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The Distribution of Cadmium, Chromium and Lead in Crabs, Clams and Oysters from Calcasieu Estuary, Louisiana

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THE DISTRIBUTION OF CADMIUM, CHROMIUM AND LEAD IN
CRABS, CLAMS AND OYSTERS FROM CALCASIEU ESTUARY,
(TITLE)
LOUISIANA

BY

Fritz Jaenke
=

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FOR THE DEGREE OF

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1979

YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

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ABSTRACT

In November 1978, specimens of oysters (Crassostrea virginica), clams (Rangia cuneata), crabs (Callinectes sapidus) and water and sediment samples were collected from Calcasieu Estuary, Louisiana. The concentrations of cadmium, chromium and lead were determined in the samples with an inductively coupled argon plasma direct reading emission spectrophotometer. Crab claw muscle, gill and carapace tissues were analyzed separately. Oysters and clams were separated into shell and whole soft tissue samples which were analyzed separately. The concentration order of the metals in crab carapace and in oyster shells was similar to that of the sediments, (Pb>Cr>Cd). The sediments and exoskeletons seem to be indicative of long-term conditions and/or of an adsorptive concentrating mechanism being present. Concentration order of the metals in water samples, in crab claw muscle and gill tissues, and in clam whole soft tissues were similar (Cr>Pb>Cd). These findings suggest that soft tissues of clams and crabs are indicative of the short-term availability of these metals to the biota and/or the presence of an absorptive concentration mechanism. It was expected that oyster soft tissues would provide similar results, however, they did not. Results show that C. sapidus, R. cuneata and C. virginica don't concentrate cadmium, chromium and lead uniformly in all anatomical body regions. Concentration of these metals seemed to be evident in the estuarine food chain in which crabs, in most cases, concentrated metals to greater extents than did clams and oysters. The possibility of a synergistic mechanism concentrating metals in each species was noted. Concentrations of cadmium, chromium and lead were independent of body weights.

INTRODUCTION

The consequential results of man's increasing industrialization on estuarine and marine environments cannot be accurately ascertained without extensive research on the organisms inhabiting these environments. The discharge of heavy metals into these environments has become of some concern to many authors (Wolfe and Rice 1972, Bryan 1971, Brooks and Rumsby 1964). The monitoring of metal concentrations in estuarine organisms can serve several purposes. The resultant concentrations can be used as indicators of existing conditions and serve as history of past conditions. Present day research can serve as reference points for future research projects. The potential danger to public health by consumption of contaminated animals may be lessened by frequent monitoring of the degree of contamination in these animals. The monitoring of metal concentrations in estuarine organisms can serve as valuable information in the understanding of the cycling of trace metals in an estuary.

Not all aquatic organisms concentrate heavy metals to the same extent or in the same manner (Jackim et al. 1977, Bryan 1971). The determination of existing conditions in an estuary with respect to trace metals, may therefore, be facilitated by comparisons of the trace metal concentrations in several species. Many aquatic organisms do not concentrate metals uniformly in all anatomical regions of the body (Wright 1977, Hutcheson 1974, Segar et al. 1971). For example, some molluscs accumulate concentrations of metals in the shell which are very reminiscent of similar concentrations in the surrounding waters; however, soft tissue concentrations of heavy

metals are more similar to metal concentrations in the sediments (Segar et al. 1971). The determination of trace metal content in separate anatomical regions of indicator organisms, therefore, will give a more complete insight of existing conditions in an estuary.

In an effort to more fully understand the existing conditions with respect to trace metals in Calcasieu Estuary, Louisiana, concentrations of cadmium, chromium, and lead were determined in the blue crab, Callinectes sapidus, the eastern oyster, Crassostrea virginica, and an estuarine clam, Rangia cuneata. Separate analyses on carapace, gill, and muscle tissues in the blue crab were conducted for each of the three metals. Oysters and clams were analyzed on the basis of concentrations of these metals in shell and whole soft body parts. Comparisons were made among concentrations of each metal in different anatomical regions of each animal, concentration differences among different species from the same location and of the same species from different locations. A series of comparisons were also completed among metal concentrations found in the sediments and water, and those found in the crabs, clams, and oysters.

LITERATURE REVIEW

The monitoring and control of heavy-metal pollution, particularly in the aquatic environment, has become very important in recent years and is the subject of a great deal of research. Man's increasing release of wastes into the aquatic environment may have a toxic affect not only on aquatic flora and fauna, but also on man (Watling and Watling 1976).

Metals such as lead, chromium, and cadmium exert their major effects on the environment by virtue of their accumulated environmental stock (Nobbs and Pearce 1976). Hence they are referred to as stock chemical pollutants. Some features of "stock" pollutants are:

- 1) Non-degradable or only slowly degraded chemicals, which persist in the environment. Each increment of the stock may be considered harmless until a threshold is reached.
- 2) They tend to be invisible in their effects up to the identified threshold. The damage caused by the stock is irreversible since the threshold is related to existing stock already present in the environment and non-recoverable.
- 3) They have direct biological effects.
- 4) Observed damage tends to occur only after certain thresholds have been reached, because the observance of the causal mechanism is complex.
- 5) Public action isn't taken because the pollutant is "invisible" and unnoticed until the threshold is reached. Individuals are unable to monitor their own consumption of pollutants.

For these reasons, heavy metals should be continuously monitored and if necessary, restrictions should be placed on the amounts of metal entering the environment by the actions of man.

Toxicity to Humans

It has been established that certain elements present at trace concentrations in human tissues are essential to health, and others have no apparent physiological function. Elements in either category may be toxic at unnatural exposure concentrations. Individual responses to a given exposure level may vary significantly; thus a designation of a "tolerable" level of exposure is often difficult (Leland et al. 1974). A heavy metal is considered toxic if it impairs growth, reproduction or metabolism of an organism when supplied above a certain concentration.

The most important mechanism of toxic action of metals is thought to be the poisoning of enzyme systems (Pringle et al. 1968). It appears likely that all divalent transition metals, as well as other electronegative metals, are poisonous by virtue of their reactivity with proteins and especially with enzymes (Passon et al. 1961). At very high concentrations metals actually act on surface tissues as protein precipitates (Bryan 1971).

