.2	77.0
2.0	$1.3x10^{-3}$**	$1.5x10^{-4}$	$4.8x10^{-5}$*	$1.6x10^{-5}$	$8.1x10^{-5}$	$3.4x10^{-6}$	23.7	50.4
4.0	$6.6x10^{-4}$*	$9.6x10^{-5}$	$3.2x10^{-5}$	$8.4x10^{-6}$	$4.1x10^{-5}$	$2.3x10^{-6}$	18.0	38.3
6.0	$4.1x10^{-4}$**	$1.0x10^{-4}$	$2.3x10^{-5}$	$2.2x10^{-6}$	$2.6x10^{-5}$	$1.6x10^{-6}$	15.6	33.2
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 67 c. 3.0 ppb Mercury + 5 ppb Cadmium + Copper

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	$8.9x10^{-4}$	$1.7x10^{-4}$	$3.0x10^{-5}$	$9.8x10^{-6}$	$5.6x10^{-5}$	$2.1x10^{-6}$	26.0	55.3
1.0	$8.4x10^{-4}$	$1.0x10^{-4}$	$2.9x10^{-5}$	$4.2x10^{-6}$	$5.3x10^{-5}$	$2.1x10^{-6}$	25.3	53.8
2.0	$1.0x10^{-3}$	$2.0x10^{-4}$	$4.5x10^{-5}$**	$7.6x10^{-6}$	$6.3x10^{-5}$	$3.2x10^{-6}$	19.4	41.3
4.0	$9.3x10^{-4}$	$4.6x10^{-5}$	$2.9x10^{-5}$	$6.0x10^{-6}$	$5.8x10^{-5}$	$2.1x10^{-6}$	28.1	59.8
6.0	$7.9x10^{-4}$	$1.6x10^{-4}$	$2.3x10^{-5}$	$1.6x10^{-5}$	$4.9x10^{-5}$	$1.6x10^{-6}$	30.0	63.8
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100.0

Table 67 d. 3.0 ppb Mercury + 20 ppb Cadmium + Copper

Copper (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
0.5	$8.3x10^{-4}$	$7.6x10^{-5}$	$1.2x10^{-5}$	$3.6x10^{-6}$	$5.2x10^{-5}$	$8.6x10^{-7}$	60.5	128.7
1.0	$6.9x10^{-4}$	$2.2x10^{-4}$	$1.9x10^{-5}$	$7.6x10^{-6}$	$4.3x10^{-5}$	$1.4x10^{-6}$	31.8	67.7
2.0	$8.9x10^{-4}$	$1.8x10^{-4}$	$8.8x10^{-5}$***	$1.4x10^{-5}$	$5.6x10^{-5}$	$6.3x10^{-6}$	8.8	18.7
4.0	$2.9x10^{-4}$***	$1.5x10^{-4}$	$8.1x10^{-6}$	$1.0x10^{-7}$	$1.8x10^{-5}$	$5.8x10^{-7}$	31.3	66.6
6.0	$8.7x10^{-4}$	$7.4x10^{-5}$	$2.3x10^{-5}$	$5.5x10^{-6}$	$5.4x10^{-5}$	$1.6x10^{-6}$	33.1	70.4
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100.0

** $P < 0.01$ *** $P < 0.001$

Tables 68 a,b,c&d. *Donax incarnatus.* *Rates of oxygen consumption (mgO_2 $h^{-1}mg^{-1}$ dry wt) and ammonia - nitrogen excretion (mg NH_4-N $h^{-1}mg^{-1}$ dry wt) under sub-lethal concentrations of cadmium (constant), copper (constant) and mercury (varying), along with the respective standard deviations, their atomic equivalents and the equivalent Oxygen : Nitrogen ratios (O : N)*

Table 68 a. *5.0 ppb Cadmium + 0.5 ppb Copper + Mercury*

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$1.5x10^{-3}$**	$2.1x10^{-4}$	$6.9x10^{-5}$**	$2.2x10^{-5}$	$9.4x10^{-5}$	$4.9x10^{-6}$	19.0	40.4
2.0	$8.7x10^{-4}$	$5.8x10^{-5}$	$3.7x10^{-5}$*	$3.5x10^{-6}$	$5.4x10^{-5}$	$2.6x10^{-6}$	20.6	43.8
3.0	$8.9x10^{-4}$	$1.7x10^{-4}$	$3.0x10^{-5}$	$9.8x10^{-6}$	$5.6x10^{-5}$	$2.1x10^{-6}$	26.0	55.3
4.0	$8.4x10^{-4}$	$1.4x10^{-4}$	$5.6x10^{-5}$***	$3.1x10^{-6}$	$5.3x10^{-5}$	$4.0x10^{-6}$	13.1	27.9
5.0	$4.7x10^{-4}$*	$1.7x10^{-4}$	$3.2x10^{-5}$	$8.6x10^{-6}$	$2.9x10^{-5}$	$2.3x10^{-6}$	12.9	27.5
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100.0

Table 68 b. *5.0 ppb Cadmium + 2.0 ppb Copper + Mercury*

	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage
Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	$3.2x10^{-4}$***	$1.1x10^{-4}$	$6.5x10^{-5}$***	$5.6x10^{-6}$	$2.0x10^{-5}$	$4.6x10^{-6}$	4.3	9.1
2.0	$1.4x10^{-3}$***	$5.8x10^{-5}$	$9.3x10^{-5}$**	$2.4x10^{-5}$	$8.8x10^{-5}$	$6.6x10^{-6}$	13.2	28.1
3.0	$1.0x10^{-3}$	$2.0x10^{-4}$	$4.5x10^{-5}$**	$7.6x10^{-6}$	$6.3x10^{-5}$	$3.2x10^{-6}$	19.4	41.3
4.0	$1.1x10^{-3}$*	$1.2x10^{-4}$	$5.8x10^{-5}$**	$6.4x10^{-6}$	$6.9x10^{-5}$	$4.1x10^{-6}$	16.6	35.3
5.0	$8.9x10^{-4}$	$1.5x10^{-4}$	$9.5x10^{-5}$***	$5.6x10^{-6}$	$5.6x10^{-5}$	$6.8x10^{-6}$	8.2	17.5
Control	$8.6x10^{-4}$	$1.3x10^{-4}$	$1.6x10^{-5}$	$1.3x10^{-5}$	$5.4x10^{-5}$	$1.1x10^{-6}$	47.0	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 68 c. 20 ppb Cadmium + 0.5 ppb Copper + Mercury

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	3.5×10^{-4}***	8.7×10^{-5}	6.9×10^{-5}***	1.7×10^{-5}	2.2×10^{-5}	4.9×10^{-6}	4.4	9.4
2.0	1.1×10^{-3}	2.7×10^{-4}	1.3×10^{-4}***	2.1×10^{-5}	6.9×10^{-5}	9.3×10^{-6}	7.4	15.7
3.0	8.3×10^{-4}	7.6×10^{-5}	1.2×10^{-5}	3.6×10^{-6}	5.2×10^{-5}	8.6×10^{-7}	60.5	128.7
4.0	7.9×10^{-4}	1.9×10^{-4}	2.9×10^{-5}	7.4×10^{-6}	4.9×10^{-5}	2.1×10^{-6}	23.8	50.6
5.0	6.3×10^{-4}	1.6×10^{-4}	6.7×10^{-5}**	1.7×10^{-5}	3.9×10^{-5}	4.8×10^{-6}	8.2	17.5
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

Table 68 d. 20 ppb Cadmium + 2.0 ppb Copper + Mercury

Mercury (ppb)	Oxygen		Ammonia		Atomic equivalents		O:N	Percentage difference
	Mean	SD	Mean	SD	O	N		
1.0	1.3×10^{-3}**	1.5×10^{-4}	4.8×10^{-5}*	1.6×10^{-5}	8.1×10^{-5}	3.4×10^{-6}	23.7	50.4
2.0	8.9×10^{-4}	1.4×10^{-4}	3.4×10^{-5}	1.4×10^{-5}	5.6×10^{-5}	2.4×10^{-6}	22.9	48.7
3.0	8.9×10^{-4}	1.8×10^{-4}	8.8×10^{-5}***	1.4×10^{-5}	5.6×10^{-5}	6.3×10^{-6}	8.8	18.7
4.0	8.9×10^{-4}	1.8×10^{-4}	8.8×10^{-5}***	1.4×10^{-5}	5.6×10^{-5}	6.3×10^{-6}	8.8	18.7
5.0	1.1×10^{-3}*	1.1×10^{-4}	5.4×10^{-5}**	8.1×10^{-6}	6.9×10^{-5}	3.9×10^{-6}	17.8	37.9
Control	8.6×10^{-4}	1.3×10^{-4}	1.6×10^{-5}	1.3×10^{-5}	5.4×10^{-5}	1.1×10^{-6}	47.0	100.0

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

or 20 ppb of cadmium, the variations in the O:N ratio was high; the exception being the presence of 20 ppb of cadmium with 1.0 ppb of mercury where the trend was reversed.

Tables 68 a to d present the data obtained on the oxygen consumption, ammonia-nitrogen excretion and the O:N ratios of Donax incarnatus exposed to combinations of 5.0 or 20ppb ofcadmium, 0.5 or 2.0 ppb of copper and 1.0 to 5.0 ppb of mercury. Here also, the difference in oxygen consumption was significantly different only in a few cases, whereas that in ammonia - nitrogen excretion, it was high in many combinations. The O:N ratio was always found to be less than that recorded for the control animals. At a few instance, very low O:N ratios were recorded. The trend in the percentage difference in the O:N ratio was maximum in three combination experiments (Tables 68 b,c and d), whereas in the experiments conducted with the minimum quantity of cadmium and copper, the trend was reversed.

4.5 DISCUSSION

The relevance of investigations on the O:N ratio to understand the physiology of stress is mainly based on the basic concept that the respiratory and the excretory physiology during stress is responsible for the ultimate mal-functioning of the organic systems, leading to either elimination from the community or death of the established populations. The behavioural response of the bivalves could to a great extend affect the rate functions. For example, long periods of valve closure is commonly associated with behavioural inactivity and cessation of pumping activity.

The most obvious effects of this are limitation of time available for feeding and consequent growth potential, cessation of aerobic respiration and accumulation of excretory products. The avoidance reaction of bivalves as a result of environmental perturbations is, however, limited to the time that the animal remains isolated from the environment by complete valve closure. This also depends on the overall physiological and biochemical condition of the animal and its basic capacity to trigger adaptive mechanisms, so as to sustain the basal metabolic processes. These adaptations will include, the utilization of anaerobic respiration to sustain basal metabolism, availability of the stored reserves and the ability to tolerate and accommodate various levels of excretory products (Akberali and Trueman, 1985).

Further, using oxygen consumption rate as an index of stress so as to relate this with the nitrogen excretion also have limitations, because of the known principles that the ability to respire anaerobically varies in bivalve tissues, depending on the easy accessibility of oxygen from the environment or the respiratory system. It is known that the deeply located tissues have a greater tendency to respire anaerobically than superficially located tissues. It has also been suggested that in bivalve molluscs, some tissues may be adapted to function anaerobically, while others which are near to the sites of gas exchange are primarily aerobic. Among this category many species of Mytilus are included. When an animal is stressed, the degree of oxygen availability to the organism need not necessarily be a function of external oxygen concentration. Bathing of the tissues with aerated water could be reduced by any failure on

the pumping mechanisms or adductor muscle in capacitations. Therefore, to compensate reduced oxygen availability, the internal tissues can easily switch over to anaerobic respiration. In the common intertidal bivalves, the ability to survive stress condition is connected to a remarkably high tissue glycogen content, together with certain adaptations of their intermediary metabolism. Under anoxic conditions, the breakdown of glycogen or glucose to the level of phospho enol pyruvate although similar to the process in vertebrates, are with different breakdown products. The end products of anaerobic glucose catabolism in intertidal bivalve molluscs are succinate and alanine.

The physiological changes in species of Mytilus induced by stress has been extensively worked out (Bayne, 1971; Bayne and Thompson, 1970; Bayne, 1973 b). In a normal animal, three levels of oxygen consumption rates are identified, namely standard, routine and active.Those animals which are fed with an energy level above maintenance energy requirement, adjust their oxygen consumption rate to a routine level, whereas those fed with less energy compared to maintenance requirement reduce their oxygen consumption rate to a standard level.

Rates of excretion of ammonia -nitrogen vary seasonally. In unstressed animals, atomic ratio of oxygen consumed to ammonia-nitrogen excretion remains about 100 for most of the year, but can be much higher when the carbohydrate levels are high. When the carbohydrate levels are low, stress results in reduced O:N values, on the other hand when carbohydrate reserves are high the O:N ratio increases during stress. There-

fore, O:N ratio has been employed as a meaningful index of stress under variable nutritive conditions.

There is little information available on the range in which environmental factors may affect either the balance between the various nitrogenous end products or the rates of excretion. The most thorough investigations have dealt with the effects of reduced salinity, which can cause an increase in the rate of ammonia excretion by Macoma inconspicua (Emmerson, 1969), Mya arenaria (Allen and Garrot, 1971) and Mytilus edulis (Bayne, 1975 b). Ansell and Sivadas (1973) have documented differences in excretion rates with differences in animal size in Donax vittatus. These studies suggest a marked variability in the rates of nitrogen excretion by bivalves and wherever recordings of oxygen consumption have been made there is evidence that these two physiological processes do not always vary in the same direction nor to the same extend in response to changes in the environment. Normally an index of balance in the catabolism between the different nutrient reserves in the tissues is provided by ratio in atomic equivalents of oxygen consumed to nitrogen excreted, in the O:N ratio (Conover and Corner, 1968). The basic concept that could be put forward to explain the low O:N ratio is that the mussels rely more heavily on the catabolism of proteins than of non-protein substrates to meet the increased demands for energy, when the animal is subjected to a stress. However, we do not yet know whether a similar response could occur in the case of animals which are in the process of gametogenic cycle as those noticed in Perna indica kept under controlled conditions. The major draw back of comparison is that, taken together, the data

indicate very variable pattern of nitrogen excretion and consequently the O:N ratios and this appears to be a feature of bivalve molluscs. The O:N ratio can serve as a useful indicator of the physiological condition of the individual only if the underlying seasonal, gametogenic /storage cycle is taken into account. Assuming that the O:N ratio recorded for the control is normal for the season for Perna indica, increase in the O:N ratio can either indicate changes in the substrate utilization or variability in the oxygen consumption. The widely publicized assumption that an O:N ratio value of 30 or less is generally indicative of a stressed condition cannot be accepted in the present instance, since the control animals recorded O:N values around 8-14. The O:N values of 30 or above have been uniformly recorded for temperate animals where the reproductive cycle is well marked and distinct. In the case of Mytilus edulis, Mytilus californianus and Mytilus galloprovincialis, the reproductive cycles are well defined and equally spaced twice a year occurring during October-November and early Summer (July-August) (Widdows et al., 1981). However, it is felt that O:N ratio is not a suitable stress index during the period of reproductive cycle which is more or less continuous in the case of Perna indica. In the present case the size of Perna indica used for the experiment ranged between 20-25 mm and at many instances they were found to be mature. The variations in the O:N ratio by animals exposed to different concentrations of heavy metals can only suggest that the catabolic process was affected and that it is not clear whether the changes in the ratio noticed are as a result of heavy metal stress of varying concentrations. It may be noted that the concentrations of heavy metals used was rather low and hence might have affected the oxygen consumption rates but

need not have affected the catabolic processes directly.

