A comparative study of the behavioural responses to heavy metal pollution populations of the common mussel mytilus edulis L, collected from a polluted site(Teesmouth) and a relatively unpolluted site (Lindisfame)

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A comparative study of the Behavioural Responses to Heavy Metal Pollution in populations of the common mussel *Mytilus edulis* L, collected from a polluted site (Teesmouth) and a relatively unpolluted site (Lindisfarne)

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Abstract

This study examined the ecological differences between two populations of the common blue mussel *Mytilus edulis* L. The project investigated the response to heavy metal pollution, administered in the form of metal salts, to individuals from populations inhabiting 'polluted' and 'relatively unpolluted' sites. First an assessment was made of morphological differences and the tissue content of copper, cadmium, lead and zinc. There were few differences in shell morphology, a slight difference in soft tissue weight, and surprisingly, a higher tissue concentration of copper and zinc at the 'unpolluted site'. Normal filtration behaviour was compared with that in the presence of solutions of salts of heavy metal, over a short time (up to 1 hour); this showed distinct differences in tolerance between the two populations in a way which was metal specific. When administered together, doses of metal salts which produced a 30% decrease in filtration rate individually, rarely showed additivity; most seemed antagonistic. There was also a distinct variation in response to equivalent doses of the same metal, and investigation of filtration rate over three hours, both in clean seawater and in the presence of copper II sulphate, showed a fluctuation over time, in a manner which was altered in response to increasing concentration of metal. It was suggested that these variations in basal filtration rate were due to alternation between an aerobic phase and an anaerobic phase. Finally an assessment was made of the rates at which copper, lead, cadmium and zinc were accumulated in the tissues, over several weeks. Doses of single metal salts producing 30% decreases in filtration rate were chosen as the test concentration, though this proved to be fatal over a period of two weeks. This section was not completed for the 'polluted site' samples, as time did not permit further collection, but the experiment was repeated for mussels from the 'unpolluted site' using doses which resulted in a 10% decrease in filtration. While the two populations could not be compared for their bioaccumulation behaviour in this instance, it was shown conclusively that all four metals were uptaken. Overall, it seemed that there was evidence for development of tolerance at both collection sites, with Lindisfarne mussels apparently able to detoxify and store zinc, and possibly copper. Due to the known high quantities of metals discharged from the Tees estuary, the suggestion was made that the Teesmouth mussel population have developed the ability to secrete metal from their bodies.

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Introduction

This thesis is concerned with the effects of mixtures of heavy metal pollutants on the common blue mussel *Mytilus edulis* L., to examine whether the effects are predictable from knowledge of responses of the mussels to individual metals.

Toxicity and marine organisms

Toxicity is an index of how poisonous a substance is; how large a dose is required to kill or damage an organism. The more toxic a substance is, the smaller the lethal dose. Although the concept of toxicity seems straightforward, its measurement is subject to many complicating factors. Toxins vary widely in the route by which they enter the body; e.g. via the mouth, digestive tract, at the gill surface, or across the integument. They also vary in the type and degree of damage they cause to any particular organism. Toxicity tests in the marine environment, or any aquatic environment for that matter, do not administer a dose as such, but expose an animal to a certain concentration of the toxin dissolved in the water. In the natural environment, toxins are rarely present in isolation, and they may interact with other substances. The combined effect of toxins may be;

i. additive
ii. containment of the effect of one within the effect of another,
iii. synergistic
iv. antagonistic.

It is often possible to detect damage by toxins to organisms at far lower concentrations than those that kill. These sub-lethal effects range from little more than physiological adjustment to changed circumstances, to major physiological stress, or developmental abnormalities which in the natural environment would likely result in early death. Pollution incidents may occur either as single events (acute exposure), or over a considerable length of time (chronic exposure). Pollutants can exert detectable effects on living organisms at several different levels: subcellular or cellular, tissue, organ, whole organism, or at the population and community levels of organisation. The definition of a substance as a pollutant requires that deleterious effects can be identified in living organisms in response to exposure to that substance. Study of these various levels together can combine to evaluate whether an organism's response is indicative of some deterioration of health; it could be merely an adjustment to changed, but not necessarily harmful, conditions. Measurement of toxicity can therefore never be a simple process.

Among the more long-lasting (conservative) pollutants are heavy metals. Extraordinarily high concentrations of such metals have been found in some animals in the sea; for instance, 2,000ppm (dry weight) of cadmium in the digestive gland of some scallops, and 57,000ppm of zinc in some oysters. If such concentrations were circulating freely in the animal, they would be lethal, but in at least some animals, methods have evolved for storing metals in the body in innocuous forms. Two methods of such detoxication are known;

1. Exposure to a low concentration of metal induces synthesis of low molecular weight proteins, metallothioneins, which form a complex with the metal, binding it and effectively rendering it inert. These are common in invertebrates; e.g. cadmium binding proteins have identified in molluscs, copper and cadmium are bound by a thionein in crabs and stored in the hepatopancreas, and metallothioneins incorporating zinc, copper and cadmium have been found in the sea urchin *Stronglyocentrus purpureus* (from Clark, 1986). This mechanism cannot deal effectively with high levels of chronic pollution.

2. Storage in granular form, enclosed within a membrane and so isolated from activity within the cell. Various forms of granule have been found; e.g. copper-containing, along with sulphur, or calcium containing, either pure for calcification of the shells of bivalve molluscs, or impure, containing manganese, magnesium, phosphorus and sometimes others such as Zn, Cd, Pb, or Fe. Impure calcium granules seem to be a system for incorporating and detoxifying metals, though the mechanism is not fully understood. Iron granules are also sometimes found, as in the heart urchin *Bryssopsis*, (cited in Clark, 1986). The commercially important shrimp *Penaeus semiculcatus* can reduce body copper and zinc levels during pre and post-moult periods, suggesting that granules are eliminated from the hepatopancreas at these times. However, such detoxication mechanisms will have only a limited capacity; once reached, the animal is then exposed to toxic effects of the heavy metal.

In some species, the capacity for dealing with certain pollutants can be induced by exposure to sub-lethal levels. This probably explains why it is sometimes possible to increase the tolerance of an organism to formerly toxic concentrations. After continuous exposure in the field, the selection of resistant strains has sometimes occurred, such as in Restronguet creek in Cornwall, where contamination with metals such as copper, zinc and arsenic from mining has occurred for more than 200 years, resulting in zinc tolerant *Carcinus maneas*, copper tolerant *Fucus vesiculosus*, and tolerance to both metals in *Nereis diversicolor* (Bryan and Hummerstone, 1973, Bryan 1971). In Bryan's study, *Nereis* sometimes contained over 1000ppm copper, much of

it being deposited in membrane bound vesicles in the epidermis. Results quoted in his 1976 paper suggested that although this type of detoxification mechanism is important, it is not the complete answer, since animals from elsewhere, also with a high level of copper, were not so tolerant to toxic concentrations of copper as found in this estuary. In addition, tolerant animals from Restronguet creek, in which the level of copper had been lowered to normal levels by dilution through increased body mass (by being kept in clean seawater in the laboratory,) retained much of their tolerance. Induction of tolerance does not seem to be a rapid process; Nöel-Lambot (1976) showed that although considerable induction of metallothionein followed the exposure of *Mytilus edulis* to 0.13ppm cadmium for 36 days, little induction followed exposure to 13ppm for 3 days.

The selection of tolerant strains means that a contaminant is exerting an effect which is greater than that to which the original population (before exposure to pollution) can adapt. Thus, comparing the tolerance of different populations of the same species to various pollutants is one way of assessing the pressures to which various ecosystems have been subjected. However it must be remembered that it is the initial accumulation of metals from seawater by phytoplankton which provides much of the momentum for their movement along food chains; organically-complexed metal ions are more readily absorbed by many animals than inorganic forms (Burton, 1979).

A given pollutant may behave differently in many respects in the sea and in fresh water. One major distinction is that a very much greater degree of dilution is available in the sea, so that concentrations are often very low. In fresh water, in comparison, concentrations may exceed lethal levels. More commonly, concentrations are recorded at levels causing sub-lethal toxicity, though it is difficult to relate these to levels as they actually occur in the environment. In the sea, except in heavily polluted coastal areas such as harbours or near waste discharges, concentrations are usually close to or beyond the limits of detection of the best available methods of analysis, and certainly at levels so low as to be impossible to maintain under experimental conditions. Oceanographers have long recognised that heavy metal concentrations in the sea are not only very low, but some orders of magnitude lower than their chemical equilibrium concentrations. This is through the rapid removal of dissolved ions from the water column, by processes of absorption, adsorption, precipitation, sedimentation, and their deposition in sediments. Presumably the same processes occur when levels are augmented by anthropogenic inputs.

The problem with experimental toxicity tests, is that concentrations of a few milligrams of heavy metal in seawater readily precipitate, and so their effective biological availability is limited. Thus there is little relevance in conventional lethal toxicity tests in the context of marine pollution. Sub-lethal toxicity testing poses difficulties in keeping the organisms in the laboratory for long periods, in the methods of measuring responses, and in the significance of those responses. The conceptual difficulties arise essentially from the fact that we cannot readily determine whether a marine organism is healthy or 'normal'. Generally, diagnostic techniques and our knowledge of the normal physiology and biochemistry of the organisms we are studying are inadequate. The range of potentially valuable techniques is wide, but the knowledge of 'normal' is frequently insufficient for identification of 'abnormal'.

Lloyd (1972) explains the difficulty with the aid of figure 1 (shown below), which demonstrates a hypothetical relationship between physiological impairment following exposure to pollutants, and the consequent disability of the organism. Measured values of physiological or biochemical variables, alteration in the behaviour of the animal or the histological appearance of the tissues, may represent conditions within the areas of the graph marked 'homeostasis' or 'normal function maintained without significant cost', even though measured values are significantly different from control values. An organism subjected to any environmental change, including the presence of a pollutant, will make physiological and biochemical adjustments to the new environmental conditions. A statistically significant difference between treated and control animals indicates only that a change has taken place; it does not necessarily indicate that the change is deleterious to the organism. Statistical significance is not the same as biological significance. The problem is to identify the point at which the value of a measured variable deviates from the control so far that it falls outside the range which is normal for a healthy animal.

Bayne et al (1979) suggest three main considerations to apply when examining the biological responses of individuals for the assessment of effects of pollution;

i. Responses for examination must give an integrated representative measure of the individual's quantitative relationship to the environmental stressor.

ii. The response must be linked quantitatively with the effect on the ecological fitness either of the individual, or the population, or both. This relationship is also complex, as it is dependent upon seasonal changes in the physiology of the animal, and the resultant spatial and temporal variability in the demands made on the animal by its ecological niche.

iii. There is a requirement for sensitivity and specificity. Cytological and biochemical responses are likely to prove more sensitive to environmental changes than some physiological or "whole animal" responses.

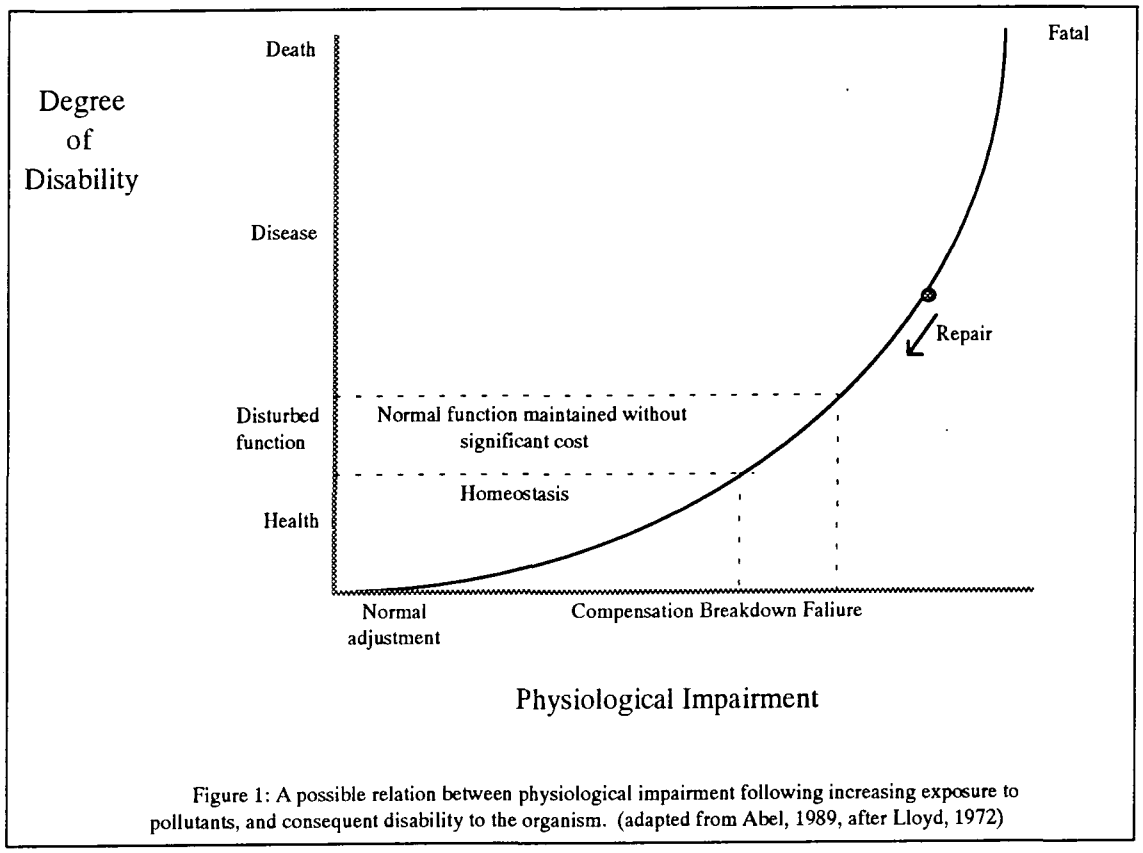

Figure 1: A possible relation between physiological impairment following increasing exposure to pollutants, and consequent disability to the organism. (adapted from Abel, 1989, after Lloyd, 1972)

Bayne et al (1979) used biochemical techniques to assess the response of the mussel *Mytilus edulis* L. to pollutants; measurement of biochemical parameters in animals from different natural populations were used to derive a model which allows prediction of growth rates. The predictions agree well with observed growth rates in the field. Some animals were transplanted from their natural environments to polluted locations, and subsequent analysis showed that they had responded biochemically to the polluted conditions as was predicted. Stebbing (1979) argued that instead of carrying out toxicity tests and seeking to compare the results with the environmental concentrations, it may be more useful in the marine situation to use suitably sensitive organisms to bioassay the seawater. He used the colonial hydroid *Campanularia flexuosa*, grown in seawater from a polluted habitat, to show that alterations in the pattern of growth occurred. Growth rates reverted to normal after the water was treated to remove all traces of the pollutant.

Mytilus edulis L. as a bioindicator

The common blue mussel *Mytilus edulis* L. is a sessile, filter-feeding bivalve mollusc, common around most of the coastline of the British Isles, and throughout the world. Various authors have investigated the response of *Mytilus* to pollution in the environment, and it was chosen as one of the target species for the "Mussel Watch" project, (Goldberg et al, 1978). With its widespread distribution, and general resilience to stressful environments, it has great potential as an indicator species. The animal is found around coastal areas wherever holdfasts are available for anchorage of the shells to a substrate, from the middle to lower tide marks. Their life span can be over 10 years in favourable conditions, so they have great potential in examination of the longer-term effects of pollutant exposure on tolerance, bioaccumulation and local adaptation. Gametogenesis occurs in late spring-summer around British shores, and the young are released into the plankton. Spat settlement can occur wherever the ocean currents will carry the young forms, and they have the ability upon settlement to relocate to more favourable conditions if those are not amenable. The muscular foot, or byssus, is the means of attachment to a solid substrate, producing proteinaceous threads which anchor the shell to the surface. These threads have been found to be one route of elimination of toxins from the visceral tissues, e.g. $^{239,240}Pu$ and often ^{241}Am in both *M. edulis* and *M. californianus* (Goldberg et al, 1978).

Mytilus feeds and respires by means of a water current drawn into its body under the influence of ctenidial cilia. The filtration rate of bivalves i.e. the volume of water per unit time drawn into the animal's body, is known to be influenced by environmental parameters such as salinity, temperature, dissolved oxygen and concentration of suspended matter (Foster-Smith, 1975, McLusky and Bryant, 1986). Bayne et al (1979) found differences in absorption efficiency of mussels from different sites, according to the organic content of the suspended particulate matter, (though this efficiency diminishes if particles less than 2-3μm in diameter). They also found that oxygen consumption varies during the year, with high values in the spring and early summer, and lower values in the winter. Seasonal variation is due largely to the annual gametic cycle of the mussels. They report that although the seasonal pattern was similar in all populations, there were differences in the intensity of metabolism.

The feeding and respiratory mechanism of the mussel, and hence the filtration rate, is impaired by the presence of pollutants in low concentrations. Abel (1976) proposed that this response was potentially suitable for use as a screening and monitoring test, by assessing the decrease in filtration rate using the absorption of

neutral red dye. Concentrations of a range of common pollutants, which reduced the filtration rate to half its control value, were approximately equal to the 96hLC_{50}. In a further study, however this time using the Mediterranean species *M. galloprovincialis*, Abel and Papanthanassiou (1986) found that the accuracy of the method was limited for screening and monitoring. However Axiak and Abel (1991) suggested that limited accuracy and even demonstrable environmental relevance are of less importance than speed and reliability, and the ease of convenience with which test organisms may be maintained in the laboratory, and routinely used in toxicity testing. They state that screening tests provide useful information, but that this cannot be used to assess environmental impact, nor to compare the toxicity of different pollutants or the effects of environmental conditions, or to determine environmentally safe levels of pollution. Bearing this in mind, in this thesis I examined two populations of *Mytilus* from comparable sections of the same coastal regime, for their responses to the same dosage ranges of the metal salts, to investigate any evidence for differences in physiological adaptations between them.

Remarkably little is known about the processes by which pollutants cross the absorptive surfaces of marine organisms. It seems likely that there is affinity between heavy metals and the transport of calcium, such as occurs in the crab *Carcinus maneas* (Wright, 1977). Most of the evidence for metals and their radionuclides points to uptake being passive, although this does not exclude some sort of carrier mediation. In filter feeding organisms, it is often quite difficult to determine experimentally the relative importance of uptake from solution, food, inorganic particles or oil droplets. It has been shown for *Mytilus* that metals such as zinc, manganese, cadmium, and selenium must largely be absorbed from suspended particles (Pentreath, 1973, Fowler and Benayoun, 1977). Schulz-Baldes (1974), who fed lead-contaminated phytoplankton to *Mytilus*, also concluded that uptake from the food was important. Uptake of particles is not confined to the digestive system; hydrated ferric oxide can be taken up at a significant rate through the gills by pinocytosis, and transferred to other tissues via circulating amoebocytes. This uptake process appears to be energy dependent, and uptake of lead and the metalloprotein ferritin has also been observed (Coombs and George, 1978).

Many of the factors that influence rates of absorption are those which have been recognised as those having an important influence on the acute toxicity of pollutants, (detailed in Bryan, 1976). The chemical, or sometimes the physical form, of a contaminant in the water, sediment or food, is a very important determinant of its rate of uptake. For *Mytilus galloprovincialis*, Fowler and Benayoun (1976), showed

that selenium in the form of selenite was absorbed from solution much more readily than selenate, and for *Mytilus edulis*, George and Coombs (1977) found that prior complexation of ionic cadmium with EDTA, humic acid, alginic acid or pectin doubled the rate of uptake. Whatever the absorption rate or mechanism, bivalve molluscs are not red to generally considered to be metal regulators, since concentrations have usually been found to reflect the availability of metals in the environment (Bryan, 1979). In this respect, they could potentially be used as a qualitative indicator of pollution, if not a quantitative one.

Metals as pollutants

Many metals are essential to the biochemistry of living organisms; metals are components of various functional protein molecules, e.g. haemoglobin (Fe), haemocyanin (Cu), Vitamin B_{12} (Co), and many enzymes; e.g. carbonic anhydrase contains zinc, and glutathione peroxidase contains selenium. The so-called "trace-elements" are definitely required by higher vertebrates, namely iron, iodine, copper, manganese, zinc, cobalt, molybdenum, selenium, chromium, nickel, vanadium, silicon and arsenic (Muntau, 1984). Other elements which might be essential at "ultra-trace" levels include lithium, aluminium, tin, fluorine, lead and cadmium (Anke et al, 1984). However, definite biochemical roles have only been identified for iron, zinc, copper, selenium, molybdenum, cobalt and iodine, and their presence is specific to a particular enzyme or metabolic pathway.

Metals exert toxic effects on animals if they enter into biochemical reactions in which they are not normally involved. The threshold concentration at which such deleterious effects occur is usually higher for essential elements than for non-essential elements, although the "window of essentiality" for some elements such as selenium, is quite narrow. Metals of biological concern can be divided into three groups (after Clark, 1986):

i. light metals (sodium, potassium, calcium etc.) transported as mobile cations in aqueous solutions;
ii. transition metals (e.g. iron, copper, cobalt and manganese), essential in low concentrations but may be toxic in high concentrations;
iii. metalloids (e.g. mercury, lead, tin selenium and arsenic), usually not required for metabolic activity and cytotoxic at quite low concentrations.

Transition metals and metalloids are usually collectively termed heavy metals, though the distinction between heavy and non-heavy metals is often unclear. Many

authors have defined a metal as heavy if it has a relative density of >5, but often the term is applied to those which are neither heavy, (e.g. aluminium), nor are metals in the strict sense (e.g. selenium). To identify their biological importance as pollutants, the classification system developed by Nieboer and Richardson (1980), which uses Lewis acid properties (i.e. "hardness" or "softness" as acids and bases) to separate metals into class A (oxygen seeking), class B (sulphur or nitrogen seeking) and borderline elements, seems the most practicable to date.

In my choice of metals for investigation, I included manganese (class A), zinc, iron, lead, chromium, cobalt, nickel (borderline) and cadmium, copper and mercury (class B), along with aluminium and selenium. The last two are not strictly heavy metals, but from a 'pollutant' perspective have significant inputs to the North Sea circulation; hence their inclusion in this study. All were used in the form of metal salts, (listed in table 3 of the methods section), to investigate their effects in inorganic form.

Metals in seawater:- some experimental considerations

An element or compound in a natural water system will generally be distributed between a variety of physico-chemical states, shown in figure 2, below. The distribution between these states, as well as the total concentration, varies between environments, e.g. rivers, coastal seas and open oceans, sediments with their associated pore waters and the overlying waters. There are also differences in physico-chemical forms between pollutant inputs, and their receiving waters and sediments. These facts place inherent limitations upon the validity of conclusions, drawn by extrapolation from findings on experimental systems, regarding the biological consequences of the presence of pollutants in natural environments, and the biological implications of variations in base-line concentrations. Some studies on bioaccumulation and toxicity have been carried out using pollutant concentrations so high as to be environmentally unrealistic.

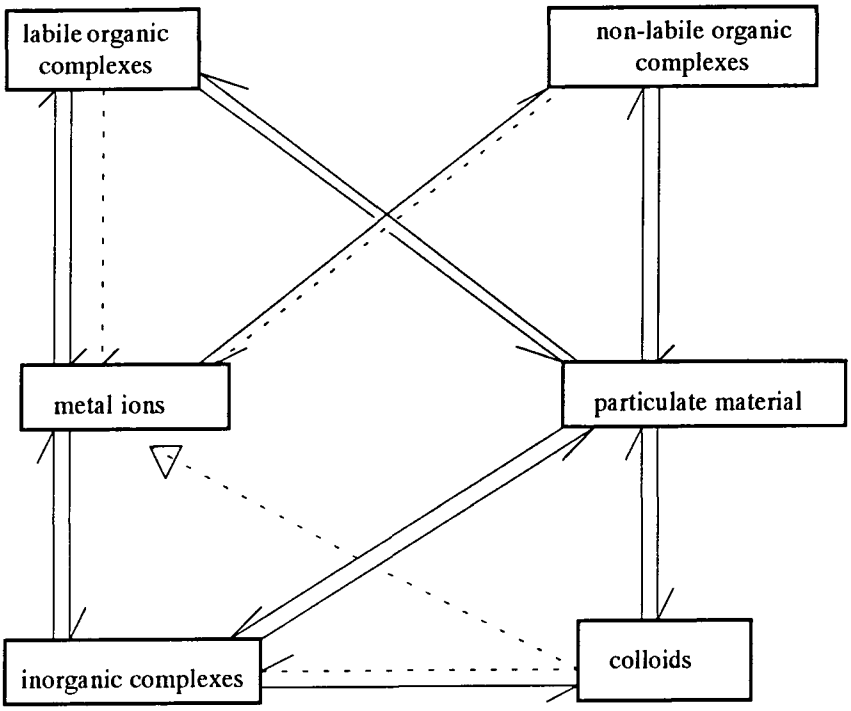

Figure 2; Principal physico-chemical interrelations between the forms in which trace metals occur in natural waters (adapted from Whitfield, 1975)

The concentration and composition of suspended particulate material varies greatly in natural waters. An average concentration of 1 mg/l has been reported for coastal water, while that for open ocean surface waters is of the order of 100µg/l (Chester and Stoner, 1972). Estuarine concentrations may rise to 1g/l or higher. Distributions of metal concentrations vary between the particulate and dissolved fractions, according to the nature of the suspended material, so aquatic systems can modify significantly the environmental consequences of discharged wastes. High concentrations of iron in acidic wastes for example, are converted to particulate ferric hydroxide, which is capable of scavenging other ions from solution. Mercury shows high associations with particulate phases in the presence of high concentrations of suspended material, and such associations influence biological availability. In experimental systems, other practical problems arise as a result of the tendency of some materials to be adsorbed onto solid surfaces, such as the container vessel for test organisms. This may cause the reservoir of dissolved metal to diminish rapidly due to uptake onto the container surfaces, the external surfaces of tissues, and faecal material, (Burton, 1979).

A number of elements can exist in more than one oxidation state in oxygenated seawater, and in many cases the distribution of an element between different oxidation states corresponds to a steady state, rather than an equilibrium condition. Reduced

forms of a number of elements may be introduced either directly into the marine environment, or formed in situ by biological processes. If the rate of oxidation is slow relative to that of input or formation, the redox speciation may differ from that corresponding to equilibrium in the sea water medium. Elderfield (1970) found that for chromium, in contrast to the equilibrium prediction that CrIV should be the only significant form of the element in seawater, from 12% to over 90% of the dissolved chromium in coastal seawater was present as CrIII, suggesting a high kinetic stability for the reduced state. Since the toxicity of metals such as selenium and chromium varies according to the oxidation state, information on environmental redox speciation is essential for the assessment of biological implications of inputs, the rates of oxidation of unstable reduced forms and their formation by biological processes. In studies of bioaccumulation or toxicity in experimental systems, the oxidation state of the metal ion may change during the course of the experiment.

In seawater, the activities of the ionic components, (both the major constituents and the trace elements), can be greatly influenced by the presence of other ions. In a natural water system, a trace metal such as copper will be associated to a significant extent with hydroxyl ions and the major dissolved anion. The activities of the free major anions, with the exception of chloride, are considerably modified by associations with major cations such as sodium, potassium, calcium etc. In average river water, dissolved copper will be almost entirely complexed with humic material, but these complexes may be less important as the salinity increases. This stresses the importance in experimental studies of reproducing closely the major composition of the medium which it is intended to simulate. The relationship between electrochemically active and inactive forms might be regarded as an approximate guide to biological availability in cases where uptake by organisms occurs in solution, though there has been no general agreement on the way in which organic complexation influences biological activity.

Input routes of pollutants to the marine environment, particularly the North Sea

Metals are natural constituents of seawater, and assessing the effect of inputs resulting from human activities is complicated by the very large natural inputs from the erosion of ore-bearing rocks, wind-blown dust, volcanic activity, forest fires and vegetation. Most river systems make a major contribution of metals to the sea, the nature of the input depending on the occurrence of metal and ore-bearing deposits in the drainage area. Where the river passes through urban or industrialised centres, the metal is augmented by human wastes and discharges.

The intense sedimentation in estuaries traps a large quantity of metals which become adsorbed onto sediment particles and carried to the bottom. Sediments in industrialised estuaries with major ports contain the legacy of a century or more of waste discharges. Regular dredging of shipping channels in such areas produces large quantities of dredging spoil, heavily contaminated with metals, which is usually dumped at sea.

A third input route is via the atmosphere; atmospheric processes are still poorly understood, and estimates of inputs are considerable, though do vary widely. Large natural inputs of some metals such as aluminium arrive in wind-blown dust derived from rocks and shales, and mercury from volcanic activity and degassing of the earth's crust. For some metals however, e.g. arsenic, cadmium, copper, nickel, lead, selenium and zinc (Clark, 1986), inputs to the atmosphere as a result of human activities are several times greater than natural inputs. Smaller quantities of metal are added to the sea by direct discharges of industrial and other wastes by pipeline, and in sewage sludge and other wastes dumped at sea. Though small, these may be locally significant if introduced to areas of poor circulation.

The North Sea is a semi-enclosed system, of 575,000 km^2. The southern end is constricted at the straits of Dover, and is shallow, with depths mostly less than 50m. The northern part is 120-145m deep, with deeper water off the Norwegian coast. It is not a homogeneous body of water, and a number of areas can be distinguished , between which water exchange is relatively slow. One of these encompasses the north-east coast of England and the east coast of Scotland, (within which both of the sample sites for this investigation lie). Flushing time in areas such as this can vary between 46 and 333 days, though surface water to a depth of 10m is exchanged more rapidly. During summer, all parts of the North Sea receive an excess of water by precipitation, and as the seawater becomes decreasingly saline, stratification of warm, less dense water often occurs. Thermoclines may develop in the eastern sections, which result in reduced concentrations of oxygen in the bottom water. The North Sea is used intensively; it is a heavily trafficked sea lane, gas and oil are extracted from central and northern areas, and gravel and sand for the construction industry is dredged in places. Much of its coasts and estuaries are highly urbanised and industrialised, and it acts as recipient for the wastes from these, together with those carried in a number of major rivers; the Rhine, Elbe, Weser, Scheldt, Ems, Thames, Trent, Tees and Tyne. It also provides the site of highly productive fisheries, which are intensively exploited. Much of the sewage input is untreated, or given only primary treatment.

Estimates of inputs of heavy metals to the North Sea vary widely, but clearly the atmosphere, rivers, and dredging spoils are the major contributors. Inputs from sewage sludge and industrial wastes are small in comparison. Sludge is discharged from the Netherlands coast, and dumped at sea by the United Kingdom, adding to the high input from rivers, along with nitrogen and phosphorus, derived from runoff from agricultural land. Input of organochlorine pesticides and PCB's is small, as both have declined in use, though leaching from agricultural land where they were formerly used, still continues, and enters via river systems. Atmospheric inputs, though large, are dispersed throughout the whole of the North Sea, and so river inputs are locally more evident. Inflows from British rivers disperse more rapidly than that from the continental river systems, which tends to remain in the coastal strip, and results in their higher metal concentrations. Mercury and lead tend to associate with particulate matter, rather than remaining in solution, and contaminate coastal sediments. Oil inputs are significant, though uncertainties exist concerning the size of atmospheric and river inputs.

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Section 1

Physical Comparison of the Two Populations

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Introduction

(a) Choice of sample site.

Polluted Site; mussel beds at Redcar, south of the Tees estuary, were chosen as sampling sites for the "polluted environment"; the coastal regime of southwards-flowing longshore drift transports effluents from the mouth of the Tees past this site. The Tees has a long history of pollution, resulting from chemical and related industries. Redcar is approximately one mile down the coast from Teesmouth; the beach has outcrops of horizontally bedded strata providing an anchoring site for the animals, of a uniform level with regard to tidal coverage. Animals of a similar size from the same area were presumed to be of roughly equivalent age, and subject to similar pollution levels. A map of the site is shown in figure 3.

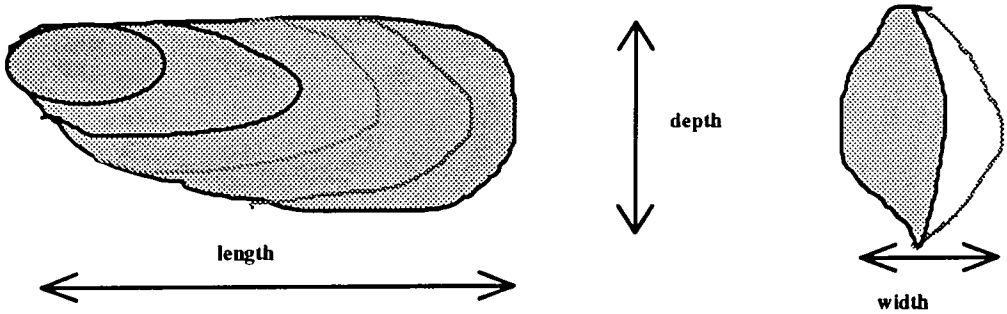
Unpolluted site; the foundations of the causeway bridge, carrying the road link between the island of Lindisfarne and the mainland, provides an anchorage site for mussels. This area is also southwards of a river estuary, so has roughly similar salinity conditions to Redcar, though it is relatively unpolluted, inputs being from the road traffic. Though possibly tolerant to the mild lead pollution from ammunition used during the shooting season, these mussels are presumed to be unused to the presence of other heavy metals in their locality. Animals similar in size to those collected from the Redcar site were harvested, presuming that these would give an appropriate sample for comparison. A map of the site is shown in figure 4.

(b) Examination of physical variables

The first question was that of any significant difference between the two populations in terms of morphology. I needed to establish whether physiological and behavioural differences investigated later tests could be affected by physical variations between the two samples. Both sets of animals were from coastal regimes subject to the same southwards longshore drift and extremes of climate; both situated south of a river outlet. Any developmental differences could be attributable to two possibilities; exposure to wave scour (the Lindisfarne site is more sheltered than the Teesmouth site), or differences in pollutant levels between the two environments.

Growth form in the common mussel is determined by the prevalent environmental conditions; different regimes can result in significant variations. Seed (1968) examined growth rate in mussels in terms of changes in length with respect to time, and also the change in one body parameter relative to other parameters; he found that *M. edulis* growth rate slows with increasing age, and changes with habitat. He also found changes in shell shape according to age and locality; and that environmental rather than phenotypic factors determined growth form. Smaller size categories of mussels from all the habitats investigated showed uniformity in gross shell morphology. Only the larger size categories had differences in form, and these were more marked in older specimens. He concludes that differences are due to the environmental conditions to which the animals are subjected, and the degree of divergence is directly proportional to the time spent under those conditions.

Age in molluscs can be measured either by the increase in dimensions, or by growth rings on the shell. These rings, found in many molluscs, are indicative of diminished or arrested growth, such as during winter when temperatures are lower and food less abundant. Seed (1968) found that in a normal year, 90% of the total annual growth occurred between April and September, giving one major ring each year, though he states that their detection may be difficult due to shell erosion. Priest (1975) found that *M. edulis* collected from areas similar to my present study, had growth rings obscured due to the energetic coastal regime. I also experienced difficulty in identification of age using the growth rings, so have instead used an index of size, comparing length, depth and area of the shells, along with shell weight, and the dry weight of soft tissue. Dimensions used are shown below; area is calculated as [½ (length x depth)].



Using mussels of similar length and area, it was hoped to eliminate physiological variations due to age, and compare only the environmentally caused variations. Coe and Fox (1942, 1943), found that *M. californianus* has deeper, less wide, and thinner shells in less exposed areas. Priest (1975) found larger weight/length ratios for *M. edulis* at Lindisfarne than at Seaton Carew, a site north of Teesmouth; concluding that the Lindisfarne site was (surprisingly),more exposed. As growth in

animals is generally logarithmic, I have also compared shell weight with length, both on logarithmic scales as weight is unlikely to depend linearly on length. (The logarithmic relationship will confirm any statistically significant differences.)

Priest found that ratios involving dry weight of soft parts/shell length and soft parts/shell weight, both give proportionally larger soft tissue mass in relation to size at Lindisfarne, as compared to Seaton Carew. She concludes that Lindisfarne mussels are larger in size, so therefore are older or faster growing, and the shells are thicker for a given length or area; also soft parts are heavier at Lindisfarne than those with comparable shell areas at Seaton Carew. The length/width ratios at Seaton Carew confirmed the idea of increased shelter. Limits to this test are the differences in age of mussels of similar size. Seed (1969) found that animals live longer where they have slow growth (e.g. the high littoral area), and also that the extent of growth is limited by environmental conditions, as the transfer of old animals (of the maximum size range for that habitat) from a less favourable to a more favourable site, produced an increase in linear growth.

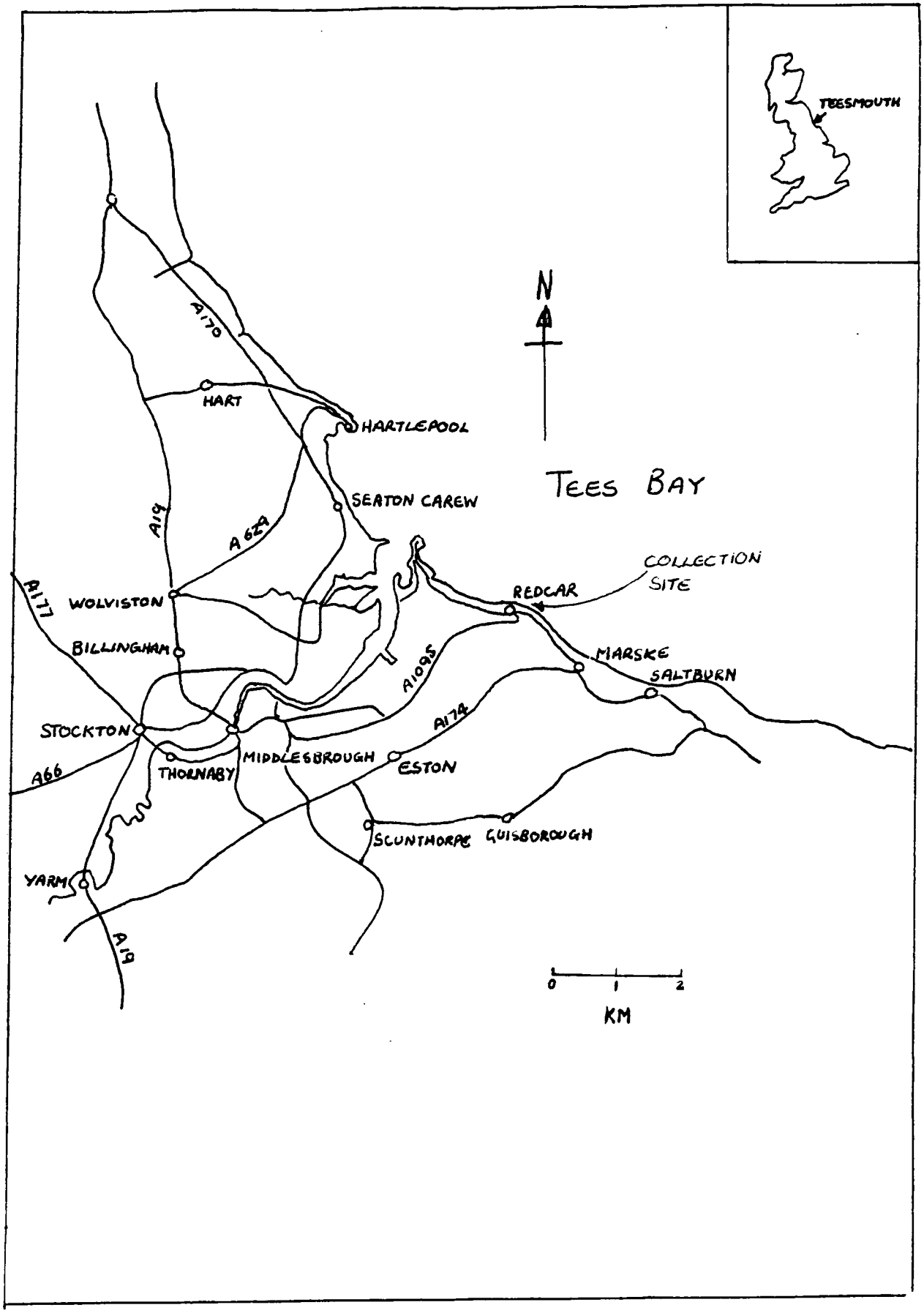


Figure 3; Map of the Tees Bay area, showing the mussel collection site (Redcar), in relation to the Tees estuary and surrounding industrial centres

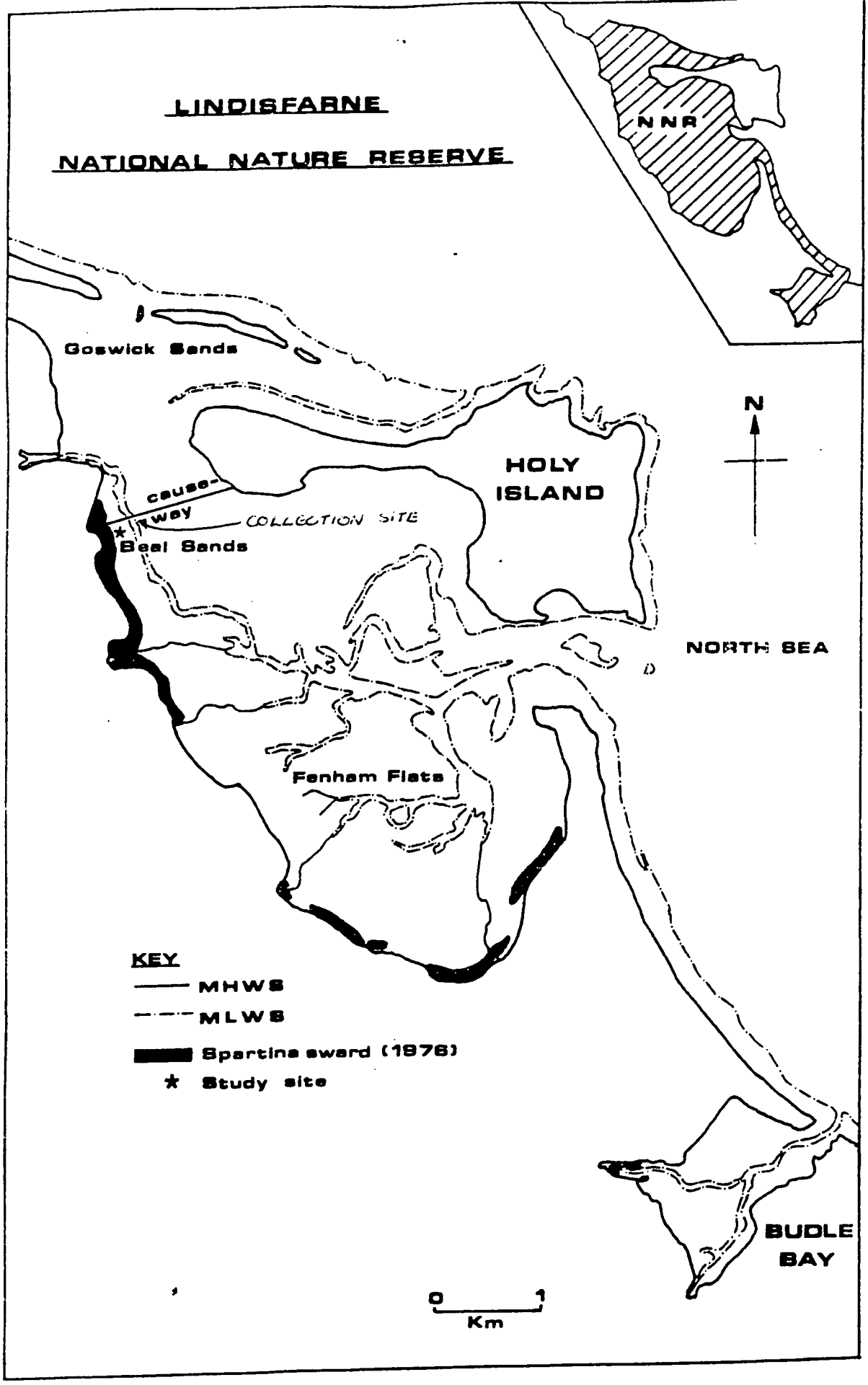


Figure 4; Map of the Lindisfarne National Nature Reserve, showing the mussel collection site (the causeway bridge)

Methods

Adult animals from Teesmouth and Lindisfarne, (the largest specimens present at each site,) were collected and kept in fresh seawater for 48 hours; this allowed the mussels to empty their guts. 30 from each sample area were treated in this way, the shells scrubbed clean, and then deep frozen. Upon thawing, the soft parts were removed from the shells, and dried in a vacuum oven at 50°C for 72 hours. Individual dry weights of soft parts, and shell weight and dimensions were recorded for each animal; the two data sets were then examined for any significant differences between the populations. F-tests were performed on the data initially, to ascertain the similarity of variances between samples, and then t-tests assuming equal or unequal variances were performed, as appropriate.