Metal-organic compounds may be either more toxic than the metal ion or much less so. Bowen (1966) divided elements into groups with regard to their pollution potentials. The very high potential pollutants group, as based on the toxicity of their metallo-organic compounds formed, included cadmium, chromium and lead.

Lead

Population exposure to lead by ingestion of water and foodstuffs and by other sources has been extensively studied. Most research on lead poisoning has been restricted to cases involving occupational overexposure, lead-based paints being ingested by children, improperly lead-glazed

earthenware vessels, discarded battery casings, and illicitly distilled whiskey (Committee on Biological Effects of Atmospheric Pollutants 1974). In most instances however, food and beverage provide the largest sources of lead for man (Goyer and Chisolm 1972).

The human capacity of lead is enhanced by inhalation of airborne lead particles, eating food contaminated with lead, and drinking from pottery and other utensils containing leachable or extractable lead (Goyer and Chisolm 1972).

U.S. citizens had concentrations of lead substantially higher than those found in any other part of the world with the exception of Hong Kong, and it was concluded that lead exposure in the U.S. from 1952-1957 exceeded the ability of the human body to excrete all the lead absorbed (Metcalf 1971).

The most prominent adverse effects of lead involve three organ systems: the nervous system, the hemopoietic system, and the excretory system. Neural tissue is very sensitive to lead. Lead encephalopathy, a swelling of the brain, can occur when lead content in the brain is relatively small. Lead can produce degenerative changes in the peripheral nervous system and interfere with the transmission of nerve impulses. The main inhibitory effect on vascular tissue by lead is thought to be due to inhibition of hemoglobin production in red blood cells. Renal ability to reabsorb small organic molecules such as amino acids, glucose, uric acid, citric acid and phosphate is decreased by lead contamination. The recognized clinical effects of lead poisoning in man include anemia, and syndromes of acute and chronic encephalopathy, peripheral neuropathy, lead nephropathy and acute abdominal colic (Goyer and Chisolm 1972).

Chromium

Relatively little is known about the transfer of chromium from air, to water, to living systems, etc. No wide-spread epidemics involving the general public have been publicized.

Chromium is an essential element in the diet of some animals including humans. It is similar to other metals which are known to be beneficial in low concentrations and harmful at higher concentrations (Smith 1972).

Chromium compounds are very useful and are widely encountered in industry. The uses of chromium include plating industries, an alloying element in steel production and stainless steel making, manufacture of chromate pigments, chrome tanning of leather, and as additives to water to prevent corrosion.

Unlike lead and several other metals, the valence state of chromium is of considerable importance to its' toxicity. It is probable that all isomers of chromium are toxic in sufficiently high concentrations, however the hexavalent form is by far the most important isomer with respect to chromium toxicity (Smith 1972).

In biological systems chromium is oxidized to its' trivalent state, thus the trivalent isomer is chiefly available through ingestion of animals containing chromium. Trivalent chromium is considerably less toxic than hexavalent; its toxicity appears to be restricted to parenteral administration (Committee on Biological Effects of Atmospheric Pollutants 1974).

Knowledge of the harmful effects of hexavalent chromium is derived almost entirely from cases of occupational overexposures where the effects are mainly on the respiratory system and skin (Committee on Biological Effects of Atmospheric Pollutants 1974). Dermatitis, skin ulcerations and

cancer of the respiratory tract has resulted from chromium overexposure (Smith 1972).

It would seem that monitoring of biological organisms would give a general outlook on environmental chromium content despite the fact that few data are available on the total ecologic cycling of chromium in the environment.

Cadmium

Cadmium has been known to be toxic to humans since 1858 (Fasset 1972). Cadmium poisoning has been identified as the cause of the epidemic outbreak of "Itai-Itai" disease among the citizens of Toyama Prefecture, Japan beginning in 1939 (Organization for Economic Cooperation and Development 1975). Approximately 100 people died from ingesting cadmium polluted rice. Conditions of malnutrition are thought to have also contributed to the fatal effects of the metal.

Cadmium is a relatively rare element in the earth's crust. Drainage, microbial activities and pH are of major importance in determining local concentrations (Fasset 1972). Industrial uses of cadmium include electroplating industries, in the production of pigments and related chemicals, plastic stabilizers, alloys and solder, nickel-cadmium batteries and pesticides.

The acute effects of oral ingestion in man are immediate nausea and vomiting which can occur at levels as small as 15 mg. of total body cadmium. There have been a considerable number of epidemics of acute nausea following the use of cadmium-plated articles as food containers (Fasset 1972).

The organs usually noted to significantly accumulate cadmium are the kidney and the liver, with the kidney usually concentrating the greater

amounts. Kidney failure is a known result of cadmium intoxication, specifically proteinuria, glucosuria, impaired concentrating abilities, impaired acid excretion, and others (Fassett 1972).

Toxicity to Aquatic Life

Heavy metals have long been recognized as serious pollutants of the aquatic environment, with deleterious effects on the associated organisms.

It appears that the range of toxic concentrations of hexavalent chromium in marine animals begins at concentrations of 1 mg/l, (1 ppm) in water. The actual concentration depends on the sensitivity of the concentrating methods and on the species of animals. For example, nereid marine worms, rainbow trout, and swimming crabs showed toxic symptoms at water concentrations of chromium of 1, 2.5, and 50 mg/l (ppm) respectively (Comm. on Biological Effects of Atmospheric Pollutants 1974). Oysters, Crassostrea virginica, exposed to .1 ppm chromium (as a chromate salt) for 20 weeks exhibited a 14% mortality, with the highest mortality rate occurring within 5-8 weeks (Shuster and Pringle 1969). It is apparent that oysters, C. virginica, can accumulate cadmium to lethal doses (Shuster and Pringle 1969). At water cadmium levels of .1 and .2 ppm, all oysters died when a body concentration of approximately 100 ppm cadmium was reached, with highest mortalities occurring during 9-12 weeks.

Oysters, C. virginica, subjected to high seawater levels of lead (0.1 and 0.2 ppm) had a general atrophy of tissues near the termination of a 10-week experimental period (Shuster and Pringle 1968). The tissue atrophy was particularly evident regarding the gonadal tissue of the oyster.

