TOXICITY, ACCUMULATION AND DEPURATION OF HEAVY METALS IN THE BROWN MUSSEL *PERNA INDICA*

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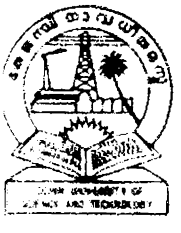

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IN MEMORY OF MY PARENTS
AND BROTHER K.N. SHAMBHULINGAPPA

CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Shri K.N. PRABHUDEVA, 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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VIII 20 87

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DECLARATION

I, Shri K.N. PRABHUDEVA, do hereby declare that this thesis, entitled "TOXICITY, ACCUMULATION AND DEPURATION OF HEAVY METALS IN THE BROWN MUSSEL PERNA INDICA" 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 associateship in any University.

20.8.87

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PREFACE

The study of the effects of pollution on marine organisms has received considerable attention from marine scientists the world over. The investigations aimed at delineating the pollutional effects, mainly centre around two broad scientific approaches viz. ecological monitoring and laboratory investigations. Ecological monitoring and the efficacy of the approach mainly depend on the *in situ* effects of pollution, which in turn are controlled by the pattern of pollutant release in space and time. Negative ecological consequences of pollution manifest themselves in detrimental deviations from the normal state of individual populations or ecosystems. The second approach namely laboratory investigations mainly take into consideration the detrimental deviation from the normal state of individuals. Such impairements can be quantified. Usually the quantifiable parameters selected for studies will be activity which can be assessed by short term studies with reasonable efficiency and accuracy.

Probably, an important aspect of laboratory studies to delineate pollutional effects, is investigations employing realistic concentrations of pollutants. The results gathered from such studies will have more applicability since the level of pollutants in areas which are not chronically affected will be much below than normal levels employed in laboratory studies. To find out the effects of toxicants on a marine organism in situations where the pollutant levels are very low, it is essential to identify species which are sensitive to lower doses of toxicants in the test media. Such studies conducted in the laboratory have shown that *Perna indica* is an excellant test organism which can be employed for toxicological assay. There are difficulties in the use of marine molluscs for pollutional assessment impact. Most important of that is the current limited understanding of the mechanisms of toxicity. The interpretation of the

significance or specificity of a measured biological response could therefore become difficult. Notwithstanding these limitations, marine mussels have been extensively used for analysing toxicological effects. The two Indian species of the genus Perna viz. Perna viridis and Perna indica have received lot of attention from this stand point. Among these, Perna indica is a well established species distributed along the relatively less polluted areas of south-west coast of India. Among the various pollutants, which have generated concern to experimental ecologists, heavy metals occupy the foremost position. The aspects mentioned above were the most important guiding principles when the present investigation was launched. It is felt that the matter presented in this Thesis will help us to widen our knowledge on toxicology in general and heavy metal toxicity in particular.

CONTENTS

		Page No.
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	5
III.	MATERIALS AND METHODS	24
	3.1 WATER	24
	3.2 TEST ANIMALS	24
	3.3 TOXICANTS	25
	3.4 ACTIVITY STUDIES	25
	3.5 ANALYSIS OF METALS	29
	3.6 JOINT TOXICITY ANALYSIS	32
	3.7 TERMINOLOGY	34
IV.	EXPERIMENTAL RESULTS	36
	4.1 LETHAL TOXICITY STUDIES	36
	4.1.1 Individual metals	36
	4.1.1.1 Copper	36
	4.1.1.2 Silver	37
	4.1.2 Metal combinations	37
	4.1.2.1 Copper + Silver	37
	4.1.2.2 Silver + Copper	38
	4.2 SUBLETHAL TOXICITY STUDIES	38
	4.2.1 Individual metals	38
	4.2.1.1 Copper (as $CuSO_4$)	38
	4.2.1.1.1 Accumulation	39
	4.2.1.1.2 Depuration	41
	4.2.1.2 OTHER SALTS OF COPPER	44
	4.2.1.2.1 Accumulation	44
	4.2.1.2.1.1 Oxygen consumption accompanying accumulation	44

4.2.1.2.1.2. Filtration accompanying accumulation 45

4.2.1.2.2 Depuration 45

4.2.1.2.2.1 Oxygen consumption accompanying depuration 46

4.2.1.2.2.2 Filtration accompanying depuration 46

4.2.1.3 Silver 47

4.2.1.3.1 Accumulation 47

4.2.1.3.1.1 Oxygen consumption accompanying accumulation 48

4.2.1.3.1.2 Filtration accompanying accumulation 48

4.2.1.3.2 Depuration 49

4.2.1.3.2.1 Oxygen consumption accompanying depuration 50

4.2.1.3.2.2 Filtration accompanying depuration 50

4.2.2 METAL INTERACTION DURING ACCUMULATION AND DEPURATION 51

4.2.2.1 SULPHATE FORMS OF COPPER AND SILVER 51

4.2.2.1.1 Effects on copper accumulation 51

4.2.2.1.1.1 Oxygen consumption accompanying accumulation 52

4.2.2.1.1.2 Filtration accompanying accumulation 53

4.2.2.1.2 Effects on copper depuration 54

4.2.2.1.2.1 Oxygen consumption accompanying depuration 55

4.2.2.1.2.2 Filtration accompanying depuration 55

4.2.2.1.3 Effects on silver accumulation 56

4.2.2.1.3.1 Oxygen consumption accompanying accumulation 57

4.2.2.1.3.2 Filtration accompanying accumulation 58

4.2.2.1.4 Effects on silver depuration 58

4.2.2.1.4.1 Oxygen consumption accompanying depuration 59

4.2.2.1.4.2 Filtration accompanying depuration 59

4.2.2.2. NITRATE FORMS OF COPPER AND SILVER 60

4.2.2.2.1 Effects on copper accumulation 60

4.2.2.2.1.1. Oxygen consumption accompanying accumulation 61

4.2.2.2.1.2 Filtration accompanying accumulation 62

4.2.2.2.2 Effects on copper depuration 62

4.2.2.2.2.1 Oxygen consumption accompanying depuration 63

4.2.2.2.2.2 Filtration accompanying depuration 64

4.2.2.2.3 Effects on silver accumulation 64

4.2.2.2.3.1 Oxygen consumption accompanying accumulation 65

4.2.2.2.3.2. Filtration accompanying accumulation 66

4.2.2.2.4 Effects of silver depuration 67

4.2.2.2.4.1 Oxygen consumption accompanying depuration 67

4.2.2.2.4.2 Filtration accompanying depuration 68

V. DISCUSSION 70

5.1 LETHAL TOXICITY 72

5.2 SUBLETHAL TOXICITY 76

5.2.1 Accumulation and depuration of copper 76

5.2.2 Accumulation and depuration of silver 83

5.2.3 Oxygen consumption accompanying accumulation and depuration 86

5.2.4 Filtration accompanying accumulation and depuration 91

VI. SUMMARY 96

VII. REFERENCES 101

I N I O N

I - INTRODUCTION

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. Because of increase in concern on the effects of heavy metals on life and activity of marine organisms, efforts are being made to collect information to compare the magnitude of natural and anthropogenic inputs. Available data show that there are instances when anthropogenic inputs exceed natural inputs by more than order of magnitude.

The metals added to the sea tend to increase transfer of them from the sea to the sediments. Therefore, direct addition of heavy metal by anthropogenic activities tend to increase the heavy metal load of estuarine and coastal marine sediments since the outfall systems are situated either in the estuaries or along the coast. Since the metals need not remain inert, depending on the hydrographic conditions, remobilisation may occur returning the metal in dissolved form to overlying water.

Evidence show that free ions are biologically the most available inorganic species of trace metals in sea water. Various factors control the concentration of ions in seawater. They are the total metal concentration, inorganic and organic complexation. It is also known that the influence of organic complexes can either result in complete prevention of metal uptake or no uptake at all. Depending on the nature and quantity of complexing elements the uptake rate could vary. Although the diet is the principal source of metals in marine animals of higher order, the lower group of marine animals can incorporate considerable quantity of heavy metal from seawater. Even now uncertainty exist as to which is the most important source of heavy metal to lower marine invertebrates. However, since mercury is most readily

absorbed from solution, it is the metal, for which water route might be expected to be most important. The process of uptake of heavy metal from water is a passive process (Bryan, 1976a). In the case of molluscs, pinocytosis has been shown to be involved in uptake and transfer of metals. Since endocytosis is a common phenomenon, it is likely to play an important role in the transfer of metals. Various factors are involved in the detoxification of heavy metals by marine invertebrates. Among these, those binding to non-specific high molecular weight proteins or to specific low molecular weight proteins of metallothionein type seem to be most important ones involved in the detoxification of metals in marine invertebrates.

In the present investigation, certain important aspects of heavy metal toxicity have been worked out. Recent studies have clearly shown that when experimental media contained more than one heavy metals, such metals could conspicuously influence the toxic reaction of the animals both in terms of quantity and nature. The experimental results available on individual metal toxicity show that, in majority of such results, unrealistically high concentrations of dissolved metals are involved. A remarkable number of factors have been shown to influence metal toxicity such as various environmental factors particularly temperature and salinity, the condition of the organism and the ability of some of the marine organisms to adapt to metallic contamination. Further, some of the more sensitive functions like embryonic and larval development, growth and fecundity, oxygen utilization and the function of various enzymes are found to be demonstrably sensitive in the presence of heavy metals. However, some of the above functions could be compensated for by adaptive process. If it is assumed that the presence of a single metal in higher concentrations could affect the life function of marine animals, more than one

metal in the experimental media should manifest such effects in a greater scale. 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 of heavy metals but at majority of such instances one of the metals will be a non-essential one. Understanding the effects involves experimentation with well recognized experimental species. Among the marine animals, marine mussels have been shown to be useful group of organisms to study the cause and effects of heavy metal pollution. Mytilus edulis and M. galloprovincialis are the two important marine mussels which have been widely used for toxicological investigations. Similarly, Perna viridis and Perna indica have been proved to be useful experimental animals to study heavy metal toxicity. Perna indica is a very sensitive marine intertidal species. This species has been used in recent years to study pollutional impacts.

The work presented in this thesis comprises lethal and sublethal toxicities of different salt forms of copper and silver on the brown mussel Perna indica.

Information on the combined toxicity of metals, petroleum hydrocarbons and pesticides with reference to tropical marine bivalve is scetchy. The present trend in toxicological investigations is to have a multifactorial approach on the response of marine organisms to be combined stress forced by the presence of more than one toxicant in the natural seawater. A clear understanding of the cause and effect could be had only if laboratory experiments are conducted employing sublethal concentrations of the above toxicants. Therefore, during the present investigation sublethal concentrations of copper and silver in their various salt forms were employed to assess concentration depen-

dent effects on survival, oxygen consumption, filtration, accumulation and depuration on Perna indica.

The results are presented under different sections to make the presentation meaningful. This sort of investigation will eventually open up a very interesting aspect of toxicology, the understanding of which would help in delineating the impact of heavy metal contamination of the coastal waters on the intertidal and subtidal biota.

REVIEW OF LITERATURE

II - REVIEW OF LITERATURE

The review of literature on the different aspects of marine pollution is a hazardous task, has tremendous proliferation of printed matter in this aspect in different parts of the world. The papers available can mainly be categorized under those relating to ecosystem damages, radio-active pollution, pollution by heavy metals and their compounds, petroleum hydrocarbons, pesticides and technical organic chemicals, domestic wastes and thermal pollution. Kinne (1984) made an exhaustive review of the various categories of literature available in his volume on Ocean Management. Various factors make a comprehensive understanding of marine pollution difficult. Among these, lack of proper concepts from the factors contributing to pollution, differences in literature reporting, utilization of non-conventional strategies, study of cause and effects are a few factors which make a clear understanding of the available data so as to plan future strategies.

The present investigation has taken into consideration only a small facet of the whole problem. This mainly centres around the toxic effects of heavy metals, singly and in combination, on Perna indica. Heavy metals are probably one of the most dangerous group of contaminants encountered in the marine ecosystem. Although many of the heavy metals are essential for the well-being of marine organisms, above optimal levels they are known to cause serious damage to the ecosystem in general and marine organisms in particular (Bryan, 1976a). Several of the metals are essential components of enzymes, haemoglobin, haemocyanin or structural body elements. Since in situ concentrations of trace metals are very low, the possibilities of increase in concentrations by anthropogenic activities are high. Marine animals usually accumulate trace metals by a concentration factor of 10^3 to 10^5 and therefore tissue concentrations of

heavy metals in those animals living in polluted waters reach toxic levels (Kinne, 1984). Hence, aspects like lethal toxicity, sublethal toxicity and accumulation and depuration are important aspects of heavy metal pollution. It is understood that the toxicity of the same heavy metal can vary depending on the salt form, which is also an integral part of heavy metal toxicity investigation. Since in the present work, where two metals and their salt forms selected are copper and silver, only those papers which have direct bearing to the type of work are reviewed in detail here. However, to present a general picture on heavy metal pollution those works which have generalised the effect of heavy metal contamination are also cited wherever necessary.

Vallee and Walker (1970) have demonstrated that silver, copper, mercury, cadmium and lead are enzyme inhibitors at toxic levels. Copper is one of the essential metals and its average natural concentrations in coastal waters is around 2-5 ppb (Davenport and Redpath, 1984). Silver is one of the most toxic heavy metals in the aquatic environment (Bryan, 1971). Average concentrations of silver, a highly toxic non-essential metal is about 0.1 ppb (Riley and Gester, 1971). Copper is essential for the action of cytochrome oxidase, lysine oxidase and tyrosenase and is known to function by forming strong complexes with protein. Although, Perna does not posses any haemolymph pigment, copper is a necessary constituent of Perna haemolymph protein. It is known that excess amount of copper can create such deleterious effects as interference with enzyme activities and electron transport reaction. Further, membrane permeability and cell divisions can also be affected (Davenport and Redpath, 1984).

Various authors have discussed the chemistry of copper in seawater (Zirino and Yamamoto, 1972; Ahrland, 1975; Lewis and Cave, 1982). Among the

factors which really affect the chemistry of copper in seawater are solubility, capacity to form complexes, colloides, adsorption and hydrogen ion concentration. Terrestrial run-off, aeolian import and geothermal addition are the main pathways through which copper enters the marine system. It is also demonstrated that sedimentary copper level is several fold higher than the overlying water.

Differences in experimental approach and criteria for toxicity have led to considerable variety in published lethal concentrations of copper to Mytilus edulis. Values as high as 200 ppb (Scott and Major, 1972) to as low as 15 ppb (Manley, 1980) have been reported to have caused 50% mortality in Mytilus edulis. The variations in these values were mainly due to the fashion of experimental exposure which varied between total static system with replenishion of test media to flow through system. Information is available in the literature on the toxicity of copper to the developmental stages of bivalves. Wisely and Blick (1967) found that 50% pediveliger was killed in 22 ppm copper in 2 h. Sheffrin (1982) proved that certain stages are more tolerant to copper. 137 ppb of copper was required to kill 50% Mytilus plantigrades in 52-53 days. Working on response of oyster embryos to copper, MacInnes (1981) found that 12.3 ppb of cupric chloride or 14.3 ppb of copper nitrate would result in 10% abnormality of the larvae.

There is a general dearth of information on acute toxicity of silver on marine invertebrates. The works available are mainly confined to sublethal toxic effects on marine bivalves. Among heavy metals, silver has received least attention although it is well known that it is one among the major toxic metals found in nature. With varying resident times, depending on the source, silver

can remain in seawater from 12,000 to 2 million hundred thousand years, although 60% of silver found in seawater is in particulate form (Riley and Chester, 1971). The presence of H_2S tend to precipitate silver leaving only small quantities in seawater. Aerated seawater of pH range 7.8-8.2 may have a silver load of 2.2-2.5 ppm. Information available on toxicity of silver is mainly confined to some of the basic functions like enzyme inhibition, respiration, development, etc. Studies on three intertidal bivalves have shown that lethal toxicity can vary from 0.05 to 0.1 ppm and juveniles are to be more sensitive than adults (Mathew, 1979). Balanus balanoides depicted LC 50 96 h value of 0.02 ppm of silver (Clarke, 1947) and Nereis diversicolor 0.50 ppm (Bryan, 1976b). On the contrary, Crassostrea virginica embryos showed 48 h LC 50 of 5.8 ppb (Calabrese et al. 1973). In the case of silver, the quantity of metal in sediment influences the overall toxicity of the metal (Bryan and Hummerstone, 1971). MacInnes and Calabrese (1978) found that lethal toxicity of silver to American oyster Crassostrea virginica could vary from 24.2 to 35.3 ppb in 48 h.

Calabrese and Nelson (1974) found inhibition of embryonic development of hard clam Marcenaria marcenaria, when they were exposed to silver. The LC 50 recorded was 21.0 ppb. Discussing on the biological effects of heavy metals on juvenile scallop Argopecten irradians, Nelson et al. (1976) stated that death of 50% of population can occur when silver was supplied in the form of nitrate ranging between 30 to 36 ppb. Calabrese et al. (1977a) found drastic variations in the lethal concentrations of silver in the case of larvae and juveniles of various molluscs. In the case of Spisula solidissima juveniles, 100 ppb was found to be lethal.

Although, numerous reports are available on the relative toxicity of individual metals on marine organisms, our present status of knowledge on the effects of metal-mixtures on marine invertebrates is limited. Since metals usually occur as mixtures rather than alone in estuarine and coastal regions, information on their interactions might provide a more realistic assessment of their toxicity to coastal organisms. Recent studies have shown that metals may interact synergistically or antagonistically on 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, 1986b; Mohan et al., 1986a&b; Prabhudeva and Menon, 1987a&b). Alderdice (1972) theorizing the effects of independent variables on biological responses, stated that the effect of more than one variable and the resultant biological response of an organism may depict plasticity, interaction, variability in tolerance and changes in capacity adaptation. Among these, the reaction of the animal facing continuous exposure to more than one environmental variable or more than one toxicant can trigger capacity adaptation.

Sprague and Ramsay (1965), working on combined toxicity of copper and zinc sulphates to juveniles of Atlantic salmon (Salmo salar) found that simple additive effect existed when copper and zinc were supplied to them. Looking into the effects of chelation on toxicity of copper, Morris and Russel (1973) suggested that complexation of metals with organic compounds may modify the properties of metals greatly. Estuarine teleosts were more affected when they were exposed to a mixture of cadmium, copper and zinc salts than they were when exposed to individual metals. Concentration of cadmium not ordinarily lethal, exerted a negative effect on survival of fish intoxicated by

the salts of copper or zinc or both (Eisler and Gardner, 1973). The work of Coombs (1974) on the nature of zinc and copper complexes in the oyster, Ostrea edulis showed that both zinc and copper were weakly complexed to the small molecular weight proteins taurine, lysine, ATP etc. Hrs-Brenko et al. (1977) suggested synergistic effects of lead, salinity and temperature on embryonic development of Mytilus galloprovincialis. They found that at comparatively high temperature the presence of lead has deleteriously affected embryonic development.

In an interesting paper on the evaluation of toxicity of a complex metal-mixture to Mya arenaria, Eisler (1977) found that the presence of more than one metal in very low concentration can result in either death or variable rates of metal uptake. Moulder (1980) discussing the effect of chlorides of mercury and copper on Gammarus duebeni, found that the presence of a sub-lethal level of cupric chloride protected G. duebeni against toxic action of mercuric chloride. He has also discussed the nature of interaction between mercury and copper. When Breittmayer and Galindo (1981) exposed mussels to a combination of zinc and mercury, antagonism was noticed when concentrations of mercury reduced considerably. Silver was found to be more toxic when Perna viridis was exposed to a mixture of these two metals(Mathew and Menon, 1983). Antagonistic effect of copper on toxicity of mercury has been observed by Roales and Perlmutter (1974) and Moulder(1980). Working on the combined toxicity of mercury and cadmium on the tropical green mussel Perna viridis, Mohan et al. (1986b) found that mercury and cadmium, in mixture, interacted more than additively in producing mortality in 96 h. It is evident that concentrations of metals in mixture also affect the toxicity to which component interactions are additive or synergistic. Comparable result was

obtained when Perna viridis were subjected to combinations of zinc and copper (Prabhudeva and Menon, 1986b).

Sublethal effects of contaminants on marine organisms are usually categorised as modifications in rate functions, behavioural responses etc., resulting from exposure of the concerned organism to contaminant levels very much below experimentally delineated lethal concentrations. The concentrations to be employed for sublethal studies should necessarily have relationships with realistic concentrations encountered in estuaries and coastal waters. It is known that the concentrations of majority of the heavy metals in estuaries and coastal waters can vary considerably depending on the status of pollution in such areas (Bryan, 1976a). However, chronic exposure to analytically undetectable levels of pollutants brings about subtle changes in the life and activity of coastal fauna. These changes could be detected only if continuous monitoring is conducted so as to assess the 'scope for growth' (Bayne, 1985). This study would involve considerable expenditure of time since, the variations negative or positive, in the rate functions and the connected aspects of the animal's biology have to be very critically followed. Planing experiments on sublethal toxicity in the laboratory should, therefore, take into consideration both concentration and time factor. Since it is difficult to keep marine organisms for longer duration under controlled conditions without interfering with the normal activity of the animal, prolonged durations are compensated with enhanced concentrations. Therefore, the concentrations employed will usually be considerably above realistic concentrations. Usually the rate functions employed to assess sublethal effects are oxygen consumption and filtration rates, two activities of any organism which have considerable physiological implications.

Oxygen consumption of bivalve molluscs have been widely studied (Kinne, 1970; Thompson and Bayne, 1972; Mackay and Shumway, 1980; Prabhudeva and Menon 1986a; Mohan et al., 1986a&b). Oxygen consumption is one of the parameters to assess stress because it is a valuable indicator of energy expanded to meet the demands of an environmental alteration. The amount of oxygen removed from seawater by marine organism varies considerably depending on many factors including the type of feeding mechanism, experimental conditions and respiratory efficiencies. Hazelhoff (1938) stated that particle feeding marine animals remove 13% oxygen from seawater passing over respiratory surface and on the other hand, many groups remove around 53%. Although a study of respiratory rate under a toxicant stress is easy and quick, its sensitivity is much lower compared to indices like growth rate, development and reproduction. Thurberg et al.(1975) employing different life stages of Spisula solidissima, observed elevated oxygen consumption on exposure to sublethal concentrations of silver. Similar oxygen intake rates under silver stress in different salinity regimes have also been noticed for four species of bivalves (Thurberg et al., 1974). Nelson et al. (1976) found toxicant dependent reduction in oxygen consumption in Argopecten irradians. MacInnes and Thurberg (1973) found silver depressed oxygen consumption in the case of Nassarius absoletus, at 0.05 ppm and above. In long-term exposure (30-90 days) 10 ppb of silver elevated oxygen consumption in bivalves. (Calabrese et al., 1976). Scott and Major (1972) found that oxygen consumption was reduced in Mytilus edulis by about 12% on exposure to 300 ppb copper. Cheng and Rodrick (1974) reported that copper sulphate at 1 ppm level decreased oxygen consumption of Nassarius absoletus by 67-75%. Brown and Newell (1972) found that copper depressed

gill tissue respiration and ciliary activity and had no effect on the respiration of the digestive gland. Oxygen consumption is a product of two important factors, namely, ventilation volume and quantity of gas withdrawn from each litre of water. Therefore, fluctuations noticed in the case of oxygen consumption is due to variations in the quantity of water propelled to the respiratory surfaces and changes in the oxygen uptake from the water by the animal (Prabhudeva and Menon, 1986a). Various reports are available on rate of oxygen consumption by Perna viridis, subjected to heavy metal stress (Murthy, 1982; Mathew and Menon, 1983; Prabhudeva and Menon, 1986a; Mohan et al., 1986 a&b). In the case of Meretrix casta and Modiolus modiolus, copper was functioned as a respiratory depressant (Mathew and Menon, 1982). Respiratory depression in Mytilus galloprovincialis could either due to valve closure or direct impairement of metabolic activity (Shapiro, 1964). Anaerobic respiration in Scrobicularia plana, when subjected to 500 ppb copper for a shorter duration was reported by Akberali and Black (1980). Cellular damage of gills and cilia bearing cells during exposure to copper and zinc for a longer duration was noticed by Prabhudeva and Menon (1986a). Baby and Menon (1986) found that among the three metals, mercury, cadmium and zinc, cadmium was least toxic and mercury the most, in bringing about declension in oxygen consumption. Further, they found that reduced oxygen consumption obtained from whole body analysis, indicated the performance of the animal. Mohan et al. (1986b) looking into the combined effects of cadmium and mercury, on oxygen consumption, found that combination of mercury and cadmium depressed oxygen uptake in Perna viridis. Salanki (1965, 1968) observed that bivalves close their shells tightly under unfavourable conditions resulting in reduction in oxygen uptake.

Abel (1976) suggested that the rate of filtration could be a useful parameter to assess the effects of contaminants on marine molluscs. Assessment of filtration rate is a non-destructive method and could be carried out effectively with minimum equipments. Abel (loc. cit.) looked into the effects of toxicants on the rate of filtration by Mytilus edulis. Filtration rate of bivalves is known to be influenced by environmental parameters such as salinity, temperature, pH, etc. (Cole and Hepper, 1954). Effects of toxicants like petroleum hydrocarbons on clearance rates of marine molluscs are worked out by Eknath and Menon (1979), Reddy and Menon (1980), Mathew and Menon (1984). Prabhudeva and Menon (1985) working on the rate of filtration of Perna viridis pre-exposed to heavy metals have shown that zinc and copper at low concentrations affect filtration deleteriously. The filtration rate was reduced to 15-25% efficiency in concentrations ranging from 40 to 80 ppb copper. These animals were kept in raw seawater during the filtration experiments, after being pre-exposed to toxicants ranging from 24 to 96 h. They concluded that pre-exposure influences the rate of filtration. Mohan et al. (1986a) found that the rate of filtration in six intertidal animals was affected by mercury and cadmium. They opined, unlike many systems of potential value as indicators of sublethal stress, the filtration rate is based on a response of ecological significance since the bivalves depend on the water they filter both for food and oxygen. They found that at high concentrations filtration was either arrested or reduced considerably. Valve closure accompanying heavy metal stress can significantly affect the amount of water drawn into the body. More than additive effect was noticed in the case of mercury and cadmium in bringing about alteration in filtration rates in Modiolus Spp and Donax spiculum (Mohan et al., 1986a). Palmer (1980)

trying to analyse behavioural and rhythmic aspects of filtration in Argopecten irradians and Crassostrea virginica, found that the tidal sequence or laboratory condition have exhibited any influence on filtration behaviour. In the case of scallop A. irradians, filtration either remained constant throughout the experimental period or stabilised after a steady initial decline. He further found that concentrations of suspended salts affected the rate of filtration. Abraham et al.(1986) found that filtration decreases exponentially with the increase in concentration of chromium, manganese, nickel, copper, cadmium and mercury in the case of Villorita cyprinoides var. cochinensis. Analysis of filtration capacity under toxicant stress has a direct ecological relevance since increase or decrease in the feeding rate is known to cause variation in growth rate in marine molluscs (Widdows et al., 1982; Stickle et al., 1984). Exposure of specimens of Mytilus edulis, to dissolved copper has led to a fall in the filtration rate when measured in whole animals and copper concentration leading to a 50% reduction in the filtration rate was found to be 94 ppb (Howell et al., 1985).

Bryan (1976b) suggested that bivalves can be used to assess the quantity of heavy metals available in the aquatic environment. The capacity of bivalves to regulate trace metal content of the body by metabolic pathways is limited (Schulz-Baldes, 1974). The ability to accumulate trace metals and other toxicants by bivalves has led to the selection of this group as an important bio-indicator. The marine pollution monitoring programme using bivalves now come to know as 'Mussel Watch' (Goldberg, 1975) envisages analysis of trace metal load of various species of bivalves from identified localities all over the world. The strategies of carrying out this programme is mainly based on the capacity and capability of marine mussels

and Coombs (1977) have carried out preliminary studies on the uptake of iron in Mytilus edulis. Pasteels (1968) demonstrated that two iron metalloproteins, ferritin and peroxidase, were pinocytosed by the gill epithelia of Mytilus edulis. Cunningham and Tripp (1973) studying the kinetics of mercury uptake in the American oyster Crassostrea virginica, found that after a very high increase of initial rise of uptake, increase of mercury concentration in the tissue dropped sharply. They found that oyster accumulates mercury 1400 and 2800 times more above the environmental concentration of 100 and 10 ppb of mercury. Total depuration was not achieved even after 6 months of maintainance in clear seawater. Pringle et al. (1968) established that Crassostrea virginica is able to accumulate and purge itself of lead under experimental conditions.

Nair et al. (1977) working on the effect of lethal concentrations of copper on the uptake by Mytilus viridis, found that the rate of uptake of copper was faster in concentrations above 100 ppb. When the animals were exposed to 50 to 1000 ppb, the rate of mortality was related to the rate of uptake. They also found that tissue level of dead animals exposed to 1 ppm, reached 47 ppm. Working out the feasibility of using Mytilus edulis as an efficient time integrator of copper, zinc, cadmium and lead over a wide variety of environmental conditions, Phillips (1976a) found that Mytilus may not be a good indicator of copper contamination. On the other hand, the rate of uptake pattern suggest that M. edulis could give a reasonable picture of the environmental load of metals like zinc, cadmium and lead. D'Silva and Qasim (1979) have worked out the kinetics of bioaccumulation and elimination of copper in the rock oyster, Crassostrea cucullata. They found that the rate of uptake of copper was dependent on metal concentra-

tion in the medium. The net rate of uptake ranged from 1.76 to 1.97 µg/gm/ week and the rate of copper loss, measured after transferring the oysters into natural seawater, was dependent on the original copper concentration in the soft parts. They further indicated that accumulation occurs in the tissues more rapidly than cleansing can eliminate it.

The uptake of silver 110m by the Tapes japonica has been worked out by Kimura and Ichikawa (1972). They found that accumulation of silver 110m by the short necked clam is relatively high in comparison with that of several other radio-active nucleids. The loss of accumulated silver from the whole clam showed a relatively slow elimination pattern. Discussing on the kinetics of iron accumulation and excretion in Mytilus edulis, George and Coombs (1977) found that iron is accumulated principally in the viscera with a smaller but significant proportions in the gill. They found that prior complexation of iron affects the uptake of this metal. Similarly, complexation affects the rate of depuration also. The citrate, EDTA and 1,10 phenanthrenine complexes have a greater level of loss. Further, they found that increased rate of uptake is compensated for by increased excretion with the result that resident time remains essentially constant inspite of changes in accumulation time.

Interpreting the mechanisms of immobilization and detoxication of toxicants in marine organisms, Coombs and George (1978) delineated an interesting aspect. Coupling uptake studies with electron microscope studies, they proved that vesicles are formed within the cell to enclose the excess metal with a membrane. This prevents contact of excess metal with vital constituents and effectively detoxify it until eliminated or passed on to other tissues as required. These authors have established the generality of this mechanism. Employing

Saccostrea glomerata, as an indicator of trace metals, Phillips (1979) showed that, rate of accumulation of cadmium, copper, iron and zinc by this bivalve gives an idea about elevated metal levels of the environment. He found out that capacity of this oyster to accumulate easily measurable levels of metals appears to be usually great. Goldberg et al.(1978) analysed the concentrations of heavy metals in the soft tissues of Mytilus edulis, M.californianus, Crassostrea virginica and Ostrea equestris, from the various localities along the western-east coast of United States as a part of 'Mussel Watch' programme. Discussing the feasibility of employing mussels by transplantation, to study heavy metal pollution, Cowell and Anderson (1980) demonstrated that one month of exposure to different environmental conditions was sufficient to allow accumulation of environmental contaminants within the animal tissue. Further, assessment of physiological reactions of the animals during this period also would give meaningful results. Klöckner (1979) made an interesting observation on the kinetics of cadmium accumulation in the polychaete Ophryotrocha diadema. He found that parentally accumulated cadmium loads were not transferred to the offspring. Trying to delineate the relationship between metallothionein like proteins and accumulation of cadmium, zinc, copper and mercury, Roesijadi (1980) demonstrated that exposure of animals to metals such as cadmium, copper, zinc and mercury in laboratory or in the field enhanced levels of metals which are associated with the metal binding proteins. Using Mytilus edulis as an indicator of trace metals in Scandinavian waters, Phillips (1978) found that this animal is a useful indicator of lead and iron in the environment whereas using the animal as an indicator of manganese seemed to be dubious. The response of the embryos of the American oyster Crassostrea virginica to various combinations of two or three metals (Copper,

mercury and zinc as nitrates and chlorides) was studied in laboratory by MacInnes (1981). He found highly significant toxic synergism in the copper-zinc mixtures and three metal-mixtures of both salts. Roesijadi (1980) found that gills were the primary organs for the concentration of copper and could be the target organ for the toxic action of the metals in the clam, Prototheca staminea. Carpene and George (1981) working on the mode of absorption of cadmium by the gills of Mytilus edulis, found that cadmium uptake increases linearly with time and was directly proportional to the external concentration. They suggested that cadmium, zinc and so forth which have long resident time in Mytilus edulis, the environmental variables such as salinity, redox potential, temperature and chelating agents which alter metal bioavailability,exert an effect on uptake process rather than excreting process. Okazaki and Panietz (1981) studied the depuration of 12 trace metals in the tissues of oyster Crassostrea gigas and C. virginica. They found that the digestive gland and kidney depurated cadmium, copper, mercury, silver and zinc more readily than the mantle and the gill. Trace metals like iron, manganese and nickel showed varying depuration patterns. Discussing on the accumulation and detoxication of copper by the mussel, Mytilus galloprovincialis, Viarengo et al. (1981) remarked that during detoxification process, the copper level decreases in the nuclear mitochondrial, soluble and microsomal fractions and the copper load increases in the lysosomal fraction. The lysosomal stability remains slow during the process. Working on the efficacy of mussels, oysters and sediments as indicator of trace metals in HongKong waters, Phillips and Yim (1981) suggested that the efficacy could vary depending on the metal. They suggested that using any animal which has partial or complete metabolic regulation as an indicator of trace metals is meaningless. Ahsanullah et al.

(1981) discussed the toxicity of zinc, cadmium and copper to the shrimp, Callianassa australiensis. They found that accumulation of cadmium was a function of metal concentration in water and of duration of exposure. Zinc and cadmium appear to enhance the uptake of each other. In a mixture of zinc and copper, the uptake of zinc was enhanced and that of copper was inhibited. Uptake and incorporation of mercury into mercury binding proteins of gills of Mytilus edulis as a function of time was analysed by Roesijadi (1982). He found that gill mercury accumulation occurred in 3 phases. There was net uptake phase initially and at the end. The intermediate stable phase was associated with induction of the predominantly mercury binding proteins and mercury incorporation into the proteins. Pre-exposure of marine mussels to low levels of mercury was found to enhance their tolerance to more toxic levels, and higher pre-exposure concentration was not effective in inducing high mercury tolerance (Roesijadi 1982). Strömgren (1982) found that during exposure to copper and zinc, there was a linear reduction in growth rate in Mytilus edulis. Reviewing different aspects of the relationships between tolerance to heavy metal pollution and metabolism in oysters, George and Frazier (1982) detailed the general mechanisms of metal tolerance, detoxication system and metabolic interactions of cadmium, copper and zinc and interrelationships between detoxication systems for copper, cadmium and zinc. Nelson et al. (1983) analysed the long-term silver effects on the marine gastropod Crepidula fornicata. This gastropod was found to accumulate large quantities of silver. George (1983) discussed the relation between the intracellular control of heavy metals in the cell and the pathway of cadmium metabolism in the kidney of Mytilus edulis. Tateda et al. (1984) found that

the abalone Haliotis discus exhibited variation in Iron-59 uptake depending on the differences in the uptake route. Viarengo et al. (1984) characterized the copper-thionein isolated from the tissues of mussels exposed to metals. They also found that soluble, heat stable copper rich protein shows characteristics common to most copper-thionein. Martincic et al. (1984) worked out the bioaccumulation of heavy metals by bivalves. They recorded very high concentration factors for zinc, cadmium, copper and lead in the mussels. Davenport and Redpath (1984), found that the tissue levels of copper increases rapidly when the mussels are exposed to hightened copper concentrations. They remarked that storage of copper by Mytilus edulis, is a somewhat obscure phenomenon. Discussing the indicator ability of Perna viridis, Phillips (1985) suggested that Perna viridis is an excellant indicator species for studies of copper and lead and its use to monitor cadmium, mercury and zinc requires further study. Phillips and Muttarasin (1985) suggested that there are evidences of moderate inter sample variability on the analytical results obtained on the heavy metal load of Perna viridis. Suggesting more reliable analytical techniques to assess trace metal load in bivalves by X-ray microprobe, Thompson et al. (1985) compared amoebocytes of copper and zinc in those of other tissue cells and found that the amoebocytes contain 5-15 fold high copper and zinc concentration, indicating that the amoebocytes are sequestering more zinc than copper and more of this metals than other tissue cells. Cain and Luoma (1985) expressed the view that some limitations in using transplants (Macoma balthica) as indicators of pollutant event or pollutant impact on resident populations. They were analysing copper and silver accumulation in transplanted and resident clam in south San Francisco Bay. Devineau and Triquet (1985), found that the level of zinc in organisms was

independent of the concentration in water suggesting that the bioaccumulation of this essential heavy metal is controlled by some physiological process in Palaemon serratus. Increased zinc concentrations in water, according to them have little or no effect on cadmium level in the prawn. Multielement analysis using electron microscopy with an energy dispursive X-ray analyser, Ishii et al. (1985a) found granules containing extremely large quantities of manganese and cadmium in the kidney of marine bivalve, Cyclosunetta menstrualis. Ishii et al. (1985b) by analytical election microscopy, found that iron, copper and sulphur were localized in granules of epithelial cells of the oyster tissue. Commenting on the high accumulation of metal in the kidney of marine bivalves, 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 center of the lumen of the kidney tubules. Tateda and Koyanagi (1986) found organ specific concentration of radionuclides in the bivalves. Cobalt and iron accumulation in the viscera and kidney, zinc in kidney and manganese in mantle.

From the review presented here it is evident that aspects like acute toxicity and sublethal effects of heavy metals with reference to behaviour, physiology, cytology and biochemistry are receiving ample attention from scientists working in different parts of the world. Many cardinal aspects are still unknown and quite a few conclusions are drawn from circumstantial evidences. A correct understanding the mechanisms involved in detoxication, storage and excretion of heavy metals could be had only if the processess are investigated by multifactorial approach employing biochemical, autoradiographical and electron microscopical studies on the various organs involved of the concerned animals.

M T E R M E T H S

III- MATERIALS AND METHODS

The present investigation deals with the toxicity of silver and copper at lethal and sublethal levels, singly and in combination, on marine mollusc Perna indica. The static bioassay methods followed were based on already accepted procedures adopted for the measurement of pollutant toxicity on marine organisms (Sprague, 1969).

3.1 WATER

The sea water used for the present investigation was collected from the unpolluted area off Cochin. The water was transported to the laboratory in polythene carbouys and kept in total darkness for one week for aging. The particulate living and non-living fractions of sea water were allowed to settle and water was filtered using fibre glass filter (length 32 cm, breadth 16 cm) containing glass wool and activated charcol and then was used for the experiments. The salinity of sea water ranged from 33.5 and 35.5‰ and pH between 8.20 and 8.40 for various sets of experiments. The seawater was aerated to saturation before use. All sets of experiments were conducted at rooom temperature (30±1 C). The addition of toxicants did not bring about appreciable variation in pH.

3.2 TEST ANIMALS

3.2.1. Perna indica

Extensive beds of this commercially important bivalve are available on the rocky beach of Shakthikulangara, (8° 56'N; 76° 35'E) along the coast of Quilon. Fresh settlement of this species occurs around September. The animals of 15-20 mm and 20-25 mm shell length were dislodged and transported to the laboratory in polythene trays with no aeration. The animals

were acclimated in the laboratory in aerated seawater (10 animals/l at 30±1° C) for 24 to 48 h prior to the setting up of experiments. Care was taken to use specimen from the same population for a single set of experiment. Contamination by pseudofaeces and metabolites was avoided by daily renewal of water. During the course of the experiment the test animals were fed with Synechosystis salina.

3.3.. TOXICANTS

3.3.1. Silver

Analar grade of silver nitrate (M.W. 169.87), silver sulphate (M.W. 311.80), silver oxide, (M.W.231.74) and silver carbonate (M.W.275.75) were the source of silver. These salts were dissolved in distilled water and added to make up the required concentrations.

3.3.2. Copper

Analar grade of copper sulphate (M.W.249.68), copper chloride (M.W. 170.48), and copper nitrate (M.W.241.60) were the source of copper. These salts were dissolved in distilled water and added to make up the required concentrations.

3.3.3. Silver and Copper

Silver and copper solutions were prepared individually as described above and added to achieve required concentrations.

3.4. ACTIVITY STUDIES

The aim of the present investigation was to study the lethal and sub-lethal effects of silver and copper, individually and in combination, on life and activity of Perna indica.

The lethal effects are the easiest to determine because of the short time between administration and affliction. Death is taken as the main cri-

teria for assessing the lethal effects. Lethal concentrations were arrived at by adopting short term still water bioassay techniques recommended by Sprague (1969).

Sublethal effects were followed employing various reactions of the animals which could be easily perceived and documented.

3.4.1. Mortality tests

Ten mussels (shell length 15-20 mm) were exposed to 5 L of the test solution in glass troughs. The test solutions were renewed every 24 h and were not aerated. The animals were not fed during the experiments. In the case of Perna indica, valve gaping beyond 5 mm and the inability of the mussels to close the valves under mechanical stimulation were the indices of death. The LC 50 values and their 95% confidence limits were calculated using Probit Analysis (Finney, 1971).

In the case of metal-combination studies, the concentrations of the toxicants (ie toxicant A&B) used were decided based on their respective 96 h LC 50 values. Here, the concentration of one toxicant was kept constant while the other varied. The additive toxicity indices were calculated as mentioned in section 3.6.

3.4.2. Accumulation of metals

Accumulation of metals by an animal is an additional response, that is important in hazard evaluation strategies. The rate of accumulation of silver and or copper salts supplied individually and in combination by Perna indica was studied as explained below. 96 mussels were exposed in polythene tubs of 50 L capacity containing 40 L of the toxicant solution. Quadruplicate experiments were conducted per toxicant concentration and control. The duration of the experiment was 16 days in the case of individual metal

salts and 7 days in the case of combination of metals. The test solutions were replenished every 24 h and the animals were fed with algal culture (Synechocystis salina) daily for 30 minutes in raw seawater. Calculated volume of the culture was added so as to get ca. $5x10^3$ algal cells ml^{-1}. The animals were exposed to the toxicant for 16 days in the case of individual metal salts and 7 days in the case of combination of metals. On 0, 4th, 8th, 12th and 16th day in the case of individual metal salts and on 7th day in the case of combination of metals, sufficient animals (12 animals) were removed from each of the quadruplate experimental tubs. The rate of accumulation of copper ($CuSO_4$) in tissues like mantle, gills, adductor muscle and remaining tissue, was studied by dissecting out the tissues from the test animals and estimating the metal load. In the case of assessing whole tissue load, done in the case of salt forms of copper other than $CuSO_4$, silver and their combinations, whole soft tissue was dissected out and washed in distilled water carefully. Analysis of the metal content of the tissue was estimated using atomic absorption spectrometry, following method explained in section 3.5

3.4.3. Depuration of metals

For studying the rate of depuration of copper and silver, individually and in combination, in Perna indica, the following experimental pattern was adopted. Those animals exposed to single metals and their salt forms for 16 days were removed every 4th day and maintained in raw seawater for 7 days. The water was replenished every 24 h. In the case of metal-combinations, in different salt forms, since the accumulation time was only 7 days, after this period the test individuals were removed and kept in raw seawater for 7 days. Here also, the raw seawater was replenished

every 24 h. The number of animals used for each concentration combination was 36. Of these 36, 12 animals were used for estimating the tissue burden of metals. The other 24 were used for filtration and oxygen consumption studies as explained in the following sections.

3.4.4. Oxygen consumption

The toxic effects of salts of silver and copper, individually and in combination, on the rate of oxygen uptake of Perna indica kept for accumulation and consequent depuration was studied. Test organisms exposed to selected concentrations of metal salts were placed in 1000 ml test solution prepared in seawater aerated to saturation, in conical flask of 1 L capacity. Gas exchange from the atmosphere was avoided by sealing containers with inert liquid paraffin. The duration of the experiment was 5 to 6 h. The rate of oxygen uptake of batches of four animals were estimated every 4th day in the case of those exposed for bioaccumulation studies and after 7th day in the case of those kept for depuration. The frequency of reading was every one hour. The water used for the estimation of dissolved oxygen was siphoned out employing a flexible polythene tube. Winkler's method was followed to estimate the dissolved oxygen. At the end of the experiments, soft tissue of mussels was scooped out, cleaned in distilled water, transferred to pre-weighed aluminium foils, dried at 60° C and then dry weight taken to constancy. The oxygen consumption is expressed as $\mu g\ O_2\ h^{-1}\ mg^{-1}$ (dry weight).

3.4.5. Filtration

The main idea behind this study was to know the trends in the rate of filtration of Perna indica kept for accumulation (of metal salts individually and in combination) and consequent depuration.

Mussels feed and respire by means of a water current drawn into the body under the influence of the ctenidial cilia. The rate of filtration was assessed by employing the dye clearance technique. This technique involves addition of a known concentration of neutral dye (Neutral red 2 ppm) to the test solution and allowing the animals to clear the dye. The quantity filtered after a specific time gap was calculated after finding out the reduction in the dye concentration with the help of a Spectrophotometer (Hitachi:model 220-20) at 435 nm. Filtration rate was estimated using equation suggested by Abel (1976) viz.

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

Where 'm' is the filtration rate; 'M' is the volume of suspension; 'n' is the number of test animals per vessel; 't' is the duration of the experiment; 'Co' is the initial concentration of suspension and 'Ct' is the final concentration. The duration of the experiment was 4 to 5 h. The rate of filtration of batches of four animals were estimated every 4th day in the case of those animals exposed for bioaccumulation and after 7th day in the case of those kept for depuration. The test animals were held in a 500 ml beaker containing 400 ml of test solution and the frequency of reading was every 1 h. The results are expressed as quantity of seawater in ml filtered by one mussel in one hour.

3.5 ANALYSIS OF METALS

Analysis of metals in the seawater, test solution and animal tissues were based on method described by Armannsson (1979).

3.5.1. Reagents and apparatus

Perkin-Elmer (Model 2030) and/or Varian Techtron (Model 1100)

Atomic Absorption Spectrometer were used in accordance with manufacturer's recommendations.

Analytical-grade reagents were used unless otherwise stated.

3.5.1.1. Dilute ammonia solution (pH 8-9).

3.5.1.2. Hydrochloric acid (constant boiling).

Concentrated hydrochloric acid and distilled water (8.25 + 6.75) were mixed and distilled. 2 M and 0.2 M solutions were prepared by dilution.

3.5.1.3. Concentrated nitric acid (distilled).

3.5.1.4. Chloroform (distilled).

3.5.1.5. Dithizone solutions

Dithizone was recrystallized by passing a current of filtered air into a nearly saturated solution of dithizone in chloroform at 40° C. The precipitate was washed with a small amount of carbon tetrachloride. Using this, a 0.2% (w/v) solution in chloroform was prepared and was cleaned by first shaking with dilute ammonia and then with 0.2 M hydrochloric acid. A 0.02% (w/v) dithizone solution was prepared daily from the 0.2% stock solution.

3.5.1.6 Filters

Membrane filters (0.45 μm pores, 4.7 mm diameter) were soaked in 2 M hydrochloric acid over night, rinsed with distilled water. 1 litre distilled water was passed through the membrane filters prior to use.

3.5.2. Procedure for seawater

Samples were collected in a plastic containers. First 500 ml filtrate was filtered and discarded. 1000 ml filtrate was transferred to a 1 litre separating funnel, pH adjusted to ca.8, and 10 ml 0.2 dithizone

solution was added shaking vigorously for 5 minutes. Organic layer was separted into a 100 ml separating funnel.

pH of the aqueous phase was brought to ca. 9.5 with 0.2 ml ammonia liquor, and extraction was repeated with 10 ml 0.2% dithizone solution. Finally, it was again extracted with 5 ml 0.02% dithizone. The combined extracts were washed with 50 ml dilute ammonia and with 5 ml chloroform. The organic phase was run into a second 100 ml separating funnel and 50 ml 0.2 M hydrochloric acid was added shaking vigorously for 2 minutes. The phases were separated and the aqueous portion was washed with 5 ml chloroform. The aqueous portion was evaporated to dryness. Sides of the beaker was washed with ca.10 ml distilled water, evaporated to dryness and residue was dissolved in 5 ml 2 M hydrochloric acid (solution A). 3 ml perchloric acid was added to the organic portion, evaporated to dryness, and 2 ml 60% perchloric acid was added to ensure that all organic matter has been oxidized. The residue after evaporating to dryness was taken up in 5 ml 2M hydrochloric acid (solution B).

For blanks, 20 ml 0.2% dithizone and 5 ml 0.02% dithizone solutions were added to a 100 ml separating funnel and washed with 50 ml dilute ammonia solution and proceeded as for the samples. Concentrated ammonia of 0.2-0.3 ml was added to each beaker before the last evaporation to dryness.

Silver and copper were determined at 328.1 and 423 nm respectively in solution B. The concentrations of the metals in seawater were expressed as ug l^{-1}.