The pollution status was compared by analysis of the dried soft tissue for the presence of certain heavy metal contaminants; lead, cadmium, copper and zinc. Each animal's dry tissue was weighed into a clean dry conical flask, and 5-10 ml of analytical grade concentrated nitric acid was added. A glass funnel was placed in the neck of each flask. These were left overnight in a fume cupboard at room temperature, and then simmered gently on a hotplate for 12-24 hours (to reflux), the glass funnels removed and the liquid evaporated off. When the residue was just dry, it was cooled, and re-dissolved in exactly 5ml of strong (3M) hydrochloric acid, filtered into a polythene bottle, (rinsed previously with acid and dried,) and stored in the cold. Samples were later analysed for heavy metals using a Pye Unicam SP9 Atomic Absorption Spectrophotometer, calibrated with solutions of known concentrations of each metal.

The SP9 was calibrated for zinc, cadmium, lead and copper, using a series of dilutions made from a concentrated stock solution of the metal salt, in distilled water. After allowing sufficient time for the lamps to warm up, the absorption maximum of appropriate wavelength for the lamp used was found, and the energy level adjusted, before setting the zero. Recordings were then made of the absorption for each standard solution, before recording the absorptions for the samples. Calibrations, followed by sample recordings were made for each metal; readings for any one metal were made on all samples at the same time, for both the 'initial' samples, and the samples from the later bioaccumulation experiment. Calibration curves were produced from averages of three readings at any one concentration, and plotted in figures 5 to 10. These averaged numbers fell on a linear scale.

Figure 6; Calibration curve for Cadmium 0-1ppm (Atomic Absorption Spectrophotometer)

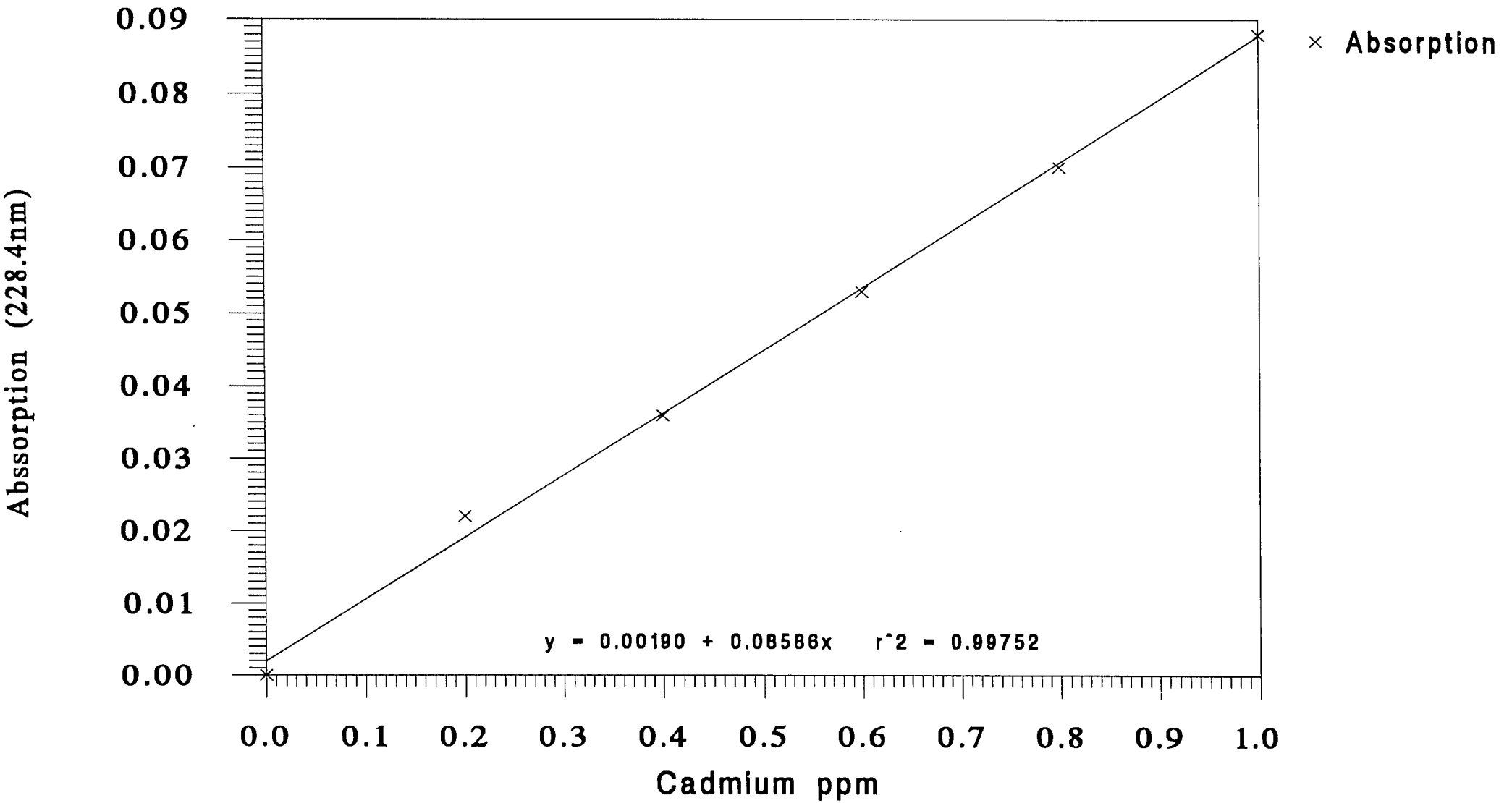


Figure 7; Calibration curve for Cadmium 0-10ppm (Atomic Absorption Spectrophotometer)

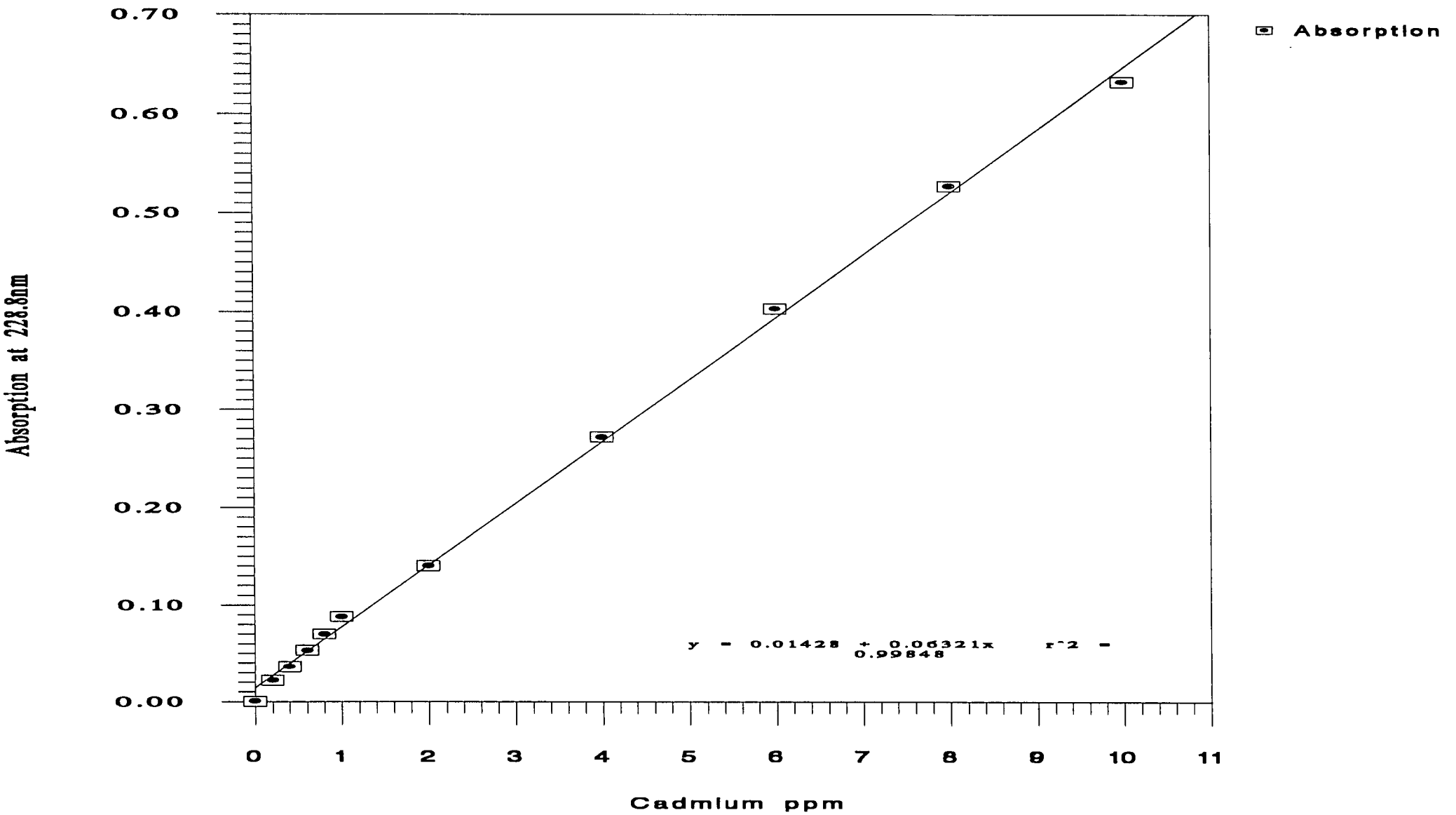


Figure 8; Calibration Curve for Copper 0-5ppm (Atomic Absorption Spectrophotometer)

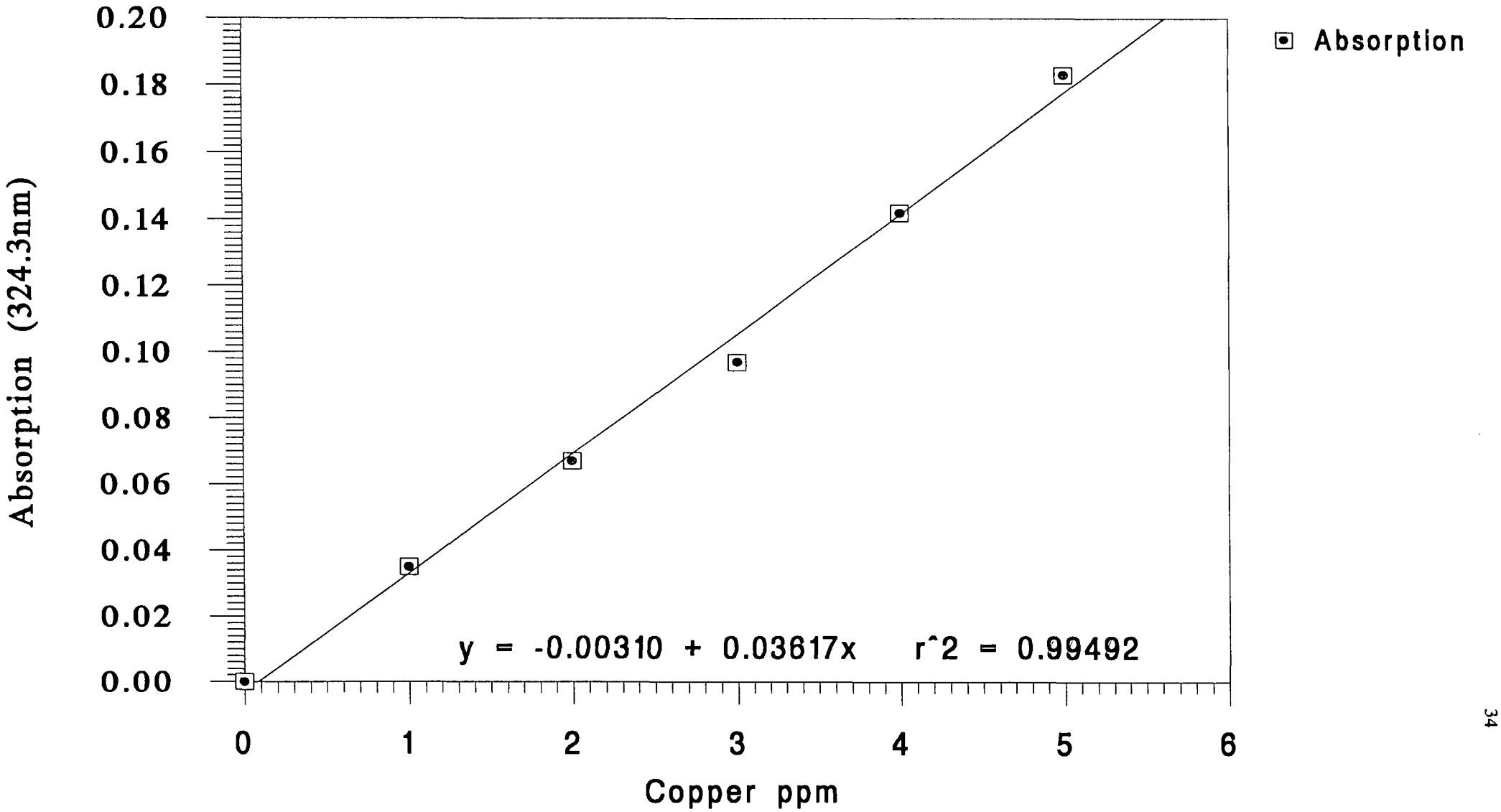


Figure 9; Calibration Curve for Lead 0-5ppm (Atomic Absorption Spectrophotometer)

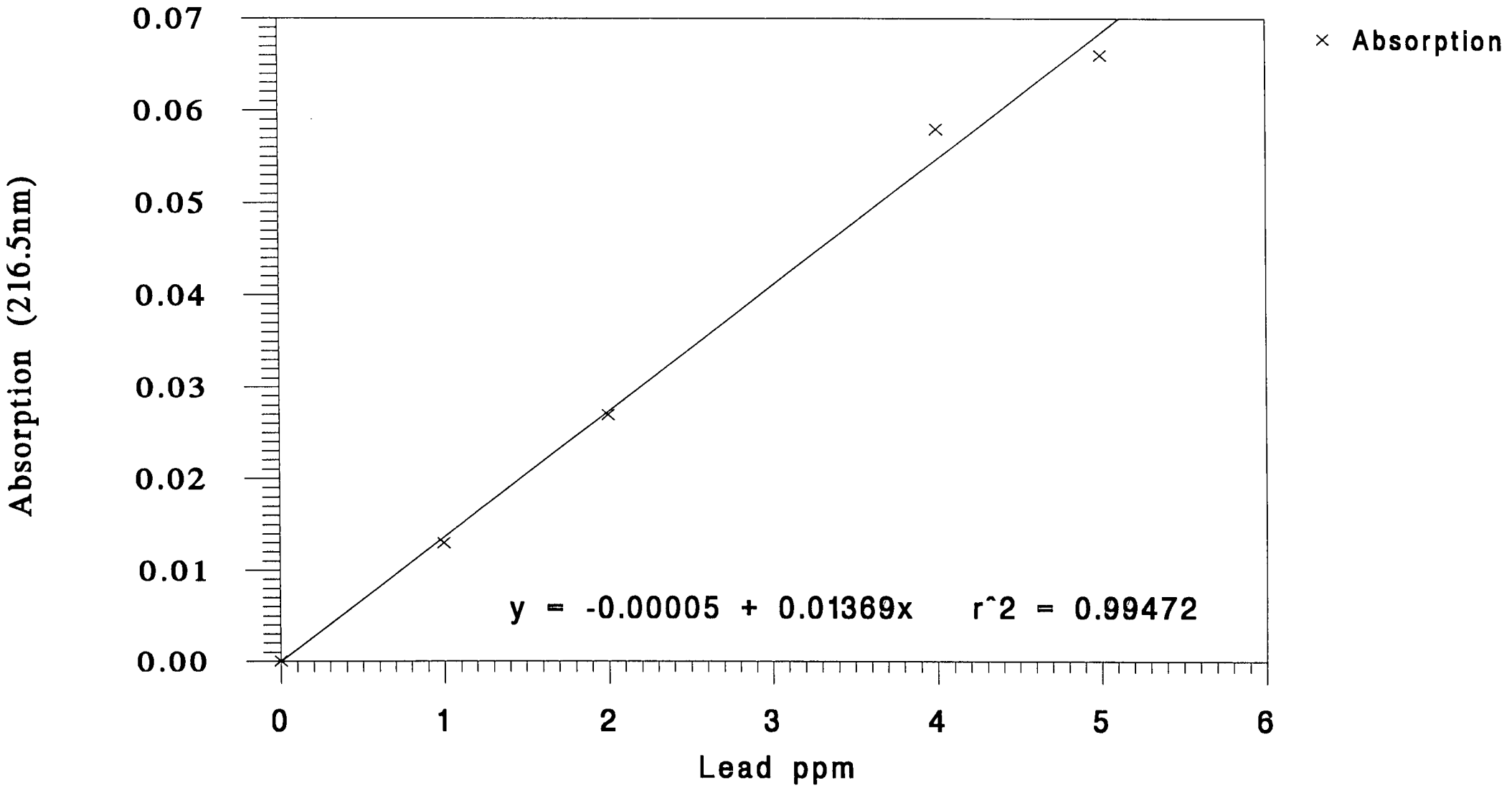


Figure 10; Calibration Curve for Zinc 0-2.5ppm (Atomic Absorption Spectrophotometer)

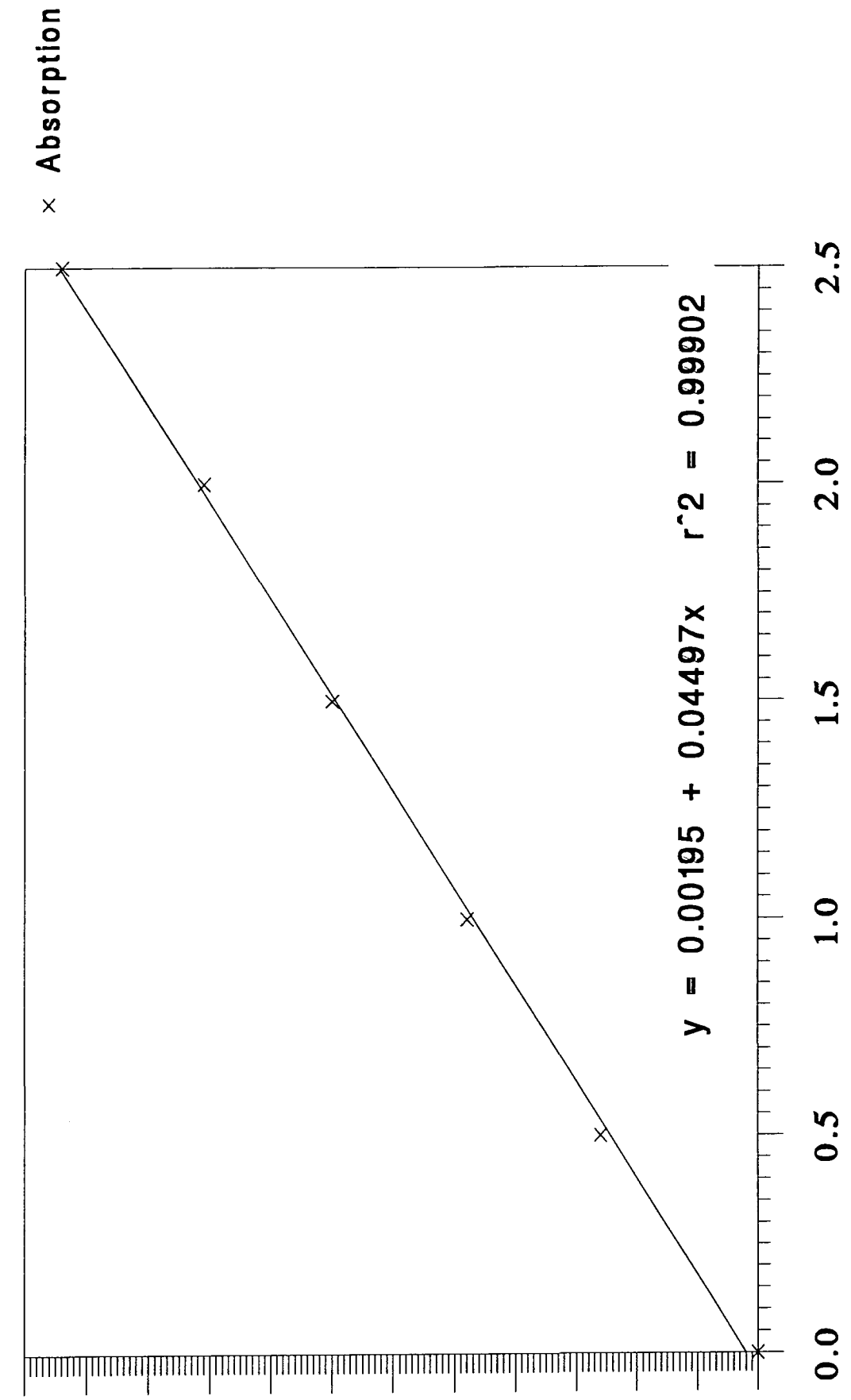
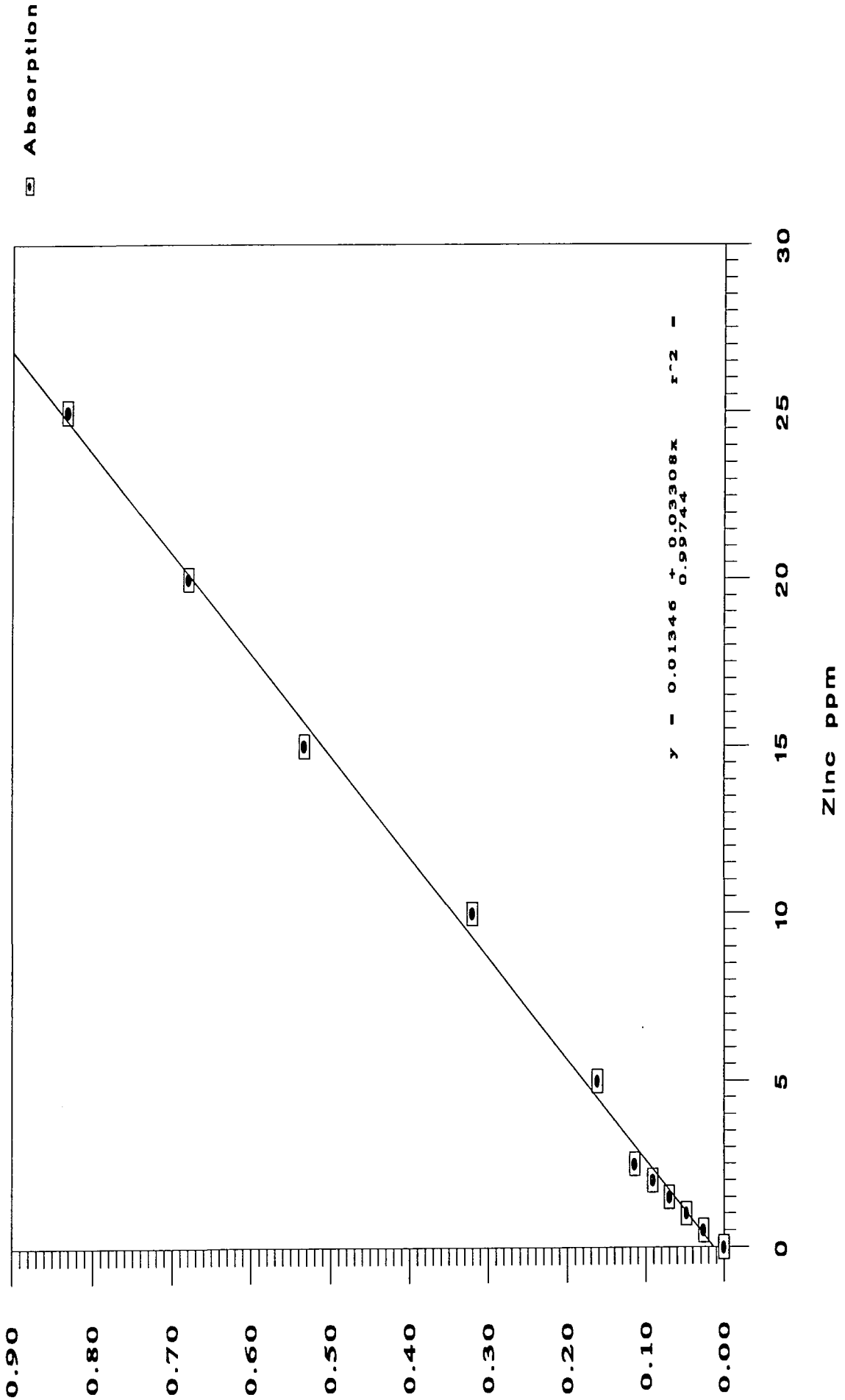


Figure 11: Calibration curve for Zinc 0-25ppm (Atomic Absorption Spectrophotometer)



Results

The results of the morphological analyses are displayed in Table 1a shown below, along with the mean values for each data set, and the standard error. The data used, and more complete information on the statistical tests are displayed in Appendix 1. Calibration curves for the spectrophotometer are shown in figures 5-10 inclusive, and the results are shown here in Table 1b. (The mean values with standard deviations are displayed graphically in figures 25 to 30, with the results from the bioaccumulation experiments.) Table 1a shows significant differences only between the dry weight of soft parts, and the ratios shell length/width and soft tissue weight/shell length. Thus the Teesmouth samples have shells which are less wide in relation to length, and also have a greater mass of soft parts in relation to shell length. The shells themselves in terms of length, weight and area did not show significant differences between samples.

Samples from the two sites showed significant differences in tissue heavy metal levels. Teesmouth mussels had greater concentrations of lead and cadmium; Lindisfarne mussels had a slightly higher level of copper in their tissues, and surprisingly, a significantly higher level of zinc, almost an order of magnitude greater than at Teesmouth. This was definitely not due to contaminated equipment, as the same batch of polythene bottles were cleaned and used concurrently for both populations. Had the bottles been contaminated, then such "freak" high values should have appeared in the Teesmouth analysis as well. The Lindisfarne samples were consistently higher.

Table 1a; Comparison of Morphology of samples of large *Mytilus edulis,* taken from sites at Lindisfarne and Teesmouth

(Statistically significant values of P are shown; others are not significant [NS])

	Lindisfarne population (mean value)	Teesmouth population (mean value)	t-test	d f.	P
soft tissue (g)	0.472 +/-0.024	0.583 +/-0.034	-2.63677	51	0.01>p>0.001**
shell length (mm)	53.191 +/-0.913	54.608 +/-0.727	-1.226	55	NS
shell width (mm)	28.478 +/-0.476	27.668 +/-0.312	1.423	46	NS
shell depth (mm)	23.354 +/-0.484	23.452 +/-0.291	-0.173	43	NS
shell weight (g)	10.322 +/-0.615	10.376 +/-0.410	-0.073	46	NS
area (mm^2)	761.985 +/-24.925	758.503 +/-17.620	0.116	55	NS
length/width	1.869 +/-0.019	1.974 +/-0.016	-4.012	55	p < 0.001***
depth/width	0.821 +/-0.012	0.850 +/-0.012	-1.699	55	NS
length/depth	2.284 +/-0.023	2.331 +/-0.026	-1.240	55	NS
shell weight./soft tissue weight	22.817 +/-1.477	19.421 +/-1.236	1.505	55	NS
weight/length	0.191 +/-0.008	0.189 +/-0.005	0.344	47	NS
log shell weight/ log length	0.576 +/-0.012	0.579 +/-0.008	-0.079	55	NS
soft tissue weight/ length	0.009 +/-0.0004	0.011 +/-0.0006	-2.091	45	0.05>p>0.01*
weight/area	0.013 +/-0.0004	0.014 +/-0.0003	-0.376	55	NS

Table 1b; Heavy metal concentrations in soft tissue of Mytilus edulis, taken from Lindisfarne and Teesmouth

(Concentrations are in mg/gram of dry weight tissue)

	Lindisfarne population (mean values)	Teesmouth population (mean values)	t-test	d.f.	P
Copper	16.74 ± 0.646	14.38 ± 2.919	0.791	31.82	0.05>p>0.01*
Lead	29.77 ± 2.374	47.72 ± 3.925	-3.915	47.05	p<0.001***
Cadmium	2.53 ± 0.088	3.72 ± 0.286	-3.980	34.37	p<0.001***
Zinc	111.73 ± 13.589	13.31 ± 1.166	7.216	26.38	p<0.001***

Discussion

Coe and Fox (1942, 1943) found that *Mytilus californianus* had deeper, less wide and thinner shells in less exposed areas, as already cited. Similarly, Williamson (1907) found mussels from wave-swept shores to be rounder, thicker and less deep. In comparing samples of the larger mussels available from Lindisfarne and Teesmouth, the main shell difference appears to be that Teesmouth shells are less deep. This could similarly be due to the more exposed site at Teesmouth. The orientation of the coast confirms this; at Lindisfarne, tidal energy would be dissipated in travelling over the sandflats to the collection site, shown in figure 1. At Redcar, the beach has a steeper morphology and would receive the full brunt of tidal scour. A second factor could be age; The Lindisfarne shells were much more encrusted with barnacles than those from Teesmouth. This could conceivably be due to greater stability for the Lindisfarne mussel population, which suffers less from freak pollution incidents and collection for human consumption. The maximum size of animals will be limited at Teesmouth, due to the intensive collecting by the local people, and also greater exposure to storm damage.

Seed (1968), in examining the effects of density on shell shape by physical compression, suggests that owing to their orientation in the mussel beds, increase in shell depth would be restricted more than length in dense populations. He also concludes that shell morphology as in relative values of length and depth, are modified by growth rate, i.e. the Lindisfarne shells could be slower growing. The two populations were of apparently similar density, so I conclude that shell differences are due more to differential exposure to tidal scour, and probably to different growth rates at the two sites.

The greater mass of soft parts in the Teesmouth sample could be attributable to the presence of pollution, selecting at the stage of spat settlement for animals with greater tolerance; perhaps conveyed via larger relative body volume to shell size, which could better deal with the toxicant. This young form of *Mytilus* is the most vulnerable to mortality induced by pollutant stress. The high output of organic effluent from the Tees river could provide an abundant source of nutrition both for the local plankton, and the shellfish, resulting in this larger body mass. Availability of oxygen may also have a part to play in this; Lindisfarne sediments have a higher clay content (and is therefore less aerobic) than Teesmouth, with a less energetic coastal regime, (though this distinction may be offset by the basal oxygen demand of pollutant discharges from the Tees). Priest (1975) found that Lindisfarne mussels had a greater soft tissue mass

than at the less exposed site of Seaton Carew. Similarly the greater mass of tissue of the Teesmouth mussel sample with respect to Lindisfarne, may be associated with its more exposed position.

A further physiological reason for the differences in body mass, could result from the variant collecting times in my study; the Lindisfarne population were sampled approximately 4 weeks after the Teesmouth population. This time could have coincided with spawning in the Lindisfarne population, resulting in a loss of body mass from release of gametes. This would give a weight loss relative to the pre-spawn state, which may appear statistically significant. Certainly physiologically, feeding rates are altered according to spawning state, which fluctuates through the season, increasing in early spring, as feeding also increases to provide for somatic growth and the sexual cycle (as reported by Bayne, 1979).

I consider one possible cause of the difference in soft tissue mass, to be a response to the evident exposure to zinc of the Lindisfarne sample, perhaps to a level which has resulted in tissue damage. Certainly the tissue concentrations of zinc have increased since 1975; Priest (1975) recorded tissue levels of between 50 and 60 ppm zinc for mussels collected from near the Lindisfarne causeway. Lead levels were then recorded as between 2 and 4 ppm for large animals, though lead is known to accumulate over time. Loss of body mass has been reported for *Mytilus* in the presence of iron as hydrated ferric oxide, facilitated through an increase in production of pseudofaecies, and loss of organic matter through the secretion of additional mucus (reported in Clark, 1986). A similar situation may have occurred at the Lindisfarne site, though presumably this has not reached the critical threshold beyond which the animals are unable to recover. Whichever explanation is appropriate, it is evident that the Lindisfarne population have adapted some mechanism of rendering the metal inert and storing it in the body; perhaps in granular form.

The surprisingly low copper and zinc concentrations in the Teesmouth mussels with respect to the Lindisfarne sample, is echoed in a study of the ragworm *Nereis diversicolor*, sampled from the same sites (Whetman, 1988). This study quoted metal concentrations (of dry weight tissue) at 26.65 ppm for lead, 0.84 ppm for cadmium, 42.00 ppm for copper and 457 ppm for zinc, in animals sampled from the causeway area at Lindisfarne. On nearby Fenham flats (shown in figure 4), these values are considerably different, at 14.10 ppm for lead, 1.58 ppm for cadmium, 35.35 ppm for copper and 238 ppm for zinc (average values). Samples taken from Teesside have 8.64 ppm lead, 1.05 ppm cadmium, 21.38 ppm copper and 335 ppm zinc (average

values). *Nereis* is a burrowing organism, and so would be expected to have higher tissue levels of such pollutants than *Mytilus,* a non-burrowing bivalve, as metals are easily accumulated in estuarine sediments. Though levels in *Nereis* are higher than my results for *Mytilus*, there is a similar unexpectedly higher tissue concentration of copper and zinc at the Lindisfarne causeway sampling site, in comparison to Teesmouth animals.

It is known that discharge from the Tees estuary contains high levels of both copper and zinc. Evans and Evans (1993) quote median concentrations of dissolved metals, recorded in surface waters of the main Tees river channel to have varied between 32.5, 51.5 and 47 μg/l for zinc, for 1990, 1991 and 1992 respectively. Median copper concentrations were recorded as 6.5, 9 and 18 μg/l for the same years; cadmium concentrations were 0.25, 0.85 and 0.19 μg/l, while lead levels were 2.5, 20.5 and 2 μg/l. They also quote maximum concentrations of dissolved metals over the Teesmouth SSSI's for 1992 over an order of magnitude higher than the channel concentrations for cadmium, and lead, while zinc and copper are over double that in the river channel; these increases illustrate what happens to pollutant loads upon entering the esturaine environment. The tissue lead levels in the Teesmouth sample are similar to the concentrations quoted as the *maxima* at the three SSSI's; copper is lower than the value recorded at Bran Sands for 1992 (59 μg/l) but higher than at Seal Sands and Greatham Harbour (both 7 μg/l), zinc varies between 2 and 8 times lower, likewise for cadmium.

In the light of these results, I suggest that the Teesmouth mussel population have undergone some evolution of tolerance mechanisms to enable tham to have lower than ambient metal levels in their tissues. Perhaps the Teesmouth population have developed the ability to excrete accumulated metal from the body. The Lindisfarne population have apparently some pollutant input which provides the zinc, possibly from the stream which drains under the causeway, though this carries only runoff from surrounding farmland and a campsite. The Lindisfarne mussels seem to have developed a storage capacity which functions as a detoxication mechanism. It could be that this is an induced response, while the apparently greater ability of the Teesmouth mussels to not accumulate high tissue metal concentrations, could be an 'evolved' function.

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Section 2

Effects of Metals in Solution on the Filtration Rate of *Mytilus edulis*

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Methods

(a) Conditions for keeping the live animals

The mussels were stored in aquarium tanks, in a constant temperature room at 8°C, producing conditions similar to those of the North Sea. A maximum of around 80 mussels were kept in each, tank in 4 litres of seawater, taken from the collection site, each with an aspirator. The water was replenished at regular intervals. Food, comprising a mixture of yeast, ground spinach (as an algal substitute) and powdered milk, (the recommended feed for fish fry) was added, in the absence of a ready supply of marine algae. Animals not exposed to heavy metals survived well on this mixture. The storage room operated with a photo-cycle of 6 hours of darkness and 18 hours daylight, and the tanks were kept covered to reduce evaporation.

(b) Procedure for measuring filtration rate

The protocol used was adapted from Abel (1979), who suggested using reductions of the filtration rate in *Mytilus* as an indicator of pollution. The vital stain Neutral Red is readily taken from solution (in seawater) by the gill tissues, upon filtration. Though not 100% efficient, the rate of uptake can be presumed to be a constant for the duration of the experiment, and so can be treated as a standard value by which to compare feeding/respiration in the presence and absence of added metal salts.

Samples of mussels of similar shell length (40-60 mm), were put into a container of seawater, aerated and effectively agitated by aspiration, and allowed to recover from handling for approximately 20 minutes. A concentrated solution of neutral red (10 mg/ml in distilled water) was added to the seawater to give a final concentration of around 5 mg/l. (This is fivefold the concentration used by Abel (1979); it was found that a 1 mg/l solution in seawater from the sample sites, precipitated so quickly that it could not be analysed effectively. Using a more concentrated solution overcame this problem, and the dye was still taken up effectively by the mussels.) After gentle mixing, taking care not to touch the animals, a 10 ml sample was taken initially, and then again at 10 minute intervals. These samples were analysed using a Pye Unicam spectrophotometer set at 425nm, with seawater as a blank. Concentrations of neutral red chloride were determined by comparison of the spectrophotometer readings with a standard curve (shown in figure 5), obtained from solutions of known concentration. These values were put into the equation shown

below, to determine the average individual filtration rate of the mussels used (after Abel, 1979).

$$M = \frac{V}{n \cdot t} \log_e \frac{Co}{Ct}$$

M = filtration rate in ml/minute
V = volume of water in test container
n = number of animals used
t = duration of experiment (10 mins)
Co = concentration at time 0 (initial)
Ct = concentration at time t (final)

As there was great individual variability in behaviour, it was decided to use a sample of 20 individuals per test vessel, to give a better estimate of the mean response of the population. Each batch of animals was kept in 2l of water for the duration of the experiment, for determination of a standard curve for the filtration behaviour in the presence of each metal salt used. A similar number, in similar volume, were also used to determine performance in the presence of mixtures of metals. Concentrated solutions of the metal salts listed in table 2, were made up in seawater, so an appropriate volume of concentrate could be added successively to each test vessel, giving the strength of solution being investigated. After settling time the filtration rate was determined. Metal chlorides and sulphates were used, to give a soluble solution of maximal availability of the metal ion, with minimal toxicity from the base compound.