Assessment of O:N ratios employing Donax incarnatus and the various metals gave information of a varying nature. In certain cases the ratios increased conspicuously in lower concentrations, suggesting lesser utilization of protein breakdown in these concentrations. It may be assumed that the animals have the option of making use of either protein or carbohydrate and that it is a matter of choice rather than availability. This is because all the animals employed for the experiments came from the same locality and presumably belonging to the same genetic structure, age and physiological grouping. Variations in the O:N ratio between metals when used singly in a way supports the assumption that the stress factor alone cannot control an animal's response reflected with reference to O:N ratios. Further, increase and decrease in the O:N ratios within the different range of concentrations also does not support the common assumption that the "end factor" can be utilised as a meaningful stressor or a parameter. Looking into the pattern of oxygen consumption makes it rather clear that the oxygen consumption showed clear cut stress effects. On the other hand, the rate of ammonia excretion did not register any specific trend. Assessing the metabolic responses of the mussels Perna viridis and Perna indica to declining oxygen tension at different salinities, Hawkins et al. (1987) stressed the importance of the response surface and natural distribution. They felt that the regulative capacity of oxygen consumption among bivalves, increases the likelihood of experiencing apoxia in nature (Bayne, 1973c;Mackey and Shumway, 1980). They recorded very low O:N ratios at full salinity in the case of Perna viridis, whereas values of about 50 in Perna indica which according to them are very

similar to temperate latitudes, which further indicates healthy nature of the animal. Commenting on the low O:N ratios exhibited by tropical bivalves, these authors felt that the requirement for carbon rich reserves may be reduced at lower latitudes, perhaps as a result of greater environmental stability. Commenting on the high rate of oxygen consumption, they felt that despite such a pronounced metabolism of protein, these animals are able to sustain high rate of oxygen consumption. The osmoregulatory capacity of tropical intertidal species is significant and hence can also influence the O:N ratio.

The O:N ratio of control animals varied between 22.3, 43.8 and 47.0. This shows that it is possible that the animals under laboratory conditions can resort to extremely varying ranges of O:N ratio. If it is to be assumed that these variations also are indices of utilization of either carbohydrate or protein, O:N ratio studies can represent meaningful results of stress. Further, very high or very low O:N ratio values as a function of stress forces us to assume that there is a clear-cut switching over of substrate utilization. It is not clear whether in the case of Donax incarnatus mobilization of stored energy plays an important role on the O:N ratios.

In the experiments with Donax incarnatus, the animals were starved before the measurement of nitrogen excretion as a precaution against contamination by faecal excretion during the experimental period. The nitrogen excretion rates as estimated did alter from high values to low values. In general, the only explanation that can be given is that where there was a reduction in the O:N ratio, the animal did resort to

protein catabolism or utilized glycogen as happens in anoxic condition, which can also produce more of ammonia and result in low O:N ratios. (Fellow and Hird, 1979).

In triad combinations, in general, the O:N ratio was lower than that of the control animals. However, here also the inter-concentration variability was unpredictable.

5
HISTOPATHOLOGY

5.1 INTRODUCTION

The effect of a toxic compound on an aquatic species could be direct, induced or indirect. A direct effect is caused by direct action of the toxic substance on the aquatic organism. The most obvious direct effect is acute and consists of irreversible damage to vital organ functions resulting in rapid morbidity and death. A chronic direct effect differs from an acute one in that the toxicant causes a sub-lethal change in the animal which may or may not be the eventual cause of death. Sub-lethal changes can occur from a single encounter or from continuous exposure to a toxicant over a long period of time. Chronic direct effects and induced effects of toxicant exposure are of particular importance to those concerned with the aquatic environment. Many toxicant induced tissue pathology examined have been non-specific and this is not surprising as aquatic toxipathology is in its infancy. As the science matures and more descriptive studies are made by combining histopathological results with the results of biochemical and physiological studies, the complete reaction of an aquatic organism to a toxicant may be defined for future diagnostic purposes. It was with this intention that histopathology was

included in the present investigation.

5.2 REVIEW OF LITERATURE

Bivalves can be used to assess the quantity of heavy metals available in the aquatic environment (Bryan, 1976 b). The capacity of the bivalves to accumulate trace metals and other toxicants has led to the selection of this group as an important bioindicator. Recently marine molluscs have been widely used to investigate the rate of accumulation and depuration of heavy metals, notwithstanding several limitations. Pasteels (1968) demonstrated that two iron metallo-protein, ferritin and peroxidase were pinocytosed by the gill epithelia of Mytilus edulis. Studies by George and Coombs (1977) showed that iron is accumulated principally in the viscera with a smaller but significant portion in the gills. Electron microscopic studies have revealed that vesicles are formed within cells to enclose the excess metal. (Coombs and George, 1978). Viarengo et al. (1984) characterised the copper thionein isolated from the tissues of mussels exposed to metals. Employing multi-element analysis using electron microscopy with an energy dispersive x-ray analyser, Ishii et al. (1985 a) found granules containing extremely large quantities of manganese and cadmium in the kidneys of marine bivalve Cyclosunetta menstrualis. Ishii et al. (1985 b) by analytical electron microscopy found that iron, copper and sulphur were localised in granules of the epithelial cells of the oyster tissue. Ishii et al. (1986) found that spherical fine granules of trace metals appear extracellularly beside microvilli and develop into larger granules while moving to the centre of the lumen of the kidney tubles.

Aquatic organisms are extremely vulnerable to toxic effects resulting from absorption or oral intake of these contaminants from the immediate environment. Various chemical compounds have been investigated to determine their potential toxicity to aquatic organisms, especially in fish and to a lesser degree in shell fishes. Unfortunately, toxicological studies of aquatic organisms have not revealed many tissue pathologies useful in diagnosing exposures to specific compounds. Since there is a general lack of information on the histopathology of molluscs subjected to heavy metal stress, the literature on fishes are extensively quoted here, to have a general idea on histopathology. Lesions have been extremely non-specific and merely indicative of toxic insult. Histopathological studies on the gills of fishes exposed to different heavy metals produced non-specific microscopic lesions which include, epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, edema with epithelial seperation from basement membranes, general necrosis and/or epithelial desquamation (Gardner and Yevich, 1970; Eisler and Gardner, 1973; Baker, 1969; Bhatnagar and Shrivastava 1975; Establier et al., 1978 a; Wobeser, 1975 a; Establier et al., 1978 b; Wobeser, 1975 b and Gilderhaus, 1966).

Heavy metal exposure of fishes have been shown to initiate several changes in the liver. They include, hepatotoxic lesions of fatty infiltration, nuclear or general hypertrophy of hepatocytes, cytoplasmic vacuolation, cellular pleiomorphism, deposition of bile or ceroid pigments, hydrophic degeneration, loss of hepatic glycogen, coagulative hepatocyte necrosis, sinusoidal and vascular congestion, loss of normal muralial architecture, degeneration or necrosis of biliary epithelium and perivascular

or periportal fibrosis (Trump et al., 1975; Tafanelli and Summerfelt, 1975; Gardner and LaRoche, 1973; Baker, 1969; Sastry and Gupta, 1978b; Establier et al., 1978 a and c; Gutierrez et al., 1978; Sastry and Gupta, 1978 a; Establier et al., 1978 b; Kendall, 1977 and Gilderhaus, 1966).

Several cytological changes are found to occur in the kidney following heavy metal stress in fishes. They are nephrotoxic lesions including the degenerative changes in tubular epithelium (cytoplasmic vacuolation, hydrophic degeneration, hyperchromatic nuclei), dilation of tubular lumina, proteinaceous or cellular casts within tubular lumina, tubular necrosis and/or epithelial desquamation, necrosis of interstitial haematopoetic tissues and excessive development of melano-macrophage centres (Gardner and Yevich, 1970; Gutierrez et al., 1978; Hawkins et al., 1980; Newman and Maclean, 1974; Tafanelli and Summerfelt, 1975; Eisler and Gardner, 1973; Gardner and LaRoche, 1973; Baker, 1969; Bhatnagar and Shrivastava, 1975; Establier et al., 1978 c; Trump et al., 1975; Establier et al., 1978 a; Wobeser, 1975 b).

The toxic lesions most commonly reported in the intestine of fishes include, hyperemia, degenerative changes in tips of villi, loss of structural integrity of mucosal folds, degeneration of mucosal epithelium and/or various smooth muscle layers, necrosis and/or desquamation of mucosal epithelium, cellular debris and excessive mucous in gut lumen, increased numbers of mucous goblet cells, vacuolation or necrosis of sub-mucosa, degeneration or necrosis of sub-mucosal vasculature, inflammatory infiltration of sub-mucosa and/or lamina propria (Gardner and Yevich, 1970; Trump et al., 1975; Newman and Maclean, 1974; Establier et

al., 1978a and c; Gutierrez et al., 1978; Sastry and Gupta, 1978 a; Establier et al., 1978 b; and Sastry and Gupta, 1978 b).

Establier et al. (1978 b) observed that following exposure to $CdCl_2$, the hepatopancreas (digestive diverticula) in some invertebrates showed atrophy, reduction in height of tubular epithelium, tubular dilation, necrosis and desquamation of tubular epithelium.

Studies by Sangalang and O'Halloran (1972) and Tafanelli and Summerfelt (1975) indicated that exposure of fishes to $CdCl_2$ produced the following changes in the testes. Stimulation of spermatogenesis and exhaustion atrophy, development of ova like cells within follicles, general atrophy and hyperspermia, necrosis of tubular boundary cells with haemorrhage, vasodilation and congestion, increased numbers of infiltrating macrophages with phagocytised debris, necrosis of primary germ cells with atrophy of seminiferous tubules, fibrosis and infiltration of mononuclear inflammatory cells.

Ovaries of fishes showed several toxic lesions resulting from heavy metal exposure. They are hyperplasia of germinal epithelium and involution of some ova, decreased frequency of oocyte maturation, cytoplasmic clumping and fragmentation and karyolysis of ova (Tafanelli and Summerfelt, 1975; Gilderhaus, 1966).

Soluble salts of mercury are noted for extreme nephrotoxicity and certain fishes are susceptible as well. [eg. hogchoker, Trinectes maculatus (Trump et al., 1975); mummichog (Wassermann and Koepp, 1977)]. Studies by Wobeser (1975 b) and Establier et al. (1978a and c) showed that mercury

and methyl mercury exposure of three species of fish resulted in both tubular and glomerular lesion.

Gills in fishes showed ultrastructural changes following exposure to $CuSO_4$ (Baker, 1969). The changes include, chloride cell degeneration with hypertrophied perinuclear spaces and smooth endoplasmic reticulum, mitochondria having disorganised cristae and ruptured membranes, formation of autophagosomes, vacuoles, mylein like bodies and apical vesicles in epithelial cytoplasm. Auffret (1988) observed that Mytilus edulis exposed to high concentrations of diesel oil and copper mixture had severe degenerative changes in the epithelia of the digestive gland. The author further noted that these mussels exhibited morphological changes in their gill filaments and severe disturbance of the ciliated epithelial cells, when compared to mussels from control and low exposure treatments. Similar lesions have been reported in mussels exposed to sub-lethal thermal stress (Gonzales and Yevich, 1976) and to copper and cadmium exposure (Sunila, 1986). Couch (1985) described atrophic epithelium sloughing of cells and necrosis as an ultimate tubular degeneration in oysters from contaminated estuaries.

Sorensen (1976) observed the following changes in the liver after exposure to Na_2HAsO_4. Distinct vacuolation of hepatocytes, enlargement and/or vesiculation of rough endoplasmic reticulum, presence of circular arrays of smooth surfaced membranes and mylein like bodies in the hepatocyte cytoplasm.

Regression of reproductive tissues in response to combinations of thermal, osmotic and nutritional stressors has been described in Mytilus

edulis (Bayne et al., 1978). A tissue response, involving the occlusion of the vascular haemolymph system of Mytilus edulis by granular blood cells has been shown to be associated with sites of chronic environmental pollution (Lowe and Moore, 1979). Presumptive neoplastic conditions have been reported in oysters and mussels from polluted areas (Farley, 1969 a and b; Farley and Sparks, 1970; Mix, 1976).

The effects of ionising radiations on the digestive and reproductive systems in oysters have been described by Mix and Sparks (1970, 1971 a and b) and Mix (1972). These effects included haemocytic infiltration, loss of digestive tubule epithelial cells, abcesses and mitotic inhibition. Investigations of tissue regeneration following brief irradiation have revealed cellular division and repopulation of digestive tubular epithelium with normal cells (Mix and Sparks 1971 b; Trenholm and Mix, 1978). Pearse (1972) suggested that metals such as copper, zinc, lead, mercury and iron can be localised in specific cellular sites.

5.3. MATERIAL AND METHODS

This section of the thesis centred around delineating the effects of exposure to sub-lethal concentrations of mercury, copper and cadmium, individually and in combination on the tissues of Perna indica and Donax incarnatus.

The details of the test animals, test media, laboratory conditioning of animals, toxicants etc. have been given earlier (Section 2.3).

5.3.1 HISTOLOGICAL STUDIES

5.3.1.1 **Exposure for periods upto 21 days**

Perna indica, pre-exposed to the highest recorded sub-lethal concentrations such as 10 ppb of mercury, 6.0 ppb of copper and 400 ppb of cadmium and Donax incarnatus to 5.0 ppb of mercury, 6.0 ppb of copper and 40 ppb of cadmium, both individually and in combination, for periods upto 21 d were utilised for the study. Ten test animals were exposed to the test concentrations in fibre glass tubs of 10 l capacity containing 5 l of the toxicant solution. The animals were fed with the algae Synechocystis salina, during the period of exposure. After termination of the exposure period, the animals were dissected and the soft tissues of the whole animal were carefully scooped out and fixed in Bouin's fixative for 24 h. The foregoing time-chart was followed for making paraffin blocks.

1. Washed overnight in running water.
2. The tissues were then treated with a saturated solution of lithium carbonate in 70% alcohol, to remove the yellow colour of the picric acid.
3. After softening, the tissues were washed in 70% alcohol and transferred to 90% alcohol for 2 h.
4. Transferred to 95% alcohol for 1 h.
5. Transferred to absolute alcohol (2 changes) for 1 h each.
6. Placed the tissues in 1:1 mixture of absolute alcohol and methyl benzoate for 30 min.
7. Cleared in methyl benzoate until the tissues became transparent.
8. Placed the tissues in benzene for 15 min.
9. The tissues were then transferred to benzene, saturated with

paraffin wax of melting point 58-60°C for 6 h.