Several metals, including copper and cadmium, have been shown to inhibit zinc metalloenzymes involved in the shell metabolism of C. virginica.

The shells of oysters in areas contaminated with metals are significantly thinner than control oysters (Frazier 1976). Although this effect may not be a direct consequence of metal lethality, this sublethal response can affect the ecological distribution of the oyster by permitting increased predation by oyster drills, boring sponges, and blue crabs.

Several factors influencing toxicity of metals to estuarine animals were indicated by Bryan (1971):

- 1) The form of the metal in the water and how easily the metal can dissociate and give up metal to the absorptive system of an animal. Precipitated metals, or those adsorbed aren't necessarily less toxic to organisms;
- 2) The rate at which the metals are absorbed;
- 3) Synergistic effects, for example, one metal increasing body porosity, with another being absorbed more readily;
- 4) Salinity or tidal changes increase or decrease the rate of metal absorption depending on the animal or metal.

Blue crabs, Callinectes sapidus, began to die at some point for three temperature-salinity regimes in which cadmium was present. The results of experiments at different temperatures and salinities at 10 ppm Cd^{+2} show that crabs concentrate cadmium more rapidly in the gills at lower salinities (Hutcheson 1974). At low salinities, rate of uptake is proportional to temperature. It is thought that crabs are hyperosmotic at lower salinities, and would have to expend more metabolic energy to maintain an osmotic gradient. Thus, less energy would be left to control, through active expulsion, influxes of heavy metals. After a threshold level of approximately 80 ppm. cadmium in the gills is reached, mortality ensues. It has been suggested that there is a fatal disruption of an essential physiological process at the gills.

The effect of salinity on acute toxicity of mercury, copper, and

chromium for an estuarine clam, Rangia cuneata, was studied by Olson and Harrel (1973). It was found that salts of each of the metals were toxic to R. cuneata at all salinities at some concentration. Generally, the more saline in the environment, the less toxic the metals. At water salinities of <1 ppt (freshwater) copper and chromium were very toxic, with concentrations of <1 ppm being lethal to half the animals tested within 48 hours. At a salinity of 22 ppt chromium was found to be the least toxic metal tested, with concentrations as high as 86 ppm being required to induce 50% mortality in the clams tested.

Accumulation of Metals in Estuarine Organisms

The accumulation of heavy metals in estuarine organisms has been well documented. It has been recognized for some time that the concentration of metals is significantly higher in marine organisms than in the surrounding waters (Brooks and Rumsby 1964, Pringle et al. 1968). Mollusca are able to concentrate chemical materials up to many hundreds of times that level found in their environment. Estuarine crabs have also been shown to concentrate trace metals (Hutcherson 1974, Wright 1977).

Several pathways by which marine animals concentrate heavy metals have been suggested (Brooks and Rumsby 1965):

- 1) Particulate ingestion of suspended material from seawater.
- 2) Ingestion of elements via their preconcentration in food material.
- 3) Complexing of metals by coordinate linkages with appropriate organic molecules.
- 4) The incorporation of these metal ions into physiologically important systems.

These pathways involve the process of absorption across a general body surface.

Aquatic organisms also adsorb trace metals onto their body surface, a process distinct from absorption. The concentration of cadmium in the exoskeleton of crabs is thought to be mainly due to adsorption, with a certain maximum level being reached when all available active sites are filled (Hutcheson 1974). The accumulation of lead in the periostracum of the clam, Mytilus edulis, is thought to be mainly an adsorptive process, with the age of the shell affecting the amount of available binding sites (Sturesson 1976). Metal uptake by exchange, for example onto the mucous sheets of oysters, has been hypothesized (Brooks and Rumsby 1975).

Many organisms actively transport metals into their bodies from the surrounding water (Wright 1977, George and Coombs 1977).

Environmental Factors Affecting Metal Uptake

The environmental concentration level to which various species may be subjected will result in different uptake rates as well as concentration levels attained, depending on the duration of exposure (Pringle et al. 1968). The concentration and uptake of metals in estuarine organisms is directly influenced by the concentration of these metals in the waters (Bryan 1971). Studies on the American oyster, Crassostrea virginica (Pringle et al. 1968, Shuster and Pringle 1969, Kopfler and Mayer 1973, Hugget et al. 1973, Frazier 1976, Zarogian and Cheer 1976), the common blue mussel, Mytilus edulis (Phillips 1976 a,b; Chow et al. 1976; Sturesson 1976; and Goldberg 1975), Rangia cuneata, an estuarine clam (Olson and Harrel 1973), blue crabs, Callinectes sapidus (Hutcheson 1974), and the shore crab, Carcinus maenas (Wright 1977), have all shown increased metal concentrations in the animals with increased concentrations of metals in the water and with increased exposure to the water.

Sediment levels of metals are not a perfect predictor of biological tissue levels, but do indicate the relative trend in availability of metals to biota (Frazier 1976). Higher metal concentrations in sediments of similar particle size do indicate greater bioavailability in estuarine organisms.

The importance of chelating agents in the uptake of trace metals by aquatic organisms has been stressed (George and Coombs 1977). It was found that naturally occurring and synthetic chelating agents facilitate the uptake of cadmium in Mytilus edulis, the blue mussel. The presence of natural and synthetic chelating agents produced a doubling in both the rate of accumulation as well as the final tissue concentrations achieved. The higher concentration of lead in the digestive gland and gills of oysters is thought to be due to the presence of many chelating organic ligands in these tissues (Pringle et al. 1968). The hypothesis that metal is transported across cell membranes only after being complexed into organic ligands rather than as simple organic species is supported by these findings. Goldberg (1957), has concluded that in general, relative enrichments for various elements in the marine biosphere follow the order of stability of complexes formed with a number of organic ligands.

In addition to levels of concentration of metals in the environment and the presence of ligands available for chelation already mentioned, other environmental factors will affect the accumulation of metals in estuarine animals. Thermo-saline regimes existing in an estuary will also affect metal accumulation in estuarine animals.