Table 2; Heavy metal compounds used for investigation

Metal Compound	Concentration of stock solution
Aluminium Potassium Chloride (BDH)	0.4g/100ml
Cadmium Sulphate (BDH)	0.4g/100ml
Chromium III Chloride hexahydrate (Sigma)	0.2g/100ml
Cobalt Chloride hexahydrate (Sigma)	0.2g/100ml
Copper II Sulphate anhydrous (BDH)	0.2g/100ml
Iron III Chloride (BDH)	0.4g/100ml
Lead II Nitrate (BDH)	0.4g/100ml
Manganese II Chloride (BDH)	0.2g/100ml
Mercuric Chloride (BDH)	0.1g/100ml
Nickel Chloride hexahydrate (Sigma)	0.2g/100ml
Selenium Dioxide (Sigma)	0.2g/100ml
Zinc Chloride (BDH)	0.4g/100ml

Metal concentrations which produced a 30% decrease in filtration rate (i.e. the 70% values from the previous determination), were used to investigate the effect of combinations of the metal salts. This was done by determining the resting initial filtration rate of a batch of mussels, followed by addition of the 30% decrease dose of one metal, determination of the decrease in rate, then further addition of a dose of the other metal, and subsequent measurement of filtration. I used only the Teesmouth mussels for these tests, as not only difficulty in collecting so much material from

Lindisfarne, but also potential variations in tolerance between the two population samples, may have made it inappropriate to compare the different results. I was interested primarily in the interaction of metal salts on filtration. Again for reasons of resource limitation, smaller numbers of animals were used; the interactive determinations were made for batches of 10 individuals, in 1 litre of water, (hence the ratio of water volume to numbers was the same as for the single metal response determinations). The results are displayed in table 6.

The filtration rate decreased roughly exponentially in response to single metal salts. Therefore additive effects (acting on the same metabolic pathways), would not appear as a linear decrease, but follow the exponential scale. Synergistic interactions (which act either on different pathways, or on convergent stages of the same pathway) would be above this value, decreasing more than a further 30%. Containment of the action of one metal by another would affect the same stage of a pathway, and not lead to a great change in filtration rate. Antagonism would mean some interaction between metals which decreases their capacity to affect a metabolic process. Although borders are somewhat arbitrary, I have set limits for these values;

90% and above	= antagonism (ant)
89% - 61%	= containment (con)
60% - 50%	= addition (add)
49% and below	= synergy (syn).

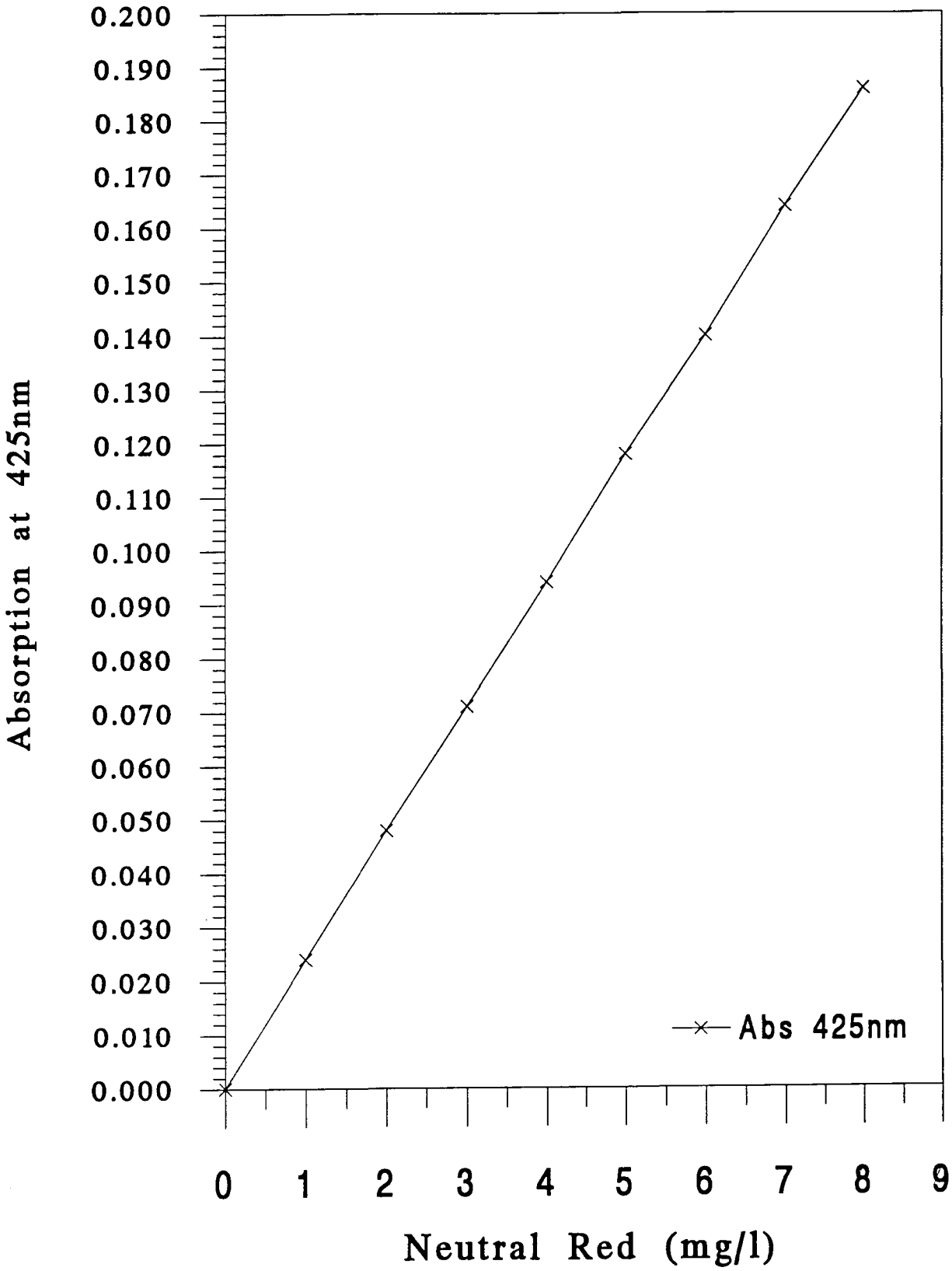
Abbreviations in brackets are used in the results table below the relevant percentage of resting filtration rate values. The difference between 'containment' and 'antagonism' is likely to be a combination of interactive and metabolic effects, so the difference between these two terms may be somewhat academic in this case.

The behavioural response to individual metals for each batch of mussels, often showed fluctuations, as can be seen from the graphed results. Rather than steady decreases in filtration rate, the measurements often fluctuate erratically, rather than producing a smooth dose-response curve. Also, the synergy/antagonism 'control' experiments tended to show similar variation. Thus I decided to investigate briefly, what fluctuations (if any), usually occurred in the filtration rate. For this I examined the response to one metal, copper II sulphate, over a period comparable to the duration of the filtration experiments with one batch of mussels; around three hours.

Sets of 10 fresh mussels were used, set up in a concentration of $CuSO_4$ as follows; 0.0 (control), 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/l, in 1 litre volumes of seawater. After allowing 20 minutes settling time in clean water, the clock

was started, and the filtration rate measured. Copper II sulphate was then introduced to each container, giving the appropriate concentration, and the filtration rate was then measured after approximately every 20 minutes, for 10 minutes each time. There was no visible amount of neutral red dye present in the water after the intervening time between determinations of filtration rate; I have presumed that any residual amount would not significantly affect the ratios of dye in subsequent determinations, and so obscure the behaviour pattern. If it had, the effect would have been an apparent slow decline in filtration, rather than the fluctuating patterns as found. After 140 minutes, the water was replenished with clean, (copper-free) sea water, and a further two subsequent measurements were taken. The results are shown in the series of graphs displayed in figure 24.

Figure 5; Calibration Curve for Neutral Red Chloride (in Seawater)



Results

(a) Pilot experiment

An initial trial of Abel's methodology, using samples of 5 mussels per test vessel, tended to give variable results with the Teesmouth mussel population. The filtration rate was determined for 6 individuals in various concentrations of zinc chloride, and the results are shown in table 3, below.

Table 3; Effect of Zinc Chloride on the filtration rate (% of value in clean seawater) of individual mussels

mussel	Concentration of Zinc Chloride (mg/l)					
	0	0.25	0.5	1.0	2.0	4.0
1 (%)	100	151	39	0	0	0
2 (%)	100	142	35	41	38	29
3 (%)	100	163	74	85	55	32
4 (%)	100	115	91	85	38	34
5 (%)	100	164	86	55	42	50
6 (%)	100	140	64	79	62	67

(b) Comparison of the effects of metals on the two populations of *Mytilus*

The procedure as outlined above was performed for the two populations, first with clean water, and then in the presence of varying concentrations of solutions of the metal salts. Standard curves were plotted as best fit lines for three determinations of filtration response for any given metal. Actual volumes of water handled during tests for initial or "resting" filtration rates varied between batches, and even varied for the same batch on different occasions. However the percentage changes for filtration rate appeared reasonably consistent, regardless of the initial volume handled, and so the graphs were constructed using these percentage rather than absolute values. Exponential curves were fitted to the pooled data from three replicate experiments, to reconstruct the type of graph obtained by Abel (1979).

From these curves, the concentration of each metal producing on average the 70% value of resting filtration rate (a 30% reduction) and the 50% value (a 50% reduction), could be determined. Though I have used the 50% value for comparison with other work, (as this is the usual value quoted,) taking both this and the 70% value gives a more appropriate idea of the response of the animals, i.e. a gradual or more steep decline in tolerance. The standard curves for behaviour in the presence of each metal were compared using the percentage decreases for increments of increasing metal concentrations, obtained from the graphs of best fit for 3 determinations. 10

percentage pairs were compared for each metal, using the "paired t-test" statistical procedure, to clarify significant differences in physiological tolerance by the two populations. Table 4 displays the 70% and 50% values along with the paired t-test results, the graphs are shown in figures 12 to 23, and the data used, with more detailed statistical information, are tabulated in Appendix 3.

It is evident that significant differences between the two populations exist in their initial response to heavy metal salts. The shape and gradient of the exponential curves appear similar, but are displaced to the right or left. No single population appears better able to handle the spectrum of pollutants, but clear distinctions exist between responses to individual metals. Responses were not significantly different for nickel and zinc, though actually fell between the 5% and 10% levels; I have used the 5% level of significance in these tests. Teesmouth mussels were better able to tolerate aluminium, cadmium, chromium, cobalt and lead, while Lindisfarne mussels have greater tolerance for copper, manganese, mercury, nickel and selenium, in the inorganic form administered. Degrees of significance varied; differences between the two sites for zinc and nickel were "not significant", for iron III was "probably significant", and for the others the difference was "highly significant". Thus differential tolerances did occur between populations as represented by my samples.

Table 4; Concentrations of metal leading to a decrease in filtration rate to 70% or 50% of the value in clean seawater.

Values are obtained from best-fit curves of graphs, each showing data from 3 determinations. (Only significant values of P are shown,; others are not significant [N/S])

Metal salt	Lindisfarne population (mg/l)		Teesmouth population (mg/l)		t	d.f.	P
	70%	50%	70%	50%			
Aluminium	16.1	25.9	18.0	28.0	17.25	9	p<0.001***
Cadmium	3.4	5.6	4.6	8.4	7.24	9	p<0.001***
Chromium	7.6	13.1	10.9	18.4	7.99	9	p<0.001***
Cobalt	3.8	8.0	4.6	9.6	10.11	9	p<0.001***
Copper	0.20	0.50	0.06	0.32	-6.56	9	p<0.001***
Iron III	6.0	10.6	7.2	12.1	2.58	9	0.05>p>0.01*
Lead	1.65	4.00	2.15	4.55	11.78	9	p<0.001***
Manganese	7.0	13.8	5.6	10.4	-10.723	9	p<0.001***
Mercury	0.036	0.074	0.004	0.023	-13.43	9	p<0.001***
Nickel	5.8	10.6	3.0	9.8	-1.97	9	N/S
Selenium	14.3	20.0	11.5	16.9	-14.46	9	p<0.001***
Zinc	3.4	8.2	3.6	7.8	0.162	9	N/S

Figure 12a; Lindisfarne sample; response to Aluminium Potassium Chloride

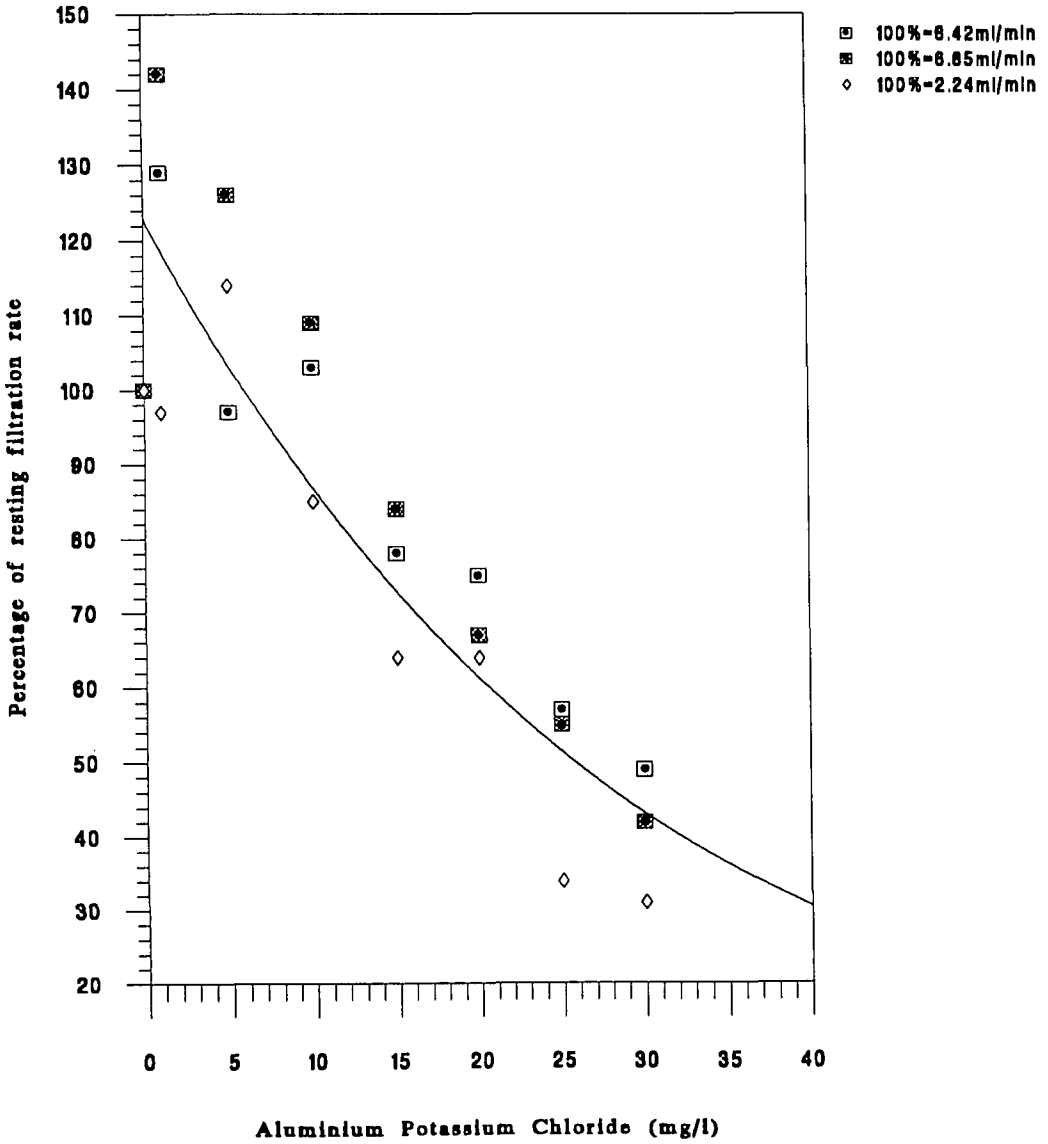


Figure 12b; Teesmouth sample; response to Aluminium Potassium Chloride

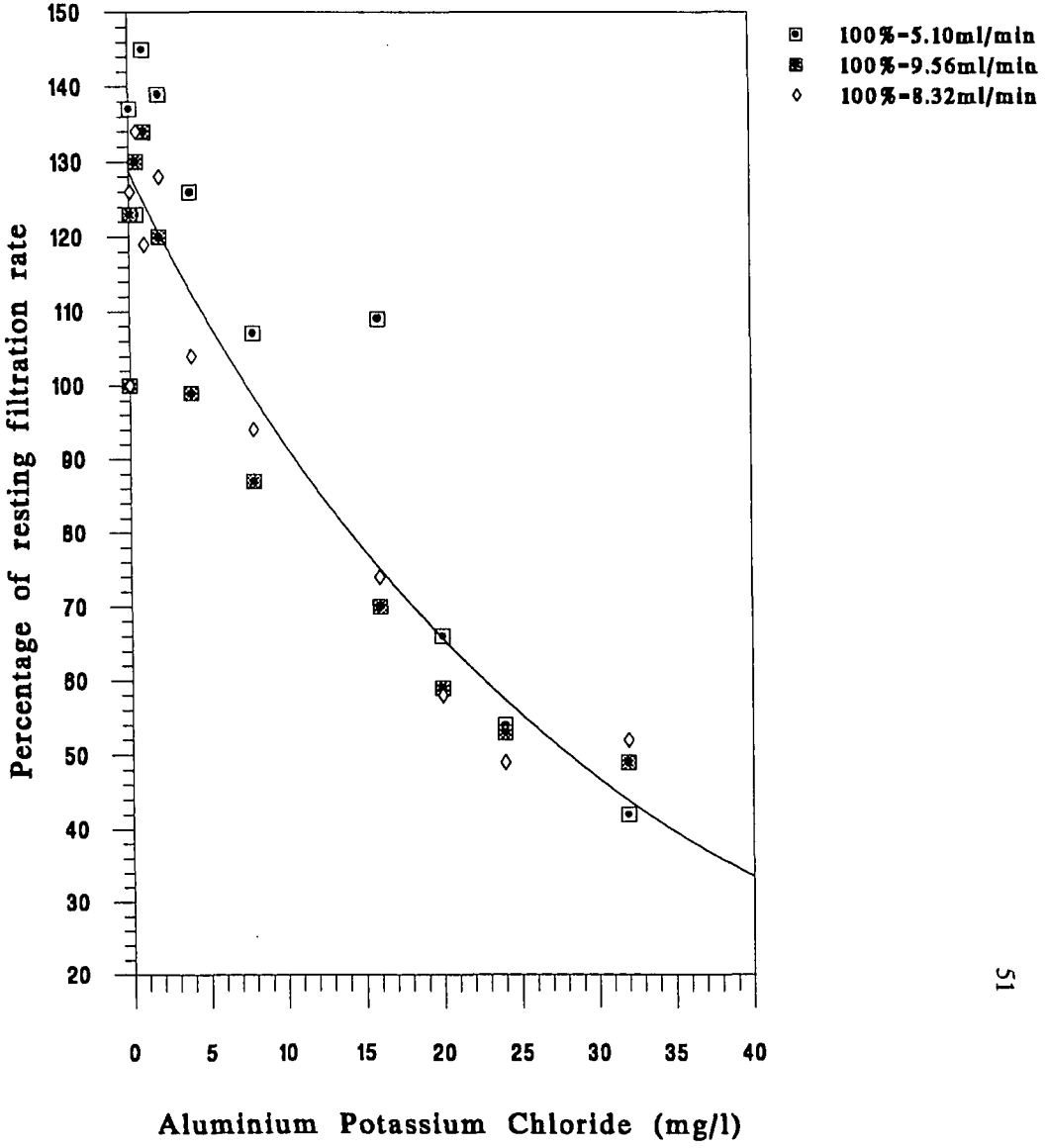
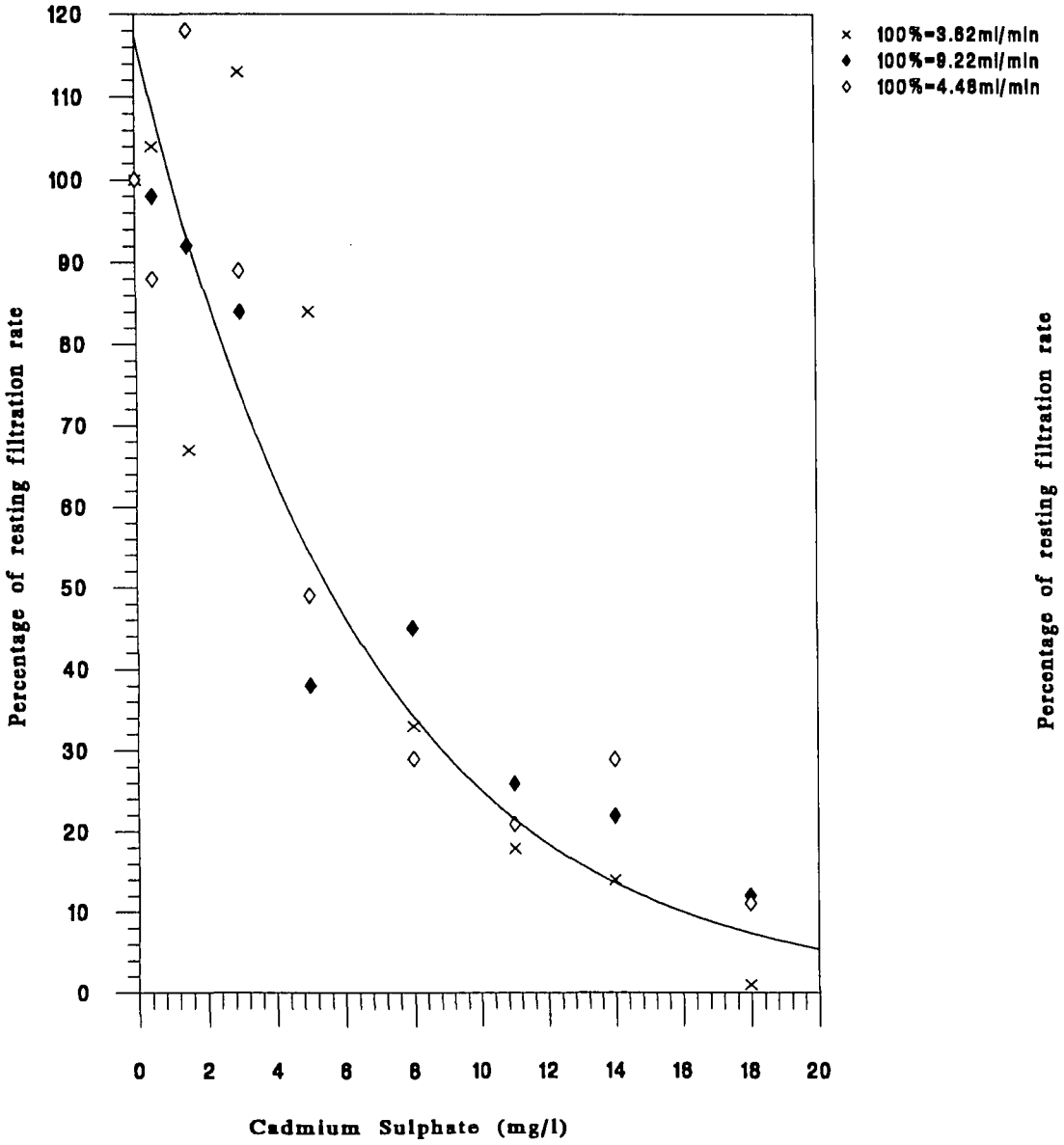