3.5.3. Procedure for animal tissues

A known weight of dry tissue was transferred into a 100 ml conical flask. 5-8 ml of nitric acid was added and left covered on a sand bath at ca. 100° C for 3 h. The cover was removed and the samples were evaporated to dryness. The treatment was repeated with 2-3 ml nitric acid until colour of the residue disappeared. 5 ml (1+1) nitric-perchloric acid was added when the solution was nearly colourless and evaporated to dryness. 50 ml of distilled water and 10 ml hydrochloric acid (constant boiling), was added and heated on a hot plate. When a residue remained, more hydrochloric acid was added until a clear solution resulted. 3 ml 50% (w/v) citric acid solution was added with sufficient ammonia to bring the pH to 8 (indicator paper). The solution was cooled and separated into a 150 ml separating funnel and the pH adjusted to 8 with ammonia and/or hydrochloric acid (constant boiling, using indicator papers. 5 ml 0.2% dithizone was added and mixed vigorously for 2 minutes. The organic layer was seperated into a 100 ml separating funnel. The pH of the solution was adjusted to 9.5 with ammonia and the extraction was repeated. Extraction was repeated with 5 ml 0.02% dithizone solution until dithizone retains its colour. The combined dithizone extracts were washed with dilute ammonia and the dithizone layer was removed into a second 100 ml separating funnel and proceeded for seawater.

Silver and copper concentrations were determined at 328.1 and 423 nm respectively and the results were expressed as µg metal g^{-1} (dry weight).

3.6 JOINT TOXICITY ANALYSIS

The concept of prediction of toxicity of mixtures of pollutants has received wide approval in aquatic toxicological studies as it provides scope

to study simultaneous effects of several pollutants in a single set of experiment, the results of which can be expressed as a single number.

To determine the toxicity of metal mixtures, at lethal and sublethal levels, additive toxicity index developed by Marking and Dawson (1975) was used. The toxicity unit or sum of the biological activity of a solution containing metal-mixtures are calculated based on the formula (when the toxicity unit is used to determine the interaction between metal mixtures)-

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

Where A and B are metals, subscripts i and m are toxicities (LC 50 or EC 50 values) of the individual metal and metal-mixtures respectively, and S is the toxicity unit or the sum of the biological activity. To arrive at an appropriate index, one of the following relationships depending on whether S is more than or less than 1 is employed.

$$\frac{1}{S} \quad 1 \text{ if } S \quad 1$$

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

when the additive indices are having negative values, it indicates less than additive, 'O' value, simple additivity and + value, more than additivity. If the 95% confidence limits overlap 'O', it is considered as simple additivity.

3.6.1. Statistical analysis

Probit Analysis (Finney, 1971) was used to calculate the LC 50 values and their 95% confidence limits.

Analysis of variance technique (ANOVA) was applied to find out

the statistically significant differences of the sublethal responses registered in respect of the test animals. Further, any significant variation (at 1 or 5% level) between two parameters was tested using Tukey's test.

3.7. TERMINOLOGY

The terminology used in the present work is those adopted by Sprague (1969, 1970, 1971). Median effective concentration (EC 50) and median lethal concentration (LC 50). These terms correspond to ED 50 and LD 50, universally used in toxicology. EC 50 and LC 50 deal with concentrations in the surrounding water. The term TLM refers to median tolerance limit. TLM can be substituted by LC 50. Incipient lethal level refer to "that level of the environmental entity beyond which fifty percent of the population can not live for an indefinite time".

Since toxicants have been used in combinations, a few approved terms are employed to express the interactions. The terminology used to describe the interaction of metal-mixtures has been reviewed by (Sprague, 1969). Joint action refers to the response produced by combination of two toxicants showing that toxicants are helping one another. Joint action can be further divided into additive, more than additive and less than additive interactions. In order to classify the interaction between a metal A and metal B, the method used is to first measure the concentration of each (1.0 A or 1.0 B) which will produce a response, say 50% mortality in the organism. The organism is then exposed to a mixture such as 0.5 A + 0.5 B or 0.3 A + 0.7 B and the response is measured. If the mixture produces the same effect as 1.0A or 1.0B then the joint action of the metals is additive, but if a stronger mixture (eg. 0.6A + 0.7B) or a weaker mixture (eg. 0.3A + 0.4B) is required to give the response then the action of the

metals ie. less than additive, or more than additive respectively. In addition to Joint action, where one metal helps the other, there is no interaction where for example 1.0 A is required to produce the response no matter what concentration of B is present in test-solution, then A alone is causing the response and B is neither helping nor inhibiting. Finally, there is antagonism where more than 1.0A is required to produce the response because of the presence of the antagonising substance B. The synergism and potentiation usually means a co-operative action. However, this is similar to more than additive.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

During the present investigation experiments were conducted to gather data on lethal toxicity of copper and silver in their different salt forms individually and in combination. Further, sublethal aspects like accumulation and depuration, oxygen uptake and filtration accompanying accumulation and depuration were also conducted. Accumulation and depuration of metals in different tissues when the animals were exposed to metals and their mixtures also formed a part of study. Variations in the rate of uptake of copper or silver when present in test solution in different salt forms were also assessed. The results obtained are presented in different sections for easy representation.

4.1. LETHAL TOXICITY STUDIES

4.1.1. Individual metals

4.1.1.1 Copper

The cumulative percentage mortality of Perna indica exposed to sulphate and nitrate forms of copper are presented in Tables 1 and 2. In general, both copper sulphate and copper nitrate did not bring about conspicuous mortality of the test organisms till the concentration reached 20 ppb. Mortality was recorded in 30 ppb of copper whether in the form of sulphate or nitrate. All the animals exposed to 80 ppb of copper (sulphate or nitrate) died within 72 h. 96 h LC 50 values worked out were 38.3 and 41.4 ppb copper in sulphate and nitrate respectively (Table 9 and Figures 1a & 2a). The different salt forms of copper did not show clear cut variations in mortality rates although sulphate was relatively more toxic than nitrate. The effective time to kill Perna indica varied considerably (between 40 and

Table 1. Perna indica. Cumulative percentage mortality of individuals exposed to varying copper ($CuSO_4$) concentrations.

Cu^{++} Concentration (ppb)	Time(h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	10
30	0	0	0	0	10	10	20	20
40	0	0	10	10	20	30	40	50
50	0	10	20	50	60	60	70	70
60	0	10	30	60	70	70	80	90
70	10	30	50	60	80	90	100	
80	20	40	70	80	90	100		

Table 2. Perna indica. Cumulative percentage mortality of individuals exposed to varying copper ($Cu(NO_3)_2$) concentrations.

Cu^{++} Concentration (ppb)	Time(h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	10	10
30	0	0	0	10	20	20	20	20
40	0	10	10	20	20	30	40	40
50	10	10	20	30	40	50	60	60
60	0	10	20	40	50	70	70	80
70	10	30	40	50	70	80	80	90
80	20	30	60	80	90	100		

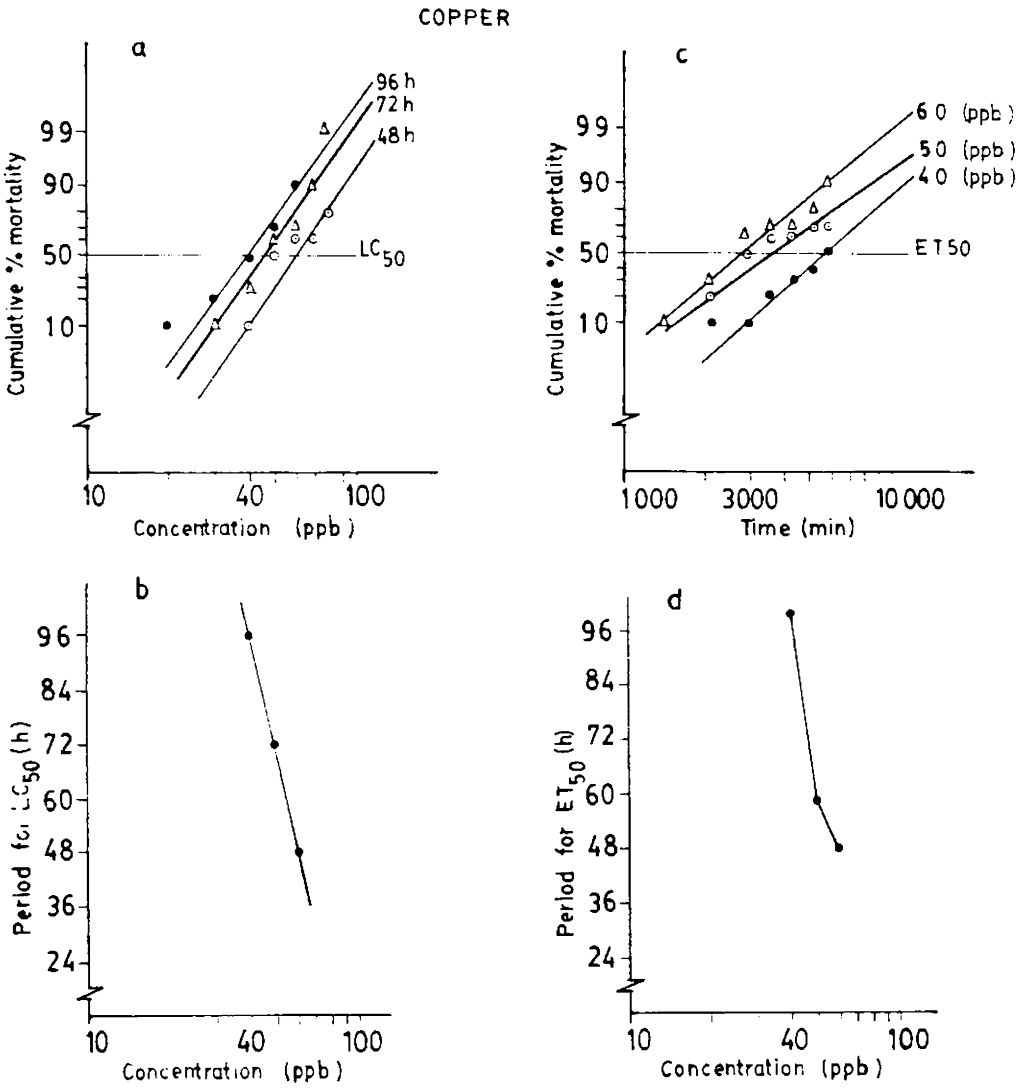

Figure 1. Perna indica. Median lethal concentrations (LC 50) for varying $CuSO_4$ exposure times (a), mortality of individuals exposed to varying $CuSO_4$ concentrations (b), and toxicity curves (c?d).

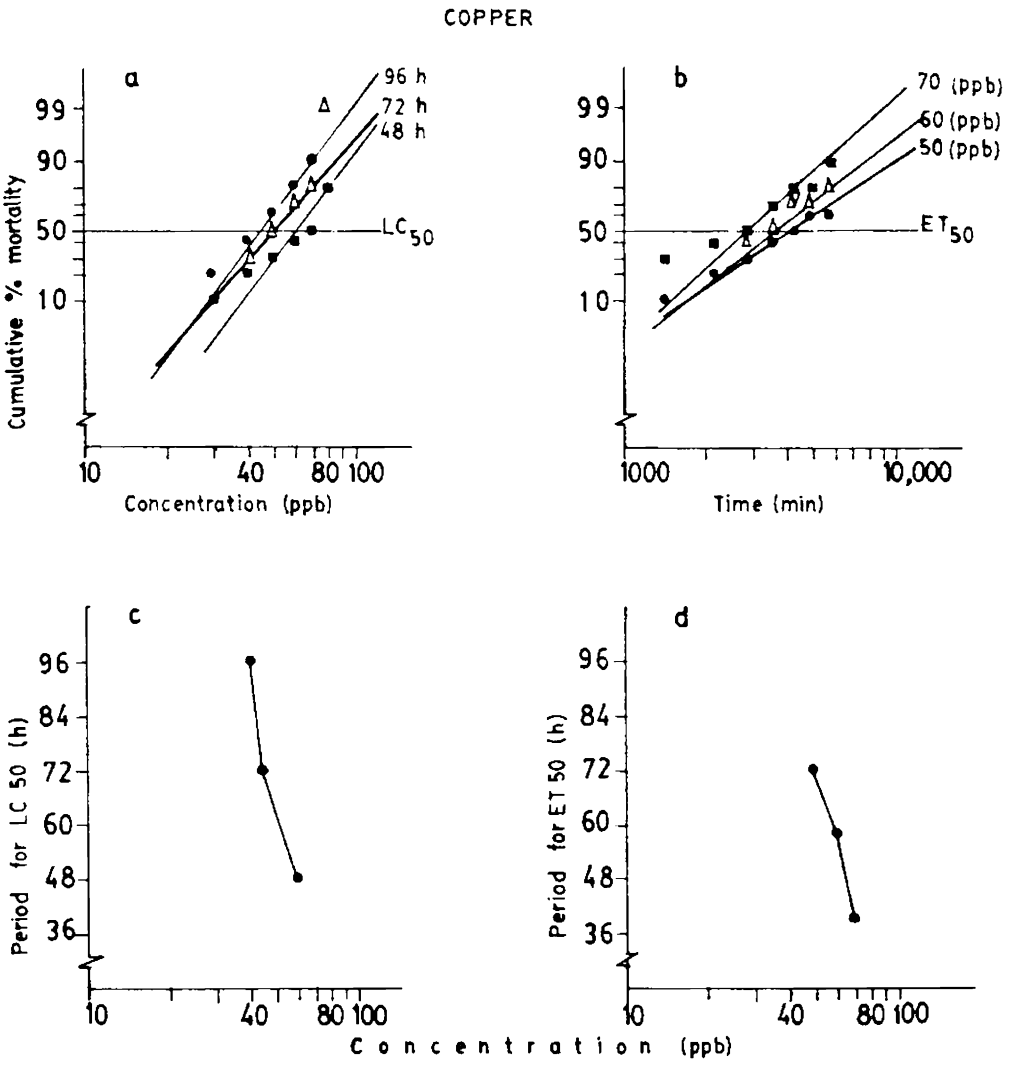

Figure 2. Perna indica. Median lethal concentrations (LC 50) for varying $Cu(NO_3)_2$- exposure times (a), mortality of individuals exposed to varying $Cu(NO_3)_2$ concentrations (b) and toxicity curves (c&d).

50 ppb) of copper in the sulphate form, whereas it was not so in the case of nitrate (Table 12 and Figures 1b & 2b).

4.1.1.2. Silver

Silver proved to be more toxic than copper (Tables 3 and 4 and Figures 3 and 4). Death was recorded when Perna indica was exposed to even such lower silver concentrations of 10 ppb supplied in the form of sulphate in test solution. In 60 ppb of silver, in sulphate form, all the test individuals died within 96 h whereas it occured only at 80 ppb when supplied in nitrate form. Clear cut variations were recorded in 96 h LC 50 values. Silver in sulphate form proved to be very toxic and 96 h LC 50 value was 24.9 ppb whereas it was 45.4 ppb when added in nitrate form to the test solution (Table 9 and Figures 3a & 4a). In the case of silver, silver sulphate recorded ET 50 of 58.49 at 60 ppb and 102.18 at 30 ppb. But in the case of silver nitrate the ET 50 at 50 ppb was 95.22 (Table 10 and Figure 3b & 4b).

4.1.2. Metal combinations

4.1.2.1. Copper + Silver

The sulphate and nitrate form of copper, in combination with the same quantity of silver were used to find out the variations in the mortality rates. The concentrations employed were 9 ppb $CuSO_4$ with 3-15 ppb Ag_2SO_4 and 9 ppb $Cu(NO_3)_2$ with 3-15 ppb $AgNO_3$. The results are documented in Tables 5 and 6. Mortality occured in both the combinations even at the lowest concentrations used. The 96 h LC 50 was 2.3 ppb Ag_2SO_4 with 9 ppb $CuSO_4$, and 6.3 ppb $AgNO_3$ with 9 ppb $Cu(NO_3)_2$. From the pattern observed on the progress of mortality it is clear that the combination of SO_4 was more lethal than that of NO_3 (Figures 4 & 5) irrespective of the metals

Table 3. Perna indica. Cumulative percentage mortality of individuals exposed to varying silver (Ag_2SO_4) concentrations.

Ag^+Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	10	10
20	0	0	10	10	10	30	30	30
30	0	0	0	10	20	30	30	50
40	0	0	10	10	30	40	60	70
50	0	0	10	20	40	50	60	80
60	0	0	20	20	30	60	80	100
70	0	10	30	50	80	100		
80	10	10	40	70	100			

Table 4. Perna indica. Cumulative percentage mortality of individuals exposed to varying silver ($AgNO_3$) concentrations.

Ag^+Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
20	0	0	0	0	0	10	10	10
30	0	0	0	0	10	10	10	10
40	0	0	0	10	10	20	20	30
50	0	0	10	10	30	40	40	50
60	0	0	0	10	20	30	50	60
70	10	10	10	20	40	60	70	70
80	10	10	20	40	50	70	90	100

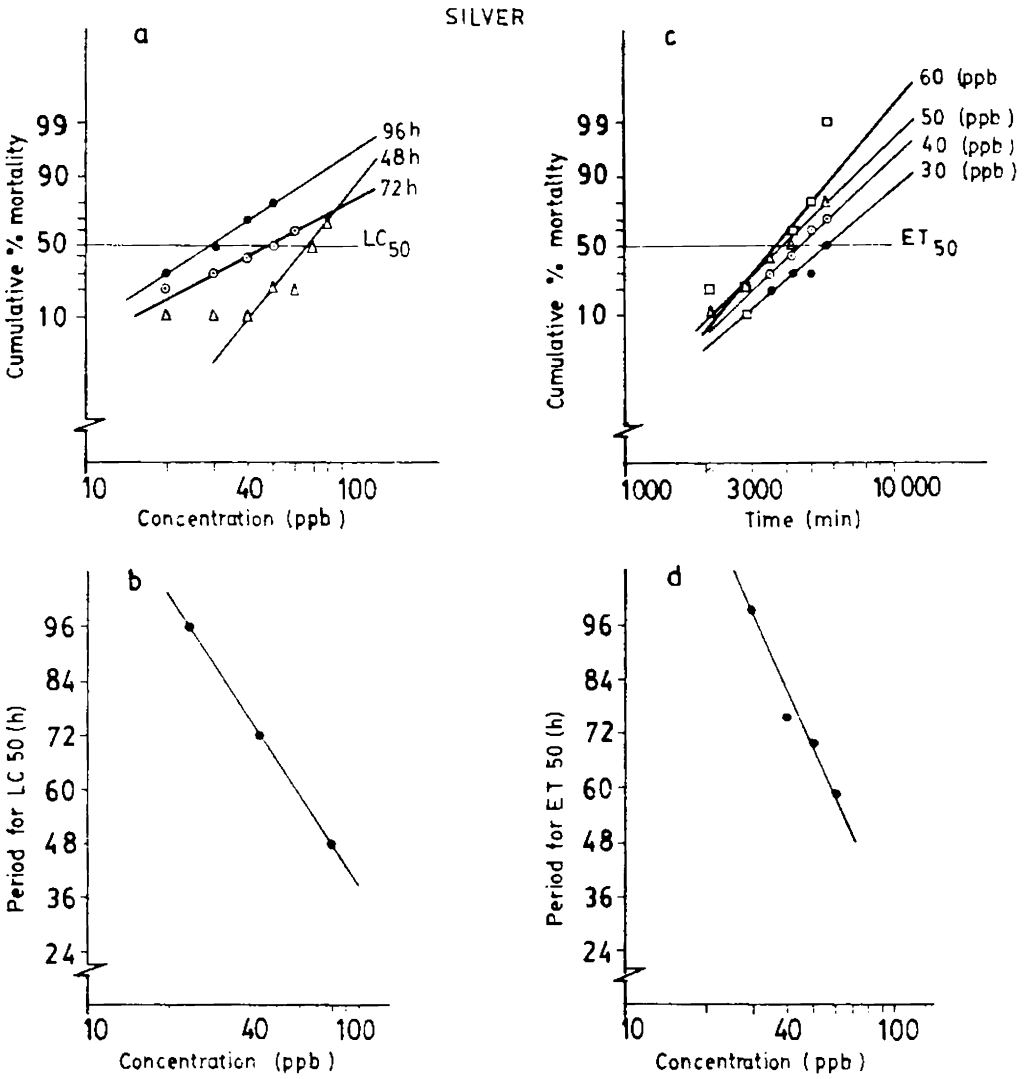

Figure 3. Perna indica. Median lethal concentrations (LC 50) for varying Ag_2SO_4 - exposure times (a), mortality of individuals exposed to varying Ag_2SO_4 concentrations (b) and toxicity curves (c?d).

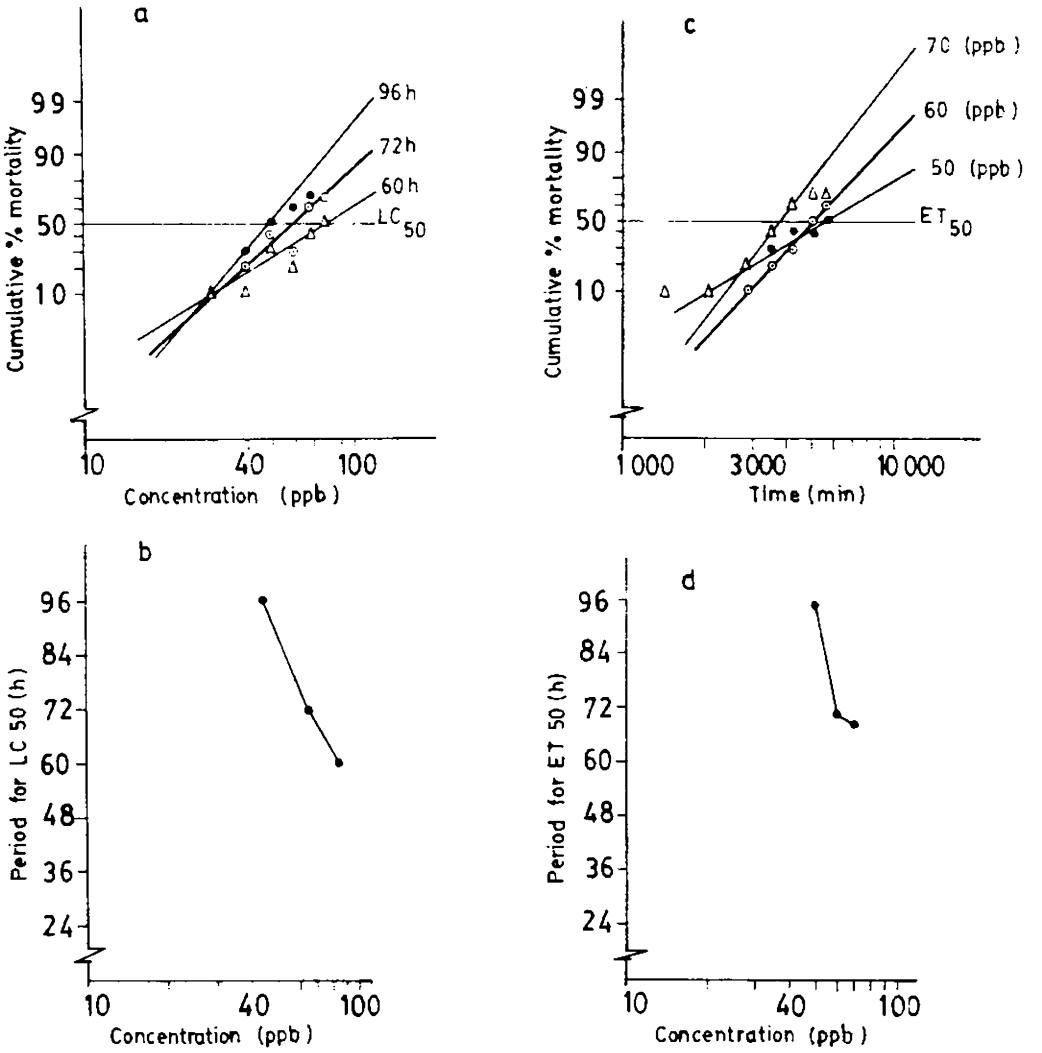

Figure 4. Perna indica. Median lethal concentrations (LC 50) for varying $AgNO_3$ exposure times (a), mortality of individuals exposed to varying $AgNO_3$ concentrations (b) and toxicity curves (c?d).

Table 5. Perna indica. Cumulative percentage mortality of individuals exposed to an unvarying overload of 9 ppb copper ($CuSO_4$) + varying silver (Ag_2SO_4) concentrations.

Ag^+ Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
3	0	0	0	0	20	30	40	60
6	0	0	0	10	20	50	60	70
9	0	0	0	20	30	60	70	80
12	0	0	10	30	50	80	90	90
15	0	0	10	30	60	90	100	

Table 6. Perna indica. Cumulative percentage mortality of individuals exposed to an unvarying overload of 9 ppb copper ($Cu(NO_3)_2$) + varying silver ($AgNO_3$) concentrations.

Ag^+ Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
3	0	0	10	20	20	20	30	30
6	0	0	10	20	20	30	40	50
9	0	10	10	30	50	60	70	70
12	0	0	10	20	40	60	90	90
15	0	0	20	60	70	80	100	

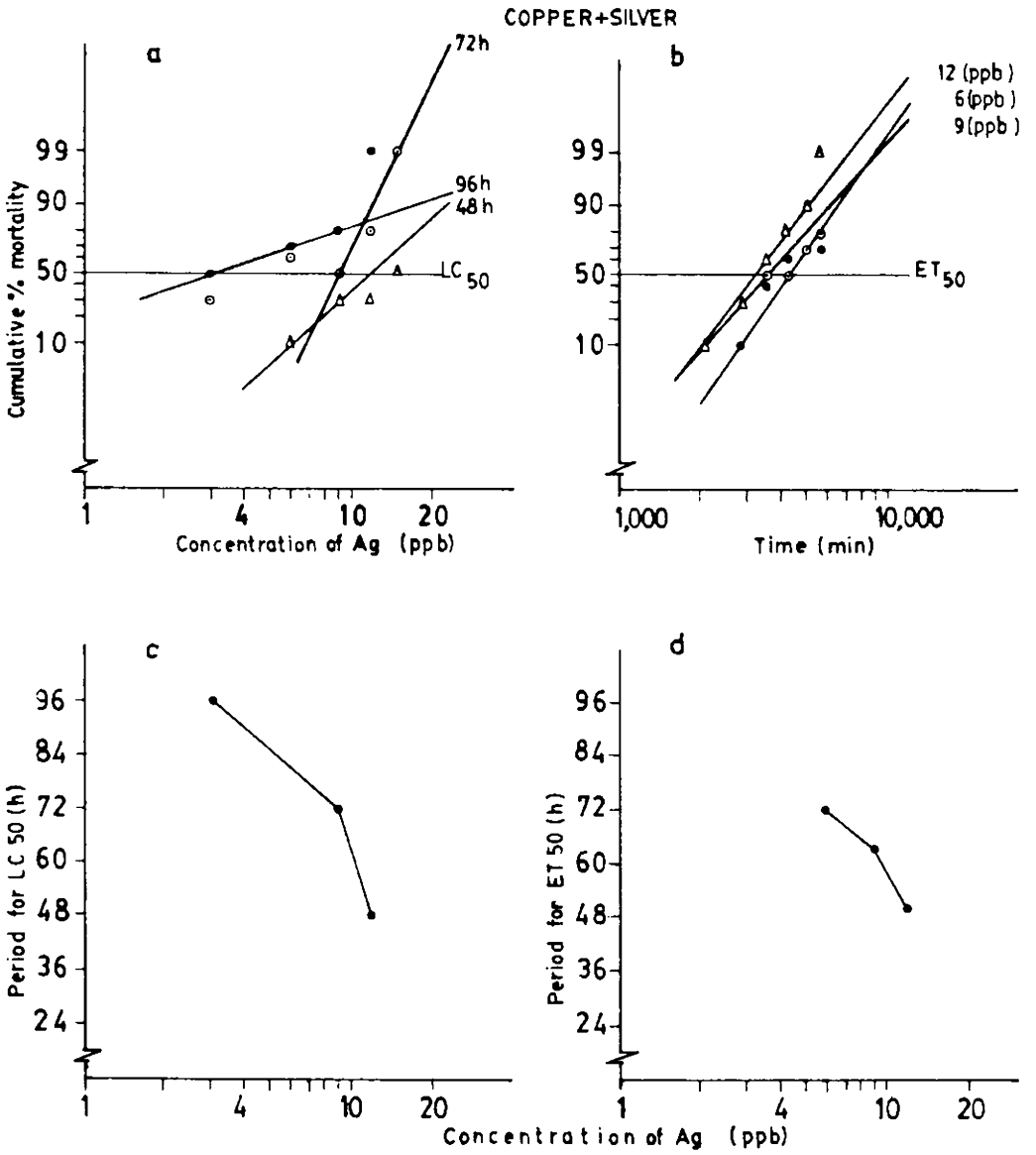

Figure 5. Perna indica. Median lethal concentrations (LC 50) for varying $AgSO_4$ + unvarying $CuSO_4$ overloads of 9 ppb exposure times (a), mortality of individuals exposed to varying $AgSO_4$ + unvarying $CuSO_4$ overloads of 9 ppb concentrations (b) and toxicity curves (c&d).

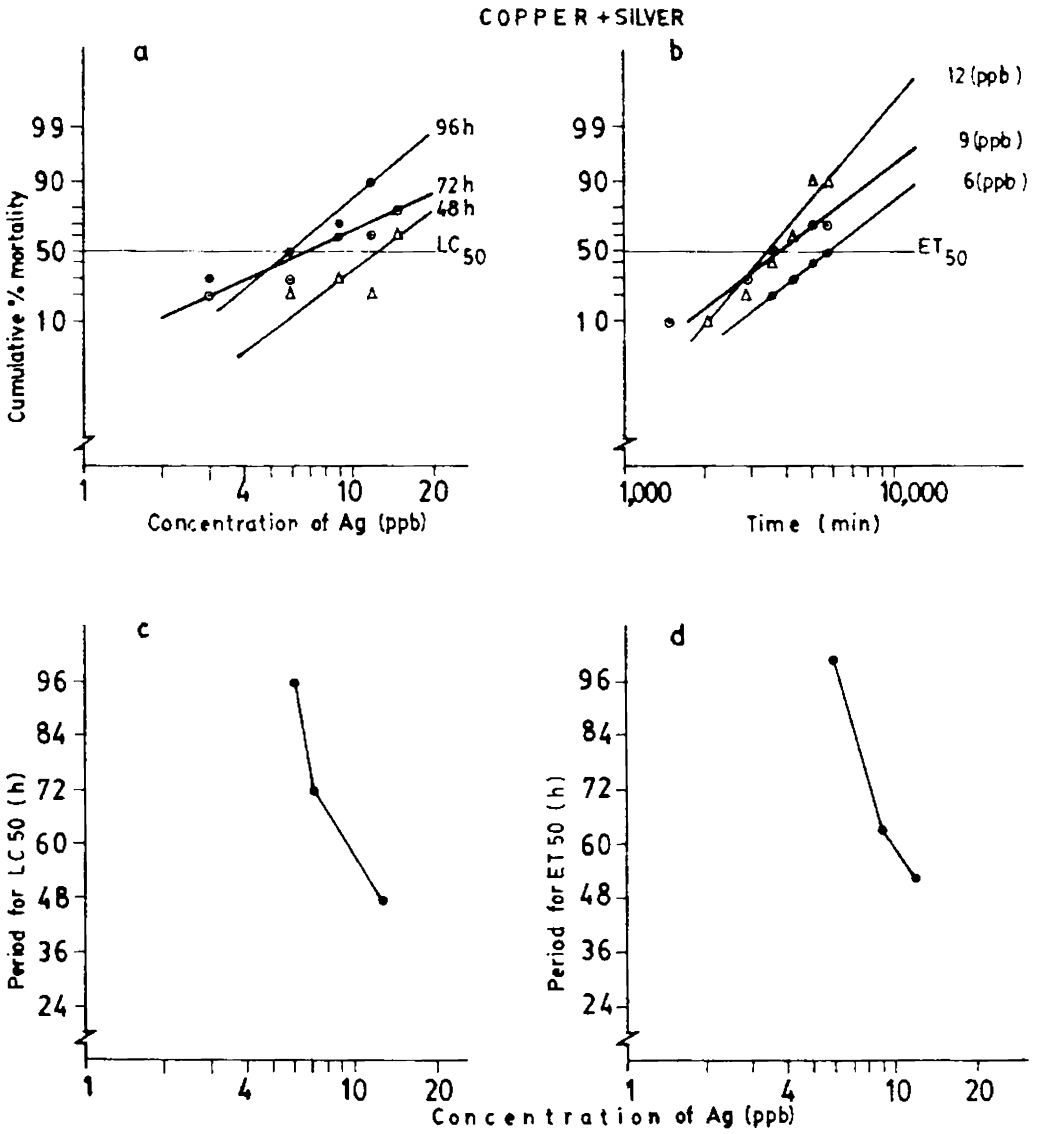

Figure 6. Perna indica. Median lethal concentrations (LC 50) for varying $AgNO_3$ + unvarying $Cu(NO_3)_2$ overloads of 9 ppb exposure times (a), mortality of individuals exposed to varying $AgNO_3$ + unvarying $Cu(NO_3)_2$ overloads of 9 ppb concentrations (b) and toxicity curves (c & d).

involved. The combinations were found to be more than additive (Table 10) in bringing about death of Perna indica. The results obtained on the combined toxicity with respect to ET 50 reflect, those obtained for individual toxicity (Table 12 and Figures 5 & 6).

4.1.2.2. Silver + Copper

In a reciprocal bioassay experiment the silver concentration was maintained constant and the copper made to vary (Tables 7 & 8 and Figures 7 & 8). Both the salt forms were employed. The results show that, here also the lowest concentration employed namely 9 ppb silver + 3 ppb of copper in SO_4 form and the same concentration of metals in NO_3 form proved lethal. The LC 50 values worked out were 5.7 ppb copper along with 9 ppb silver in SO_4 form and 7.3 ppb copper with 9 ppb silver in NO_3 form. Here also, the combinations were found to be more than additive (Table 11) in bringing about death of Perna indica. The results obtained on the combined toxicity with respect to ET 50 reflect those obtained for individual toxicity (Table 12 and Figures 7b & 8b).

4.2 SUBLETHAL TOXICITY STUDIES

In this part, the results obtained on accumulation and depuration of copper and silver in their different salt forms and sublethal effects on oxygen consumption and rate of filtration accompanying accumulation and depuration are presented.

4.2.1. Individual metals

4.2.1.1. Copper (as $CuSO_4$)

Different salts of copper namely $CuSO_4$, $Cu(NO_3)_2$ and $CuCl_2$ were used to find out the nature of accumulation and depuration by Perna indica. Among these salts, $CuSO_4 \cdot 5H_2O$ which was found to be more toxic, was

Table 7. Perna indica. Cumulative percentage mortality of individuals exposed to an unvarying overload of 9 ppb silver (Ag_2SO_4) + varying copper ($CuSO_4$) concentrations.

Cu^{++} Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
3	0	0	0	0	10	10	20	20
6	0	0	0	10	10	20	20	40
9	0	0	10	20	20	30	30	50
12	0	0	0	10	20	30	40	60
15	0	0	10	20	40	60	80	90

Table 8. Perna indica. Cumulative percentage mortality of individuals exposed to an unvarying overload of 9 ppb silver ($AgNO_3$) + varying copper ($Cu(NO_3)_2$) concentrations.

Cu^{++} Concentration (ppb)	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
3	0	0	10	10	30	40	40	40
6	0	0	0	20	40	50	50	50
9	0	10	10	30	50	60	60	70
12	0	0	10	30	50	50	70	80
15	0	10	30	70	80	90	90	100

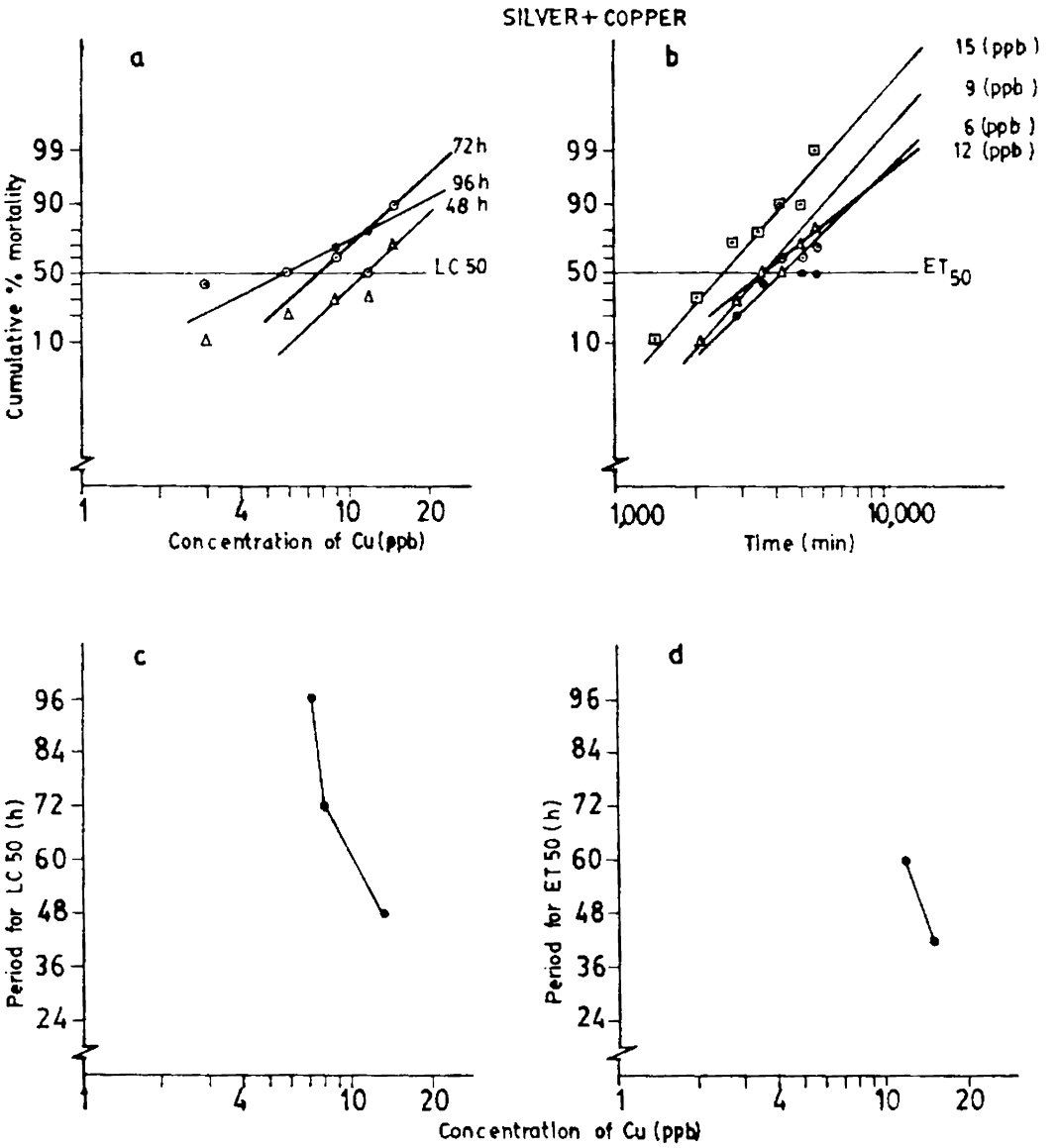

Figure 7. Perna indica. Median lethal concentrations (LC 50) for varying $CuSO_4$ + unvarying Ag_2SO_4 overloads of 9 ppb exposure times (a), mortality of individuals exposed to varying $CuSO_4$ + unvarying Ag_2SO_4 overloads of 9 ppb concentrations (b) and toxicity curves (c&d).

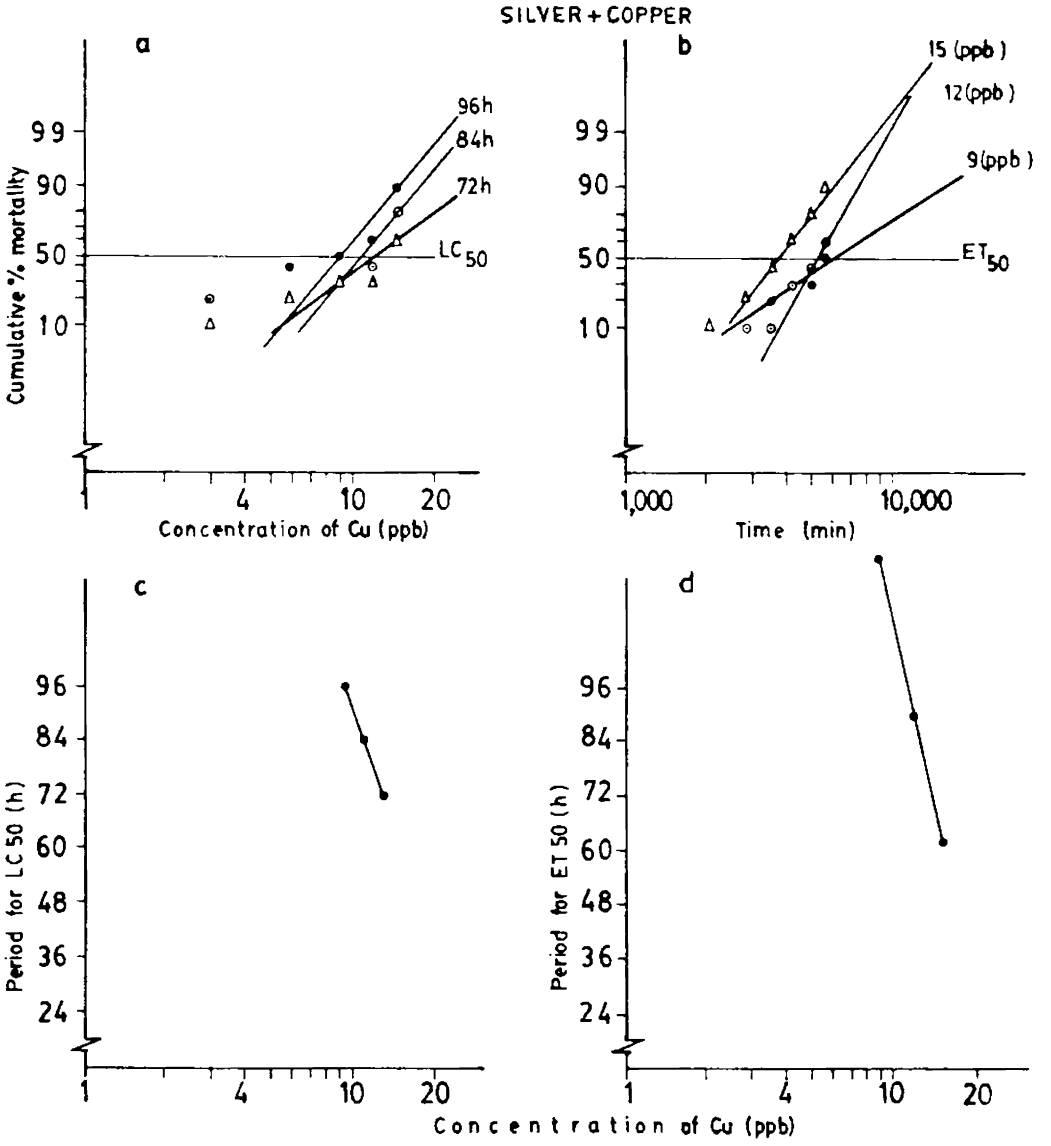

Figure 8. Perna indica. Median lethal concentrations (LC 50) for varying $Cu(NO_3)_2$+ unvarying $AgNO_3$ overloads of 9 ppb exposure times (a), mortality of individuals exposed to varying $Cu(NO_3)_2$+ unvarying $AgNO_3$ overloads of 9 ppb concentrations (b) and toxicity curves.

Table 9. Perna indica. LC 50 (ppb), upon exposure to copper and silver (SO_4 and NO_3 form) salts over periods upto 96 h, along with respective 95% confidence limits and slope functions.

Metal	48 h		96 h	
	LC 50 (ppb)	Slope 'b'	LC 50 (ppb)	Slope 'b'
Copper ($CuSO_4.5H_2O$)	57.9 (52.2-64.9)*	6.1	38.3 (34.7-42.1)*	5.3
Copper ($Cu(NO_3)_2$)	61.0 (58.1-64.0)*	4.3	41.4 (32.2-44.8)*	4.7
Silver (Ag_2SO_4)	81.6 (54.0-123.3)*	2.8	24.9 (19.4-32.0)*	4.0
Silver ($AgNO_3$)			45.4 (37.0-55.7)*	5.0

* 95% confidence limits.

Table 10. Perna indica. 96 h LC 50 (ppb), upon exposure to copper + silver (SO_4 and NO_3 form), salts along with respective 95% confidence limits, slope functions and additive indices.

Copper (ppb)	Silver 96 h LC 50 (ppb)	Slope 'b'	Additive index
9 (SO_4)	2.3 (1.5-3.4)*	1.6	+ 0.32**
9 (NO_3)	6.3 (2.3-7.4)*	17.0	+ 0.65**

* 95% confidence limits

** More than additive

Table 11. Perna indica. 96 h LC 50 (ppb), upon exposure to silver + copper (SO_4 and NO_3 form), salts along with respective 95% confidence limits, slope functions and additive indices.

Silver (ppb)	Copper 96 h LC 50 (ppb)	Slope 'b'	Additive index
9 (SO_4)	5.7 (4.4-7.5)*	5.4	+ 0.60**
9 (NO_3)	7.3 (4.3-12.2)*	16.2	+ 0.63**

* 95% confidence limits

** More than additive

Table 12. Perna indica. ET 50 (h) (effective time to kill 50% of test population) when exposed to copper, silver, copper + silver and silver + copper (SO_4 and NO_3 form) as a function of time.

Metal	Concentration (ppb)	Time (h)
INDIVIDUAL		
Copper ($CuSO_4$)	40	103.70 (87.5 120.3)
	50	58.25 (52.3 64.0)
	60	48.16 (44.4 51.3)
Copper ($Cu(NO_3)_2$)	50	72.47 (69.5 76.10)
	60	57.04 (54.47 60.26)
	70	39.26 (35.27 43.23)
Silver (Ag_2SO_4)	30	102.18 (90.20 116.23)
	40	77.45 (70.20 86.32)
	50	69.39 (66.36 73.39)
	60	58.49 (49.37 70.26)
Silver ($AgNO_3$)	50	95.22 (80.05 112.32)
	60	56.57 (54.46 59.05)
	80	43.50 (32.44 58.33)
COMBINATIONS		
Copper + Silver		
9ppb Cu+Ag (sulphate)	6	76.04 (72.12 80.01)
	9	69.19 (63.13 75.50)
	12	57.38 (41.27 73.22)
9ppb Cu+Ag (nitrate)	6	104.22 (91.22 119.12)
	9	64.36 (57.49 72.04)
	12	56.21 (59.53 69.21)
Silver + Copper		
9ppb Ag+Cu (sulphate)	9	114.18 (75.23 173.30)
	12	90.29 (85.03 95.02)
	15	62.58 (47.27 83.28)
9ppb Ag+Cu (nitrate)	6	58.17 (49.23 - 69.31)
	9	45.05 (39.59 50.38)
	15	42.32 (38.32 46.28)

Values in parantheses represent 95% confidence limits.

used to delineate the nature of uptake, storage and depuration in various tissues such as mantle, gill, adductor muscle and remaining tissue.