Cadmium concentrations in estuarine mussels and crabs are greatly affected by differences in water temperature and salinity regimes (Hutcheson 1974,

Jackim et al. 1977, and Wright 1977). In four species of estuarine bivalves tested, it was found that a decrease in water salinity from 30 ppt to 20 ppt, caused an increase in cadmium uptake of between 24-400% by all species tested. Wright (1977) found that cadmium accumulation by the haemolymph, gills and carapace of the shore crab, Carcinus maenas, was significantly higher in dilute seawater. There was no significant salinity effect noted for hepatopancreas or muscle cadmium concentrations. Generally, it is assumed that metal uptake increases with decreased water salinities (Jackim et al. 1977).

In the blue crab, Callinectes sapidus, it was found that concentration and rates of uptake of cadmium were greatest at low water salinities and high water temperatures. The major sites of localization were in the gills, hepatopancreas, and carapace. Claw muscle was comparatively low in cadmium content (Hutcherson 1974). Huggett et al. (1973) suggest that a source of oysters from low salinity areas would be potentially more dangerous to those that consume them than oysters from higher salinity areas because of the effect of salinity on the accelerated metal accumulation. A study involving M. edulis also found that as salinity decreased, metal uptake increased generally. Lead was found to be an exception, as salinity decreased lead concentration also decreased (Phillips 1976).

Besides the environmental factors mentioned, many organismal differences will affect the rate and extent of metal accumulated by estuarine animals. The sampling of natural populations with their high levels of biological variability causes difficulties in interpretation of data for identifying metal contaminated areas (Frazier 1976). Much variation exists among individuals in a given population of organisms. Huggett (1973) found that

oysters from the same location often differed in metal concentration as much as 100% and occasionally by 300%. Although statistical computations are weakened, it is suggested that a sample mean be taken and assumed to approximate the population mean.

The body size of an organism may affect its' metal uptake and concentration (Boyden 1974 and 1977). It was found for estuarine molluscs, that metal concentrations can be either dependent or independent of body size. When concentrations are independent of body size, it is possible to calculate mean concentrations for a population. When metal concentrations are influenced by size, the data may be considered as (1) absolute quantities or content in micrograms, or as (2) a concentration ($\mu\text{g/g}^{-1}$ tissue weight). In the oyster, Ostrea sp., cadmium concentrations were found to be independent of body size, whereas lead was found in greater concentrations in smaller individuals. It has been suggested (Boyden 1977) that other species of the genus Crassostrea may be expected to behave similarly to Crassostrea gigas. In the mussel, M. edulis cadmium was independent of body size and lead showed a greater concentration in smaller animals, while in the clam, Mercenaria mercenaria, the exact reverse was found, lead was independent of body size while cadmium was found to be more concentrated in smaller individuals. It has been suggested that when metal content is dependent on body size, regression analyses for the species being compared should be considered.

Species differences exist in accumulation of trace metals within a community of organisms. In a study involving lead uptake in three molluscan species, the eastern oyster, the soft, and the hard-shelled clams, it was found that under the same experimental conditions, the oyster

accumulated trace metals most effectively followed by the soft-shelled clam. The hard-shelled clam was least effective (Pringle et al. 1968).

An animal's feeding mode, filter feeder versus deposit feeder, will affect cadmium uptake. The filter feeders accumulate greater amounts of cadmium (Jackim et al. 1977). Surface silt layers of high organic content may be a major source of elements to deposit feeders in detritus-based food webs. In turbulent estuaries, these surface deposits may become suspended and be ingested by filter feeding organisms (Wolfe and Rice 1972). Bacteria, which may be filtered from the water as food by molluscs can accumulate metals (Tornabene and Edwards 1972, Doyle et al. 1975). Both of these studies indicated that microorganisms could introduce metals into the aquatic food web.

Blue crabs, Callinectes sapidus, feed mainly on molluscs, fish and crustaceans (Tagatz 1969). The molluscs consumed included the estuarine clam, Rangia cuneata and oyster spat of Crassostrea virginica among others. It would seem logical that crabs consuming molluscs which had concentrated metals would also tend to concentrate these metals.

Estuarine animals vary in their treatment of accumulated metals. Differing physiological activities of estuarine animals can affect their rate of uptake and extent of concentration of trace metals from their environment. For example, the amount of metal an organism accumulates is greatly affected by the amount of metal it can excrete per unit time (Pringle et al. 1968).

In a study involving rate of depletion of metals in the eastern oyster, C. virginica, the northern quahaug, Mercenaria mercenaria, and the soft-shelled clam, M. arenaria, it was found that lead was depleted at a

faster rate by the oyster than by the quahang, but at a slower rate by the oyster than the soft-shelled clam (Pringle et al. 1968). The rate of excretion of cadmium in the blue mussel, Mytilus edulis, was found to be eighteen times slower than the rate of uptake when exposed to seawater containing 1 ppm cadmium (George 1977).

Blue crabs were found not to experience any significant loss of accumulated or associated metals within 96 hours when removed from water containing 10 ppm cadmium and placed in uncontaminated water (Hutcheson 1974). However, Wright (1977) found the shore crab, Carcinus maenas, lost 50% of the cadmium accumulated during a 37-day exposure period in 11 days. Losses from the carapace and gills were important components of this reduction in cadmium concentration.

Because estuarine animals can't efficiently excrete trace metals as quickly as they accumulate them, many species store and detoxify the metals until they can be excreted slowly.

The mussel, M. edulis, immobilizes the cadmium it takes up by binding it to a protein, this prevents it from interacting with essential enzymes. When the protein becomes saturated with metal, toxic effects begin to occur (George 1977). The high concentration of metals in oyster soft tissue is thought to be due to metal binding proteins which may act as detoxifying agents (Frazier 1976).

The oyster, C. virginica, can store lead in blood and tissues; they can't control the amounts entering their systems, then lose lead when water concentrations are lower (Bryan 1971). Oysters can also store some metals as granules in leukocytes.

The hepatopancreas is virtually a temporary storage area for metals

In some bivalve molluscs until the excess can be lost to the blood or through diffusion from the body during low environmental concentrations (Bryan 1971). Segar (1971) studied the metal accumulated by eleven species of bivalve molluscs from the Irish Sea. In those organisms which were dissected, gut and digestive glands contained the highest concentrations, mantle, gills and gonads contained moderate concentrations and muscle and shell contained the lowest concentrations of all trace metals tested in all organisms. These findings may indicate generalized storage areas. In the mussel M. edulis, the cadmium concentration in various anatomical regions usually followed the trend of kidney>viscera>gills >mantle>muscle (George 1977).