Figure 13a; Lindisfarne sample; response to Cadmium Sulphate



Figuree 13b; Teesmouth sample; response to Cadmium Sulphate

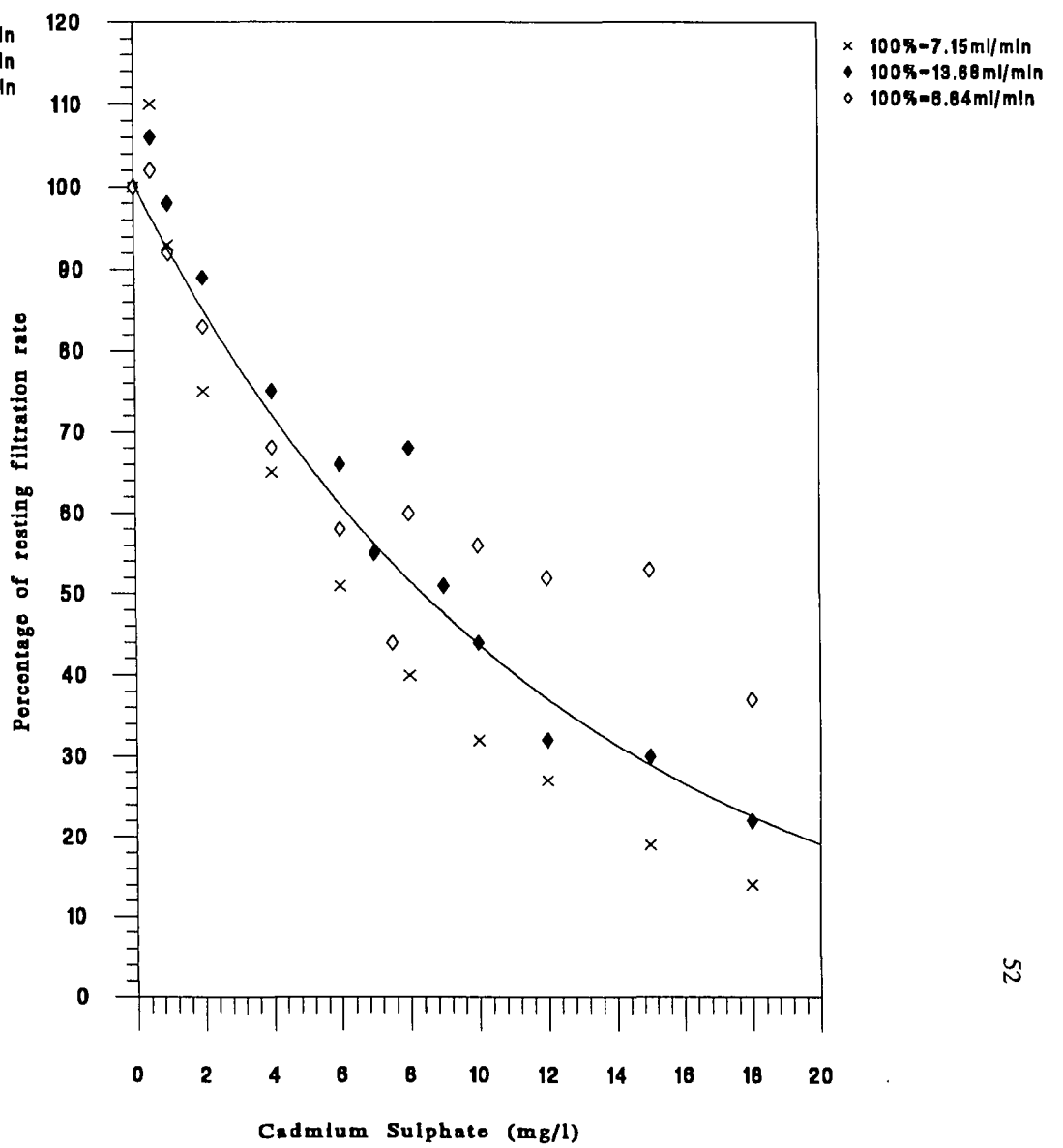


Figure 14a; Lindisfarne sample; response to Chromium III Chloride

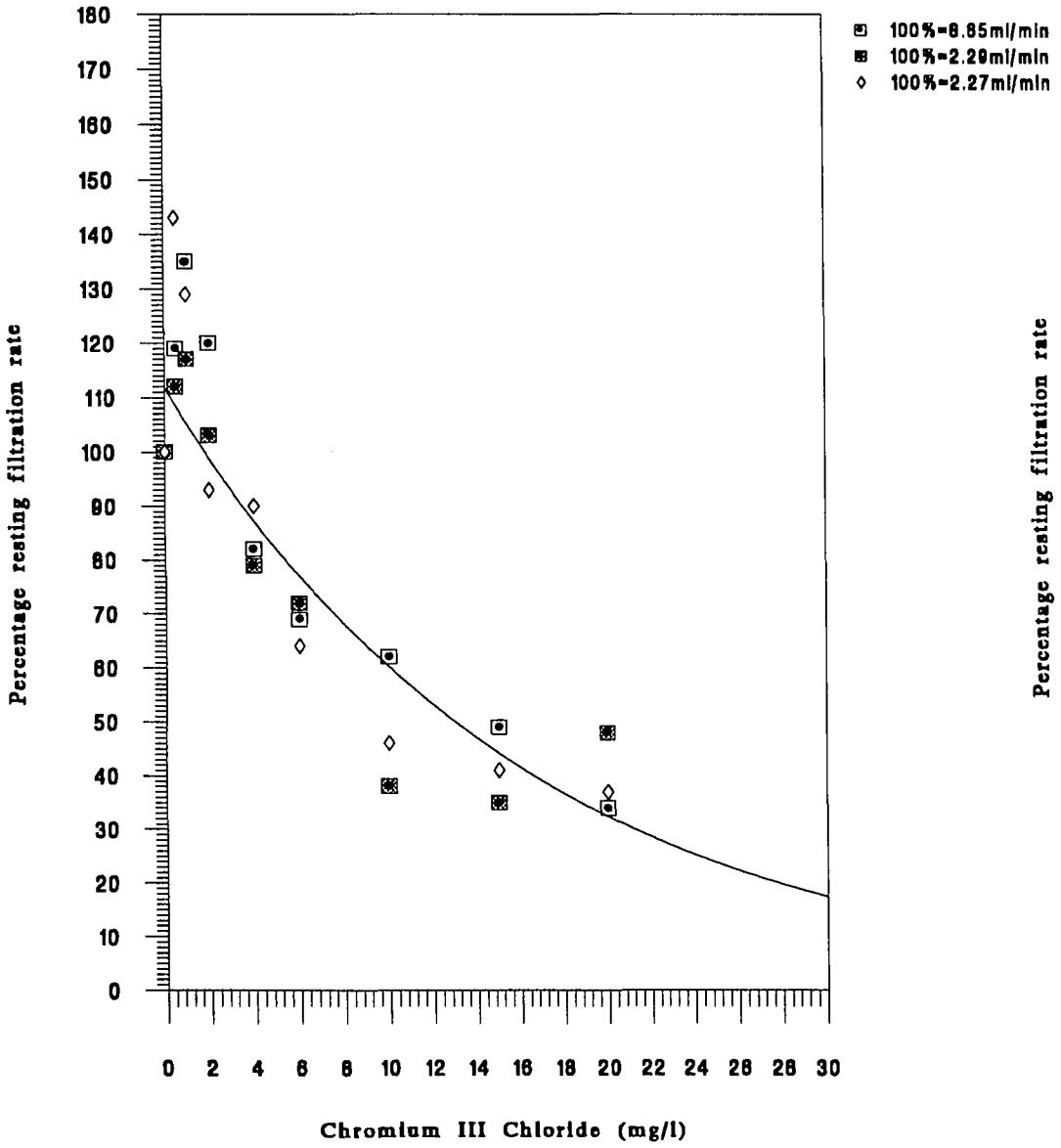


Figure 14b; Teesmouth sample; response to Chromium III Chloride

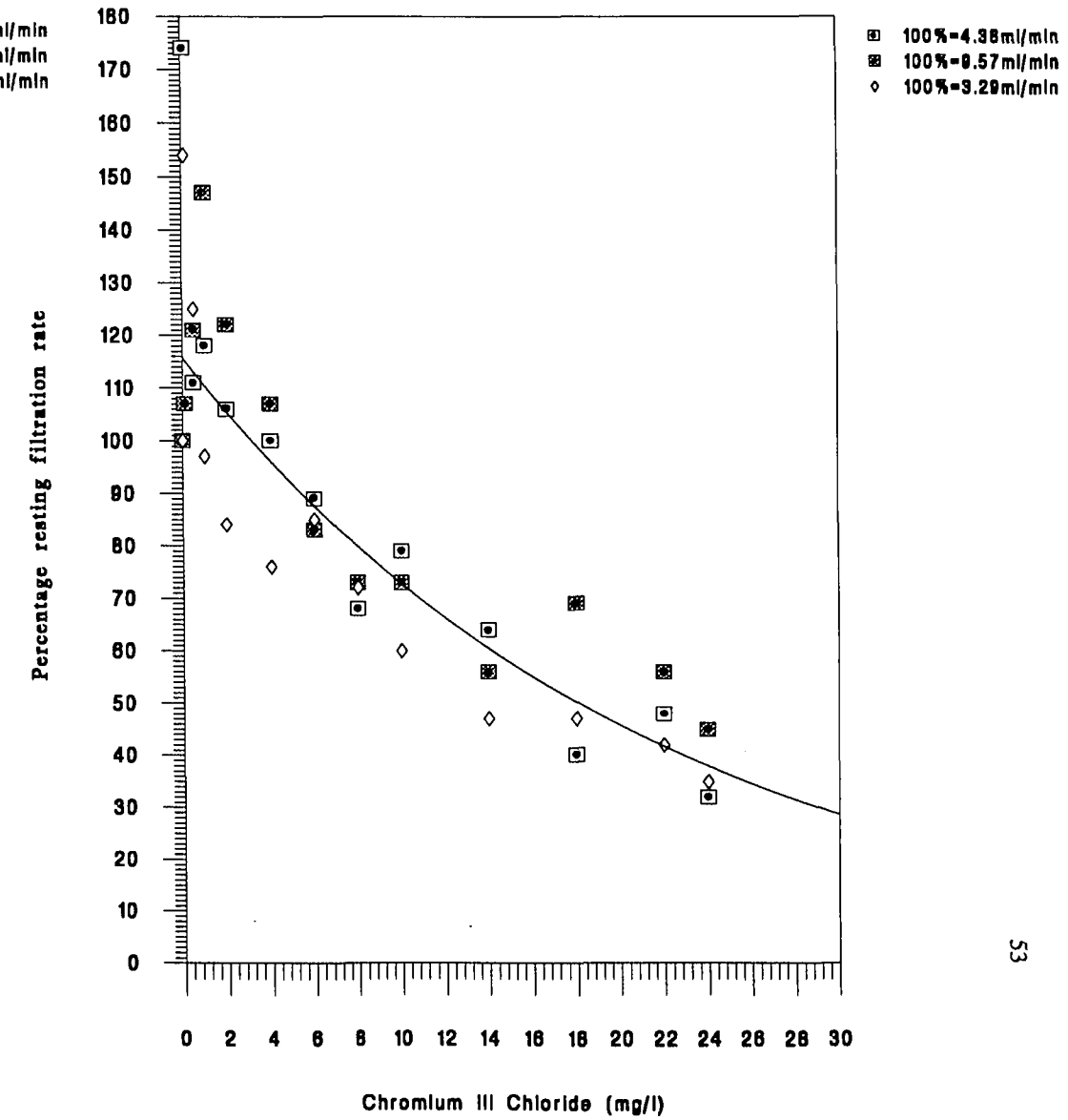


Figure 15a; Lindisfarne sample; response to Cobalt Chloride

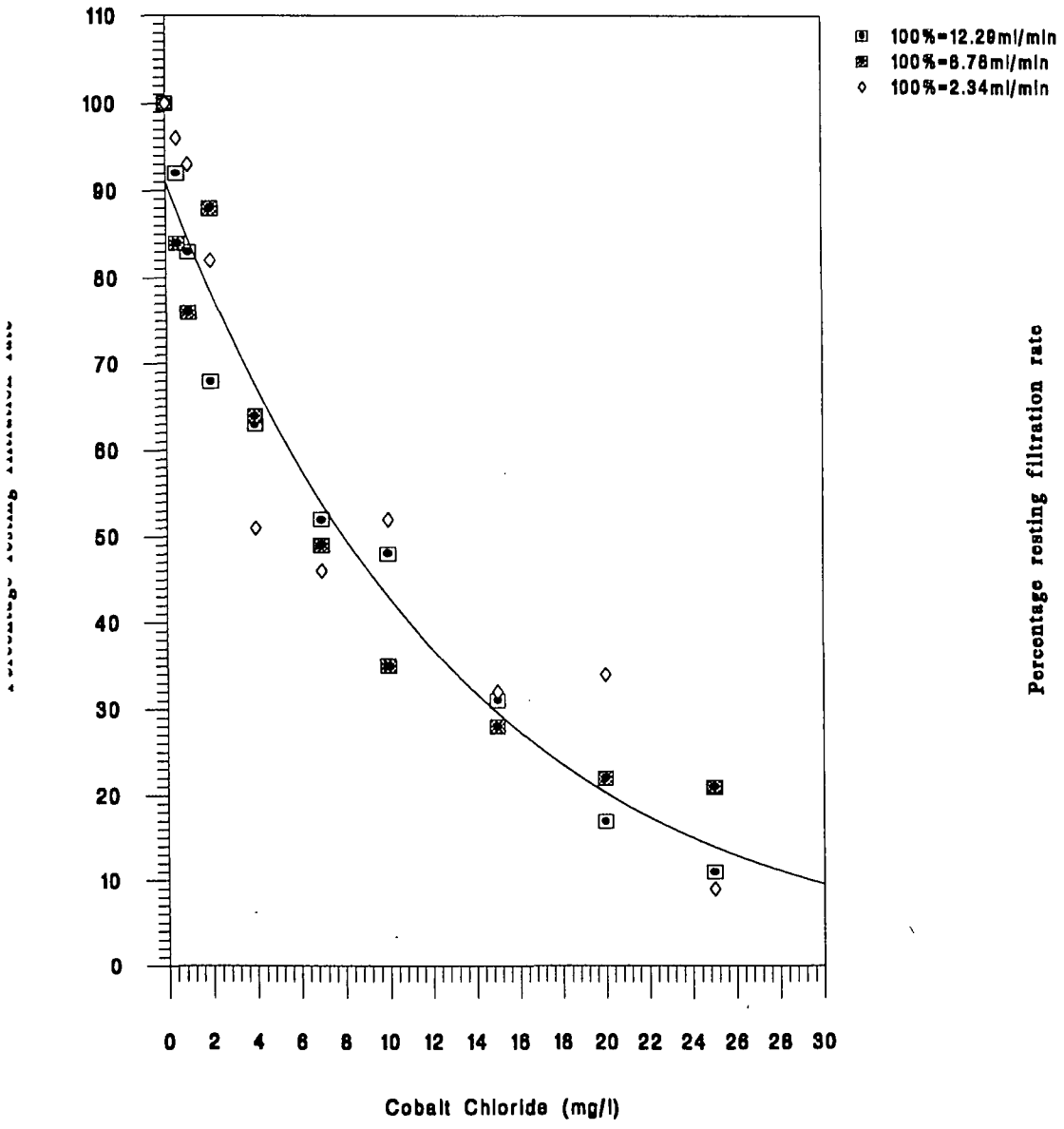


Figure 15b; Teesmouth sample; response to Cobalt Chloride

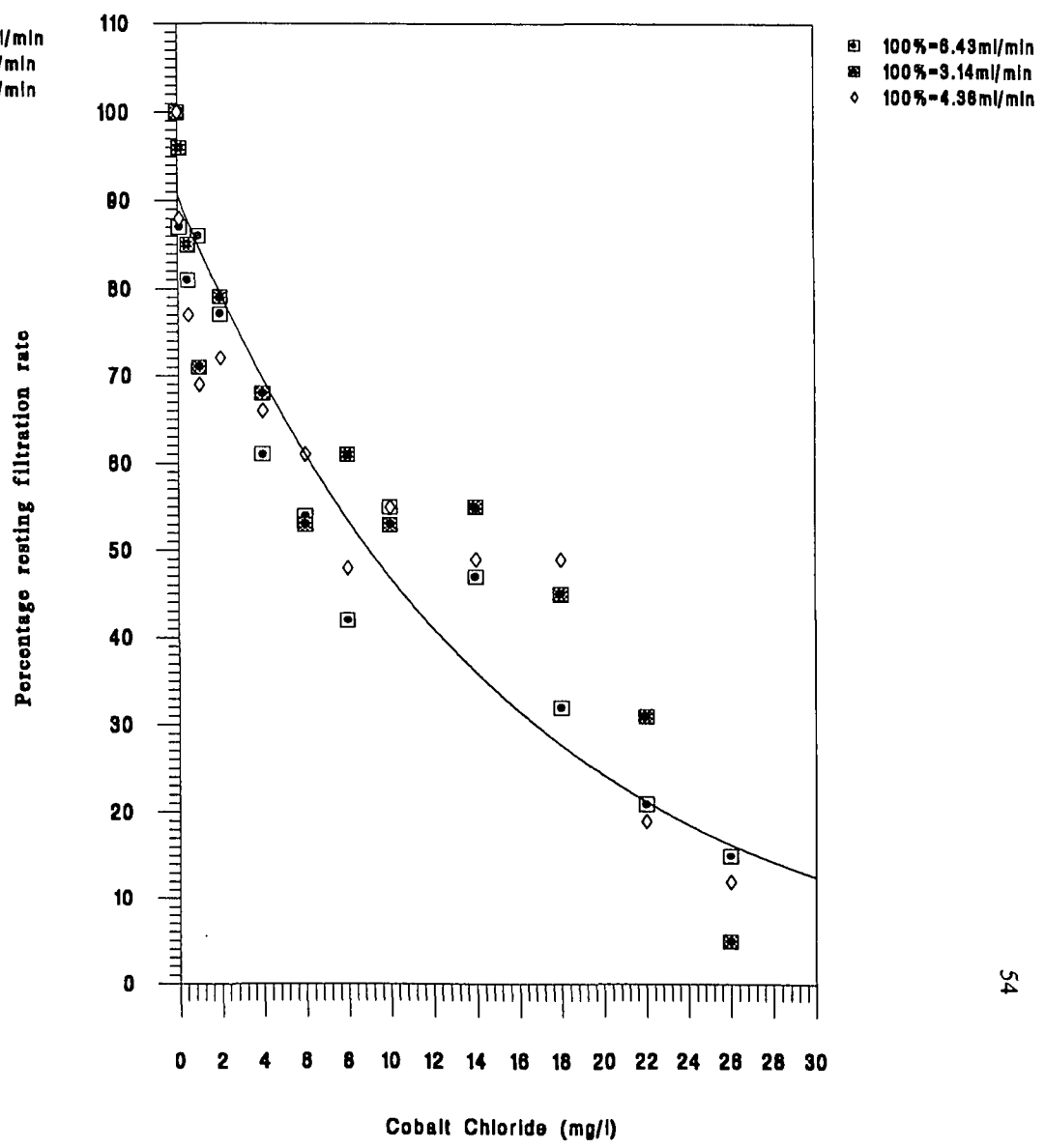


Figure 16a; Lindisfarne sample; response to Copper II Sulphate

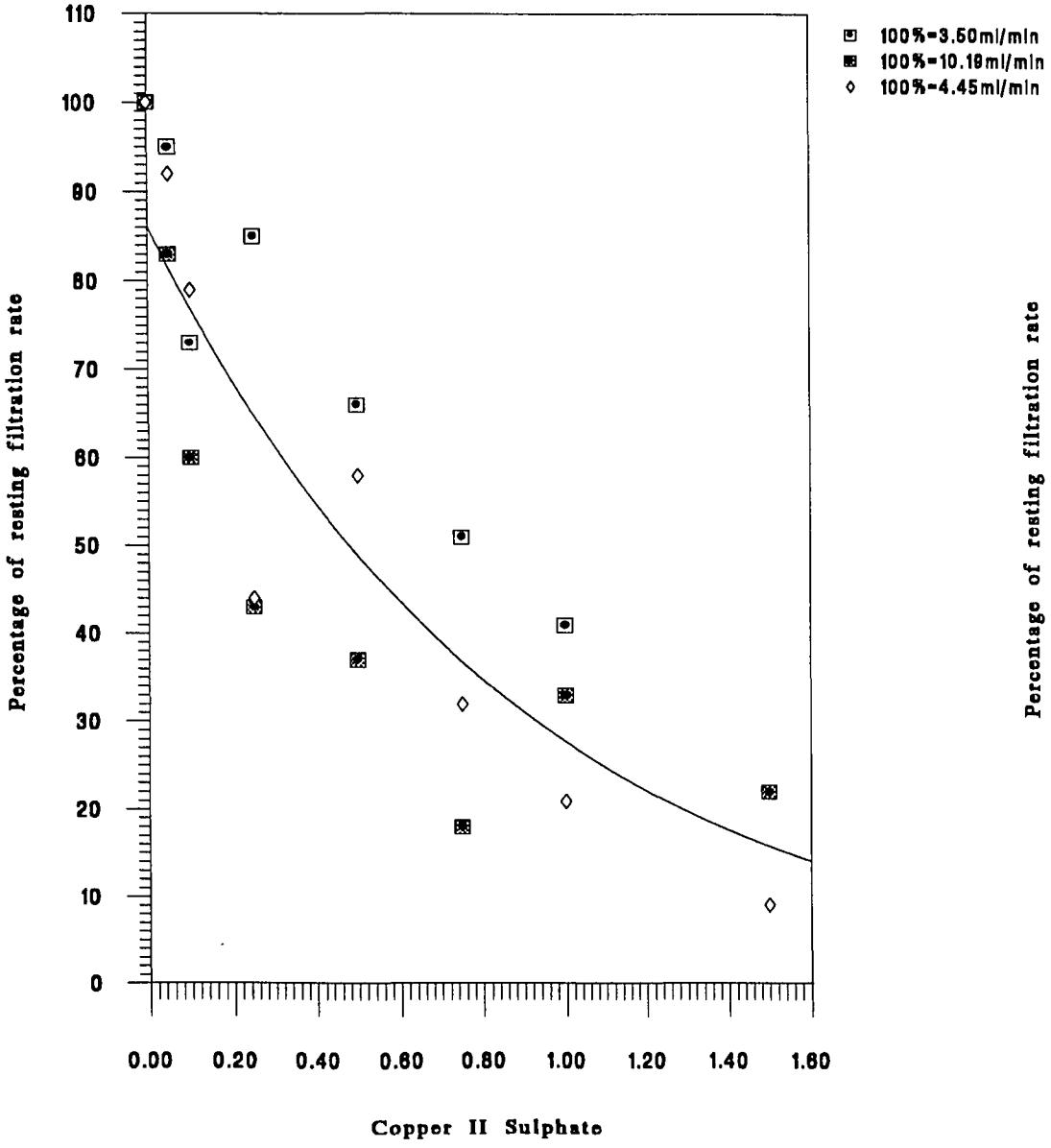


Figure 16b; Teesmouth sample; response to Copper II Sulphate

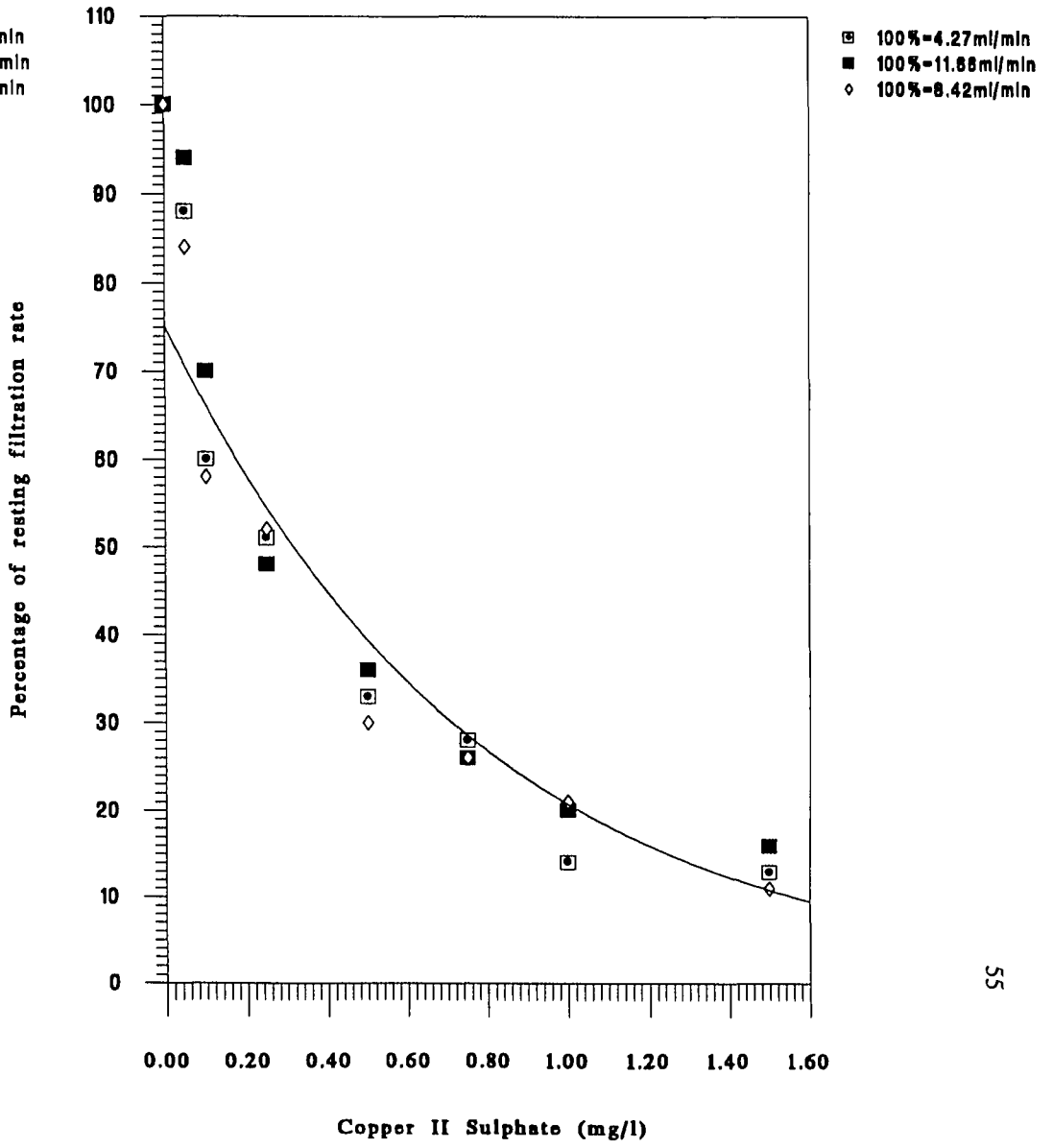


Figure 17a; Lindisfarne sample; response to Iron III Chloride

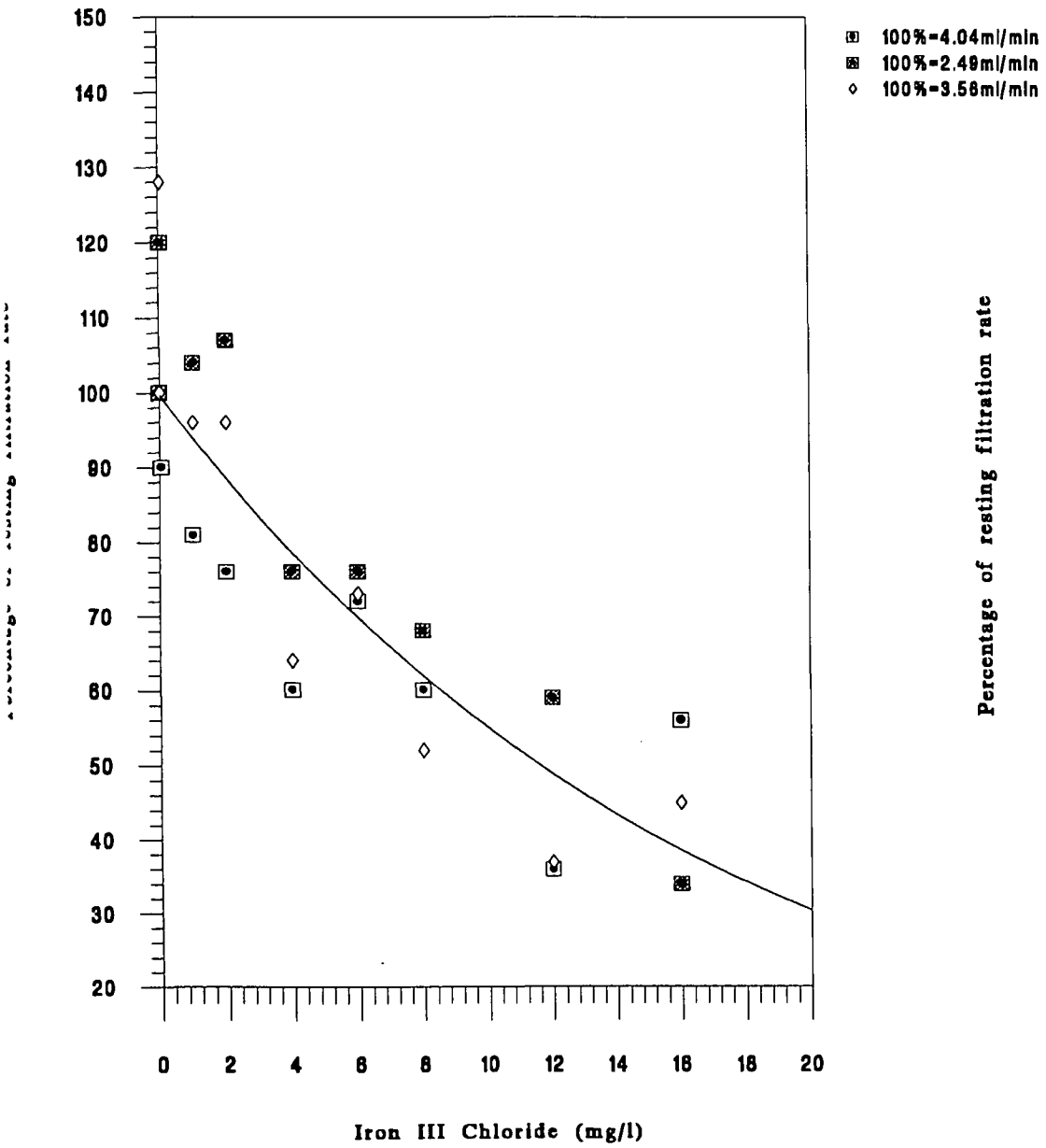


Figure 17b; Teesmouth sample; response to Iron III Chloride

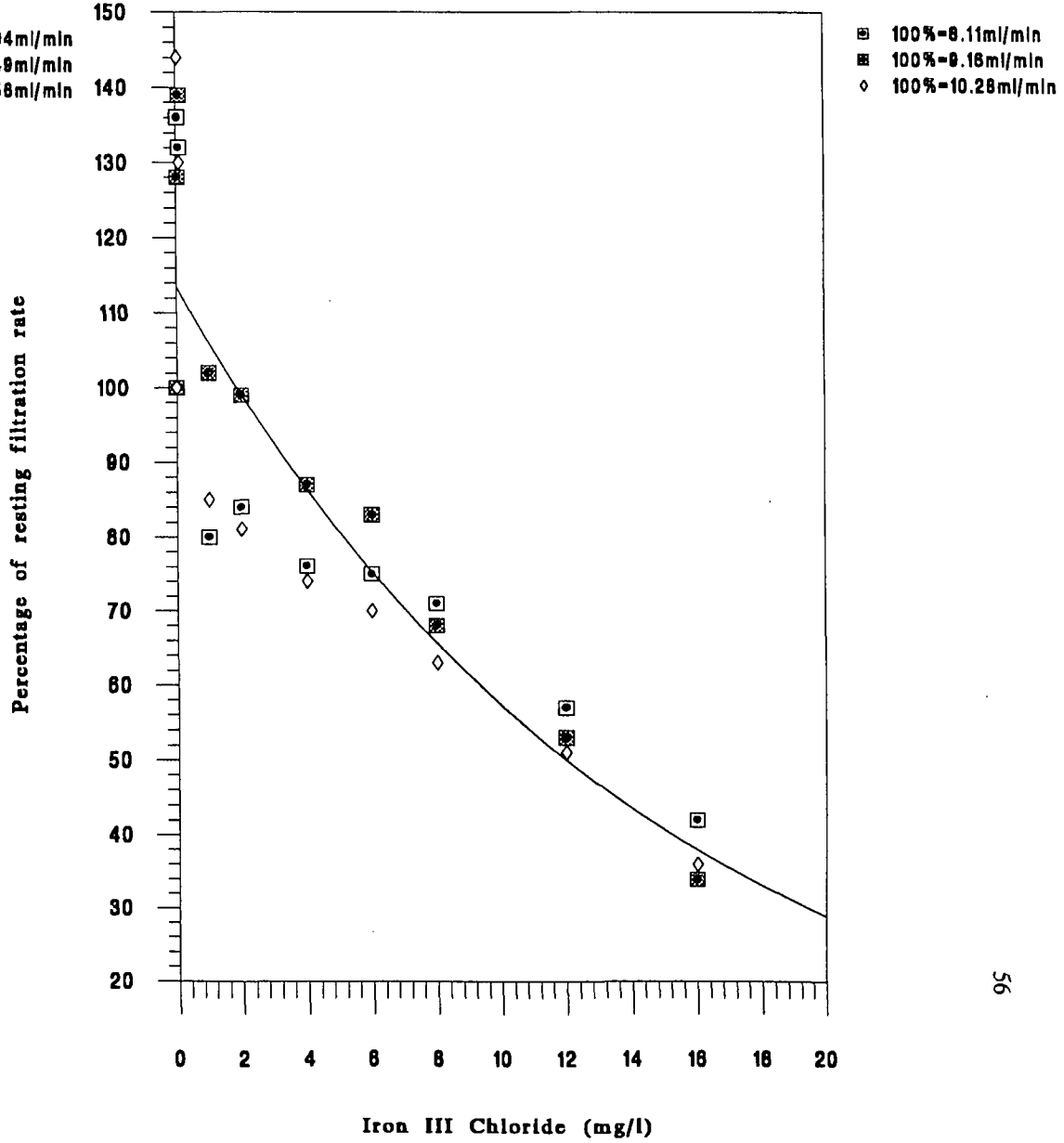


Figure 18a; Lindisfarne sample; response to Lead II Nitrate

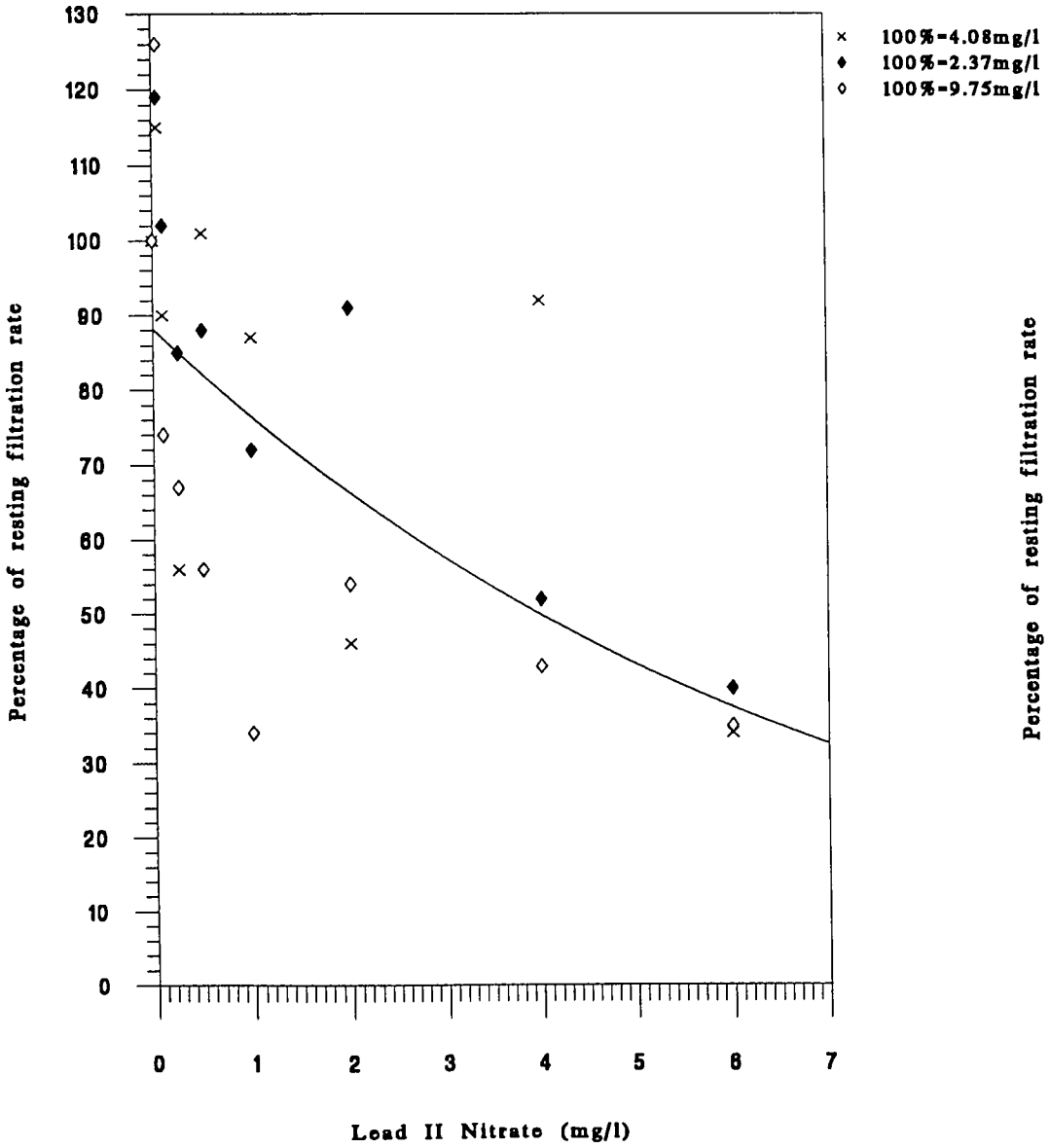


Figure 18b; Teesmouth sample; response to Lead II Nitrate

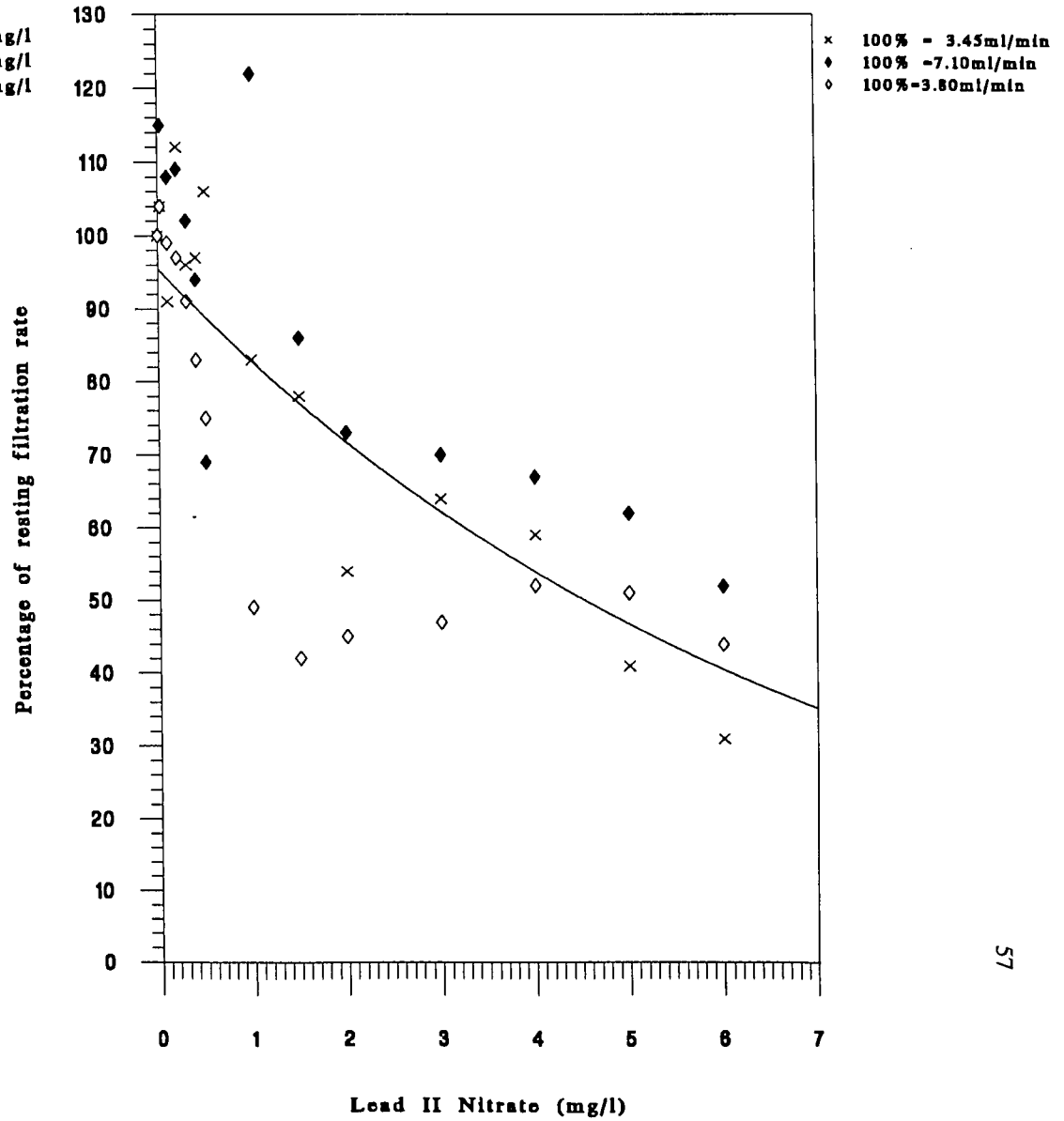


Figure 19a; Lindisfarne sample; response to Manganese II Chloride

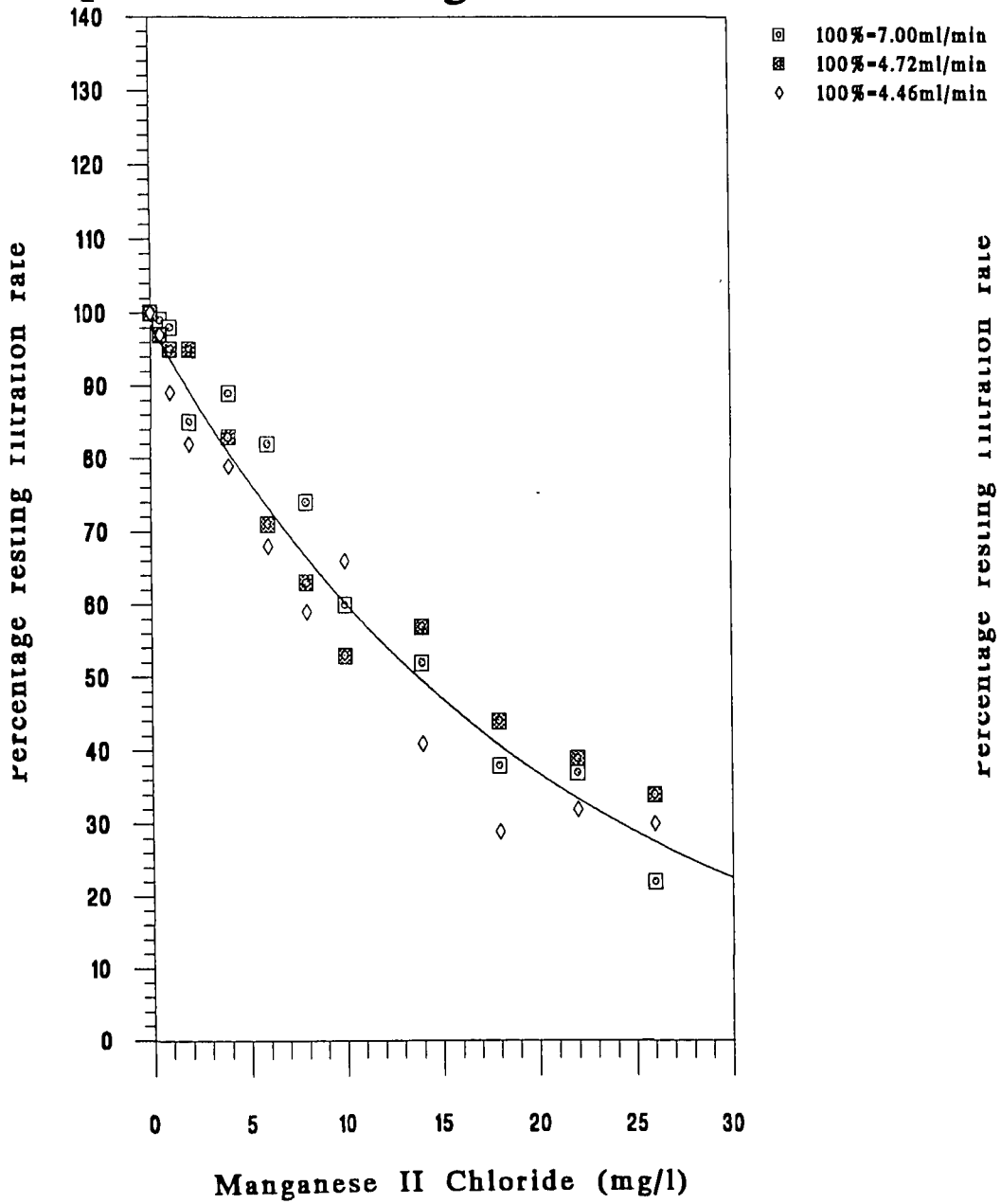


Figure 19b; Teesmouth sample; response to Manganese II Chloride

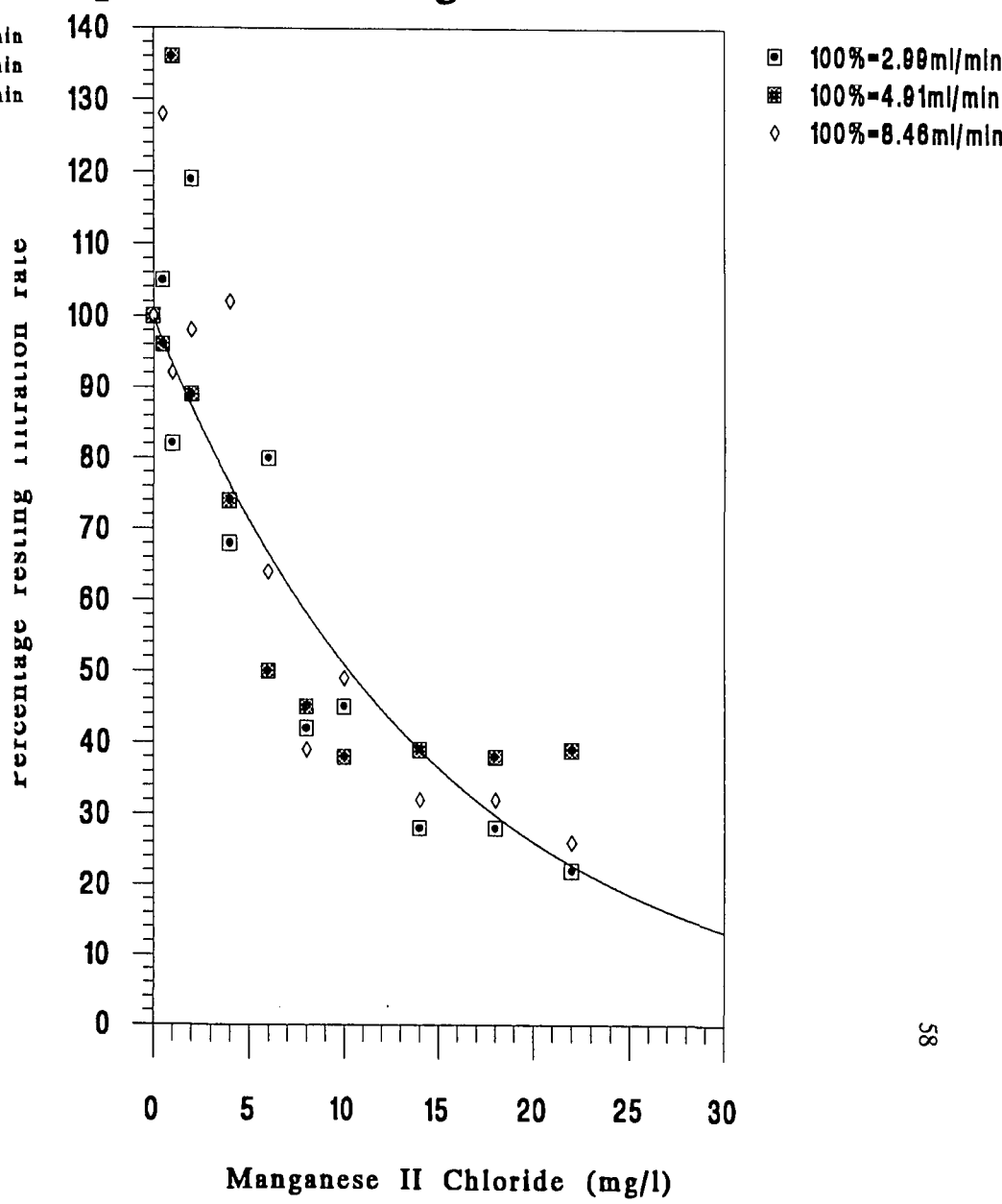


Figure 20a; Lindisfarne sample; response to Mercuric Chloride

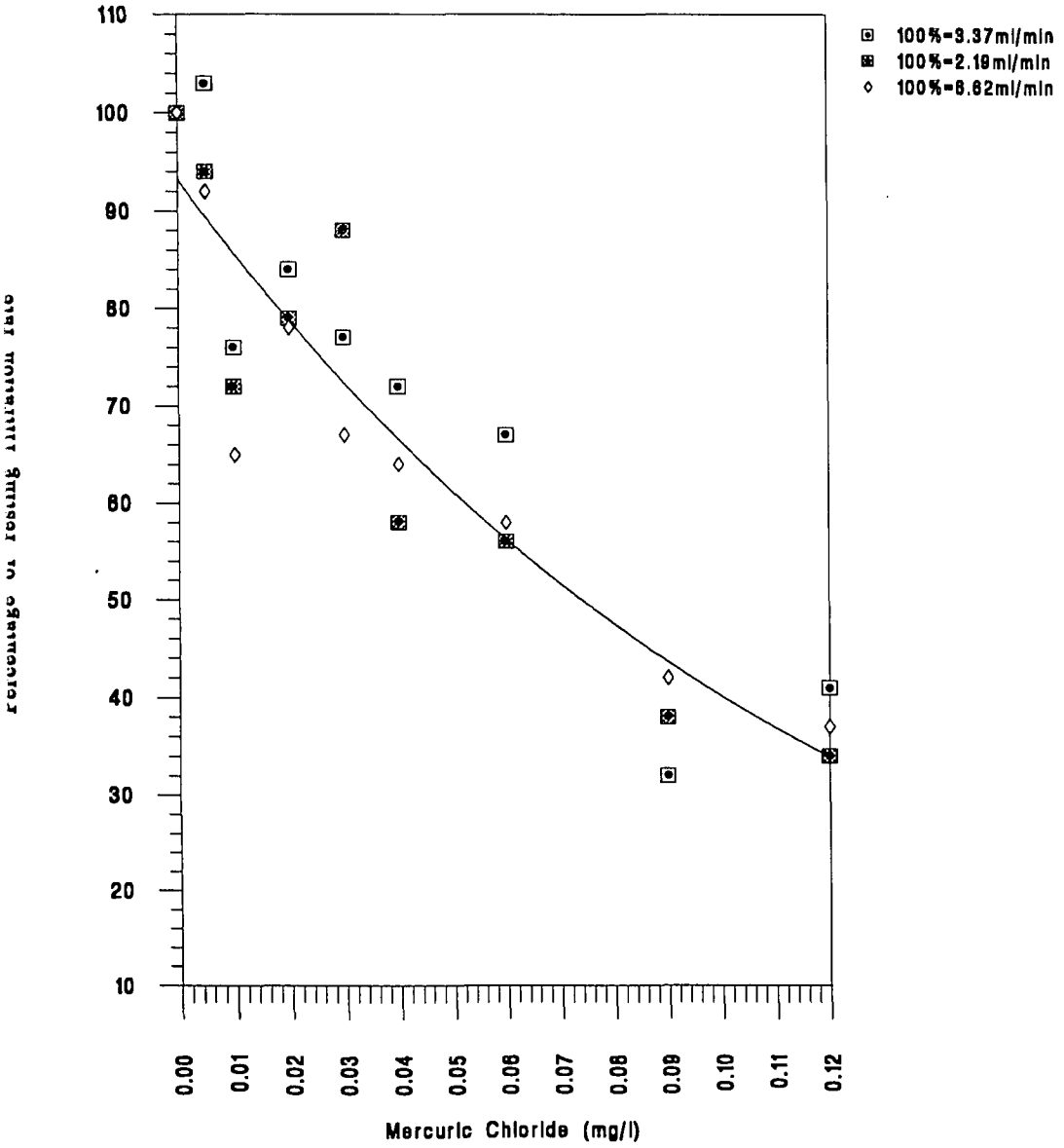


Figure 20b; Teesmouth sample; response to Mercuric Chloride

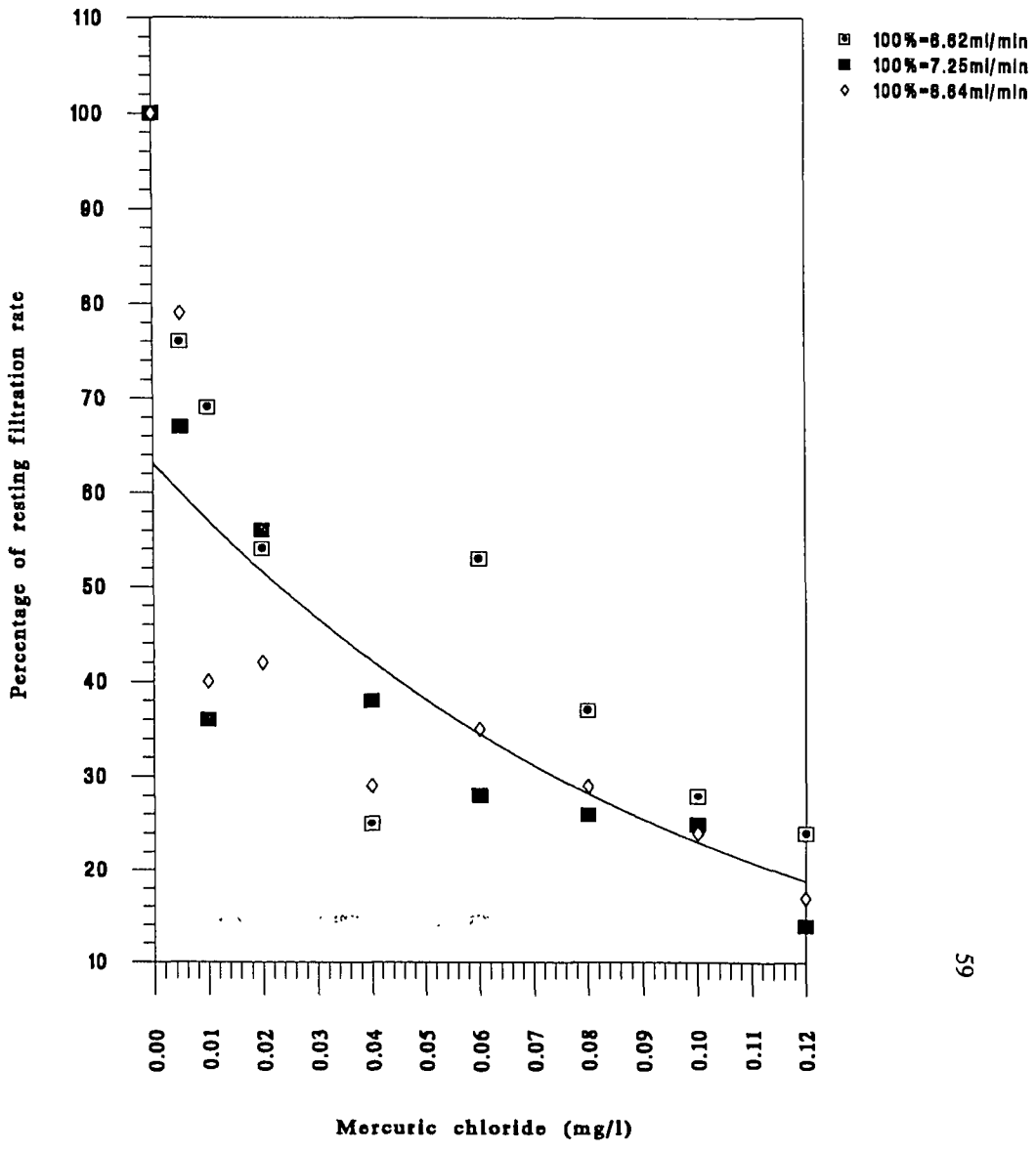


Figure 21a; Lindisfarne sample; response to Nickel Chloride

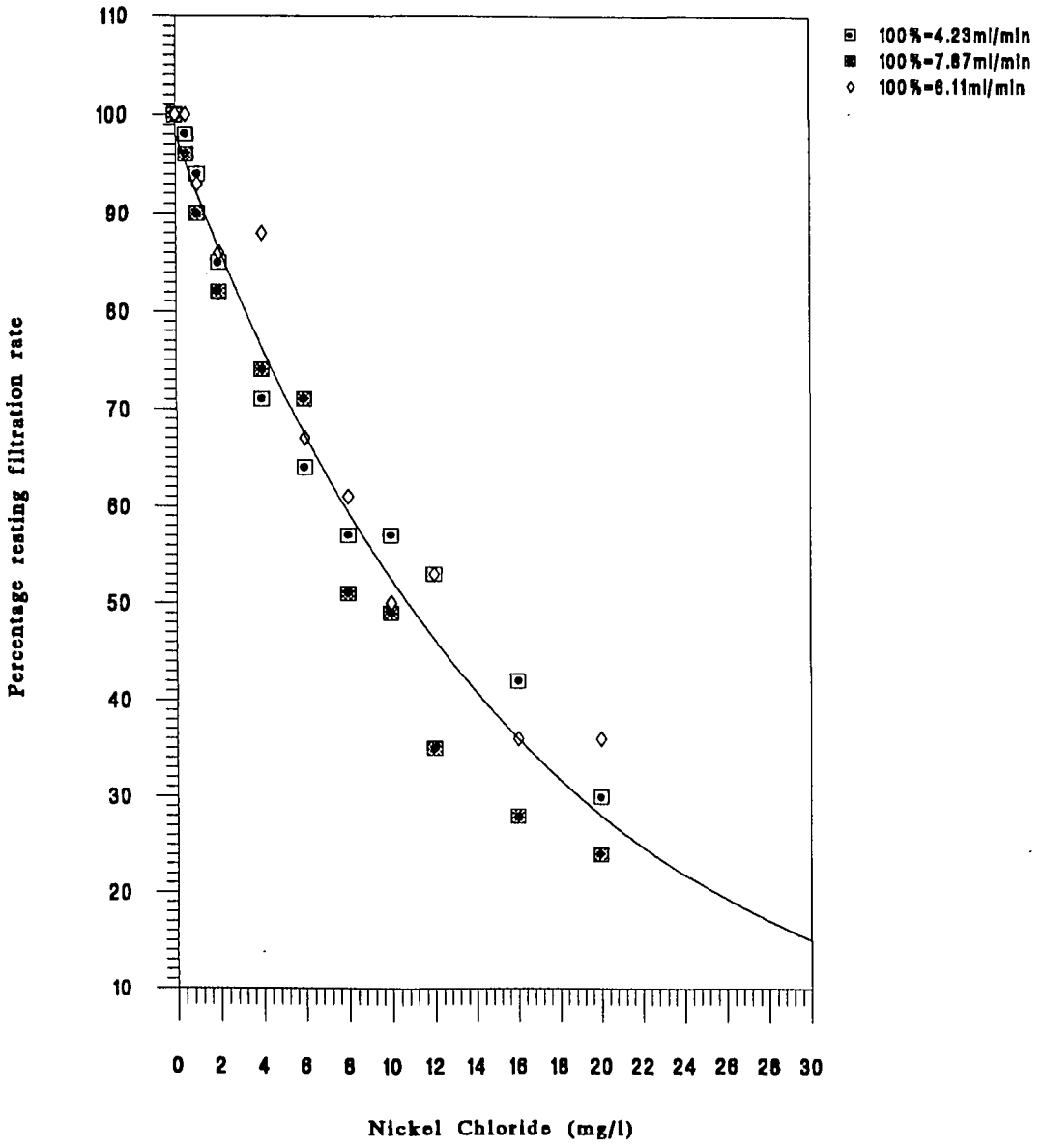


Figure 21b; Teesmouth sample; response to Nickel Chloride

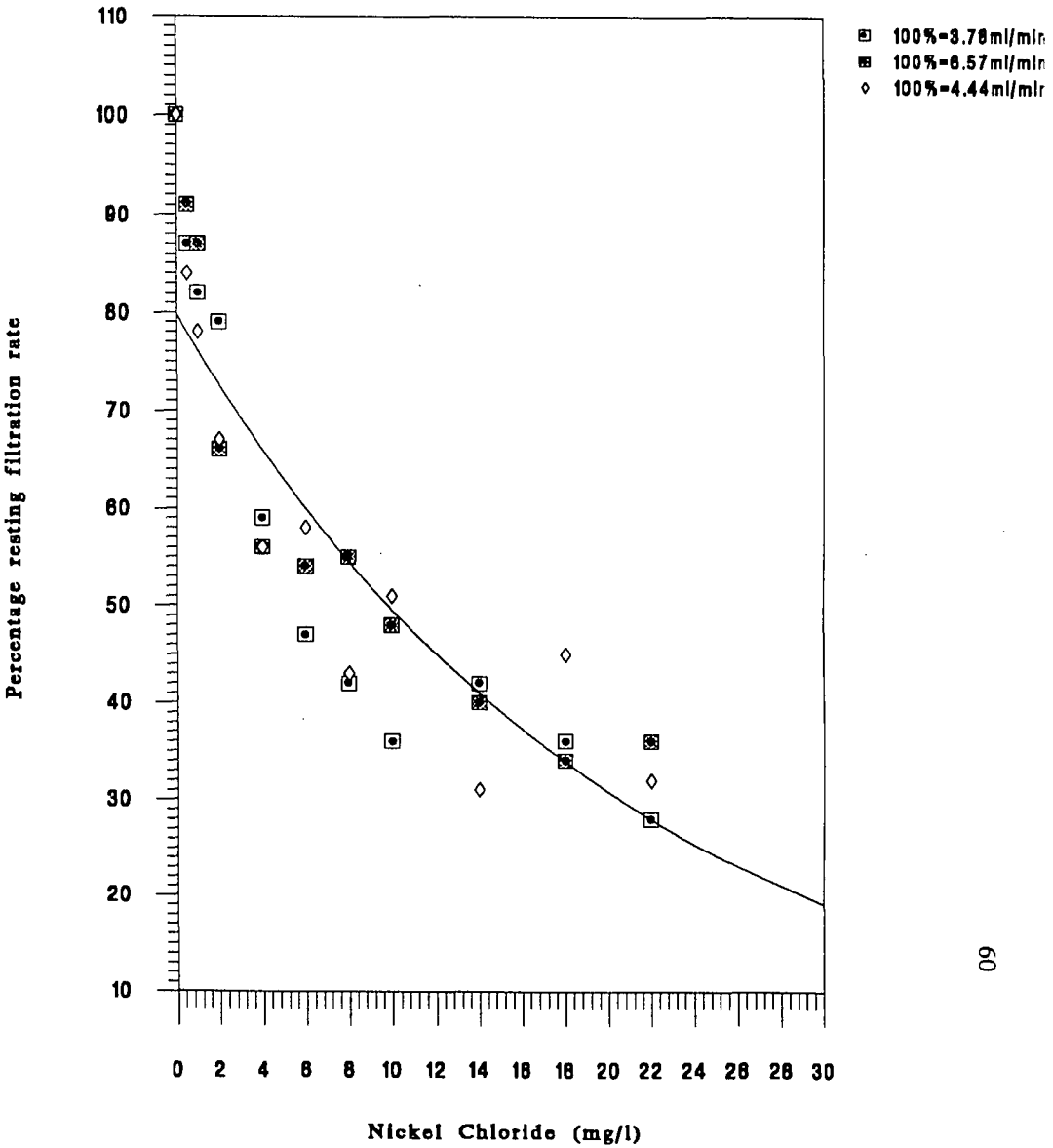
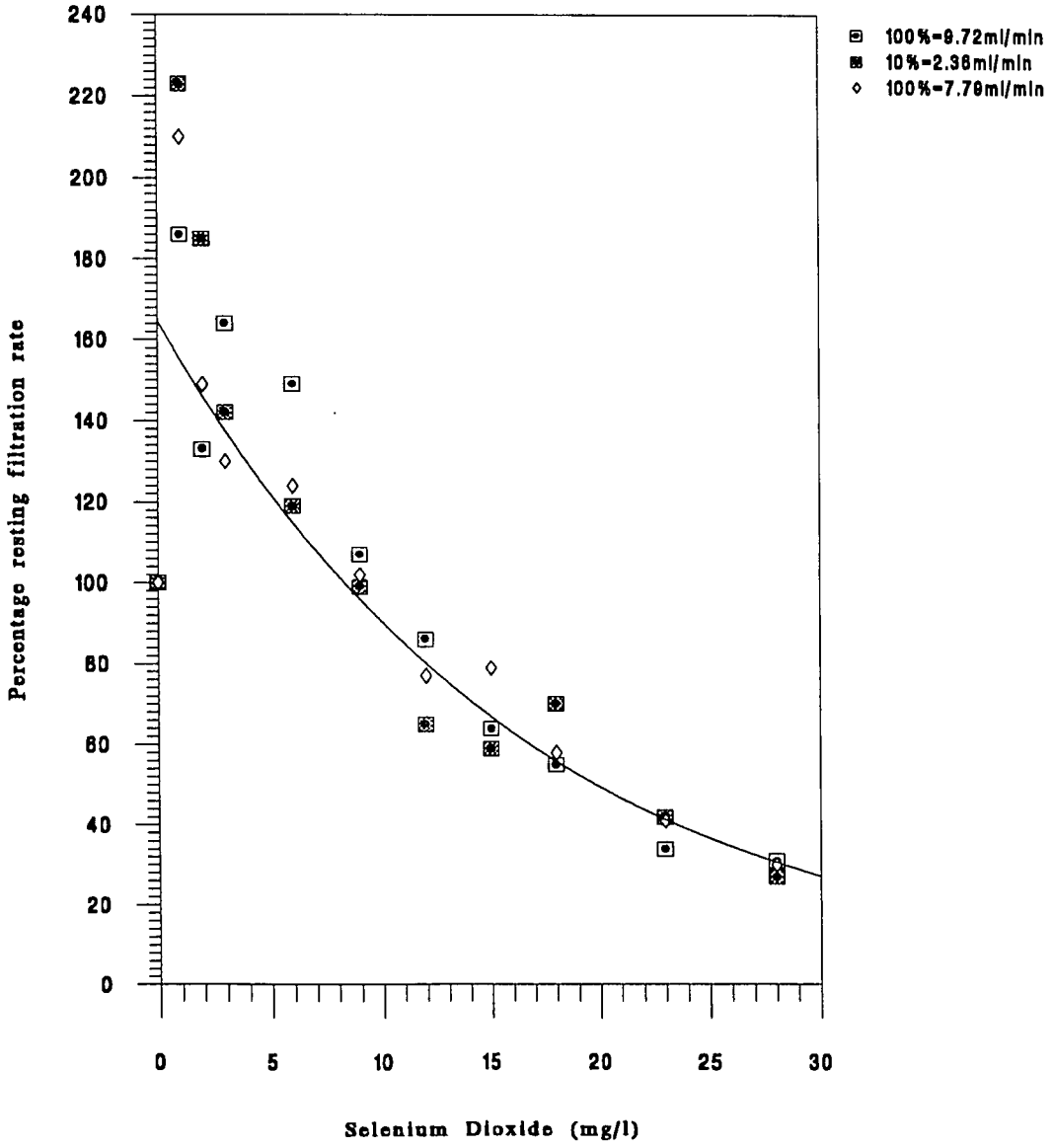


Figure 22a; Lindisfarne sample; response to Selenium Dioxide



Figuree 22b; Teesmouth sample; response to Selenium Dioxide

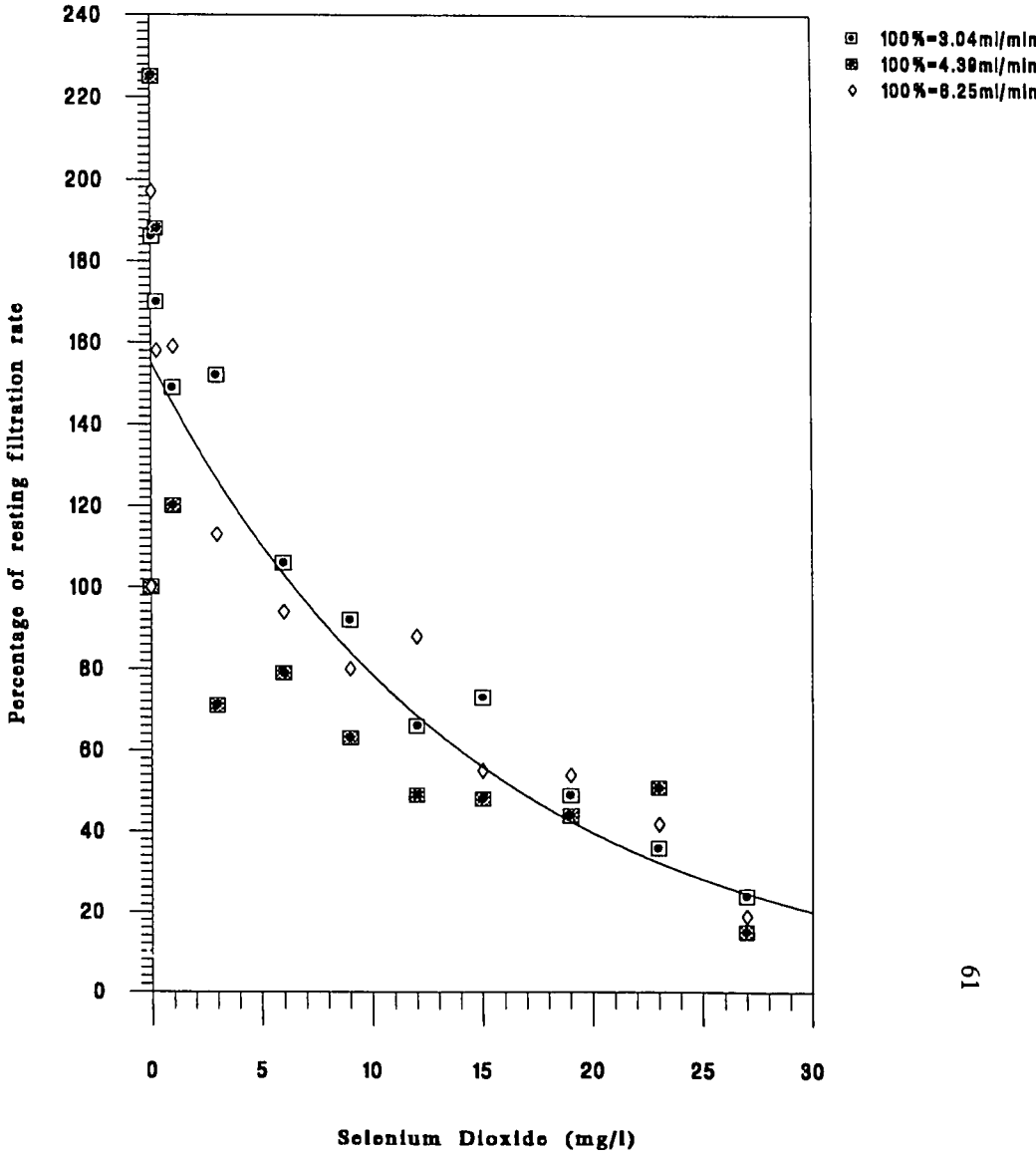


Figure 23a; Lindisfarne sample; response to Zinc Chloride

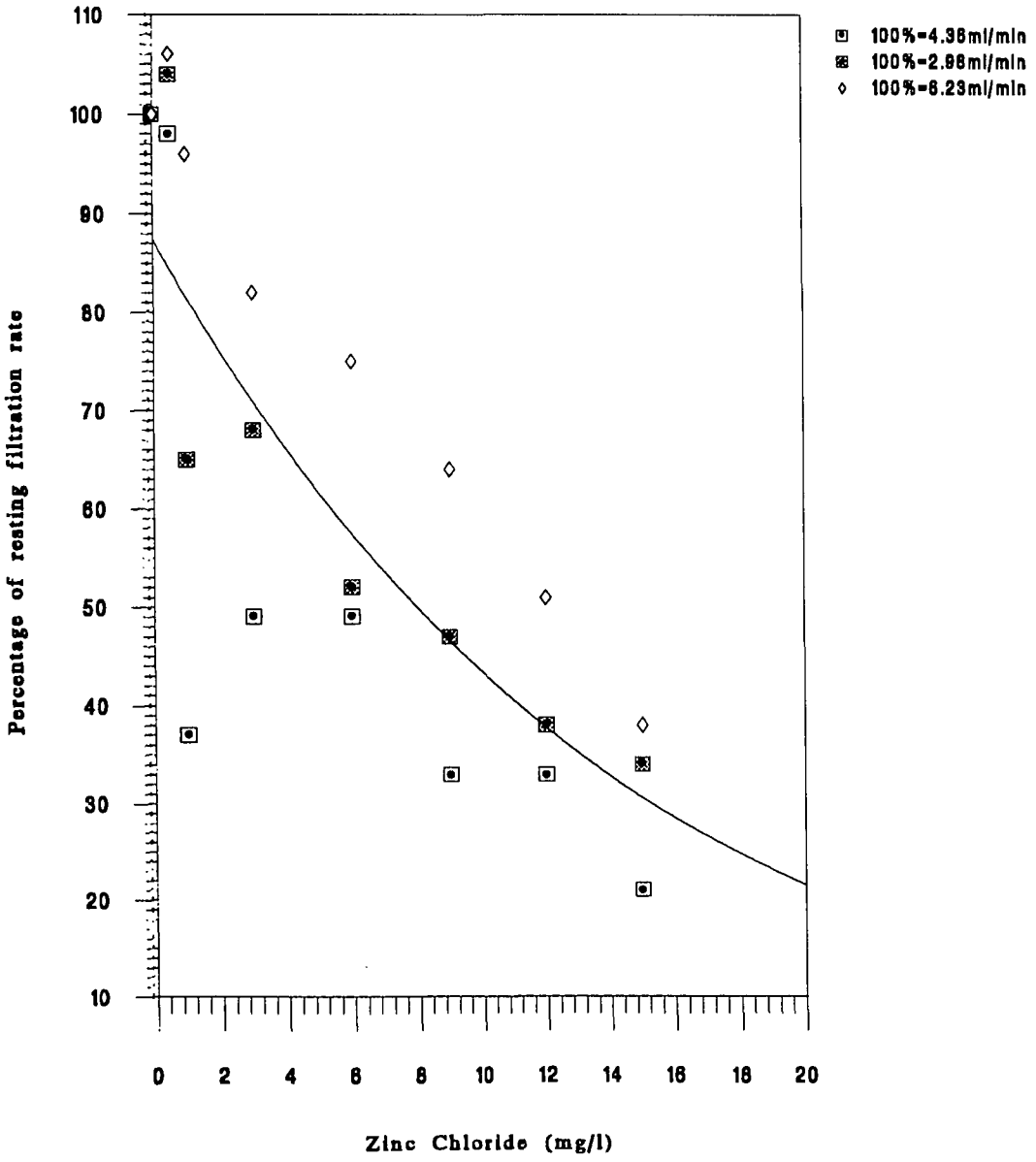
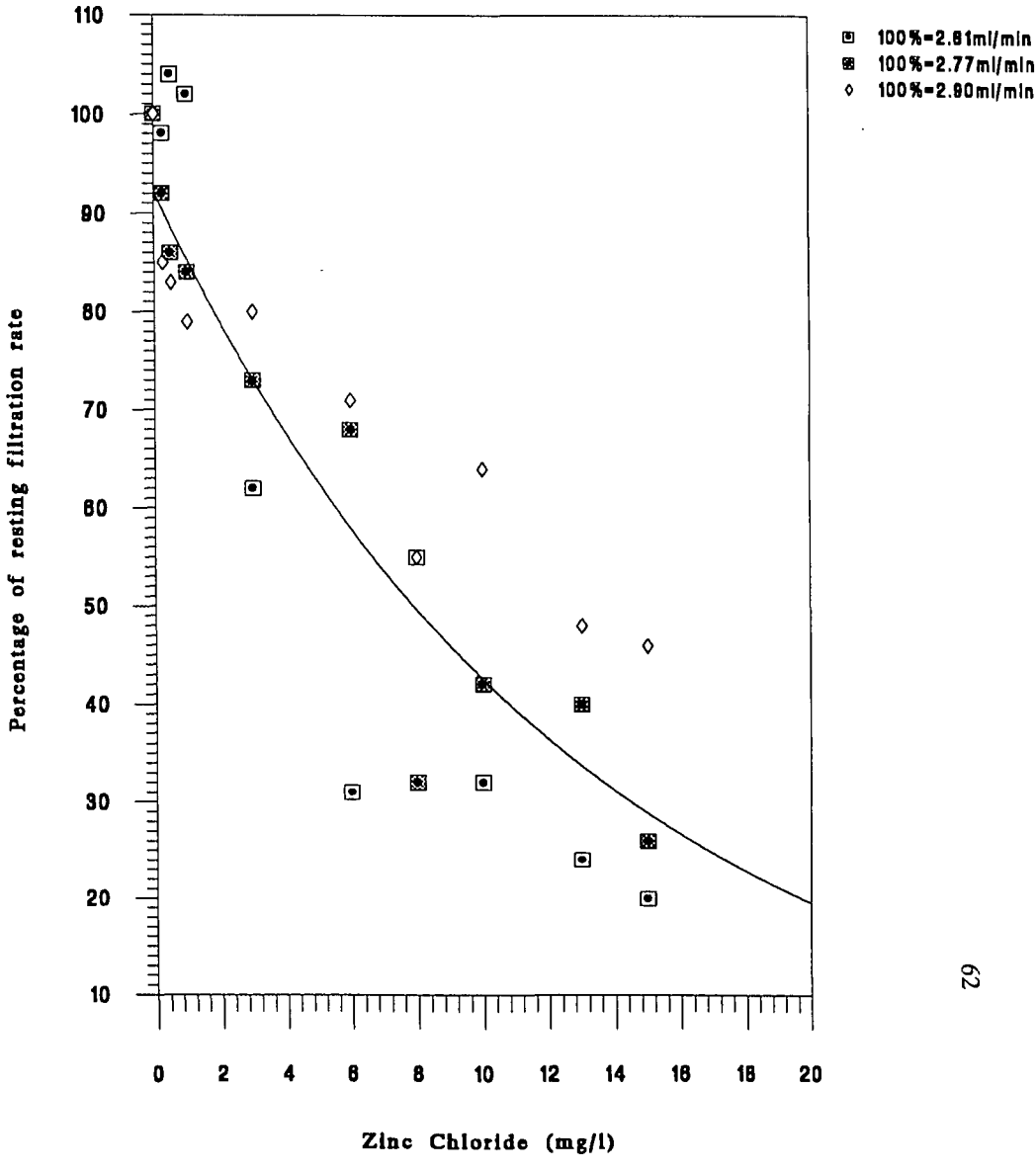


Figure 23b; Teesmouth sample; response to Zinc Chloride



Equasions of exponential best fit lines, for figures 12-23

Figure 12a;	y=122.95	10•(-0.015x)	r^2= 0.81
Figure 12b;	y=128.82	10•(-0.015x)	r^2= 0.90
Figure13a;	y=117.17	10•(-0.067x)	r^2= 0.81
Figure13b;	y=100.46	10•(-0.036x)	r^2= 0.79
Figure14a;	y=111.77	10•(-0.027x)	r^2= 0.84
Figure14b;	y=115.64	10•(-0.021x)	r^2= 0.84
Figure15a;	y=90.99	10•(-0.033x)	r^2= 0.92
Figure15b;	y=90.65	10•(-0.028)	r^2= 0.80
Figure16a;	y=86.09	10•(-0.049)	r^2= 0.77
Figure16b;	y=75.11	10•(-0.560)	r^2= 0.92
Figure17a;	y=99.65	10•(-0.026x)	r^2= 0.78
Figure17b;	y=113.51	10•(-0.029x)	r^2= 0.88
Figure18a;	y=88.17	10•(-0.062x)	r^2= 0.50
Figure18b;	y=33.45	10•(-0.082x)	r^2= 0.02
Figure19a;	y=98.32	10•(-0.021x)	r^2= 0.94
Figure19b;	y=99.68	10•(-0.029x)	r^2= 0.83
Figure20a;	y=92.20	10•(-0.069x)	r^2= 0.87
Figure20b;	y=63.07	10•(-4.373x)	r^2= 0.71
Figure21a;	y=97.89	10•(-0.027x)	r^2= 0.93
Figure21b;	y=79.76	10•(-0.021x)	r^2= 0.82
Figure22a;	y=164.61	10•(-0.026x)	r^2=0.87
Figure22b;	y=95.45	10•(-0.062x)	r^2=0.63
Figure23a;	y=97.42	10•(-0.030x)	r^2=0.67
Figure23b;	y=97.45	10•(-0.032x)	r^2=0.66

---ooOOoo---

(c) Evidence for synergistic and antagonistic interactions between metal salts

The results for the interaction experiments are presented in table 5. Out of 62 possible combinations, 43 appear antagonistic according to my definition of boundaries, and a further 11 show containment. Interactions between these chemicals in saline solution thus typically produced lower toxicities to the animals. 6 of them showed synergistic interactions, while a further 5 appeared additive. Synergistic interactions occurred between copper and aluminium, manganese, selenium and zinc; and additivity between copper and mercury. Synergy also occurred between aluminium and iron and zinc, whilst additivity occurred between nickel and selenium, zinc and manganese, and mercury and cadmium, iron and copper.