4.2.1.1.1. Accumulation

Table-13 gives data gathered on copper concentrations in the mantle of Perna indica, when they were exposed from 0.50 to 4 ppb copper for 16 days. Increase in the concentration of copper in the test media affected the rate of uptake. It was noticed that the quantity that accumulated during the first 4 days by Perna indica, exposed to 3 and 4 ppb copper was higher than the quantity recorded in the mantle even after 16 days in those animals exposed to 0.5 to 2 ppb for 16 days. However, both in 3 ppb and 4 ppb, the difference between the quantities accumulated between 4th and 16th day was relatively more in 4 ppb. Figure 9 shows the pattern of accumulation of copper in the mantle and clearly indicates that in the higher concentration, the quantity of copper accumulated was very high during the initial 4 days of accumulation. From the analysis of variance, the following generalisations can be made based on least square difference using Tukey's test (Table 14). Between treatment combinations, the quantity accumulated by those animals exposed to 0.5 ppb copper was significantly different from those kept in 1,2,3 and 4 ppb copper. When the duration of exposure reached 12 and 16 days, the animals kept in 4 ppb copper accumulated it in such a fashion which was significantly different from that of the rest. Those animals kept in 0.5 ppb for 8 and 12 days and those kept in 1 ppb for 4 days had comparable quantity of copper in their mantle. Depending on the concentrations, the duration varied to achieve comparable concentrations in the mantle. Between concentrations, the animals kept at different concentrations accumulated copper in a fashion significantly

Table 13. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the mantle ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to sublethal concentration (0.5 to 4 ppb) of copper for periods upto 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	7.58	6.37	13.30	17.88	11.28	4.62
0.50	8	20.66	24.25	17.67	22.80	21.34	2.47
	12	27.37	27.12	28.56	28.51	27.89	0.65
	16	29.30	29.06	34.07	34.45	31.72	2.54
	4	23.56	21.97	23.14	37.10	26.44	6.18
1.00	8	32.62	33.67	34.53	33.69	33.62	0.67
	12	37.83	35.22	38.19	36.49	36.93	1.17
	16	42.53	46.91	42.12	39.43	42.74	2.68
	4	44.94	46.32	46.32	46.26	45.96	0.58
2.00	8	50.91	52.33	53.86	53.81	52.72	1.21
	12	51.72	51.37	51.84	49.03	50.99	1.14
	16	56.75	51.06	56.72	56.87	55.35	2.47
	4	58.04	50.63	56.03	59.55	56.06	3.37
3.00	8	61.94	64.85	66.02	62.82	63.90	1.61
	12	72.17	73.09	73.94	71.31	72.62	0.98
	16	78.86	77.45	75.12	73.32	76.18	2.12
	4	74.51	71.94	69.56	69.11	71.28	2.15
4.00	8	82.97	79.60	82.98	75.38	80.23	3.12
	12	88.91	91.22	96.06	88.05	91.06	3.11
	16	108.05	104.15	100.46	94.31	101.74	5.06

Table 14. Perna indica. Accumulation: Analysis of variance for changes in copper concentration in the mantle, when the animals were exposed to various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	11.9844	3.9948	0.3623(NS)
2. Between treatment combinations	19	45101.1000	2373.7421	215.3288**
a.Between concentrations	4	40323.9000	10080.9750	914.4736**
b.Between periods	3	4079.3800	1359.7933	123.3506**
c.Interaction	12	697.7970	58.1497	5.2749**
3. Error	57	628.3590	11.0238	
4. Total	79	45741.4434		

NS - Not significant

** Significant at 1% level.

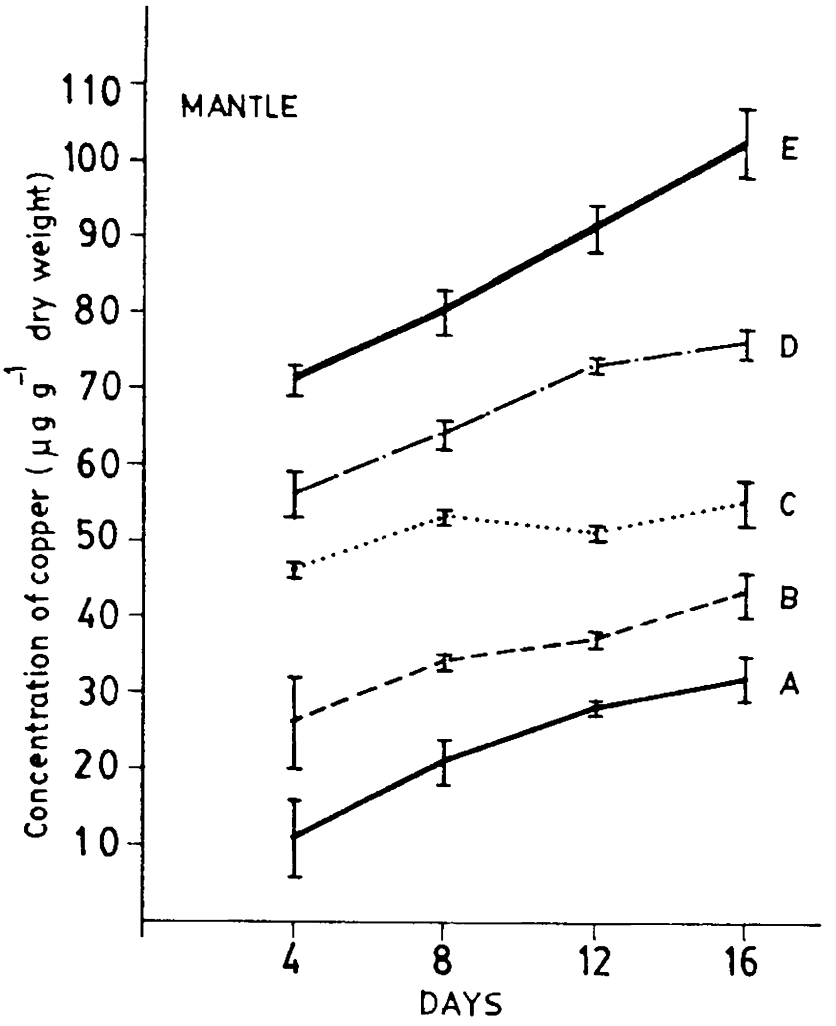

Figure 9. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the mantle, when the animals were exposed to sublethal concentrations (A = 0.5 ppb, B = 1 ppb, C = 2 ppb, D = 3 ppb and E = 4 ppb) of copper, for period from 4 to 16 days.
Vertical bars show standard deviation.

different from each other. Similarly, the same pattern was observed between periods also.

The copper concentrations of gill of Perna indica exposed to various concentrations for periods upto 16 days is given in Table 15. Among the four groups of tissues examined, gills accumulated more copper than mantle and adductor muscle. Here also, the animals exposed to 4 ppb copper accumulated maximum copper in the gill and those at 0.5 ppb the least. The pattern of uptake (Figure 10) between days, show that animals exposed to 4 ppb copper, continued to accumulate more throughout the period of exposure. On the other hand, in concentrations 0.5, 1 and 2 ppb the rate of uptake was slower in the gill. The examination of the analysis of variance (Table 16) gives the following generalisations. The variations between treatment combinations were tested for significance using least square difference based on Tukey's test. The quantity accumulated in the gill by those animals exposed to 0.5 ppb for 16 days was comparable to those kept in 2 ppb for 4 days. The animals kept at 4 ppb for 4 days recorded comparable accumulation rates with those kept at 3 ppb for a period of 16 days. The rate of uptake by those animals kept in a copper concentration of 4 ppb was significantly different in between the periods. The differences noticed between concentrations and between periods were significantly different from each other (Table 16).

The details of concentrations of copper in the adductor muscle of Perna indica, exposed to various concentrations are presented in Table 17 and Figure 11. Comparatively those animals exposed to 0.5 to 3 ppb accumulated very low quantities of copper after enhanced uptake during the initial 4 days (Figure 11). On the other hand, those animals kept in 4 ppb

Table 15. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the gill ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to sublethal concentrations (0.5 to 4 ppb) of copper for periods upto 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	24.52	26.58	21.43	20.14	23.16	2.53
0.50	8	29.18	32.25	30.14	33.50	31.26	1.70
	12	29.92	34.36	31.08	26.26	30.40	2.89
	16	34.16	36.26	40.10	37.45	36.99	2.14
	4	48.03	45.20	46.50	40.09	44.95	2.98
1.00	8	51.33	49.25	49.74	46.33	49.16	1.80
	12	49.87	50.14	51.81	47.70	49.88	1.48
	16	53.06	55.95	50.95	53.60	53.39	1.77
	4	36.07	29.11	30.57	35.19	32.73	2.95
2.00	8	40.67	39.09	45.56	47.07	43.09	3.30
	12	48.46	42.90	35.22	57.15	45.93	8.00
	16	56.13	54.32	46.76	46.98	51.04	4.22
	4	61.56	64.15	66.54	64.39	64.16	1.76
3.00	8	68.86	67.58	69.73	70.57	69.18	1.10
	12	76.66	76.67	73.30	69.09	73.93	3.11
	16	79.85	85.87	84.47	80.58	82.69	2.54
	4	93.30	94.97	90.83	91.34	92.61	1.64
4.00	8	109.61	92.09	108.89	103.23	103.45	7.01
	12	121.07	113.71	114.64	122.42	117.96	3.82
	16	136.94	128.60	125.09	135.19	131.45	4.81

Table 16. Perna indica. Accumulation: Analysis of variance for changes in copper concentration in the gill, when the animals were exposed to various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	37.2188	12.4062	0.7298(NS)
2. Between treatment combinations	19	70296.6000	3699.8210	217.6582**
a. Between concentrations	4	64861.0000	16215.2500	953.9336**
b. Between periods	3	4030.2200	1343.4066	79.0318**
c. Interaction	12	1405.3800	117.1150	6.8898**
3. Error	57	968.9060	16.9983	
4. Total	79	71302.7248		

NS - Not significant
** - Significant at 1% level.

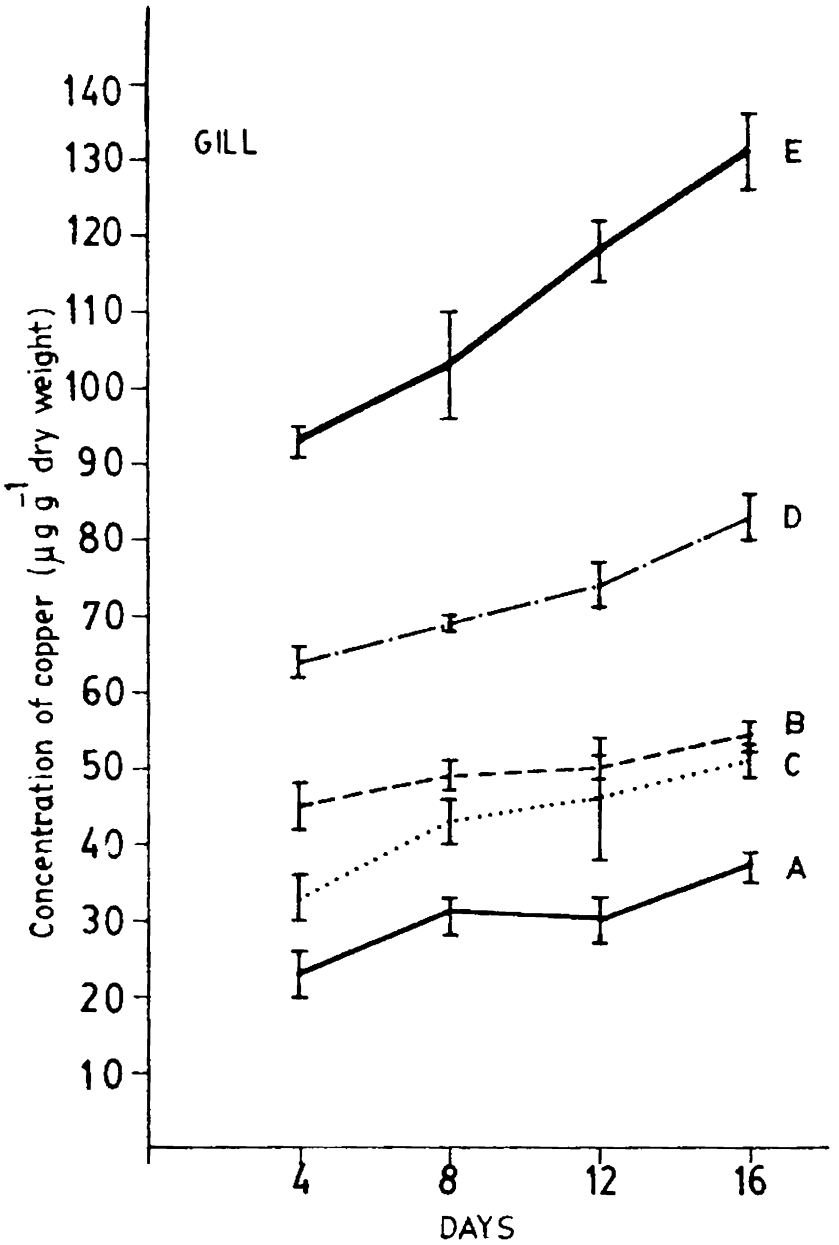

Figure 10. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the gill, when the animals were exposed to sublethal concentrations (A = 0.5 ppb, B = 1 ppb, C = 2 ppb, D = 3 ppb and E = 4 ppb) of copper, for a period from 4 to 16 days. Vertical bars show standard deviation.

comparatively accumulated more copper. The data was subjected to analysis of variance (Table 18) and the results show that although there were significant differences between periods, the uptake pattern was mainly controlled by concentration and exposure duration, especially at lower concentrations. Those animals kept at 4 ppb of copper showed comparable uptake pattern after 12 and 16 days.

Table 19 explains the concentrations of copper in the remaining tissues of Perna indica, exposed to various concentrations of copper. Among the four tissues examined, the remaining tissue which contained the viscera had the maximum concentration of copper. Concentration dependent increase in the uptake pattern (Figure 12) was observed. Those animals kept at 4 ppb accumulated large quantities of copper during the first 4 days of exposure. Those animals in 0.5 and 1 ppb accumulated very low quantity even after 16 days. Between 8th and 12th day, animals exposed at 3 ppb registered a high rate of uptake, whereas this happened during 12th and 16th day in 4 ppb. From the analysis of variance, it is evident that the pattern of uptake between concentrations and between periods were significant (Table 20).

4.2.1.1.2. Depuration

After exposing Perna indica to various sublethal concentrations of $CuSO_4$ for periods ranging from 4 to 16 days, the animals in each set of experimental exposure category namely 4 days, 8 days, 12 days and 16 days were immediately transferred to raw seawater and retained there for a uniform period of 7 days to assess the rate of depuration. The main idea behind this schedule of experiment was to find out the capacity of the animals to detoxify accumulated heavy metals in various tissues when

Table 17. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the aductor muscle (µg g^{-1} dry weight), when the animals were exposed to sublethal concentration (0.5 to 4 ppb) of copper for periods upto 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of exposure (days)	Replicates				Mean	SD
		1	2	3	4		
	4	18.73	15.44	12.97	16.52	15.91	2.07
0.50	8	20.65	16.66	15.02	16.57	17.22	2.08
	12	20.13	20.67	15.60	17.73	18.53	2.02
	16	20.98	24.13	16.79	18.84	20.18	2.71
	4	21.48	21.16	21.95	20.72	21.32	0.44
1.00	8	24.57	23.50	22.32	21.73	23.03	1.09
	12	30.07	28.09	30.60	25.82	28.64	1.88
	16	30.60	29.75	30.24	27.30	29.47	1.29
	4	24.22	23.54	28.17	23.06	24.74	2.01
2.00	8	27.05	28.59	30.03	38.57	31.06	4.46
	12	32.95	35.17	37.17	36.49	35.44	1.61
	16	37.08	40.66	42.32	38.38	39.61	2.02
	4	34.18	27.79	27.42	29.22	29.65	2.69
3.00	8	37.08	30.79	29.22	24.44	30.38	4.51
	12	40.05	36.30	40.13	47.06	40.88	3.88
	16	42.97	41.25	46.36	46.80	44.34	2.32
	4	68.99	57.31	51.51	57.33	58.78	6.35
4.00	8	75.53	62.55	71.02	63.66	68.19	5.34
	12	78.82	75.82	78.76	77.05	77.61	1.25
	16	82.50	91.73	80.72	81.07	84.00	4.50

Table 18. Perna indica. Accumulation: Analysis of variance for changes in copper concentration in the adductor muscle, when the animals were exposed to various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	58.4609	19.4869	1.5277(NS)
2. Between treatment combinations	19	30777.3000	1619.8578	126.9948**
a. Between concentrations	4	27937.4000	6984.3500	547.5645**
b. Between periods	3	2197.6800	732.5600	57.4318**
c. Interaction	12	642.2270	53.5189	4.1958**
3. Error	57	727.0550	12.7553	
4. Total	79	31562.8159		

NS - Not significant
** - Significant at 1% level.

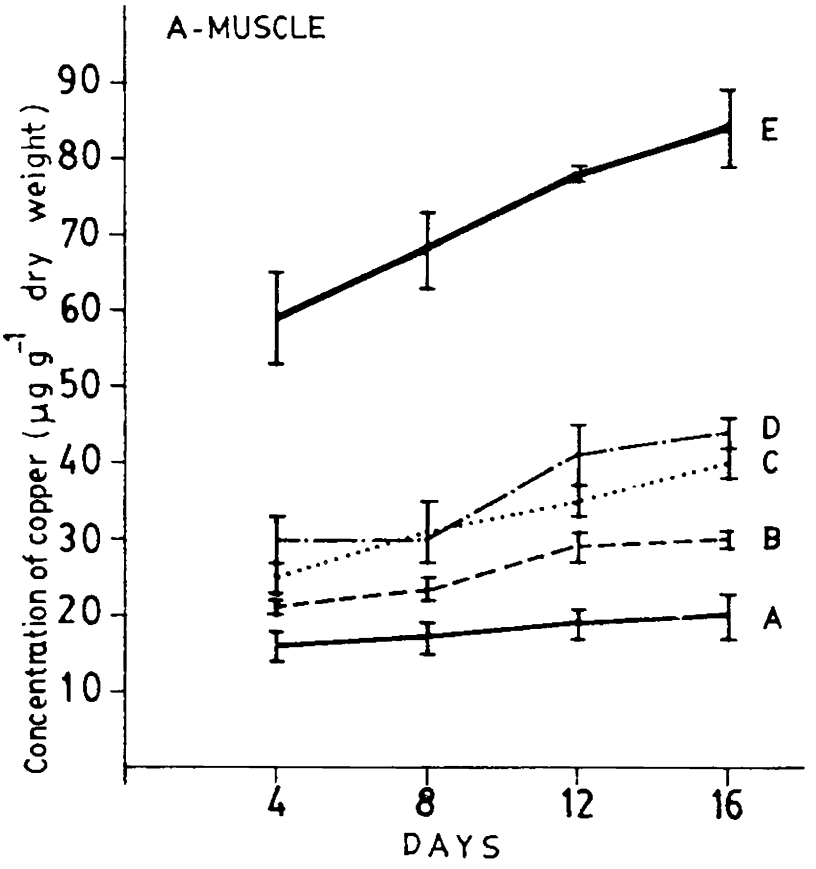

Figure 11. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the adductor muscle, when the animals were exposed to sublethal concentrations (A = 0.5 ppb, B = 1 ppb, C = 2 ppb, D = 3 ppb and E = 4 ppb) of copper, for period ranging from 4 to 16 days. Vertical bars show standard deviation.

Table 19. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the remaining tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to sublethal concentration (0.5 to 4 ppb) of copper for periods upto 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of exposure (days)	Replicates				Mean	SD
		1	2	3	4		
0.50	4	10.26	12.57	4.98	3.91	7.93	3.59
	8	6.50	9.15	11.34	10.96	9.48	1.91
	12	20.81	27.53	27.96	24.94	25.31	2.84
	16	26.15	27.37	31.52	26.41	27.86	2.16
1.00	4	15.94	11.71	17.96	13.62	14.80	2.35
	8	27.43	24.53	23.27	22.52	24.43	1.87
	12	29.79	29.11	35.53	31.52	31.48	2.49
	16	33.15	34.00	31.19	30.40	32.18	1.44
2.00	4	34.00	36.68	35.22	32.96	34.71	1.38
	8	45.65	44.94	47.13	47.85	46.39	1.15
	12	49.30	50.20	46.72	51.94	49.54	1.88
	16	40.64	48.73	52.63	46.43	47.10	4.34
3.00	4	53.60	60.39	50.80	47.85	53.16	4.64
	8	66.95	59.92	48.85	48.73	56.11	7.73
	12	76.97	75.38	69.64	70.68	73.16	3.08
	16	76.86	80.85	80.14	90.50	82.08	5.08
4.00	4	91.22	92.05	93.32	88.29	91.22	1.84
	8	92.62	99.17	95.85	91.46	94.77	3.00
	12	112.57	103.14	94.10	93.30	100.77	7.82
	16	138.65	133.91	144.98	156.53	143.51	8.47

Table 20. Perna indica. Accumulation: Analysis of variance for changes in copper concentration in the remaining tissue, when the animals were exposed to various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	24.4219	8.1406	0.3530(NS)
2. Between treatment combinations	19	95413.7000	5021.7736	217.7755**
a. Between concentrations	4	83439.5000	20859.8750	904.6148**
b. Between periods	3	7927.1900	2642.3966	114.5908**
c. Interactions	12	4046.9700	337.2475	14.6251**
3. Error	57	1314.3900	23.0594	
4. Total	79	96752.5119		

NS Not significant
** Significant at 1% level.

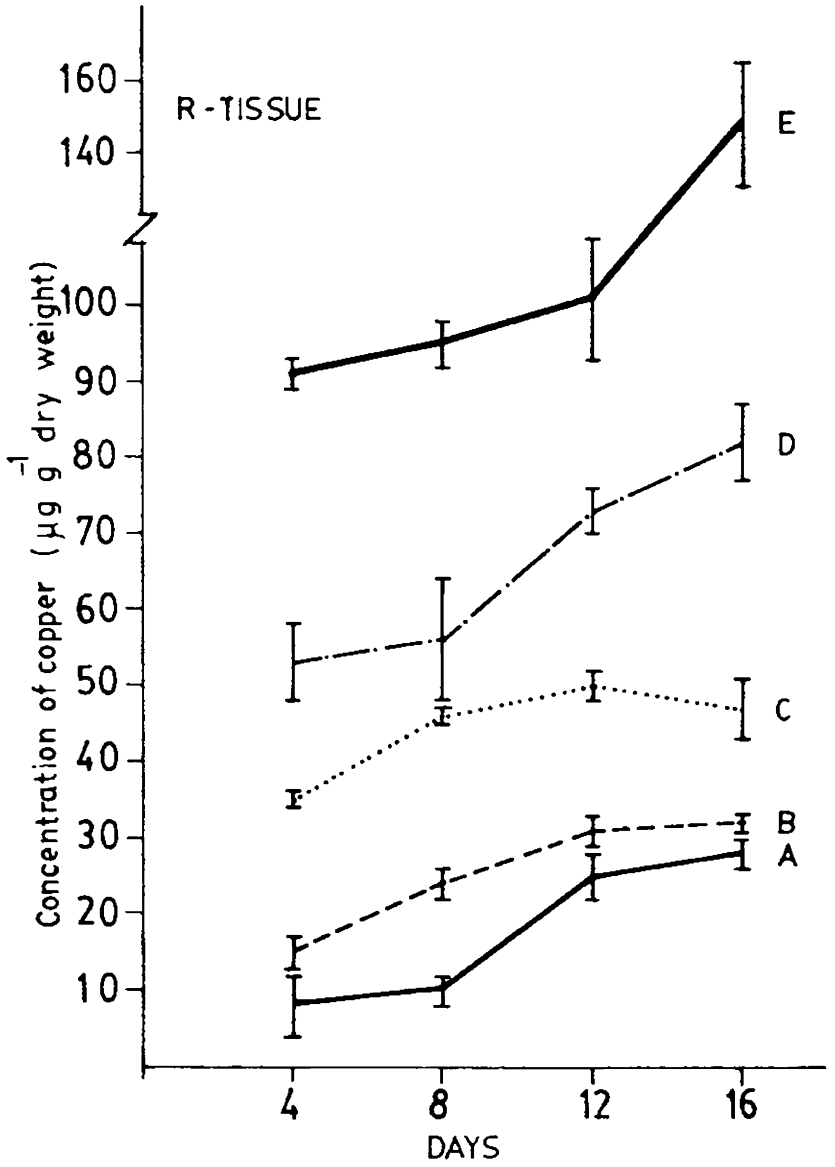

Figure 12. Perna indica. Accumulation: Copper ($CuSO_4$) concentration in the remaining tissue, when the animals were exposed to sublethal concentrations (A = 0.5 ppb, B = 1 ppb, C = 2 ppb, D = 3 ppb and E = 4 ppb) of copper, for period ranging from 4 to 16 days. Vertical bars show standard deviation.

exposed to the constant concentrations and different durations.

Tables 21-23 and Figure 13 give the information on the concentrations of copper in the mantle of Perna indica after 7 days depuration, the quantity depurated by the animals from the mantle and the analysis of variance. A clear cut feature of the results obtained was that, the quantity of copper depurated from the mantle was more a function of the load in the tissue at the beginning of depuration process than the duration for which the animals were exposed to heavy metals. Further, the quantity depurated by the animals earlier exposed to 0.5 ppb copper was very low. Thus the values obtained ranged between 1.55 to 2.01 ppm. Maximum quantity of copper was depurated by the mantle of those animals exposed to 4 ppb. There was no difference in the quantity depurated based on the duration of exposure (Table 22). The analysis of variance data (Table 23) show that the rate of depuration between treatment combinations and concentrations were significant while between periods were not significant. The depuration rate in those animals which were exposed to 1,2 and 3 ppb copper is comparable.

Tables 24-26 and Figure 14 give the details of depuration of copper from the gill by Perna indica, exposed to various sublethal concentrations of copper for periods ranging from 4 to 16 days. The rate of depuration of copper from the gill by those animals exposed to 0.5, 1 and 2 ppb was comparable irrespective of the duration of exposure. The load maintained in the gill seems to be having some relationship with the rate of depuration of copper from the gill. This is especially so in the case of gill tissue of Perna indica, exposed to 4 ppb of copper. The rate of depuration by those animals exposed to 0.5 to 2 ppb was more or less uniform, whereas

Table 21. Perna indica. Depuration: Copper ($CuSO_4$) concentration in the mantle (ug g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of pre-exposure (days)	Replicates				Mean	SD
		1	2	3	4		
	4	6.25	3.63	10.29	16.90	9.26	5.00
0.50	8	18.35	22.07	15.76	21.75	19.48	2.59
	12	25.64	26.04	26.25	27.40	26.33	0.65
	16	28.19	26.82	32.18	33.25	30.11	2.67
	4	20.29	16.48	20.26	30.23	21.81	5.09
1.00	8	28.01	30.37	30.77	28.77	29.48	1.13
	12	31.95	31.11	32.88	30.55	31.62	0.88
	16	38.66	40.53	37.93	35.55	38.16	1.78
	4	41.13	42.24	41.92	42.77	42.01	0.59
2.00	8	46.55	49.45	49.18	47.81	48.24	1.16
	12	47.14	47.45	46.20	44.45	46.31	1.16
	16	54.33	46.60	52.36	52.96	51.56	2.95
	4	52.26	43.97	52.27	53.48	50.49	3.79
3.00	8	57.63	60.46	61.35	58.99	59.60	1.41
	12	65.49	68.01	67.25	66.91	66.91	0.91
	16	75.50	74.14	70.00	68.64	72.07	2.83
	4	64.15	62.13	58.13	62.27	61.67	2.19
4.00	8	75.54	70.51	76.63	67.61	72.57	3.67
	12	76.53	83.95	90.07	81.75	83.07	4.85
	16	96.38	94.97	91.57	85.71	92.15	4.11

Table 22. Perna indica. Depuration: Quantities of copper ($CuSO_4$) depurated from the mantle (µg g^{-1} dry weight), when the animals were exposed to raw seawater for 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Concentration (ppb)	Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	1.33	2.74	3.01	0.98	2.01	0.87
0.50	8	2.31	2.18	1.91	1.05	1.86	0.49
	12	1.73	1.08	2.31	1.11	1.55	0.50
	16	1.11	2.24	1.89	1.20	1.61	0.47
	4	3.27	5.49	2.88	6.87	4.62	1.63
1.00	8	4.61	3.30	3.76	4.92	4.14	0.64
	12	5.88	4.11	5.31	5.94	5.31	0.73
	16	3.87	6.38	4.19	3.88	4.58	1.04
	4	3.81	4.08	4.40	3.49	3.94	0.33
2.00	8	4.36	2.88	4.68	6.00	4.48	1.10
	12	4.58	3.92	5.64	4.58	4.68	0.61
	16	2.42	4.46	4.36	3.91	3.78	0.81
	4	5.78	6.66	3.76	6.07	5.56	1.09
3.00	8	4.31	4.39	4.67	3.83	4.30	0.30
	12	6.68	5.08	6.69	4.40	5.71	1.00
	16	3.36	3.31	5.12	4.68	4.11	0.79
	4	10.36	9.81	11.43	6.84	9.61	1.70
4.00	8	7.43	9.09	6.35	7.77	7.66	0.97
	12	12.38	7.27	5.99	6.30	7.98	2.58
	16	11.67	9.18	8.89	8.60	9.58	1.22

Table 23. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated from the mantle, when the animals were exposed to raw seawater for 7 days, after being maintained in various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	1.9715	0.6571	0.4065 (NS)
2. Between treatment combinations	19	424.5630	22.3454	13.8250**
a. Between concentrations	4	397.9530	99.4882	61.5530**
b. Between periods	3	5.4764	1.8254	1.1294(NS)
c. Interaction	12	21.1342	1.7611	1.0896(NS)
3. Error	57	92.1325	1.6163	
4. Total	79	518.6670		

NS - Not significant
** - Significant at 1% level.

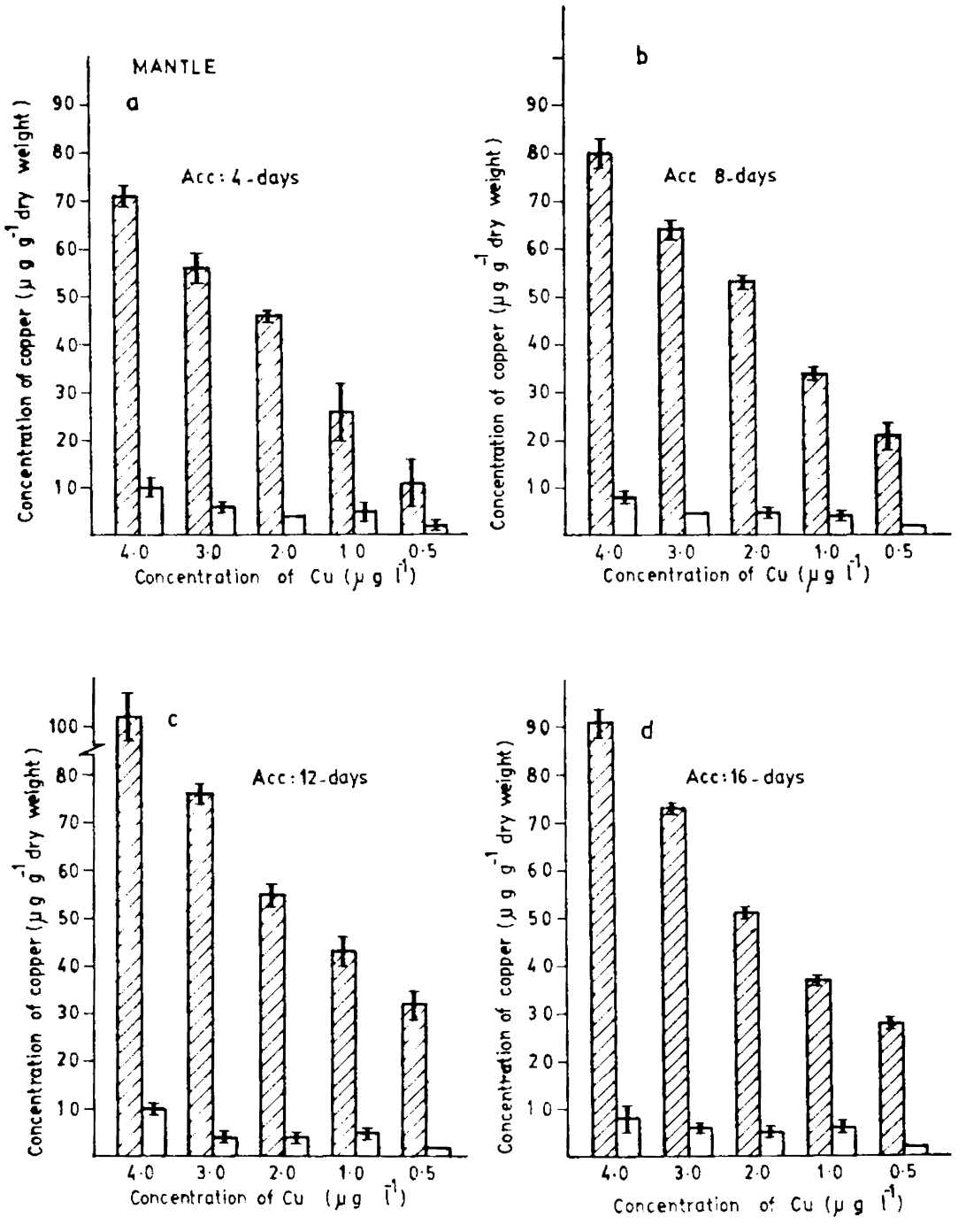

Figure 13. Perna indica. Quantities of copper accumulated (hached histogram) and quantities depurated in 7 days (open histogram) from the mantle.

Table 24. Perna indica. Depuration: Copper ($CuSO_4$) concentration in the gill ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of pre-exposure (days)	Replicates				Mean	SD
		1	2	3	4		
0.50	4	18.52	18.94	18.38	16.67	18.12	0.86
	8	23.60	23.36	25.37	29.37	25.42	2.40
	12	25.56	28.98	24.17	20.09	24.70	3.18
	16	31.00	29.55	36.12	33.43	32.52	2.49
1.00	4	42.98	40.75	40.75	36.71	40.29	2.26
	8	47.02	45.48	43.42	39.85	43.94	2.68
	12	45.24	46.83	47.06	41.16	45.07	2.36
	16	45.88	49.34	45.90	45.66	46.69	1.53
2.00	4	32.85	23.66	24.37	30.63	27.87	3.94
	8	34.55	35.20	40.79	39.17	37.42	2.62
	12	41.39	36.24	31.24	49.95	39.70	6.91
	16	52.85	48.45	40.64	41.80	45.93	4.98
3.00	4	51.45	56.02	57.66	56.29	55.35	2.33
	8	59.74	58.56	63.59	63.38	61.31	2.20
	12	68.20	70.45	65.65	64.73	67.25	2.23
	16	73.69	80.00	80.27	75.29	77.31	2.88
4.00	4	77.78	78.17	74.04	77.46	76.86	1.64
	8	101.89	81.70	102.46	92.65	94.67	8.44
	12	112.42	104.04	106.37	113.35	109.04	3.94
	16	126.20	118.61	113.12	125.30	120.80	5.31

Table 25. Perna indica. Depuration: Quantities of copper ($CuSO_4$) depurated from the gill (µg g^{-1} dry weight), when the animals were exposed to raw seawater for 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Concentration (ppb)	Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	6.29	7.64	3.05	3.47	5.11	1.91
0.50	8	5.58	8.89	4.77	4.13	5.84	1.83
	12	4.36	5.38	6.91	6.17	5.70	0.94
	16	3.16	6.71	3.98	4.02	4.33	1.33
	4	5.05	4.45	5.75	3.38	4.65	0.86
1.00	8	4.31	3.77	6.32	6.48	5.22	1.19
	12	4.63	3.31	4.75	6.54	4.80	1.14
	16	7.18	6.61	5.05	7.94	6.69	1.06
	4	3.22	5.45	6.20	4.56	4.85	1.10
2.00	8	6.12	3.89	4.77	7.90	5.67	1.51
	12	7.07	6.66	3.98	7.20	6.22	1.31
	16	3.28	5.87	6.14	5.18	5.11	1.11
	4	10.11	8.13	8.88	8.10	8.80	0.81
3.00	8	9.12	9.02	6.14	7.19	7.86	1.25
	12	8.46	6.22	7.65	4.36	6.67	1.55
	16	6.16	5.87	4.20	5.28	5.37	0.75
	4	15.52	16.80	16.79	13.88	15.74	1.19
4.00	8	7.72	10.39	6.43	10.58	8.78	1.76
	12	8.65	9.67	8.27	9.07	8.91	0.51
	16	10.74	9.99	11.97	9.89	10.64	0.83

Table 26. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated from the gill, when the animals were exposed to raw seawater for 7 days, after being maintained in various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	4.3686	1.4562	0.6800(NS)
2. Between treatment combinations	19	560.2540	29.4870	13.7699**
a. Between concentrations	4	386.4370	96.6092	45.1149**
b. Between periods	3	26.0266	8.6755	4.0513**
c. Interaction	12	147.7910	12.3159	5.7513**
3. Error	57	122.0600	2.1414	
4. Total	79	686.6826		

NS - Not significant
** Significant at 1% level.

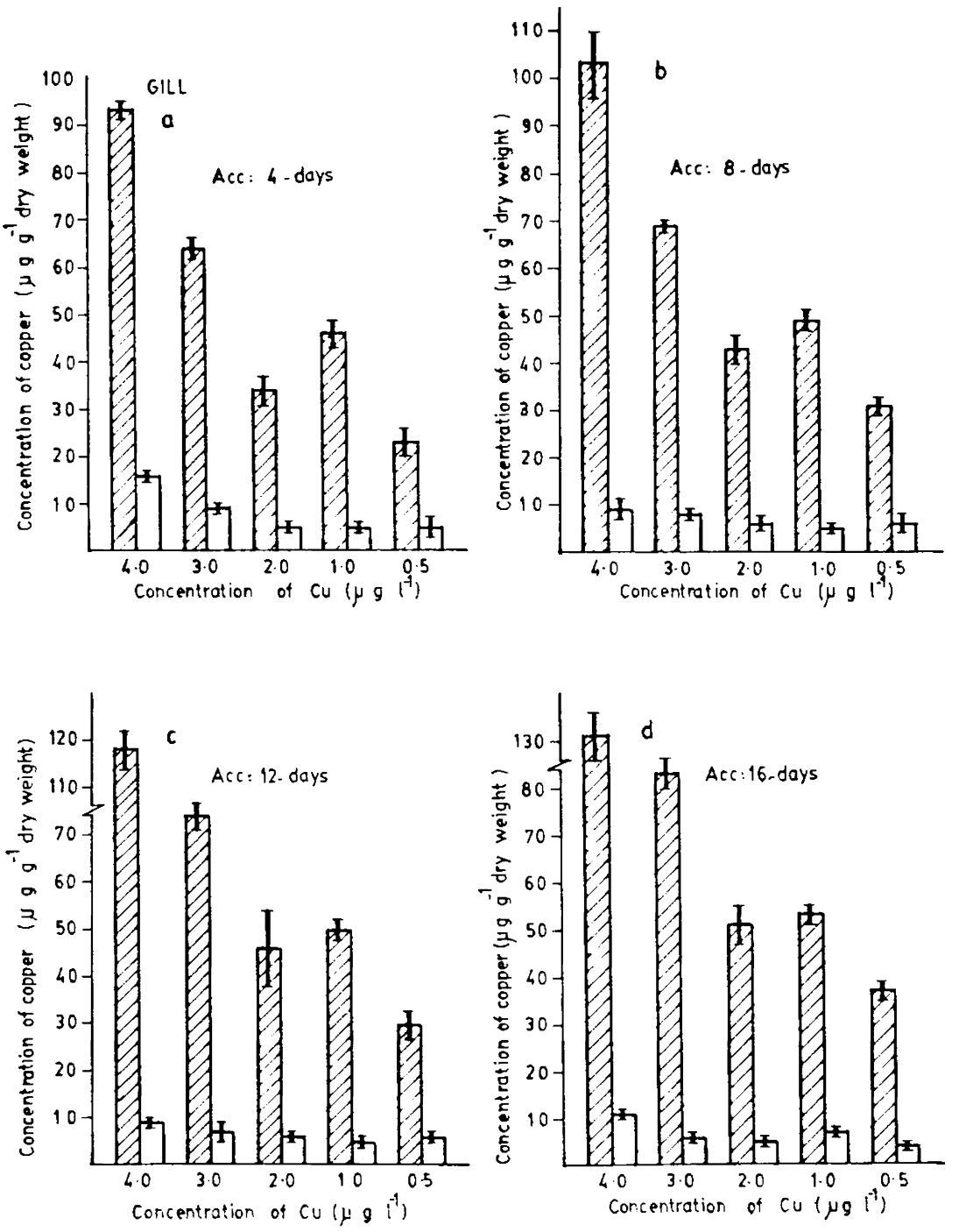

Figure 14. Perna indica. Quantities of copper accumulated (hached histogram) and quantities depurated in 7 days (open histogram) from the gill.

in the case of 4 ppb exposed animals the depuration showed clear cut difference when the animals were pre-exposed to only for 4 days. The analysis of variance (Table 26) shows that between treatment combinations, between concentrations and between periods, the results obtained were highly significant.

Tables 27-29 and Figure 15 show the details regarding the load of copper in the adductor muscle of Perna indica, exposed to 0.5 to 4 ppb of copper for durations ranging from 4 to 16 days, the quantity depurated after exposure to raw seawater for 7 days and analysis of variance data. The quantity depurated from adductor muscle by the animals was minimum. Slight increase was noticed in the case of those animals exposed to 4 ppb. Here also, inter-concentration variability was less. In general, rate of depuration from the adductor muscle by the animals exposed to various durations did not show variability probably the reduced load in the adductor muscle in the lower concentration exposed animals could be one of the reasons. The data obtained between treatment combinations, and between concentrations were significant whereas between periods and interactions were not significant (Table 29).

Tables 30-32 and Figure 16 give information on the copper load subsequent to depuration of 7 days, the quantity depurated during the 7 days and the analysis of variance for the remaining tissue of Perna indica. The load of copper that remained in the remaining tissue was uniformly more as a function of duration of exposure to the toxicant irrespective of concentrations. The quantity depurated by those animals exposed to 0.5 ppb did not show much variation, dependent on increase in the duration. The values ranged between 2.36 and 3.26 ppm. The same trend was main-

Table 27. Perna indica. Depuration: Copper ($CuSO_4$) concentration in the aductor muscle (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being exposed to various sub-lethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	17.45	14.45	11.86	15.65	14.85	2.03
0.50	8	19.63	15.59	13.93	13.96	15.77	2.32
	12	19.15	16.92	13.44	16.76	16.56	2.03
	16	20.31	22.97	15.81	17.26	19.08	2.76
	4	19.08	17.40	18.14	15.68	17.57	1.24
1.00	8	22.59	20.95	18.16	17.92	19.90	1.95
	12	27.55	24.31	26.97	23.11	25.48	1.83
	16	27.72	25.08	27.63	23.35	25.94	1.83
	4	21.09	19.17	24.84	20.08	21.29	2.15
2.00	8	24.29	25.50	24.95	34.70	27.36	4.25
	12	30.00	31.18	33.08	31.82	31.51	1.10
	16	33.42	36.18	40.10	35.30	36.25	2.43
	4	30.80	23.78	21.66	24.61	25.21	3.40
3.00	8	32.19	26.98	26.02	19.76	26.23	4.41
	12	35.00	31.58	37.97	43.80	37.08	4.48
	16	41.20	37.96	43.90	42.58	41.41	2.20
	4	63.11	54.10	44.59	52.43	53.55	6.58
4.00	8	70.59	57.59	67.90	59.02	63.77	5.57
	12	75.84	72.57	74.18	68.38	72.74	2.77
	16	79.49	86.07	74.31	75.04	78.87	4.56

Table 28. Perna indica. Depuration: Quantities of copper ($CuSO_4$) depurated from the adductor muscle (µg g^{-1} dry weight), when the animals were exposed to raw seawater for 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Concentration (ppb)	Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	1.28	0.99	1.11	0.87	1.06	0.15
0.50	8	1.02	1.07	1.09	2.61	1.44	0.67
	12	0.98	3.75	2.16	0.97	1.96	1.13
	16	0.67	1.16	0.98	1.58	1.09	0.32
	4	2.40	3.76	3.81	5.04	3.75	0.93
1.00	8	1.98	2.55	4.16	3.81	3.12	0.89
	12	2.52	3.78	3.63	2.71	3.16	0.55
	16	2.88	4.67	2.61	3.95	3.52	0.82
	4	3.13	4.37	3.33	2.98	3.45	0.54
2.00	8	2.76	3.09	5.08	3.87	3.70	0.89
	12	2.95	3.99	4.11	4.67	3.93	0.62
	16	3.66	4.48	2.22	3.08	3.36	0.82
	4	3.38	4.01	5.77	4.61	4.44	0.88
3.00	8	4.89	3.81	3.20	4.68	4.14	0.67
	12	5.05	4.72	2.16	3.26	3.79	1.16
	16	1.77	3.29	2.46	4.22	2.93	0.91
	4	5.88	3.21	6.92	4.90	5.22	1.36
4.00	8	4.94	4.96	3.12	4.64	4.41	0.75
	12	2.98	3.25	4.58	8.67	4.87	2.27
	16	3.01	5.66	6.41	6.03	5.27	1.33

Table 29. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated from the aductor muscle, when the animals were exposed to raw seawater for 7 days, after being maintained in various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	9.3339	3.1113	2.5783(NS)
2. Between treatment combinations	19	117.3360	6.1755	5.1177**
a. Between concentrations	4	106.3310	26.5827	22.0292**
b. Between periods	3	1.5636	0.5212	0.4319(NS)
c. Interaction	12	9.4417	0.7868	0.6520(NS)
3. Error	57	68.7831	1.2067	
4. Total	79	195.4530		

NS - Not significant
** - Significant at 1% level.

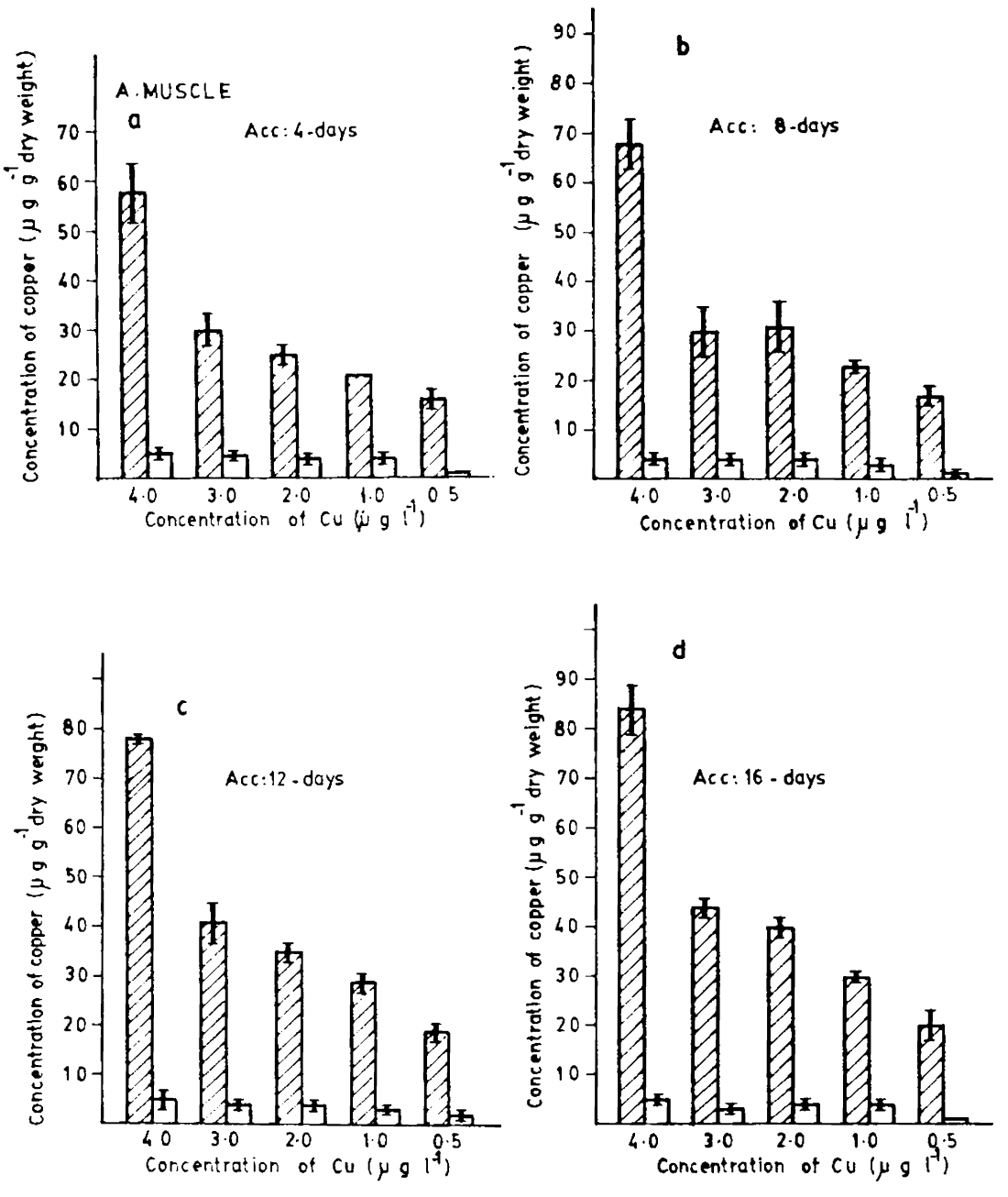

Figure 15. Perna indica. Quantities of copper accumulated (hached histogram) and quantities depurated in 7 days (open histogram) from the adductor muscle.

Table 30. Perna indica. Depuration: Copper ($CuSO_4$) concentration in the remaining tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Copper Concentration (ppb)	Duration of pre-exposure (days)	Replicates				Mean	SD
		1	2	3	4		
	4	6.78	10.17	3.00	2.72	5.66	3.05
0.50	8	2.46	7.01	9.22	9.43	7.03	2.80
	12	16.42	22.34	25.77	22.86	21.84	3.39
	16	22.44	23.71	29.06	24.43	24.86	2.55
	4	11.72	7.63	15.72	8.64	10.92	3.15
1.00	8	23.55	21.73	19.31	20.56	21.28	1.56
	12	24.76	25.79	30.90	27.78	27.30	2.34
	16	29.41	28.43	28.46	25.53	27.95	1.45
	4	28.45	31.94	31.24	26.82	29.61	2.07
2.00	8	38.49	40.28	43.14	44.78	41.67	2.44
	12	42.64	46.81	44.91	48.28	45.66	2.11
	16	35.77	43.45	49.74	43.11	43.01	4.94
	4	44.62	55.00	42.00	40.98	45.65	5.55
3.00	8	59.31	53.20	41.91	42.98	49.35	7.24
	12	70.99	68.95	63.98	62.98	66.72	3.34
	16	70.84	72.58	72.98	85.59	75.49	5.88
	4	78.80	81.86	84.25	77.86	80.69	2.53
4.00	8	82.81	83.59	88.94	81.76	84.27	2.77
	12	101.91	95.49	86.88	82.72	91.75	7.45
	16	126.97	123.33	137.33	149.42	134.26	10.14

Table 31. Perna indica. Depuration: Quantities of copper ($CuSO_4$) depurated from the remaining tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater for 7 days, after being exposed to various sublethal concentrations (0.5 to 4 ppb) of copper from 4 to 16 days, along with standard deviation.