In the shore crab Carcinus maenas, the cadmium concentration was highest in the hepatopancreas, followed by the gills, carapace, haemolymph, and muscle, respectively (Wright 1977). The hepatopancreas and gills were also found to be the major sites of cadmium accumulation in the blue crab (Hutcheson 1974). As previously mentioned, the hepatopancreas appears to be a storage area for metals in crabs as well as many bivalves.

The lead content in M. edulis was found to be very high in the shell, surpassed only by the gills (Chow et al. 1976). This may represent a storage area for the metal in these mussels.

Estuarine Animals as Indicators of Metal Pollution

An estuary is a fluctuating system and statistical problems arise when determining water quality with respect to trace metals by use of direct water analysis (Frazier 1976). Trace metal concentrations will fluctuate with tidal stages, amounts of freshwater runoff and variations in discharges containing trace elements (Shuster and Pringle 1969).

The use of sediment analyses as an alternative to direct water analyses can demonstrate local sources of contamination. However many complications arise when considering the physical-chemical processes which control the partition of metals between the aqueous overlying phase and the solid sediment phase (Bender et al. 1972, Frazier 1976).

Since estuarine animals accumulate trace metals and can concentrate them, they are indicators of existing conditions in their environment. Many marine and estuarine animals have been used as indicator organisms. The mussel, M. edulis, has been used in many studies (Segar et al. 1971; Goldberg 1975; Alexander et al. 1976; Chow et al. 1976; Fowler and Oregoni 1976; Phillips 1976 a,b,; Stuesson 1976; George and Coombs 1977; and Jackim et al. 1977). The american oyster, C. virginica, as an indicator organism has been used by many authors (Pringle et al. 1968; Shuster and Pringle 1969; Kopfler and Mayer 1972; Huggett et al. 1973; Frazier 1976; and Zarogian and Cheer 1976).

Estuarine molluscs are useful as indicator organisms because they fit guidelines for monitoring organisms. Some guidelines proposed by Watling and Watling (1976) are that indicator organisms should be plentiful, sedentary, accessible, of reasonable size and of known taxonomy. Several other characteristics of indicator organisms as well as preferred methods for sampling have been proposed by Phillips (1976).

Pelagic crabs, such as the blue crab, C. sapidus, are less useful as indicator organisms largely because of their motility. Blue crabs are known to have extensive seasonal migrations (Tagatz 1969). Crabs are however a major seafood source of man and deserve attention because of their role in food chains. As previously mentioned, estuarine molluscs, including

oyster spat and clams, are a principal food source of the blue crab (Tagatz 1969); it can be seen that these crabs may be an important link in the cycling of metals back to man.

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METHODS AND MATERIALS

Description of Study Area

The Calcasieu River Basin in southwestern Louisiana is the state's largest industrialized area (United States Environmental Protection Agency 1972). Industries in the lower portion of the Calcasieu River Basin are involved in the production of chemicals, petrochemicals, and petroleum products. All of these industries are discharging waste waters into the Calcasieu River and its' tributaries.

The area studied was the Calcasieu Estuary (Fig. 1). It is defined as the area from the salt water barrier north of the city of Lake Charles, to the Gulf of Mexico, approximately 50 miles to the south. A ship channel, maintained by the Army Corps of Engineers, connects the Gulf of Mexico with the Port of Lake Charles. This channel is navigated by ships and barges of various sizes and types and acts as a barrier between certain parts of the estuary.

No distinct saline regimes exist between the northern and southern portions of the estuary because of irregular mixing patterns due to lunar and wind-dominated tides. Although no clear-cut saline regimes exist, the southern portion of the estuary, adjacent to the Gulf of Mexico, is usually considered to be more saline. The Calcasieu Estuary is relatively shallow and very productive. Crabs, shellfish, shrimp and fish flourish in this estuary and support a large commercial and sport fishery.

Field Sampling

Six sampling stations were chosen because they represented excellent sources of at least one of the species chosen for study (Fig. 1). The possible presence of chemical pollution was not a consideration. Each sampling station was visited once during November 16 and 17, 1978. At each station a dredge tow of approximately 2-5 minutes was taken for collection of shellfish. A simple oyster dredge was used. Clams of the species Rangia cuneata, and oysters, Crassostrea virginica, were sorted and labeled. At least 10 specimens of each species were collected. When crab traps were available at a station, blue crabs, Callinectes sapidus were also collected. All specimens were put on ice and frozen for later analysis.

Water was sampled by submerging an acid washed and rinsed 200 ml polyethylene bottle at each sampling station. Water samples were preserved with the addition of laboratory reagent grade nitric acid. Water temperature, salinity and dissolved oxygen readings were obtained using a Beckman in situ potentiometer. Depth of collection of each set of samples was also recorded.

A sediment sample was taken at each station using a Peterson dredge grab. All water and sediment samples were put on ice for later determination of trace metals.

Trace Metal Determination

All metal analyses were conducted at the Illinois Natural History Survey, Urbana, Illinois by emission spectrometry using a Jarrel-Ash Model 975 Plasma Atom Comp. A radio-frequency inductively coupled argon plasma was used as the source of radiation, The computer controlled