10. Infiltered the tissues in 2-3 changes of molten paraffin wax of melting point 58-60°C for 1 h each.

11. Embedded in paraffin wax of melting point 60-62°C.

The blocks were sectioned at 10 µm thickness. For histological studies, the stain was Papanicolaou stain.

5.3.1.2 **Staining technique followed with Papanicolaou stain**

1.	Dewax sections in xylene	: 5	min.
2.	Rinse in absolute alcohol	: 2	"
3.	Rinse in 70% alcohol	: 2	"
4.	Rinse in tap water	: 5	"
5.	Stain in Harris' haematoxylin	: 3	"
6.	Wash in tap water until sections are blue	: 5	"
7.	Differentiate in 0.1% HCl solution		
8.	Blue in ammonia water		
9.	Wash in tap water	: 2	"
10.	Rinse in 70% alcohol	: 1	"
11.	Rinse in 95% alcohol	: 1	"
12.	Stain in Papanicolaou O.G.C. solution	: 3	min.

13.	Rinse in 95% alcohol	:	1 min.
14.	Stain in Papanicolaou E.A. 50 solution	:	3 "
15.	Rinse in 95% alcohol	:	1 "
16.	Rinse in absolute alcohol	:	2 "
17.	Clear in xylene	:	2 "
18.	Mount in Canada balsam.		

5.4 RESULTS

The animals exposed to various concentrations and combinations of metals have been subjected to a thorough histological study with reference to gill and gastric diverticula (digestive tubule) to assess morphological variations, if any. The photomicrographs obtained from control animals are tallied with those of stressed animals and the inferences are presented here.

It is known that it is possible to observe structural alterations in individual cells or groups of cells at an early stage of a stress response, before an integrated cellular alteration would manifest itself at the level of the whole animal's physiological processes. However, some of these changes are generative whereas others are indicative of a specific or a particular type of stressor. Therefore, histopathological studies form an important aspect of investigations of stress response in marine invertebrates. When cells are stressed, they undergo a series of irreversible biochemical and cellular changes. To a very great extend, these changes are indicative

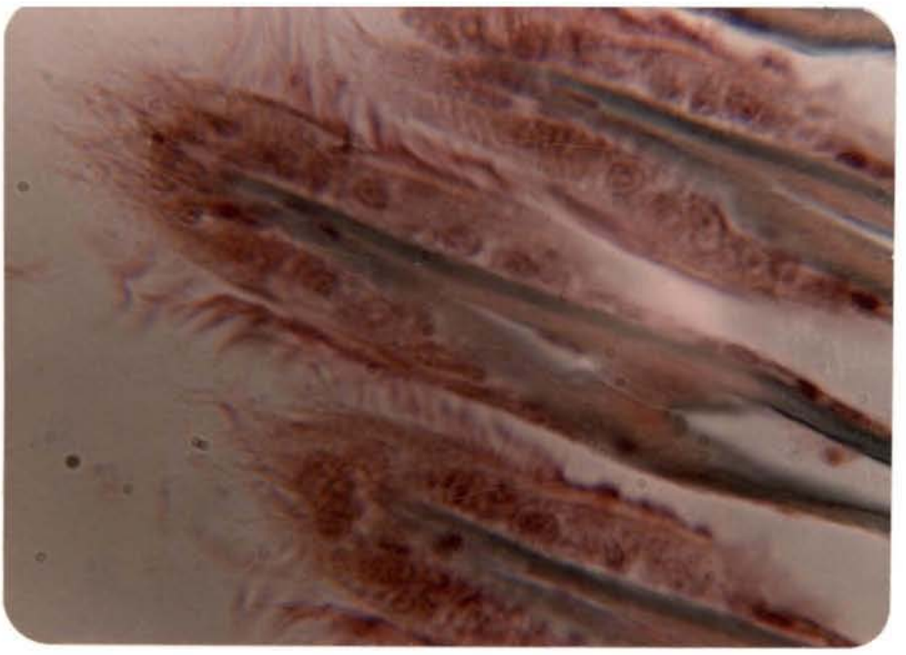
of the degree of stress and the adaptive capacity of the organisms.

Respiration and digestion are important physiological responses of marine invertebrates used to assess stress response. Therefore, it is logical to study variations in the tissues responsible for the above functions to assess the effects of pollutional stress.

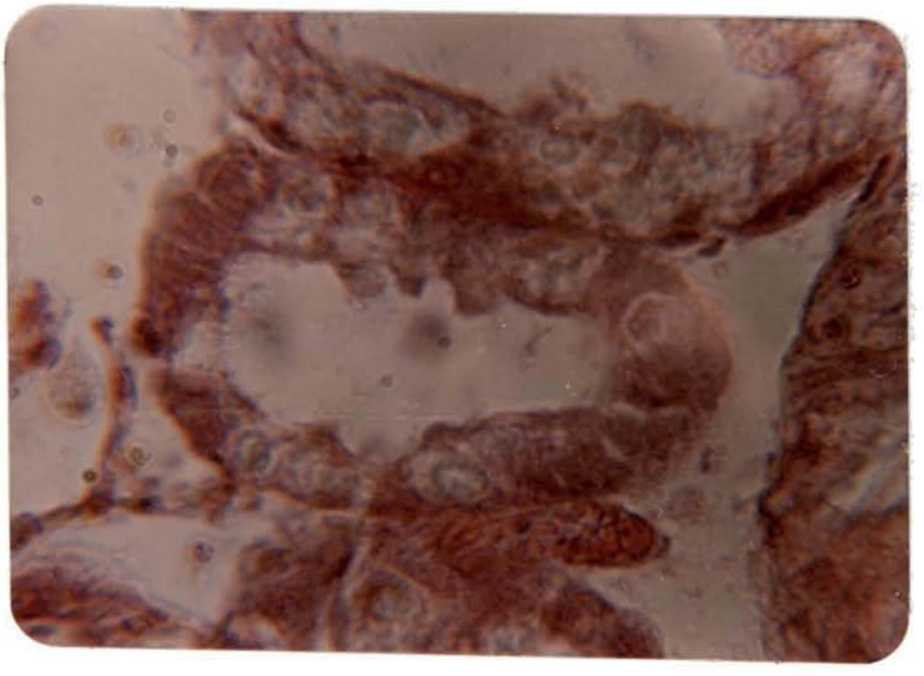
5.4.1 PERNA INDICA

Photomicrographs 1 and 2 indicate the normal structure of the gill filaments and gastric tubules prepared from the tissues of control animals. It is evident from the photomicrograph that the gill filaments show a normal structure of a bivalve ctenidium with the central core tissue of the filaments and well arranged epithelial cells having lateral and frontal cilia. It is also noticed that the lateral cilia more or less overlap with the adjacent filaments. The epithelial cells are normal with laterally placed nuclei. The epithelial cells of the digestive gland tubules are arranged normally and there is no enlargement of primary lysosomes or the appearance of secondary lysosomes.

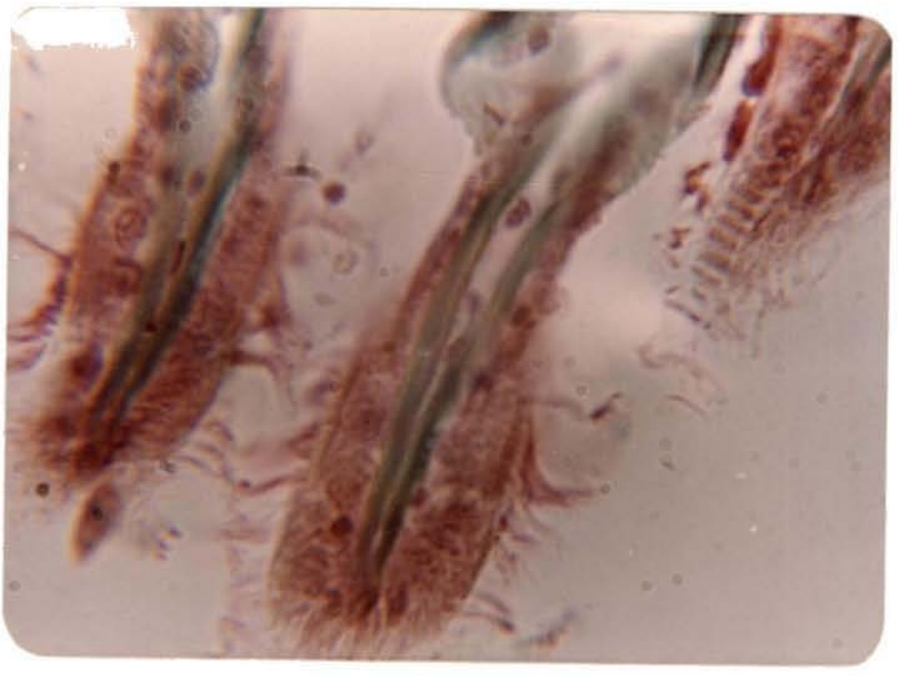
The structure of the gill filaments and digestive tubules of Perna indica exposed to 10 ppb of mercury for a period of 21 d is depicted in photomicrographs 3 and 4. It is evident from the sections that the lateral and frontal cilia have sloughed off and the lumen of the gill filaments had blood cells. Further, spaces have developed between the gill lamina and the cells. The digestive tubule showed degeneration and the vacuolar nature of the cells indicate atrophy which may be an effect of metal toxicity.



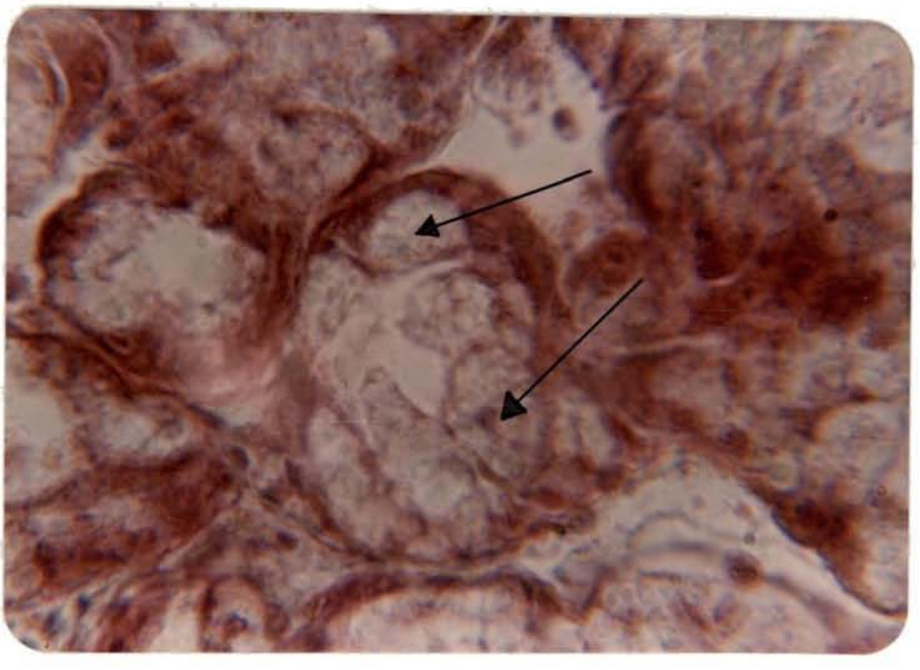
Photomicrograph 1. C.S. of gill of Perna indica (control) x 1000.



Photomicrograph 2. C.S. of digestive tubule of Perna indica (control) x 1000.



Photomicrograph 3. C.S. of gill of Perna indica exposed to 10 ppb of mercury for a period of 21 d x 1000.

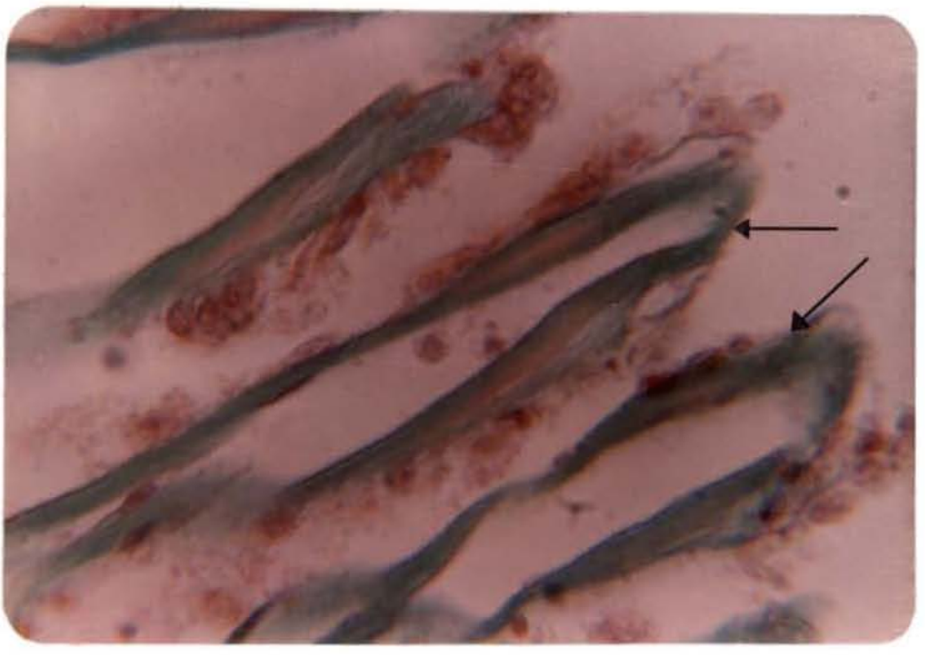


Photomicrograph 4. C.S. of digestive tubule of Perna indica exposed to 10 ppb of mercury for a period of 21 d x 1000. Arrows indicate vacuolated epithelial cells.