Concentration (ppb)	Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
	4	3.48	2.40	1.98	1.19	2.26	0.82
0.50	8	4.04	2.14	2.12	1.53	2.45	0.94
	12	4.39	5.19	2.19	2.08	3.46	1.35
	16	3.71	3.66	2.46	1.98	2.95	0.75
	4	4.22	4.08	2.24	4.98	3.88	1.00
1.00	8	3.88	2.80	3.96	1.96	3.15	0.82
	12	5.03	3.32	4.63	3.74	4.18	0.68
	16	3.74	5.57	2.73	4.87	4.22	1.08
	4	5.55	4.74	3.98	6.14	5.10	0.81
2.00	8	7.16	4.66	3.99	3.07	4.72	1.51
	12	6.66	3.39	1.81	3.66	3.88	1.75
	16	4.87	5.28	2.89	3.32	4.09	1.00
	4	8.98	5.39	8.80	6.87	7.51	1.47
3.00	8	7.64	6.72	6.94	5.75	6.76	0.67
	12	5.98	6.43	5.66	7.70	6.44	0.77
	16	6.02	8.27	7.16	4.91	6.59	1.25
	4	12.42	10.19	9.07	10.53	10.53	1.20
4.00	8	9.81	8.46	6.91	9.70	8.72	1.17
	12	10.66	7.65	7.22	10.58	9.02	1.60
	16	11.68	10.58	7.65	7.11	9.25	1.92

Table 32. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated from the remaining tissue, when the animals were exposed to raw seawater for 7 days, after being maintained in various sublethal concentrations of copper from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	35.4919	11.8306	8.6830**
2. Between treatment combinations	19	468.5300	24.6594	18.0986**
a. Between concentrations	4	448.0130	112.0032	82.2042**
b. Between periods	3	5.0234	1.6744	1.2289(NS)
c. Interaction	12	15.4939	1.2911	0.9476(NS)
3. Error	57	77.6670	1.3625	
4. Total	79	581.6889		

NS Not significant
** - Significant at 1% level.

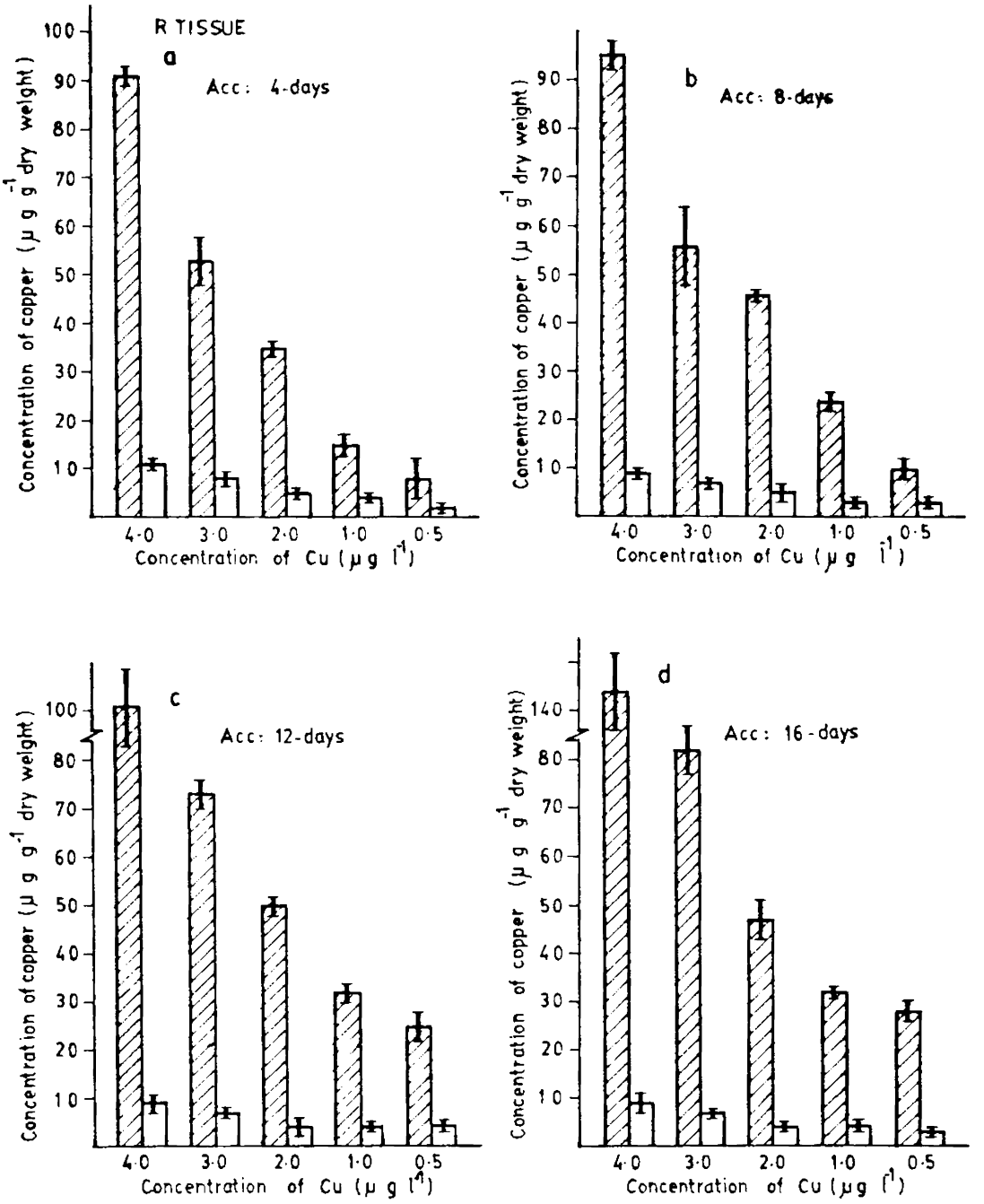

Figure 16. Perna indica. Quantities of copper accumulated (hached histogram) and quantities depurated in 7 days (open histogram) from the remaining tissue.

tained by those animals exposed to 1 to 4 ppb. The only difference was that the quantity increased as a function of increased pre-exposure concentration. Convincingly the data obtained on the interaction and the variability between periods was found to be not significant (Table 32).

4.2.1.2. OTHER SALTS OF COPPER

4.2.1.2.1. Accumulation

Copper nitrate and copper chloride were the other salts which were used to find out the rate of accumulation by Perna indica. The results obtained are presented in Table 33 and Figure 17. Copper irrespective of the two salt forms employed, showed a time bound increase in the rate of accumulation. Thus, copper (supplied as $Cu(NO_3)_2$) load in the whole tissue was 18.74 ppm after 4 days and reached 28.36 ppm after 16 days. The quantity of copper accumulated when supplied in the form of $CuCl_2$ was comparatively more and the copper load was 21.24 ppm after 4 days and reached 29.03 ppm after 16 days. Analysis of variance of the data obtained shows that between treatment combinations the variations noticed in the rate of uptake of copper between salts were significantly different (Table 34).

4.2.1.2. Oxygen consumption accompanying accumulation

Perna indica, which have accumulated various quantities of copper on exposure to 0.5 ppb of copper supplied in the form of nitrate and chloride, was subjected to oxygen consumption studies. The animals were selected from the lot exposed for 4,8, 12 and 16 days. The data obtained are presented in Tables 35, 36a&b and Figure 18. It is evident from the results that the animals exposed to $Cu(NO_3)_2$ consumed lesser quantities of oxygen although the load in the whole tissue was low. On the contrary, the ani-

Table 33. Perna indica. Accumulation: Copper concentration in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days, along with standard deviation.

Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD
Copper nitrate						
4	19.33	17.52	20.97	17.14	18.74	1.53
8	20.99	22.06	21.27	22.07	21.59	0.47
12	22.25	25.34	26.83	23.65	24.51	1.72
16	27.26	30.89	28.30	27.00	28.36	1.53
Copper chloride						
4	22.50	22.67	21.76	18.06	21.24	1.87
8	24.19	24.67	24.58	23.76	24.30	0.36
12	27.75	26.27	27.41	25.67	26.77	0.84
16	28.75	32.05	27.14	28.20	29.03	1.83

Table 34. Perna indica. Accumulation: Analysis of variance for changes in copper concentration in the whole tissue, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	18.1465	6.0488	2.8632(NS)
2. Between treatment combinations	7	370.3770	52.9110	25.0454**
a. Between salts	1	33.1074	33.1074	15.6714**
b. Between periods	3	332.1130	110.7043	52.4019**
c. Interaction	3	5.1562	1.7187	0.8135(NS)
3. Error	21	44.3652	2.1126	
4. Total	31	432.8887		

NS Not significant
** Significant at 1% level.

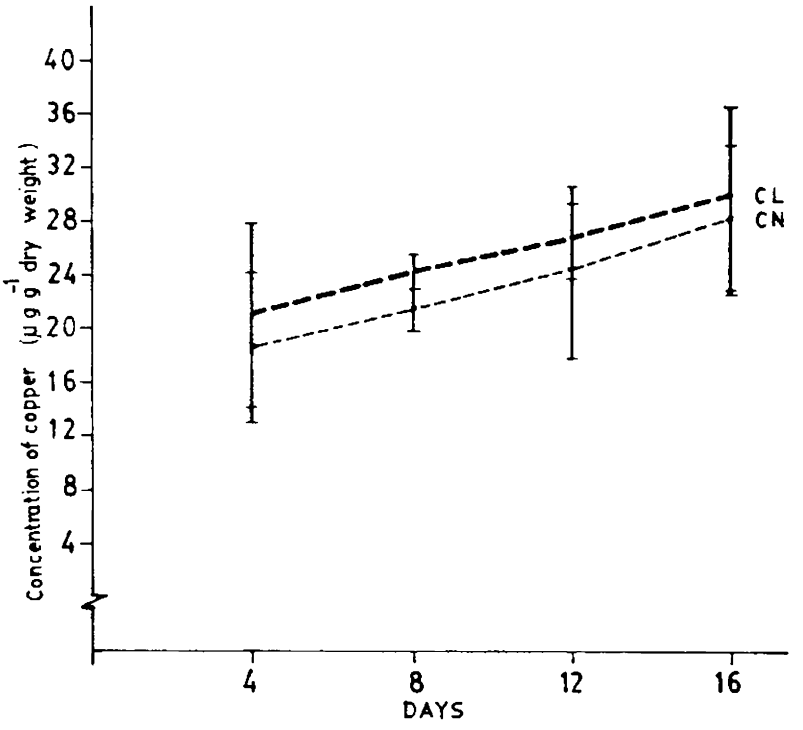

Figure 17. Perna indica. Accumulation: Copper concentration in the whole tissue, when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation.

Table 35. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to 0.5 ppb of copper nitrate and copper chloride from 4 to 16 days, along with standard deviation.

Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD	% of control
Control							
4	2.18	2.36	2.83	1.98	2.33	0.31	
8	2.98	2.80	3.98	2.67	3.10	0.51	
12	2.71	3.98	2.99	2.05	2.93	0.69	
16	3.67	2.58	3.67	1.98	2.98	0.72	
Copper nitrate							
4	2.51	3.46	3.05	1.42	2.61	0.76	111.52
8	2.75	1.86	3.09	2.96	2.66	0.48	85.79
12	1.81	1.43	2.16	1.33	1.68	0.32	57.41
16	0.91	0.71	1.89	0.40	0.97	0.55	33.03
Copper chloride							
4	4.79	3.16	1.83	3.81	3.39	1.07	145.18
8	3.12	1.52	4.46	2.09	2.79	1.11	89.99
12	2.95	2.03	2.99	3.18	2.78	0.44	94.97
16	1.18	2.18	1.87	1.68	1.72	0.36	58.23

Table 36. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days.

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	8.6309	2.1577	7.8093*
Between replicates	3	1.9387	0.6462	2.3388(NS)
Error	12	3.3161	0.2763	
Total	19	13.8857		

NS Not significant
* Significant at 5% level.

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	5.8882	1.4720	1.7039(NS)
Between replicates	3	0.8896	0.2965	0.3432(NS)
Error	12	10.3677	0.8639	
Total	19	17.1455		

NS Not significant

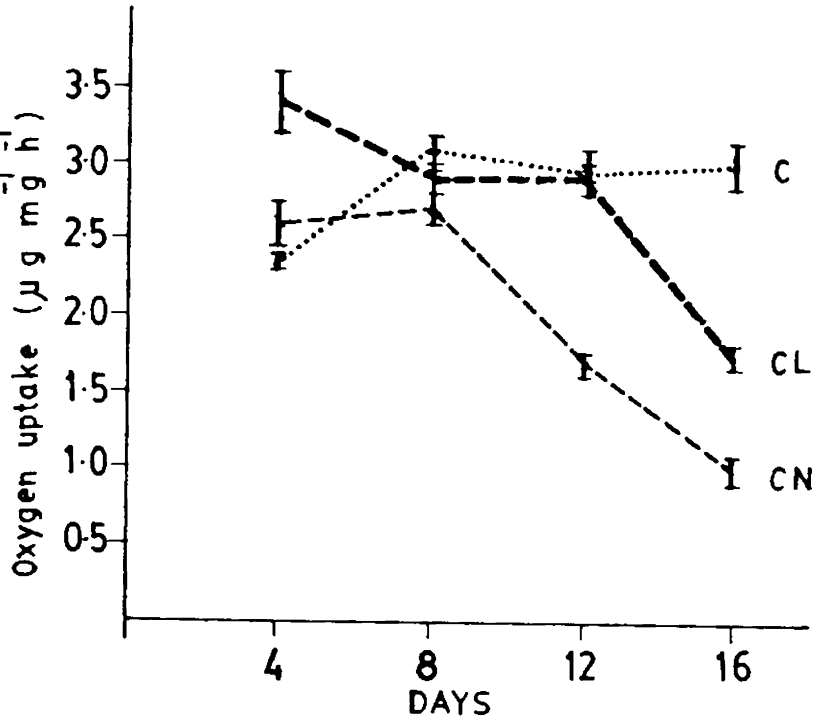

Figure 18. Perna indica. Accumulation: Mean oxygen consumption, when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation.

mals exposed to $CuCl_2$ consumed more oxygen and the whole tissue had relatively large quantities of copper. In both the cases the exposure for longer duration reduced oxygen consumption. To find out the variation in oxygen consumption and its significance, the results were subjected to analysis of variance (Table 36a&b). In the case of those animals exposed to $CuCl_2$, there was no significant difference in the oxygen consumption between periods, whereas significant difference between periods was noticed when the animals were exposed to $CuNO_3$. The apparent variations noticed in the oxygen uptake between animals exposed to the two salts was found to be insignificant statistically.

4.2.1.2.1.2. Filtration accompanying accumulation

Perna indica, was exposed to 0.5 ppb of $Cu(NO_3)_2$ and $CuCl_2$ for periods ranging from 4 to 16 days. Representative individuals were removed to raw seawater after 4, 8, 12 and 16 days and their filtration rate was assessed (Table 37, 38a&b and Figure 19). In both the cases, the rate of filtration decreased as a function of increase in time. When the results were analysed for significance (Table 38a&b), the following points became apparent. The rate of filtration was significantly different between periods in the case of those animals maintained in $Cu(NO_3)_2$. Tukey's test was carried out to find out the significance. The same trend was noticed in the case of those animals maintained in $CuCl_2$. In both the cases, the decrease in filtration rate as time progressed was significantly different.

4.2.1.2.2. Depuration

Individuals of species of Perna indica after being subjected to accumulation phase of 16 days in $Cu(NO_3)_2$ and $CuCl_2$ were uniformly exposed to raw seawater for a period of 7 days to find out the rate of depuration.

Table 37. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to 0.5 ppb of copper nitrate and copper chloride from 4 to 16 days, along with standard deviation.

Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD	% of control
Control							
4	30.83	32.49	38.64	29.86	32.95	3.41	
8	35.36	33.61	34.06	35.91	34.73	0.93	
12	34.73	36.66	33.98	32.63	34.50	1.45	
16	35.93	36.73	34.87	35.43	35.74	0.68	
Copper nitrate							
4	38.16	43.89	34.01	36.06	38.03	3.68	115.41
8	34.67	34.16	25.79	41.93	34.13	5.71	98.27
12	28.07	27.62	23.37	29.80	27.21	2.36	78.86
16	20.38	27.20	22.28	28.44	24.57	3.34	68.74
Copper chloride							
4	41.12	48.80	36.46	40.54	41.73	4.45	126.64
8	33.44	39.78	37.15	32.78	35.78	2.84	103.02
12	23.43	27.26	26.46	40.78	29.48	6.67	85.44
16	22.01	22.17	24.80	26.33	23.82	1.81	66.64

Table 38. Perna indica. Accumulation: Analysis of variance for changes in filtration rate, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days.

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	462.0600	115.5150	7.1029*
Between replicates	3	87.1659	29.0553	1.7865(NS)
Error	12	195.1560	16.2630	
Total	19	744.3819		

NS Not significant
* Significant at 5% level.

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	742.5040	185.6260	8.0385*
Between replicates	3	56.5761	18.8587	0.8166(NS)
Error	12	277.1040	23.0920	
Total	19	1076.1841		

NS Not significant
* Significant at 5% level.

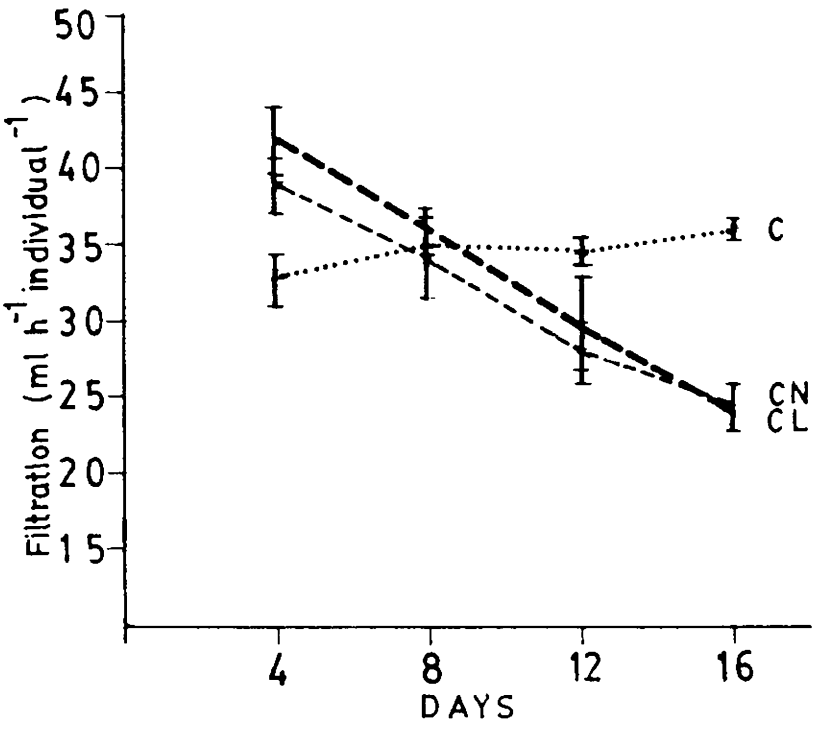

Figure 19. Perna indica. Accumulation: Mean rate of filtration when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation.

The reason for this experiment was to assess the relationship between duration and quantity of accumulated metal and subsequent depuration rate. Therefore, here the animals exposed to raw seawater belong to 4 categories, those subjected to 4 days, 8 days, 12 days and 16 days of accumulation. The results obtained are presented in Tables 39 and 40 and Figure 20. The rate of depuration of copper was more or less comparable irrespective of the previous accumulation history of the animal. It is seen from the Table 40 that after 7 days of depuration, 4 days animals pre-exposed to $Cu(NO_3)_2$ gave out 5.17 ppm and those pre-exposed to 16 days 7.50 ppm. Similarly, in the case of $CuCl_2$ the corresponding values ranged between 5.53 and 7.18 ppm.

4.2.1.2.2.1 Oxygen consumption accompanying depuration

The rate of oxygen consumption of Perna indica during the depuration process was assessed to find out the trend in energy expenditures. Those animals retained for shorter duration i.e. 4 to 8 days in both $Cu(NO_3)_2$ and $CuCl_2$ were found to consume more oxygen after 7 days, whereas those exposed to 12 and 16 days were found to consume less oxygen (Table 41 and Figure 21). The results were analysed statistically to find out the significance noticed (Table 42). The results show that there is no significant difference in the oxygen uptake of the test individuals pre-exposed to $Cu(NO_3)_2$ and $CuCl_2$ for periods ranging from 4 to 16 days after a recovery period of 7 days.

4.2.1.2.2.2. Filtration accompanying depuration

The rate of filtration of Perna indica subjected to pre-exposure periods of 4 to 16 days in 0.5 ppb copper (as $Cu(NO_3)_2$ and $CuCl_2$) and subsequent exposure to raw seawater was analysed to find out the effects of

Table 39. Perna indica. Depuration: Concentration of copper in the whole tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)	Duration of exposure to raw seawater 7 days	Replicates 1	2	3	4	Mean	SD
Copper nitrate							
4		13.34	13.83	13.87	13.21	13.56	0.29
8		14.24	15.49	16.87	15.55	15.53	0.93
12		17.57	17.22	17.53	17.88	17.55	0.23
16		21.62	20.57	20.92	20.31	20.85	0.49
Copper chloride							
4		15.63	15.11	15.53	16.58	15.71	0.53
8		17.07	16.97	17.37	16.67	17.02	0.25
12		17.65	18.49	17.46	17.68	17.82	0.37
16		21.65	24.62	20.51	20.64	21.85	1.65

Table 40. Perna indica. Depuration: Quantities of copper depurated (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)		Replicates 1	2	3	4	Mean	SD
Copper nitrate							
4	Duration of exposure to raw seawater: 7 days	5.99	3.69	7.10	3.93	5.17	1.42
8		6.75	6.57	4.40	6.52	6.06	0.96
12		4.68	8.12	9.30	5.77	6.96	1.83
16		5.64	10.32	7.38	6.69	7.50	1.73
Copper chloride							
4		6.87	7.56	6.23	1.48	5.53	2.38
8		7.12	7.70	7.21	7.09	7.28	0.24
12		10.10	7.78	9.95	7.99	8.95	1.07
16		7.10	7.43	6.63	7.56	7.18	0.35

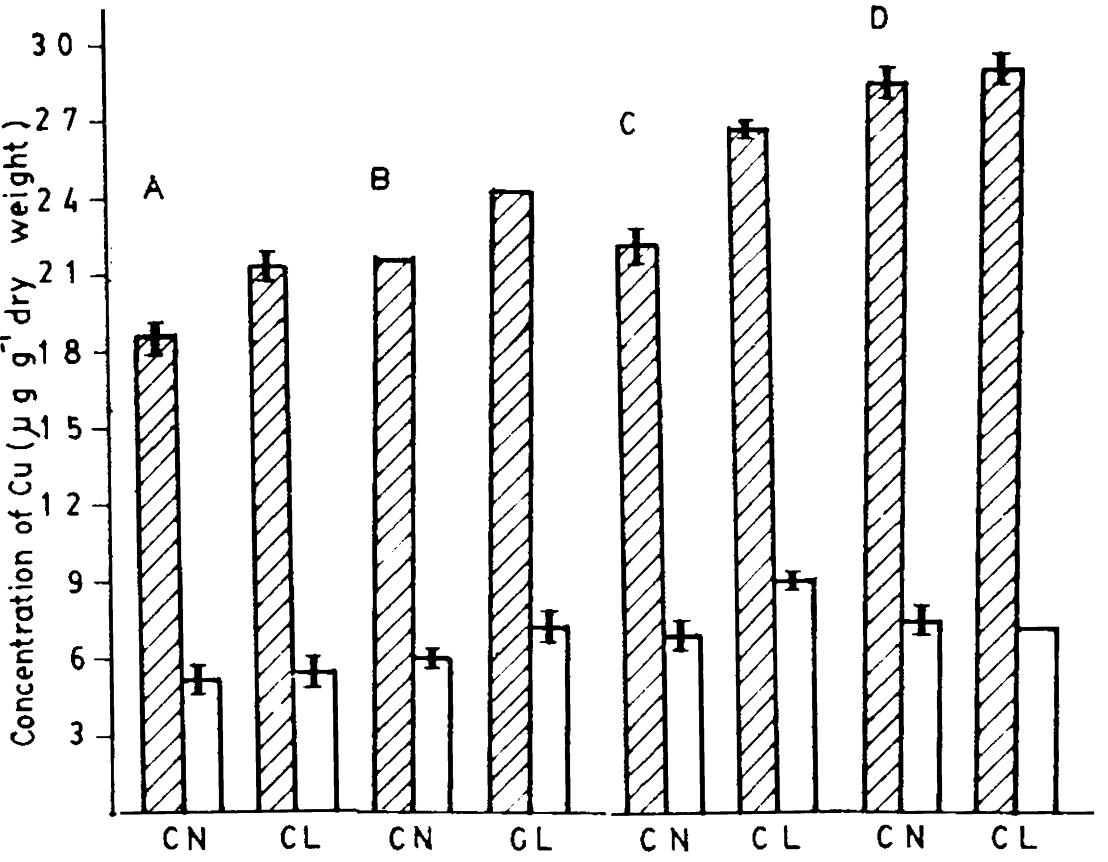

Figure 20. Perna indica. Quantities accumulated in the whole tissue (hached histogram) and quantities depurated in 7 days (open histogram).

A Fourdays accumulation C Twelve days accumulation

B Eight days accumulation D Sixteen days accumulation

Table 41. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)		Replicates 1	2	3	4	Mean	S D	% of control
Control								
4	raw seawater 7 days	2.98	2.05	3.96	2.18	2.79	0.76	
8		2.08	3.98	2.80	2.11	2.74	0.77	
12		3.98	2.08	2.09	2.13	2.57	0.81	
16		3.97	3.68	1.42	2.09	2.79	1.06	
Copper nitrate								
4	exposure to	3.78	4.52	0.93	2.25	2.87	1.38	102.75
8		2.90	1.16	1.89	1.68	2.15	0.46	78.80
12		2.06	2.43	2.12	1.01	1.90	0.53	74.24
16		1.05	1.37	1.96	1.33	1.42	0.33	51.41
Copper chloride								
4	Duration of	3.77	3.10	4.83	2.20	3.47	0.96	124.31
8		2.68	2.49	4.24	3.45	3.21	0.69	117.27
12		3.31	1.79	2.89	2.98	2.74	0.57	106.76
16		2.54	2.44	1.99	2.02	2.24	0.24	80.83

Table 42. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days.

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	4.8382	1.2095	1.7504(NS)
Between replicates	3	2.1705	0.7235	1.0470(NS)
Error	12	8.2920	0.6910	
Total	19	15.3007		

NS Not significant

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	4.1000	1.0250	2.3018(NS)
Between replicates	3	2.1376	0.7125	1.6001(NS)
Error	12	5.3445	0.4453	
Total	19	11.5821		

NS Not significant

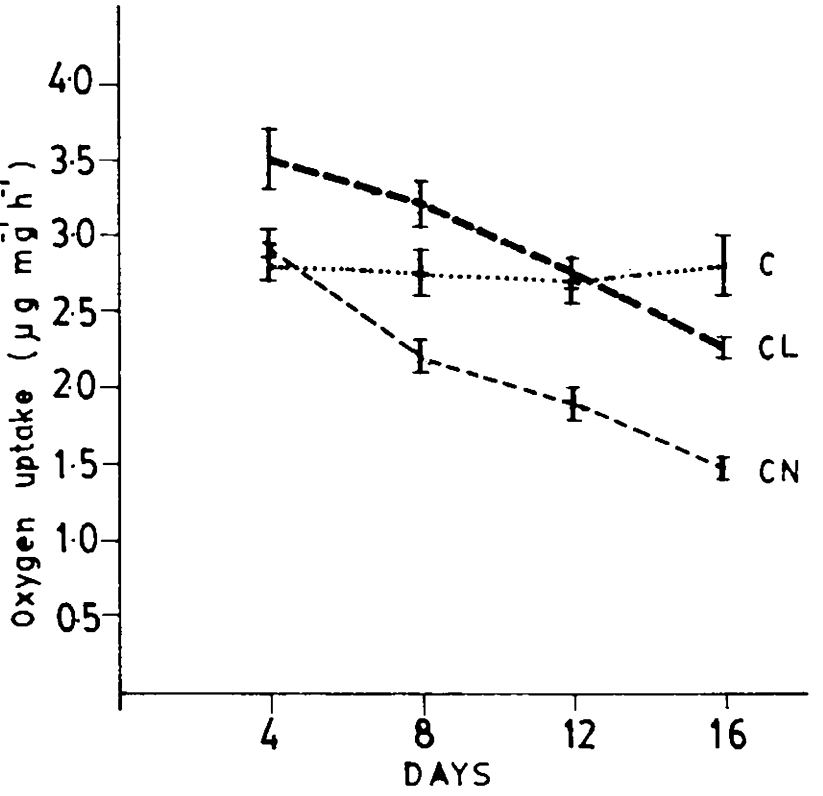

Figure 21. Perna indica. Depuration Mean oxygen consumption, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.50 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days.

depuration (Table 43 and Figure 22). Clear cut variations were noticed in the rate of filtration. Animals pre-exposed to $Cu(NO_3)_2$ for 4 days, filtered more water after they were maintained in raw seawater for 7 days. The same trend was recorded by those animals pre-exposed to $CuCl_2$. Both in the case of $Cu(NO_3)_2$ and $CuCl_2$ 16 days pre-exposure and subsequent 7 days maintainance in clean seawater did not result in increase in the rate of filtration. The animals filtered 28.52 and 24.79 ml h^{-1} ind^{-1}. When the data was subjected to analysis of variance (Table 44a&b), the following generalisations could be arrived at. Results obtained show that between periods the rate of filtration by those animals exposed to $CuCl_2$ were significant. This was further tested using least significant difference based on Tukey's test and it became apparent that the rate of filtration of those animals pre-exposed to 4 days were significantly different from those pre-exposed to 8,12 and 16 days and those of control. The same trend was maintained by those animals exposed to $Cu(NO_3)_2$ also.

4.2.1.3. Silver

Silver in two forms, carbonate and oxide was used to find out the rate of accumulation, rate of depuration, oxygen consumption and filtration rates by Perna indica. The results are presented in Tables 45 to 56 and Figures 23 to 28.

4.2.1.3.1. Accumulation

Silver carbonate and silver oxide at realistic concentrations of 0.5 ppb of silver were used to assess the rate of accumulation by Perna indica. The animals were exposed in this concentration for a period of 16 days and the load in the whole tissue was assessed after 4, 8, 12 and 16 days. The data are presented in Tables 45, 46 and Figure 23. The average quan-

Table 43. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)		Replicates 1	2	3	4	Mean	SD	% of control
Control	Duration of exposure to raw seawater:7days							
4		29.21	33.63	34.42	32.06	32.33	1.99	
8		30.81	33.69	32.79	33.63	32.73	1.16	
12		34.64	32.83	33.47	43.41	36.08	4.27	
16		34.35	36.41	35.08	30.69	34.13	2.12	
Copper nitrate								
4		45.50	60.93	44.46	51.24	50.53	6.53	156.29
8		37.15	39.73	33.33	28.54	34.68	4.21	105.95
12		28.24	41.94	31.01	34.44	33.90	5.13	93.95
16		29.80	23.78	29.50	31.01	28.52	2.79	83.56
Copper chloride								
4		43.33	49.48	53.78	54.86	50.36	4.53	155.76
8		36.53	37.15	38.46	33.43	36.39	1.84	111.18
12		24.24	28.01	17.16	30.93	25.08	5.15	169.51
16		23.33	27.62	20.18	28.04	24.79	3.23	72.63

Table 44. Perna indica. Depuration: Analysis of variance for changes in filtration rate, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days.

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	1228.4640	307.1160	12.4403**
Between replicates	3	112.4667	37.4889	1.5185(NS)
Error	12	296.2440	24.6870	
Total	19	1637.1747		

NS Not significant
** Significant at 1% level

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	1803.4080	450.8520	23.9117**
Between replicates	3	47.9103	15.9701	0.8470(NS)
Error	12	226.2576	18.8548	
Total	19	2077.5759		

NS - Not significant
** - Significant at 1% level

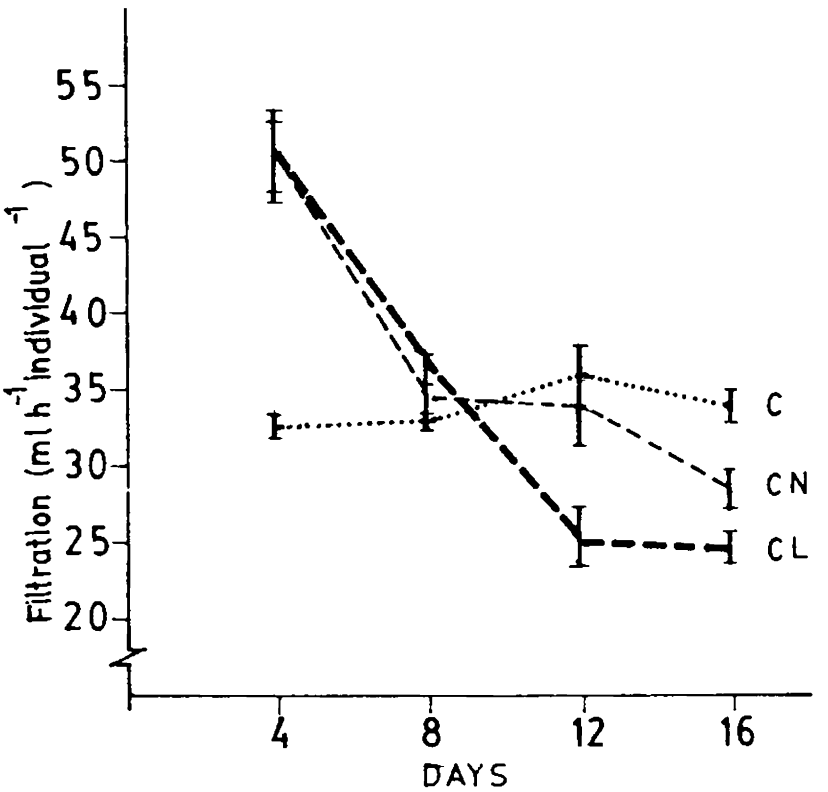

Figure 22. Perna indica. Depuration Mean filtration, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.50 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days.

tity of silver in the whole tissue ranged from 4.72 to 8.74 ppm when supplied in carbonate form and 5.45 to 9.96 when supplied in oxide form. Apparently the raw data gives marginal variations in the rate of uptake as a function of time. The data was subjected to analysis of variance and the results show that the variations noticed between treatment combinations, between salts and periods were highly significant (Table 46).

4.2.1.3.1.1. Oxygen consumption accompanying accumulation

The rate of oxygen consumption by Perna indica, when the animals had internal load of silver, was assessed. The results are presented in Tables 47-48 and Figure 24. Perna indica consumed more oxygen than the control animals when the internal load was 4.72 and 5.45 ppm of silver. However, increase in the silver concentration, that was achieved after 8th day, resulted in slight reduction in oxygen consumption. But when the results of oxygen consumption pattern is examined, it becomes clear that the rate of oxygen consumption reduced with increase in duration of exposure. However, the body load did not show any significant influence on oxygen consumption for, animals exposed to silver oxide, consumed more oxygen than those exposed to silver carbonate although the body load was higher.

4.2.1.3.1.2. Rate of filtration accompanying accumulation

Tables 49-50 and Figure 25 depict the rate of filtration of Perna indica exposed to 0.5 ppb of silver (carbonate and oxide). As in the case of oxygen consumption, the animals after an accumulative phase of 4, 8, 12 and 16 days were tested for their filtration capacity. Those animals exposed to silver carbonate filtered less water when compared to those exposed to silver oxide. It may be recalled here that the internal silver

Table 45. Perna indica. Accumulation: Silver concentration in the whole tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to 0.5 ppb silver carbonate and silver oxide from 4 to 16 days, along with standard deviation.

Exposure period (days)	Replicates 1	2	3	4	Mean	SD
Silver carbonate						
4	4.74	4.66	4.97	4.53	4.72	0.16
8	5.16	6.12	5.39	4.46	5.28	0.59
12	6.23	6.22	6.72	6.43	6.40	0.20
16	8.46	7.65	10.58	8.27	8.74	1.10
Silver oxide						
4	5.75	5.77	4.08	6.20	5.45	0.81
8	6.41	6.32	6.48	6.45	6.41	0.06
12	7.20	8.04	6.77	7.97	7.49	0.53
16	10.93	10.46	10.22	8.26	9.96	1.01

Table 46. Perna indica. Accumulation: Analysis of variance for changes in silver concentration in the whole tissue, when the animals were exposed to 0.5 ppb silver carbonate and silver oxide from 4 to 16 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	0.6092	0.2030	0.3073(NS)
2. Between treatment combinations	7	91.9574	13.1367	19.8861**
a. Between salts	1	8.7255	8.7255	13.2084**
b. Between periods	3	82.9420	27.6473	41.8518**
c. Interaction	3	0.2897	0.0965	0.1461(NS)
3. Error	21	13.8737	0.6606	
4. Total	31	106.4403		

NS - Not significant
** - Significant at 1% level

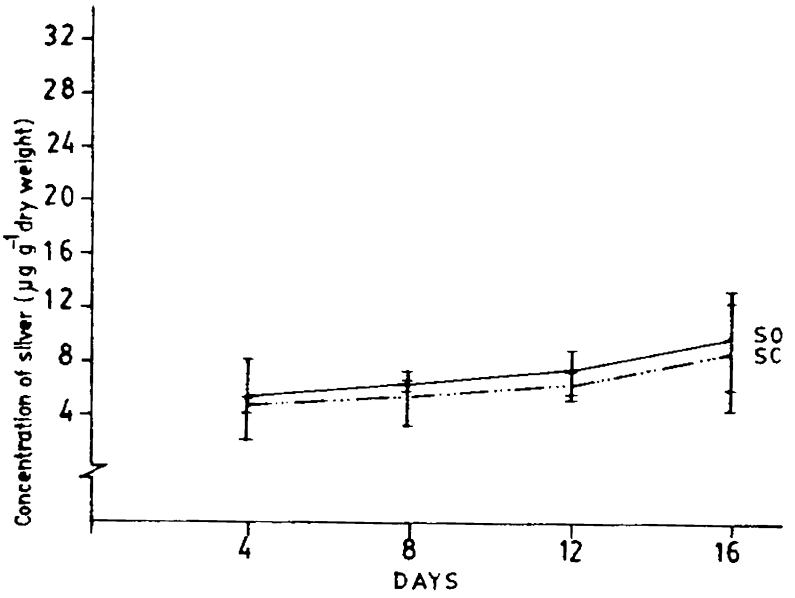

Figure 23. Perna indica. Accumulation: Silver concentration in the whole tissue, when the animals were exposed to 0.5 ppb of silver carbonate (SC) and silver oxide (SO) for period ranging from 4 to 16 days, along with standard deviation.

Table 47. Perna indica. Accumulation: Mean oxygen consumption (μg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to 0.5 ppb of silver carbonate and silver oxide from 4 to 16 days, along with standard deviation.

Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD	% of Control
Control							
4	2.18	2.36	2.83	1.98	2.33	0.31	
8	2.98	2.80	3.98	2.67	3.10	0.51	
12	2.71	3.98	2.99	2.05	2.93	0.69	
16	3.67	2.58	3.67	1.98	2.97	0.72	
Silver carbonate							
4	3.81	2.91	4.22	2.18	3.28	0.79	140.20
8	4.79	3.49	3.29	1.34	3.22	1.23	103.85
12	3.15	3.16	3.13	1.49	2.73	0.71	93.13
16	1.17	1.67	0.98	1.31	1.28	0.25	43.28
Silver oxide							
4	2.26	2.85	1.73	2.13	2.24	0.40	95.98
8	2.44	1.19	3.17	1.86	2.16	0.72	69.70
12	4.46	1.17	0.92	1.37	1.98	1.44	67.57
16	2.16	1.37	1.34	2.95	1.95	0.66	65.84

Table 48. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption, when the animals were exposed to 0.5 ppb silver carbonate and silver oxide from 4 to 16 days.

A. Silver carbonate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	10.4674	2.6168	4.7040**
Between replicates	3	4.5480	1.5160	2.7251(NS)
Error	12	6.6761	0.5563	
Total	19	21.6915		

NS - Not significant
** Significant at 5% level

B. Silver oxide

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	0.7459	0.1864	0.2004(NS)
Between replicates	3	1.9977	0.6659	0.7157(NS)
Error	12	11.1646	0.9303	
Total	19	13.9082		

NS - Not significant

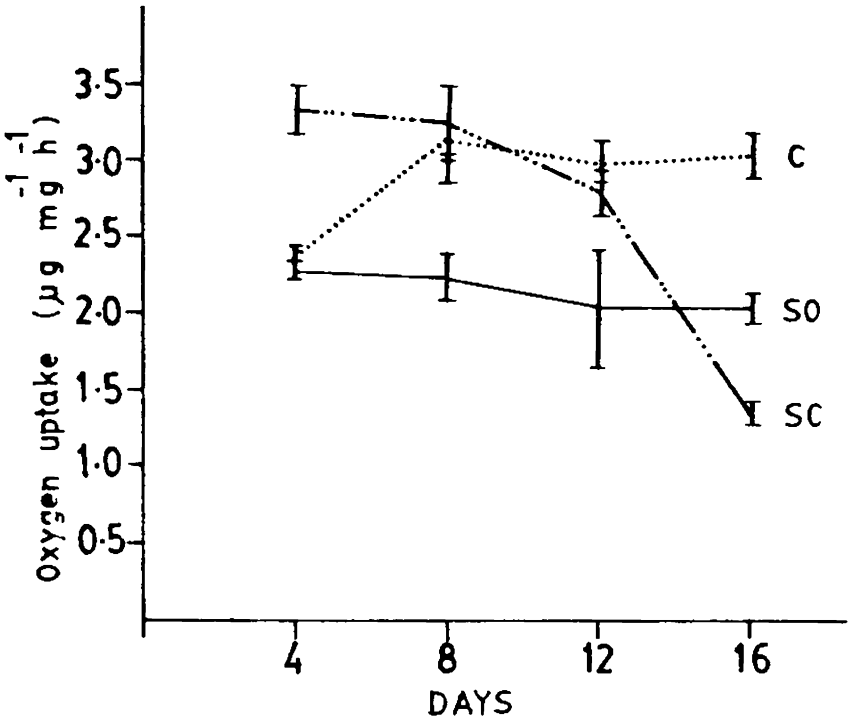

Figure 24. Perna indica. Accumulation: Mean oxygen consumption, when the animals were exposed to 0.5 ppb of silver carbonate (SC) and silver oxide (SO) for period ranging from 4 to 16 days, along with standard deviation.

Table 49. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to 0.5 ppb of silver carbonate and silver oxide from 4 to 16 days, along with standard deviation.

Duration of exposure (days)	Replicates 1	2	3	4	Mean	SD	% of control
Control							
4	30.83	32.49	38.64	29.86	32.95	3.41	
8	35.36	33.61	34.06	35.91	34.73	0.93	
12	34.73	36.66	33.98	32.63	34.50	1.45	
16	35.93	36.73	34.87	35.43	35.74	0.68	
Silver carbonate							
4	27.38	21.32	28.39	30.28	26.84	3.34	81.45
8	20.16	25.67	29.32	23.06	24.55	3.37	70.68
12	16.73	14.27	18.42	19.11	17.13	1.86	49.65
16	17.95	23.59	15.68	13.24	17.64	3.83	49.27
Silver oxide							
4	33.61	38.42	32.95	26.14	32.78	4.37	99.48
8	32.63	33.36	28.25	24.64	29.84	3.47	85.91
12	26.58	31.48	26.86	26.09	27.75	2.16	80.43
16	22.64	21.19	17.61	23.79	21.30	2.32	59.59

Table 50. Perna indica. Accumulation: Analysis of variance for changes in filtration rate, when the animals were exposed to 0.5 ppb silver carbonate and silver oxide from 4 to 16 days.

A. Silver carbonate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	522.2480	130.5620	9.3901**
Between replicates	3	26.4580	8.8193	0.6342(NS)
Error	12	166.8492	13.9041	
Total	19	715.5552		

NS Not significant
** - Significant at 1% level

B. Silver oxide

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	299.3680	74.8420	8.1229**
Between replicates	3	85.0947	28.3649	3.0785(NS)
Error	12	110.5654	9.2137	
Total	19	495.0281		

NS - Not significant
** - Significant at 5% level

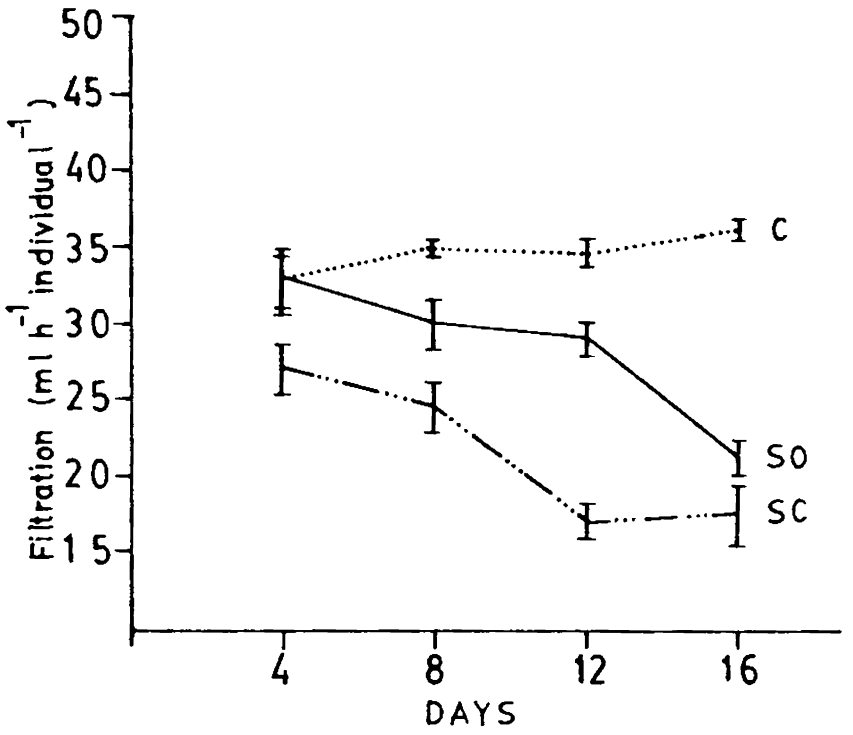

Figure 25. Perna indica. Accumulation: Mean rate of filtration when the animals were exposed to 0.5 ppb of silver carbonate (SC) and silver oxide (SO) for period ranging from 4 to 16 days, along with standard deviation.

load of Perna indica exposed to silver carbonate was comparatively less when compared to those exposed to silver oxide. When the data was subjected to analysis of variance showed (Table 50), that the variations between periods was significant.