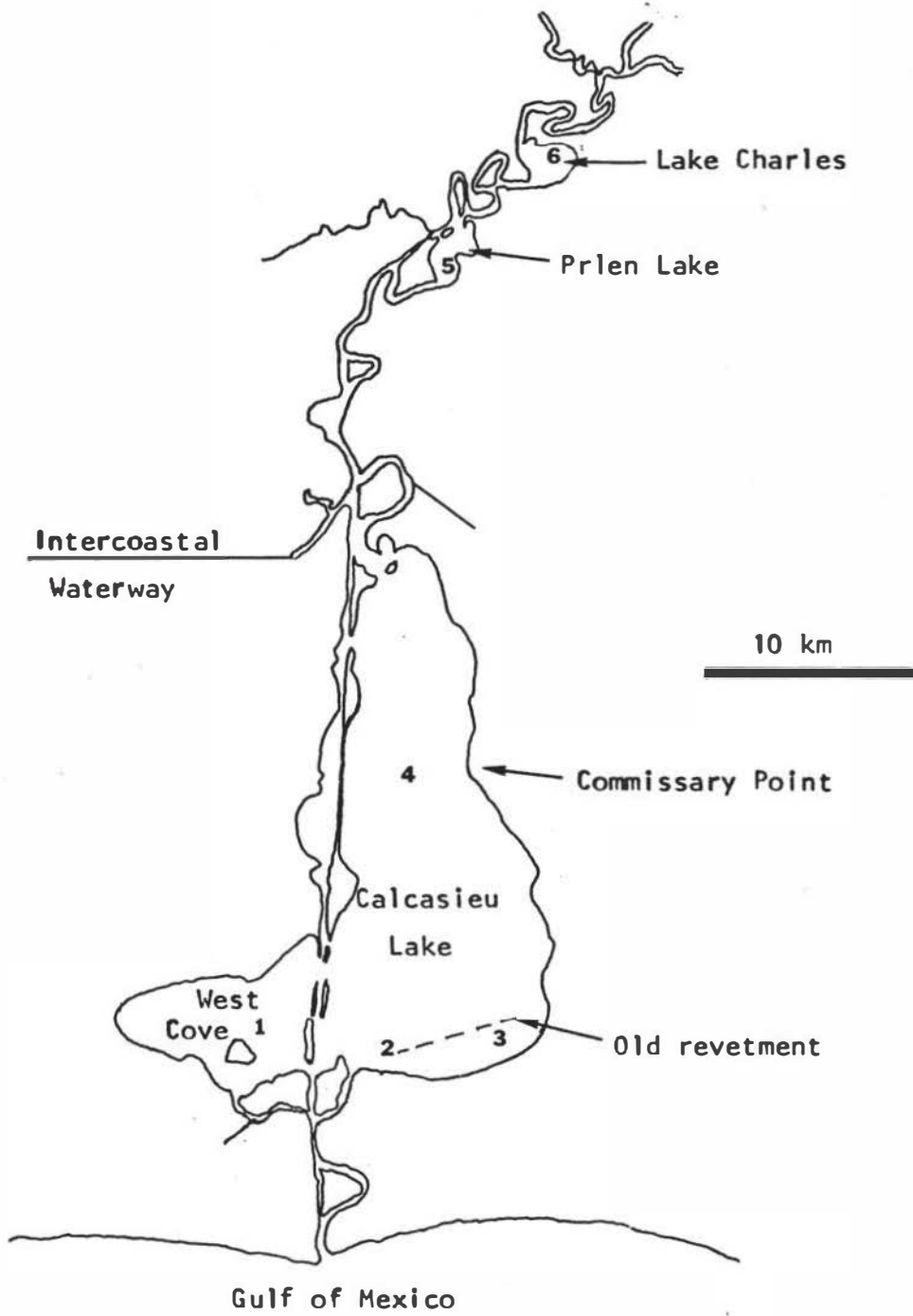


Figure 1. Map of Calcasieu Estuary, La. with stations.

spectrometer was calibrated using standard pure metal solutions of cadmium, chromium and lead.

All water samples were unfiltered and analyzed for cadmium, chromium and lead by emission spectrometry. The procedures used follow those prescribed by the United States Environmental Protection Agency (1974).

Sediments were analyzed for trace metals according to methods used by Perkin-Elmer (1976). Total surface area of each sediment sample was determined with ethylene glycol retention as described by Bower and Gschwend (1952), to eliminate bias when comparing sediment metal content between stations. The metal content for each sample was then calculated in $\mu\text{g}/\text{m}^2$.

Shellfish were weighed and their shell length determined before the shells were removed. Soft body parts were weighed individually and frozen for later analysis. Procedures used for determination of cadmium, chromium, and lead were developed by Dr. Kenneth E. Smith of the Illinois Natural History Survey. All samples were digested in a heated nitric-perchloric acid mixture, after being thawed and re-weighed, by refluxing under a fume eradicator hood for approximately three hours. The cooled solutions were diluted to 25 mls using redistilled, polished water, centrifuged and analyzed by emission spectrometry. Whole shells, after being measured were fragmented and random samples of 2.0 grams each were digested in the same manner as the soft body parts.

Crabs were dissected after whole-body weights and measurements were determined. For each crab, tissue samples of carapace, gill and claw muscle were removed and weighed, then frozen for analysis. All crab tissues were analyzed using the same procedure followed for shellfish metal

determination.

Because organisms were not dry-ashed or oven-dried, the weights and concentrations determined to be present represent the amounts of metals normally available to natural predators or human consumers.

Statistics

All statistics were computed in conjunction with the Computing Services office at the University of Illinois at Urbana-Champaign. Analysis of variance was computed using the SOUPAC statistical system and multiple comparisons of the data were analyzed using the Sheffe post-hoc test.

RESULTS

Water and Sediments

Data for water temperature, dissolved oxygen, salinity and depth of collection of samples for all stations are listed in Table 1. Depth of collection of samples was fairly uniform, with values ranging from 0.9 - 1.8 meters. Temperature of water was also quite homogenous with readings ranging between 20-24°C. Station 4 exhibited a higher dissolved oxygen reading than any other station (12.0 ppt), the other stations were very similar with respect to dissolved oxygen. Station 4 also had the greatest salinity, 19.0 ppt, while station 1 had the lowest salinity found in the areas sampled (10 ppt). Metal content in water samples by station, is illustrated in Figure 2. Station 6 had the lowest concentrations of all three metals tested. Cadmium was least concentrated and chromium was most concentrated in all water samples.

Sediment metal concentrations (ug metal/gm sediment), total surface areas, and corrected metal concentrations (ug metal/m² sediment surface area) are listed in Table 2. A comparison of sediment metal concentrations (ug/m²) is illustrated in Figure 3. Lead was found in greatest concentrations in all sediment samples in contrast to the values from the water samples. Cadmium was still found in least concentrations at all stations. Station 4 exhibited the greatest concentrations of all metals of all stations sampled. Station 2 had the lowest concentrations of lead and chromium of all stations sampled. Cadmium was found to be least concentrated in the sediments at station 1.