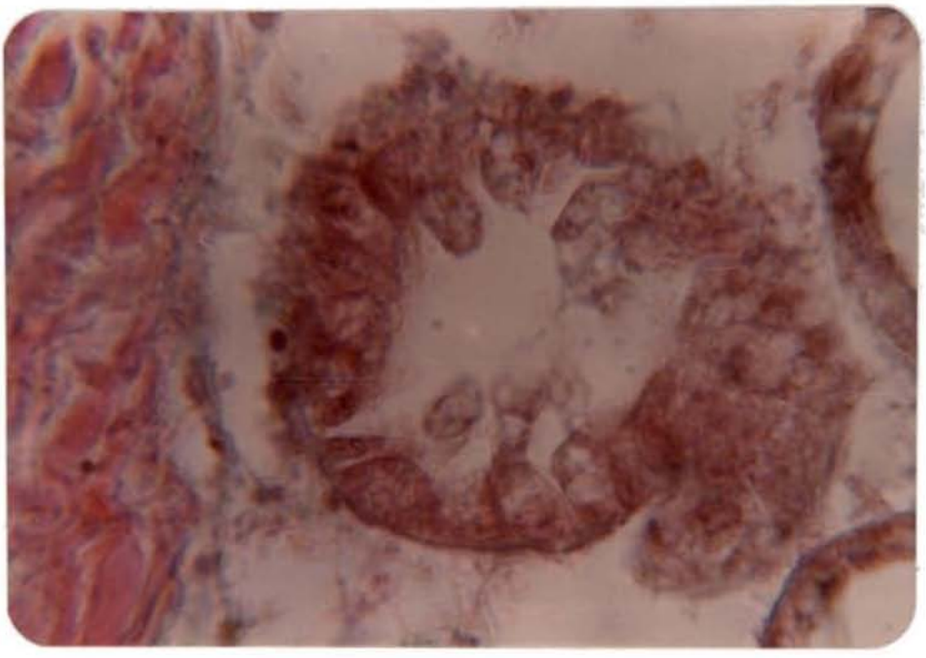
The structural deformity of the gill and digestive tubules of animals exposed to 6.0 ppb of copper for longer duration (21 d) is shown in photomicrographs 5 and 6. It is clear from the micrographs that the gill filaments have been thoroughly damaged and the cells have been completly destroyed with unusual bulging of the lumen of the gill filaments. This is a major damage in the gills. The changes that occurred in the digestive tubule in response to copper exposure mainly relates to total damage of cells. However, it is not clear whether secondary lysosomes have developed although vacuolar regions are seen in the sections.

Photomicrographs 7 and 8 show the sections of gills and digestive tubules of animals exposed to 400 ppb of cadmium for 21 d. A conspicuous feature of the gill filaments was the bulging of the distal ends. This evidently was the effect of formation of space inside the filamentar lumen, together with the atrophy and sloughing off of the median and proximal cells on the filaments. The distal cilia were found missing although lateral cilia were found clumped together. The damage that occurred to the digestive tubules was not very clear although certain cells in the tubule showed atrophy.

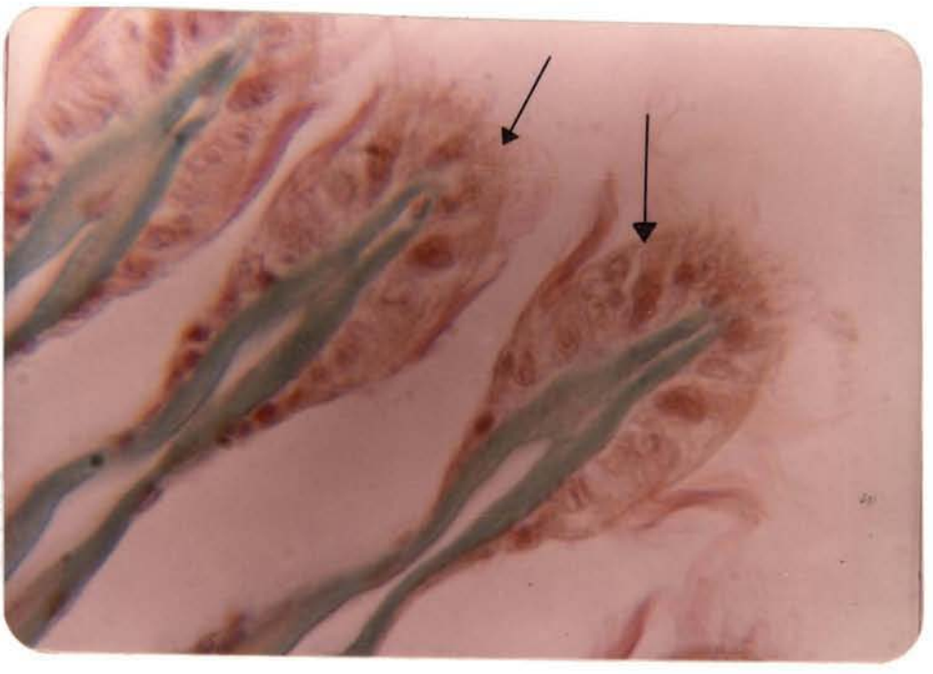
Perna indica was exposed to a combination of mercury and copper for 21 d and the morphological variations or pathological effects on the gills and digestive tubules assessed. Here in the case of gill filaments, it was noticed that the filaments have become distorted, especially the central lumen. No conspicuous cellular damage was noticed although distal cilia were found to be damaged. Total degeneration of digestive tubules was indicated and the lumen contained dislodged cells. However,



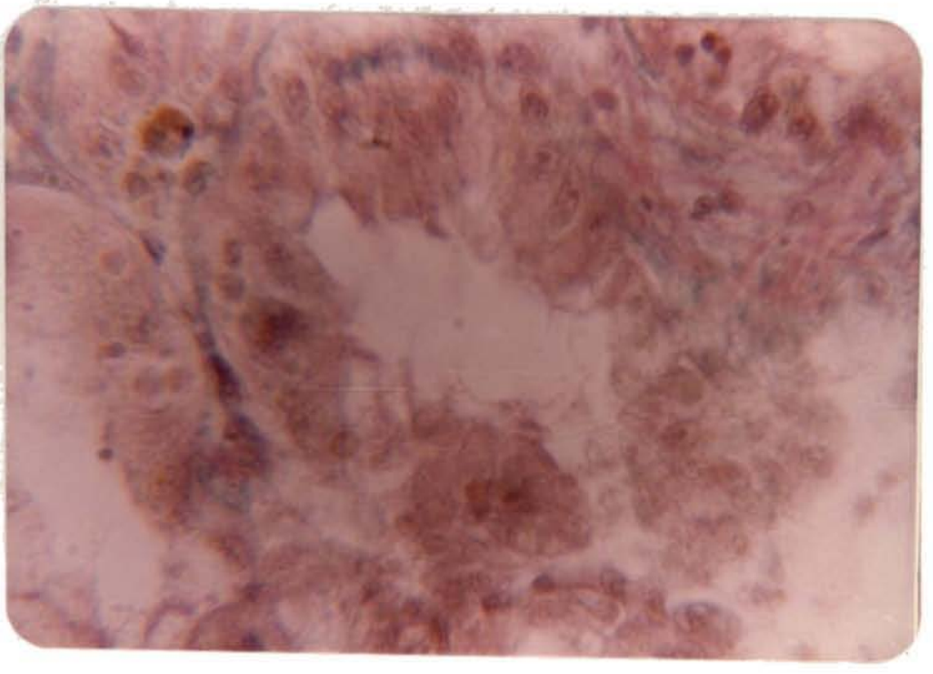
Photomicrograph 5. C.S. of gill of Perna indica exposed to 6.0 ppb of copper for a period of 21 d x 1000. Arrows indicate damaged gill filaments.



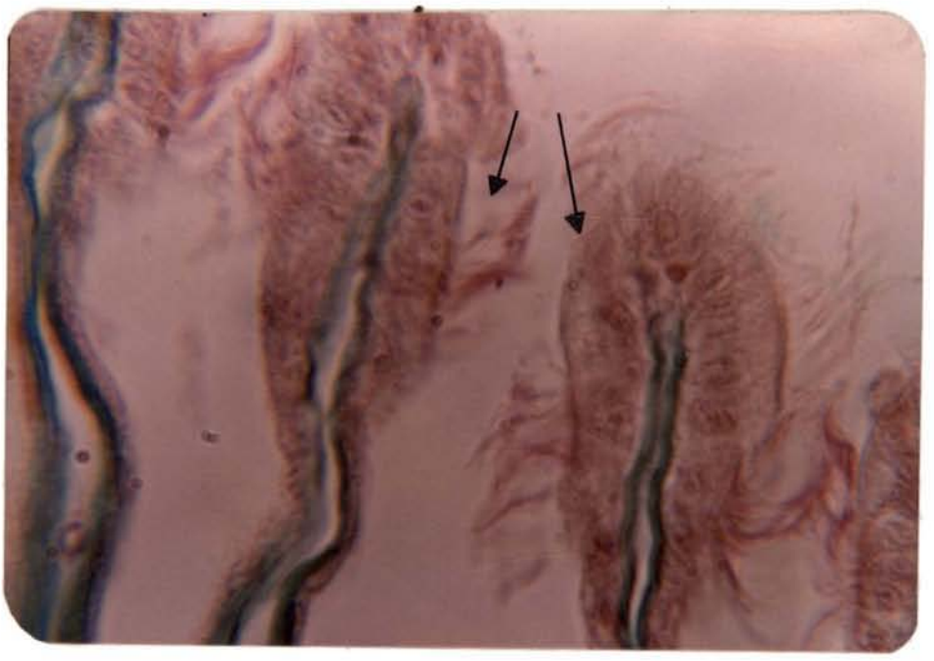
Photomicrograph 6. C.S. digestive tubule of Perna indica exposed to 6.0 ppb of copper for a period of 21 d x 1000.



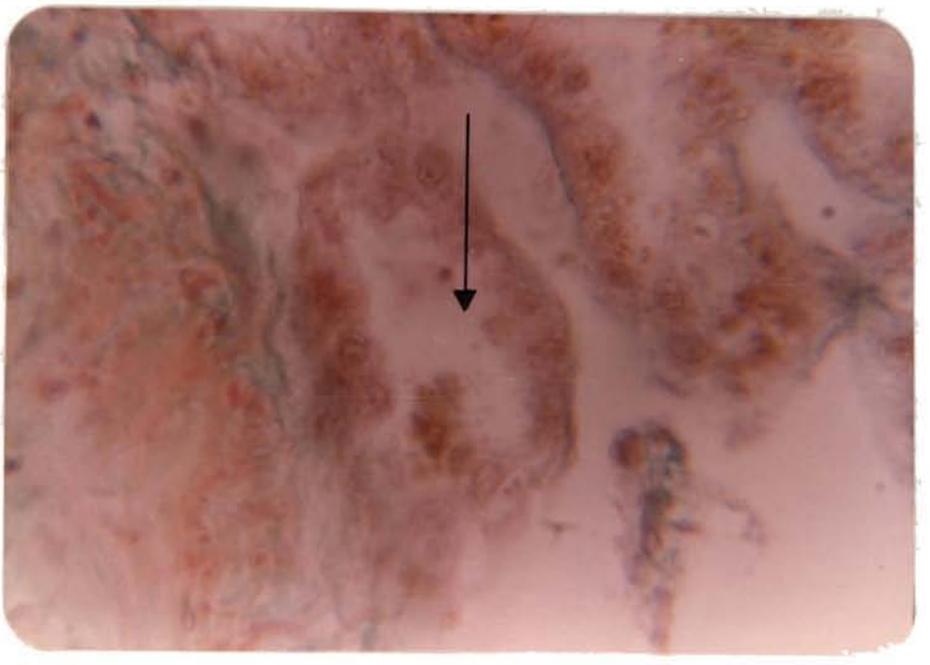
Photomicrograph 7. C.S. of gill of Perna indica exposed to 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate enlarged epithelial cells.



Photomicrograph 8. C.S. of digestive tubule of Perna indica exposed to 400 ppb of cadmium for a period of 21 d x 1000.



Photomicrograph 9. C.S. of gill of Perna indica exposed to 10 ppb of mercury, along with 6.0 ppb of copper for a period of 21 d x 1000. Arrows indicate damaged gill filaments.



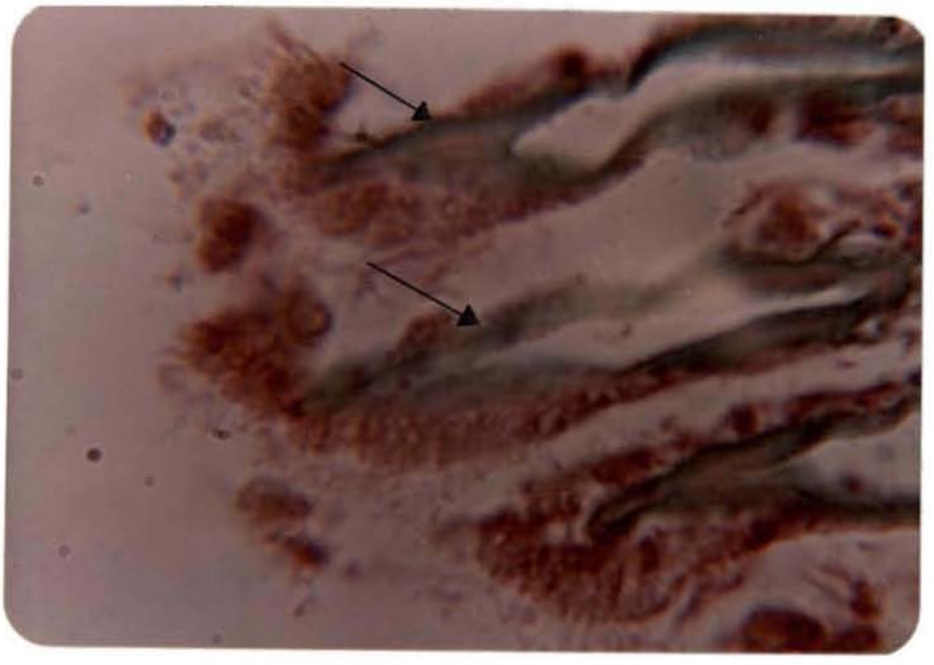
Photomicrograph 10. C.S. of digestive tubule of Perna indica exposed to 10 ppb of mercury, along with 6.0 ppb of copper for a period of 21 d x 1000. Arrows indicate disintegrated digestive tubules.

enlargement of cells was not evident (Photomicrographs 9 and 10).

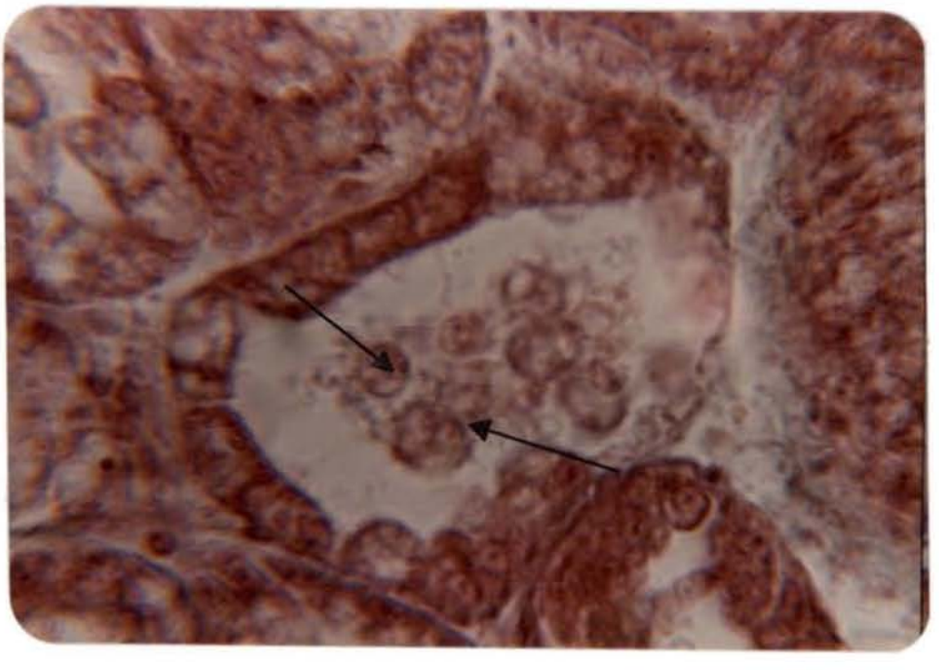
When exposed to a combination of mercury and cadmium for a longer duration, the damage of the gill filaments was conspicuous which ranged from a total atrophy of cells, removal of cilia and enlargement of the lumen of the gill filaments. It is clear from the photomicrographs 11 and 12 that the cells of the filaments were totally damaged, resulting in partial removal of cells from the filaments. In the case of gastric tubules, the formation of secondary lysosomes in the form of large vacuoles and dislodgement of cells, the same being deposited in the lumen were the two conspicuous effects.