4.2.1.3.2. Depuration

As in the case of the exposure studies employing Perna indica and copper, here also the test animals were exposed continuously for 16 days under 0.5 ppb of silver (carbonate and oxide). From the animals so exposed, representatives were removed after 4,8,12 and 16 days, assuming that there is a concomitant increase in the silver concentration in the tissue as a function of time, and such selected animals were allowed to depurate continuously for 7 days in raw seawater. Therefore, the period of depuration was uniform irrespective of the different duration of accumulative phase. The results obtained on the rate of depuration, the quantity retained in the whole tissue after 7 days depuration are presented in Tables 51-52 and Figure 26. Table 51, depicts the concentration of silver in the whole tissue. An important finding from the results obtained was that the rate of depuration and thereby the subsequent load in the whole tissue did not show internal concentration dependence. Thus, after 4 days of accumulation, the whole tissue contained 4.72 ppm and 8.74 ppm after 16 days when silver was supplied in the form of silver carbonate. Comparative values using silver oxide ranged between 5.45 and 9.96 ppm (Table 45). The rate of depuration ranged between 1.89 to 2.51 ppm and 1.86 to 2.28 ppm (Table 52). This shows that the quantity thrown out of the tissue showed only marginal differences and irrespective of the salt forms the depurative rate was com-

Table 51. Perna indica. Depuration: Concentration of silver in the whole tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)		Replicates 1	2	3	4	Mean	SD
Silver carbonate							
4	Duration of exposure to raw seawater: 7 days	2.86	2.47	3.17	2.83	2.83	0.24
8		3.80	4.26	3.97	3.61	3.91	0.23
12		5.29	5.28	4.60	4.74	4.97	0.31
16		6.00	5.49	7.78	5.62	6.22	0.91
Silver oxide							
4		3.68	3.63	3.13	3.90	3.58	0.28
8		4.76	3.92	4.24	4.73	4.41	0.35
12		6.68	6.85	6.26	5.09	6.22	0.68
16		9.40	6.80	7.70	6.85	7.68	1.05

Table 52. Perna indica. Depuration: Quantities of silver depurated (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)	Replicates 1	2	3	4	Mean	SD
Silver carbonate						
4	1.88	2.19	1.80	1.70	1.89	0.18
8	1.36	1.86	1.42	0.85	1.37	0.35
12	0.94	0.94	2.12	1.69	1.42	0.50
16	2.46	2.16	2.80	2.65	2.51	0.23
Silver oxide						
4	2.07	2.14	0.95	2.30	1.86	0.53
8	1.65	2.40	2.24	1.72	2.00	0.32
12	0.52	1.19	0.51	2.88	1.27	0.96
16	1.53	3.66	2.52	1.41	2.28	0.90

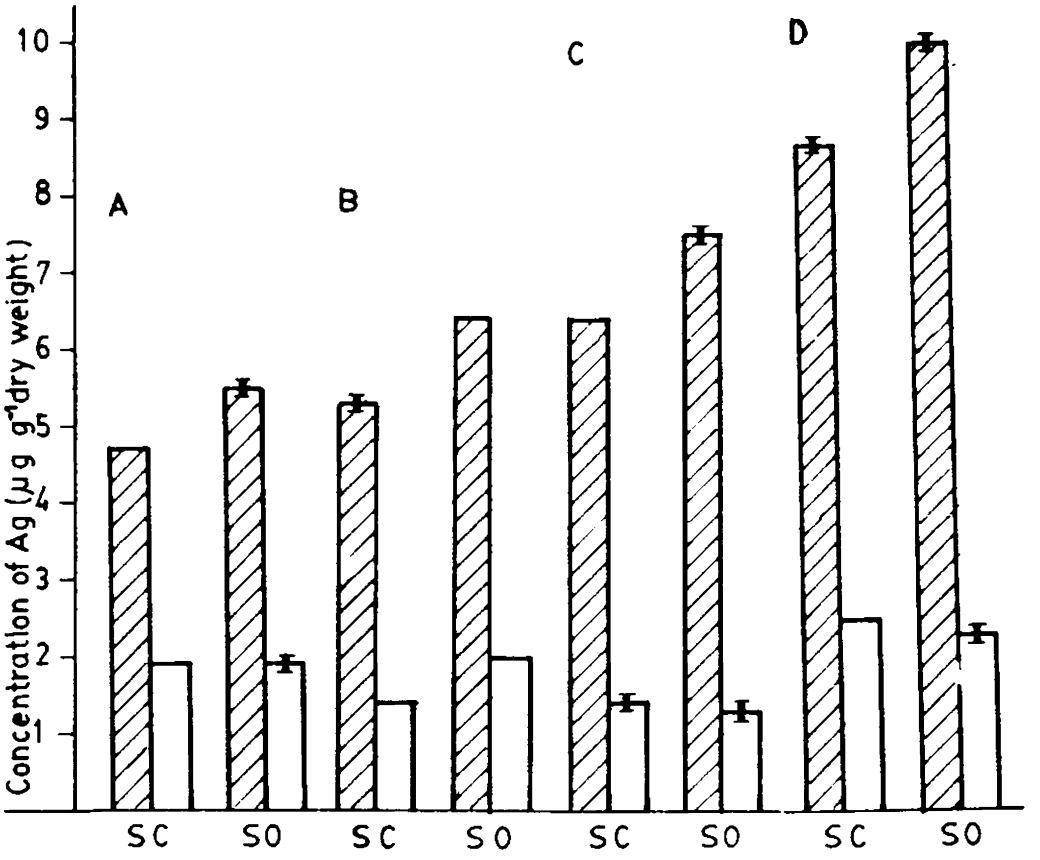

Figure 26. Perna indica. Quantities accumulated in the whole tissue (hached histogram) and quantities depurated in 7 days (open histogram).

A Four days accumulation C Twelve days accumulation

B Eight days accumulation D Sixteen days accumulation

parable. No clear cut increase in the rate of depuration was evinced vis-a-vis internal concentration. This is a very significant finding which gives an idea on the relationship between external concentration or environmental concentration, rate of accumulation, rate of depuration and duration of exposure.

4.2.1.3.2.1. Oxygen consumption accompanying depuration

The trend in the rate of oxygen consumption by Perna indica having an accumulative history of periods 4, 8, 12 and 16 days when subjected to a depurative phase of 7 days is presented in Tables 53-54 and Figure 27. Compared to the control animals, the animals pre-exposed to silver, either in silver carbonate or silver oxide form, consumed more oxygen. However, among the two lots, those which received silver carbonate as a source of silver to their test medium consumed more oxygen compared to those faced with silver oxide. Irrespective of these variations, those animals which encountered silver as either silver carbonate or silver oxide, for 16 days decidedly consumed less oxygen after 7 days depuration. When the data was subjected to analysis of variance only at one instance (silver carbonate exposed animals) the variation in oxygen consumption was found to be significant (Table 54).

4.2.1.3.2.2. Rate of filtration accompanying depuration

As in the case of oxygen consumption studies, the animals were tested for their capacity to filter after being exposed to 0.5 ppb silver carbonate and silver oxide for 4, 8, 12 and 16 days and then exposed to raw seawater for 7 days uniformly. The results are presented in Tables 55-56 and Figure 28. One conspicuous difference noticed was, those animals pre-exposed to silver carbonate for 4 days filtered more water than control

Table 53. Perna indica. Depuration: Mean oxygen consumption (µg mg^{-1} h^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)	Duration of exposure to raw seawater: 7 days	Replicates 1	2	3	4	Mean	SD	% of control
Control								
4		2.98	2.05	3.96	2.18	2.79	0.76	
8		2.08	3.98	2.80	2.11	2.74	0.77	
12		3.98	2.08	2.09	2.13	2.57	0.81	
16		3.97	3.68	1.42	2.09	2.79	1.06	
Silver carbonate								
4		4.89	3.94	2.16	3.50	3.62	0.98	129.63
8		3.07	4.64	3.16	2.42	3.32	0.81	121.23
12		4.17	3.05	2.93	2.93	3.27	0.52	129.05
16		2.41	2.09	1.35	1.59	1.86	0.41	66.90
Silver oxide								
4		3.78	2.20	3.18	2.52	2.92	0.60	104.55
8		3.26	1.76	2.72	3.47	2.80	0.66	102.3 1
12		3.93	2.72	3.41	2.06	3.03	0.70	117.85
16		2.85	2.86	0.98	0.70	1.84	1.01	66.47

Table 54. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days.

A. Silver carbonate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	8.4969	2.1242	4.3618*
Between replicates	3	2.6664	0.8888	1.8250(NS)
Error	12	5.8441	0.4870	
Total	19	17.0074		

NS - Not significant
* - Significant at 5% level

B. Silver oxide

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	3.6167	0.9041	1.4771(NS)
Between replicates	3	2.3042	0.7680	1.2548(NS)
Error	12	7.3454	0.6121	
Total	18	13.2663		

NS - Not significant

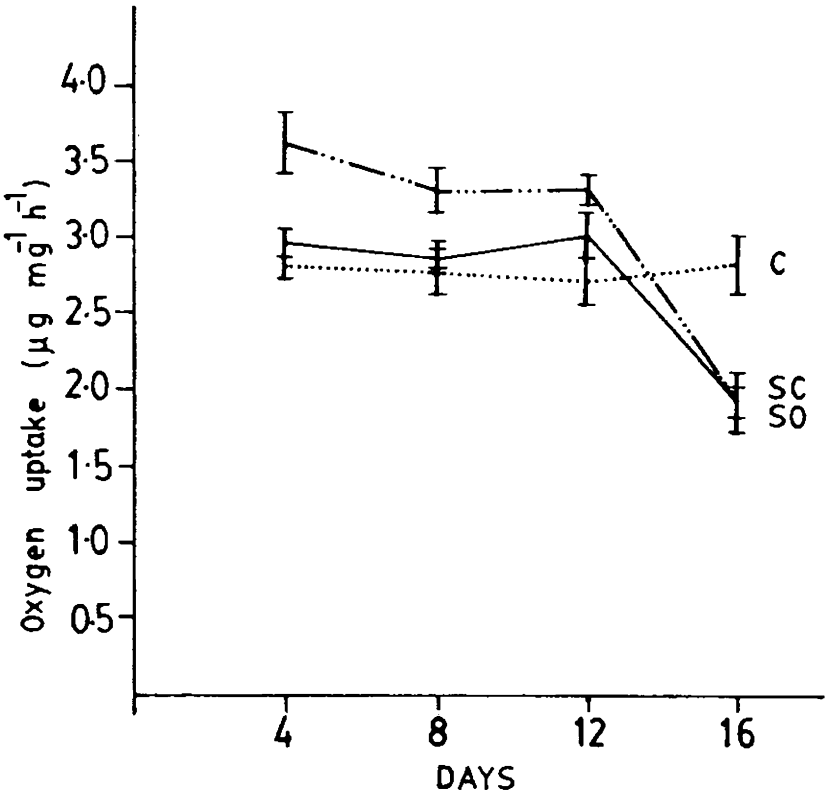

Figure 27. Perna indica. Depuration Mean oxygen consumption, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.50 ppb of silver carbonate (SC) and silver oxide (SO) for period ranging from 4 to 16 days.

Table 55. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days (accumulation), along with standard deviation.

Duration of pre-exposure (days)		Replicates 1	2	3	4	Mean	SD	% of control
Control								
4	raw seawater:	29.21	33.63	34.42	32.06	32.33	1.99	
8		30.81	33.69	32.79	33.63	32.73	1.16	
12		34.64	32.83	33.47	43.41	36.08	4.27	
16		34.35	36.41	35.08	30.69	34.13	2.12	
Silver carbonate								
4	exposure to 7 days	30.05	38.33	36.25	33.21	34.46	3.13	106.58
8		28.37	26.63	19.46	24.66	24.78	3.34	75.71
12		18.18	22.66	21.41	20.92	20.79	1.63	57.62
16		18.49	19.11	26.34	21.47	21.35	3.08	62.55
Silver oxide								
4	Duration of	33.73	31.63	31.14	26.16	30.66	2.77	94.83
8		36.34	24.94	28.14	23.06	28.12	5.08	85.91
12		23.91	18.86	24.39	27.59	23.68	3.12	65.63
16		27.58	26.75	28.17	25.65	27.03	0.94	79.19

Table 56. Perna indica. Depuration: Analysis of variance for changes in filtration rate, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.5 ppb silver carbonate and silver oxide from 4 to 16 days.

A. Silver carbonate

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	552.2840	138.0710	11.7182**
Between replicates	3	33.4326	11.1442	0.9458(NS)
Error	12	141.3912	11.7826	
Total	19	727.1078		

NS Not significant
** Significant at 1% level

B. Silver oxide

Source of variation	DF	SSQ	MSSQ	F. ratio
Between periods	4	123.9012	30.9753	2.4624(NS)
Between replicates	3	56.0265	18.6755	1.4846(NS)
Error	12	150.9516	12.5793	
Total	19	330.8793		

NS - Not significant

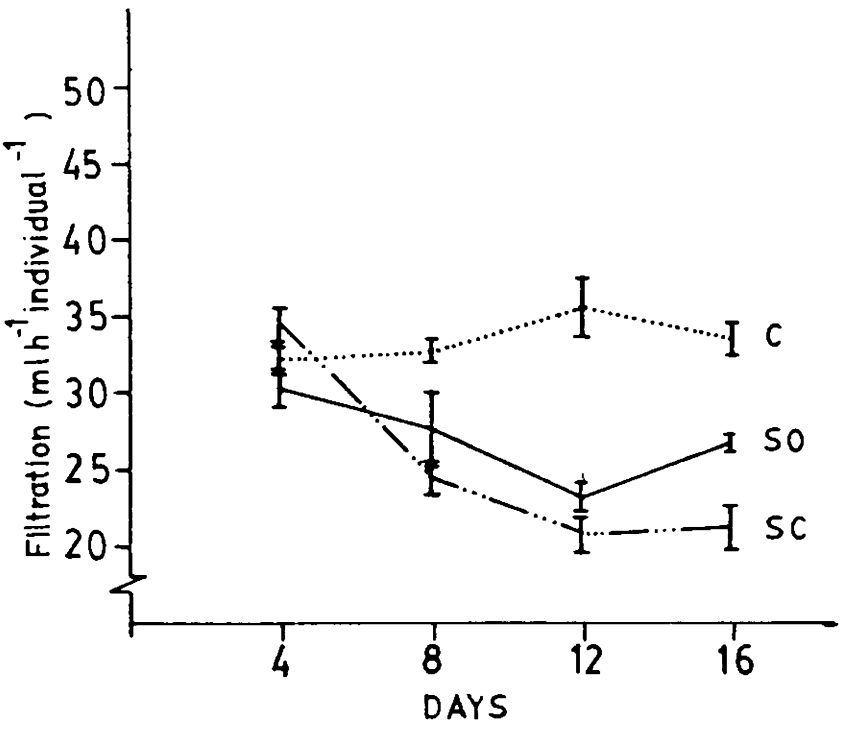

Figure 28. Perna indica. Depuration Mean filtration, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.50 ppb of silver carbonate (SC) and silver oxide (SO) for period ranging from 4 to 16 days.

even after 7 days of depuration. In the rest of the experiments all the animals filtered less quantity of water. Here also the silver carbonate pre-exposed animals seems to be more affected and filtered comparatively less quantities of water after 7 days of depuration. Consequently, the above finding showed significant variation between periods in the case of silver carbonate pre-exposed animals. It may be added here that the internal concentration of silver had positive relationship with reduction in filtration rate. The results evidently show that marginal variations in the rate of filtration can also occur.

4.2.2. METAL INTERACTION DURING ACCUMULATION AND DEPURATION

A series of experiments were conducted to delineate the effects of combinations of copper and silver in different salt forms on accumulation, depuration and oxygen consumption and filtration accompanying the above two processess. The main objective of the experiments was to analyse the effects of prolonged exposure (7 days) to test media of combinations of sulphate and nitrate forms of copper and silver at realistic concentrations of 0.25, 0.50 and 0.75 ppb.

4.2.2.1. SULPHATE FORMS OF COPPER AND SILVER

4.2.2.1.1. Effects on copper accumulation

Perna indica was exposed to $CuSO_4$ individually for a period of 7 days to find out the rate of copper uptake. Depending on the concentration of copper in the test medium there was variation in the rate of uptake (Table 57 and Figure 29). Subsequently, combinations of Ag_2SO_4 along with $CuSO_4$ were introduced into the test medium to analyse the rate of uptake of copper. Three series of combinations were employed, details of which are given in Table 57. The most important finding from the experiments

Table 57. Perna indica. Accumulation: Concentration of copper (as $CuSO_4$) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to varying concentrations of copper (as $CuSO_4$) individually and in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD
Ag 0.00 + Cu 0.25	17.12	16.70	16.23	16.68	0.36
+ Cu 0.50	21.49	20.46	20.59	20.84	0.45
+ Cu 0.75	24.35	23.95	23.57	23.95	0.31
Ag 0.25 + Cu 0.25	24.33	22.62	19.26	22.07	2.10
+ Cu 0.50	26.65	31.49	32.34	30.16	2.50
+ Cu 0.75	31.91	32.56	34.56	33.01	1.12
Ag 0.50 + Cu 0.25	15.43	13.81	13.39	14.21	0.87
+ Cu 0.50	15.55	17.45	18.77	17.25	1.32
+ Cu 0.75	18.19	18.78	19.71	18.89	0.62
Ag 0.75 + Cu 0.25	10.13	10.32	10.19	10.21	0.07
+ Cu 0.50	11.21	11.43	11.63	11.42	0.17
+ Cu 0.75	10.84	14.00	14.04	12.96	1.49

Table 58. Perna indica. Accumulation: Analysis of variance for changes in copper (as $CuSO_4$) concentrations in the whole tissue when exposed to varying concentrations of copper and three unvarying concentrations of silver (as Ag_2SO_4)

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	2.5449	1.2724	0.5565(NS)
2. Between treatment combinations	11	1678.6300	152.6027	66.7465**
a. Between silver concentrations	3	1360.4700	453.4900	198.3510**
b. Between copper concentrations	2	253.1910	126.5955	55.3713 **
c. Interaction	6	64.9727	10.8287	4.7363**
3. Error	22	50.2998	2.2863	
4. Total	35	1731.4747		

NS - Not significant
** - Significant at 1% level

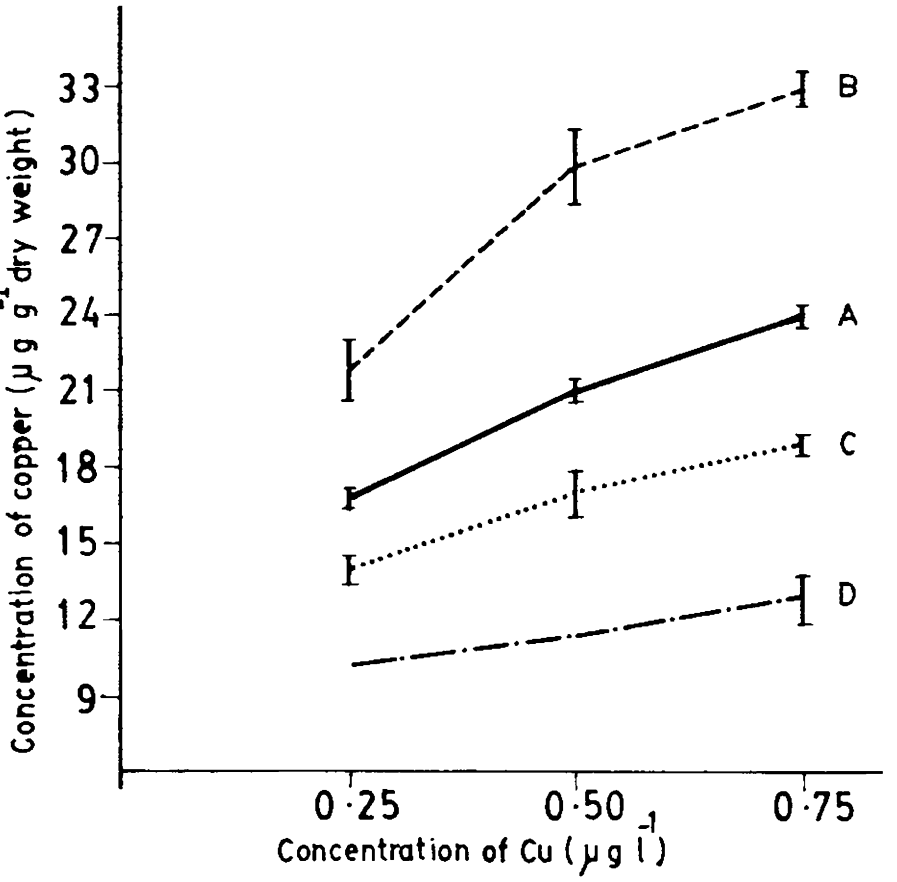

Figure 29. Perna indica. Accumulation: Concentration of copper (as $CuSO_4$) in the whole tissue ,when the animals were exposed to copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

was that the presence of increased concentrations of silver (0.5 and 0.75 ppb) reduced the uptake of copper. Whereas in that combinations which contained an unvarying concentration of 0.25 ppb silver and varying concentration of copper 0.25, 0.50 and 0.75 ppb, the uptake of copper was more. On the other hand, when the silver concentration was increased to 0.50 and 0.75 ppb, the rate of uptake of copper came down. In the highest concentration of 0.75 ppb silver and 0.75 ppb of copper, the quantity of copper accumulated was 12.96 ppm, whereas 0.25 ppb silver with 0.75 ppb copper resulted in a load of 33.01 ppm in the whole tissue of Perna indica. The results were subjected to analysis of variance (Table 58). It is clear that the variations between treatment combinations, between silver concentrations, between copper concentrations and their interaction, were highly significant. The results clearly indicate that when the medium contained both high concentrations of copper and silver, the rate of uptake of copper was low, whereas when the experimental medium contained a low level of silver and high level of copper, the rate of uptake of copper was high.

4.2.2.1.1.1. Oxygen consumption accompanying accumulation

Perna indica after having exposed to various concentrations of copper individually and in combination with silver (as sulphates) for 7 days was subjected to oxygen consumption studies in raw seawater. The results are presented in Tables 59-62 and Figure 30. When the internal tissue of Perna indica contained only copper, those animals which were pre-exposed to higher concentrations of copper, consumed less quantities of oxygen. The results were significant (Table 60). But after a pre-exposure history to silver and copper, the oxygen consumption pattern was erratic. In general, 0.25 ppb silver with 0.25, 0.50 and 0.75 ppb of copper did not conceivably affected

Table 59. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were maintained in varying concentrations of copper (as $CuSO_4$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.83	2.98	3.05	2.95	0.09	
0.25	1.98	2.51	1.42	1.97	0.44	66.67
0.50	2.18	2.24	1.16	1.86	0.49	62.99
0.75	1.13	0.77	0.98	1.06	0.27	36.07

Table 60. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption when exposed to varying concentrations of copper (as $CuSO_4$) for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	7.5141	2.5047	15.4325*
Between replicates	2	1.1744	0.5872	3.6179(NS)
Error	6	0.9738	0.1623	
Total	11	9.6623		

NS Not significant
* - Significant at 1% level

Table 61. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), recorded after maintenance in varying concentrations of copper (as $CuSO_4$) in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	1.90	1.35	1.15	1.46	0.31	49.80
+ Cu 0.50	1.09	2.01	0.86	1.32	0.49	44.72
+ Cu 0.75	1.41	1.42	0.98	1.27	0.20	43.14
Ag 0.50 + Cu 0.25	1.95	0.91	1.64	1.50	0.43	50.80
+ Cu 0.50	1.80	1.98	1.52	1.76	0.18	59.73
+ Cu 0.75	1.34	1.56	2.81	1.90	0.64	64.47
Ag 0.75 + Cu 0.25	0.79	2.77	3.67	2.41	1.20	81.61
+ Cu 0.50	1.81	2.18	0.98	1.65	0.50	56.02
+ Cu 0.75	0.85	0.93	0.82	0.86	0.04	29.54

Table 62. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption when exposed to varying concentrations of copper (as $CuSO_4$) and three unvarying concentrations of silver (as Ag_2SO_4) for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	0.2687	0.1343	0.2713(NS)
2. Between treatment combinations	8	4.5615	0.5701	1.1514(NS)
a. Between silver concentrations	2	0.8895	0.4447	0.8981(NS)
b. Between copper concentrations	2	0.6797	0.3398	0.6862(NS)
c. Interaction	4	2.9922	0.7480	1.5106(NS)
3. Error	16	7.9240	0.4952	
4. Total	26	12.7542		

NS - Not significant

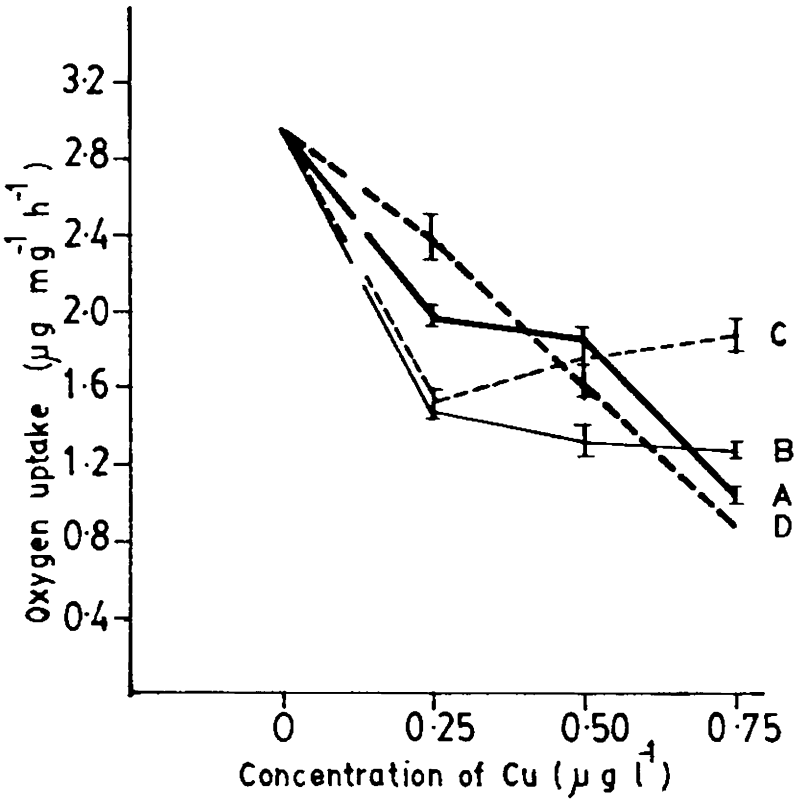

Figure 30. Perna indica. Accumulation: Mean oxygen consumption when the animals were exposed to copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

Table 63. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of copper which caused a 50% reduction in the oxygen consumption upon exposure to copper and three unvarying concentrations of silver + varying concentrations of copper (as sulphates) for a period of 7 days, along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Silver	+	Copper	
		0.55 (0.30 - 1.02)*	
0.25		0.23 (0.19 - 0.27)*	+ 0.29 (MA)
0.50		0.91 (0.52 1.59)*	1.37 (LA)
0.75		0.51 (0.50 0.53)*	0.0072 (SA)

* 95% confidence limits

MA More than additive

LA Less than additive

SA - Simple additive

oxygen consumption, although the accumulation results show that the whole tissue contained very high concentrations of copper. Compared to the oxygen consumption rates of control animals all the experimental animals having a previous history of exposure to silver and copper continued to consume less quantity of oxygen. When the results were subjected to analysis of variance (Table 62) it was found that there was no significance.

Since the usual method to explain the reaction of an animal with reference to sublethal response in the presence of toxicant combinations is by the analysis of additive index and effective concentrations, the data on oxygen consumption were subjected to this analysis (Table 63). Drastic variations in the effective concentrations of copper in the presence of silver were noticed. Copper alone, exemplified an EC 50 of 0.55 ppb, whereas it became to 0.23 ppb in the presence of 0.25 ppb silver and shot upto 0.91 ppb copper in the presence of 0.50 ppb silver. The three combinations of silver and copper depicted more than additivity to simple additivity.

4.2.2.1.1.2. Filtration accompanying accumulation

The rate of filtration of Perna indica pre-exposed to copper alone, and a combination of three unvarying concentrations of silver and varying concentrations of copper was assessed. As in the case of oxygen consumption experiments, here also the animal tissue contained copper and silver. The details are given in Tables 64-67 and Figure 31. The main idea was to delineate whether the body burden of metal has any influence on filtration capacity of Perna indica compared to control. All the animals uniformly filtered less quantity of water and the reduction in quantity was proportional to increase in the copper and silver concentrations in the external medium at the time of phase of accumulation. The same was the

Table 64. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were maintained in varying concentrations of copper (as $CuSO_4$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	31.86	33.94	33.39	34.06	1.85	
0.25	24.69	18.19	16.36	19.74	3.57	57.95
0.50	11.46	10.81	18.31	13.52	3.39	39.69
0.75	10.66	15.04	8.85	11.51	2.59	33.79

Table 65. Perna indica. Accumulation: Analysis of variance for changes in filtration rate when exposed to varying concentrations of copper (as $CuSO_4$) for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	796.6440	265.5480	15.5208**
Between replicates	2	0.5932	0.2966	0.0173(NS)
Error	6	102.6546	17.1091	
Total	11	899.8918		

NS Not significant
** - Significant at 1% level

Table 66. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), recorded after maintenance in varying concentrations of copper (as $CuSO_4$) in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	28.36	23.16	27.66	26.39	2.30	77.48
+ Cu 0.50	15.89	16.82	14.39	15.70	1.00	46.09
+ Cu 0.75	11.30	9.08	10.11	10.16	0.90	29.82
Ag 0.50 + Cu 0.25	25.73	18.69	16.48	20.30	3.94	59.60
+ Cu 0.50	13.81	15.14	12.64	13.86	1.00	40.69
+ Cu 0.75	7.74	8.16	8.84	8.24	0.45	24.19
Ag 0.75 + Cu 0.25	18.25	14.89	14.97	16.03	1.56	47.06
+ Cu 0.50	12.53	13.41	16.08	14.00	1.50	41.10
+ Cu 0.75	3.81	7.81	5.07	5.56	1.66	16.32

Table 67. Perna indica. Accumulation: Analysis of variance for changes in filtration rate when exposed to varying concentrations of copper (as $CuSO_4$) and three unvarying concentrations of Ag_2SO_4 for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	1.9511	0.9755	0.2408(NS)
2. Between treatment combinations	8	827.3520	103.4190	25.5311**
a. Between silver concentrations	2	463.7080	231.8540	57.2380 **
b. Between copper concentrations	2	321.6000	160.8000	39.6968**
c. Interaction	4	42.0444	10.5111	2.5948(NS)
3. Error	16	64.8125	4.0507	
4. Total	26	894.1156		

NS - Not significant
** Significant at 1% level

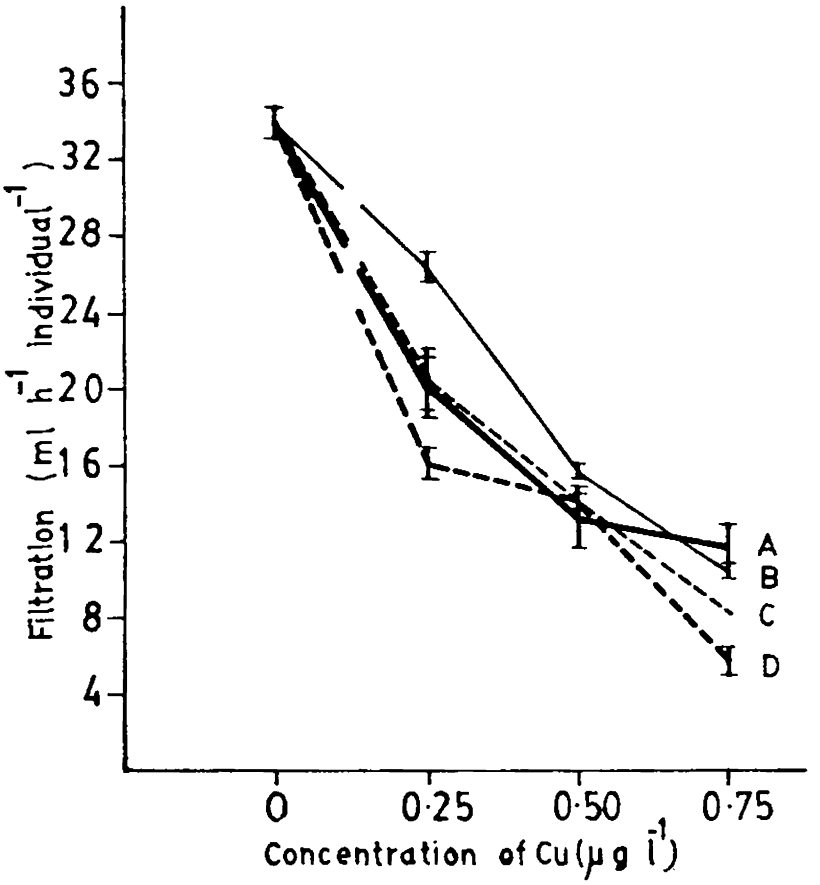

Figure 31. Perna indica. Accumulation: Mean rate of filtration when the animals were exposed to copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

Table 68. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of copper which caused a 50% reduction in the filtration rate upon exposure to copper and three unvarying concentrations of silver + varying concentrations of copper (as sulphates) for a period of 7 days, along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Silver	+	Copper	
		0.34 (0.30 - 0.39)*	
0.25		0.47 (0.46 0.48)*	0.74 (LA)
0.50		0.34 (0.29 - 0.40)*	0.73 (LA)
0.75		0.25 (0.11 - 0.57)*	0.83 (LA)

* 95% confidence limits

LA Less than additive

case when the animals were exposed to copper alone. In the case of copper exposed animals, the decrease in filtration coincided with increase in body burden of the animal. When the results were subjected to analysis of variance (Table 67), the data obtained between treatment combinations, between silver concentrations, between copper concentrations and their interactions were found to be significant. Table 68 explains effective concentrations which caused a 50% reduction in the filtration rate. The interaction of the two metals on filtration was less than additive. Here, with increase in silver concentration, the copper concentration that brought about 50% reduction in filtration was found to go down.

4.2.2.1.2. Effects on copper depuration

After exposing Perna indica to various concentrations of $CuSO_4$ alone and in combination with Ag_2SO_4 for a period of 7 days, they were maintained in raw seawater for 7 days, to find out the rate of depuration. A significant result obtained was the drastic variations in the rate of depuration of copper irrespective of the variation in silver concentration. Further, those animals maintained with copper alone in the test medium depurated more quantity of copper depending on increase in body burden. On the other hand, when exposed to a combination of copper and silver, the quantity depurated was more when silver concentrations were high. Only in one instance when the external medium contained 0.25 ppb silver, the rate of depuration of copper was found to be erratic and found no relationship with body burden (Tables 69-70 and Figure 32). Analysis of variance data (Table 71) show that the results between treatment combinations, between silver concentrations, and between copper concentrations were highly significant.

Table 69. Perna indica. Depuration: Concentration of copper (as $CuSO_4$) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $CuSO_4$) individually and in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD
Ag 0.00 + Cu 0.25	13.43	12.48	12.09	12.66	0.56
+ Cu 0.50	15.50	14.74	14.58	14.94	0.40
+ Cu 0.75	17.87	18.87	16.66	17.80	0.90
Ag 0.25 + Cu 0.25	18.51	21.05	15.65	18.40	2.20
+ Cu 0.50	24.75	24.82	27.94	25.83	1.48
+ Cu 0.75	29.50	31.43	32.53	31.15	1.25
Ag 0.50 + Cu 0.25	14.96	10.22	10.56	11.91	2.15
+ Cu 0.50	10.16	11.13	11.91	11.06	0.71
+ Cu 0.75	11.01	12.34	10.12	11.15	0.91
Ag 0.75 + Cu 0.25	9.81	9.38	9.50	9.56	0.18
+ Cu 0.50	7.57	7.34	6.91	7.27	0.27
+ Cu 0.75	7.86	7.42	7.86	7.71	0.20

Table 70. Perna indica. Depuration: Quantity of copper depurated (as $CuSO_4$) from the whole tissue ($\mu g\ g^{-1}$ dry weight) when the animals were exposed to raw seawater after being maintained in varying concentrations of copper (as $CuSO_4$) individually and in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentration of metal (ppb)		Replicates 1	2	3	Mean	SD
Ag 0.00	+ Cu 0.25	3.69	4.22	4.14	4.01	0.23
	+ Cu 0.50	5.99	5.72	6.01	5.90	0.13
	+ Cu 0.75	6.48	5.08	6.91	6.15	0.78
Ag 0.25	+ Cu 0.25	5.82	1.57	3.61	3.66	1.73
	+ Cu 0.50	1.90	6.67	4.40	4.32	1.94
	+ Cu 0.75	2.41	1.13	2.03	1.85	0.53
Ag 0.50	+ Cu 0.25	0.47	3.59	2.83	2.29	1.32
	+ Cu 0.50	5.39	6.32	6.86	6.19	0.60
	+ Cu 0.75	7.18	6.44	9.59	7.73	1.34
Ag 0.75	+ Cu 0.25	0.32	0.94	0.69	0.65	0.25
	+ Cu 0.50	3.64	4.09	4.72	4.15	0.44
	+ Cu 0.75	2.98	6.58	6.18	5.24	1.61

Table 71. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated (as $CuSO_4$), when the animals were exposed to raw seawater after being maintained in varying concentrations of copper (as $CuSO_4$) and three unvarying concentrations of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	5.7160	2.8580	1.6547(NS)
2. Between treatment combinations	11	138.2400	12.5672	7.2760**
a. Between silver concentrations	3	38.5859	12.8619	7.4467**
b. Between copper concentrations	2	51.6049	25.8024	14.9388**
c. Interaction	6	48.0494	8.0082	4.6365**
3. Error	22	38.0001	1.7272	
4. Total	35	181.9561		

NS Not significant
** - Significant at 1% level

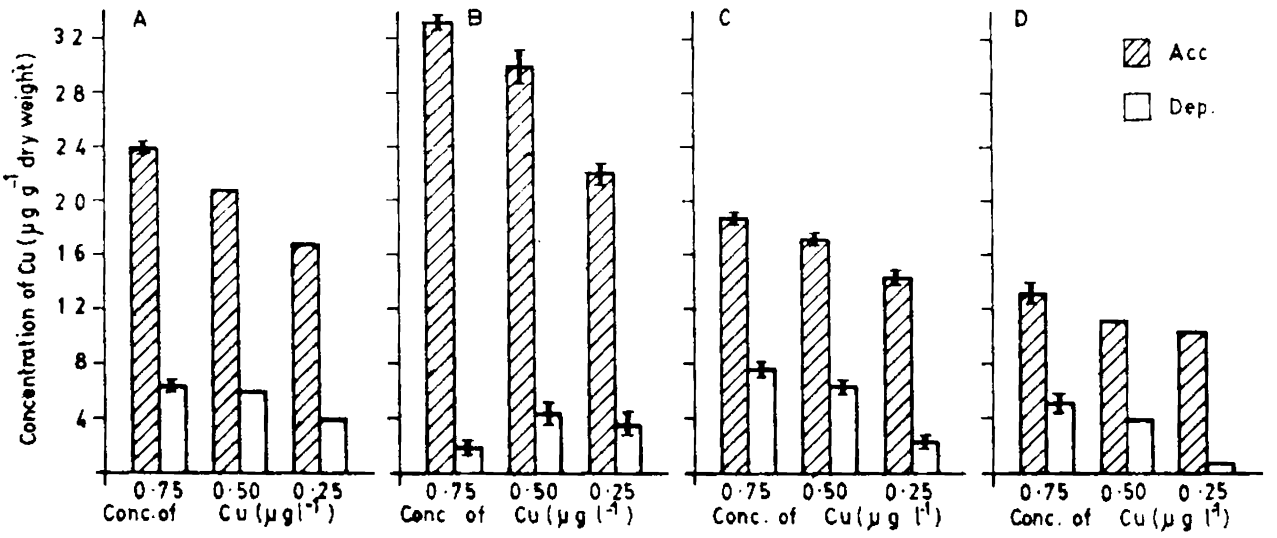

Figure 32. Perna indica. Quantities accumulated in the whole tissue after 7 days and depurated subsequent to exposure to raw seawater for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

4.2.2.1.2.1. Oxygen consumption accompanying depuration

The rate of oxygen consumption by Perna indica, which had an exposure history of 7 days in a medium containing copper alone or copper in combination with silver and subsequently in raw seawater for 7 days, were tested. The results are presented in Tables 72-75 and Figure 33. Depending on the copper concentrations to which the animals were pre-exposed, the rate of oxygen consumption varied. In the highest concentrations maintained animals (0.75 ppb), the rate of oxygen consumption was lowest even after 7 days of depuration. On the other hand, the data obtained, on the rate of oxygen consumption in the case of animals exposed to silver and copper combination, were erratic and inconclusive. This is significant in the light of results obtained from experiments conducted with animals exposed to copper alone. Here, higher the exposure concentration, lower the rate of oxygen consumption of the trend continued even after 7 days of depuration. Analysis of variance data (Table 75) showed that the variations observed were not significant.

4.2.2.1.2.2. Filtration accompanying depuration

The results on the rate of filtration are presented in Tables 76-79 and Figure 34. Those animals pre-exposed to 0.25, 0.50 and 0.75 ppb of copper for 7 days and subsequently maintained in raw seawater for 7 days, did not regain the capacity to filter in a appreciable manner. The capacity to regain normalcy was found to be viciated by the exposure of the animals to higher concentrations of copper for longer duration. The observed variations were statistically highly significant (Table 77). Quite contrary to the results obtained for oxygen consumption, here also, those animals which encountered a combination of copper and silver showed

Table 72. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $CuSO_4$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.89	2.89	3.06	2.94	0.08	
0.25	2.18	1.58	3.86	2.54	0.96	92.50
0.50	3.10	2.69	1.08	2.29	0.87	83.40
0.75	2.10	1.79	2.18	2.02	0.16	73.81

Table 73. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of copper (as $CuSO_4$)

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	0.8740	0.2913	0.3291(NS)
Between replicates	2	0.1886	0.0943	0.1065(NS)
Error	6	5.3111	0.8851	
Total	11	6.3737		

NS - Not significant

Table 74. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $CuSO_4$) in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations of metal (ppb)	Replicates					
	1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	3.88	2.45	2.58	2.97	0.64	108.18
+ Cu 0.50	1.16	3.08	1.42	1.88	0.85	68.79
+ Cu 0.75	2.00	1.72	1.61	1.77	0.16	64.74
Ag 0.50 + Cu 0.25	2.86	1.89	2.09	2.28	0.41	83.08
+ Cu 0.50	1.98	2.16	1.89	2.01	0.11	73.42
+ Cu 0.75	1.87	1.65	2.18	1.90	0.21	69.26
Ag 0.75 + Cu 0.25	1.65	1.42	2.10	1.72	0.28	62.92
+ Cu 0.50	2.00	2.75	1.72	2.15	0.43	78.63
+ Cu 0.75	1.06	0.95	1.01	1.00	0.04	36.84

Table 75. Perna indica. Depuration: Analysis of variance for changes in oxyg consumption by those animals pre-exposed to varying concentrations copper (as $CuSO_4$) and three unvarying concentrations of silver (Ag_2SO_4

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	0.2162	0.1081	0.3597(NS)
2. Between treatment combinations	8	6.5020	0.8127	2.7046(NS)
a. Between silver concentrations	2	2.6607	1.3303	4.4271*
b. Between copper concentrations	2	1.6404	0.8202	2.7294(NS)
c. Interaction	4	2.2009	0.5502	1.8310(NS)
3. Error	16	4.8094	0.3005	
4. Total	26	11.5276		

NS Not significant
* Significant at 5% level

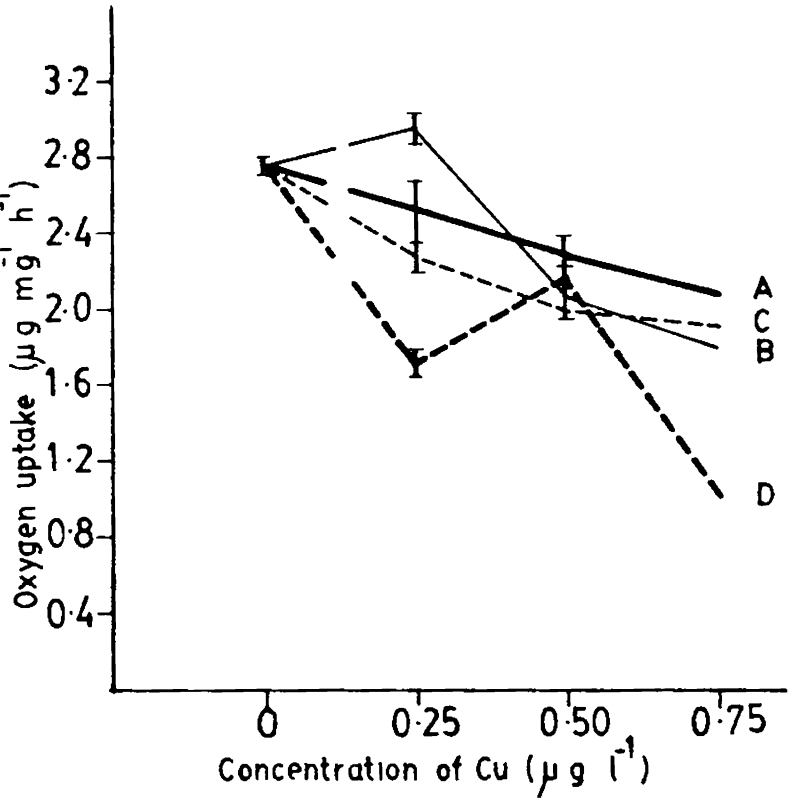

Figure 33. Perna indica. Depuration: Mean oxygen consumption when the animals were exposed to raw seawater for 7 days, after being maintained in copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

Table 76. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $CuSO_4$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentration (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	30.38	36.36	33.42	33.38	2.44	
0.25	25.96	11.68	13.81	17.15	6.29	51.37
0.50	16.77	14.87	15.64	15.76	0.78	47.21
0.75	11.84	17.83	14.65	14.77	2.44	44.24

Table 77. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentration of copper (as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	697.0080	232.3360	9.3299**
Between replicates	2	6.9482	3.4741	0.1395(NS)
Error	6	149.4138	24.9023	
Total	11	853.3700		

NS Not significant
** - Significant at 1% level

Table 78. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $CuSO_4$) in combination with three unvarying Ag_2SO_4 overloads for a period of 7 days, along with standard deviation and percentage variation from control (100% readings.

Pre-exposure concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	32.18	26.98	26.97	28.71	2.45	86.00
+ Cu 0.50	18.34	17.63	18.20	18.05	0.30	54.07
+ Cu 0.75	14.71	11.90	12.83	13.14	1.16	39.36
Ag 0.50 + Cu 0.25	26.15	23.46	20.83	23.48	2.17	70.34
+ Cu 0.50	14.71	16.23	14.74	15.22	0.70	45.59
+ Cu 0.75	10.08	8.75	8.90	9.24	0.59	27.68
Ag 0.75 + Cu 0.25	20.58	16.32	24.97	20.62	3.53	61.77
+ Cu 0.50	16.87	16.26	15.61	16.24	0.51	48.65
+ Cu 0.75	9.70	6.85	9.09	8.54	1.22	25.58

Table 79. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of copper (as $CuSO_4$) and three unvarying concentration of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	16.6533	8.3266	1.5665(NS)
2. Between treatment combinations	8	1000.1300	125.0162	23.5196**
a. Between silver concentrations	2	605.1360	302.5680	56.9229**
b. Between copper concentrations	2	283.8570	141.9285	26.7013**
c. Interaction	4	111.1330	27.7832	5.2269*
3. Error	16	85.0479	5.3154	
4. Total	26	1101.8312		

NS Not significant
* Significant at 5% level
** Significant at 1% level

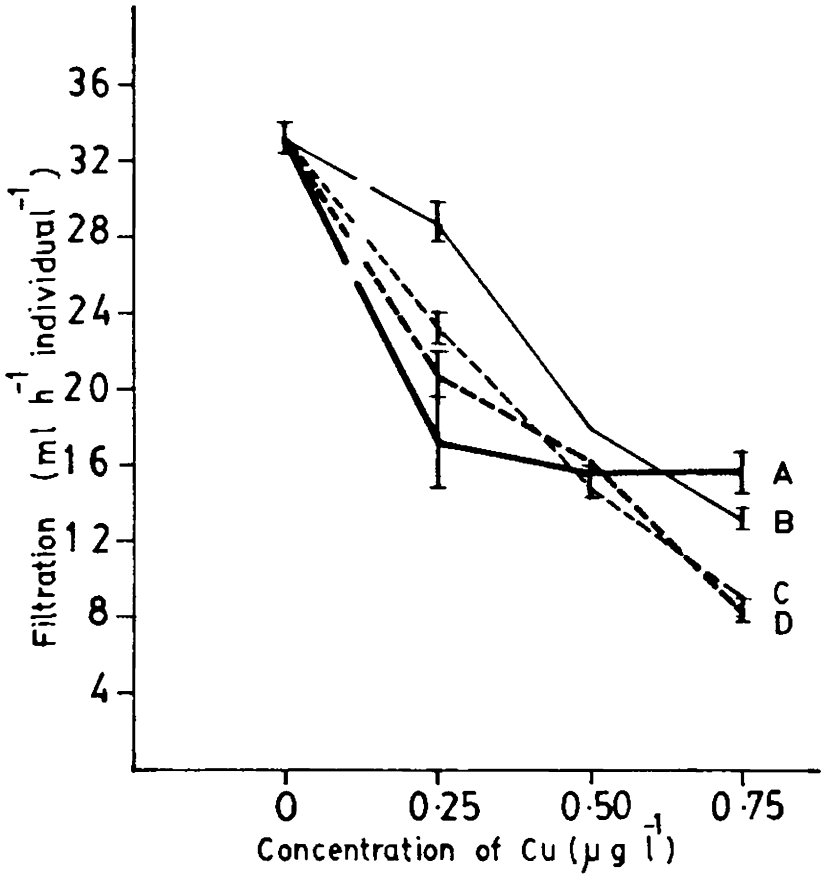

Figure 34. Perna indica. Depuration: Mean rate of filtration when the animals were exposed to raw seawater for 7 days, after being maintained in copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

clear cut concentration dependent filtration rate (Table 78). Those animals which encountered 0.75 ppb copper along with 0.25, 0.50 and 0.75 ppb silver for 7 days, uniformly filtered less quantity of water. Those animals which were exposed to 0.75 ppb copper with 0.75 ppb silver for 7 days and subsequent exposure to raw seawater for 7 days did not appreciably regain their capacity. A similar situation was found in the case of animals which experienced 0.75 ppb copper and 0.75 ppb silver.

4.2.2.1.3. Effects on silver accumulation

The pattern of accumulation of copper supplied in the form of sulphate along with three unvarying concentrations of Ag_2SO_4, in the whole tissue of Perna indica was analysed to find out the combined effects on accumulation. The three unvarying concentrations of copper employed were 0.25, 0.50 and 0.75 ppb. To compare the rate of uptake of copper when supplied in combination with silver, another set of experiments was also conducted to gather base line information on silver accumulation individually. The concentrations employed were similar.

The rate of uptake of silver by Perna indica, was concentration dependent. Thus, Perna indica accumulated most quantity of silver when exposed for 7 days to a concentration of 0.75 ppb of silver supplied in SO_4 form (Tables 80-81 and Figure 35). A conspicuous feature of the results obtained employing the combination was, reduction in the uptake of silver in the presence of unvarying concentrations of copper. In the presence of 0.25 ppb of copper along with 0.25, 0.50 or 0.75 ppb of silver resulted in accumulation of silver from 4.01 ppm to 19.76 ppm. Whereas when the copper concentration was increased to 0.50 and 0.75 ppb silver uptake came down. Thus combination 0.75 ppb copper and 0.75 ppb silver

Table 80. Perna indica. Accumulation: Concentration of silver (as Ag_2SO_4) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to varying concentrations of silver (as Ag_2SO_4) individually and in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation.