Copper is a known inhibitor of respiration in mussels. It seems here to act on a different component of the filtration process than the other metals quoted, but which are components of the same metabolic process, such as e.g. enzymes in two divergent branches of a biochemical pathway.

---ooOOoo---

Table 5; Interaction of metal salts; effects on filtration rates of the Teesmouth mussels

	Al	Cd	Cr	Co	Cu	Fe	Pb	Mn	Hg	Ni	Se	Zn
Al	85											
Cd	104 (ant)	59										
Cr	190 (ant)	159 (ant)	66									
Co	137 (ant)	159 (ant)	70 (ant)	82								
Cu	40 (syn)	120 (ant)	105 (ant)	190 (ant)	62							
Fe	45 (syn)	81 (con)	82 (con)	90 (ant)	142 (ant)	85						
Pb	102 (ant)	70 (con)	148 (ant)	183 (ant)	72 (con)	149 (ant)	76					
Mn	67 (con)	63 (con)	157 (ant)	171 (ant)	30 (syn)	109 (ant)	117 (ant)	59				
Hg	72 (con)	56 (add)	116 (ant)	133 (ant)	58 (add)	59 (add)	119 (ant)	63 (con)	69			
Ni	72 (con)	143 (ant)	128 (ant)	116 (ant)	97 (ant)	119 (ant)	91 (ant)	114 (ant)	94 (ant)	79		
Se	129 (ant)	115 (ant)	208 (ant)	167 (ant)	31 (syn)	115 (ant)	189 (ant)	110 (ant)	103 (ant)	54 (add)	67	
Zn	29 (syn)	118 (ant)	146 (ant)	152 (ant)	42 (syn)	96 (ant)	43 (ant)	60 (add)	112 (ant)	80 (con)	68 (con)	71

(Results represent percentage values of resting filtration rate, with the specified dosed of the two metal salts specified. The numbers in boxes are the results from the dose of the single metal salt.)

(d) Study of filtration rate over a three hour period

Figure 24 shows the series of graphed results for this study. For each graph, the vertical line represents the point at which the bathing solution was replenished with clean water. The graphs show that filtration rate was not constant over time, even for the control batch. Batches kept in copper II sulphate, (known to act as an inhibitor of respiration,) displayed a variety of apparent responses, as follows; (letters refer to the graph displaying the respective result, and they are presented in order of increasing copper concentration).

(a.) Respiration in clean sea water fluctuated over time.

(b.) At 0.005 mg/l, there was a decrease in filtration rate, though this did not come into effect immediately; perhaps there is an "active site" of some enzymic system which takes a short time to encounter enough water at this concentration to obtain an inhibitory dose.

(c.) At 0.01 mg/l, the response was more immediate, increasing the filtration rate above the resting level. This is possibly an attempt to clear the toxin from the system. This has diminished after 60 minutes, perhaps as inhibition takes effect; this suggests that a minimum concentration of the pollutant is required for recognition in order to elicit an active response. Upon introduction of clean water, the clearance rate elevated once more as the inhibitor is removed.

(d.) At 0.025 mg/l the filtration rate increases and decreases even more rapidly, perhaps from a tension between an active clearance response and the increased inhibitory effect of copper. This shows a slower recovery upon introduction of clean water.

(e.) At 0.05 mg/l there is a damped response, similar in pattern to that at 0.025 mg/l, though with a slightly elevated recovery peak.

vi. 0.1 mg/l shows a depression of filtration, and a heightened recovery in clean water.

vii. 0.2 mg/l shows further decreases in steady filtration rate, and a heightened recovery response.

viii. At 0.4 mg/l this respiratory decrease is further pronounced, and the recovery clearance heightened to over 200% of the resting filtration rate.

Thus filtration, and hence feeding and respiration, were not constant over time for the mussels, but vary between heightened and lessened phases. The metal salt copper II sulphate affected this pattern, presumably by eliciting a physiological detoxication type response.

Figure 24; A study of filtration rate over time for the Lindisfarne mussel sample, in response to various concentrations of copper II sulphate (mg/l)

Figure 24a; clean seawater control (0mg/l)