Perna indica, when exposed to a combination of copper and cadmium for 21 d showed deformation of the gill filaments and extensive vacuolization of digestive tubules (See photomicrographs 13 and 14). The damage of the gills was mainly enlargement of the gill filamentar tips and rupture of the filamentar lumen. While the general shape of the digestive tubules remained unaltered, large vacuoles were found inside the cells. Certain portions of the tubules were damaged in the sense that the cells were found dislodged.

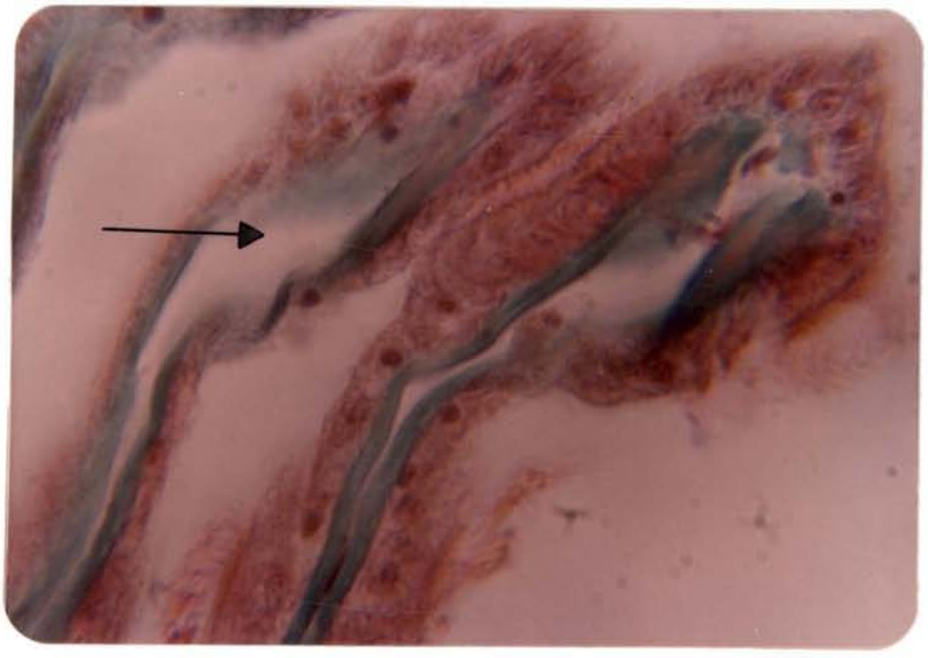
When the animals were exposed to a triad combination of mercury, copper and cadmium for a period of 21 d, it was noticed that the gill filaments were totally damaged and would have become non-functional. No epithelial cells were found intact on the gill filaments. Further, rupture of the filaments occurred because of excessive enlargement of the lumen. The digestive tubule also showed extensive disintegration of cells, giving the indication that the number of funtional cells was rather limited. There



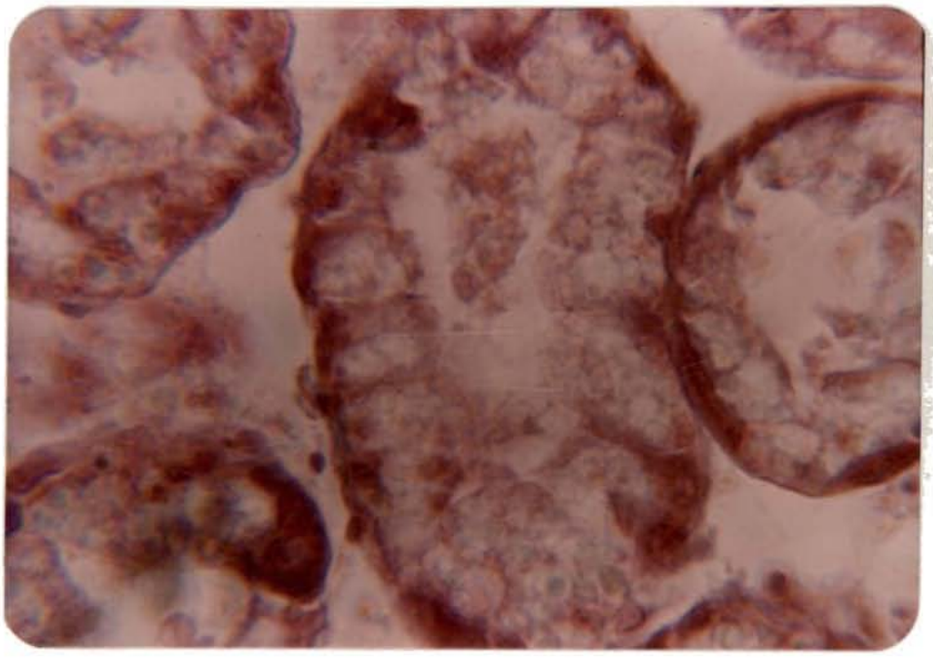
Photomicrograph 11. *C.S. of gill of <u>Perna indica</u> exposed to 10 ppb of mercury, along with 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate denuded gill filaments.*



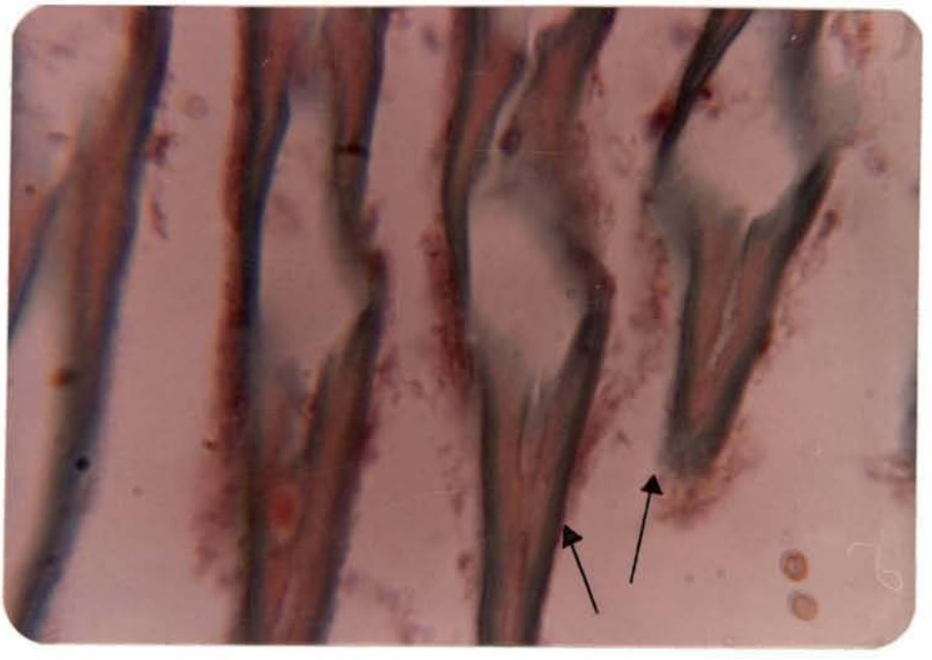
Photomicrograph 12. *C.S. of digestive tubule of <u>Perna indica</u> exposed to 10 ppb of mercury, along with 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate sloughing off of epithelial cells.*



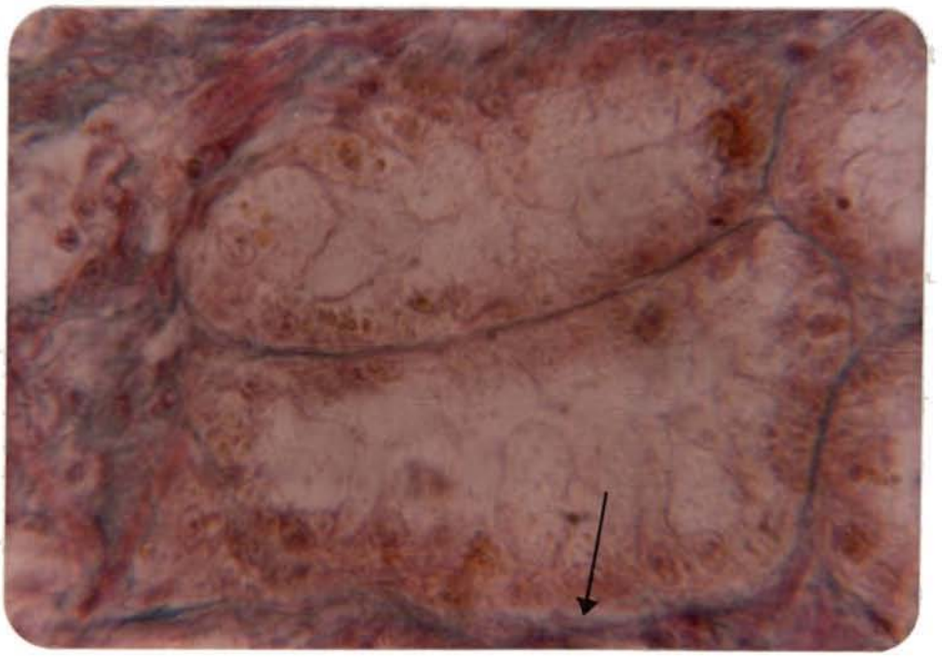
Photomicrograph 13. C.S. of gill of Perna indica exposed to 6.0 ppb of copper, along with 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate enlarged filamentar tips.



Photomicrograph 14. C.S. of digestive tubule of Perna indica exposed to 6.0 ppb of copper, along with 400 ppb of cadmium for a period of 21 d x 1000.



Photomicrograph 15. C.S. of gill of Perna indica exposed to 10 ppb of mercury, 6.0 ppb copper, along with 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate denuded gill filaments.



Photomicrograph 16. C.S. of digestive tubule of Perna indica exposed to 10 ppb of mercury, 6.0 ppb of copper, along with 400 ppb of cadmium for a period of 21 d x 1000. Arrows indicate dislodged epithelial layer.

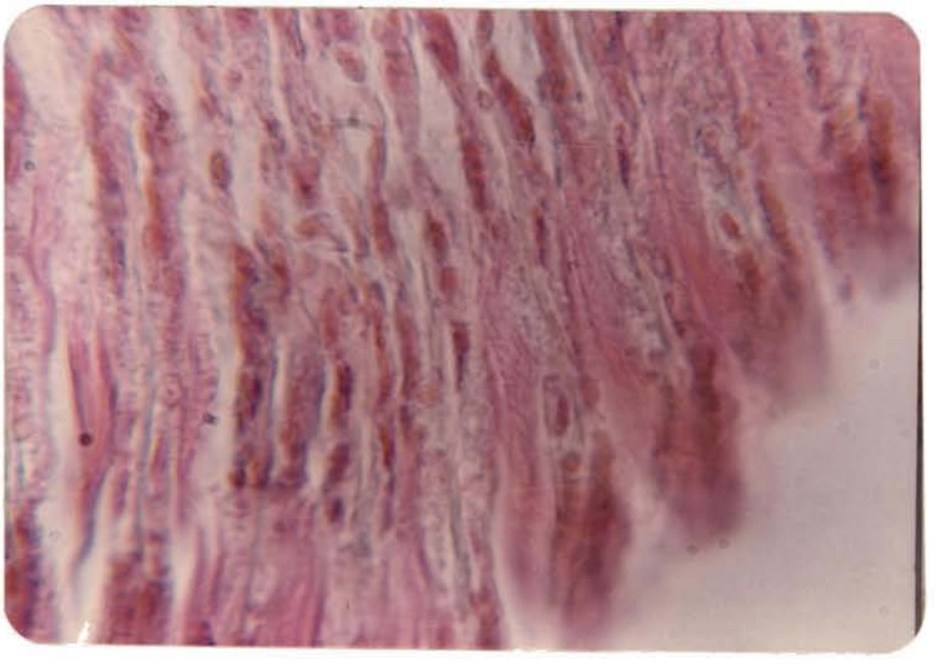
are indications of extensive vacuolization of some cells (Photomicrographs 15 and 16).

5.4.2 DONAX INCARNATUS

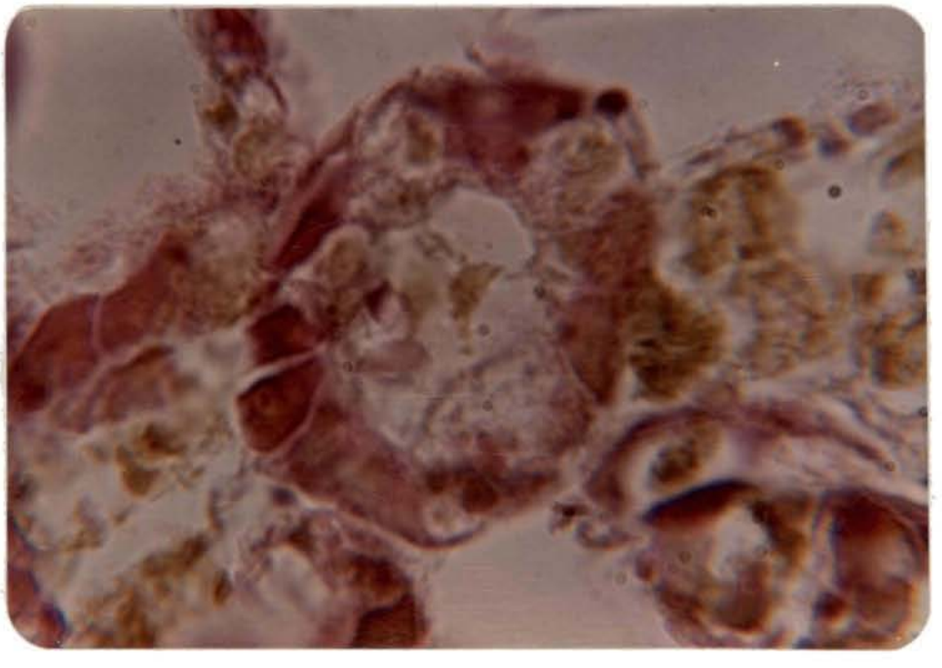
Information on the histology of Donax species from the tropical regions is totally lacking. Therefore, the results obtained here are explained just based on the photomicrographs.