Concentration of metal (ppb)		Replicates 1	2	3	Mean	SD
Cu 0.00	+ Ag 0.25	9.86	8.13	9.56	9.18	0.75
	+ Ag 0.50	20.06	25.90	27.80	24.58	3.29
	+ Ag 0.75	26.89	30.01	28.89	28.59	1.29
Cu 0.25	+ Ag 0.25	2.93	4.81	4.31	4.01	0.79
	+ Ag 0.50	16.77	15.67	15.66	16.03	0.52
	+ Ag 0.75	18.26	21.69	19.33	19.76	1.43
Cu 0.50	+ Ag 0.25	7.67	6.93	4.95	6.51	1.14
	+ Ag 0.50	9.80	9.70	8.54	9.34	0.57
	+ Ag 0.75	14.22	12.77	11.45	12.81	1.13
Cu 0.75	+ Ag 0.25	6.14	5.33	4.20	5.22	0.79
	+ Ag 0.50	9.54	9.07	8.30	8.97	0.51
	+ Ag 0.75	8.39	10.89	9.19	9.49	1.04

Table 81. Perna indica. Accumulation: Analysis of variance for changes in silver (as Ag_2SO_4) concentrations in the whole tissue when exposed to varying concentrations of silver (Ag_2SO_4) and three unvarying concentrations of copper (as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	5.2535	2.6267	1.0010(NS)
2. Between treatment combinations	11	2018.1900	183.4718	69.9179**
a. Between copper concentrations	3	1754.4900	584.8300	222.8687**
b. Between silver concentrations	2	50.0532	25.0266	9.5372**
c. Interaction	6	213.6480	35.6080	13.5696 **
3. Error	22	57.7305	2.6241	
4. Total	35	2081.1740		

NS - Not significant
** - Significant at 1% level

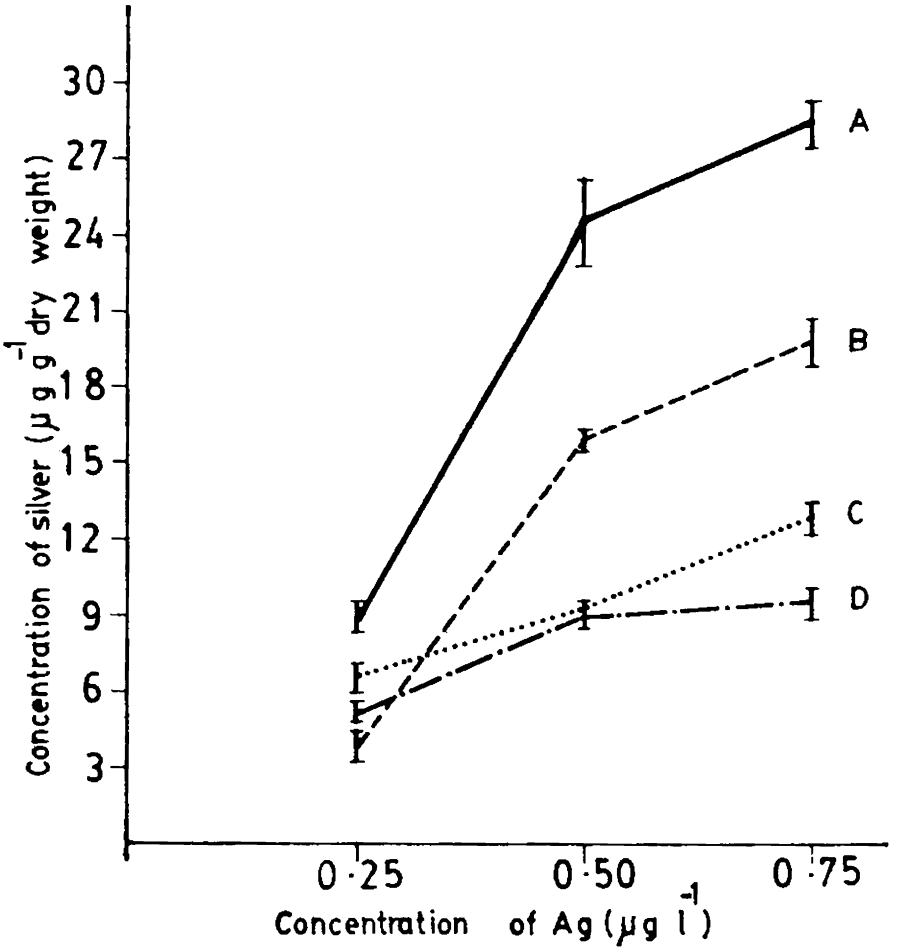

Figure 35. Perna indica. Accumulation: Concentration of silver (as Ag_2SO_4) in the whole tissue when the animals were exposed to silver and silver + copper (as sulphates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

resulted in a 9.49 ppm silver after 7 days. To analyse the significance of the results, the data was subjected to analysis of variance (Table 81). The variations obtained between treatment combinations such as copper concentrations and silver concentrations were significant at 1% level.

4.2.2.1.3.1. Oxygen consumption accompanying accumulation

To find out the effect of accumulation on oxygen consumption of Perna indica, representative samples of animals exposed to unvarying concentrations of copper and varying concentrations of silver were subjected to oxygen consumption analysis. The base line data was provided employing silver alone for comparison (Tables 82-83 and Figure 36). Increase in concentration of silver both in the external medium and the tissue resulted in reduction in oxygen consumption, showing a direct significance. Similarly, in the other groups of animals a 7 days accumulative period of silver in the presence of copper also resulted in the concomitant reduction in oxygen consumption with increase in silver concentrations (Table 84 and Figure 36). However, it may be noted here that the concentration of silver in the tissue of Perna indica exposed to an unvarying concentration of copper (0.75 ppb) and varying concentration of silver, was low (Table 80). The results were subjected to analysis of variance (Table 85). None of the results obtained were found to be significant. The data obtained on the effective concentrations of silver which resulted in a 50% reduction in oxygen consumption varied between 0.26 to 0.68 ppb silver. Although individually the EC 50 of silver was 0.69 ppb, statistically reduction in silver concentration showed only a less than additive reaction with unvarying concentrations of copper (Table 86).

Table 82. Perna indica. Accumulation: Mean oxygen consumption (μg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to varying concentrations of silver (as Ag_2SO_4) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.83	2.98	3.05	2.95	0.09	
0.25	3.49	2.79	1.97	2.75	0.62	93.01
0.50	2.23	1.88	1.08	1.73	0.48	58.59
0.75	1.88	0.98	1.76	1.54	0.39	52.10

Table 83. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption by those animals exposed to varying concentrations of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	6.1332	2.0444	12.4355**
Between replicates	2	1.9415	0.9707	5.9048**
Error	6	0.9865	0.1644	
Total	11	9.0612		

** - Significant at 1% level

Table 84. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), recorded after maintenance in varying concentrations of silver (as Ag_2SO_4) in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)		Replicates 1	2	3	Mean	SD	% of control
Cu 0.25	+ Ag 0.25	1.90	1.35	1.15	1.46	0.31	49.80
	+ Ag 0.50	1.95	0.91	1.64	1.50	0.43	50.80
	+ Ag 0.75	0.79	2.77	3.67	2.41	1.20	81.61
Cu 0.50	+ Ag 0.25	1.09	2.01	0.86	1.32	0.49	44.72
	+ Ag 0.50	1.80	1.98	1.52	1.76	0.18	59.73
	+ Ag 0.75	1.81	2.18	0.98	1.65	0.50	56.02
Cu 0.75	+ Ag 0.25	1.41	1.42	0.98	1.27	0.20	43.14
	+ Ag 0.50	1.34	1.56	2.81	1.90	0.64	64.47
	+ Ag 0.75	0.85	0.93	0.82	0.86	0.04	29.54

Table 85. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption by those animals exposed to varying concentrations of silver (as Ag_2SO_4) and three unvarying concentrations of copper(as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	1.2306	0.6153	1.6959(NS)
2. Between treatment combinations	8	7.5134	0.9391	2.5886(NS)
a. Between copper concentrations	2	3.4716	1.7358	4.7844*
b. Between silver concentrations	2	2.0817	1.0408	2.8689(NS)
c. Interaction	4	1.9600	0.4900	1.3506(NS)
3. Error	16	5.8050	0.3628	
4. Total	26	14.5490		

NS - Not significant
* - Significant at 5% level

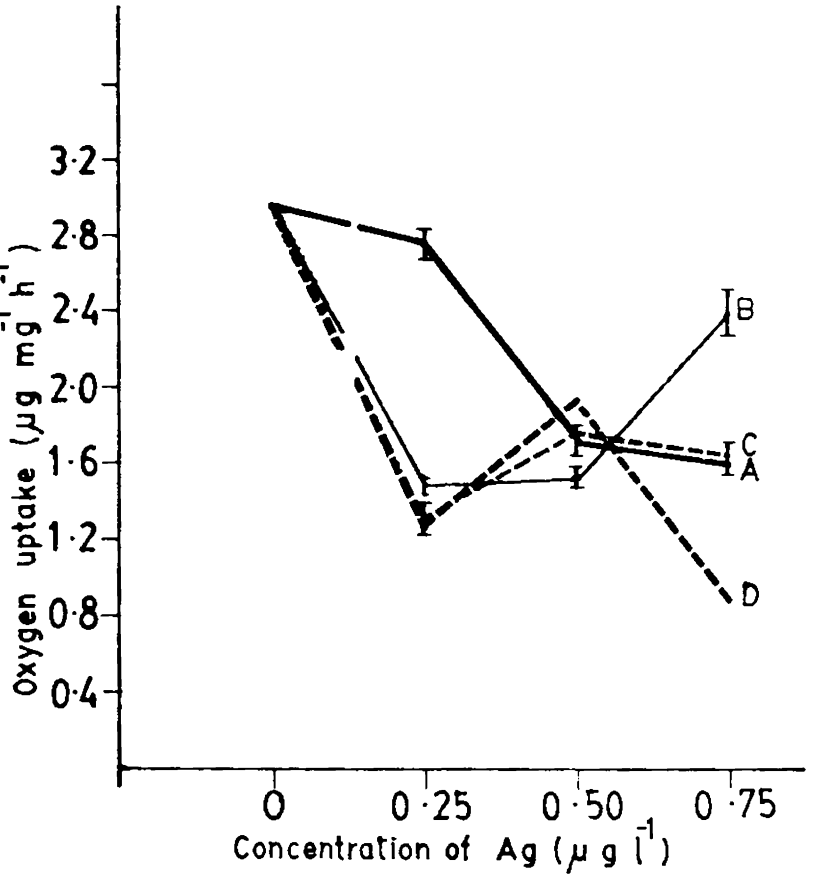

Figure 36. Perna indica. Accumulation: Oxygen consumption when exposed to silver and silver + copper (as sulphates) for 7 days.

A : Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 86. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of silver which caused a 50% reduction in the oxygen consumption upon exposure to silver and three unvarying concentrations of copper + varying concentrations of silver (as sulphates) for a period of 7 days, along with 95% confidence limits and additive indices.

	EC 50 (ppb)	Additive index
Copper	Silver	
	0.69 (0.50 0.97)*	
0.25	0.68 (0.27 1.69)*	- 0.48 (LA)
0.50	0.61 (0.29 1.29)*	0.78 (LA)
0.75	0.26	- 0.73 (LA)

* 95% confidence limits

LA Less than additive

4.2.2.1.3.2. Filtration accompanying accumulation

Experiments to analyse the effects of accumulation of silver in the tissue when supplied individually and in combination with unvarying concentrations of copper were conducted to assess the impact of silver and copper in the tissue on the rate of filtration of Perna indica. The data are presented in Tables 87-91 and Figure 37. The increase in the body burden of silver resulted in the decrease in the rate of filtration. The results obtained were found to be significant (Table 88). Irrespective of reduced body burden by silver in the presence of copper, the rate of filtration was reduced. Those animals which were subjected to an accumulative phase of 7 days in a medium with 0.75 ppb of copper with 0.25, 0.50 or 0.75 ppb of silver reduced filtration considerably. However, in other silver and copper combinations where the silver value was 0.75 ppb and copper 0.25 and 0.50 ppb, the reduction was not that conspicuous. The variations in filtration rates when subjected to analysis of variance (Table 90), showed that the treatment combinations of silver and copper were either highly significant or significant.

4.2.2.1.4. Effects on silver depuration

Perna indica, after exposure to concentrations of silver alone, unvarying concentrations of copper and varying concentrations of silver, the metals supplied in sulphate form for 7 days were subsequently exposed to raw seawater for 7 days. The test individuals were analysed for the residual concentration of silver. The data obtained are presented in Tables 92-94 and Figure 38. The rate of depuration of silver was found to have some relationship with internal concentration. The quantity depurated was more when the tissue burden was more. Further, in the case of animals

Table 87. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to varying concentrations of silver (as Ag_2SO_4) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	31.86	33.94	33.39	34.06	1.85	
0.25	27.73	23.87	22.06	24.55	2.36	72.07
0.50	19.81	20.25	24.17	21.41	1.95	62.85
0.75	14.73	17.66	13.28	15.22	1.82	44.68

Table 88. Perna indica. Accumulation: Analysis of variance for changes in filtration rate by those animals exposed to varying concentrations of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	641.3070	213.7690	10.0011**
Between replicates	2	17.0971	8.5485	0.3999 (NS)
Error	6	128.2470	21.3745	
Total	11	786.6511		

NS - Not significant
** Significant at 1% level

Table 89. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), recorded after maintenance in varying concentrations of silver (as Ag_2SO_4) in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Cu 0.25 + Ag 0.25	28.36	23.16	27.66	26.39	2.30	77.48
+ Ag 0.50	25.73	18.69	16.48	20.30	3.94	59.60
+ Ag 0.75	18.25	14.89	14.97	16.03	1.56	47.06
Cu 0.50 + Ag 0.25	15.89	16.82	14.39	15.70	1.00	46.09
+ Ag 0.50	13.81	15.14	12.64	13.86	1.00	40.69
+ Ag 0.75	12.53	13.41	16.08	14.00	1.50	41.10
Cu 0.75 + Ag 0.25	11.30	9.08	10.11	10.16	0.90	29.82
+ Ag 0.50	7.74	8.16	8.84	8.24	0.45	24.19
+ Ag 0.75	3.81	7.81	5.07	5.56	1.66	16.32

Table 90. Perna indica. Accumulation: Analysis of variance for changes in filtration rate by those animals exposed to varying concentrations of silver (as Ag_2SO_4) and three unvarying concentrations of copper(as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	8.5615	4.2807	0.7982(NS)
2. Between treatment combinations	8	915.9350	4.4918	21.3488**
a. Between copper concentrations	2	751.0730	375.5365	70.0248**
b. Between silver concentrations	2	140.1560	70.0780	13.0671**
c. Interaction	4	60.7056	15.1764	2.8298(NS)
3. Error	16	85.8071	5.3629	
4. Total	26	1010.3036		

NS Not significant
** Significant at 1% level

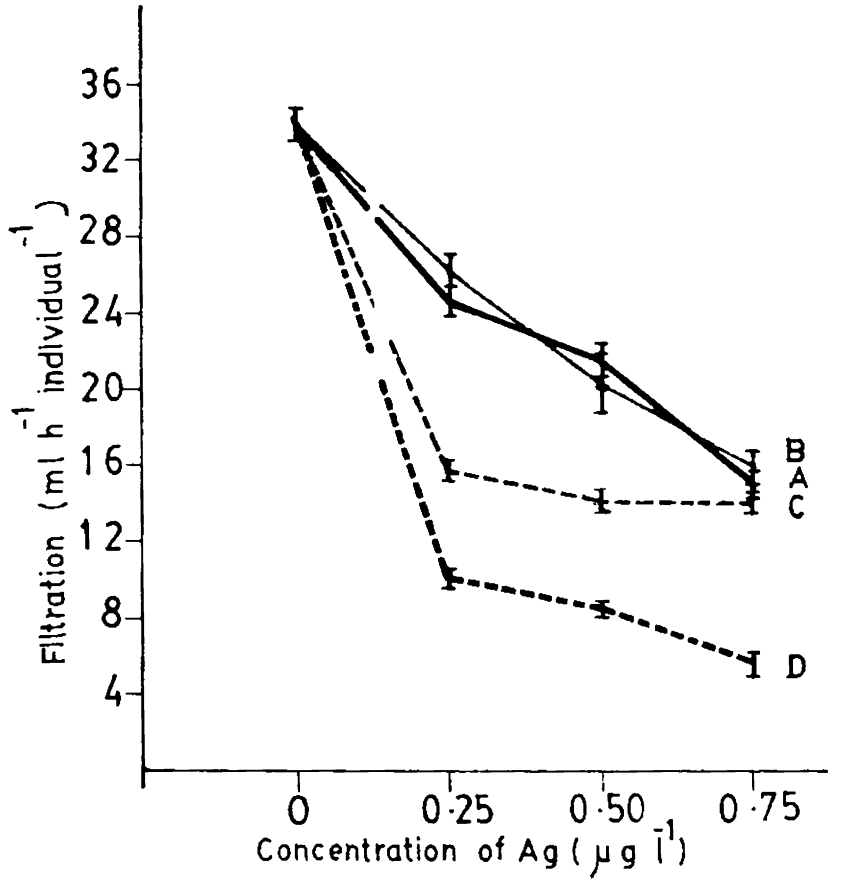

Figure 37. Perna indica. Accumulation: Rate of filtration when exposed to silver and silver + copper (as sulphates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.50 ppb Cu

Table 91. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of silver which caused a 50% reduction in the filtration rate upon exposure to silver and three unvarying concentrations of copper + varying concentrations of silver (as sulphates) for a period of 7 days, along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Copper	+	Silver	
		0.68 (0.47 1.01)*	
0.25		0.68 (0.67 - 0.69)*	- 0.73 (LA)
0.50		0.10 (0.06 - 0.15)*	- 0.61 (LA)
0.75		0.069 (0.02 - 0.21)*	1.30 (LA)

* - 95% confidence limits

LA - Less than additive

which had accumulated silver along with copper, the silver concentration of the tissue was less (Table 80), subsequently the quantity depurated was also less (Table 93 and Figure 38). The quantity depurated varied from 15.98 ppm to 0.75 ppm. The data when subjected to analysis of variance (Table 94) showed that between treatment combinations and between copper concentrations, the depuration rate was highly significant, whereas the variation between silver concentrations was not significant.

4.2.2.1.4.1. Oxygen consumption accompanying depuration

The rate of consumption of oxygen by animals after subjected to a depurative phase of 7 days was assessed. The data is presented in Tables 95-98 and Figure 39. In the case of those animals which received only 0.50 ppb silver in the medium during an accumulative phase, the reduction in oxygen consumption was not concentration dependent. Thus, those animals exposed to 0.25 ppb of silver consumed more oxygen. Slight reduction was noticed only in the case of those animals which had been exposed to 0.75 ppb silver. Those animals exposed to a combination of silver and copper, the oxygen consumption did not show declension and at some instances, the results were erratic. Analysis of the data statistically, showed that the variations observed between treatment combinations, between copper concentrations and between silver concentrations were significant whereas their interactions were not significant (Table 98).

4.2.2.1.4.2. Filtration accompanying depuration

Perna indica after being exposed to raw seawater for 7 days, subsequent to exposure to various concentrations of silver alone and copper and silver in sulphate form were tested for their filtration capacity. The data is presented in Tables 99-102 and Figure 40. Increased body burden

Table 92. Perna indica. Depuration: Concentration of silver (as Ag_2SO_4) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as Ag_2SO_4) individually and in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentrations of metal (ppb)		Replicates				
		1	2	3	Mean	SD
Cu 0.00	+ Ag 0.25	6.46	7.66	8.68	7.60	0.90
	+ Ag 0.50	6.19	8.08	13.60	9.29	3.14
	+ Ag 0.75	21.51	18.95	14.92	18.46	2.71
Cu 0.25	+ Ag 0.25	1.03	4.04	3.83	2.96	1.37
	+ Ag 0.50	14.08	14.83	13.04	13.98	0.73
	+ Ag 0.75	12.90	12.31	11.08	12.09	0.75
Cu 0.50	+ Ag 0.25	6.81	6.55	3.94	5.76	1.29
	+ Ag 0.50	8.29	7.62	4.91	6.94	1.46
	+ Ag 0.75	11.58	10.49	9.83	10.63	0.72
Cu 0.75	+ Ag 0.25	4.04	4.90	3.12	4.02	0.72
	+ Ag 0.50	7.70	7.93	7.98	7.87	0.12
	+ Ag 0.75	7.98	8.35	6.89	7.74	0.61

Table 93. Perna indica. Depuration: Quantity of silver (as Ag_2SO_4) depurated from the whole tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater after being maintained in varying concentrations of silver (as Ag_2SO_4) individually and in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD
Cu 0.00 + Ag 0.25	3.40	0.47	0.88	1.58	1.29
+ Ag 0.50	13.87	17.82	14.20	15.29	1.78
+ Ag 0.75	5.38	11.06	13.97	10.13	3.56
Cu 0.25 + Ag 0.25	1.90	0.77	0.48	1.05	0.61
+ Ag 0.50	2.69	0.84	2.62	2.05	0.85
+ Ag 0.75	5.36	9.38	8.25	7.66	1.69
Cu 0.50 + Ag 0.25	0.86	0.38	1.01	0.75	0.26
+ Ag 0.50	1.51	2.08	3.63	2.40	0.89
+ Ag 0.75	2.64	2.28	1.62	2.18	0.42
Cu 0.75 + Ag 0.25	2.10	0.43	1.08	1.20	0.68
+ Ag 0.50	1.84	1.14	0.32	1.10	0.62
+ Ag 0.75	0.41	2.54	2.30	1.75	0.95

Table 94. Perna indica. Depuration: Analysis of variance for changes in the quantity of silver (as Ag_2SO_4) depurated by those animals pre-exposed to varying concentrations of silver (as Ag_2SO_4) and three unvarying concentrations of copper (as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	3.4436	1.7218	0.5427(NS)
2. Between treatment combinations	11	704.3540	64.0321	20.1860**
a. Between copper concentrations	3	607.3110	202.4370	63.8179**
b. Between silver concentrations	2	27.5384	13.7692	4.3407*
c. Interaction	6	69.5049	11.5841	3.6518*
3. Error	22	69.7883	3.1721	
4. Total	35	777.5859		

NS - Not significant
* - Significant at 5% level
** - Significant at 1% level

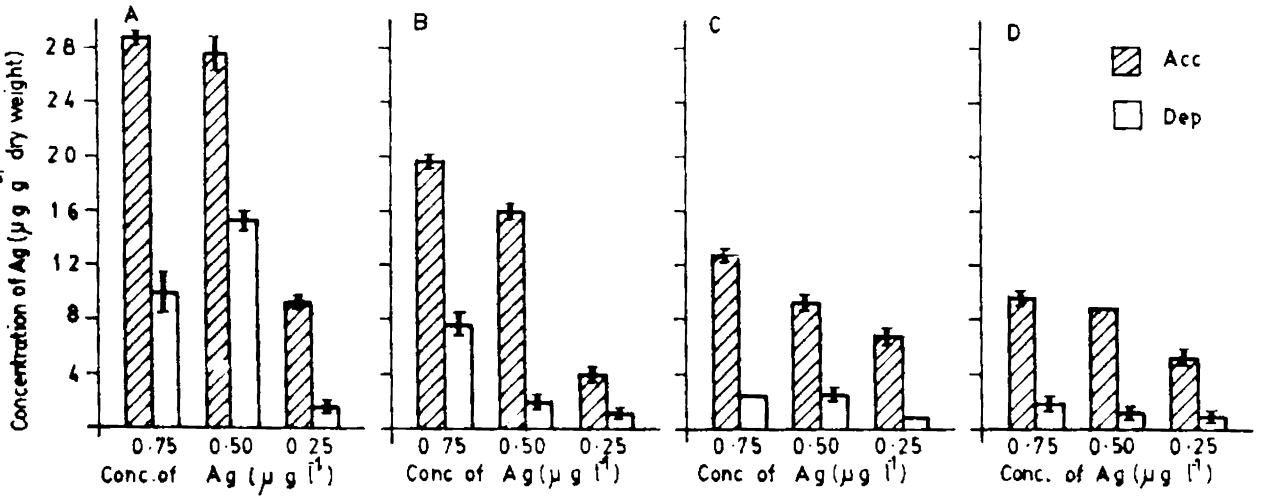

Figure 38. Perna indica. Quantities accumulated in the whole tissue after 7 days and depurated subsequent to exposure to raw seawater for 7 days.

A Ag alone
B Ag + 0.25 ppb Cu
C Ag + 0.50 ppb Cu
D Ag + 0.75 ppb Cu

Table 95. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as Ag_2SO_4) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.89	2.89	3.06	2.94	0.08	
0.25	3.41	3.95	2.79	3.38	0.47	123.21
0.50	4.08	2.63	1.37	2.69	1.10	98.04
0.75	2.81	1.88	1.96	2.21	0.42	80.78

Table 96. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	2.0666	0.6888	1.1076(NS)
Between replicates	2	1.4844	0.7422	1.1934(NS)
Error	6	3.7319	0.6219	
Total	11	7.2829		

NS - Not significant

Table 97. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw sea-water for a period of 7 days, after being maintained in varying concentrations of silver (as Ag_2SO_4) and in combination with three unvarying $CuSO_4$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Cu 0.25 + Ag 0.25	3.88	2.45	2.58	2.97	0.64	108.18
+ Ag 0.50	2.86	1.89	2.09	2.28	0.41	83.08
+ Ag 0.75	1.65	1.42	2.10	1.72	0.28	62.92
Cu 0.50 + Ag 0.25	1.16	3.08	1.42	1.88	0.85	68.79
+ Ag 0.50	1.98	2.16	1.89	2.01	0.11	73.42
+ Ag 0.75	2.00	2.75	1.72	2.15	0.43	78.63
Cu 0.75 + Ag 0.25	2.00	1.72	1.61	1.77	0.16	64.74
+ Ag 0.50	1.87	1.65	2.18	1.90	0.21	69.26
+ Ag 0.75	1.06	0.95	1.01	1.00	0.04	36.84

Table 98. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of silver (as Ag_2SO_4) and three unvarying concentrations of copper (as $CuSO_4$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	0.6282	0.3141	1.2469(NS)
2. Between treatment combinations	8	9.3833	1.1729	4.6562*
a. Between copper concentrations	2	3.1910	1.5955	6.3338*
b. Between silver concentrations	2	5.0741	2.5370	10.0716**
c. Interaction	4	1.1181	0.2795	1.1095(NS)
3. Error	16	4.0316	0.2519	
4. Total	26	14.0431		

NS - Not significant
* Significant at 5% level
** Significant at 1% level

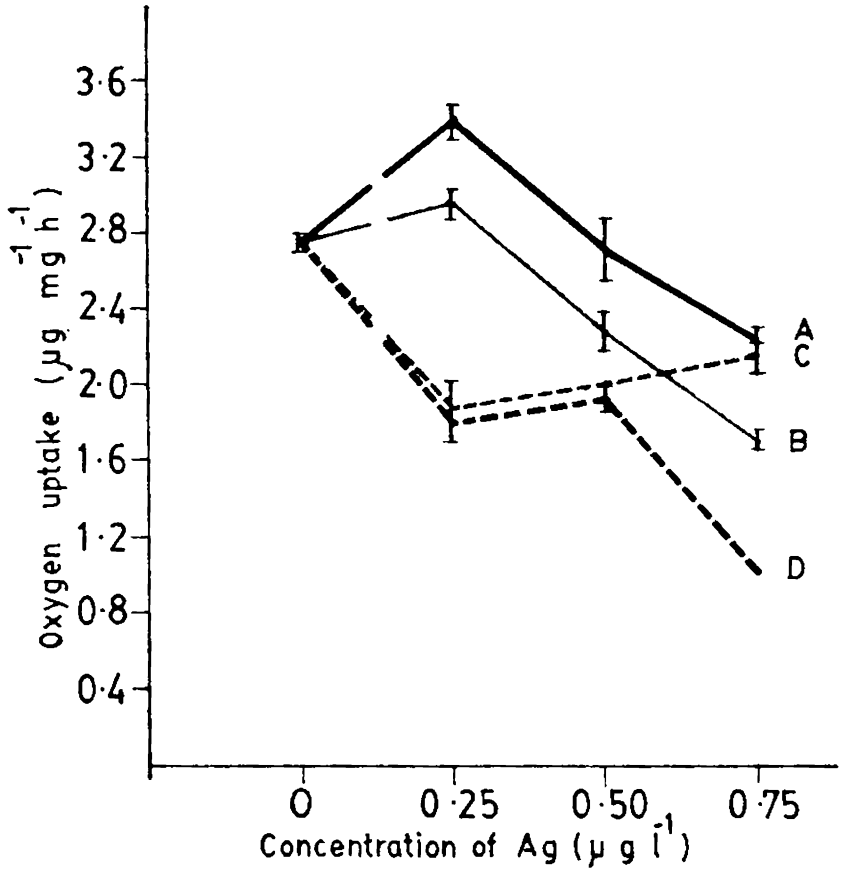

Figure 39. Perna indica. Depuration: Oxygen consumption when the animals were exposed to raw sea-water for 7 days, after being maintained in silver and silver + copper (as sulphates) for seven days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 99. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}) when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as Ag_2SO_4) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	30.38	36.36	33.42	33.38	2.44	
0.25	28.77	24.93	23.68	25.79	2.16	77.26
0.50	27.11	16.49	25.59	23.06	4.68	69.08
0.75	13.47	24.08	19.05	18.86	4.33	56.50

Table 100. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of silver (as Ag_2SO_4).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	336.0810	112.0270	4.3777(NS)
Between replicates	2	0.7158	0.3579	0.0139(NS)
Error	6	153.5400	25.5900	
Total	11	490.3368		

NS Not significant

Table 101. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as Ag_2SO_4) in combination with three unvarying $CuSO_4$ overloads for a period of 7 days along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentration of metal (ppb)			Replicates			Mean	SD	% of control
			1	2	3			
Cu	0.25	+ Ag 0.25	32.18	26.98	26.97	28.71	2.45	86.00
		+ Ag 0.50	26.15	23.46	20.83	23.48	2.17	70.34
		+ Ag 0.75	20.58	16.32	24.97	20.62	3.53	61.77
Cu	0.50	+ Ag 0.25	18.34	17.63	18.20	18.05	0.30	54.07
		+ Ag 0.50	14.71	16.23	14.71	15.22	0.70	45.59
		+ Ag 0.75	16.87	16.26	15.61	16.24	0.51	48.65
Cu	0.75	+ Ag 0.25	14.71	11.90	12.83	13.14	1.16	39.36
		+ Ag 0.50	10.08	8.75	8.90	9.24	0.59	27.68
		+ Ag 0.75	9.70	6.85	9.09	8.54	1.22	25.58

Table 102. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of silver (as Ag_2SO_4) and three unvarying concentrations of copper (as $CuSO_4$)

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	20.8442	10.4221	2.7407(NS)
2. Between treatment combinations	8	1031.4600	128.9325	33.9055**
a. Between copper concentrations	2	880.7390	440.3695	115.8044**
b. Between silver concentrations	2	120.3880	60.1940	15.8292**
c. Interaction	4	30.3311	7.5827	1.9940(NS)
3. Error	16	60.8442	3.8027	
4. Total	26	1113.1484		

NS Not significant
** - Significant at 1% level

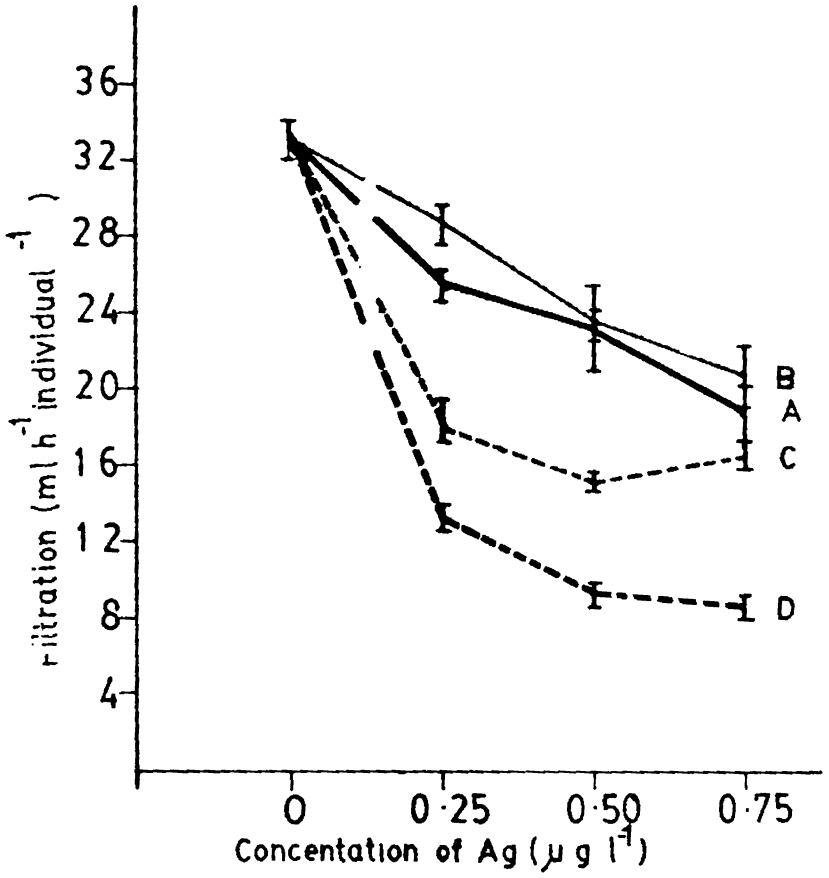

Figure 40. Perna indica. Depuration: Rate of filtration when the animals were exposed to raw seawater for 7 days, after being maintained in silver and silver + copper (as sulphates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

because of silver alone, resulted in reduction in filtration rate (Table 99). However, reduction in filtration rate was found to be affected by factors other than body burden also. Analysis of the data statistically, showed that the variations observed between treatment combinations, between copper concentrations and between silver concentrations were highly significant whereas their interactions were not significant (Table 102).

4.2.2.2. NITRATE FORMS OF COPPER AND SILVER

Experiments, to find out, the rate of uptake of copper individually and in combination with silver when the experimental medium contained these metals in nitrate form, were conducted employing Perna indica as test organism. The main objective behind this series of experiments was to delineate the rate of uptake, rate of depuration and effects of these on the pattern of oxygen consumption and filtration. Here, the animals employed for oxygen consumption and filtration studies belong to two categories, one set that had body burden of heavy metals, and the other given opportunity to depurate and hence with reduced heavy metal load. The results obtained are categorised and given in Tables and Figures.

4.2.2.2.1. Effects on copper accumulation

$Cu(NO_3)_2$ of concentrations containing 0.25, 0.50 and 0.75 ppb of copper were prepared and Perna indica was exposed to these solutions for 7 days. The rate of uptake of copper was assessed. It was found that the quantity incorporated into the tissues increased as a function of external concentrations. When Perna indica, was subjected to an accumulative phase for 7 days in the test medium containing three unvarying concentrations of silver and varying concentration of copper, a totally different picture was obtained. When the medium contained more of silver,

Table 103. Perna indica. Accumulation: Concentration of copper (as $Cu(NO_3)_2$) in the whole tissue ($\mu g\ g^{-1}$ dry weight), when exposed to varying concentrations of copper (as $Cu(NO_3)_2$ individually and in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation.

Concentration of metal (ppb)		Replic ates 1	2	3	Mean	SD
Ag 0.00	+ Cu 0.25	16.75	14.67	13.97	15.13	1.18
	+ Cu 0.50	22.48	25.67	23.07	23.74	1.38
	+ Cu 0.75	27.80	28.81	26.68	27.76	0.87
Ag 0.25	+ Cu 0.25	18.31	16.54	17.25	17.36	0.72
	+ Cu 0.50	19.23	20.72	18.71	19.55	0.85
	+ Cu 0.75	22.87	21.39	20.43	21.56	1.00
Ag 0.50	+ Cu 0.25	11.23	9.48	8.31	9.67	1.19
	+ Cu 0.50	13.88	13.99	14.93	14.26	0.47
	+ Cu 0.75	14.17	15.78	15.17	15.04	0.66
Ag 0.75	+ Cu 0.25	9.72	10.45	10.86	10.34	0.47
	+ Cu 0.50	11.22	11.52	11.68	11.47	0.19
	+ Cu 0.75	13.75	12.03	12.09	12.62	0.79

Table 104. Perna indica. Accumulation: Analysis of variance for changes in copper (as $Cu(NO_3)_2$) concentrations in the whole tissue when exposed to varying concentrations of copper and three unvarying concentrations of silver ($AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	3.6230	1.8115	1.6343(NS)
2. Between treatment combinations	11	1046.0175	95.0925	85.7925**
a. Between silver concentrations	3	711.5280	237.1760	213.9805**
b. Between copper concentrations	2	233.7000	116.8500	105.4222**
c. Interaction	6	100.7880	16.7980	15.1551**
3. Error	22	24.3848	1.1084	
4. Total	35	1704.0253		

NS Not significant
** Significant at 1% level

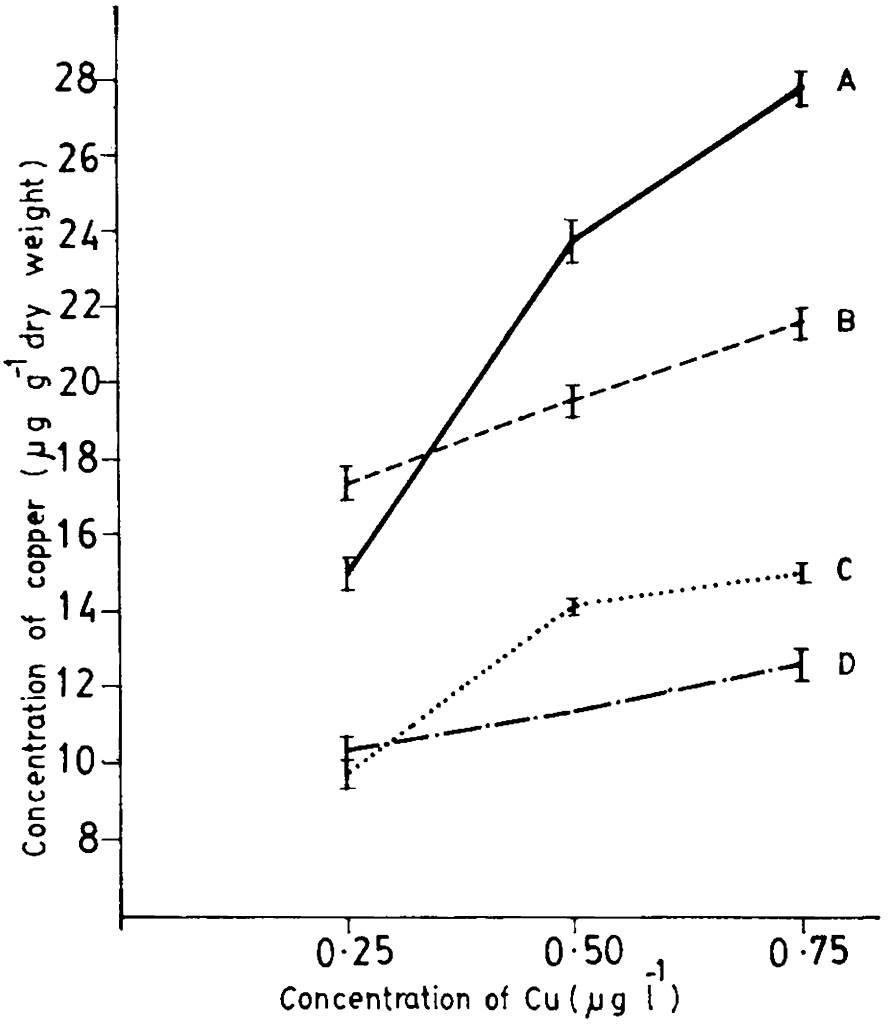

Figure 41. Perna indica. Accumulation: Concentration of copper (as $Cu(NO_3)_2$) in the whole tissue when the animals were exposed to copper and copper + silver (as sulphates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

the quantity of copper accumulated was low (Table 103 and Figure 41). The data was subjected to analysis of variance (Table 104). These are the observations based on Tukey's test. Copper accumulation, in combinations 0.50 ppb copper and 0.50 ppb silver was significantly different from those exposed to individual concentrations of copper and other combinations (ie. 0.25 ppb Cu + 0.25 to 0.75 ppb Ag, 0.50 ppb Cu + 0.25 and 0.75 ppb Ag and 0.75 ppb Cu + 0.25 to 0.75 ppb Ag). The rate of uptake of copper when present alone at a concentration of 0.25 ppb, was comparable with those animals exposed to a combination of 0.50 ppb of copper and silver and 0.75 ppb of copper and silver. All the silver concentrations reacted in different manner from each other to affect copper uptake (Table 104).

4.2.2.2.1.1. Oxygen consumption accompanying accumulation

The rate of uptake of oxygen by Perna indica, exposed to various concentrations of copper alone and unvarying concentrations of silver and varying concentrations of copper, supplied to the medium in the form of nitrate, was assessed. The results are presented in Tables 105-108 and Figure 42. When present alone, the declension in oxygen consumption was controlled by increase in concentration of copper (Table 105 and Figure 42). Similarly, in those combinations of silver and copper, the rate of oxygen consumption came down when the animals were exposed either to a higher unvarying silver concentration along with higher concentration of varying copper or the highest concentration of copper with 0.25 and 0.50 ppb of silver (Table 107). The results also show that the internal burden of copper had some influence on the rate of oxygen uptake. When

Table 105. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were maintained in varying concentrations of copper (as $Cu(NO_3)_2$) for a period of 7 days, along with standard deviation and percentage variation from the control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.83	2.98	3.05	2.95	0.09	
0.25	2.55	3.10	1.98	2.54	0.45	86.13
0.50	1.98	2.48	1.81	2.09	0.28	70.74
0.75	2.05	1.85	1.55	1.81	0.20	61.56

Table 106. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption when exposed to varying concentrations of copper (as $Cu(NO_3)_2$) for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	3.6243	1.2081	9.6186*
Between replicates	2	0.8358	0.4179	3.3272(NS)
Error	6	0.7536	0.1256	
Total	11	5.2137		

NS - Not significant
** Significant at 5% level

Table 107. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), recorded after maintenance in varying concentrations of copper (as $Cu(NO_3)_2$) in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation and percentage variation from the control (100%) readings.

Concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	3.39	1.78	1.25	2.14	0.91	72.48
+ Cu 0.50	1.93	2.68	2.41	2.34	0.31	79.26
+ Cu 0.75	1.27	2.99	1.08	1.78	0.85	60.20
Ag 0.50 + Cu 0.25	2.16	2.00	1.88	2.01	0.11	68.17
+ Cu 0.50	2.81	1.15	2.58	2.18	0.73	73.80
+ Cu 0.75	0.60	2.45	1.85	1.63	0.77	54.85
Ag 0.75 + Cu 0.25	1.92	0.98	2.32	1.74	0.56	59.02
+ Cu 0.50	1.30	1.29	2.58	1.72	0.60	58.35
+ Cu 0.75	1.63	0.98	1.09	1.23	0.28	41.87

Table 108. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption when exposed to varying concentrations of copper (as $Cu(NO_3)_2$) and three unvarying concentrations of silver (as $AgNO_3$) for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	1.9632	0.9816	1.9624(NS)
2. Between treatment combinations	8	2.8088	0.3511	0.7019(NS)
a. Between silver concentrations	2	0.6631	0.3315	0.6628(NS)
b. Between copper concentrations	2	1.6389	0.8194	1.6382(NS)
c. Interaction	4	0.5068	0.1267	0.2532(NS)
3. Error	16	8.0038	0.5002	
4. Total	26	12.7758		

NS - Not significant

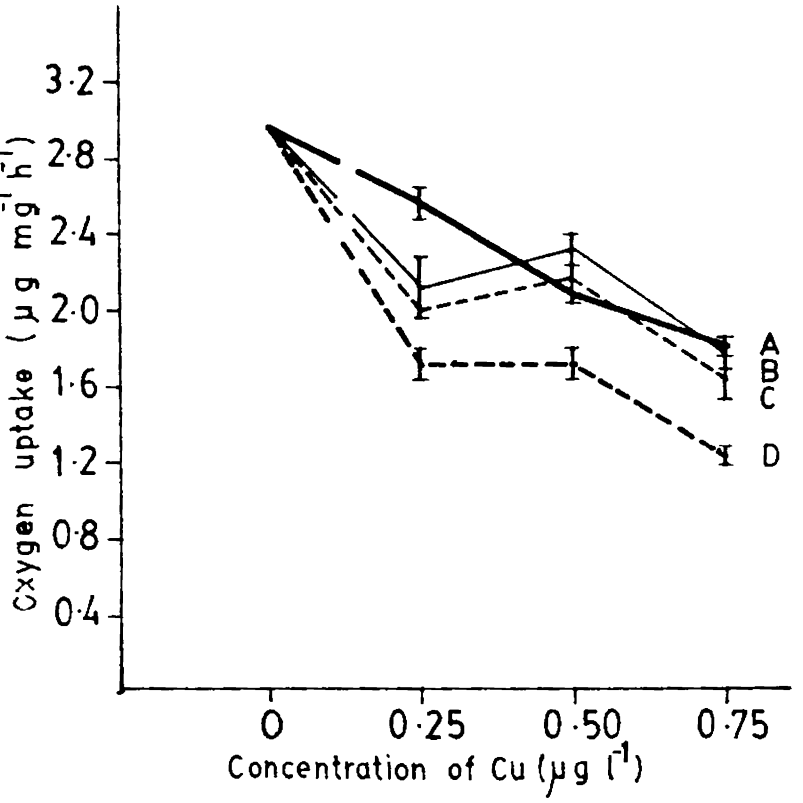

Figure 42. Perna indica. Accumulation: Oxygen consumption when the animals were exposed to copper and copper + silver (as nitrates) for 7 days.

A	Cu alone	B	Cu + 0.25 ppb Ag
C	Cu + 0.50 ppb Ag	D	Cu + 0.75 ppb Ag

Table 109. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of copper which caused a 50% reduction in the oxygen consumption upon exposure to copper and three unvarying concentrations of silver + varying concentrations of copper (as nitrates) for a period of 7 days, along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Silver	+	Copper	
		1.09 (0.64 1.87)*	
0.25		0.94	0.18 (LA)
0.50		0.83	0.79 (LA)
0.75		0.56 (0.42 - 0.78)*	0.47 (LA)

* 95% confidence limits

LA Less than additive

the data was subjected to analysis of variance (Table 108), the variations noticed were found to be not significant. The EC 50 values indicate that in the metal-mixture medium, increased concentrations of silver brought down the EC 50 of copper (Table 109).

4.2.2.2.1.2. Filtration accompanying accumulation

Similar to the experiments conducted to assess the oxygen consumption, a series of tests were performed to assess filtration rate also. The data is presented in Tables 110-113 and Figure 43. The rate of filtration was found to be drastically affected by the presence of $Cu(NO_3)_2$. However, the variations between the highest and lowest concentrations of copper was not significant (Table 111). The presence of silver, however, modified the pattern (Table 112 and Figure 43). In those concentrations which contained 0.25, 0.50 and 0.75 ppb silver with 0.25 ppb of copper, the rate of filtration was higher, while when the copper concentrations increased to 0.50 and 0.75 ppb the rate of filtration came down. This resulted in lower EC 50 values (Table 114). Notwithstanding this, combined action of these two metals in bringing down filtration, was found to be less than additive (Table 114).

4.2.2.2.2. Effects on copper depuration

Tests, to find out the rate at which copper was removed from the whole tissue of Perna indica, after being exposed to copper individually and in combination with silver, were conducted. The results are presented in Tables 115-117 and Figure 44. A conspicuous feature of the results was increased rate of depuration of copper, by those animals which were exposed to unvarying concentrations of silver (0.25 and 0.50 ppb) and varying concentrations of copper. When the internal load was examined, it

Table 110. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to varying concentrations of copper (as $Cu(NO_3)_2$ for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	31.86	33.94	36.39	34.06	1.85	
0.25	24.08	13.48	18.61	18.72	4.32	54.96
0.50	25.04	16.81	15.14	18.99	4.32	55.75
0.75	18.01	14.13	11.49	14.54	2.67	42.68

Table 111. Perna indica. Accumulation: Analysis of variance for changes in filtration rate when exposed to varying concentrations of copper (as $Cu(NO_3)_2$ for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	541.7130	180.5710	13.3339**
Between replicates	2	62.8834	31.4417	2.3217(NS)
Error	6	81.2532	13.5422	
Total	11	685.8496		

** - Significant at 1% level

NS - Not significant

Table 112. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}) when the animals were exposed to varying concentrations of copper (as $Cu(NO_3)_2$ in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation and percentage variation from the control (100%) readings.

Concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Ag 0.25 + Cu 0.25	29.73	24.59	18.74	24.35	4.48	71.49
+ Cu 0.50	19.64	19.65	15.56	18.28	1.92	53.66
+ Cu 0.75	16.75	13.12	10.19	13.35	2.68	39.19
Ag 0.50 + Cu 0.25	25.68	27.43	22.89	25.33	1.86	74.36
+ Cu 0.50	17.40	13.26	15.72	15.36	1.70	45.09
+ Cu 0.75	7.81	9.48	12.42	9.90	1.90	29.06
Ag 0.75 + Cu 0.25	20.37	22.78	17.66	20.81	2.09	61.09
+ Cu 0.50	12.98	9.28	11.63	11.29	1.52	33.14
+ Cu 0.75	4.46	5.84	3.97	4.75	0.79	13.94

Table 113. Perna indica. Accumulation: Analysis of variance for changes in filtration rate when exposed to varying concentrations of copper (as $Cu(NO_3)_2$ and three unvarying concentrations of $AgNO_3$ for a period of 7 days.

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	45.6919	22.8459	2.6812(NS)
2. Between treatment combinations	8	851.8040	106.4755	12.4962**
a. Between silver concentrations	2	635.9400	317.9700	37.3177**
b. Between copper concentrations	2	179.2890	89.6445	10.5209**
c. Interaction	4	36.5747	9.1436	1.0731(NS)
3. Error	16	136.3310	8.5206	
4. Total	26	1033.8269		

NS - Not significant
** Significant at 1% level

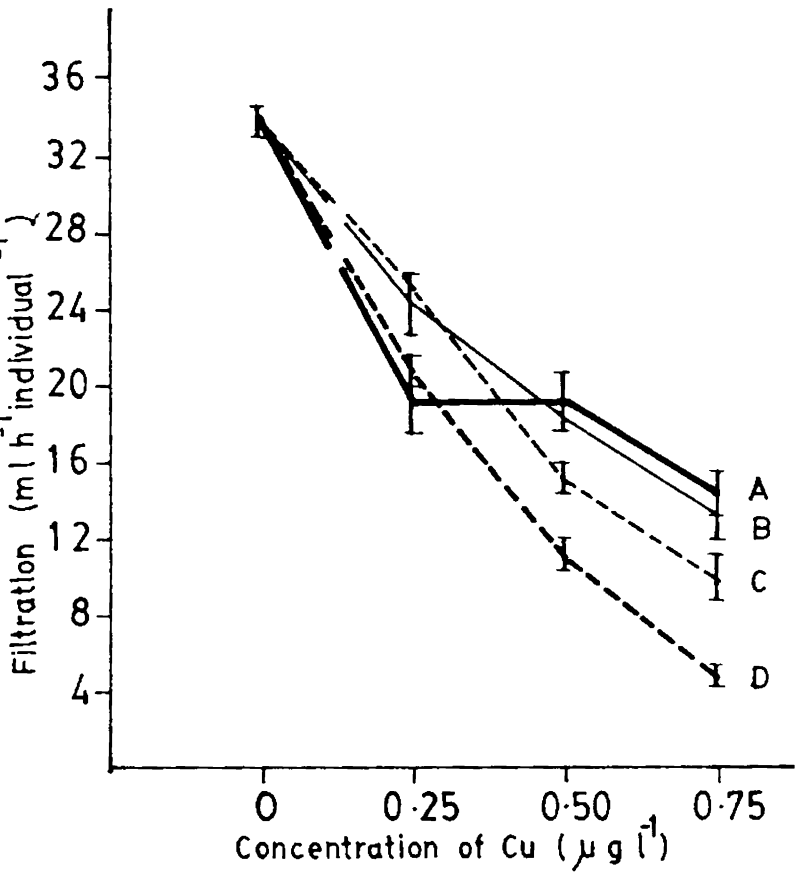

Figure 43. Perna indica. Accumulation Rate of filtration when the animals were exposed to copper and copper + silver (as nitrates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

Table 114. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of copper which caused a 50% reduction in the filtration rate upon exposure to copper and three unvarying concentrations of silver + varying concentrations of copper (as nitrates) for a period of 7 days, along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Silver	+	Copper	
		0.50 (0.23 - 1.12)*	
0.25		0.53 (0.50 0.57)*	- 0.33 (LA)
0.50		0.45 (0.45 0.45)*	0.44 (LA)
0.75		0.32 (0.28 - 0.36)*	0.46 (LA)

* 95% confidence limit

LA Less than additive

was noticed that irrespective of the internal load there was uniformity in depuration rate. However, at a few instances results were erratic, which do not comply with this generalisation. For instance, those animals exposed to 0.25 ppb silver and 0.75 ppb copper removed only 0.86 ppm of copper in 7 days while the internal load was 18.17 ppm (Table 115). On the other hand, in another instance when the body load was 6.53 ppm (when the animals exposed to 0.25 ppb copper and 0.75 ppb silver) the quantity removed was 3.81 ppm. When the data was subjected to analysis of variance (Table 117) none of the results were found to be significant.

4.2.2.2.2.1. Oxygen consumption accompanying depuration

A series of experiments were conducted to find out the rate of oxygen consumption by those animals exposed to unvarying concentrations of silver with varying concentrations of copper for 7 days and subjected to depuration for 7 days. As mentioned earlier, the reason for this experiment was to assess the capacity of the animals to regain normal function after depuration for 7 days. The results are presented in Tables 118-121 and Figure 45. Apparently, those animals which depurated copper when encountered singly regained capacity to consume oxygen normally after 7 days when they were earlier exposed to 0.25 and 0.50 ppb of copper. In the presence of silver, the results obtained on oxygen consumption after depuration were erratic by almost all the animals. None of the results obtained was found to be significant (Tables119 and 121). This fact can be easily seen in Tables 120 and 121. In general, it seems that the animals regain its capacity to respire normal although they encountered combined effects of silver and copper in varying concentrations.

Table 115. Perna indica. Depuration: Concentration of copper (as $Cu(NO_3)_2$) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying sublethal concentration of copper(as $Cu(NO_3)_2$) individually and in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentration of metal (ppb)		Replicates				
		1	2	3	Mean	SD
Ag 0.00	+ Cu 0.25	13.43	12.72	11.31	12.48	1.07
	+ Cu 0.50	18.66	19.53	18.40	18.86	0.59
	+ Cu 0.75	22.59	23.58	21.76	22.64	0.91
Ag 0.25	+ Cu 0.25	14.82	12.23	12.31	13.12	1.47
	+ Cu 0.50	15.22	14.12	14.03	14.45	0.66
	+ Cu 0.75	18.28	18.78	17.46	18.17	0.66
Ag 0.50	+ Cu 0.25	5.69	7.56	6.55	6.60	0.93
	+ Cu 0.50	11.61	12.08	10.09	11.26	0.84
	+ Cu 0.75	11.36	11.24	10.51	11.03	0.46
Ag 0.75	+ Cu 0.25	5.36	7.95	6.29	6.53	1.31
	+ Cu 0.50	7.16	8.26	8.93	8.11	0.89
	+ Cu 0.75	9.04	9.49	9.31	9.28	0.18

Table 116. Perna indica. Depuration: Quantity of copper depurated(as $Cu(NO_3)_2$) from the whole tissue (µg g^{-1} dry weight), when the animals were exposed to raw seawater after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$) individually and in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentration of metal (ppb)		Replicates				
		1	2	3	Mean	SD
Ag 0.00	+ Cu 0.25	3.32	1.95	2.66	2.64	0.55
	+ Cu 0.50	3.82	6.14	4.06	4.67	1.04
	+ Cu 0.75	5.21	5.23	4.92	5.12	0.14
Ag 0.25	+ Cu 0.25	3.49	4.31	4.94	4.24	0.59
	+ Cu 0.50	4.01	6.60	4.68	5.09	1.09
	+ Cu 0.75	2.61	2.97	3.39	0.86	
Ag 0.50	+ Cu 0.25	5.54	1.92	1.76	3.07	1.74
	+ Cu 0.50	2.27	1.91	4.84	3.00	1.30
	+ Cu 0.75	2.81	4.54	4.66	4.00	0.84
Ag 0.75	+ Cu 0.25	4.36	2.50	4.57	3.81	0.93
	+ Cu 0.50	4.06	3.26	2.75	3.35	0.53
	+ Cu 0.75	4.71	2.54	2.78	3.34	0.97

Table 117. Perna indica. Depuration: Analysis of variance for changes in the quantity of copper depurated (as $Cu(NO_3)_2$) when the animals were exposed to raw seawater after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$) and three unvarying concentrations of silver (as $AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	0.9289	0.4644	0.3104(NS)
2. Between treatment combinations	11	22.4959	2.0450	1.3670(NS)
a. Between silver concentrations	3	5.3508	1.7836	1.1922(NS)
b. Between copper concentrations	2	2.4997	1.2498	0.8354(NS)
c. Interaction	6	14.6453	2.4408	1.6316(NS)
3. Error	22	32.9133	1.4960	
4. Total	35	56.3381		

NS - Not significant

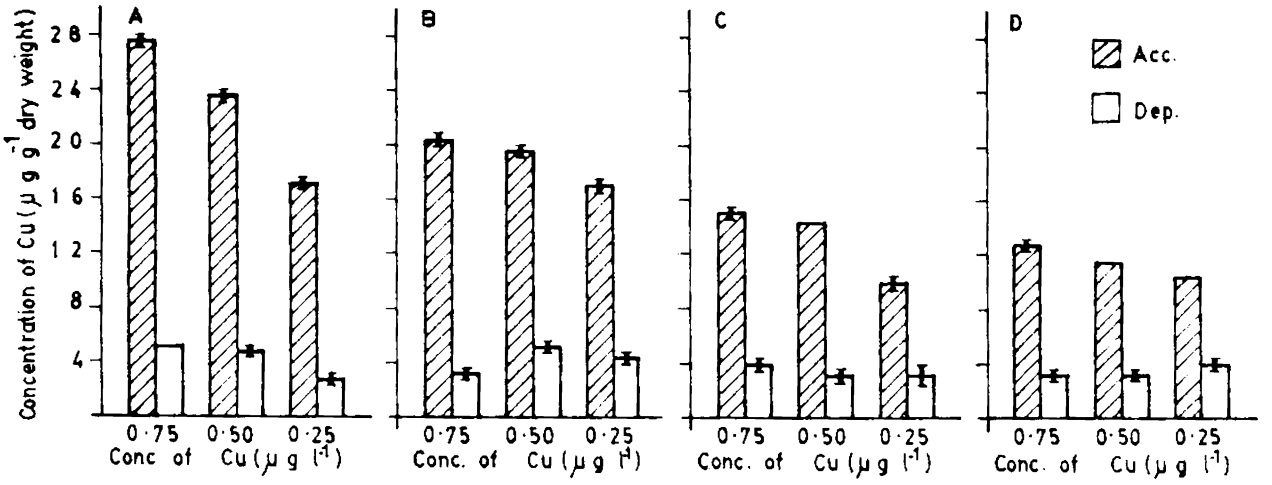

Figure 44. Perna indica. Quantities accumulated in the whole tissue after 7 days and depurated subsequent to exposure to raw seawater for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

Table 118. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.28	2.89	3.06	2.74	0.33	
0.25	3.66	2.58	1.98	2.74	0.69	100.00
0.50	2.80	1.98	3.40	2.72	0.58	99.63
0.75	1.98	1.93	0.79	1.56	0.54	57.15

Table 119. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	3.0839	1.0279	1.8274(NS)
Between replicates	2	0.3338	0.1669	0.2967(NS)
Error	6	3.3753	0.5625	
Total	11	6.7930		

NS Not significant

Table 120. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight) when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$) in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations of metal (ppb)		Replicates			Mean	SD	% of control
		1	2	3			
Ag 0.25	+ Cu 0.25	2.20	3.81	2.01	2.67	0.80	97.45
	+ Cu 0.50	1.63	2.68	3.69	2.66	0.84	97.27
	+ Cu 0.75	0.96	2.98	2.88	2.27	0.92	82.84
Ag 0.50	+ Cu 0.25	2.16	3.38	2.61	2.71	0.50	98.94
	+ Cu 0.50	4.04	1.15	3.17	2.78	1.21	101.46
	+ Cu 0.75	0.53	2.18	1.90	1.53	0.72	56.11
Ag 0.75	+ Cu 0.25	1.73	1.38	1.98	1.69	0.24	61.87
	+ Cu 0.50	2.26	3.81	1.81	2.62	0.85	95.67
	+ Cu 0.75	1.73	1.30	1.91	1.64	0.25	60.05

Table 121. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of copper (as $Cu(NO_3)_2$) and three unvarying concentrations of silver ($AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	1.3185	0.6592	0.9387(NS)
2. Between treatment combinations	8	8.7090	1.0886	1.5500(NS)
a. Between silver concentrations	2	6.2064	3.1032	4.4186**
b. Between copper concentrations	2	0.7916	0.3958	0.5635(NS)
c. Interaction	4	1.7109	0.4277	0.6090(NS)
3. Error	16	11.2383		
4. Total	26	21.2658		

NS Not significant
** Significant at 1% level

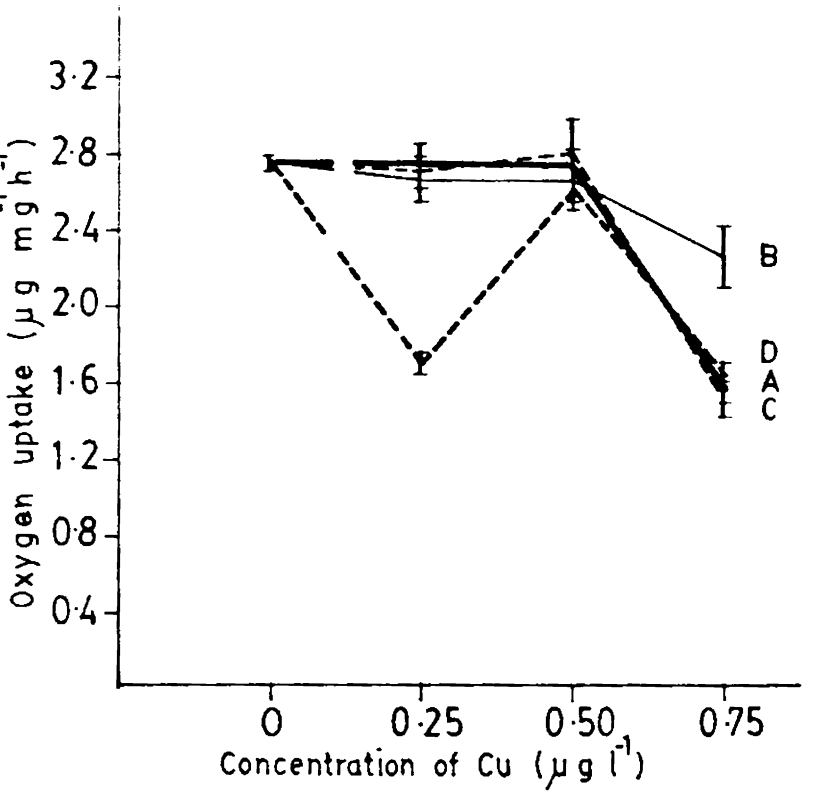

Figure 45. Perna indica. Depuration Oxygen consumption when the animals were exposed to raw seawater for 7 days, after being maintained in copper and copper + silver (as nitrates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

4.2.2.2.2.2. Filtration accompanying depuration

The results obtained on the rate of filtration by Perna indica, after allowing a depurative phase of 7 days gave a totally different picture (Tables 122-125 and Figure 46) from that of oxygen consumption. Those animals exposed to copper alone before depuration, regained normalcy only slowly. Even after 7 days of cleaning, those exposed to 0.75 ppb of copper filtered only 58.68% of that of control. Those test individuals, exposed to higher concentrations of silver and copper earlier did not regain normalcy even after 7 days of depuration. A clear indication of this was shown by those animals exposed to 0.75 ppb of silver with 0.75 ppb of copper, both in nitrate form. The pace with which normalcy regained was directly proportional to the external concentrations of silver and copper the animals encountered during their accumulative phase. Therefore, analysis of variance showed that the noted variations between treatment combinations, between silver concentrations and between copper concentrations were highly significant (Table 125).

4.2.2.2.3. Effects on silver accumulation

As in the case of the previous set of experiments, another set of experiments were conducted to delineate the rate of uptake of silver by Perna indica, when the metal was supplied along with an unvarying concentration of copper. After accumulation, the animals were used to assess the capacity to take oxygen and to filter water. Representatives of Perna indica, which have accumulated silver in their tissue were exposed to raw seawater and the rate of depuration after 7 days was assessed. Further, to find out how far the animals have regained normalcy, the respiration rate and filtration capacity of the animals was also assessed. The results

Table 122. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$ for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	30.38	36.36	33.42	33.38	2.44	
0.25	28.28	30.69	22.30	27.09	3.52	81.15
0.50	23.98	18.31	29.81	24.03	4.69	71.98
0.75	19.02	22.91	16.85	19.59	2.50	58.68

Table 123. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	301.9860	100.6620	4.5205(NS)
Between replicates	2	6.5815	3.2907	0.1477(NS)
Error	6	133.6068	22.2678	
Total	11	442.1743		

NS - Not significant

Table 124. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}) when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of copper (as $Cu(NO_3)_2$) in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations of metal (ppb)		Replicates			Mean	SD	% of control
		1	2	3			
Ag 0.25	+ Cu 0.25	30.88	24.09	16,48	23.81	5.88	71.33
	+ Cu 0.50	20.87	18.18	17.91	18.98	1.34	56.86
	+ Cu 0.75	17.73	14.11	9.00	13.61	3.58	40.77
Ag 0.50	+ Cu 0.25	24.73	23.34	26.98	25.01	1.49	74.92
	+ Cu 0.50	16.56	17.73	14.27	16.18	1.43	48.47
	+ Cu 0.75	8.91	10.30	12.88	10.69	1.64	32.02
Ag 0.75	+ Cu 0.25	21.49	20.13	16.53	19.38	2.09	58.05
	+ Cu 0.50	12.47	10.37	10.74	11.19	0.91	33.52
	+ Cu 0.75	6.68	3.81	6.69	5.06	1.20	15.15

Table 125. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of copper (as $Cu(NO_3)_2$) and three unvarying concentrations of silver (as $AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	44.6543	22.3271	2.2073(NS)
2. Between treatment combinations	8	818.5640	102.3205	10.1156**
a. Between silver concentrations	2	621.8990	310.9495	30.7411**
b. Between copper concentrations	2	191.5810	95.7905	9.4700**
c. Interaction	4	5.0839	1.2709	0.1256(NS)
3. Error	16	161.8430	10.1151	
4. Total	26	1025.0613		

NS Not significant
** - Significant at 1% level

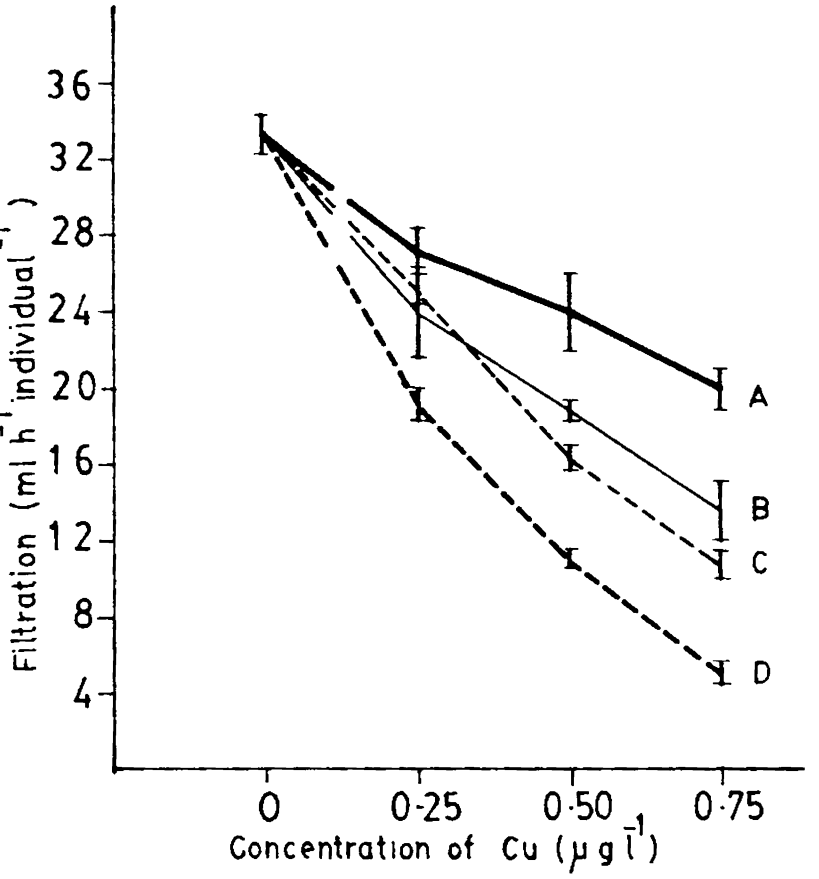

Figure 46. Perna indica. Depuration: Rate of filtration when the animals were exposed to raw seawater for 7 days, after being maintained in copper and copper + silver (as nitrates) for 7 days.

A Cu alone B Cu + 0.25 ppb Ag

C Cu + 0.50 ppb Ag D Cu + 0.75 ppb Ag

are presented in Tables 126-148 and Figures 47-53.

Perna indica, accumulated different quantities of silver, when they were exposed to varying concentrations of silver and unvarying concentrations of copper for a period of 7 days. The data is presented in Tables 126-127 and Figure 47. When silver was present alone, the rate of accumulation was external concentration dependent. On the other hand, 0.25 ppb of copper uptake of silver when the experimental concentration was 0.25 silver. In the case of those animals which were exposed to 0.50 ppb copper and 0.25 and 0.50 ppb silver, the rate of uptake was uniformly low. Elevation of copper concentration to 0.75 ppb only with 0.25 and 0.50 ppb of silver also resulted in reduced uptake of silver. The data was subjected to analysis of variance (Table 127). It was found that the results obtained between treatment combinations, between copper concentrations, between silver concentrations and their interactions were highly significant, showing that the presence of copper significantly affects silver accumulation and different concentrations also affects accumulation.

4.2.2.2.3.1. Oxygen consumption accompanying accumulation

The data on oxygen consumption of Perna indica, after being exposed to varying concentrations of silver for a period of 7 days is presented in Tables 128-129 and Figure 48. In the lowest concentration of silver, the animals respired at a level very similar to that of controls. Only those animals which were maintained with the medium containing 0.75 ppb of silver, the rate of oxygen consumption was low. The results obtained were not significant (Table 129). The pattern of oxygen consumption obtained from those animals which were maintained in a combination of

Table 126. Perna indica. Accumulation: Concentration of silver (as $AgNO_3$) in the whole tissue (µg g^{-1} dry weight), when the animals were exposed to varying concentrations of silver (as $AgNO_3$) individually and in combination with three unvarying $AgNO_3$ overloads for a period of 7 days, along with standard deviation.

Concentration of metal (ppb)			Replicates 1	2	3	Mean	SD
Cu 0.00	+	Ag 0.25	12.58	14.69	12.50	13.25	1.01
	+	Ag 0.50	16.12	18.12	19.63	17.95	1.43
	+	Ag 0.25	26.41	29.06	28.57	28.01	1.15
Cu 0.25	+	Ag 0.25	7.15	8.31	6.97	7.47	0.59
	+	Ag 0.50	11.44	10.77	10.63	10.94	0.35
	+	Ag 0.75	27.13	27.47	26.57	27.05	0.37
Cu 0.50	+	Ag 0.25	4.33	3.74	6.97	5.01	1.40
	+	Ag 0.50	6.06	6.79	6.94	6.59	0.38
	+	Ag 0.75	8.39	8.21	8.61	8.40	0.16
Cu 0.75	+	Ag 0.25	2.28	2.06	2.11	2.15	0.09
	+	Ag 0.50	12.40	13.30	12.85	12.85	0.36
	+	Ag 0.75	11.35	11.51	11.03	11.29	0.19

Table 127. Perna indica. Accumulation: Analysis of variance for changes in silver ($AgNO_3$) concentrations in the whole tissue when exposed to varying concentrations of silver ($AgNO_3$) and three unvarying concentrations of copper ($Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	3.6024	1.8012	2.1478(NS)
2. Between treatment combinations	11	2180.3760	198.2160	236.3653**
a. Between copper concentrations	3	966.6360	322.2120	384.2260**
b. Between silver concentrations	2	828.4240	414.2120	493.9327**
c. Interaction	6	385.3116	64.2186	76.5783**
3. Error	22	18.4492	0.8386	
4. Total	35	2202.4276		

NS Not significant
** - Significant at 1% level

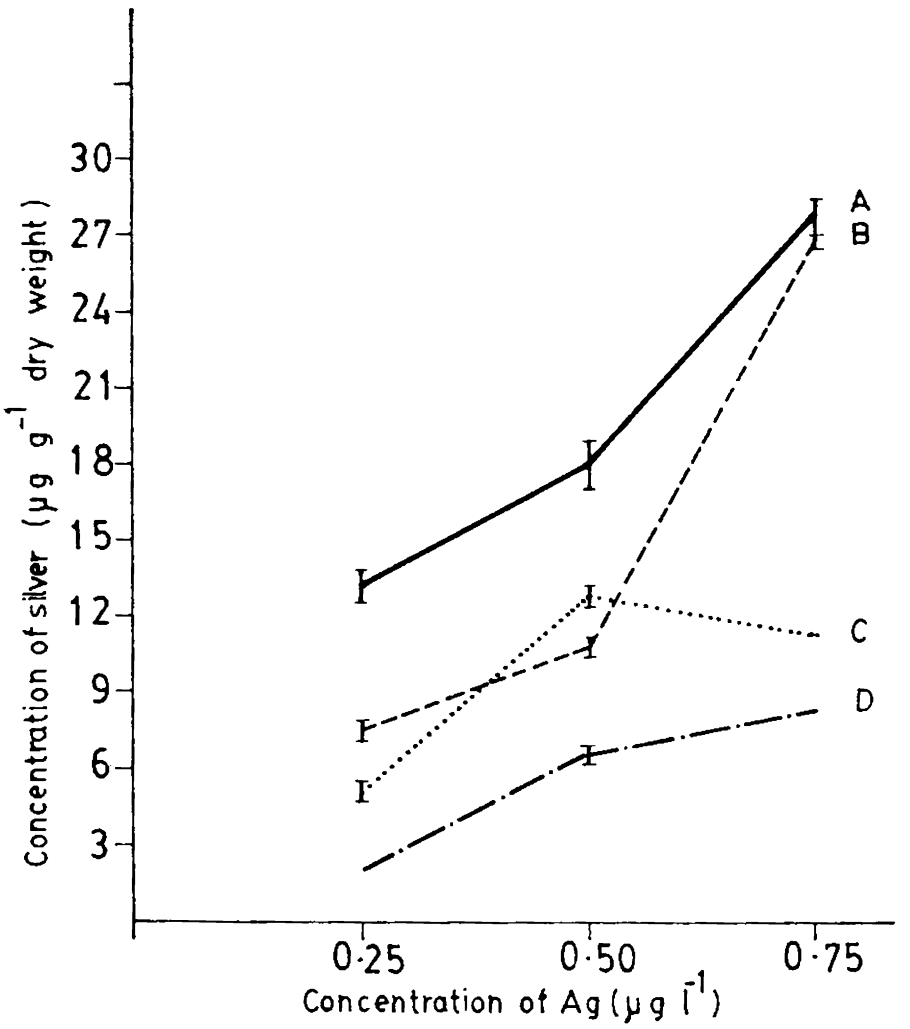

Figure 47. Perna indica. Accumulation: Concentration of silver (as $AgNO_3$) in the whole tissue when the animals were exposed to silver and silver + copper (as nitrates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 128. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to varying concentration of silver (as $AgNO_3$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.83	2.98	3.05	2.95	0.09	
0.25	2.85	2.81	3.16	2.94	0.15	99.50
0.50	2.79	3.81	1.09	2.56	1.12	86.65
0.25	0.98	2.08	1.54	1.53	0.44	51.96

Table 129. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption by those animals exposed to varying concentrations of silver ($AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	5.1009	1.7003	2.6365(NS)
Between replicates	2	1.1741	0.5870	0.9103(NS)
Error	6	3.8694	0.6449	
Total	11	10.1444		

NS Not significant

Table 130. Perna indica. Accumulation: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to varying concentrations of silver (as $AgNO_3$) in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentrations of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Cu 0.25 + Ag 0.25	3.39	1.78	1.25	2.14	0.91	72.48
+ Ag 0.50	2.16	2.00	1.88	2.01	0.11	68.17
+ Ag 0.75	1.92	0.98	2.32	1.74	0.56	59.02
Cu 0.50 + Ag 0.25	1.93	2.68	2.41	2.34	0.31	79.26
+ Ag 0.50	2.81	1.15	2.58	2.18	0.73	73.80
+ Ag 0.75	1.30	1.29	2.58	1.72	0.60	58.35
Cu 0.75 + Ag 0.25	1.27	2.99	1.08	1.78	0.85	60.20
+ Ag 0.50	0.60	2.45	1.85	1.63	0.77	54.85
+ Ag 0.75	1.63	0.98	1.09	1.23	0.28	41.87

Table 131. Perna indica. Accumulation: Analysis of variance for changes in oxygen consumption by those animals exposed to varying concentrations of silver (as $AgNO_3$) and three unvarying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	0.0381	0.0190	0.0286(NS)
2. Between treatment combinations	8	2.7508	0.3438	0.5165(NS)
a. Between copper concentrations	2	1.4076	0.7038	1.0572(NS)
b. Between silver concentrations	2	1.2991	0.6495	0.9757(NS)
c. Interaction	4	0.0440	0.0110	0.0165(NS)
3. Error	16	10.6527	0.6657	
4. Total	26	13.4416		

NS - Not significant

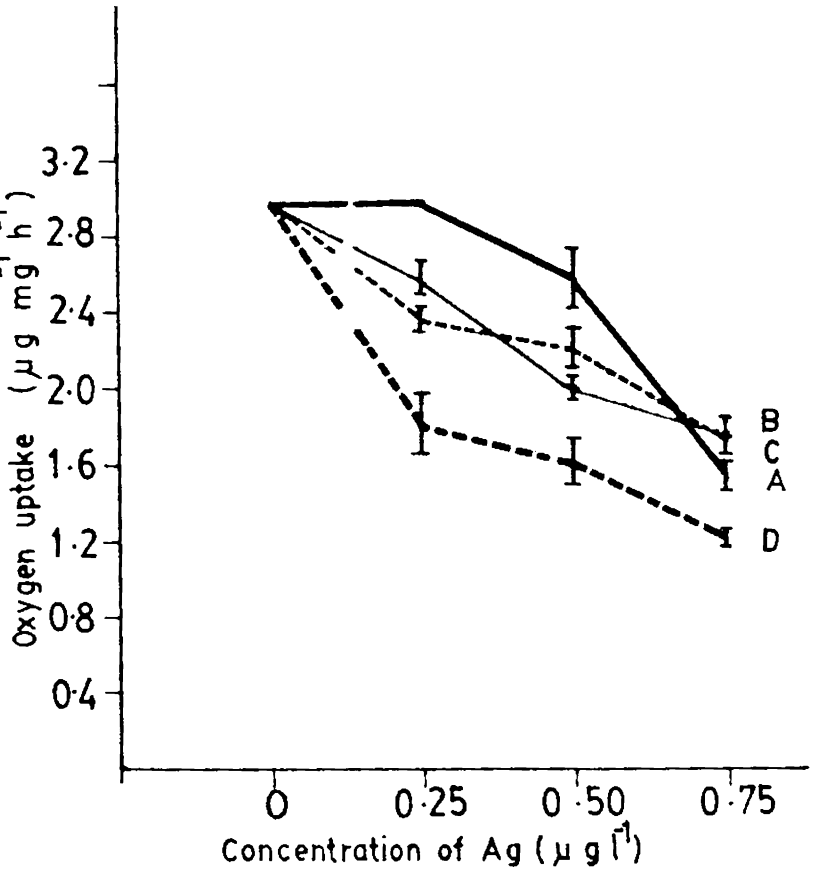

Figure 48. Perna indica. Accumulation: Oxygen consumption when the animals were exposed to silver and silver + copper (as nitrates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 132. Perna indica. Accumulation: Effective concentrations (EC 50 ppb) of silver which caused a 50% reduction in the oxygen consumption upon exposure to silver and three unvarying concentrations of silver + varying concentrations of copper (as nitrates) for a period of 7 days (accumulation), along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Copper	+	Silver	
0.00		0.78 (0.75 0.81)*	
0.25		1.76 (0.54 5.72)*	1.47 (LA)
0.50		1.32 (0.53 3.30)*	1.14 (LA)
0.75		0.52 (0.36 0.76)*	- 0.34 (LA)

* 95% confidence limits

LA Less than additive

copper and silver for 7 days, showed uniformity, that was reduction in oxygen consumption compared to those of controls. Higher silver concentration along with copper usually brought about maximum reduction in quantity of oxygen consumption (Table 130). The results when tested for analysis of variance showed no significance (Table 131). The effective concentrations with reference to oxygen consumption were worked out (Table 132). The values show that the combinations of silver and copper brought out a less than additivity reaction. Further, in the presence of copper at 0.25 and 0.50 ppb levels in the medium, silver was found to become less toxic with reference to oxygen consumption.

4.2.2.2.3.2. Filtration accompanying accumulation

Rate of filtration by Perna indica, after subjected to an accumulative phase of 7 days, either in media contaminated with silver alone or with silver and copper, was assessed. The resutls are presented in Tables 133-137 and Figure 49. When present alone, increased concentration of silver resulted in decrease in the rate of filtration. The observed decrease between concentrations was highly significant (Table 134). Further, it was observed that the mean rate of filtration of Perna indica, showed declension when they were exposed to a combination of copper and silver during the accumulative phase. The declension was concentration dependent. However, in those concentrations namely 0.50 and 0.75 ppb copper with 0,25, 0.50 and 0.75 ppb silver, the decrease in the rate was irrespective of the internal concentration of silver in the tissue. The data was subjected to analysis of variance (Table 136). Variations between treatment combinations, between copper concentrations and between silver concentrations were found to be highly significant. Considerable decrease was noti-

Table 133. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to varying concentrations of silver (as $AgNO_3$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	31.86	33.94	36.39	34.06	1.85	
0.25	23.81	27.16	18.93	23.30	3.37	68.40
0.50	12.96	18.28	19.61	16.95	2.87	49.76
0.25	21.76	15.37	21.46	19.53	2.94	57.33

Table 134. Perna indica. Accumulation: Analysis of variance for changes in filtration rate by those animals exposed to varying concentrations of silver (as $AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	410.877	136.9590	9.0753**
Between replicates	2	4.6049	2.3024	0.1525(NS)
Error	6	90.5478	15.0913	
Total	11	506.0297		

NS Not significant
** - Significant at 1% level

Table 135. Perna indica. Accumulation: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to varying concentrations of silver (as $AgNO_3$) in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Concentration of metal (ppb)			Replicates 1	2	3	Mean	SD	% of control
Cu 0.25	+	Ag 0.25	29.73	24.59	18.74	24.35	4.48	71.49
	+	Ag 0.50	25.68	27.43	22.89	25.33	1.86	74.36
	+	Ag 0.75	20.37	22.78	17.66	20.81	2.09	61.09
Cu 0.50	+	Ag 0.25	19.64	19.65	15.56	18.28	1.92	53.66
	+	Ag 0.50	17.40	13.26	15.72	15.36	1.70	45.09
	+	Ag 0.75	12.98	9.28	11.03	11.29	1.52	33.14
Cu 0.75	+	Ag 0.25	16.75	13.12	10.19	13.35	2.68	39.19
	+	Ag 0.50	7.81	9.48	12.42	9.90	1.90	29.06
	+	Ag 0.75	4.46	5.84	3.97	4.75	0.79	13.94

Table 136. Perna indica. Accumulation: Analysis of variance for changes in filtration rate by those animals exposed to varying concentrations of silver (as $AgNO_3$) and three unvarying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	38.6426	19.3213	2.8993(NS)
2. Between treatment combinations	8	1119.6700	139.9587	21.0022**
a. Between copper concentrations	2	889.994	444.9970	66.7762**
b. Between silver concentrations	2	207.1280	103.5640	15.5408**
c. Interaction	4	22.5508	5.6377	0.8459(NS)
3. Error	16	106.6240	6.6640	
4. Total	26	1264.9366		

NS Not significant
** - Significant at 1% level

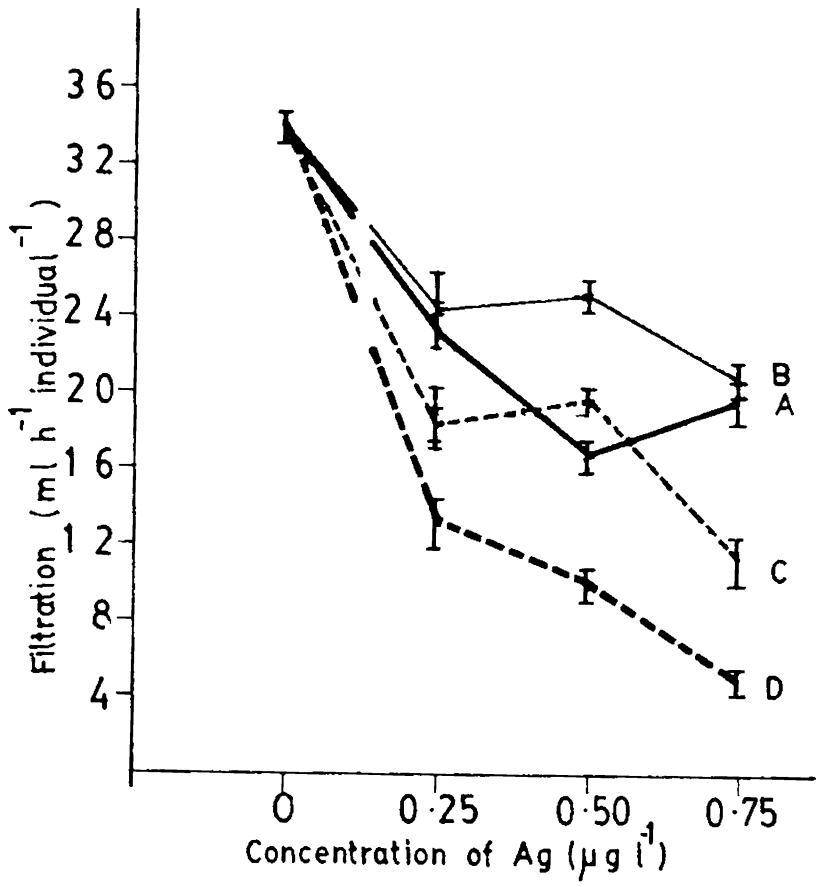

Figure 49. Perna indica. Accumulation Rate of filtration when the animals were exposed to silver and silver + copper (as nitrates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 137. Perna indica. Accumulation: Effective concentration (EC 50 ppb) of silver which caused a 50% reduced in the filtration rate upon exposure to silver and three unvarying concentrations of silver + varying concentrations of copper (as nitrates) for a period of 7 days (accumulation), along with 95% confidence limits and additive indices.

		EC 50 (ppb)	Additive index
Copper	+	Silver	
0.00		0.91 (0.18 4.50)*	
0.50		0.32 (0.24 - 0.43)*	- 0.35 (LA)
0.75		0.18 (0.06 0.54)*	- 0.69 (LA)

* 95% confidence limits

LA Less than additive

ced in the effective concentrations also (Table 137). In the presence of 0.75 ppb copper, 0.18 ppb of silver resulted in 50% reduction in filtration rate. Notwithstanding this drastic drop in silver concentration with reference to filtration, the combined action of copper and silver was found to be less than additive.

4.2.2.2.4. Effects on silver depuration

After exposing Perna indica, to three unvarying concentrations of copper and varying concentrations of silver for a period of 7 days, during which duration the animals had accumulated both the metals, their rate of depuration was assessed by exposure to raw seawater for 7 days. The results obtained are presented in Tables 138-140 and Figure 50. The rate of depuration of silver was found to vary drastically. At some instances, the rate of depuration showed relationship with internal concentrations. In fact higher body burden of silver within resulted in increased rate of depuration. In such cases, where, the rate of depuration was low, the internal load was also found to be less (Table 126 and Figure 47). The rate of depuration, worked out in quantities of silver, therefore, show wider fluctuations (Table 139 and Figure 50). When the data was subjected to analysis of variance (Table 140) it was noticed that the quantity depurated, by those animals, with reference to between treatment combinations, between silver concentrations, between copper concentrations and their interactions was found to be highly significant. Only those organisms exposed to different concentrations of silver have depurated significant amount of silver.

4.2.2.2.4.1. Oxygen consumption accompanying depuration

To find out, to what a extent Perna indica had regained normalcy

Table 138. Perna indica. Depuration: Concentration of silver (as $AgNO_3$) in the whole tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as $Cu(NO_3)_2$) individually and in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentrations of metal (ppb)		Replicates				
		1	2	3	Mean	SD
Cu 0.00	+ Ag 0.25	6.38	6.92	5.61	6.30	0.65
	+ Ag 0.50	9.41	9.13	12.60	10.38	1.92
	+ Ag 0.75	16.56	16.09	14.11	15.58	1.30
Cu 0.25	+ Ag 0.25	6.20	7.92	5.78	6.63	1.13
	+ Ag 0.50	10.31	10.07	9.82	10.06	0.24
	+ Ag 0.75	25.04	27.18	22.92	25.04	2.13
Cu 0.50	+ Ag 0.25	3.15	2.91	3.78	3.28	0.44
	+ Ag 0.50	5.09	4.80	5.20	5.03	0.20
	+ Ag 0.75	5.36	5.78	7.57	6.23	1.17
Cu 0.75	+ Ag 0.25	1.98	1.72	2.06	1.92	0.17
	+ Ag 0.50	10.96	9.74	9.44	10.01	0.74
	+ Ag 0.75	8.32	9.20	7.58	8.36	0.81

Table 139. Perna indica. Depuration: Quantity of silver (as $AgNO_3$) depurated from the whole tissue ($\mu g\ g^{-1}$ dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentration of silver (as $AgNO_3$) individually and in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD
Cu 0.00 + Ag 0.25	6.20	7.77	6.89	6.95	0.64
+ Ag 0.50	6.71	8.99	7.03	7.57	1.00
+ Ag 0.75	9.85	12.97	14.46	12.42	1.92
Cu 0.25 + Ag 0.25	0.95	0.39	1.19	0.84	0.33
+ Ag 0.50	1.13	0.70	0.81	0.88	0.18
+ Ag 0.75	2.09	0.29	3.65	2.01	1.37
Cu 0.50 + Ag 0.25	1.18	0.83	3.19	1.73	1.03
+ Ag 0.50	0.97	1.99	1.74	1.56	0.43
+ Ag 0.75	3.03	2.43	1.04	2.16	0.83
Cu 0.75 + Ag 0.25	0.30	0.34	0.05	0.23	0.12
+ Ag 0.50	1.54	3.56	3.41	2.83	0.91
+ Ag 0.75	1.81	2.31	3.45	2.52	0.68

Table 140. Perna indica. Depuration: Analysis of variance for changes in the quantity of silver (as $AgNO_3$) depurated by those animals pre-exposed to varying concentrations of silver (as $AgNO_3$) and three unvarying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	5.2837	2.6418	2.2335(NS)
2. Between treatment combinations	11	435.1080	39.5552	33.4420**
a. Between copper concentrations	3	365.8540	121.9513	103.1039**
b. Between silver concentrations	2	34.1769	17.0884	14.4474**
c. Interaction	6	35.0793	5.8465	4.9429**
3. Error	22	26.0231	1.1828	
4. Total	35	466.4148		

NS - Not significant
** Significant at 1% level

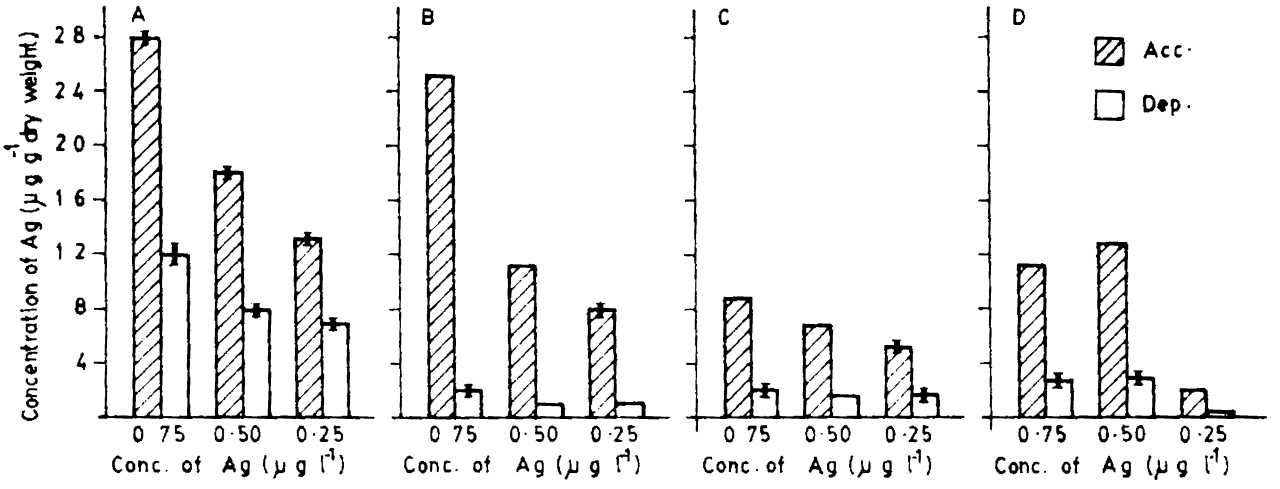

Figure 50. Perna indica. Quantities accumulated in the whole tissue after 7 days and depurated subsequent to exposure to raw seawater for 7 days.

A Ag alone

B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu

D Ag + 0.75 ppb Cu

after being subjected to depuration for 7 days representative samples were tested for their oxygen consumption rates. The data is given in Tables 141-144 and Figure 51. Those animals which were maintained in media containing only silver during accmulative phase, showed concentration dependent decrease when the silver level was 0.75 ppb. Even after 7 days depuration those who were maintained at 0.25 and 0.50 ppb consumed more oxygen than that of control. However, this variation was not statistically significant (Table 142). The pattern of oxygen consumption by Perna indica after depuration, before being exposed to combinations of copper and silver, was erratic. Therefore, little variations noticed between treatment combinations, between copper concentrations, between silver concentrations and their interactions were not statistically significant (Table 144).

4.2.2.4.2. Filtration accompanying depuration

The capacity to regain normal rate of filtration by Perna indica, after being subjected to an accumulative phase of 7 days to silver contaminated test media and copper and silver contaminated test media, and depuration for 7 days was tested. The data is given in Tables 145-148 and Figure 53. In the case of those animals, which contaminated only silver in the media, the capacity to filter normally exemplified by rates similar to that of control was shown only by those animals maintained in 0.25 ppb of silver. The others did not increase the rate of filtration even after 7 days of depuration. The differences obtained were found to be significant (Table 145 and 146). On the other hand, encountering combined concentration of copper and silver brought about different results (Table 147 and 148). Those animals which accumulated silver from a metal combination of copper and silver, the former being at higher unvarying

Table 141. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days (depuration), after being maintained in varying concentrations of silver (as $AgNO_3$) for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	2.28	2.89	3.06	2.74	0.33	
0.25	3.15	4.20	2.89	3.41	0.56	124.29
0.50	5.16	2.63	1.37	3.05	1.57	111.19
0.75	3.10	2.25	1.67	2.34	0.58	85.30

Table 142. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed varying concentration of silver (as $AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	1.8678	0.6226	0.5364(NS)
Between replicates	2	2.8232	1.4116	1.2161(NS)
Error	6	6.9643	1.1607	
Total	11	11.6553		

NS - Not significant

Table 143. Perna indica. Depuration: Mean oxygen consumption (µg O_2 mg^{-1} h^{-1} dry weight), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as $AgNO_3$) in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation and percentage variation from control (100%) readings.

Pre-exposure concentrations of metals (ppb)	Replicates 1	2	3	Mean	SD	% of control
Cu 0.25 + Ag 0.25	2.20	3.81	2.01	2.67	0.50	97.45
+ Ag 0.50	2.16	3.38	2.61	2.71	0.50	98.94
+ Ag 0.75	1.73	1.38	1.98	1.69	0.24	61.87
Cu 0.50 + Ag 0.25	1.63	2.68	3.69	2.66	0.84	97.27
+ Ag 0.50	4.04	1.15	3.17	2.78	1.21	101.46
+ Ag 0.75	2.26	3.81	1.81	2.62	0.85	95.67
Cu 0.75 + Ag 0.25	0.96	2.98	2.88	2.27	0.92	82.84
+ Ag 0.50	0.53	2.18	1.90	1.53	0.72	56.11
+ Ag 0.75	1.73	1.30	1.91	1.64	0.25	60.05

Table 144. Perna indica. Depuration: Analysis of variance for changes in oxygen consumption by those animals pre-exposed to varying concentrations of silver (as $AgNO_3$) and three unvarying concentrations of copper (as $Cu(NO_3)_2$).