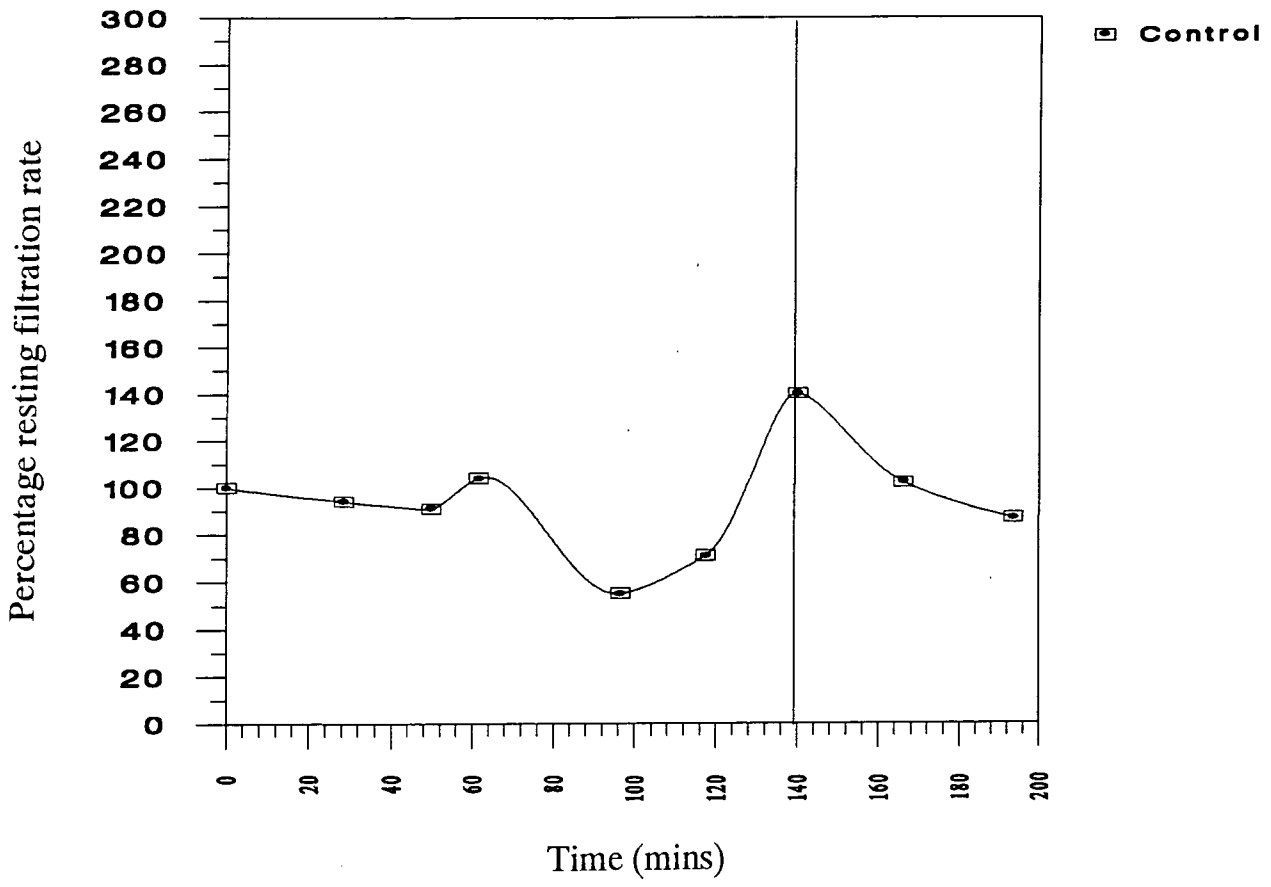


Figure 24b; 0.005mg/l

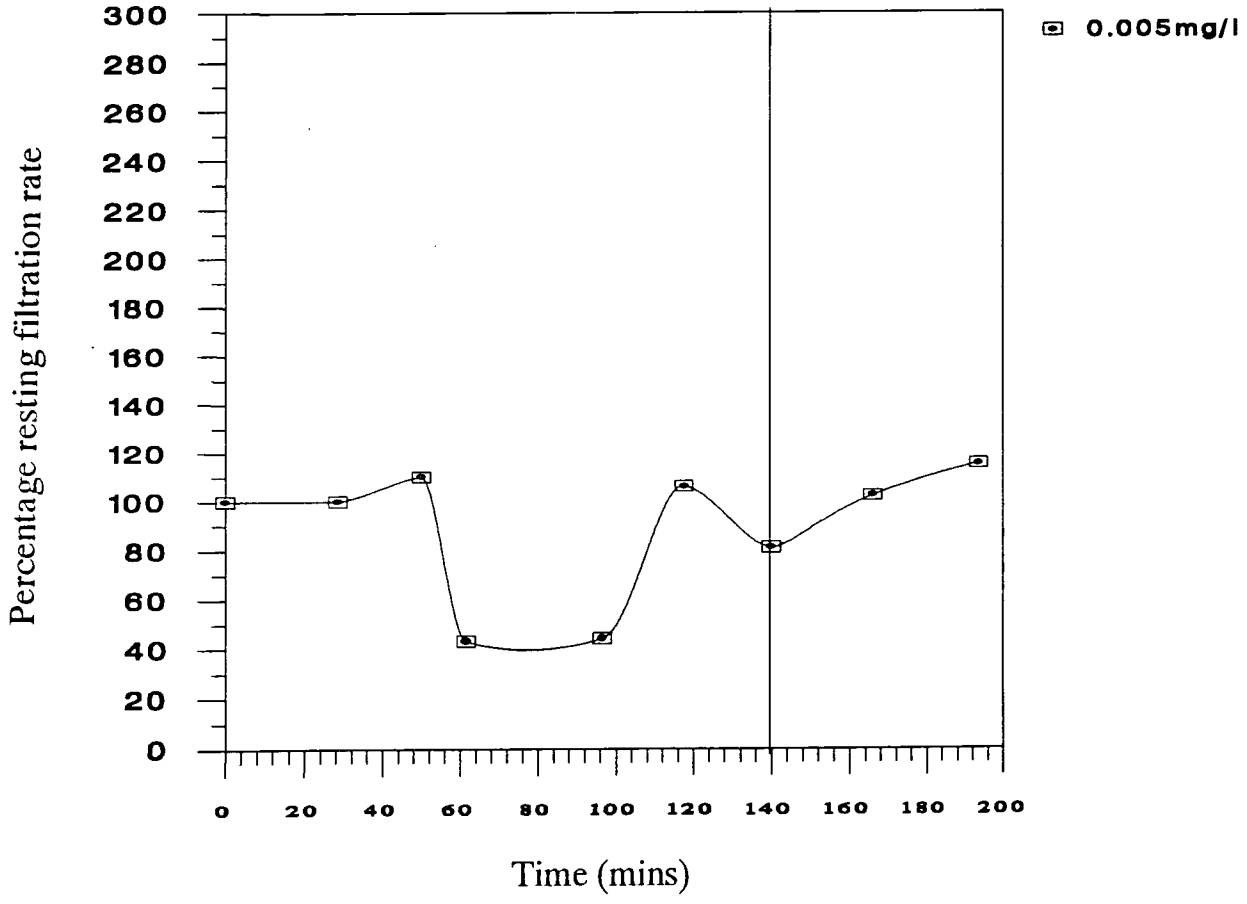


Figure 24c; 0.01mg/l

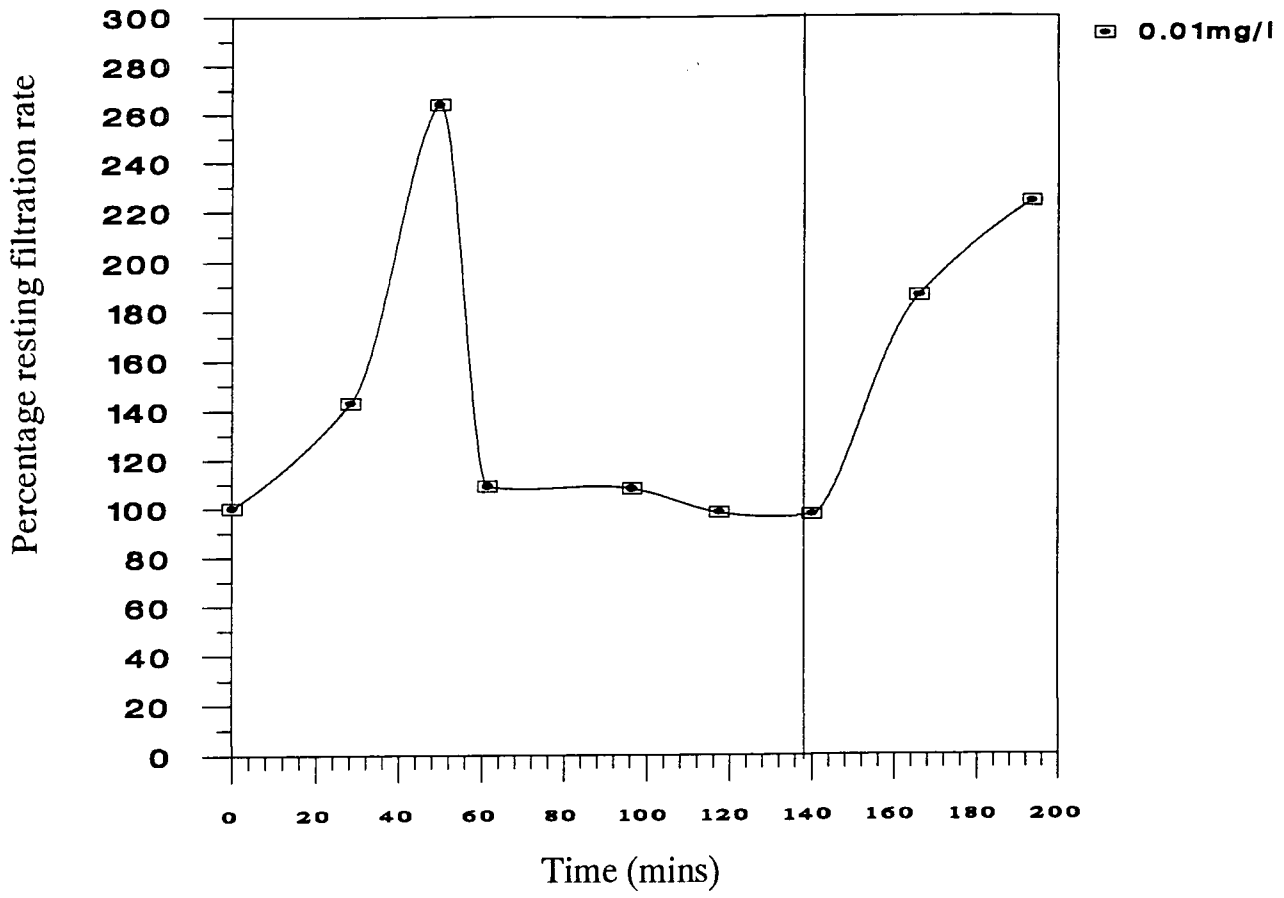


Figure 24d; 0.025mg/l

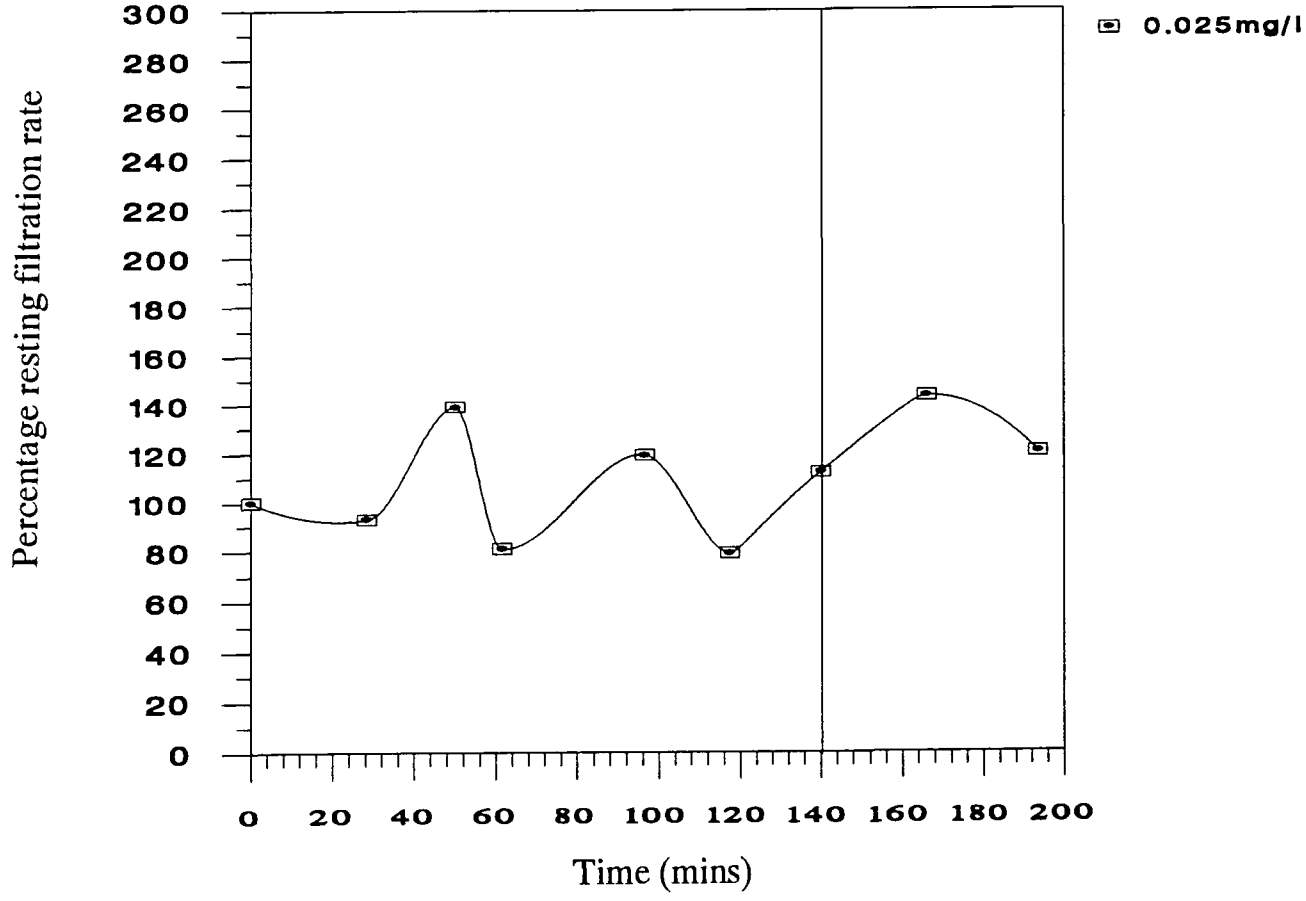


Figure 24e; 0.05mg/l

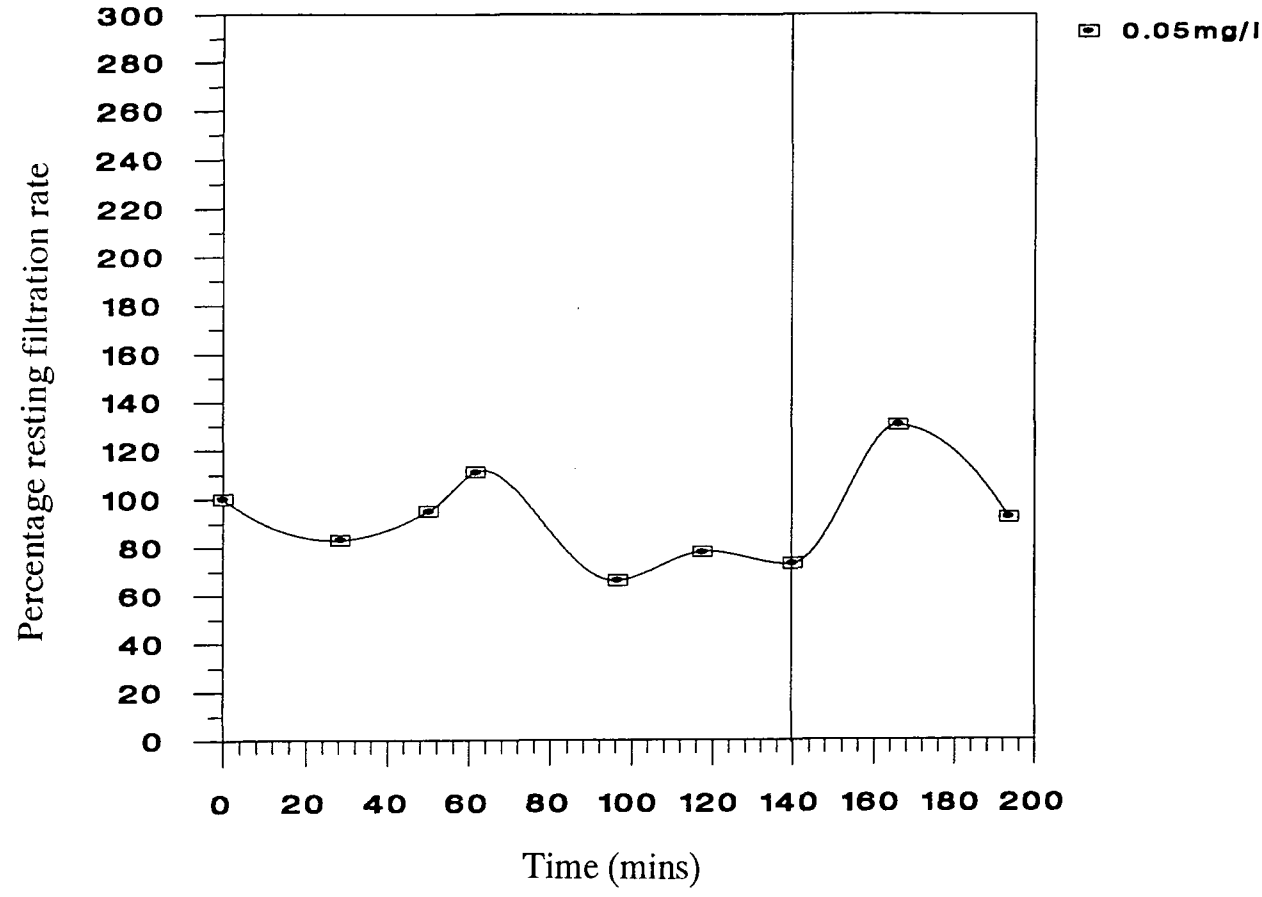


Figure 24f; 0.1mg/l

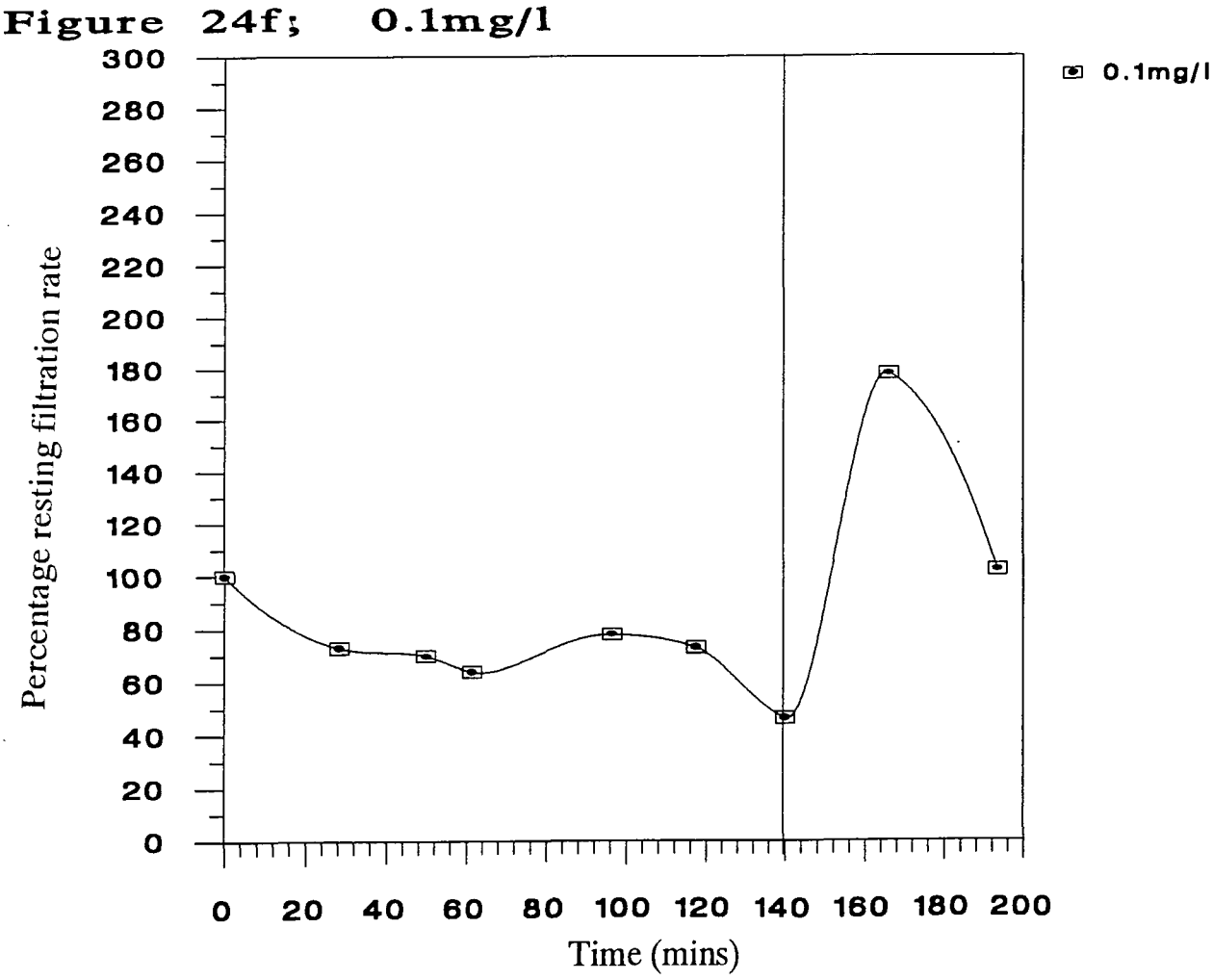


Figure 24g; 0.2mg/l

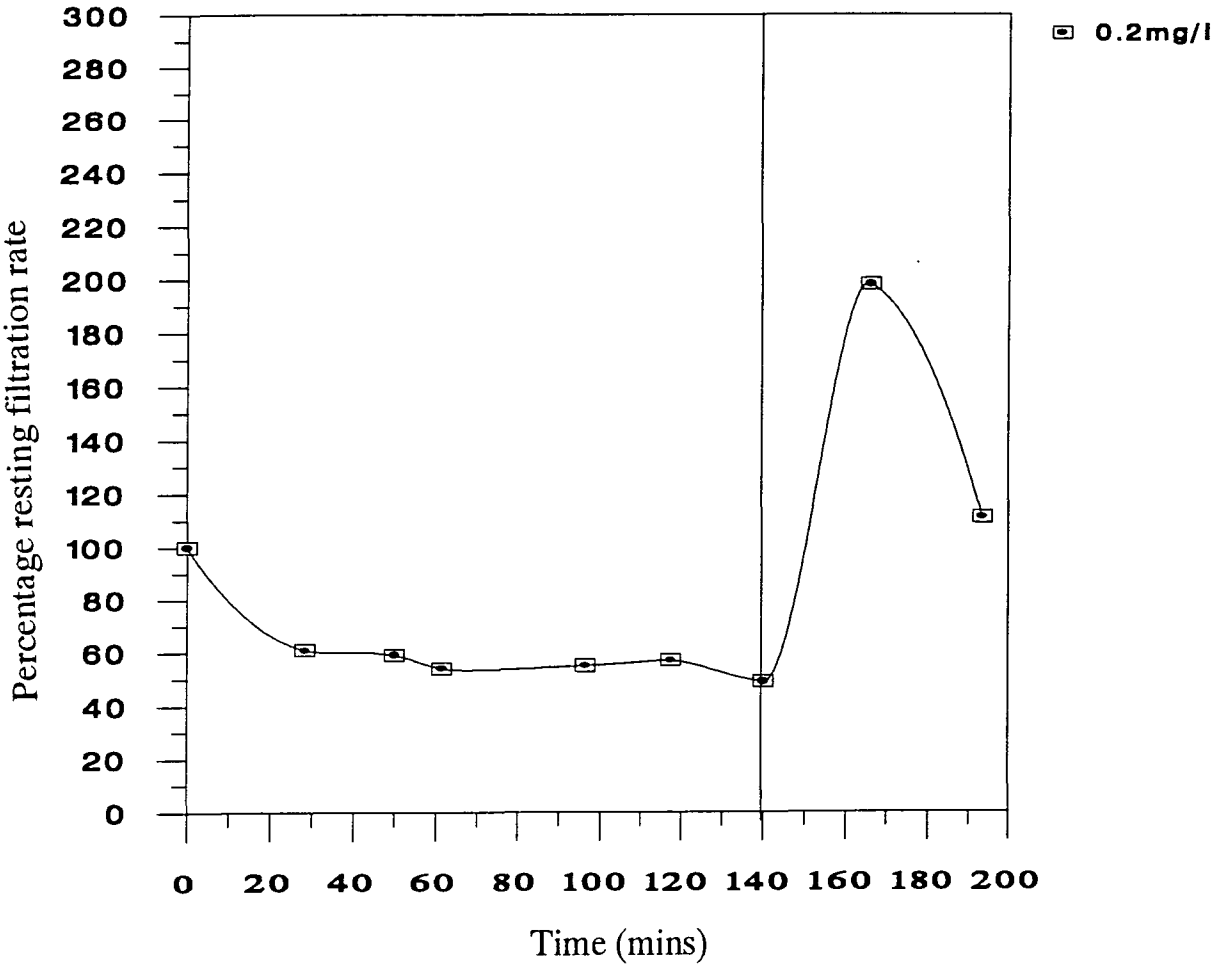
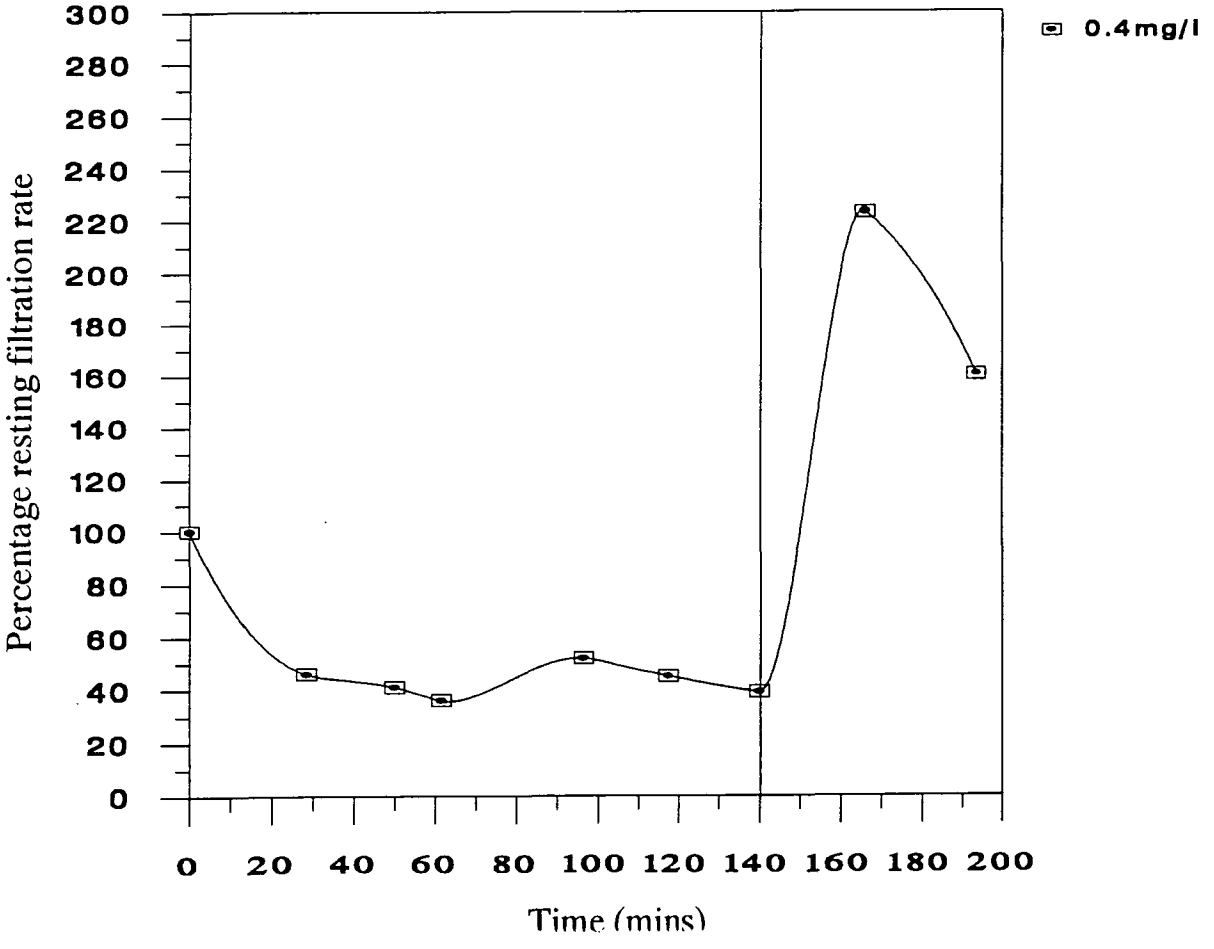


Figure 24h; 0.4mg/l



Discussion

(a) The effects of single metal salts on filtration behaviour

Variations in tolerance between the two populations, as represented by these samples, do show significant differences. The greater tolerance of the Lindisfarne population to metals not commonly present in the environment of either population could be accounted for if the Lindisfarne mussel samples were actually older than the Teesmouth population, as suggested in section 1. Growth patterns alter with age, and this is likely reflected in the general physiology; studies of freshwater fish for instance, have shown that "older" or "adult" forms are generally less sensitive to pollutants than "younger" or "embryonic" forms, (Macek and Sleight, 1977). So there could be selection for tolerance to pollutants at the stage of spat settlement at Teesmouth; this stage is the most sensitive to pollutant toxicity, so clearly a selection pressure will be in operation.

Mytilus edulis release their gametes into the sea, where fertilisation occurs and zygotic forms join the plankton. This is such an effectively large reservoir for mixing, it is to be expected that the population of spat is effectively a "constant", within which evolution would be slow. However as *Mytilus* species are so widespread, it seems likely that as a species, there will be a wide phenotypic expression of continuous characters such as tolerance to the various pollutant stresses. According to the "Mussel Watch", (an American government and industrial joint initiative, to monitor samples of shellfish from around the coast of the USA for the result of long term effects of pollution, the results of which are reported by Goldberg et al, 1978), the global population of *Mytilus* species form an interbreeding system, but they do cite an example of a relatively "closed system" in a fjord inlet, which has resulted in a morphologically distinct sub-population. Such isolation could conceivably occur elsewhere according to the local regime. The North Sea is a semi-enclosed system, which is why this area is prone to problems such as "red tides". There will likely be some selectivity for pollution-tolerant individuals, as many mussel populations around the North Sea coastlines are subjected to a locally polluted regime. The larvae they produce would not only have phenotypic stress resistance, but have potential to be immediately subjected to selection on their basis of tolerance. This embryological stage is the point in the life cycle most susceptible to toxicity. Comparison of these results with populations from further afield, such as the west coast of Britain, would show more clearly how far such selection pressures have taken the Teesmouth (and Lindisfarne) populations.

In table 6, I compare the values obtained here with comparable experiments as documented in the published literature. Results documenting the effect of pollution on marine invertebrates were not available for all of the metals in my study; this highlights the relatively sparse coverage of ecotoxicology within the marine environment, in comparison to freshwater systems. Of those where alternative data exists, this is often with regard to EC50 tests (which determine the concentration of toxin which upon a singly administered dose, causes 50% mortality of the test organisms within a specified time), or growth rates, which examine responses over several days, rather than the duration of these experiments. In cases where data is available regarding a similar protocol to that used here, quoted values vary; e.g. for cadmium, my study used cadmium sulphate, and found values of 5.6 mg/l for Lindisfarne and 8.4 mg/l for Teesmouth mussels. The comparison available is for a different species; the brown horse mussel *Perna perna*, which decreases its average filtration rate by 50% in the presence of 28 mg/l of cadmium, but this time in the form of the chloride (Watling and Watling, 1982). There is growing evidence for an influence of the associated ions having a significant effect on uptake into the tissues; I discuss this further in section 3.

In other instances, researchers have used the same compound, e.g. Nickel, as nickel chloride. In this case my results quote concentrations an order of magnitude larger than those obtained by Watling and Watling. Mercuric chloride in their test however is quoted at a concentration of 0.025 mg/l, which is practically identical to my value for the Teesmouth sample, of 0.023 mg/l. The Lindisfarne population showed far greater tolerance; their value is 0.074. For copper sulphate, there is a twofold difference between my findings and those of Abel (1976), who also worked on *M. edulis*. This difference could be significant, though is likely affected by the different temperatures at which the experiments were conducted, and also possibly salinity differences,(which were not measured),. affecting the composition of the ionic species present. The lack of standards for such variables in toxicity testing of a sub-lethal nature for the marine environment, make comparisons of experimental findings either very difficult, or inappropriate.

In terms of application to the marine environment, several criticisms of this experimental protocol can be made. Firstly, in most instances the range of metal concentrations used here is far in excess of any realistic concentration that the mussels would encounter in their natural habitats. Thus, concentrations cited in such studies so far use concentrations far in excess of the amounts detected in the water column of polluted coastal environments, (though not necessarily in excess of pollution incidents in the estuarine environment). Secondly, such pollutants would rarely impinge upon

the population in isolation as they are presented here; the variation of hydrated ionic species formed, and the ratios between particulate or ionic forms are characteristically altered. This in turn affects the biological availability. Thirdly, the experimental environment is not really realistic of the ecosystem that the mussels inhabit, where metal pollutants would be processed by a variety of organisms both in the plankton and the sediments. This could drastically alter their toxicity; mercury is one example. Salts of the organic form mono-methyl mercury, are much more toxic and have a greater biological "availability"; as shown by the findings of Watling and Watling (1982), displayed in table 6.

Further problems conceivably arise from the lack of tidal regime on the mussel population; physiology is probably affected when the animals are no longer exposed to periods out of the water, and the resulting fluctuations in temperature. In a study by Bayne and Widdows (1978) of the physiological ecology of two mussel populations from British shores, they found that the animals had their shells open for around 90% of the submersion time. and in the intervening exposed time, (during which respiration did continue, though at a reduced rate,) would undergo temperature fluctuations of up to 8°C. Of course, these variations will be specific to any one population at a particular location, but such differences will exist. For the time out of the water, anaerobic activity will likely occur, which may result in an elevation of filtration and hence respiration, for a time after initial re-submersion, in the repayment of an 'oxygen debt'. Alleviation of the stresses of a changed regime such as this will affect the animal's physiology in comparison to the in vivo situation.

In this study, statistically significant differences appear between the Teesmouth and Lindisfarne populations, but whether these are biologically significant is unclear. As I used seawater for each population, collected at the sample site, the differences that arise for the response to metals such as manganese, mercury and selenium, could either be the result of greater resilience of the Lindisfarne population, and so are better able to handle metals to which no evolution of tolerance could have occurred, through their not being present in the environment in quantities causing toxic effects, or could result from an effectively higher dose for the Teesmouth mussels due to some traces of pollutant already in their bathing water. If the bathing water from the two sites is of different salinity, or contains variant components as anions and cations, then there could be an effectively different ratio of the various complex ions formed by the metals upon dilution. This in itself potentially contributes to the variations observed.

Tolerance could have developed in the Teesmouth population for aluminium, cadmium, chromium, cobalt and lead, though the result for lead is not as pronounced as the others. The Lindisfarne site is known to incur some lead pollution, which both provides a confirmation of the likelihood of some evolution of tolerance, and an explanation of why the difference between this and the Teesmouth population's response is not large. With lead usually present, then settlement of mussel spat which are not in some inheritable way predisposed to cope with elevated lead levels in their environment, would be prevented; selection for tolerance exerts a strong pressure at this stage of the life cycle. When only tolerant animals are permitted to live in a particular environment, those among them with a greater degree of tolerance will have a higher fecundity, or a greater success rate in the survival of young. Over successive generations in the presence of the pollutant, there would be a refinement of the particular allelic characteristic or characteristics, which produce that tolerance, resulting in a local variety which has developed a mechanism for detoxification, probably by storage or excretion in the case of heavy metals.

Differences in filtration behaviour between samples from the two populations did not appear significant for iron or zinc, so there seems little difference in the ability of the two populations to tolerate these metals in some capacity. With reference to zinc, this is not surprising, when bearing in mind the high bodily zinc content of the Lindisfarne mussels; any inducible mechanisms for dealing with pollutant concentrations will have been activated. It would be interesting to be able to further extend this work to incorporate tissue levels of other metals such as iron, in order to clarify whether the equivalent responses of the two populations arise from a similar 'tolerance'.

Table 6; Comparison of results with published literature

('Lindisfarne' and 'Teesmouth' refer to the results from the respective population samples)

Metal	Test species	Chemical form	Conc. (mg/l)	Time (days)	Effect	Reference
Aluminium	Lindisfarne	Aluminium potassium	25.9	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth	chloride	28.0	1 h		
Cadmium	Lindisfarne	Cadmium sulphate	5.6	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		8.4	1 h		
	Mytilus edulis (larva)	cadmium dichloride	1.20	2	EC_{50} development abnormal	Martin et al, 1981
	Mytilus edulis (adult)	cadmium dichloride	0.002	>4	growth stimulated	Stromgren, T., 1982
			0.010	>4	growth reduced	
	Perna perna	cadmium dichloride	28.0	1 h	filtration rate reduced 50%	Watling & Watling, 1982
Chromium	Lindisfarne	chromium III chloride	13.1	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		18.4	1 h		
	Mya arenia	potassium chromate	57	4	LC_{50}	Eisler & Henneky, 1977
	Mytilus edulis (larva)	potassium chromate	4.47± 0.74	2	EC_{50} development abnormal	Martin et al, 1981
	Perna perna	chromium trichloride	2.0 (1.8-2.6)	1 h	filtration rate reduced 50%	Watling & Watling, 1982
		sodium dichromate	1.6 (1.0-2.0)	1 h	filtration rate reduced 50%	
Cobalt	Lindisfarne	cobalt chloride	8.0	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		9.6	1 h		
Copper	Lindisfarne	copper II sulphate	0.50	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		0.32	1 h		
	Mytilus edulis	copper II sulphate	0.15 (ave.)	1 h	Filtration rate reduced 50%	Abel, P.D. 1976
	Mya arenia	Cu2+	0.035	7	50% mortality	Eisler, R. 1977
			0.039	4	LC_{50}	
	Mytilus edulis (larva)	copper II sulphate	0.005	2	EC_{50} development abnormal	Martin et al, 1981
	Mytilus edulis (adult)	copper II sulphate	0.001	14	unaffected	Stromgren, T., 1982
			0.003	5-6	growth reduced	
			0.005	1	valves closed	
			0.008	1	growth stopped	
			0.01	21	no mortality	
			0.04	14	50% mortality	

Iron III	Lindisfarne	iron III chloride	10.6	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		12.1	1 h		
Lead	Lindisfarne	lead II nitrate	4.0	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		4.55	1 h		
	Mya arenia	lead II nitrate	8.8	7	LC_{50}	Lloyd, R. 1984
	Mytilus edulis		>5.0	39	LC_{50}	
	Mytilus edulis (adult)	$Pb(CH_3)_4$	0.27	4	} LC_{50}	Maddock and Taylor, 1977
		$Pb(C_2H_5)_4$	0.1	4		
		$Pb(CH_3)_3$	0.5	4		
		$Pb(C_2H_5)_3$	1.1	4		
	Mytilus edulis (larva)	lead II nitrate	0.476 ±0.001	2	EC_{50} development abnormal	Martin et al, 1981
	Bullia digitalis	lead II nitrate	0.5	4	no effect	Brown, A.C. 1982
			1.0	4	stops burrowing	
			1.5	4	decreased oxygen uptake	
Manganese	Lindisfarne	manganese IV chloride	13.8	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		10.4	1 h		
Mercury	Lindisfarne	mercuric chloride	0.074	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		0.023	1 h		
	Mytilus edulis	mercuric chloride	0.04	1 h	Filtration rate reduced 50%	Abel, P.D. 1976
	Bullia digitalis	mercuric chloride	0.5	4	no effect	Brown, A.C. 1982
			2.0	4	stops burrowing	
			5.0	4	20% decrease in oxygen uptake	
	Mytilus edulis (larva)	mercuric chloride	0.0058 ±0.001	2	EC_{50} development abnormal	Martin et al, 1981
	Mytilus edulis (adult)	mercuric chloride	0.0003	5	growth reduced	Stromgren, T., 1982
			0.0016	4	growth halted	
			0.025	1	some mortalities	
	Perna perna	mercuric chloride	0.025	1 h	} filt. ↓	Watling & Watling, 1982
		$CH_3.HgCl_2$	0.050			
		$C_2H_4.HgCl_2$	0.030			
		phenyl-mercuric acetate	0.020			
		Na-acetate	1.0		(no effect)	
		mercuric chloride + selenium chloride 0.2mg/l)	0.040		filter rate reduced 50%	

Nickel	Lindisfarne	nickel chloride	10.6	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		9.8	1 h		
	Mercenaria mercenaria	nickel chloride	0.310 0.571	4 8-10	LC_{50} EC_{50} larval growth	Calabrese et al, 1974, 1977
	Mya arenia	nickel chloride	320.0	4	LC_{50}	Eisler and Henneky, 1977
	Mytilus edulis (larvae)	nickel sulphate	0.891± 0.21	2	EC_{50} development abnormal	Martin et al, 1981
	Mytilus edulis (adult)	nickel chloride	72.0	4	LC_{50}	Eisler and Henneky, 1977
	Perna perna	nickel chloride	0.7 (0.6-1.0)	1 h	Filtration rate reduced 50%	Watling and Watling, 1982
Selenium	Lindisfarne	selenium dioxide	20.0	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		16.9	1 h		
	Bullia digitalis	selenium dioxide	1.0 4.0 5.0 7.0	4 4 4 4	no effect stops burrowing 15%↓ O_2 uptake irreversible stress	Brown A.C. 1982
	Mytilus edulis (larva)	selenium dioxide	10.0	2	EC_{50} not reached	Martin et al, 1981
	Perna perna	selenium oxide	0.2 (0.1-0.3)	1 h	Filtration rate reduced 50%	Watling and Watling, 1982
Zinc	Lindisfarne	zinc chloride	8.2	1 h	Filtration rate reduced 50%	(This thesis)
	Teesmouth		7.8	1 h		
	Mytilus edulis	zinc sulphate	1.6 (ave.)	1 h	Filtration rate reduced 50%	Abel, P.D. 1976
	Mya arenia	zinc chloride	5.2 7.7 1.55, 3.1	4 4 7	LC_{50}	Eisler and Henneky, 1977, Eisler 1977
	Mytilus edulis (larva)	zinc sulphate	0.175	2	EC_{50} development abnormal	Martin et al, 1981
	Mytilus edulis (adult)	zinc chloride	0.05 0.025 0.01	1 2 22	growth reduced	Stromgren, T. (1982)
	Mytilus edulis (adult)	zinc chloride	2.5, 4.3	4	LC_{50}	Aubert et al, 1975
	Nyssarius obsoletus	zinc chloride	50.0 7.4	4 7	LC_{50}	Eisler and Henneky, 1977

(b) Filtration behaviour in response to combinations of metal salts

Though well documented for freshwater systems, the interaction of pollutants in the marine environment has been little investigated. Rather more factors than merely the presence of the different pollutants exert an affect on the biological consequences of any interaction. Predominantly, the interactions between metals as shown in my study were antagonistic. Interactions between cations, and complex ion formation will occur in the introduction of the metal salts into the same saline solution, so that there may be either precipitation of the salt compound, or particulate formation, which possibly renders the metal "inert" in terms of affecting the mussels. This would effectively lower the proportion of the administered dose which is biologically available, and could explain many of the responses shown here. Selenium as selenium chloride, 0.2 mg/l, when added to mercuric chloride in the brown horse mussel *Perna perna*, increased the quantity of mercuric chloride which was required to produce a 50% decrease in filtration, from 0.025 mg/l for mercuric chloride alone, to 0.040 mg/l in combination (Watling and Watling, 1982). Antagonism was likewise demonstrated in this determination.

In my findings copper showed a synergistic interaction with four other metals present, and additivity with a further one. Of these five, four of them (aluminium, manganese, selenium and zinc) are thought to be essential as trace elements, and known to have a role in metabolism. It is likely that these metals act on different enzymic components of the same metabolic pathway as copper inhibits, in which these other metals compete to displace trace metals from other enzymes, and in binding, reduce enzymic functionality or rate of activity. Another metal, mercury, had an additive effect with copper, cadmium, iron and zinc.

Mercury is readily uptaken into the tissues and is adsorbed onto the surfaces of particulate matter in solution; *Mytilus* feeds by removing particulate material from the water, and so when in the presence of a readily precipitated compound such as a sparingly soluble salt of heavy metal, adsorption onto the surface of the particles of precipitate may help to increase uptake and access into the body as the animal gathers material from suspension. Aluminium precipitates easily in saline solution, and may appear synergistic with iron and zinc because they too have the ability to adsorb onto particles, hence accelerating their uptake. This may also be true of the additive action of selenium with nickel; nickel is typically of a quite low solubility, and thus lower toxicity. Nickel is also an enzymic component. Certain enzymes which have a trace-metal component, may have that cofactor displaced from its binding site to the protein,

when encountering such elevated levels of other metal ions. At equilibrium at these concentrations, the foreign metal ion may have a greater binding affinity to the protein, resulting in displacement of the usual metal from the enzyme; this would probably decrease the protein's biochemical activity. This could also be true for cadmium and iron, which also readily precipitate, (and which show additivity with mercury).

Cadmium has been shown to inhibit the movement of latero-frontal cilia (Capuzzo and Sasner, 1977), so perhaps this could reduce the water flow through the gills, and hence the access of other metals to the body. Cadmium shows additivity only with mercury, has a containing effect on iron, lead and manganese, and is antagonistic with all others. Hence the order of addition of the metals could exert an effect over the mechanism of their combined behaviour. In this test, because time and animal material was limiting, I did not have the resources to perform reciprocal determinations on fresh batches of mussels, to examine the relative behaviour for any one interaction in terms of the order of introduction of the metal salt to the test animals. Individual metal salts were added sequentially 'one way', i.e. one metal salt was added first to six batches of mussels, and then six other metals were added, one to each batch. This test, that is one metal combining with six others, was performed for all metals, and in this way the array of possible combinations covered.

The doses of single metal salts, which produced 70% of the resting filtration rate in the earlier determinations, however did not show repeatability with a great degree of accuracy. Perhaps this confirms the assertions of Abel and Papanthanassiou (1986), the assessment of pollutant toxicity by this method is not as repeatable, at least for this smaller sample size (10), and so is not reliable quantitatively. However there is a variation in filtration behaviour which seems to be prevalent under normal or 'control' conditions, which is not taken into account in these determinations. The combination of the apparent temporal fluctuations in filtration rate in the presence of these many chemicals, along with the unpredictability of the complex ion formation in the interacting metal solutions, make interpretation of these results difficult. Perhaps the only physiological significance is in terms of the few which appear to act synergistically together, or possibly those which appear additive.

(c) Variation in filtration rates over time

The respiration and feeding rate of *Mytilus edulis* fluctuates over time, and this pattern is affected by the presence of pollutants in the body.

The results appeared to be similar to those found over a longer period, looking at growth rates in the colonial hydroid *Campanularia flexuosa* in the presence of different concentrations of copper II sulphate (Stebbing and Hiby, 1978). My experimentation also investigated the response to this pollutant. In *Mytilus*, as in the pattern of hydroid growth, an active response is elicited at a certain critical level of the pollutant, which results in increased metabolic activity. In *Campanularia*, this was demonstrated in alteration of growth rate. This stress response in *Campanularia* possibly indicates an attempt to deal with the toxin by a metabolic process. In *Mytilus*, this response is presumably an elevation of through-flow to clear the contaminant from the body, and can be damped by an inhibitory concentration of the pollutant. On introduction to the lower concentrations, it may take some time to encounter enough of the metal to have this inhibitory effect, which results in a cumulative reduction in filtration rate over time. After being in the presence of an inhibitory concentration for a short time, as shown in these experiments, there is a heightened filtration response upon introduction to clean water, in an attempt to eliminate the toxicant from the body. This could be also due to 'repayment of an oxygen debt', after a period of anaerobic activity, perhaps induced by the presence of the pollutant.

Short-term fluctuations in metabolic activity have been recorded for other marine invertebrates. In a study of oxygen consumption, measured at hourly intervals in the common cockle *Cardium edule*, respiration was found to alternate between periods of aerobic activity and quiescent (anaerobic) respiration (Newell, 1966). A mean of his results gave a steady value for oxygen consumption, above and below which the point values fluctuated, with respect to time. He cites similar alternation between aerobic and anaerobic activity for other intertidal animals, specifically the anemone *Actinia equina*, the polychaete *Nephthys hombergi*, the winkle *Littorina littorea* and the barnacle *Balanus balanoides* (Newell and Northcroft, 1965).

Newell's experiment showed respiratory behaviour in *Cardium edule* at different temperatures; the 5°C determination showed a period of aerobic activity, followed by a period of quiescence which lasted for two hours (at the resolution of hourly sampling), followed by further aerobic activity. The mussels could have a similar pattern of aerobic and anaerobic respiration, which is manifest in high, then low

filtration rates respectively. This pattern would easily be obscured in the sampling of a batch of 10 individuals, resulting in the fluctuations over time, as seen for the control experiment, displayed in figure 22(a). This would also explain the erratic filtration activity shown in the pilot experiment, which determined rates for individual mussels, (displayed in table 3.) There was a rapid cessation of filtration activity of individual no. 1, from high to immeasurably low, at concentrations of zinc chloride which the other individuals tolerated with over 50% of their resting filtration rates. It now seems probable that this individual moved from an aerobic to an anaerobic phase during the course of that experiment.

(d) Summary

The wide variations that occur between toxicological studies as cited in the literature, illustrate the difficulty in attempting to attach biological significance to measured physiological responses to pollutant stress. In the case of the marine environment, there is no overall standardisation regarding the kind of impact assessment which is most appropriate. Often there are discrepancies in the quantitative experimental results between research laboratories, of an order of magnitude or more. Abel's (1976) test as it was performed here, comparing filtration rates for batches of mussels, is arguably applicable for the in-vitro comparison performed here, but quantitatively these values may not be comparable, particularly as variations in local regime and phase of the reproductive cycle will exacerbate differences.

However, it seems that a greater understanding of the normal physiological behaviour of *Mytilus* may yield a further possibility of a quantitative criterion, in terms of the behaviour of the individual. It seems that toxicity is perhaps directly measurable for periods of aerobic activity in terms of a dose-response curve, as Abel originally intended. There may be alteration in the length of time spent in the anaerobic phase. If determinations are performed on an array of individuals, then not only a quantitative measurement of impact of the toxicant could be made, but also an idea could be gained of the spectrum of responses exhibited by a population to a pollutant or combination of pollutants. The fluctuating patterns observed in the filtration activity in the presence of individual metal salts, could result from the tendency of individuals within the batch at levels according to individual tolerance, to lapse into a period of anaerobic respiration, hoping to wait for the subsidence of a sudden pollution incident. Such behaviour is likely to be highly individual, and could offer an explanation as to the different dose-response behaviour as found in the 70% 'control' determinations of the experiment investigating synergy and antagonism between solutions of two metal salts.

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Section 3

Measurement of Bioaccumulation of Metal Salts

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Methods

The concentrations of single metal salts which reduced the resting filtration rate in *Mytilus* to 70% of its former level, were initially chosen as the sub-lethal dose for investigation of the longer-term behaviour in the presence of these compounds. Four of the metals were chosen for this; copper, lead, cadmium and zinc. Uptake levels in *Mytilus* for two of these (Cd and Pb) were cited as being reliable, and two (Cu and Zn), as being unreliable indicators of the presence of pollutant concentrations of that metal in the animal's environment (Barnett, 1990). This part of the study was intended to compare bioaccumulation in the two populations, over a period of 6 weeks. Batches of 60 *Mytilus* were kept in the presence of these metals, to be sampled at two-week intervals.