A normal gill filament of Donax incarnatus compares well with Perna indica in that the cilia, both frontal and lateral are profusely distributed along the tips of the gill filaments. However, from the nature of the cilia, they look to be smaller and more in number and hence indistinct when less enlarged. The digestive tubules have structure very close to that of Perna indica, containing cells which are either digestive or secretory in nature, the former performing phagocytosis and the latter supplying digestive juices for luminar digestion (See photomicrographs 17 and 18).

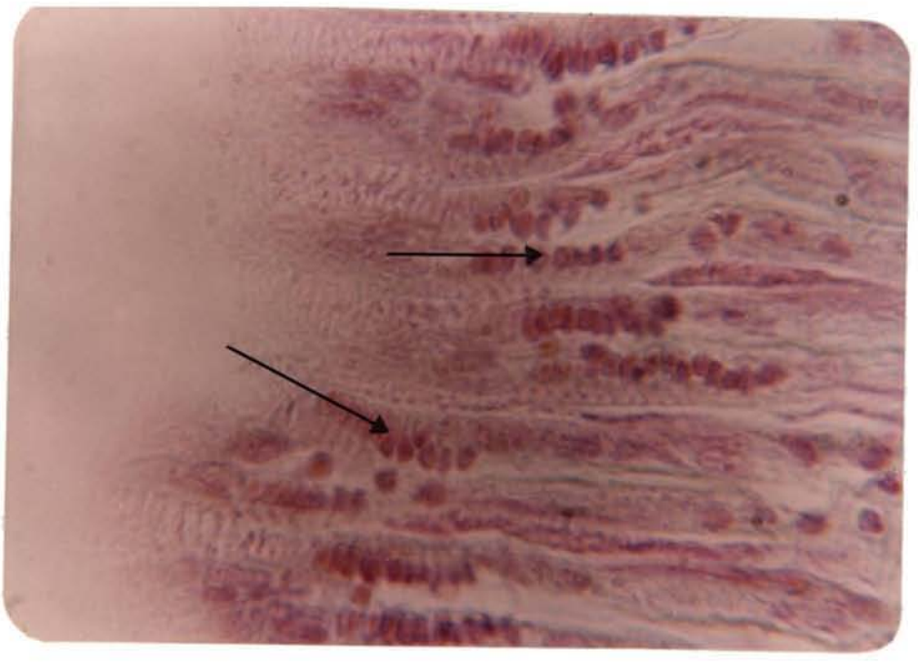
The photomicrographs 19 and 20 are those of animals exposed to 5.0 ppb of mercury for 14 d. The damage that has occurred to the gill is confined to the internal morphology, in that the lumen has shown slight enlargement. Dislodgement of cilia was not noticed. The digestive tubule contained cells which seems to have been pinched off from the tubular wall. However, the tubule contained cells with food at various stages of digestion. The lumen is filled with cells nipped off from the tubular walls. No conspicuous enlargement of secondary lysosomes could be seen.



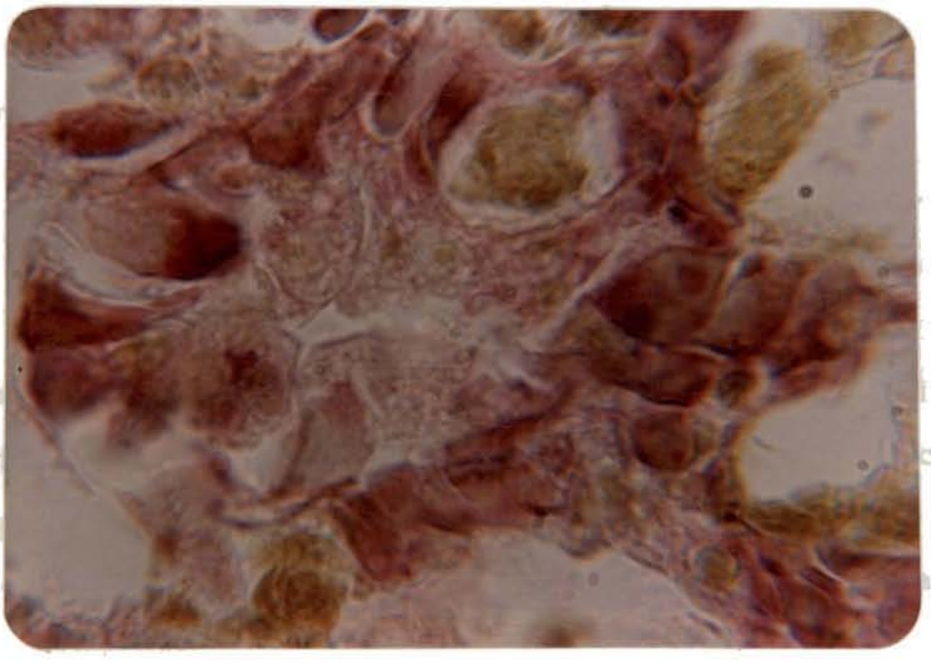
Photomicrograph 17. C.S. of gill of Donax incarnatus (control) x 1000.



Photomicrograph 18. C.S. of digestive tubule of Donax incarnatus (control) x 1000 .



Photomicrograph 19. C.S. of gill of Donax incarnatus exposed to 5.0 ppb of mercury for a period of 14 d x 1000. Arrows indicate wandering haemocytes.



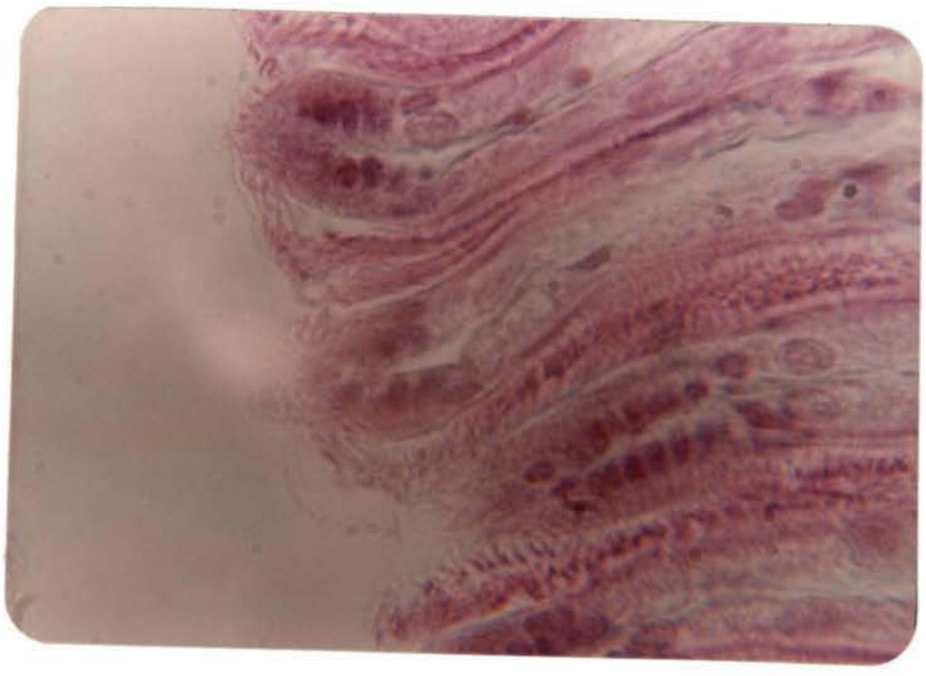
Photomicrograph 20. C.S. of digestive tubule of Donax incarnatus exposed to 5.0 ppb of mercury for a period of 14 d x 1000.

Contrary to the findings obtained from mercury exposed animals, Donax incarnatus exposed to copper (6.0 ppb) gave indications of more extensive cellular damage. This was very true in the case of digestive tubules. Vacuolization of tubular cells was rampant and the digestive processes were at low key evidenced from the digested cells. In the case of gills, sloughing off of cilia was noticed on the lateral side of the gill filaments. The filamentar lumen showed only limited extend of enlargement (See photomicrographs 21 and 22).

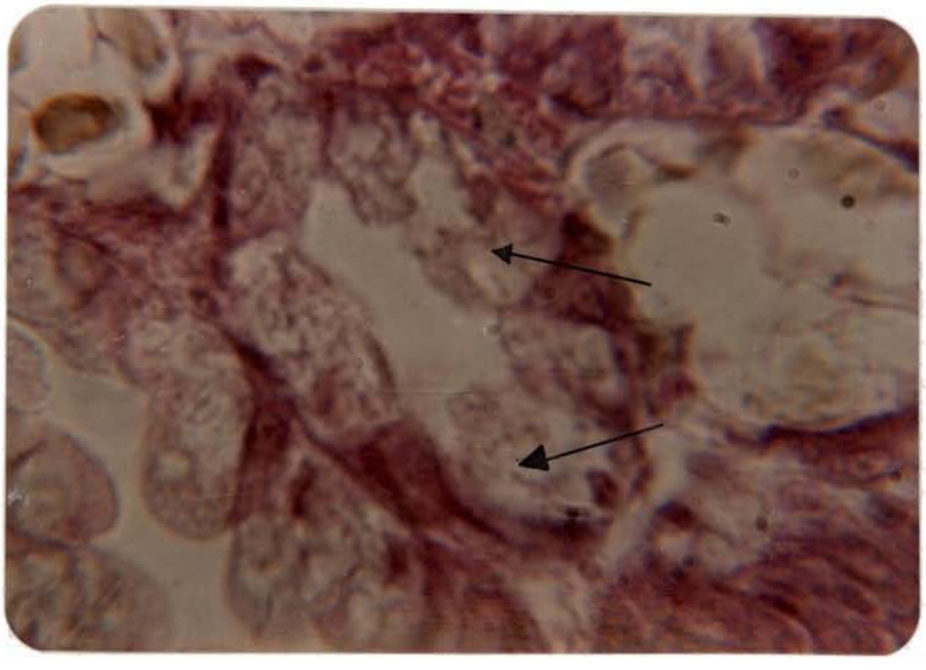
Curiously enough, the animals exposed to cadmium (40 ppb) showed extensive damage of gills and gastric digestive tubules. Sloughing off of the frontal cilia and the enlargement of gill filamentar cells and the enlargement of blood cells were distinct in the sections. The digestive tubule depicted extensive damage with majority of cells at various stages of destruction. It is assumed that these cells are secretory in nature (Photomicrographs 23 and 24).

Donax incarnatus exposed to a combination of mercury and copper also showed extensive damage of both gills and tubules (Photomicrographs 25 and 26). In the case of gills, enlargement of blood cells, lumen of the gill filaments and sloughing off of frontal cilia were indicated. In the case of digestive tubules, many cells had developed vacuoles. Dislodged cells were found in the lumen of the digestive tubules. The empty cells might be indicative of the fact that they are incapaciated for normal digestion.

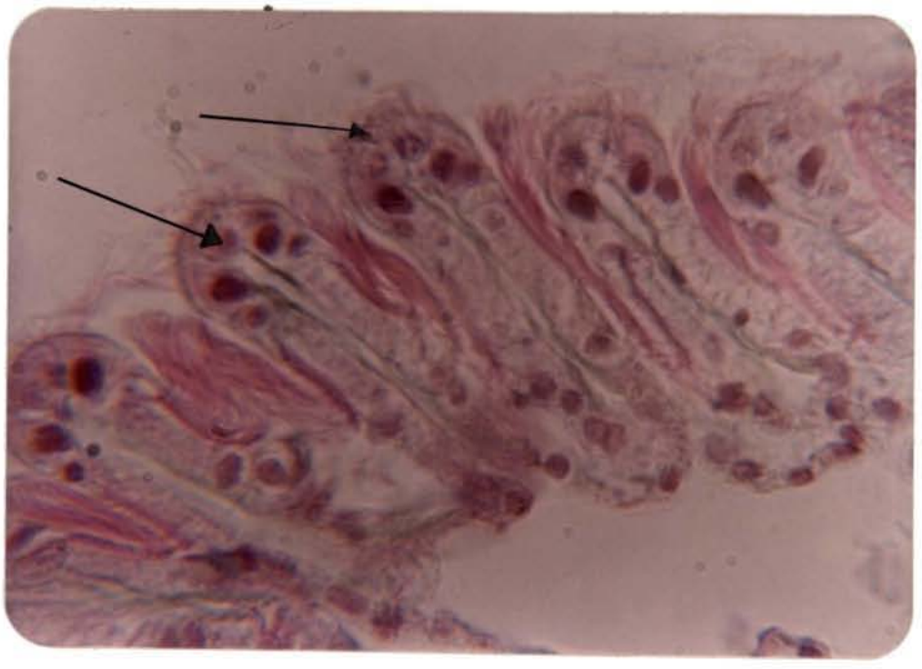
A combination of mercury and cadmium seems to produce more damage to the tissues of Donax incarnatus. In the case of gills, the damage



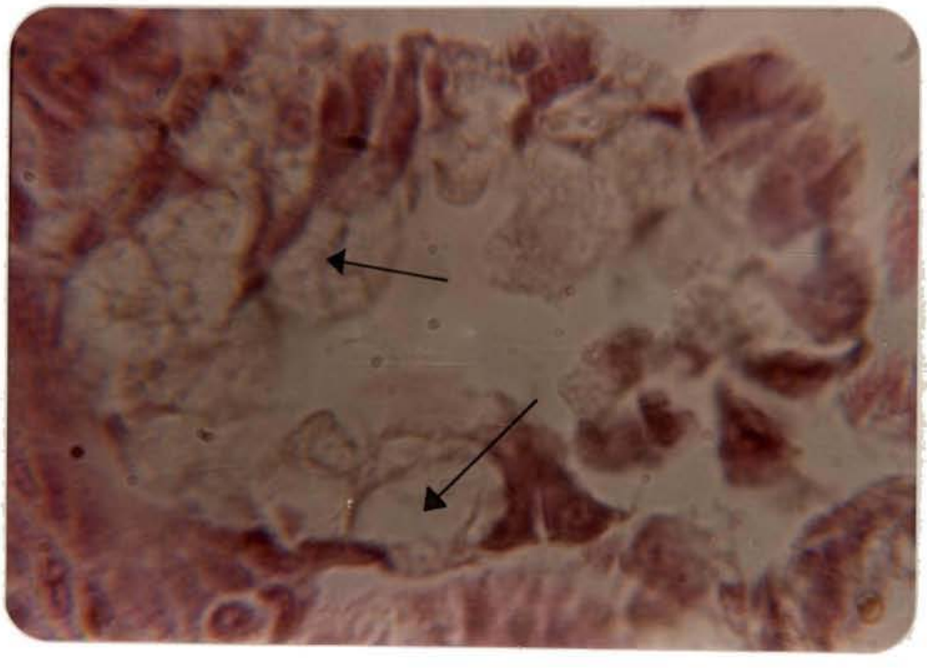
Photomicrograph 21. C.S. of gill of Donax incarnatus exposed to 6.0 ppb of copper, for a period of 14 d x 1000.