Source of variation		DF	SSQ	MSSQ	F. ratio
1.	Between replicates	2	1.9343	0.9671	1.1015(NS)
2.	Between treatment combinations	8	6.4918	0.8114	0.9242(NS)
	a. Between copper concentrations	2	2.5086	1.2543	1.4285(NS)
	b. Between silver concentrations	2	1.3999	0.6999	0.7972(NS)
	c. Interaction	4	1.5832	0.3958	0.4507(NS)
3.	Error	16	14.0492	0.8780	
4.	Total	26	22.4753		

NS - Not significant

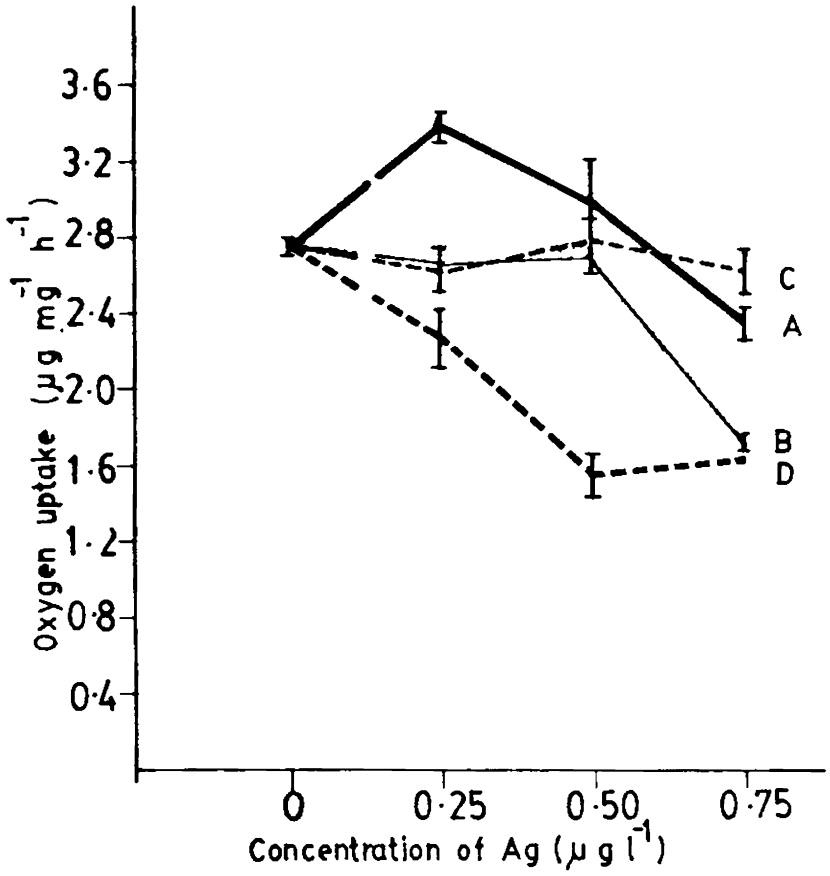

Figure 51. Perna indica. Depuration Oxygen consumption when the animals were exposed to raw seawater for 7 days, after being maintained in silver and silver + copper (as nitrates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

Table 145. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver (as $AgNO_3$) for a period of 7 days, along with standard deviation and percentage variation from the control (100%) readings.

Pre-exposure concentration (ppb)	Replicates 1	2	3	Mean	SD	% of control
Control	30.38	36.36	33.42	33.38	2.44	
0.25	26.66	33.44	24.63	28.24	3.76	84.60
0.50	28.92	19.11	17.49	21.84	5.04	65.42
0.75	20.38	16.85	19.81	19.01	1.54	56.95

Table 146. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of silver ($AgNO_3$).

Source of variation	DF	SSQ	MSSQ	F. ratio
Between concentrations	3	375.4230	125.1410	6.0064*
Between replicates	2	19.1264	9.5632	0.4590(NS)
Error	6	125.0076	20.8346	
Total	11	519.5570		

NS Not significant
* Significant at 5% level

Table 147. Perna indica. Depuration: Mean filtration (ml h^{-1} ind^{-1}), when the animals were exposed to raw seawater for a period of 7 days, after being maintained in varying concentrations of silver ($AgNO_3$) in combination with three unvarying $Cu(NO_3)_2$ overloads for a period of 7 days, along with standard deviation and percentage variation from the control (100%) readings.

Pre-exposure concentration of metal (ppb)	Replicates 1	2	3	Mean	SD	% of control
Cu 0.25 + Ag 0.25	30.88	24.09	16.48	23.81	5.88	71.33
+ Ag 0.50	24.73	23.34	26.98	25.01	1.49	74.92
+ Ag 0.50	21.49	20.13	16.53	19.38	2.09	58.05
Cu 0.50 + Ag 0.25	20.87	18.18	17.91	18.98	1.34	56.86
+ Ag 0.50	16.56	17.73	14.27	16.18	1.43	48.47
+ Ag 0.75	12.47	10.37	10.74	11.19	0.91	33.52
Cu 0.75 + Ag 0.25	17.73	14.11	9.00	13.61	3.58	40.77
+ Ag 0.50	8.91	10.30	12.88	10.69	1.64	32.02
+ Ag 0.75	6.68	3.81	6.69	5.06	1.20	15.15

Table 148. Perna indica. Depuration: Analysis of variance for changes in filtration rate by those animals pre-exposed to varying concentrations of silver ($AgNO_3$) and three unvarying concentrations of copper ($Cu(NO_3)_2$).

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	2	53.4434	26.7217	3.1612(NS)
2. Between treatment combinations	8	1018.1100	127.2637	15.0556**
a. Between copper concentrations	2	758.4760	379.2380	44.8646**
b. Between silver concentrations	2	238.6860	119.3430	14.1185**
c. Interaction	4	20.8506	5.2126	0.6166(NS)
3. Error	16	135.2470	8.4529	
4. Total	26	1206.8004		

NS Not significant
** Significant at 1% level

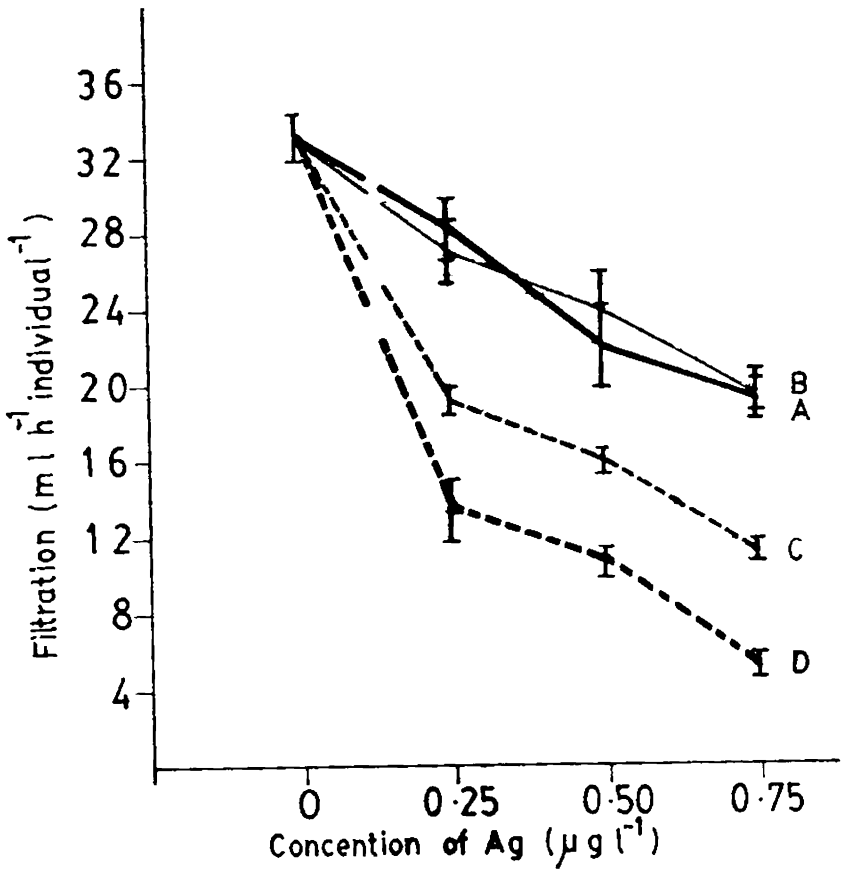

Figure 52. Perna indica. Depuration Rate of filtration when the animals were exposed to raw seawater for 7 days, after being maintained in silver and silver + copper (as nitrates) for 7 days.

A Ag alone B Ag + 0.25 ppb Cu

C Ag + 0.50 ppb Cu D Ag + 0.75 ppb Cu

concentration, did not regain normalcy even after 7 days of depuration. As a matter of fact, those animals exposed to 0.25 ppb copper and 0.50 ppb and 0.75 ppb silver, 7 days depuration resulted in marginal regaining of filtration capacity only. Representatives of Perna indica, which were maintained in 0.75 ppb copper and 0.75 ppb silver for 7 days, nearly did not regain capacity to filter even after 7 days of depuration. When the results were analysed for variance (Table 148), it was found that the variations noticed between copper concentrations and between silver concentrations were highly significant.

DISCUSSION

V DISCUSSION

Heavy metals in trace quantities have been normal constituents of the aquatic environment since the beginning of biological time. Industrialisation and increase in anthropogenic activities have lead to elevation of environmental concentrations of heavy metals (Klein and Goldberg, 1970). However, literature shows a great deal of variability in the base line concentration in seawater. Various reasons have been assigned for this, such as variations in natural input of heavy metals into the sea, changes in the distribution pattern affected by agencies such as biological transport and sample contamination and use of different analytical methods. Probably, discripencies in literature are mainly due to the use of different method of analysis. It is clear that the concentrations of several metals formerly regarded as normal are too high. Atmospheric input from anthropogenic sources has been evoked to explain elevated levels of all heavy metals including mercury and copper in both continental shelf surface waters (Windom and Smith, 1979; Windom and Taylor, 1979) and ocean surface waters (Gardner, 1975; Boyle, et al., 1977).

Although metals are known to be toxic, atleast eleven of them are essential for life and activity of living organisms. These are iron, copper, zinc, cobalt, magnesium, manganese, chromium, molybdenum, selenium, nickel and tin (DaSilva, 1978). These metals always function in combination with organic molecules and most commonly with proteins, either firmly bound in metallo-proteins or more loosely entrapped in metal-protein complexes (Vallee and Walker, 1970). The copper and iron form important components in oxygen carrying metallo-protein, haemocyanin and haemoglobin respectively. The optimum concentrations of these

metals presumably are those occurring naturally in the ecosystem.

Generally speaking, the most rapidly absorbing metals like mercury, copper and silver are the most toxic. In the case of others, the toxicities are extremely variable depending not only on permeability of particular species to the metal and also its capacity to detoxify the metal. This is an important aspect to be considered in short term experiments which might give clear results when more toxic metals are used than the less toxic ones. Various factors have been assigned to explain the toxicity dependence of a metal. Some of them are the form of the metal which will govern its availability for bioaccumulation; the presence of other metals; environmental factors; condition of the organism; ability to avoid metals and ability to adapt to metals (Bryan, 1984).

Experimental studies are probably one of the best methods to assess the toxicity of heavy metals to marine organisms. The investigation would involve assessment of stress on the animal exemplified by death or modified physical or physiological activities of the test organisms when exposed to various realistic concentrations of heavy metals. Analysis of toxicological responses are usually engineered by subjecting the animal to a stress and analysing the response. Delineation of such responses is an important aspect which requires selection of suitable indices which in turn will provide the necessary clues to the stress phenomenon.

One of the most important areas in pollution bioassay experiments is the analysis of combined toxicity of mixtures of pollutants. Effluents are mixtures of chemicals rather than a single chemical. Pollutants of dissimilar chemical nature interact in a variety of ways in waters of

various composition. Many workers have stressed the need in this regard for establishing water quality criteria because interaction between pollutants may aggravate or alleviate the toxicity. Gray (1974), stated that the discovery of mixture of chemicals that act antagonistically so that mixture is less toxic than the component chemical of a mixture is an important and neglected aspect of pollution research.

5.1. LETHAL TOXICITY

Uptake of soluble copper by plankter is the principle pathway for entry of the metal into the food web, although ingestion of copper containing non-biotic particulate may also lead to copper accumulation by marine organisms (Smith, 1978). Copper was found to be highly toxic to Perna indica resulting in rather low LC 50 values. Another species of the genus, Perna viridis was found to be comparatively less sensitive. Menon et al. (1987) recorded 105 ppb when copper was supplied in SO_4 form to Perna viridis. Perna indica was found to be more sensitive to $CuSO_4$ than $CuNO_3$. Nair et al. (1977) suggested that higher tolerance of Mytilus viridis to copper, may be owing to elevated natural levels of copper in the estuarine waters of Goa from where the test organisms were collected. Mathew and Menon (1983) found species specificity of metal toxicity. Higher tolerance to copper has been reported for bivalves especially those existing in the Cochin backwater areas where the natural load of copper is as high as 3 ppb (unpublished data). Villorita cyprinoides tolerated 2 ppm of copper (Lakshmanan and Nambisan, 1977). The mean tolerance limit of copper to larvae of Crassostrea virginica and Mercenaria mercenaria was 32.8 and 16.4 ppb respectively (Calabrese et al., 1978). Variations in toxicity depending on the mode of experiments was noticed

in the case of Crassostrea gigas. The 96 h LC 50 values to this oyster when found out with flow through system ranged between 0.3 ppm (Harrison and Rice, 1978) and 0.56 ppm (Okazaki, 1976). Similarly, drastically different variations could be seen with reference to 96 h LC 50 values in the literature. Kumaraguru and Ramamoorthi (1979) found that Meretrix casta recorded 96 h LC 50 of 72 ppb, Kumaraguru et al.(1980) 500 ppb and Mathew and Menon (1983) 75 ppb. MacInnes and Calabrese (1979) observed a profound influence of salinity and temperature on copper toxicity to Crassostrea virginica. It is clear that the chronic exposure to very low levels of copper in the environment could drastically affect the lethal toxicity of copper in a cosmopolitan or widely distributed species.

Silver is known to be a highly toxic metal, although it has received little attention in aquatic environmental study, especially in marine waters (Nelson et al., 1983). The average silver concentrations in seawater is known to be 0.1 ppb (Riley and Chester, 1971). Although, it is generally assumed to be a widespread pollutant, it does occur in industrial discharges and should be listed in any classification of highly toxic pollutants (Saila and Segar, 1979). The present findings show that silver is more toxic than copper. Among the two salt forms, $AgSO_4$ was distinctly more toxic than $AgNO_3$. Only scanty attention has so far been paid towards acute toxicity of silver. Studies of Clarke(1947) and Bryan (1976a) showed a high level of resistance to silver by the oyster, C. virginica and the polychaete, Nereis diversicolor. Calabrese et al. (1980) working on juvenile scallop, Argopecten irradians recorded a 96 h LC 50 of 33 ppb of silver. MacInnes and Calabrese (1978) found that, 48 h median lethal concentration of silver to the American oyster, Crassostrea virginica could vary from 24.2. to

35.3 ppb depending upon temperature. The increased toxicity at 30° C was attributed to great solubility of metal salts and elevated rate of water and solid movement across cell membrane due to increased activity which is thermal controlled. Pointing to the complexcity on influence of toxicity on aquatic organisms, MacInnes and Calabrese (1978) have stated that many toxicants that influence the energy metabolism or that alter respiration may have its potential influenced by temperature increase. The sensitivity of silver to different life stages of many commercially important bivalves has been reported by many workers. Calabrese and Nelson (1974) found that 21 ppb of silver was the LC 50 values for the eggs of Mercenaria mercenaria whereas that of larvae was 32.4 ppb.

Sprague (1970) remarked "probably the most exciting and potentially useful recent development in pollution biology has been a method of predicting toxicity of mixtures of toxicants". The methodology developed helps to measure the simultaneous effects of several pollutants and this can be expressed as numbers. Observations of combined toxicity could be used at lethal or sublethal levels. Sprague and Ramsay (1965) used the toxic unit method of predict the toxicity of copper and silver to Atlantic salmon, Salmo salar. Other techniques for evaluating the toxicity of mixtures have also been advanced. Most of these follow mathematical model for additive joint toxicity that yields their harmonic mean of the LC 50 of the components (Finney 1971). This model tests the hypothesis that the toxicity of chemical mixture is simply additive. Smyth et al. (1969) normalised the value 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 seawater

and the resultant benefit or hazard is fairly new and only a few methods have been investigated.

The outcome of the experiments employing Perna indica and combinations of realistic concentrations of copper and silver indicated that when in combination, the toxicity of individual metals increases. Further, the combinations of SO_4 salts were much more toxic than the NO_3 salts. The reasons for this could be multifarious. Selective absorption of metals, disruption of detoxifying mechanisms, impediment on the transport of the metal ions after entry into the cells, reaction of the metal ions of both metals on the same site in the cell thereby increase in the stress could be factors. Another conspicuous feature of the finding was that, when in combination with varying concentrations of copper, the toxicity of silver reduced while, when silver was in combination with unvarying concentration of copper its toxicity increased. Therefore, the same metal can show differences in toxicity in nature when it is present along with other metal salts in varying concentrations. Such findings have not been reported so far in the literature. In a revealing study on long-term silver effects on Crepidula fornicata, Nelson et al. (1983) recorded preferential uptake of copper in the presence of silver. They found that at comparatively low silver exposure concentration body burden in mussels increased. Analysis of slope function showed interesting aspects on the above results. Slope functions increased gradually only when the animals were exposed to single metal contaminated test media. On the other hand, it was found to fluctuate considerably when the LC 50 results on combinations were plotted. Slope function is the factor by which dose must be multiplied or divided to produce a standard deviation in response. Therefore, variations in the slope functions give information on the relative variability

in the LC 50s obtained. From the data it is clear that the slope functions varied between concentrations and between combinations, indicating that the death of Perna indica was caused not only by increase or decrease in the toxicant concentrations employed in a combination but also, in a particular series of experiments, where one of the components was constant and the other varying, death would have occurred either at concentrations with less variability or more variability. The reason for this variability is unknown.

5.2 SUBLETHAL TOXICITY

5.2.1. Accumulation and depuration of copper

The main concern about metallic contamination centres around the capacity of the marine animal to accumulate abnormal concentrations of metals in the tissues. Mussels, although do not normally contain high concentrations of heavy metals have been recognised as potential indicators of metallic contaminations. Experimental studies with several metals including zinc, manganese, cadmium and selenium have indicated that particulate matter may be a more significant source of metals than the dissolved species (Pentreath, 1973; Fowler and Benayoun, 1974, 1976). Experimental studies suggest that for most metals, the concentration in the mussel will come to reflect that of environment. However, erratic behaviour has been reported for copper in mussels by Phillips (1976 a&b); and Bryan and Hummerstone (1977), expressed doubt about the ability of mussels to reflect changes in the availability of silver.

The concentration of copper in the various tissues of Perna indica, showed that the remaining tissue which includes the gastric diverticula contained the maximum, followed by gills and the adductor muscle the least. The rate of accumulation was found to be influenced by the external concen-

tration only when the concentration exceeded 2 ppb. A variation from this generalisation was found only in mussels where those animals exposed to 4 ppb showed increase in copper in the adductor muscle when the environmental concentration of copper ranged between 2 to 5 ppb, (Delhaye and Cornet, 1975; Simkis et al., 1982). These authors thought that the concentrating power of Mytilus was impressive, although it was considerably less than mussel's ability to concentrate iron, cadmium or zinc. Scott and Major (1972), exposed mussels to 300 ppb of copper in a static system and found that tissue copper level rose from 10 ppm to 25 ppm (dry weight) after 24 h and subsequent reduction to initial tissue concentration after 24 h. However, this result was not confirmed by Delhaye and Cornet (1975). The work of Scott and Major (1972) probably indicates that their mussels had removed all of the copper from static solution surrounding them. Boyden (1977), who transferred mussels from uncontaminated area to contaminated area found that the rate of uptake of copper was not that high as reported by Scott and Major (1972). Scott and Major's (1972) finding that there was declension in the copper concentration in the tissue suggest that there is a very clear cut excretory process. However, the present findings clearly indicate that, when Perna indica was exposed to very low concentrations of copper the rate of excretion was considerably low. This is very clearly indicated from the studies conducted on depuration. Although there was variations on concentration dependent rates differences in depuration, the total quantity that ultimately left the body of animals after 7 days depuration was very low. Therefore, conclusions drawn from studies employing higher concentrations of copper, totally unrealistic from natural situations, might not give a clear idea on capacity of mussels to depurate copper. The findings of Phillips (1976 a&b), that copper concen-

trations in mussels varied with season, mainly because of wet weight changes caused by the build up and loss of gonadal materials need not necessarily reflect the normal depuration capacity of mussels. Phillips (1976 a&b) hypothesised that there might be specific copper transporting sites which have evolved because of biological importance of copper to Mytilus. It is interesting to note in this connection that the findings of Longston and Zhoe (1987, in press), who propounded in the case of cadmium accumulation, distribution and elimination in Macoma balthica, neither metallotheonein nor metallo-proteins were involved. Variation in elution profile of copper binding protein in laboratory exposed and environmental exposed oyster, Crassostrea virginica, have been noticed by Engel and Brouwer (1982). The presence of double copper peaks in laboratory exposed versus the single peak in the environmental exposed animals, according to Engel and Brouwer (1982) might have been caused by differences in the metal concentration in the water, duration of exposure, the mode of presentation of metal and the interaction of the metals. The point regarding the influence of differences in metal concentration and accumuation, is well established in the present series of experiments. The realistic concentration of copper under which the laboratory animals were maintained gave indications that there could be clear cut concentration dependent variation in uptake even when the external concentration of copper ranged from 0.5 ppb to 4 ppb. Another important finding in the present study was that the rate at which copper was removed from the tissues had some relationship with the copper burden of the respective tissues. Perna indica removed more copper from those

tissue which had final high concentration of copper after 16 days of exposure.

It has been proved that the chemical state, as well as the kinetics of transformation between different species of chemical salts dissolved in water, influence the rate of bioaccumulation of heavy metals (Stumm and Bilinski, 1972; Branica, 1978; Mantoura et al., 1978; Allen et al., 1980; Nurnberg, 1982, 1983). In another series of experiments, Coombs (1977), Coombs and George (1978) and George and Coombs (1977) proved that the shellfishes living in estuaries can be affected by the salt form of metals with reference to uptake. To elaborate this, they used chelating agents to alter the quantum biologically available to the animal. The results show that differences in the rate of availability may not drastically influence the pattern of distribution of the concerned metals over the various tissues although it can significantly increase the quantity accumulated in each tissue. When supplied in very low quantities the rate of accumulation of copper available in nitrate and chloride forms showed variations. The rate of uptake of copper in chloride form was higher than that from nitrate by Perna indica. Discussing on salt dependent variations in uptake, Martincic et al. (1984), have suggested various points which influence the differences. They are the chemical species in which the trace metals exist and variations in metal species depending on the ligand type which might in turn may or may not favour uptake. Discussing on increased uptake of copper in the oyster, Martincic et al. (loc. cit.) suggested that between different species of copper available, the oysters pick out the larger part. It is possible that oyster needs higher quantities of copper for saturation of specific copper-metallo-enzyme and or a higher protein binding sites

which are strong copper acceptors. During the last decade studies have been carried out in the field as well as in laboratory on uptake kinetics of metals by marine animals. In all the experiments the metal was supplied either in the water or in the diet. Many authors are of the opinion that the metal uptake from the diet is of more importance than the direct uptake from the water. If this is so it is intriguing to consider salt variations and rate of uptake. Therefore, information gathered on salt variation on uptake based on experiments conducted by inoculating the salts directly into the water is more meaningful. On the other hand, from the enrichment factors evaluated by comparison of metal concentrations of different organs of bivalves with total dissolved metals, it was found that accumulation of zinc, cadmium, and copper by bivalves occur predominantly from the dissolved state (Martincic et al., 1984). Discussing on the relative merits of using labelled metals and stable metals to analyse rate of accumulation and depuration, Romeril (1971), has stressed the importance of taking into account the different physico-chemical forms in which zinc exists in seawater. Bernhard et al.(1967), found that the rate of uptake of stable zinc was significantly different from that observed for radioactive zinc. This failure to observe equillibriation between added ionic Zn^{65} and stable zinc in seawater even after several months might explain the discripancy in the uptake results observed between stable and ionic forms of metals. Therefore, it would not be logical to use stable metal observations from the environment to predict the behaviour of ionic metal forms under experimental conditions. It is quite likely that by supplying trace metal in stable and ionic form directly we are influencing the biological half life of metals. It is known that biological half life of a trace metal does influence accumulation and depuration of a metal.

The results on depuration show that time limit given for depuration namely, 7 days, did not bring about any clear cut differences in the depuration rate. Irrespective of the previous accumulation history, the quantity thrown out was always more when the body burden was high. This indicates that irrespective of the salt forms of metals, the rate of depuration was controlled by the quantity of copper rather than salt forms supplied. Cunningham and Tripp (1973) have recognised three types of metal release by bivalves, viz., increase in biological half life with increase in body burden of trace metal, stable biological half life when an equillibrium is maintained by a proprotionate increase in the rate of trace metal loss when body burden increases and decrease in biological half life with increase in body burden of trace metal. The present findings on depuration show that the short-biological half life evidence the present case are related to rapid elimination of trace metals after their trapping by wandering amoebocytes and mucus. It is interesting to note that Korringa (1952) suggested elimination of trace metals by mucus sheets. Trace metal depuration may be related to deposition or binding characteristic of each metal. Therefore, decrease in the copper concentration from the tissue after the animals were kept in clear seawater for 7 days clearly indicate the copper is eliminated quickly.

Very little information is available on the rate of accumulation and depuration of heavy metals when the animal was exposed to a combination of these. The finding that the presence of increased concentrations of silver reduced the uptake of copper is a significant one. The various factors involved in the uptake of a metal might have played a

role in this case. Elliott et al. (1986) working on the effects of metal combinations on metal uptake found that cadmium accumulation can be influenced by the presence of zinc. Further, they suggested that such interactions occur only at high concentration of interacting metals, although they found enhanced cadmium accumulation in lower concentrations of copper and zinc. Negilski et al. (1981) found that both cadmium and zinc reduced copper accumulation by the shrimp Callianassa australiensis. Phillips (1976b) also found a reduction in copper in the presence of cadmium and zinc in Mytilus edulis. The present finding that the concentrations of silver above 0.50 ppb can drastically reduce the uptake of copper clearly substantiates the previous findings with reference to other bivalves. Increased zinc concentration in water had little or no effect on cadmium level in Palaeomon serratus (Devineau and Triquet, 1985). The most important interaction between these two metals was antagonism exerted by cadmium on biological uptake of zinc (Divenaeu and Triquet, 1985). According to Jackim (1974), in marine bivalves, Mytilus edulis and Mulinia lateralis, zinc in amounts as low as 5 ppb lowers cadmium uptake. According to Bryan (1971), the concentration of mercury, silver or lead in organisms depend on their concentration in the environment. Bryan (1976a), hypothesised that essential trace element can be regulated while non-essential ones cannot. Moulder (1980), found that the presence of 1.8 ppb of copper in the uptake medium significantly reduced mercury uptake in Gammarus duebeni by a similar amount to that present in ordinary 100% seawater. Possibility of two metals acting antagonistically or synergistically at different concentrations has also been suggested by Moulder

(loc. cit.). In the present experiments, the concentration of metal mixtures were very low and usually antagonism appears only when concentrations exceeded certain thresholds. Irrespective of the salt forms, the presence of copper as $CuSO_4$ along with $AgSO_4$ and $CuNO_3$ along with $AgNO_3$ reduced copper accumulation when the silver concentrations increased beyond particular level. Analysis of the data further revealed that lower concentrations of silver in the presence of copper increases copper uptake and the uptake rate of copper from sulphate form is more than that from nitrate form. There is no comparable data of this sort for comparison.

Discussing on the accumulation and depuration of copper, Viarengo et al. (1985), demonstrated a increase in the level of copper binding proteins during the first 6 days of detoxification. This increase regained normalcy only after 12 days. The high level of these proteins in the mussels indicate increased detoxification during initial 3 to 6 days and establishment of an equillibrium between synthesis and elimination later. The present findings give the following information. Irrespective of the internal load of copper accumulated by Perna indica, in the presence of silver, the rate of depuration of copper was found to be more only in such cases where the accumulative phase provided higher concentration of silver and copper.

5.2.2. Accumulation and depuration of silver

The main feature obtained from the experiments on the rate of accumulation of silver supplied in different salt forms was that the quantity accumulated was distinctly less than those accumulated when copper

was supplied in different salt forms. Further, the animals exposed to silver oxide accumulated more silver than those exposed to silver carbonate. In a paper discussing the effects of silver on life history of Crepidula fornicata, Nelson et al. (1983), found that silver was accumulated rapidly in a parental C. fornicata during the 6 months of exposure to 1, 5 and 10 ppb of silver and there was no increase after 12th month. After prolonged exposure for 24 months there was significant reduction in silver accumulation. Curiously enough, Nelson et al. (loc. cit.) found that although there was no increase in the silver concentration after 12 months, the copper concentration in the tissue showed an increase. Incidently copper was not added to the experimental media but the background concentration of the incoming seawater ranged between 2 to 4 ppb. Greig (1979), exposed filter feeding bivalve molluscs, Arctica islandica, Spisula solidissima and Crassostrea virginica simultaneously to silver, cadmium, and copper at concentration of 10 ppb of each metal for a maximum period of 43 days. Among the three bivalve molluscs used, oyster concentrated maximum quantity of silver. Previous studies have shown that Mytilus edulis exposed to silver for 21 months exhibited a preferential uptake of copper in the presence of silver. The experiments conducted with Perna indica where the same concentrations of silver and copper were introduced into the culture media, the animals distinctly accumulated less quantity of silver. This difference was more conspicuous when the copper concentrations increased. Similarly, when different salts of both the metals were used in combinations, the uptake pattern remained the same. This clearly indicate that irrespective of the form of the metal, two relationships are indicated in the selective uptake

of metal by Perna indica subjected to exposure to a mixture of copper and silver. They are, 1. increased uptake of copper in the presence of silver and 2. concentration dependent variation in copper uptake in the presence of silver. Popham and D'Auria (1982), in a study correlating the effects of seawater concentrations on trace metal concentration in tissue of Mytilus edulis, suggested that copper concentrations in mussels were partially a function of concentration of zinc and or lead in the seawater. It is quite likely that this is true of silver also. Stenner and Nickless (1974), Phillips (1976b) and Lobel et al. (1982) also suggested metal interactions with reference to metal uptake by Mytilus edulis. But majority of such information are speculations arrived at after analysing the tissue load for trace metals in marine molluscs from natural populations inhabiting polluted localities. It is understood that even those localities which are chronically polluted, the concentration of heavy metals can vary at any point of time. Further, the concentrations for which the animals are exposed would be considerably low. Therefore, comparison of result obtained from laboratory studies with that of monitoring studies need not give a clear cut comparative idea.

Along with studies on accumulation dependent on metal combinations, the rate of depuration was also looked into. The study was mainly sought to follow the dynamics and interactions of copper and silver on the rate of depuration. Okazaki and Panietz (1981) discussing on the rate of depuration of cadmium, mercury, silver and iron in Crassostrea gigas showed that the presence of one metal can decrease the biological half life and the rate of decrease is more when the burden by other metal is more. If this idea is fitted into the present observation, it may be seen that the increased load of copper did not decrease the rate of depuration of silver,

whereas the rate of depuration of copper increased with increase in body burden of silver. Therefore, it may be assumed that the biological half life of any trace metal can vary depending on the presence and status of body burden of other metal. It may be pointed out here that when the animals are exposed to very low concentrations of heavy metals the rate of uptake will be less and the animals get enough time to detoxify them and tuck them away in areas from where throwing out requires more time. In the case of those trace metals which are concentrated in more quantities in the gills and the mantle in the case of bivalve molluscs depurative process could be quicker. On the other hand, transporting heavy metals to internal organs may not facilitate quick removal eventhough the animals are exposed to clean seawater. In this connection it is interesting to note the findings of Ishii et al. (1986) on Cyclosunetta menstrualis. They found that large size granules formed of various trace elements like iron, copper, zinc, tin and lead can be concentrated inside the kidney. They attributed that the presence of large quantities of trace metals in kidney may be owing to the existance of metal containing granules. They also found that metals exist in various chemical forms such as chlorides, carbonates, or oxide in addition to phosphate.

5.2.3. Oxygen consumption accompanying accumulation and depuration

Laboratory investigations on the sublethal effects of pollution are usually performed by assessing four categories of responses that could be reflected in physiology, biochemistry and cell structure, behaviour and neurophysiology, and reproduction of concerned organism. Waldichuk (1979) remarked that 'a response is not linear with pollutant concentration'. Labo-

ratory experimentation of sublethal stress can delineate linear or nonlinear responses, the concentration usually positioned in between measurable sublethal threshold and incipient threshold. Trace metals have irrivocably proved to affect the rate of oxygen consumption and filtration rate of marine bivalves. Majority of the available information pertain to oxygen consumption or filtration rate of marine bivalves subjected to exposure to various concentrations of trace metals. In the present case, these activities were measured employing animals which have either an elevated body burden of heavy metals, copper and or silver, subsequent to exposure to an accumulative phase or to depurative phase. The main objective of this study was to find out the effects of body burden on oxygen consumption or filtration.

Rate of oxygen uptake is a useful index to assess the toxicant stress in animals as this indicates the energy expenditure to meet the demand of an environmental alteration (Thurberg et al., 1975). Heavy metals can either depress or elevate rate of oxygen consumption in marine bivalves. Mathew and Menon (1983) reported that sublethal concentrations of silver, copper and zinc functioned as respiratory depressants in Perna viridis and Meretrix casta. Baby and Menon (1986) also found that mercury, cadmium and zinc at sublethal concentrations induced reduction in the rate of oxygen consumption in Perna indica.

The load of copper and or silver in the tissue of animals subjected to oxygen consumption studies derived the metal supplied in different salt forms. The rate of oxygen consumption was more when the body burden of copper was high. The fact that the rate of oxygen consumption reduced,

irrespective of internal load when the animals were exposed for a longer duration show that prolonged exposure distinctly reduces oxygen consumption indicating increased stress. Further, regaining normalcy evidenced by increased rate of oxygen consumption after a depurative process, was recorded only in the case of those animals which had shorter accumulative phase. This indicate that irrespective of the load, exposure for a longer duration tampers with the mechanism or the organs involved in respiratory process, which could be either at the cellular level or tissue level and possibly behavioural level also. Perna indica has only limited capacity to regulate oxygen consumption (Hawkins et al., 1986). Discussing the pattern of oxygen consumption in Perna indica, Hawkins et al. (loc. cit.), found that the reduced oxygen uptake during the stress period indicate metabolic inhibition.

The interesting aspects of the results obtained are that the effective concentrations to reduce oxygen consumption showed great differences. While EC 50 was more when the metals were present alone, the combination of metals reduced this value considerably. However, this reduction did not always signify a more than additive reaction indicating that the effect of two metals in the exposure medium could also be independent. Discussing on the combined toxicity of cadmium and mercury on the tropical green mussel, Perna viridis Mohan et al. (1986b) also found similar results.

Various explanations have been put forward by different authors to explain the reasons for decreased oxygen consumption in bivalves in the presence of heavy metals. Mathew and Menon (1983), suggested that changes in respiratory rate induced by the presence of heavy metals depend on the type of metal and experimental animal species. It is apparent that

the reduction in oxygen consumption obtained from whole body analysis indicate the overall performance of the mussels, qualified by behavioural responses like shell valve closure, siphonal activity and the rate of gill irrigation. Therefore, it has been assumed that the recorded reduction in oxygen consumption is a compensation for modified or altered behavioural responses. Brown and Newell (1972) concluded that the reduction in the Mytilus in the presence of copper was due to supression in ciliary activity, rather be the direct inhibition of the respiratory rate. The present finding that prolonged exposure even to lower concentrations of copper and silver reduced oxygen consumption rates even after the animals have been given chance to depurate part of the accumulated metal indicate that the animals are incapaciated to perform normal respiratory action. Various factors are involved which can modify or alter the respiratory rate of a bivalve. Inhibition of ciliary activity in the presence of low quantities of copper ion has not been found by Davenport and Fletcher (1978). While working out the relationship between copper toxicity and oxygen consumption in Mytilus edulis. While working out oxygen tension of the mantle fluid of Mytilus edulis, exposed to different concentrations of copper, Manley (1983), recorded immediate decrease in oxygen tension of mantle fluid and that the decrease was concomitant with increase in copper concentration. Such a thing would not have happened in the present experiment, as the animals were exposed to raw seawater after varying duration of exposure to copper, silver and their combinations in different salt forms. The results therefore, indicate the performance of the animals after increase in the body burden or decrease in the body burden as the case may be. Among the salts employed,

$CuNO_3$ was more toxic than $CuCl_2$. In the case of silver, it was proved that there could be an elevation in oxygen consumption when mussels are exposed to sublethal levels of silver. The present findings support this but continuous exposure to even low concentrations of silver can result in reduction in oxygen consumption. Effects of different salts on oxygen consumption rates is a field which has not been properly experimented on. Apart from direct effect of internal concentrations of metals on oxygen consumption, shell closure ability, rate of ciliary beat, oxygen tension of the mantle fluid can directly influence the rate of oxygen uptake. The ventilation activity of Mytilus could be controlled by periodic closure of exhellant siphon and constriction of external aperture. Widdows et al. (1980), measuring the physiological conditions of mussel transplanted along a pollution gradient found that continuous exposure to a chronically polluted area can result in reduction in oxygen consumption rates and thereby by declension in 'scope for growth'. The condition of the present experiments are comparable since the oxygen uptake rates were assessed in clean seawater after the animals were exposed to polluted media. The results obtained on the additive index show that the interaction between silver and copper or copper and silver in bringing about oxygen consumption responses are less than additive. It may be noted here that copper reduces oxygen consumption and silver increases oxygen consumption. This could be the reason for less than additive reaction.

The trend in the oxygen consumption rates by those animals which have been exposed to experimental conditions so as to result in accumulation of heavy metals in the tissues, and subsequently transfered to raw seawater

offers information regarding two major aspects of the animals. They are, effects brought about by irrepairable damage of respiratory tissue and other morphological features that influence respiratory behaviour; the temperory tissue repair and effects of reduced body burden. Information on such studies is totally lacking in the literature. The present results help us to make a few generalisations only. After depuration, those animals subjected to single metal exposure consume more oxygen when the duration of exposure was less. While in metal combinations irrespective of different salt forms the trend in respiratory rate was erratic. It has been proved that silver alone enhance respiratory rate at lower concentrations. Similarly, low concentration of $CuNO_3$ and $CuCl_2$ enhances respiratory rate when the animals were exposed for a shorter period. This clear cut trend was disrupted depending on either with increase in duration of exposure or the presence of more than one metal salt. With the available literature it is not possible to explain the reasons for such observations. A clear cut picture could be obtained only if the real processes of gas exchange, behavioural response of the animal, the extent of damage of respiratory surfaces and variability in oxygen tension in the mantle fluid which could be influenced by the shell closure abilities and the functional responses of the siphons are properly assessed.

5.2.4. Filtration accompanying accumulation and depuration

Effects of body burden of copper and silver supplied in different salt forms, singly and together, during the process of accumulation and subsequent exposure to raw seawater for depuration was assessed. The objective was to delineate the effects of these metals in the tissue on the rate

of filtration. It was demonstrated that the feeding of mussels was impaired in the presence of pollutants (Abel, 1976). The effects can be measured by simple technique which are potential for rapid evaluation of toxicity test based on sublethal response of ecological significance. Unlike in the case of oxygen consumption studies, the rate of filtration was found to have a direct relationship with the body burden of heavy metals. The presence of copper and silver, singly or in combination, in the whole tissue influence filtration rate. When the quantity in the tissue was more, the rate of filtration was less. No work on combined toxicity and its influence on filtration rate is available for comparison with reference to tropical bivalves. It may be assumed that internal toxicity caused by heavy metals, distinctly affect the filtration rate. The effective concentrations give a clear cut idea on this. Silver in the presence of copper functioned in such a fashion that an higher concentration of silver always resulted in reducing the concentration of copper in bringing about reduction in filtration rate. However, silver in combination with copper produced only less than additive response. Similarly, copper in combination with silver also produced less than additive response. From the results it become apparent that even very low concentration of silver along with copper can result in 50% reduction in filtration activity. The variation in the values resulted in highly significant variation in the rate of filtration. This was so between metal concentrations and between salt concentrations of the same metal.

Mohan et al. (1986b), opined that unlike many systems of potential value as indicators of sublethal stress, the filtration rate is an important one since this activity gives a direct idea on the quantity of water pro-

pelled 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 (1983), Stickle et al. (1984), Prabhudeva and Menon (1985), Mohan et al. (1986b). The rate of filtration can be affected by various factors. To understand the details, it is necessary to delineate the various processes involved in filtration. 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, lying at the posterior end of the mantle. Both the siphons posses velum, which can regulate the current flow. Usually the bivalves reduce the effective area of lamellar contact with the water by means of mucus. Mytilus edulis does not filter in very dilute suspensions. Filtration is initiated at a critical particle concentration. Davids (1964) found that the pumping rate of Mytilus edulis was reduced when the cell concentration increased beyond a particular level. It has been proved that the scope for activity for a mussel varies with environmental parameters. Bayne et al. (1973) found a close agreement between scope for activity and filtration rate. Reduction in feeding rate or energy acquisition by molluscs exposed to petroleum hydrocarbons has been reviewed by Moore et al. (1987, in press). The inhibition of feeding rate in the presence of petroleum hydrocarbons according to various authors in due to the narcotic effects of hydrocarbons (Widdows et al., 1982; Stickle et al., 1984; Johnson, 1977). The present study indicate that Perna indica is more sensitive to the presence of toxicants. The

decrease in the quantity of water filtered and increase in oxygen consumption show that the animals probably retain the water that enter the pallial cavity for a longer duration. Repeated circulation of water within the pallial cavity and water bathing the gills which has considerable coating of mucus would have resulted in reducing the dye concentration. Stepping down of particle number below a threshold limit would have eventually resulted in the cessation of filtration and the animals would have used only the dissolved oxygen in water. How far the behavioural responses of Perna indica, in influencing filtration activity at very low sublethal concentrations of heavy metals, singly or in combination, is unknown. These are areas which should receive further attention.

The principle behind estimating the rate of filtration, by those animals exposed to heavy metals so as to result in accumulation in the tissue and subsequent depuration for a constant duration, was to find out whether the process of accumulation and subsequent depuration affect the rate of filtration. A conspicuous feature of the results obtained in the case of those animals exposed to $CuNO_3$ and $CuCl_2$ even after depuration was that the rate of filtration did not increase in the case those animals which had maximum duration of exposure to toxicants. On the other hand, exposure to 0.5 ppb of copper, either in nitrate or chloride, enhanced filtration rate in the case of those animals exposed for a shorter duration. It may be added here that the body burden of animals exposed for longer duration even after depuration was high. In the case of those animals which had a chance to depurate silver, the rate of filtration was always low. In this respect these animals behaved very similar to those exposed for copper

for longer duration preceeding depuration. However, irrespective of the combinations, the salt form of metals produced significant variation in the rate of filtration by animals even after the depurative phase. The results obtained from the combinations of $CuSO_4$ and $AgSO_4$ when compared with that of $CuNO_3$ and $AgNO_3$ give this idea. Further, the presence of silver, either in sulphate or nitrate form, was much more deleterious than sulphate or nitrate form of copper. This clearly show that in nature when the animals encountered very low concentrations of copper and silver, the quantities of silver would influence the life and activity much more than copper. It may further be added that the locality from where Perna indica was collected recorded a background value of 0.18 ppb of copper and negligible quantities of silver. However, silver has been proved to be more deleterious to Perna indica than copper.

The information available on the effect of depuration on regaining normalcy by marine bivalves is rather scarce. In this respect the present finding on the behaviour of Perna indica subjected to accumulation and depuration are of pioneering nature. It is seen that the results have rised quite a few interesting questions regarding the efficacy of depuration on clencing the animal and bringing back normal activity regimes. This proved that the previous accumulation and depuration history should be throughly followed to arrive at better results. A few questions rised during the course of this discussion require further detailed investigation both at behavioural, cellular and suscellular levels.

SUMMARY

VI SUMMARY

The matter presented in this thesis relates to toxicity of heavy metals like copper and silver, singly and in combination, on the life and activity of Perna indica, the common brown mussel extensively distributed along south-west coast of India. Information is gathered on aspects like individual toxicity, combined toxicity, effects of sublethal exposure on rate functions, accumulation and depuration of toxicants, singly and in combination and performance of the animal when there was body burden by heavy metals. Further, the activity of the animals after depuration is also worked out and presented.

The chapter in Introduction broadly outlines the present status of information on heavy metal toxicity with special reference to marine molluscs.

The Review of Literature which is the next chapter gives information on the important scientific work carried out on heavy metal toxicity with special reference to marine molluscs. The important aspects brought out in the review are lethal effects of heavy metals on marine molluscs. Acute toxicity of metal mixtures and variation in relative toxicity also reviewed. A few papers which deal with toxicity of complex metal-mixtures are also reviewed. Papers dealing with modification of rate functions and behavioural responses are reviewed and the highlights of the work outlined. The capacity of bivalves to regulate trace metal content of body either by accumulation, detoxification or depuration has been discussed from the available literature on these aspects.

The chapter on Materials and Methods gives information on the test

media, test animals and the toxicants. The methodology employed to assess the different rate functions are outlined. Analytical methods involved in the assessment of heavy metal load in water and different tissues are outlined. The statistical methods used to work out the analysis of variance, effective concentrations etc. are also recorded in this chapter.

The chapter on Results presents toxicity studies on individual metals and metal combinations and salt forms of different metals. The results show that among the metals employed silver was more toxic than copper and the sulphate form of the metal is more poisonous than the others.

Accumulation pattern of copper supplied in the form of sulphate in the tissues showed that the remaining tissue contained the maximum copper followed by gills. The rate of depuration was found to be influenced by the internal load. The pace with which copper was depurated from the gill was quicker than adductor muscle.

Depending on the salt forms, there were variations in the rate of uptake of copper. The rate of oxygen consumption and filtration did not show significant difference depending on accumulation resulting from exposure to nitrate and chloride forms of copper. Regaining normalcy, after depuration of copper evidenced by oxygen consumption and filtration, was slow.

Different salt forms of silver influence the rate of accumulation and depuration. Similarly, the rate of oxygen consumption and filtration accompanying accumulation and after depuration also was found to be influenced by the salt forms which were the source of silver. It seems that the animals exposed to silver carbonate were more affected than those exposed to silver oxide.

The metal interaction during accumulation and depuration was analysed. The most important finding was that the presence of increased concentration of silver ($AgSO_4$) reduced uptake of copper ($CuSO_4$). Further, the rate of variation was found to be influenced by concentration of silver in the combinations. This was evidenced by the fact that when the experimental media contained low level of silver and low level of copper, the rate of uptake of copper was high. Drastic variations in the effective concentrations of copper which resulted in 50% reduction in oxygen consumption was noticed when copper was supplied along with silver. The interaction of two metals on the rate of filtration was less than additive.

The rate of depuration of copper and silver was found to be influenced by the internal load of these two metals. The rate of oxygen consumption was found to be influenced by the internal load of these two metals. The rate of oxygen consumption was found to be erratic in the case of those animals which had chance to cleanse their body burden of copper or silver after depuration. Clear cut concentration dependent filtration rate was noticed even after depuration by Perna indica pre-exposed to copper and silver concentrations. Varying concentration of silver and unvarying concentration of copper resulted in enhanced up take of silver. This gives the idea that when the external concentration of silver was high and copper was low, copper uptake was more. Whereas equal concentrations of copper and silver resulted in comparative reduction in silver uptake. Increase in concentration of silver both in the external media and tissue resulted in increase in oxygen consumption, on the other hand, this reduced filtration rate.

The quantity of silver depurated was more when the tissue burden was high. The rate of oxygen consumption by Perna indica exposed to a combination of copper and silver was erratic even after depuration. Further, those animals subjected to depuration for 7 days after pre-exposure did not regain normalcy in filtration rate.

As in the case of sulphate, nitrate forms of copper and silver also depicted similar results. Some relationship exists between copper and silver on the uptake of these metals by Perna indica. Among the three concentrations used viz. 0.25, 0.50 and 0.75 ppb, the trend varies depending on the external concentration. It seems that a concentration dependent reciprocal relationship exists.

There was uniformity in the rate of depuration of copper and silver by Perna indica, when the metals were supplied in nitrate forms, irrespective of the internal load.

The effects on accumulation of silver in the presence of unvarying concentration of copper was assessed. When silver was present alone, the rate of accumulation was external concentration dependent. On the other hand, the presence of copper increase the uptake of silver when the copper concentration was low. The pattern of oxygen consumption obtained from those animals which were maintained in a combination of copper and silver for 7 days, was reduction in the rate. Higher silver concentration along with copper brought about the maximum reduction in oxygen consumption rate. The mean rate of filtration by Perna indica showed declension when they were exposed to a combination of copper and silver.

The rate of depuration of silver was found to vary drastically after the animals had accumulated silver along with copper. Perna indica main-

tained in medium containing only silver during accumulative phase consumed less oxygen only when the silver level was high. Even after depuration this trend was continued. But the trend in oxygen consumption was erratic even after depuration when the animals faced silver and copper together during the accumulative phase.

The chapter on Discussion, enlightens the results obtained in the light of the available literature. It becomes clear that metal combinations, salt forms of heavy metals and same salt forms of different metals influence uptake and depuration processes. Exposure history may have an influence on the reaction of the animal even after depuration. The present findings support already known fact that silver is a highly toxic metal and even at such low concentration of 0.25 ppb can be highly toxic in the presence of elevated concentrations of copper. Further, the findings support the notion that the sulphate salts are more toxic than nitrate salts.

The important papers referred to are listed in the chapter on References.

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