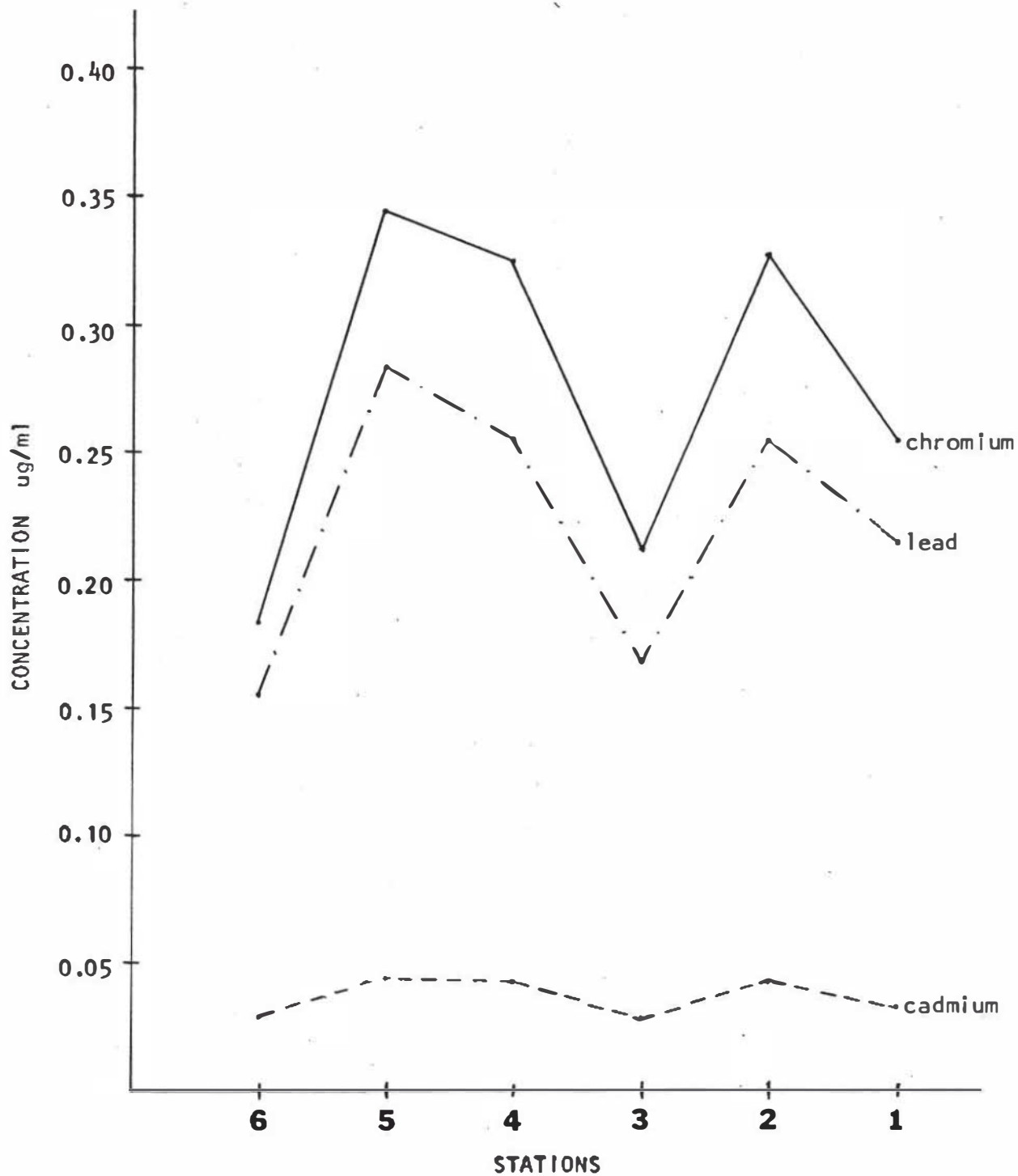


Figure 2. Concentrations of cadmium, chromium and lead (ug/ml) in the waters at each station sampled in Calcasieu Estuary, La.