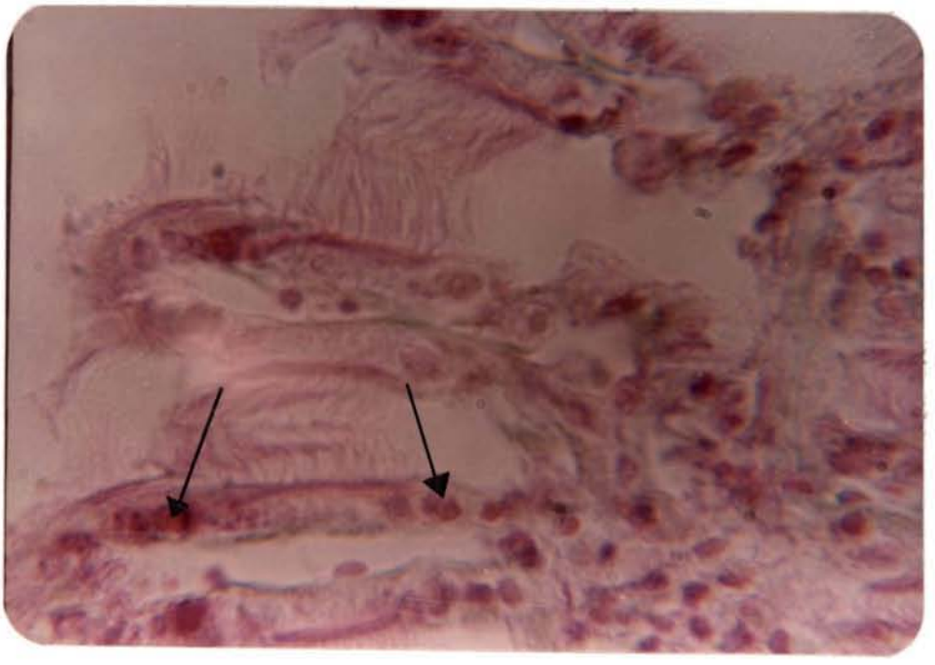
Photomicrograph 22. C.S. of digestive tubule of Donax incarnatus exposed to 6.0 ppb of copper, for a period of 14 d x 1000. Arrows indicate vacuolated digestive cells.



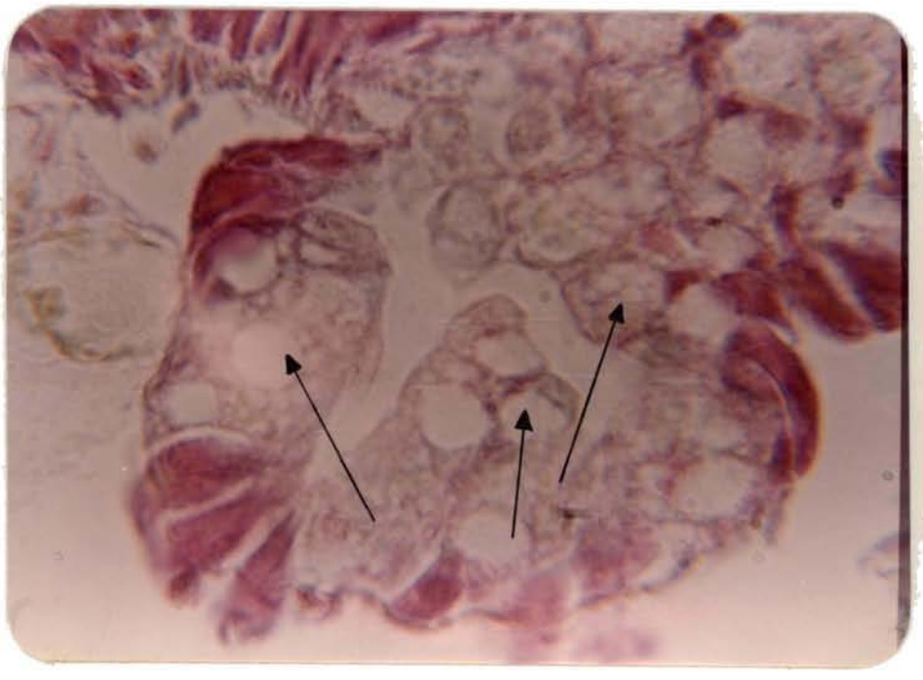
Photomicrograph 23. C.S. of gill of Donax incarnatus exposed to 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate enlarged gill filaments.



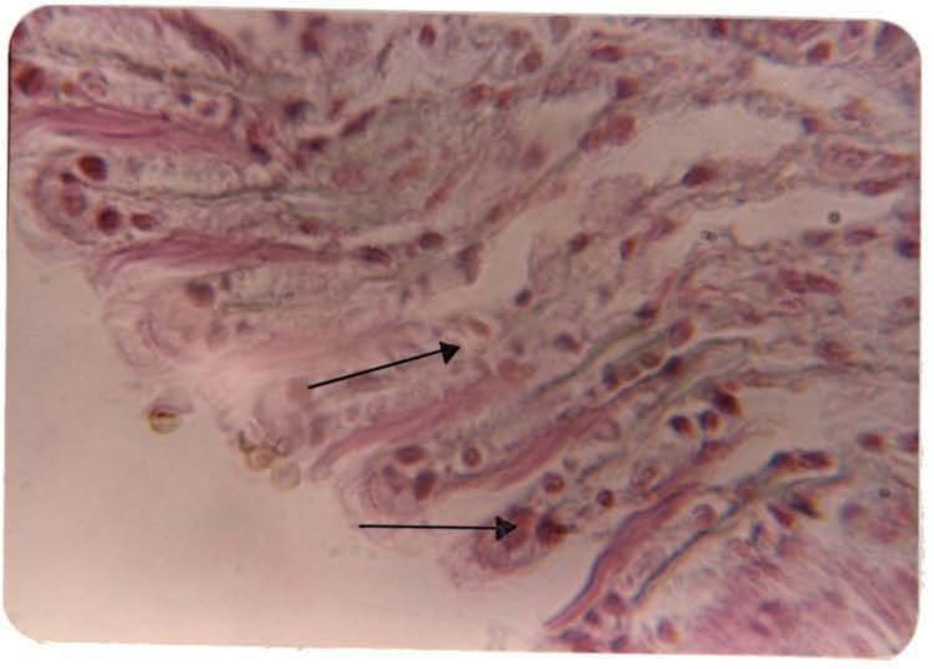
Photomicrograph 24. C.S. of digestive tubule of Donax incarnatus exposed to 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate damaged epithelial cells.



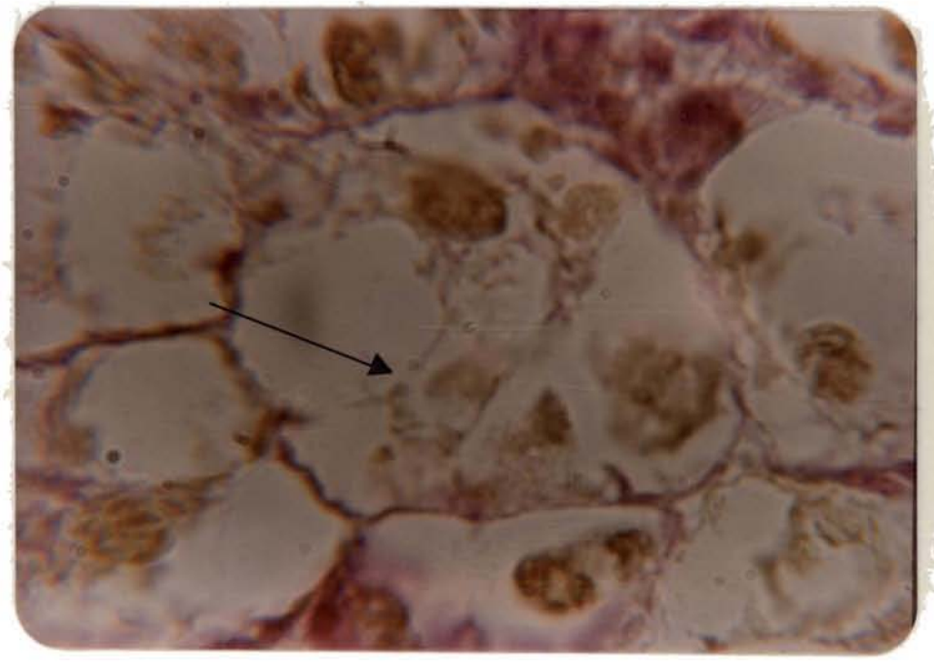
Photomicrograph 25. C.S. of gill of Donax incarnatus exposed to 5.0 ppb of mercury, along with 6.0 ppb of copper for a period of 14 d x 1000. Arrows indicate wandering haemocytes.



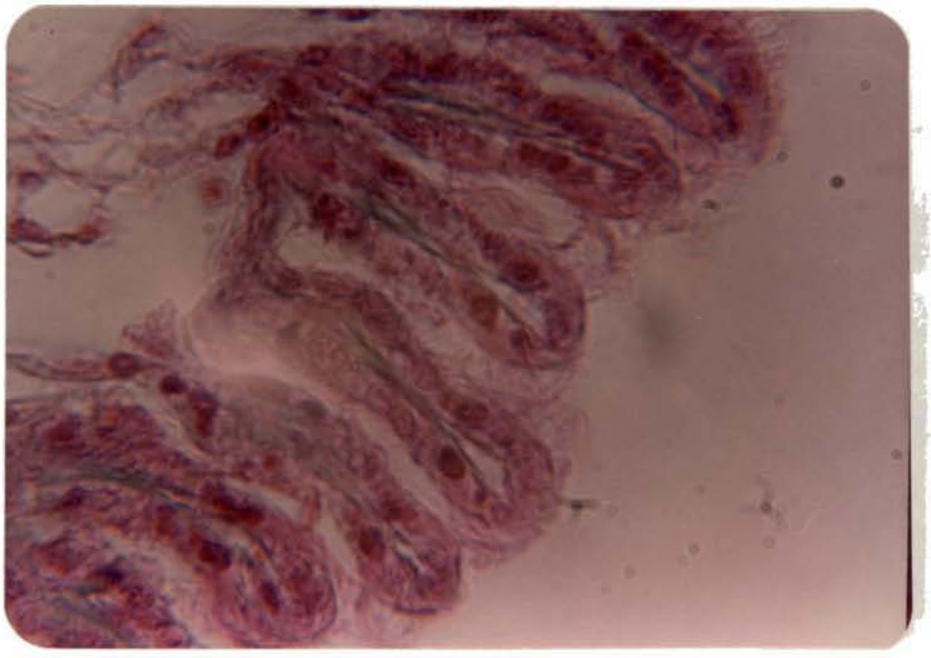
Photomicrograph 26. C.S. of digestive tubule of Donax incarnatus exposed to 5.0 ppb of mercury, along with 6.0 ppb of copper for a period of 14 d x 1000. Arrows indicate dislodged vacuolated cells.



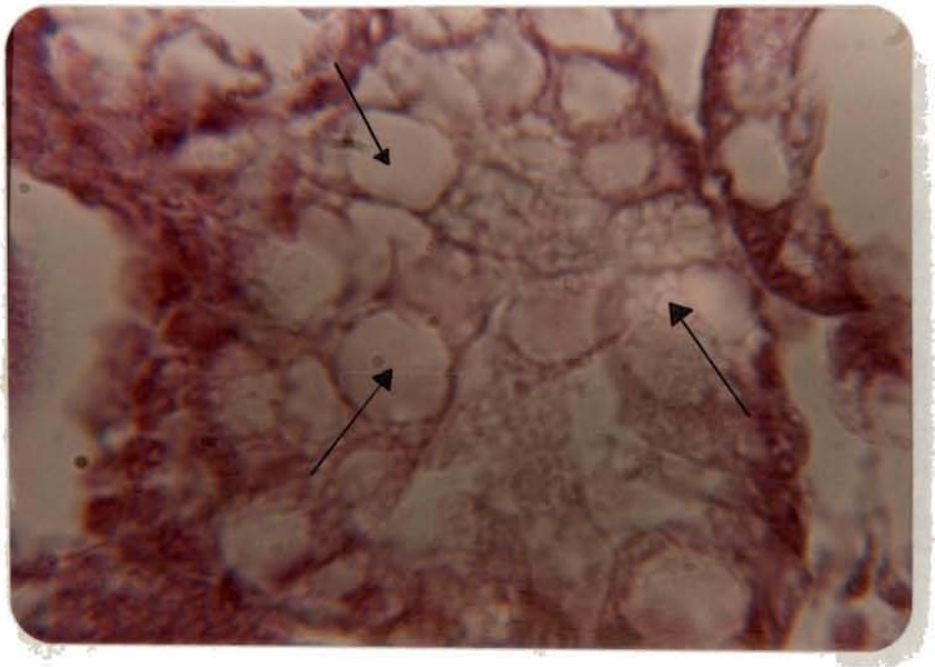
Photomicrograph 27. C.S. of gill of Donax incarnatus exposed to 5.0 ppb of mercury, along with 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate damaged gill filaments.



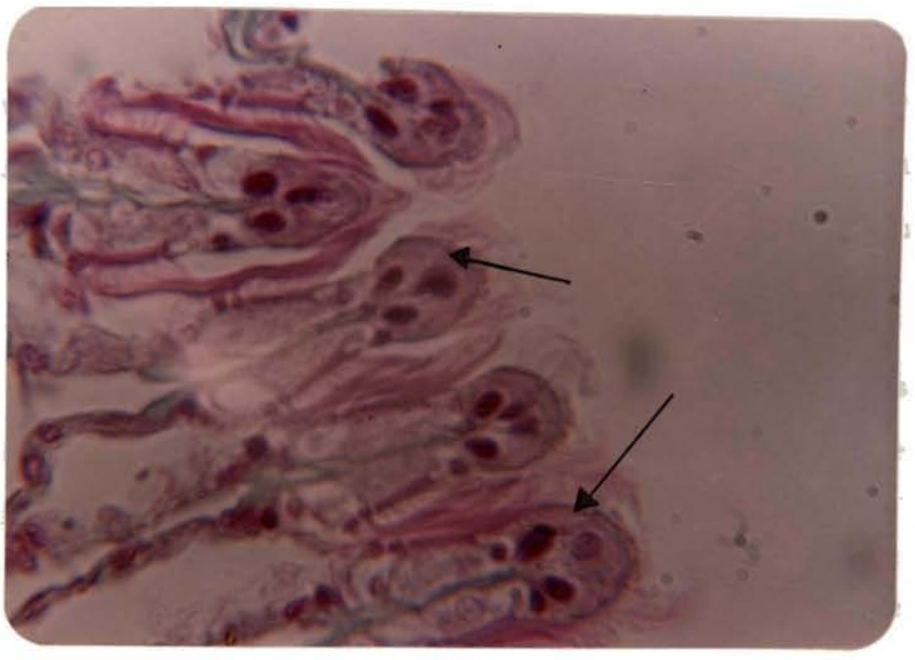
Photomicrograph 28. C.S. of digestive tubule of Donax incarnatus exposed to 5.0 ppb of mercury, along with 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate totally destroyed digestive tubule.