Unfortunately, the Teesmouth sample did not survive well under these conditions, and after steady mortality, all had died completely after 17 days. After 14 days, small samples (of 10-15 individuals), were taken for analysis; the results are presented in figure 25. The Lindisfarne sample, were not collected until after beginning the investigation of the Teesmouth sample, and so was given a much reduced dose, approximating to that producing 90% of the resting filtration rate according to their dose-response curves, in an attempt at avoiding a similar fate. These survived well, and were sampled at ten day intervals for 30 days. Time and resources unfortunately did not permit further collection for a repetition of this test with the Teesmouth population.

Results

Samples of 20 individuals were taken at each stage, frozen overnight, the soft parts removed, dried and analysed as for the initial population study, using the atomic absorption spectrophotometer, (outlined in section 1). The results of the analyses are presented below in table 7, along with the bathing concentrations of metal in which the animals were kept. (The 'initial' figures are those as presented in section 1; the preliminary analysis.) These results are presented graphically in figures 26-29, for individual metals over time in the Lindisfarne population, and figure 30 for all four metals. The data, with more detailed statistics, can be seen in tabulated form in Appendix 4.

Table 7; Soft tissue concentrations of 4 heavy metals, for *Mytilus edulis*, collected from Lindisfarne and Teesmouth and kept for the specified time in solutions of the metal salt. (Concentrations quoted are in mg/g tissue [dry weight], showing mean and standard deviations.)

	lead	cadmium	copper	zinc
Bathing conc. (mg/l)	0.5	1.5	0.05	1.25
Lindisfarne; initial sample	29.77 ± 1.233	2.53± 0.046	16.72 ± 0.335	111.76 ± 7.062
10 days	21.77 ± 3.48	38.57 ± 8.61	26.90 ± 4.10	404.52 ± 93.75
20 days	25.00 ± 6.00	29.60 ± 8.14	26.10 ± 6.49	406.36 ± 94.82
30 days	27.55 ± 6.11	200.77 ± 42.53	23.17 ± 4.56	464.65 ± 184.94
Bathing conc. (mg/l)	2.0	4.5	0.1	3.5
Teesmouth; initial sample	47.72 ± 2.15	3.72 ± 0.03	1.234 ± 0.62	13.31 ± 0.11
14 days	93.71 ±29.17	266.70 ± 107.1	66.37 ± 48.13	585.29 ± 284.73

Figure 25; Teesmouth sample; Bioaccumulation over time for 4 heavy metals

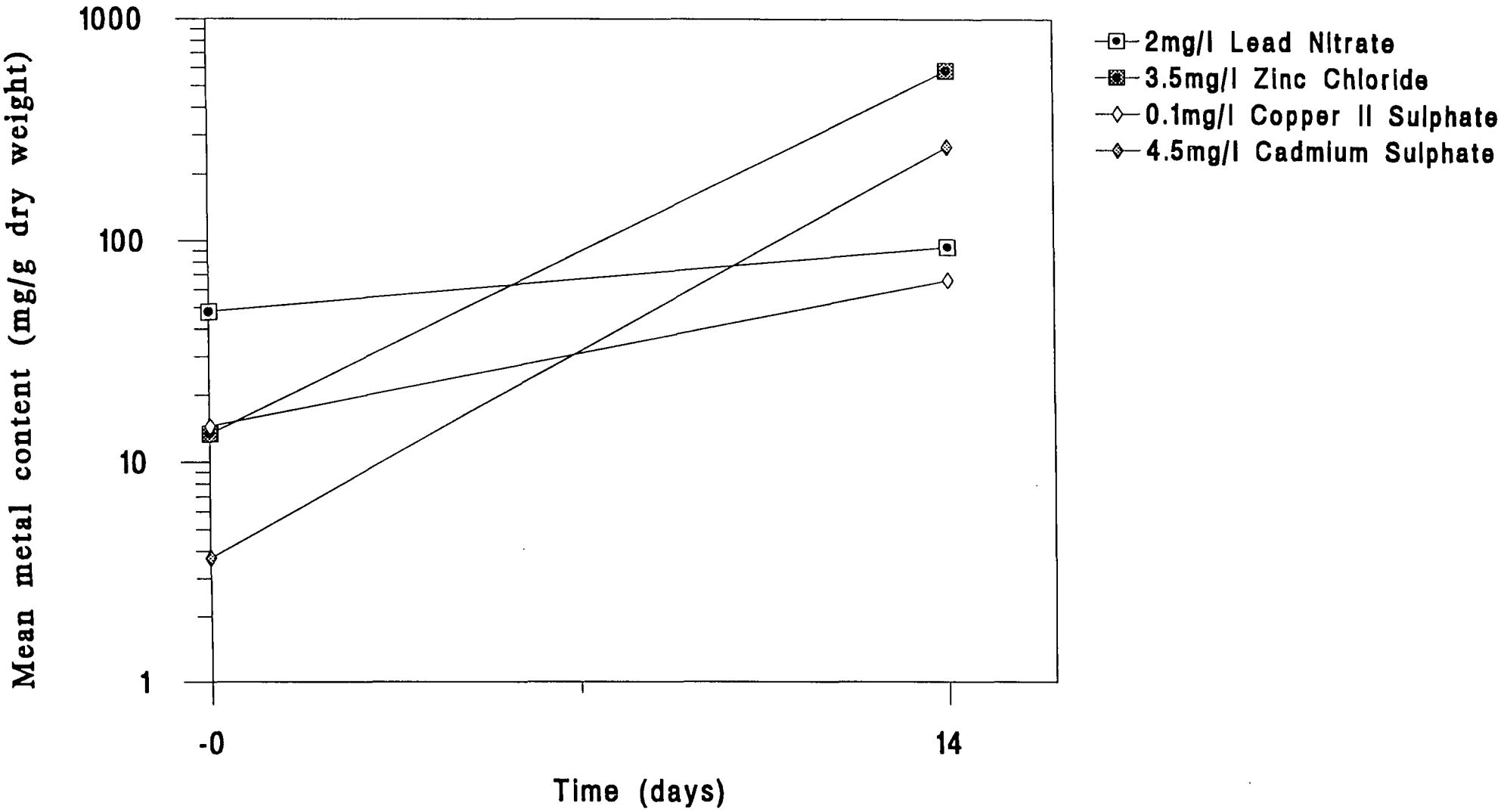


Figure 26; Lindisfarne sample; change in mean cadmium content for mussels kept in 3.0 mg/l cadmium sulphate

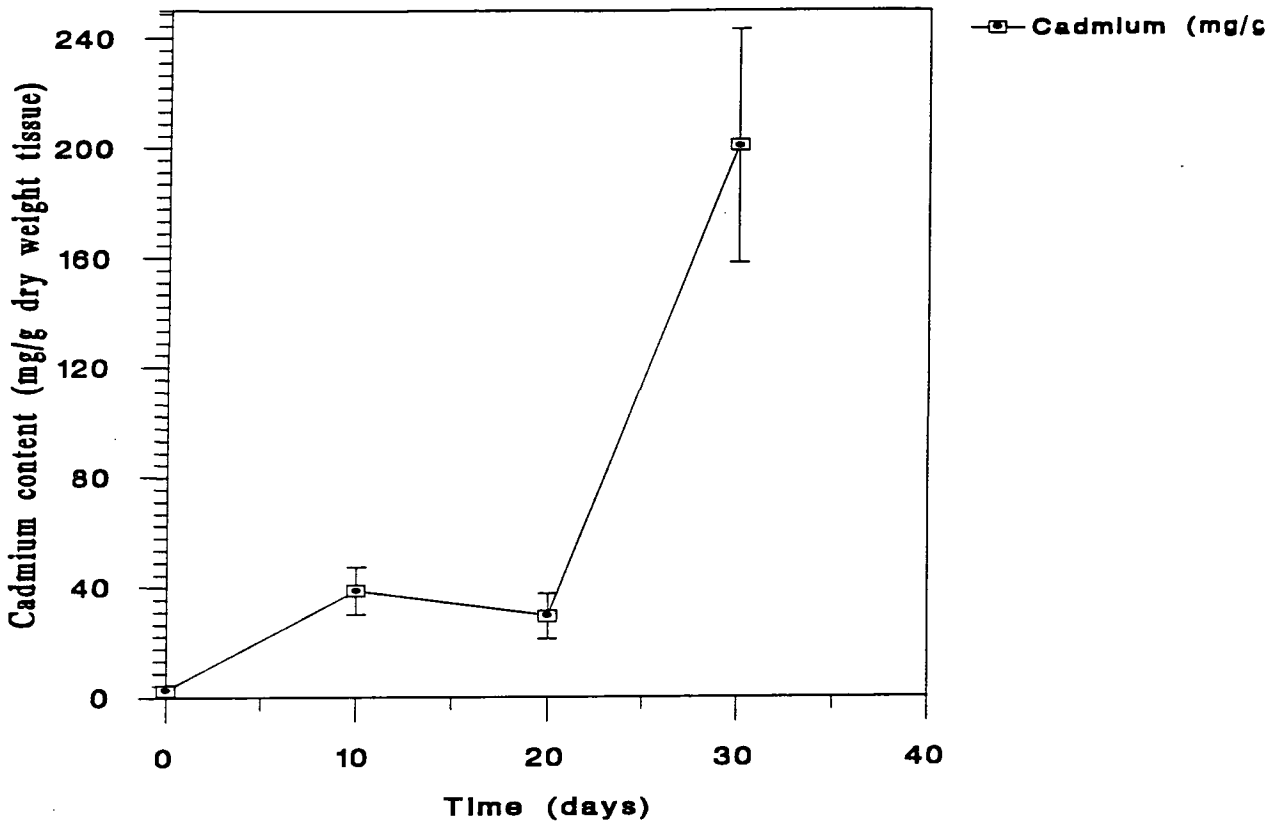


Figure 27; Lindisfarne sample; change in mean copper content for mussels kept in 0.05 mg/l copper II sulphate

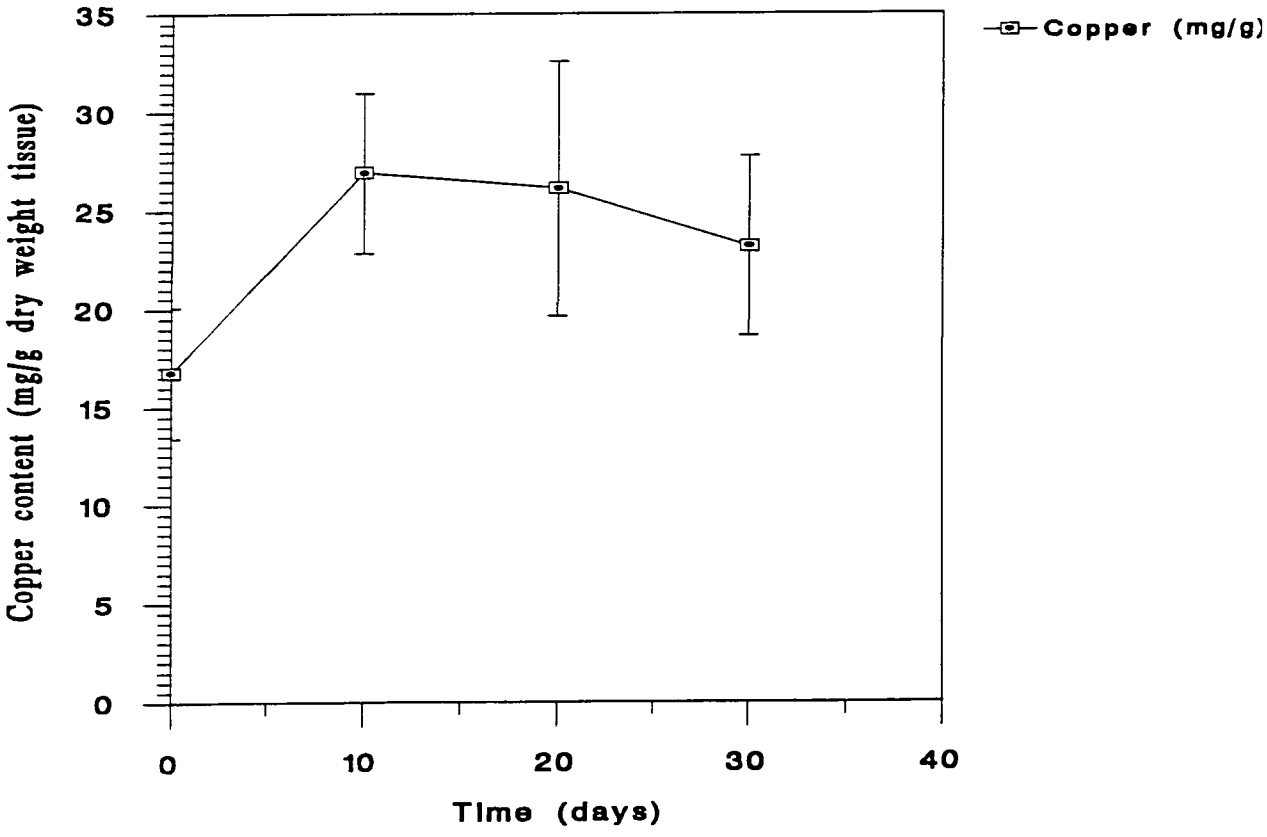


Figure 28; Lindisfarne sample; change in mean lead content for mussels kept in 1.25 mg/l lead II nitrate

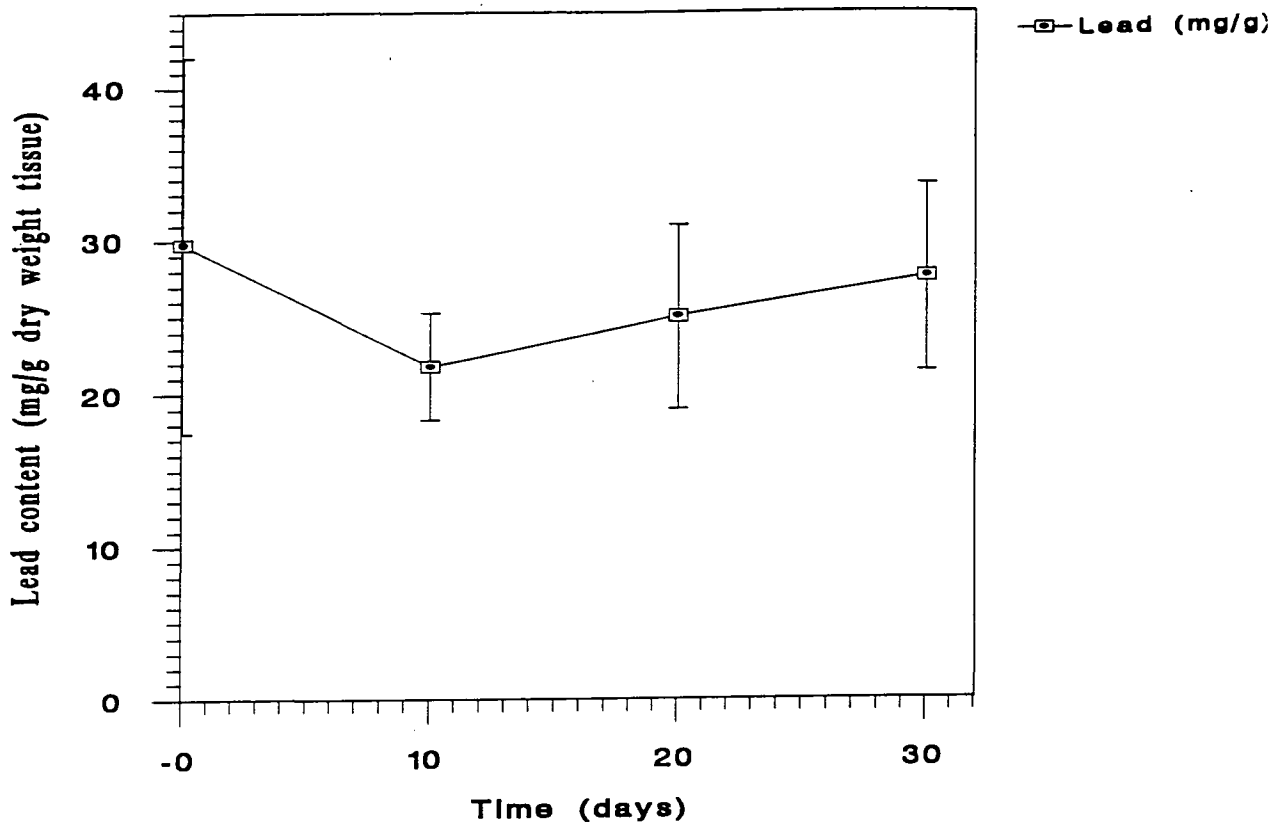


Figure 29; Lindisfarne sample; change in zinc content for mussels kept in 1.6 mg/l zinc chloride

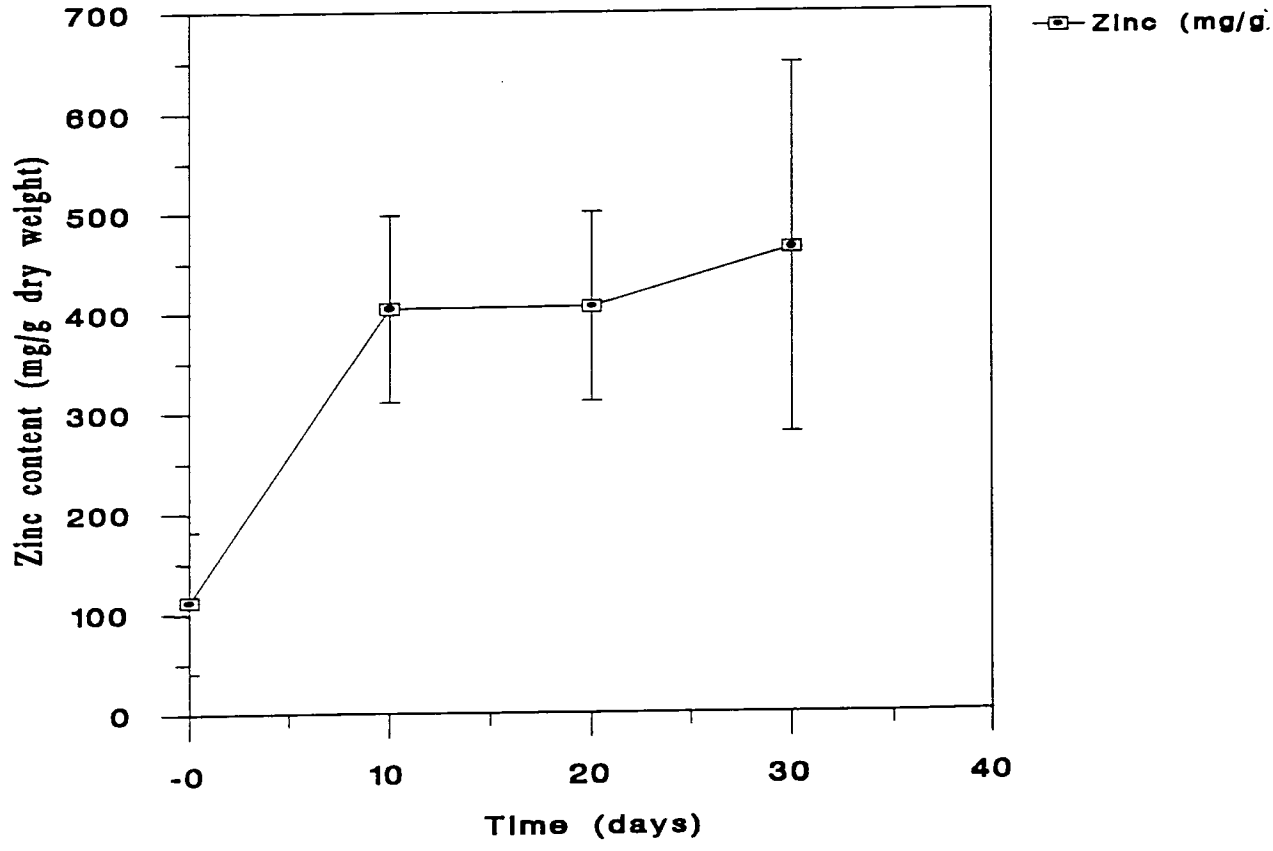
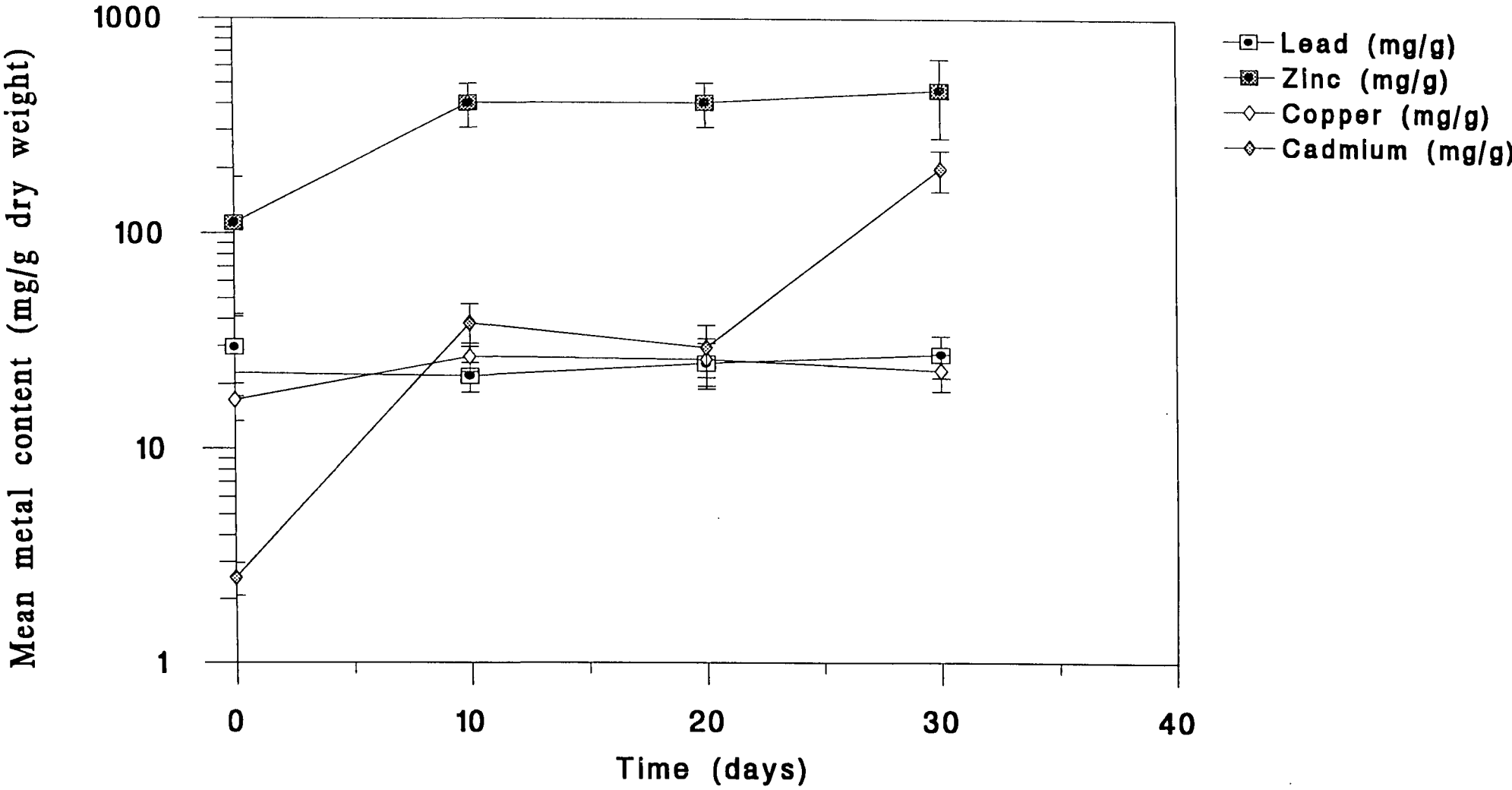


Figure 30; Lindisfarne sample; Bioaccumulation over time for 4 heavy metals



(c) Discussion

It seems that all four heavy metals examined here are potentially bioaccumulated from sub-lethal background concentrations. The results from the Teesmouth sample kept in higher concentrations, show a surprisingly large accumulation in the tissues; for copper and zinc these are well over an order of magnitude above the administered dose. This is clear from the graph in figure 25. The 70% filtration concentrations, though quite low, are arguably higher than would be normally encountered in an in-vivo situation, even in the case of a localised pollutant discharge, so ecological significance can only be supposed with caution. The dose administered was of a lethal rather than sub-lethal nature over the duration of the experiment, and meant that the filtration response is a short-term stress response which can only be tolerated for a short length of time. Presumably in an in-vivo situation, such high discharge concentrations would be rapidly dissipated by the tide, and so this is a response in the 'disturbed function' phase of tolerance to pollutant stress (see figure 1; Lloyd 1972).

For the Lindisfarne population at the lower concentrations of metal salt, there is different bioaccumulatory behaviour. Cadmium and zinc appear to be accumulated effectively, while copper and lead show comparatively little change. This result does not necessarily mean that copper and lead are not taken up into the tissues. The bathing concentration of copper is very low, and the final tissue concentration is over two orders of magnitude above ambient levels, almost a doubling of the concentration already present. This appears indistinct on the graph when compared to the others, because the numbers are so small. Copper II sulphate as administered here, seems to have quantitatively a greater toxicity than the other metals used, and concentrations are thus necessarily very low.

The stipulations of Barnett (1990), in citing the presence in the tissues of cadmium and lead as reliable indicators of pollution, and copper and zinc as unreliable, seem to disagree with the findings here. *Mytilus edulis* are known bioaccumulators of lead, (Schulz-Baldes, 1974, 1978), and this has also been demonstrated with iron as hydrated ferric oxide (George, Pirie and Coombs, 1977). Uptake and storage of zinc has also been recorded (Coombs, 1977). Davenport and Manley (1978) in investigating the valve closure mechanisms of *Mytilus edulis* in exposure to copper sulphate, suggested from their findings that mussels would be reasonably efficient indicators of copper pollution up to concentrations of 160-200µg/l. Coombs and George (1978) combined electron microscope studies of tissues with experiments

investigating metal uptake in *Mytilus edulis* of lead and iron, and *Ostrea edulis* of copper and zinc. This study reveals a storage mechanism which showed vesicle formation within the cell, enclosing uptaken metal within a membrane.

Lead levels in the Lindisfarne population seemed not to have changed; the range within a single standard deviation from the mean incorporated all the attained values, so differences are likely due to an insufficiently large sample size. Clearly though, the levels of lead had not increased. Perhaps the concentration of lead in the bathing solution was either below a certain threshold at which elimination mechanisms could deal with further lead ions effectively, or the lead ions were in a complex chemical form which either were not absorbed, or could be efficiently eliminated, rather than requiring detoxification and storage in the body. Alternatively, rates of uptake were either so slow as to not be apparent in my present study, or had reached a physiologically determined maximum value for this particular population. Lead stored in the body is not eliminated when transplanted to an environment with a lower lead content; indeed, tissue lead concentration has been suggested as a reliable means of estimating the age of mussels when the annual ring patterns are indistinct. Thus the presence of lead merely confirms that lead has been in the environment for some time, rather than as an indicator of shorter term pollution incidents.

Schulz-Baldes (1974) recorded a linear uptake over time for Mytilus in the presence of lead, though later suggested that as the concentration factor should reach a maximum value at equilibrium conditions, this apparently linear relationship has to be viewed as an initial phase of an exponential relationship (Schulz-Baldes, 1978). The time to reach equilibrium seems to be a metal-specific value, and in most cases is a very slow process. The oyster *Crassostrea virginica*, was found to continue uptake of cadmium at 5μg/l after more than 10 months (Zarrooigan and Cheer, 1976). Schulz-Baldes (1978) found that absorption of lead occurs immediately into the gills and blood, and somewhat slower, into the kidney. He suggests that these are the internal transport routes, with transport within the animal to the kidney followed by uptake into excretory cells, and formation of granules, as the rate-limiting step.

Cadmium is also cited as being reliably absorbed by the tissues from low levels in the environment. Uptake in this experiment was marked upon exposure to the metal, (which showed little difference between the Lindisfarne 30 day sample and the Teesmouth 14 day sample). Coombs and George (1978) found that whereas absorption of lead was altered significantly by complexation of the ions, cadmium gave equal absorption of both low and high molecular weight complexes. They performed

in-vitro experiments examining uptake by gill tissues from *Mytilus edulis*, which gave results that would agree with those expected from a passive accumulation process such as facilitated diffusion.

Van Haren et al (1990), found that in the western Scheldt estuary of the Dutch Delta area, where severe contaminations of trace metals and organic pollutants occur, both cadmium and copper concentrations were elevated in *Mytilus edulis*. Cadmium concentrations here reached 874 nmol/g adw (ashfree dry weight) in 1981, and 22 nmol/g adw in 1987; this was with a cadmium load to the locality of around 10 tons /year in 1981, and 6 tons/year in 1987. Likewise for copper, concentrations of 346 nmol/g adw (1981) and 197 nmol/g adw in 1987, with local pollution levels reaching 62-73 tons/year for 1981 and 1987. They contrast this with the findings of DeKock and Marquenie (1981), who assessed background levels of 5.78 nmol/g adw for cadmium, and 122 nmol/g adw for copper, measured at Killary Harbour near Leenaun in western Ireland. Though I am unable to compare my results quantitatively to theirs, they both agree in a qualitative sense; that cadmium and copper are both accumulated by *Mytilus*, the copper from a very low bathing concentration (0.05 mg/l) in my assessment.

Mytilus edulis is cited as an unreliable indicator of zinc in the environment by Barnett (1990); this contrasts not only to my findings for the initial samples from Lindisfarne, but also other accounts in the published literature, e.g. Pentreath, 1973. Coombs (1977) found that lead and zinc are stored in granular form in the kidney of *Mytilus*, and also occur in the gills and gut, though this may be just stages of internal transport rather than storage, as suggested for similar findings for lead (Schulz-Baldes, 1978).

An investigation by Abel (1975) into bioaccumulation of metals by *Mytilus edulis*, kept samples of mussels in known concentrations of suspended sediments from the Teesmouth estuary, for which metal contents had been determined. Upon sampling over 6 weeks, Abel found that copper, nickel and cadmium were not uptaken reliably at these concentrations, which were low. Manganese, zinc and lead levels increased markedly to between 2 and 6 times the control concentration, and uptake from the suspended sediments was similar to that found from solutions of comparable concentration. Iron, existing in colloidal form or as a precipitate in the sediments, was not uptaken. These findings were compared to a field experiment, where mussels of three size groups were introduced to the south gare of the Tees estuary, and sampled over 6 weeks. The zinc, copper and iron concentrations increassed makedly in the

mussel tissues, in all size groups, while manganese and cadmium showed no increases. Copper uptake is one example where the laboratory experiments were inconclusive, but metal was clearly accumulated in the field situation. This highlights not only the ability of *Mytilus* to uptake metals, but also that experimental conclusions regarding the significance to an organism of a metal pollutant need modification in the light of both experimental results from other organisms, and evidence of changes in the phase distribution of metals in the effluent discharge.

Detailed knowledge of metabolism of essential and trace metals is sparse. The essential step in any metabolic pathway must be the transport of the metal ion across a cellular or organelle membrane. Coombs and George (1978) schematised the possibilities for this, shown here in figure 31. These are;

(a) 'Pore' theory; transport of ions down a potential gradient through a pore, whose geometry may confer some cation specificity.

(b) Carrier protein; a ligand which complexes with some degree of specificity with the metal ion, forming a neutrally charged hydrophobic complex, able to diffuse through the phospholipid membrane bilayer.

(c) Carrier-mediated transport; where the complexing ligand is attached to the membrane. Complexation with a suitable metal ion induces a conformational change in the membrane, allowing the complex to flip over to the cytoplasmic side, and releasing the metal ion by another conformational change.

A pollutant metal can interfere with any of these processes, by competing with essential metals, and either gain entry into the cell, affecting the intracellular system, or induce a deficiency of the displaced functional metal ion. Alternatively;

(d) Organic pollutants, which can posses complexing groups, can also affect metal transport by preferentially complexing the metal and altering its normal metabolic pathway. This in turn either an excess or a deficiency within the cell.

The results I obtained for Teesmouth, do illustrate that above a certain concentration, (which will be specific for each metal and determined by the constitution of the environment,) all regulatory mechanisms for limiting tissue accumulation of metal are overwhelmed.

Thus the chemical form of the metal presented to the cell, i.e. its biological availability, may have a profound effect on its transport across the membrane, and hence its absorption. It is generally agreed that metal ions in seawater are not present as free hydrated ions but as multi-complexed to inorganic and organic ligands. Changes in salinity can alter uptake, likewise naturally occurring or synthetic complexing agents. Coombs (1977) found that in *Mytilus*, lead added to the water at

0.1 mg/l as nitrate, was uptaken into all of the tissues, with the highest concentration being found in the kidney (300μg/g dry weight tissue). When the lead was complexed with citrate, a 3-4 fold increase in both the rate of accumulation and final tissue concentration was seen. The high molecular weight complexes humic and alginic acids and pectin however, were not as effective, and produce at best a 1.5-2 fold increase.

Complexation of metal ions can therefore significantly affect the absorption rate, with or without changing the tissue distribution. This varies between metals; Coombs and George (1978) found that complexation increases lead absorption, but for cadmium, both low and high molecular weight complexes gave an equal increase in the rate of absorption. For iron, depending on the type of complex used, either an increase or a decrease was obtained, and changes in the tissue distributions were observed. They conclude that for cadmium, a carrier assisted means of diffusive entry, (as in route (b), figure 31), is in operation. For the other metals in their study, a variety of routes, according to the form of the ion, are potentially possible. In their studies of *Mytilus edulis* and *Ostrea edulis*, however, they were unable to detect any localised storage of this metal in vesicles like the other metals in their study. Instead, there appears to be a more generalised spread throughout the cytoplasm. Detoxication and storage in this case is by complexation to cytoplasmic thionein, and could represent a different immobilisation mechanism.

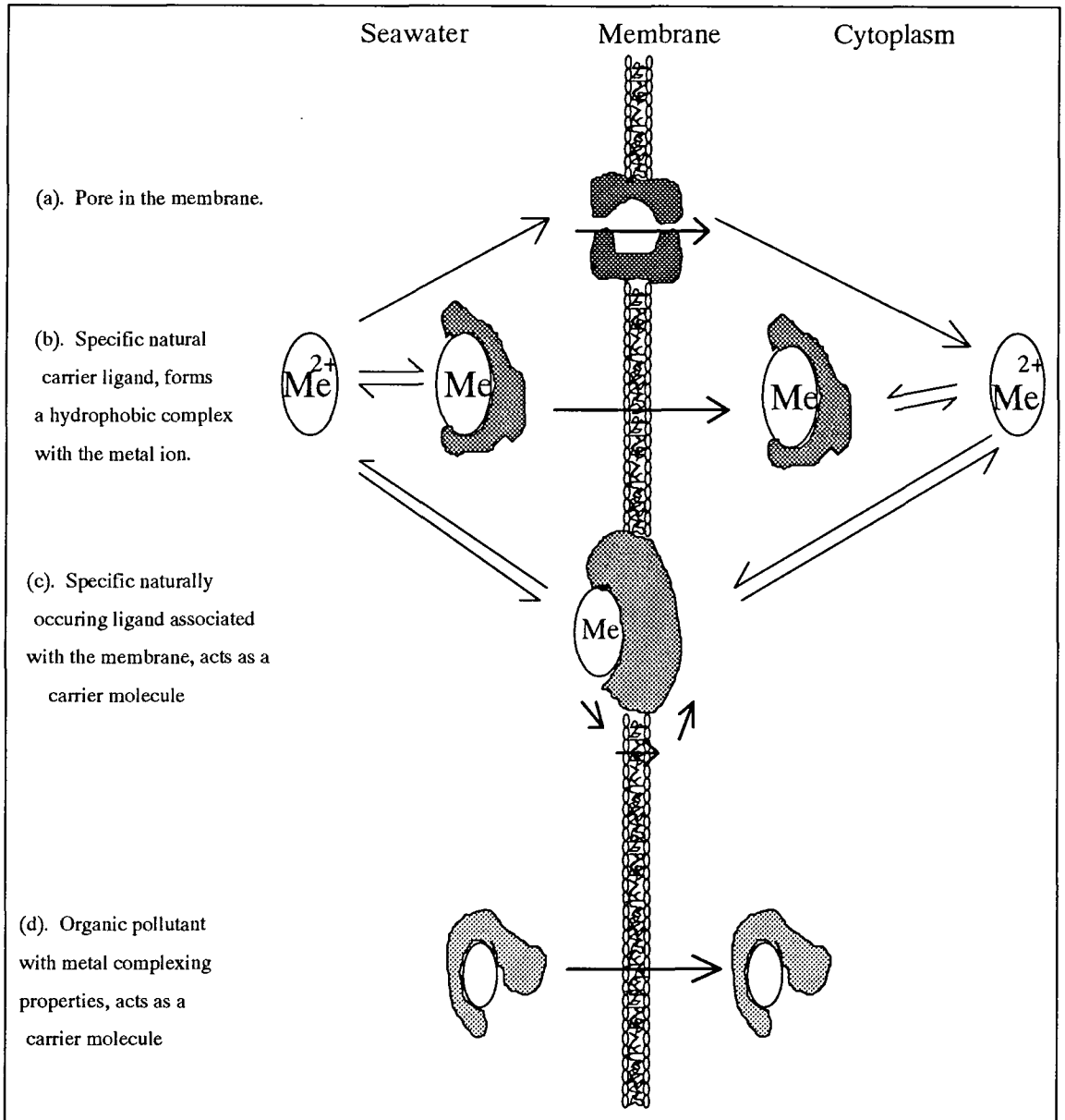


Figure 31; Schematic representation of possible routes across a phospholipid membrane, for a metal ion. (after Coombs and George, 1978).

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Summary

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This experiment has studied in some detail, the sub-lethal responses of *Mytilus edulis* to pollution from heavy metal salts, in inorganic form. It seems apparent that reduction of filtration rates in response to stresses such as this, constitute a rather more serious problem for a population of these bivalves than is indicated by a lowering of metabolic activity for an individual organism, or small sample of organisms. A study by Capuzzo and Sasner (1977) examining the effects of chromium on *Mytilus* both as sedimentary forms and free in solution, found that ciliary activity was inhibited; thus the ability to feed is reduced. Other studies have shown that physiological stress in various marine bivavles has resulted in decreased growth rates (Galstoff et al, 1947), losses of carbohydrate and protein reserves (Bayne and Thompson, 1970), interference with spawning time, and greater production of abnormal offspring (Bayne, 1975). Clearly, metal accumulation in areas affected by industrial wastes results in serious consequences to filter-feeding bivalves such as *Mytilus*.

The North Sea system as a whole may have a variant population that are more tolerant of pollution than is found in less enclosed, less polluted seas. Growth form between the two sample sites here showed slight differences. Other studies already cited have found large differences; the animals seem to have a capacity for adaptation of life-history strategy according to the locality. This is also true for tolerance of pollutants in physiological terms, with respect to the inducible detoxification mechanisms possible in these and other bivalves.

It is also apparent that *M. edulis* have a surprising capacity for resilience to such stresses. They are present world-wide, and only the more extreme effluent emissions have defined areas which they cannot tolerate. This study has highlighted some differences in adaptation between the populations from Lindisfarne and Teesmouth, in terms of bioaccumulatory behaviour between their respective ambient environments, and also behavioural tolerance to inorganic heavy metal. I conclude that these differences result from evolution of a mechanism of metal pollution tolerance in the Teesmouth population, which possibly facilitates the active excretion of metal from the body, so enabling tissue concentrations to stay below ambient levels. Given further time, I would have liked to complete this study regarding the bioaccumulatory behaviour over time in known metal concentrations for the Teesmouth population sample, in order to ascertain if this hypothesis is true.

In areas where pollutant discharges have been prevalent for some time, the adaptive tolerances of organisms from that locality could give the impression that pollution in these areas does not have quite the biological significance which could more accurately be apportioned to it. Also, the place in the ecosystem of the monitored organism will also give different significance to pollution impact. While invertebrates such as mussels may be able to survive a certain level of discharge, as a population they have a greater ability to recover than e.g. many species of fish, in which the bioconcentration of metals and other effluent ingredients will be much more marked, due to their position at a higher trophic level.

New biochemical techniques looking at sub-lethal responses of organisms to pollution include examining production of various stress-related proteins, e.g. metallothioneins; proteins of low molecular weight, which appear to be induced in response to elevated levels of heavy metals, in all invertebrates so far studied. Metallothioneins offer at least the possibility that we can define the 'acceptable' level of heavy metal in the environment, in terms of the level of metallothionein production. However caution is necessary, as some investigators have reported that metallothioneins, or similar proteins, appear in animals subjected to various other kinds of environmental stress (Abel, 1991).