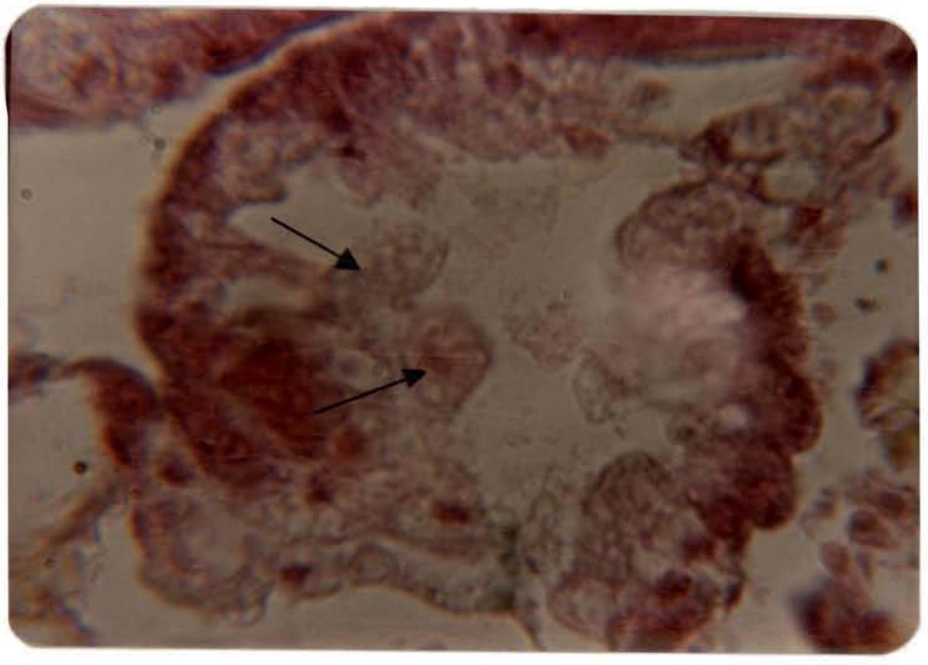
Photomicrograph 29. C.S. of gill of Donax incarnatus exposed to 6.0 ppb of copper, along with 40 ppb of cadmium for a period of 14 d x 1000.



Photomicrograph 30. C.S. of digestive tubule of Donax incarnatus exposed to 6.0 ppb of copper, along with 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate vacuolated digestive cells.



Photomicrograph 31. C.S. of gill of Donax incarnatus exposed to 5.0 ppb of mercury, 6.0 ppb of copper, along with 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate swollen gill filamentar tips.



Photomicrograph 32. C.S. of digestive tubule of Donax incarnatus exposed to 5.0 ppb of mercury, 6.0 ppb of copper, along with 40 ppb of cadmium for a period of 14 d x 1000. Arrows indicate dislodged epithelial cells.

caused was comparable to that happened in the previous cases, whereas in the case of digestive tubules, total destruction of cells was noticed which indicated profound reduction in the digestive processes (Photomicrographs 27 and 28).

Copper and cadmium also produced very comparable results. It is clear from the photomicrographs that the damage of the digestive tubules was extensive (See photomicrographs 29 and 30).

Animals exposed to the triad combination showed enlargement of the gill filamentar tips and blood cells (Photomicrographs 31 and 32). Similarly, the digestive tubules also showed damage of cells which probably would have affected the digestive processes.

5.5 DISCUSSION

While analysing the results obtained from histological studies, a basic distinction has to be taken into consideration. Toxicological investigations at individual level normally centre around physiological mal-functioning and morphological abberations. It is understood that the effects of damaged tissues detected through histological studies will be reflected on the physiology of the animal. However, the distinction between these two types of observations is that physiology is mainly assessed by rate functions and histopathology by actual in situ damage. Histochemical studies or molecular biology would explain more clearly the variations in rate functions. Such a scenario cannot be expected from histopathological investigations. Therefore, in the ensuing discussion only the extent of damage of major tissues could be assessed with reference to toxicity

induced damage.

The tissues studied are gill and digestive tubules. Controversy still exists regarding the main site of entry and the mode of entry of heavy metals into the tissues of marine mussels. Investigations conducted employing dissolved and particulate fractions of copper have shown that copper in dissolved form is much more toxic than particulate form. This has a direct reflection in the mode of entry. Copper would have entered in the dissolved form mainly through the respiratory surface while that in particulate form through the gut. Therefore, the damage of tissues, if it is a direct effect of heavy metal entry, should vary in extend and dimension depending on the nature of availability of the toxicant (Menon and Widdows, in press).

The present experimental animals were fed in situ during the course of the experiments which lasted 14 d in case of Donax incarnatus and 21 d in case of Perna indica. Therefore, the heavy metals would have entered the animal tissues through water as well as through food. It is known that diatoms have the capacity to chelate heavy metal ions by adsorption on to their surfaces (Menon et al., in press). Therefore, to have a comparison on the extend of damage owing to the entry of heavy metals through respiratory surfaces or through digestive surfaces, the gill and the digestive tubules of the affected animals were examined.

Microscopical observation of tissues has shown that there were pathological changes which corresponded mainly to inflammatory and degenerative processes. The epithelium of the digestive tract as well as the gills showed inflammation and necrosis. Brown coloured secondary and tertiary

lysosomes are known to be conspicuous features in the digestive cells of the tubules (Auffret, 1988). The incidence of these lysosomes are known to be xenobiotic induced cellular pathology, disturbing the structure and function. (Moore, 1982; 1985). The most obvious pathological condition is the granulocytomas occurring throughout the interstitial tissue and according to Lowe and Moore (1979), there is a relationship between a particular type of inflammatory response and chronic pollution. Rasmussen et al. (1983b) have recorded similar pathology in mussels after chronic exposure to chemicals. Sloughing off of epithelial cells from the gill filaments seem to be a normal pathological indicator of heavy metal exposure. This should be the after effects of initial mal-functioning and death of cells leading to sloughing off.

Severe degeneration of epithelial cells of the digestive gland has been recorded as an indicator of exposure to high concentrations of copper. The appearance of such cellular disturbances in the sections obtained during this investigation support the hypothesis that autolytic processes are a consequence of full lysosomal destabilization, put forward by Moore (1985). Necrosis and infiltration of severely damaged tubules by haemocytes has also been reported as effects of chronic exposure to various chemical compounds (Rasmussen et al., 1983 a and b). The presence of thoroughly damaged epithelial layer of the digestive tubules is an indication of atrophy leading to sloughing off of cells and necrosis. This is likely to lead to tubular degeneration. It may be assumed here that chronically induced injuries are responsible for such lesions. Changes in the morphology of gill filaments accompanied by severe degeneration of ciliated epithelial cells have been reported in the case of mussels exposed to sub-lethal

thermal stress and to contamination by heavy metals (Gonzales and Yevich, 1976; Sunila, 1986). The fact that these pathological indications have occurred both in the case of natural and man made disturbances clearly indicate that the observed damage of gills reported during the present investigation is a general phenomenon that could occur to the mussels exposed to a stress and that it is not restricted to chronically induced toxication.

The presence of granulocytomas can be used as an index of haemocytic response to aquatic contamination. Experimental induction of cellular damage of internal organs in mussels, can normally be brought about only by subjecting the mussels either to relatively higher concentrations of contaminants or to chronic exposure. Definition of histopathological evidences as indicators of chronic pollutional stress could be achieved only if the method is standardized by incorporating this as a component of ecosystemic evaluation studies of chronically polluted areas. On the other hand, examination of animals subjected to severe stress in laboratory conditions based on histological studies cannot fully reflect the effects brought about by contaminants alone. Auffret (1988) has suggested that poor health condition of mussels inhabiting chronically polluted areas may be indicative of xenobiotic effects and an examination of gill and digestive tubules of mussels from such localities has indicated a severe damage of epithelial cells.

Auffret (1988) has been sceptical about dose - dependent histopathological evidences in mussels, although he did assume that histopathology could give useful information on sub-lethal effects of chemical conta-

minants. Epithelial cell shrinkage and erosion of cells noticed in the present instance have been suggested as the effects of chemical contamination as suggested by Lowe (1988) in Mytilus edulis. He concluded that the most conspicuous effect of chemical contamination will be indicated in the histopathology of digestive tubules and germinal cells. According to him, strict lack of agreement between the biological evidence of pollution on tissue chemistry and histopathology may be due to a lack of understanding of the more subtle effects of combined toxicity. In the present instance it was found difficult to quantify histopathological evidences in relation to nature of metal mixtures. The only evidence noticed was greater damage of tissues when more than one metal was responsible for the insult. In general, it may be assumed that as in the case of physiological rate functions, more than additivity, simple additivity or less than additivity are involved in bringing about histological damage also. A more detailed analysis may help to quantify the damage based on toxicity indices.

SUMMARY

The subject matter of the thesis centres around pollutional impacts and accompanying physiological and morphological effects or alterations in the case of two well known intertidal bivalve molluscs namely, Perna indica and Donax incarnatus. The work presented in this thesis is the result of experimentation using these bivalves collected from recognised unpolluted beaches of Kerala. Perna indica known as brown mussel, is a well established species for pollution research whereas Donax incarnatus is a novice, ever to be used for stress analysis when subjected to heavy metal insult.

A short preface synoptically explains the relevance of the present work in the context of environmental impact analysis. The thesis is presented in five chapters comprising INTRODUCTION, ACUTE TOXICITY, VENTILATION RATE, OXYGEN : NITROGEN RATIO and HISTOPATHOLOGY. Each chapter has been divided into various sections such as INTRODUCTION, REVIEW OF LITERATURE, MATERIAL AND METHODS, RESULTS and DISCUSSION. This was done so, as it was felt during the course of the preparationof the thesis that clearcut description and explanation could be provided only if the chapters are singled out as the subject matter relates to different themes.

The general INTRODUCTION describes the present status of the problem from the Indian context.

The chapter on ACUTE TOXICITY provides an extensive review of literature. The papers reviewed are mainly those dealing with stress

as a function, whether induced naturally or through anthropogenic influences. The work involving toxic effects of heavy metals on bivalves have been reviewed in detail. It may be seen from the review that the work carried out employing species such as Mytilus edulis, Mytilus galloprovincialis, Crassostrea virginica and Modiolus spp. form the major chunk of the papers reviewed.

The section on material and methods describes in detail the nature of test organisms employed, the locality of occurrence, the laboratory conditioning, toxicants employed and the parameters judged to assess toxicity.

The results are presented under the species heads. Simple additivity or less than additivity happened to be the major nature of reaction of these molluscs subjected to either double or triad metal combinations. Clear cut idea could be obtained to hypothesise that it is not possible to ignore the quantity of toxicants administered and the quantity reaching the site of action, so as to cause mortality. The results obtained are discussed and conclusions have been drawn. The most important finding is that factors that have influenced toxicity could be selective absorption of metals, disruption of detoxifying mechanisms and impediment of transport of metal ions.

Ventilation in bivalves has been recognised as a behavioural and physiological function of great relevance. Ventilation is known to get affected due to various factors, among which stress induced by toxicants is an important one. Dye clearance technique, a well established method of estimation of ventilation in bivalves developed in accordance

with the work conducted by Winter and Abel has been used to assess this rate function. The review of literature has taken into consideration the published information on the causative factors influencing this rate function and behavioural pattern of bivalves. Papers dealing with filtration and metal toxicity have been extensively reviewed wherever possible to bring forth explanations for the observed variations. The papers dealing with synergism or antagonism with reference to filtration efficiency is uniformly limited, although locally available information from papers and thesis gave information on this comparatively unknown field of investigation. The material and methods employed to assess the ventilation rate are explained. The results treated under different species heads are described in the light of the data presented in the tables. Increase or declension in the ventilation rate noticed as a function of varying concentrations of metals are presented. The statistical significance of the results obtained have also been worked out. Conspicuous variations in ventilation rate are noticed in the case of both Perna indica and Donax incarnatus. Curiously enough, populational differences are also recorded. More than additive reaction as well as simple additivity are exemplified when different populations are tested. It is seen that, variations noticed in the ventilation rate is more influenced by the concentration factor rather than the quality of the metal employed.

The chapter on O : N RATIO encompasses information on the relevance of this parameter to understand pollution impact. It is known that O : N ratio could be used as a useful index in studying the energetics of marine bivalves. In majority of cases, it is used to explain variations in energy budget accompanying growth and reproduction of cultivable bivalve

molluscs. Howfar this technique can be employed to analyse anthropogenic stress is not properly understood. However, it has been proved that O : N ratio could be an important tool to explain stress effects. The literature available on this topic mainly deals with factors connected with basic physiology. Very few papers are available which have looked into O : N ratio and heavy metal impact. The material and methods employed are detailed out and the technique followed listed. The results obtained show that oxygen consumption is a more reliable index of heavy metal insult than rate of nitrogen excretion. The rate of nitrogen excretion is found to show wider fluctuations thereby influencing the O : N ratio drastically in some cases. In the case of Donax incarnatus, the results are more convincing. This is mainly because of the fact that compared to the two animals, Donax incarnatus is more sensitive and hence prone to show convincing concentration dependent fluctuations. Discussion of O : N ratio has shown that increase and decrease in the O : N ratio within the different range of concentrations, need not support the common assumption that this "end factor" could be utilised as a meaningful stressor or parameter.

The chapter on HISTOPATHOLOGY is a useful addition to the information available on the effect of heavy metal ions on bivalves. The animals used for histopathology are those exposed to the highest sub-lethal concentrations.

The papers reviewedincludethosewhich have looked into the histopathology of bivalves living in chronically polluted areas. This is essential to understand variations that could occur in tissue characteristics of animals,

fish or bivalves inhabiting chronically polluted localities. Histopathological effects accompanying exposure to low level pollutants in the laboratory are rather scarce in which context, the present information would be very useful for further studies. The material and methods employed for the study are explained. A modified method of staining is used to have a better idea on the effects of heavy metal stress on gills and digestive tubules/the gastric diverticula. The results obtained are explained with the help of suitable photomicrographs of the affected regions. Both the gills and digestive tubules are found to be affected by heavy metals. The most important change noticed is in the structure of gill filaments and digestive tubules. Severe degeneration of epithelial cells of the digestive glands, erosion of epithelial cells, vacuolisation of cells on the internal lining of gastric tubules, sloughing off of cells in the case of gill filaments are the noticeable effects of heavy metal pollution in the case of Perna indica and Donax incarnatus.

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