An alternative approach is the use of an experimental ecosystem, which instead of exposing a single species to a pollutant, populations of two or more species, ideally representing different trophic levels, are maintained and exposed to the pollutant together. The ecology of a species is governed not only by its relationship with the physical and chemical environment and associated endogenous processes, but also by the relationships it has with other species, with which it shares its habitat. In this way, the various possible input routes to the environment may be covered, and also an accurate assessment of what the sublethal impact of a pollutant means in terms of decreasing the chances of survival of an individual or population in the field.

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Appendices

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Appendix 1

Lindisfarne initial sample;

Body	Shell;					Ratios							
soft tissue	length	width	depth	weight	area	l/w	d/w	l/d	wt/st	wt/l	logwt/logl	st/l	wt/area
0.397	45.8	26.4	19.1	6.13	604.6	1.73	0.72	2.40	15.44	0.13	0.47	0.01	0.01
0.534	52.4	26.9	23.4	8.72	704.8	1.95	0.87	2.24	16.33	0.17	0.55	0.01	0.01
0.594	45.2	24.2	20.2	6.16	546.9	1.87	0.83	2.24	10.37	0.14	0.48	0.01	0.01
0.621	51.9	27.8	22.8	10.15	721.4	1.87	0.82	2.28	16.34	0.20	0.59	0.01	0.01
0.369	48	27.2	21.8	7.57	652.8	1.76	0.80	2.20	20.51	0.16	0.52	0.01	0.01
0.404	54.4	25.9	22.2	8.39	704.5	2.10	0.86	2.45	20.77	0.15	0.53	0.01	0.01
0.174	51.5	27.2	23.6	8.95	700.4	1.89	0.87	2.18	51.44	0.17	0.56	0.00	0.01
0.419	48.4	27.3	22.6	8.32	660.7	1.77	0.83	2.14	19.86	0.17	0.55	0.01	0.01
0.368	52.3	28.7	21.75	9.17	750.5	1.82	0.76	2.40	24.92	0.18	0.56	0.01	0.01
0.475	55.5	28.5	23.6	10.95	790.9	1.95	0.83	2.35	23.05	0.20	0.60	0.01	0.01
0.411	52.9	29.3	23.9	9.29	775	1.81	0.82	2.21	22.60	0.18	0.56	0.01	0.01
0.613	60.4	28.9	26.5	15.48	872.8	2.09	0.92	2.28	25.25	0.26	0.67	0.01	0.02
0.558	56.4	30.3	24.3	11.45	854.5	1.86	0.80	2.32	20.52	0.20	0.60	0.01	0.01
0.493	57.6	29.5	24.8	12.6	849.6	1.95	0.84	2.32	25.56	0.22	0.63	0.01	0.01
0.447	50.3	26.5	21.7	7.02	666.5	1.90	0.82	2.32	15.70	0.14	0.50	0.01	0.01
0.378	53.5	30	22.8	9.93	802.5	1.78	0.76	2.35	26.27	0.19	0.58	0.01	0.01
0.708	54.9	30.6	22.85	9.35	840	1.79	0.75	2.40	13.21	0.17	0.56	0.01	0.01
0.617	61.15	31.7	26.5	15.1	969.2	1.93	0.84	2.31	24.47	0.25	0.66	0.01	0.02
0.293	45.2	25.4	20	6.37	574	1.78	0.79	2.26	21.74	0.14	0.49	0.01	0.01
0.517	51.3	27.9	24	10.03	715.6	1.84	0.86	2.14	19.40	0.20	0.59	0.01	0.01
0.433	50.3	26.9	23.3	11.45	676.5	1.87	0.87	2.16	26.44	0.23	0.62	0.01	0.02
0.548	61.5	31.3	31.5	17.81	962.5	1.96	1.01	1.95	32.50	0.29	0.70	0.01	0.02
0.356	53.3	28.4	23.1	9.94	756.9	1.88	0.81	2.31	27.92	0.19	0.58	0.01	0.01
0.414	52.7	27.7	23.25	12.45	729.9	1.90	0.84	2.27	30.07	0.24	0.64	0.01	0.02
0.498	58.7	31.2	23.1	11.65	915.7	1.88	0.74	2.54	23.39	0.20	0.60	0.01	0.01
0.73	60.9	36.5	27.4	17.32	1111.4	1.67	0.75	2.22	23.73	0.28	0.69	0.01	0.02
0.381	49.7	26.7	20.5	6.95	663.5	1.86	0.77	2.42	18.24	0.14	0.50	0.01	0.01

Descriptive Statistics

	soft tissue	*length*	*width*	*depth*	*weight*	*area*	*l/w*	*d/w*	*l/d*	*wt/st*	*wt/l*	*logwt/logl*	*st/l*	*wt/area*
Mean	0.47	53.19	28.48	23.35	10.32	761.99	1.87	0.82	2.28	22.82	0.19	0.58	0.01	0.01
St. Error	0.02	0.91	0.48	0.48	0.61	24.93	0.02	0.01	0.02	1.48	0.01	0.01	0.00	0.00
Median	0.45	52.70	27.90	23.10	9.93	729.90	1.87	0.82	2.28	22.60	0.19	0.58	0.01	0.01
st. dev	0.13	4.74	2.47	2.51	3.19	129.52	0.10	0.06	0.12	7.68	0.04	0.06	0.00	0.00
Variance	0.02	22.49	6.12	6.32	10.20	16774.6	0.01	0.00	0.01	58.93	0.00	0.00	0.00	0.00
Kurtosis	0.18	-0.59	3.02	3.30	0.36	0.71	0.90	2.35	1.37	6.75	0.02	-0.52	0.98	-0.24
Skewness	0.07	0.17	1.24	1.28	0.90	0.78	0.50	0.95	-0.41	1.90	0.77	0.22	0.05	0.77
Range	0.56	16.30	12.30	12.40	11.68	564.50	0.43	0.28	0.59	41.07	0.16	0.23	0.01	0.01
Minimum	0.17	45.20	24.20	19.10	6.13	546.90	1.67	0.72	1.95	10.37	0.13	0.47	0.00	0.01
Maximum	0.73	61.50	36.50	31.50	17.81	1111.40	2.10	1.01	2.54	51.44	0.29	0.70	0.01	0.02
Sum	12.75	1436.15	768.90	630.55	278.70	20573.6	50.48	22.16	61.67	616.06	5.16	15.55	0.24	0.36
Count	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00

Appendix 1 cont...

Teesmouth initial sample;

Body	Shell					Ratios							
soft tissue	length	width	depth	weight	area	l/w	d/w	l/d	wt/st	wt/l	lgwt/ lgl	st/l	wt/area
0.637	50.15	25.55	22	8.8	640.6	1.96	0.86	2.28	13.81	0.18	0.56	0.01	0.01
0.407	52.7	26.2	24.7	11.64	700.9	2.01	0.94	2.13	28.60	0.22	0.62	0.01	0.02
0.73	51.85	27.6	21	8.39	715.5	1.88	0.76	2.47	11.49	0.16	0.54	0.01	0.01
0.415	59.6	27.6	24	12.71	822.5	2.16	0.87	2.48	30.63	0.21	0.62	0.01	0.02
0.594	58.8	29.6	25.05	11.27	870.2	1.99	0.85	2.35	18.97	0.19	0.59	0.01	0.01
0.694	61.8	28.4	25.2	14.37	877.6	2.18	0.89	2.45	20.71	0.23	0.65	0.01	0.02
0.428	53.05	28	21.4	7.74	742.7	1.89	0.76	2.48	18.08	0.15	0.52	0.01	0.01
0.415	62	31.55	24.95	14.34	978	1.97	0.79	2.48	34.55	0.23	0.65	0.01	0.01
0.488	53.6	27.6	21.85	9.42	739.7	1.94	0.79	2.45	19.30	0.18	0.56	0.01	0.01
0.756	55.95	26.8	25.25	11.25	749.7	2.09	0.94	2.22	14.88	0.20	0.60	0.01	0.02
0.785	57.15	30.15	21.5	9.25	861.5	1.90	0.71	2.66	11.78	0.16	0.55	0.01	0.01
0.987	56.85	29.3	25.45	12.22	832.8	1.94	0.87	2.23	12.38	0.21	0.62	0.02	0.01
0.197	46.85	24.95	22.1	6.49	584.5	1.88	0.89	2.12	32.94	0.14	0.49	0.00	0.01
0.544	57.1	29.1	24	8.99	830.8	1.96	0.82	2.38	16.53	0.16	0.54	0.01	0.01
0.436	50.45	27.05	22.5	8.03	682.3	1.87	0.83	2.24	18.42	0.16	0.53	0.01	0.01
0.471	60.2	30	25.25	14.73	906	2.01	0.84	2.38	31.27	0.24	0.66	0.01	0.02
0.698	58.75	30.65	24.95	13.43	900.3	1.92	0.81	2.35	19.24	0.23	0.64	0.01	0.01
0.468	55.85	28.25	23.4	10.54	788.9	1.98	0.83	2.39	22.52	0.19	0.59	0.01	0.01
0.755	55.4	27.75	26.65	12.69	768.7	2.00	0.96	2.08	16.81	0.23	0.63	0.01	0.02
0.301	53.85	26.8	21.1	8.18	721.6	2.01	0.79	2.55	27.18	0.15	0.53	0.01	0.01
0.463	54.1	26.1	24.3	9.85	706	2.07	0.93	2.23	21.27	0.18	0.57	0.01	0.01
0.653	49.9	25.15	23.7	8.97	627.5	1.98	0.94	2.11	13.74	0.18	0.56	0.01	0.01
0.473	50	25.85	21.8	7.72	646.3	1.93	0.84	2.29	16.32	0.15	0.52	0.01	0.01
0.524	48.4	25.25	21.8	8.62	611.1	1.92	0.86	2.22	16.45	0.18	0.56	0.01	0.01
0.722	52.6	26.85	23.05	8.46	706.2	1.96	0.86	2.28	11.72	0.16	0.54	0.01	0.01
0.753	50.75	28.6	21.95	8.88	725.7	1.77	0.77	2.31	11.79	0.17	0.56	0.01	0.01
0.464	52.8	25.75	23.5	10.97	679.8	2.05	0.91	2.25	23.64	0.21	0.60	0.01	0.02
0.461	52.45	27.8	22.25	9.29	729.1	1.89	0.80	2.36	20.15	0.18	0.56	0.01	0.01
0.811	58.1	28.2	23.5	11.53	819.2	2.06	0.83	2.47	14.22	0.20	0.60	0.01	0.01
0.945	57.2	27.6	25.4	12.51	789.4	2.07	0.92	2.25	13.24	0.22	0.62	0.02	0.02

Descriptive Statistics

	soft tissue	*length*	*width*	*depth*	*weight*	*area*	*l/w*	*d/w*	*l/d*	*wt/st*	*wt/l*	*logwt/ logl*	*st/l*	*wt/area*
Mean	0.58	54.61	27.67	23.45	10.38	758.50	1.97	0.85	2.33	19.42	0.19	0.58	0.01	0.01
s.e.	0.03	0.73	0.31	0.29	0.41	17.62	0.02	0.01	0.03	1.24	0.01	0.01	0.00	0.00
Median	0.53	53.98	27.60	23.50	9.64	741.20	1.96	0.84	2.33	18.25	0.18	0.57	0.01	0.01
s.d.	0.19	3.98	1.71	1.60	2.24	96.51	0.09	0.06	0.14	6.77	0.03	0.05	0.00	0.00
Variance	0.03	15.85	2.91	2.55	5.03	9314.47	0.01	0.00	0.02	45.86	0.00	0.00	0.00	0.00
Kurtosis	-0.33	-0.76	-0.40	-1.23	-0.85	-0.45	0.39	-0.56	-0.38	-0.23	-1.12	-0.96	-0.70	-1.22
Skewness	0.27	0.08	0.35	0.09	0.42	0.28	0.32	-0.03	0.17	0.86	0.22	0.01	0.17	0.00
Range	0.79	15.15	6.60	5.65	8.24	393.50	0.40	0.25	0.58	23.06	0.11	0.17	0.01	0.01
Minimum	0.20	46.85	24.95	21.00	6.49	584.50	1.77	0.71	2.08	11.49	0.14	0.49	0.00	0.01
Maximum	0.99	62.00	31.55	26.65	14.73	978.00	2.18	0.96	2.66	34.55	0.24	0.66	0.02	0.02
Sum	17.48	1638.25	830.05	703.55	311.28	22755.10	59.22	25.49	69.96	582.65	5.66	17.37	0.32	0.41
Count	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

Appendix 1 cont...

Soft Tissue Mass

F-Test: Two-Sample for Variances

	farne st	*tees st*
Mean	0.4722	0.5825
Variance	0.0159	0.0347
Observations	27	30
df	26	29
F	2.172153	
P(F<=f) one-tail	0.024533	
F Critical one-tail	1.651099	

t-Test: Two-Sample, Unequal Variances

	farne st	*tees st*
Mean	0.4722	0.5825
Variance	0.0159	0.0347
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		3.5
df		51.28814
t		-2.63677
P(T<=t) one-tail		0.005533
t Critical one-tail		1.675285
P(T<=t) two-tail		0.011065
t Critical two-tail		2.007582

Shell length

F-Test: Two-Sample for Variances

	farne l	*tees l*
Mean	53.1907	54.6083
Variance	22.4877	15.8455
Observations	27	30
df	26	29
F	1.419182	
P(F<=f) one-tail	0.179882	
F Critical one-tail	1.88302	

t-Test: Two-Sample Assuming Equal Variances

	farne l	*tees l*
Mean	53.1907	54.6083
Variance	22.4877	15.8455
Observations	27	30
Pooled Variance		18.98547
Hypoth'd Mean Diff.		0
df		55
t		-1.22644
P(T<=t) one-tail		0.112629
t Critical one-tail		1.673034
P(T<=t) two-tail		0.225258
t Critical two-tail		2.004044

Shell width

F-Test: Two-Sample for Variances

	farne w	*tees w*
Mean	28.4778	27.6683
Variance	6.1171	2.9104
Observations	27	30
df	26	29
F	2.101814	
P(F<=f) one-tail	0.027091	
F Critical one-tail	1.88302	

t-Test: Two-Sample, Unequal Variances

	farne w	*tees w*
Mean	28.4778	27.6683
Variance	6.1172	2.9104
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		18.98547
df		45.54643
t		1.422979
P(T<=t) one-tail		0.080819
t Critical one-tail		1.679427
P(T<=t) two-tail		0.161638
t Critical two-tail		2.014103

Appendix 1 cont...

Shell depth

F-Test: Two-Sample for Variances

	farne d	*tees d*
Mean	23.3537	23.4516
Variance	6.3238	2.5454
Observations	27	30
df	26	29
F	2.484388	
P(F<=f) one-tail	0.00944	
F Critical one-tail	1.88302	

t-Test: Two-Sample, Unequal Variances

	farne d	*tees d*
Mean	23.3537	23.4517
Variance	6.3238	2.5454
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		18.98547
df		43.17038
t		-0.17343
P(T<=t) one-tail		0.431564
t Critical one-tail		1.681071
P(T<=t) two-tail		0.863128
t Critical two-tail		2.016691

Shell weight

F-Test: Two-Sample for Variances

	farne wt	*tees wt*
Mean	10.3222	10.376
Variance	10.1999	5.03326
Observations	27	30
df	26	29
F	2.026505	
P(F<=f) one-tail	0.03344	
F Critical one-tail	1.88302	

t-Test: Two-Sample, Unequal Variances

	farne wt	*tees wt*
Mean	10.3222	10.376
Variance	10.1999	5.0333
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		18.98547
df		46.07458
t		-0.07281
P(T<=t) one-tail		0.471137
t Critical one-tail		1.678659
P(T<=t) two-tail		0.942274
t Critical two-tail		2.012894

Shell area

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	761.98	758.503
Variance	16774.6	9314.46
Observations	27	30
df	26	29
F	1.80092	
P(F<=f) one-tail	0.06294	
F Critical one-tail	1.88302	

t-Test: Two-Sample Assuming Equal Variances

	farne	*tees*
Mean	761.98	758.503
Variance	16774.6	9314.46
Observations	27	30
Pooled Variance		12841.08
Hypoth'd Mean Diff.		0
df		55
t		0.115828
P(T<=t) one-tail		0.454105
t Critical one-tail		1.673034
P(T<=t) two-tail		0.908211
t Critical two-tail		2.004044

Appendix 1 cont...

Length; weight ratio

F-Test:

	farne	*tees*
Mean	1.8694	1.9704
Variance	0.0094	0.0077
Observations	27	30
df	26	29
F	1.211543	
P(F<=f) one-tail	0.314126	
F Critical one-tail	1.929212	

t-Test: Two-Sample Assuming Equal Variances

	L; l/w	*T; l/w*
Mean	1.8694	1.9704
Variance	0.0094	0.0077
Observations	27	30
Pooled Variance		0.008559
Hypoth'd Mean Diff.		0
df		55
t		-4.01169
P(T<=t) one-tail		9.69E-05
t Critical one-tail		1.674689
P(T<=t) two-tail		0.000194
t Critical two-tail		2.006645

Depth; width ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	0.8206	0.8493
Variance	0.0036	0.0041
Observations	27	30
df	26	29
F	1.143329	
P(F<=f) one-tail	0.367644	
F Critical one-tail	1.665661	

t-Test: Two-Sample Assuming Equal Variances

	farne	*tees*
Mean	0.8206	0.8493
Variance	0.0036	0.0041
Observations	27	30
Pooled Variance		0.003843
Hypoth'd Mean Diff.		0
df		55
t		-1.69965
P(T<=t) one-tail		0.047586
t Critical one-tail		1.674689
P(T<=t) two-tail		0.095171
t Critical two-tail		2.006645

Length; depth ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	2.2839	2.3287
Variance	0.0141	0.02108
Observations	27	30
df	26	29
F	1.493064	
P(F<=f) one-tail	0.156531	
F Critical one-tail	1.665661	

t-Test: Two-Sample Assuming Equal Variances

	farne	*tees*
Mean	2.2838	2.3286
Variance	0.0141	0.02108
Observations	27	30
Pooled Variance		0.017599
Hypoth'd Mean Diff.		0
df		55
t		-1.24029
P(T<=t) one-tail		0.110217
t Critical one-tail		1.674689
P(T<=t) two-tail		0.220435
t Critical two-tail		2.006645

Appendix 1 cont...

Shell weight; Soft Tissue weight ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	22.817	19.816
Variance	58.9251	48.4594
Observations	27	30
df	26	29
F	1.215967	
P(F<=f) one-tail	0.310874	
F Critical one-tail	1.929212	

t-Test: Two-Sample Assuming Equal Variances

	L; wt/st	*T; wt/st*
Mean	22.817	19.816
Variance	58.9251	48.4594
Observations	27	30
Pooled Variance		53.69225
Hypoth'd Mean Diff.		0
df		55
t		1.504608
P(T<=t) one-tail		0.069238
t Critical one-tail		1.674689
P(T<=t) two-tail		0.138475
t Critical two-tail		2.006645

Weight;Length ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	0.1911	0.1875
Variance	0.0019	0.0009
Observations	27	30
df	26	29
F	1.990041	
P(F<=f) one-tail	0.042613	
F Critical one-tail	1.929212	

t-Test: Two-Sample, Unequal Variances

	L; wt/l	*T; wt/l*
Mean	0.1911	0.1875
Variance	0.0019	0.0009
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		53.69225
df		46.86222
t		0.344073
P(T<=t) one-tail		0.36618
t Critical one-tail		1.678659
P(T<=t) two-tail		0.732359
t Critical two-tail		2.012894

Log Weight; Log Length ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	0.5759	0.5771
Variance	0.0039	0.0022
Observations	27	30
df	26	29
F	1.842401	
P(F<=f) one-tail	0.062838	
F Critical one-tail	1.929212	

t-Test: Two-Sample Assuming Equal Variances

	farne	*tees*
Mean	0.5759	0.5771
Variance	0.0039	0.0022
Observations	27	30
Pooled Variance		0.003067
Hypoth'd Mean Diff.		0
df		55
t		-0.07906
P(T<=t) one-tail		0.468643
t Critical one-tail		1.674689
P(T<=t) two-tail		0.937287
t Critical two-tail		2.006645

Appendix 1 cont...

Soft tissue weight; Length ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	0.0088	0.0104
Variance	4.46E-06	1.01E-05
Observations	27	30
df	26	29
F	2.261329	
P(F<=f) one-tail	0.021022	
F Critical one-tail	1.665661	

t-Test: Two-Sample, Unequal Variances

	farne	*tees*
Mean	0.0088	0.0104
Variance	4.46E-06	1.01E-05
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		0.003067
df		45.23399
t		-2.09137
P(T<=t) one-tail		0.021085
t Critical one-tail		1.679427
P(T<=t) two-tail		0.042171
t Critical two-tail		2.014103

Shell Weight; Shell Area Ratio

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	0.0133	0.0136
Variance	5.31E-06	3.91E-06
Observations	27	30
df	26	29
F	1.358636	
P(F<=f) one-tail	0.219936	
F Critical one-tail	1.929212	

t-Test: Two-Sample Assuming Equal Variances

	farne	*tees*
Mean	0.0133	0.0136
Variance	5.31E-06	3.91E-06
Observations	27	30
Pooled Variance		4.61E-06
Hypoth'd Mean Diff.		0
df		55
t		-0.37642
P(T<=t) one-tail		0.354069
t Critical one-tail		1.674689
P(T<=t) two-tail		0.708138
t Critical two-tail		2.006645

---ooOOoo---

Appendix 2;

Heavy Metals in initial samples from Lindisfarne and Teesmouth

Lindisfarne initial sample

Lead (mg/g)	Cadmium (mg/g)	Copper (mg/g)	Zinc (mg/g)
34	2.3	20.2	144.8
29	2.1	17.8	126.2
26.9	1.9	12.6	105.2
25.8	2.6	18.5	249.6
35.2	2.2	16.3	170.7
39.6	3.2	14.9	207.9
80.4	3.7	24.5	286.8
36.5	2.6	11.9	207.6
50.3	3.3	19	146.7
35.8	2.5	12.6	149.5
26.8	2.2	18.2	121.7
26.1	2.6	13.9	71.8
22.4	2.3	18.8	111.1
26.3	2.8	19.3	131.1
24.6	2.9	19.3	125.2
25.1	2.4	18.5	103.1
17.7	2	10.6	146.9
21.1	2.6	14.6	29.2
25.6	2.4	23.9	44.4
21.3	1.9	16.9	40.6
24.2	2.3	17.3	35.8
24.6	2.4	15.5	52.6
26.7	3.1	16.9	43.5
29	3.1	15.7	50.7
24	2.4	15.1	45.2
17.1	1.9	12.3	28.1
27.6	2.6	17.1	40.7

	Lead	Cadmium	Copper	Zinc
Mean	29.76	2.53	16.75	111.73
Standard Error	2.37	0.088	0.65	13.59
Median	26.3	2.4	16.9	111.1
Mode	29	2.6	12.6	#N/A
Standard Deviation	12.32	0.456	3.35	70.61
Variance	151.87	0.21	11.25	4986.28
Kurtosis	10.98	0.31	0.29	0.049
Skewness	2.97	0.75	0.35	0.76
Range	63.3	1.8	13.9	258.7
Minimum	17.1	1.9	10.6	28.1
Maximum	80.4	3.7	24.5	286.8
Sum	803.7	68.3	452.2	3016.7
Count	27	27	27	27

Appendix 2 cont...

Teesmouth initial sample;

Lead (mg/g)	Cadmium (mg/g)	Copper (mg/g)	Zinc (mg/g)
34.5	5.2	15.7	14.9
49.1	5.9	12.3	18.4
25.3	3.3	4.8	10.3
71.1	7.5	21.7	19.3
49.7	4	6.7	10.1
23.8	4	10.1	10.1
47.9	4.3	93	14.1
72.3	7.2	12	19.3
53.3	4.9	12.3	14.3
45.6	3.3	6.6	11.2
29.3	3.9	12.7	10.2
42.5	1.7	8.1	7.6
111.7	4.6	2.5	38.1
55.1	3.3	34.9	11.1
40.1	2.8	13.8	19.5
60.5	5.1	17	15.9
29.4	2.4	12.9	10.7
26.7	0.3	10.7	15
29.1	2.8	6.6	15
48.2	5.3	16.6	19.9
95	3.9	9.7	1.3
39.8	2.8	7.7	6.9
50.7	2.5	10.6	12.7
86.8	2.7	9.6	14.3
24.2	2.3	6.9	10.4
33.2	2.3	8.6	10
45.3	4.3	14	10.8
38.5	3	8.7	10.8
45.5	3.9	14.1	11.9
27.5	2.1	10.6	5.3

	Lead	Cadmium	Copper	Zinc
Mean	47.72	3.72	14.38	13.31
Standard Error	3.93	0.29	2.92	1.167
Median	45.4	3.6	10.65	11.55
Mode	#N/A	3.3	12.3	19.3
Standard Deviation	21.50	1.57	15.99	6.39
Variance	462.25	2.46	255.63	40.81
Kurtosis	1.92	0.67	21.62	7.18
Skewness	1.39	0.51	4.43	1.90
Range	87.9	7.2	90.5	36.8
Minimum	23.8	0.3	2.5	1.3
Maximum	111.7	7.5	93	38.1
Sum	1431.7	111.6	431.5	399.4
Count	30	30	30	30

Appendix 2 cont...

LEAD

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	29.77	47.73
Variance	151.87	462.25
Observations	27	30
df	26	29
F		3.043688
P(F<=f) one-tail		0.002672
F Critical one-tail		1.651099

t-Test: Two-Sample Assuming Unequal Variances

	farne	*tees*
Mean	29.77	47.73
Variance	151.87	462.25
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		3.5
df		47.04499
t		-3.9154
P(T<=t) one-tail		0.000145
t Critical one-tail		1.677927
P(T<=t) two-tail		0.00029
t Critical two-tail		2.011739

CADMIUM

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	2.53	3.72
Variance	0.21	2.45
Observations	27	30
df	26	29
F		11.81735
P(F<=f) one-tail		6.21E-09
F Critical one-tail		1.651099

t-Test: Two-Sample Assuming Unequal Variances

	farne	*tees*
Mean	2.53	3.72
Variance	0.21	2.45
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		3.5
df		34.37083
t		-3.98023
P(T<=t) one-tail		0.000171
t Critical one-tail		1.690923
P(T<=t) two-tail		0.000343
t Critical two-tail		2.032243

Appendix 2 cont...

COPPER

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	16.75	14.38
Variance	11.25	255.62
Observations	27	30
df	26	29
F		22.72127
P(F<=f) one-tail		3.04E-12
F Critical one-tail		1.651099

t-Test: Two-Sample Assuming Unequal Variances

	farne	*tees*
Mean	16.75	14.38
Variance	11.25	255.62
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		3.5
df		31.82078
t		0.791029
P(T<=t) one-tail		0.217469
t Critical one-tail		1.695519
P(T<=t) two-tail		0.434938
t Critical two-tail		2.039515

ZINC

F-Test: Two-Sample for Variances

	farne	*tees*
Mean	111.73	13.31
Variance	4986.28	40.81
Observations	27	30
df	26	29
F		122.1866
P(F<=f) one-tail		2.74E-23
F Critical one-tail		1.88302

t-Test: Two-Sample Assuming Unequal Variances

	farne	*tees*
Mean	111.73	13.31
Variance	4986.28	40.81
Observations	27	30
Pearson Correlation		#N/A
Pooled Variance		3.5
df		26.38315
t		7.215506
P(T<=t) one-tail		5.79E-08
t Critical one-tail		1.705616
P(T<=t) two-tail		1.16E-07
t Critical two-tail		2.055531

Appendix 3

T-test; paired two sample for means, comparing response of Mytilus edulis, to solutions of salts of heavy metal

Aluminium

(mg/l)	Tees (%)	Farne (%)
3	114	110
5	108	103
7	102	96
10	91	86
15	78	72
20	65	61
25	56	52
30	47	43
35	40	36
40	35	31

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	73.6	69
Variance	850.49	825.11
Observations	10	10
Pearson Correlation		0.99969
Pooled Variance		837.4444
Hypothesized Mean Difference		0
df		9
t		17.25
P(T<=t) one-tail		1.67E-08
t Critical one-tail		1.833114
P(T<=t) two-tail		3.33E-08
t Critical two-tail		2.262159

Cadmium

(mg/l)	Tees (%)	Farne (%)
2	85	85
4	71	63
6	60	46
8	51	34
9	46	29
10	43	25
12	37	19
14	32	14
16	27	10
18	18	8

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	47	33.3
Variance	420.89	618.23
Observations	10	10
Pearson Correlation		0.983457
Pooled Variance		501.6667
Hypothesized Mean Difference		0
df		9
t		7.241799
P(T<=t) one-tail		2.43E-05
t Critical one-tail		1.833114
P(T<=t) two-tail		4.86E-05
t Critical two-tail		2.262159

Chromium

(mg/l)	Tees (%)	Farne (%)
2	102	100.5
4	95	89
6	87	79
8	80	70
10	72	61
14	61	47
16	56	42
18	51	37.5
20	46	34
22	42	29

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	69.2	58.9
Variance	448.18	618.6
Observations	10	10
Pearson Correlation		0.997226
Pooled Variance		525.0778
Hypothesized Mean Difference		0
df		9
t		7.989005
P(T<=t) one-tail		1.12E-05
t Critical one-tail		1.833114
P(T<=t) two-tail		2.24E-05
t Critical two-tail		2.262159

Appendix 3 cont...

Cobalt

(mg/l)	Tees (%)	Farne (%)
2	79	78
4	70	68
6	62	59
8	54	50
10	47	43
14	36.5	33
16	32	28
18	28	24.5
20	25	21
22	22	18

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	45.55	42.25
Variance	401.2472	436.1806
Observations	10	10
Pearson Correlation		0.999596
Pooled Variance		418.1806
Hypothesized Mean Difference		0
df		9
t		10.10415
P(T<=t) one-tail		1.64E-06
t Critical one-tail		1.833114
P(T<=t) two-tail		3.28E-06
t Critical two-tail		2.262159

Copper

(mg/l)	Tees (%)	Farne (%)
0.2	57.5	68
0.3	52	62
0.4	45.5	54
0.5	38	48
0.6	35	43
0.8	27	34.5
1	21	20.75
1.2	16	22
1.4	13	18
1.6	10	14

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	31.5	38.425
Variance	280.2222	377.4451
Observations	10	10
Pearson Correlation		0.993983
Pooled Variance		323.2639
Hypothesized Mean Difference		0
df		9
t		-6.56123
P(T<=t) one-tail		5.19E-05
t Critical one-tail		1.833114
P(T<=t) two-tail		0.000104
t Critical two-tail		2.262159

Iron

(mg/l)	Tees (%)	Farne (%)
2	99	88
4	86	79
6	75	69
8	65	62
7	72	65
10	57	55
12	50	49
14	43	44
16	38	39
18	33	34

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	61.8	58.4
Variance	463.2889	305.3778
Observations	10	10
Pearson Correlation		0.998694
Pooled Variance		375.6444
Hypothesized Mean Difference		0
df		9
t		2.579181
P(T<=t) one-tail		0.014868
t Critical one-tail		1.833114
P(T<=t) two-tail		0.029737
t Critical two-tail		2.262159

Appendix 3 cont...

Lead

(mg/l)	Tees (%)	Farne (%)
1	81	76
1.5	76	72
2	71	66
2.5	66	63
3	62	58
3.5	57	54
4	53	50
5	47	43
6	41	38
7	35	33

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	58.9	55.3
Variance	228.7667	207.3444
Observations	10	10
Pearson Correlation		0.999066
Pooled Variance		217.5889
Hypothesized Mean Difference		0
df		9
t		11.78377
P(T<=t) one-tail		4.49E-07
t Critical one-tail		1.833114
P(T<=t) two-tail		8.99E-07
t Critical two-tail		2.262159

Manganese

(mg/l)	Tees (%)	Farne (%)
3	82	85
5	71	77
8	58	66
10	51	61
13	42	52
18	30	41
21	27.5	35
24	20	31
26	18	28
30	15	24

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	41.45	50
Variance	546.4694	462.4444
Observations	10	10
Pearson Correlation		0.997162
Pooled Variance		501.2778
Hypothesized Mean Difference		0
df		9
t		-10.7225
P(T<=t) one-tail		9.98E-07
t Critical one-tail		1.833114
P(T<=t) two-tail		2E-06
t Critical two-tail		2.262159

Mercury

(mg/l)	Tees (%)	Farne (%)
0.01	57	84
0.02	51	79
0.03	37	72
0.04	42	66
0.05	38	61
0.06	35	56
0.07	31	51
0.08	28	47
0.09	26	44
0.1	23.5	41

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	36.85	60.1
Variance	116.225	222.3222
Observations	10	10
Pearson Correlation		0.959863
Pooled Variance		154.2944
Hypothesized Mean Difference		0
df		9
t		-13.4327
P(T<=t) one-tail		1.47E-07
t Critical one-tail		1.833114
P(T<=t) two-tail		2.93E-07
t Critical two-tail		2.262159

Appendix 3 cont...

Nickel

(mg/l)	Tees (%)	Farne (%)
2	72.5	87.5
4	66	77
6	60	68
8	54	59.5
10	49.5	52.5
12	45	46
14	41	41
16	37	36
18	34	32
20	31	28

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	49	52.75
Variance	195.6111	397.9028
Observations	10	10
Pearson Correlation		0.999247
Pooled Variance		278.7778
Hypothesized Mean Difference		0
df		9
t		-1.97757
P(T<=t) one-tail		0.039685
t Critical one-tail		1.833114
P(T<=t) two-tail		0.07937
t Critical two-tail		2.262159

Selenium

(mg/l)	Tees (%)	Farne (%)
3	127	137
5	109	123
8	90	102
10	78	92
13	65	76
18	46	57
21	38	47
24	30	40
26	28	36
30	21	28

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	63.2	73.8
Variance	1351.289	1468.4
Observations	10	10
Pearson Correlation		0.998955
Pooled Variance		1407.156
Hypothesized Mean Difference		0
df		9
t		-14.4545
P(T<=t) one-tail		7.78E-08
t Critical one-tail		1.833114
P(T<=t) two-tail		1.56E-07
t Critical two-tail		2.262159

Zinc

(mg/l)	Tees (%)	Farne (%)
2	79	75
4	68	65
6	58	57.5
8	49.5	50
9	46	46
10	43	43.5
12	36.5	38
14	32	32.5
16	27	28.5
18	23	25

t-Test: Paired Two-Sample for Means

	Tees (%)	*Farne (%)*
Mean	46.2	46.1
Variance	323.7889	261.1
Observations	10	10
Pearson Correlation		0.999221
Pooled Variance		290.5333
Hypothesized Mean Difference		0
df		9
t		0.161749
P(T<=t) one-tail		0.437538
t Critical one-tail		1.833114
P(T<=t) two-tail		0.875076
t Critical two-tail		2.262159

Appendix 4

Successive accumulations of metal

Copper ppm/g dry weight tissue

Farne 10 dys	Farne 20 dys	Farne 30 dys	Tees 14 dys
25.6	16.1	20.9	55
25.2	27.8	19.9	17.7
25.9	20.9	23.6	42.3
32.1	33.4	24.7	57.6
31.8	23.5	26.2	160.5
31.4	21.3	27.8	43.5
29.5	35.5	21.7	110.4
22.9	25.7	23.5	20.7
30.2	15.7	35.4	33.2
35.6	26.2	24.8	122.8
22.4	22.1	21.7	
22.6	26.6	15.9	
20.7	22.1	26.5	
26.1	36.6	20.6	
24.2	36.8	25	
25.8	28.6	19.2	
29.1	24.8	27.3	
25.5		19	
21.7		16.5	

	Farne 10 dys	*Farne 20 dys*	*Farne 30 dys*	*Tees 14 dys*
Mean	26.9	26.1	23.16842	66.37
Standard Error	0.909338	#N/A	#N/A	#N/A
Median	25.85	25.7	23.5	49.25
Mode	#N/A	22.1	21.7	#N/A
Standard Deviation	4.066681	6.493458	4.559733	48.12807
Variance	16.53789	42.165	20.79117	2316.311
Kurtosis	-0.63813	-0.61036	1.611582	-0.10015
Skewness	0.389592	0.284328	0.781754	1.026589
Range	14.9	21.1	19.5	142.8
Minimum	20.7	15.7	15.9	17.7
Maximum	35.6	36.8	35.4	160.5
Sum	538	443.7	440.2	663.7
Count	20	17	19	10

Lead mg/g dry weight tissue

Farne 10 dys	Farne 20 dys	Farne 30 dys	Tees 14 dys
24.2	28	39	93.2
23.5	29.1	28.5	39.7
19.5	22.1	30.2	87.6
20.7	23.8	22.9	112.1
20.7	21.6	19.9	85.8
24.8	21.8	31	122.1
23.7	40.5	17	143.8
17.2	24.7	21.5	113.3
18.4	25.1	33.5	75.6
20.8	22.8	30.8	121.5
24.3	24.8	23.5	128.8
25.1	24.3	28.2	55.9
21.9	37.4	25.7	71.6
31.9	27.4	34	83.1
22.3	24.3		71.5
16.7	16.8		
21.7	20.4		
20.7	29.3		
18.3	15.4		
18.9	20.3		

	Farne 10 dys	*Farne 20 dys*	*Farne 30 dys*	*Tees 14 dys*
Mean	21.765	24.995	27.55	93.70667
Standard Error	0.777928	1.341964	#N/A	#N/A
Median	21.25	24.3	28.35	87.6
Mode	20.7	24.3	#N/A	#N/A
Standard Deviation	3.479001	6.001445	6.119295	29.16709
Variance	12.10345	36.01734	37.44577	850.7192
Kurtosis	2.537254	1.832762	-0.49153	-0.65959
Skewness	1.118008	1.10468	0.02293	-0.04698
Range	15.2	25.1	22	104.1
Minimum	16.7	15.4	17	39.7
Maximum	31.9	40.5	39	143.8
Sum	435.3	499.9	385.7	1405.6
Count	20	20	14	15

Cadmium mg/g dry weight tissue

Farne 10 dys	Farne 20 dys	Farne 30 dys	Tees 14 dys
32.5	39	237.2	149.5
47	31.5	211.7	246.5
49	21.7	331.4	141.5
45.3	25.2	185.6	493.3
43.4	22.3	223.8	307.3
32.3	31.3	212.6	443
39.4	46.3	211.8	239.6
48	28.2	196.1	177.5
29.3	16	236.1	311.4
35.4	26.4	220.7	282.5
55.2	37.8	158.2	268.3
27.7	40.5	191.6	154.5
30.7	25.9	163.4	252.2
29.7	24.5	187.8	
41.2	23.5	202.9	
39.3	25.7	136.2	
32.6	37.4	183.2	
51	21	147.2	
25.9	38.2	177.3	
36.5			

	Farne 10 dys	*Farne 20 dys*	*Farne 30 dys*	*Tees 14 dys*
Mean	38.57	29.6	200.7789	266.7
Standard Error	1.92488	#N/A	#N/A	#N/A
Median	37.9	26.4	196.1	252.2
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	8.608325	8.140775	42.53384	107.0969
Variance	74.10326	66.27222	1809.127	11469.75
Kurtosis	-1.0499	-0.69665	4.163642	0.544137
Skewness	0.321295	0.441132	1.426578	0.92598
Range	29.3	30.3	195.2	351.8
Minimum	25.9	16	136.2	141.5
Maximum	55.2	46.3	331.4	493.3
Sum	771.4	562.4	3814.8	3467.1
Count	20	19	19	13

Zinc mg/g dry weight tissue

Farne 10 dys	Farne 20 dys	Farne 30 dys	Tees 14 dys
624.6	307.1	611.3	265.8
410.1	453	512.9	523.7
338.7	450.1	606	261
338.1	418.7	411.9	1181.2
618.4	304.6	358.4	598
280.5	456.4	369.2	789
373	284.2	462.4	367.7
382	380.1	519.9	417.9
315.9	414.9	431.8	661.7
472.9	510.2	420.5	786.9
377.5	316.4	297.3	
391.6	485	223.1	
379.5	532.4	429.8	
362.9	366.7	410.1	
329.5	329.3	360.3	
398.3	345	1117.2	
480.3	370.3	425.7	
315.8	306.2	617.6	
513.2	658.6	352.2	
387.5	438	355.3	

	Farne 10 dys	*Farne 20 dys*	*Farne 30 dys*	*Tees 14 dys*
Mean	404.515	406.36	464.645	585.29
Standard Error	20.96379	21.20194	41.3537	#N/A
Median	380.75	397.5	423.1	560.85
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	93.75293	94.81798	184.9394	284.7317
Variance	8789.612	8990.449	34202.56	81072.13
Kurtosis	1.200225	1.034885	8.045542	0.775468
Skewness	1.279155	0.932849	2.42658	0.883065
Range	344.1	374.4	894.1	920.2
Minimum	280.5	284.2	223.1	261
Maximum	624.6	658.6	1117.2	1181.2
Sum	8090.3	8127.2	9292.9	5852.9
Count	20	20	20	10

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