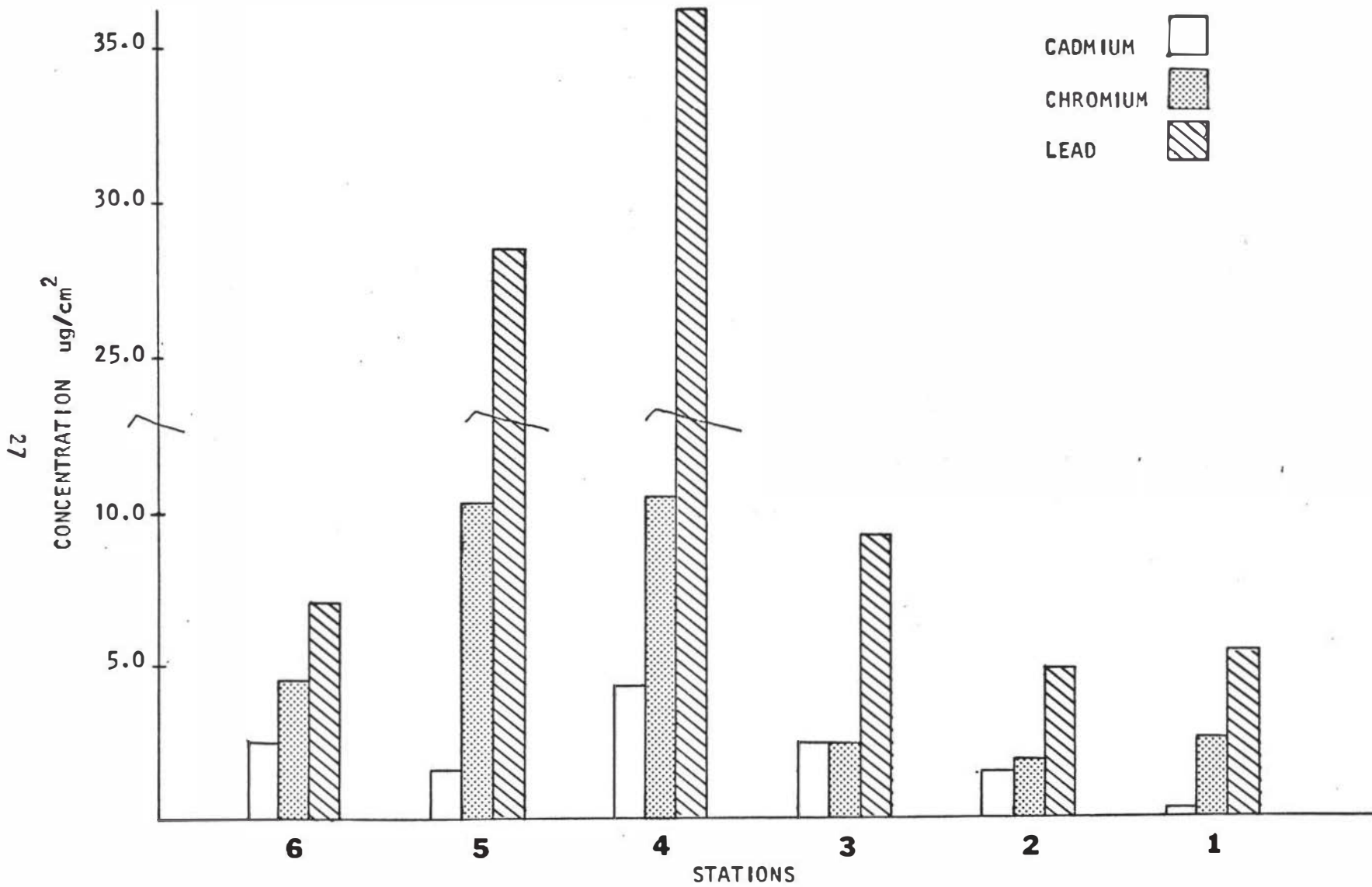


Figure 3. Concentrations of cadmium, chromium and lead ($\mu\text{g metal}/\text{m}^2$ sediment) in the sediments at each station sampled in Calcasieu Estuary, La.

Table 1. Water temperature, dissolved oxygen, salinity, and depth of collection of samples for 6 stations sampled in Calcasieu Estuary, Louisiana.

Station	Temperature (° C)	Salinity (ppt)	Dissolved Oxygen (ppt)	Depth of Collection (meters)
1	21.0	10.0	9.0	1.2
2	20.5	18.0	9.2	1.1
3	20.0	18.5	9.5	0.9
4	21.0	19.0	12.0	1.9
5	24.0	15.0	9.2	1.8
6	24.0	12.5	9.0	1.8

Table 2. Metal concentration in sediments from 6 sampling stations in Calcasieu Estuary, Louisiana.

Location	Metal	Metal concentration (ug/g. wet wt. sediment)	Total Surface area (m ² /g)	Metal Concentration (ug/cm ²)
Station 1	Cadmium	0.190	100.69	0.2
	Chromium	2.416		2.4
	Lead	5.398		5.4
Station 2	Cadmium	2.879	204.09	1.4
	Chromium	3.511		1.7
	Lead	9.520		4.7
Station 3	Cadmium	4.990	210.39	2.4
	Chromium	5.077		2.4
	Lead	19.071		9.1
Station 4	Cadmium	3.875	91.53	4.2
	Chromium	9.438		10.3
	Lead	33.108		36.2
Station 5	Cadmium	1.170	72.21	1.6
	Chromium	7.294		10.1
	Lead	20.410		28.3
Station 6	Cadmium	5.955	256.37	2.3
	Chromium	11.601		4.5
	Lead	18.136		7.1

INTRASPECIFIC RELATIONSHIPS

Crabs

Specimens of the crab, Callinectes sapidus, were found at four stations in the estuary (Table 3). Claw muscle of this species contained the lowest concentrations of the tissues analyzed of all metals at all stations. Carapace exhibited the highest concentrations and gill tissue was found to have intermediate concentrations of all metals at all stations at the 95 percent confidence level (Table 3).

General observation of all data shows that the concentration of all metals tested were found to be independent of the size of the crab at the 95 percent confidence level.

Chromium was found in significantly greater concentrations than either cadmium or lead in all muscle and gill tissues analyzed at all stations ($\alpha = .05$), Table 3. Cadmium was present in significantly lower concentrations than lead or chromium in all muscle, gill and carapace tissues analyzed from all stations. Lead was present in significantly greater concentrations in crab carapace than chromium and cadmium.

All metals were present in significantly greater concentrations in crab carapace at station 3 than at stations 1 and 6 at the 95 percent confidence level. In crab gill, chromium was found to be significantly higher in concentration at station 5 than at stations 1 and 6. Cadmium and lead were not found to be significantly different at any stations in crab gill. Cadmium and lead were more concentrated in crab muscle at station 3 than at station 1. Chromium was not found in significantly different concentrations at stations 3 and 1 at the 95 percent confidence

Table 3. Callinectes sapidus. Mean concentrations \pm standard deviations of cadmium, chromium and lead in muscle, gill and carapace tissues of crabs from 4 locations in Calcasieu Estuary, Louisiana. Data for wet weights and carapace width are also included.

Location	n	Tissue	Metal Concentration (ug/gm wet weight)			Wet Weights (g)	Carapace Width (mm)
			Cadmium	Chromium	Lead		
Station 1	3	Muscle	0.130 \pm 0.120	2.657 \pm 0.112	0.160 \pm 0.172	221.0 \pm 49.6	156.0 \pm 7.5
		Gill	0.143 \pm 0.132	2.764 \pm 0.280	0.662 \pm 0.672		
		Carapace	1.513 \pm 0.272	9.780 \pm 0.567	21.435 \pm 2.920		
Station 3	4	Muscle	0.240 \pm 0.670	3.241 \pm 0.113	1.628 \pm 0.418	234.84 \pm 36.9	164.3 \pm 9.8
		Gill	0.566 \pm 0.311	3.873 \pm 0.671	3.471 \pm 2.436		
		Carapace	2.432 \pm 0.451	12.366 \pm 0.638	28.138 \pm 3.549		
Station 5	4	Muscle	0.175 \pm 0.596	1.574 \pm 0.211	1.546 \pm 0.476	297.75 \pm 37.5	176.3 \pm 13.8
		Gill	0.336 \pm 0.441	4.088 \pm 0.614	2.449 \pm 0.238		
		Carapace	2.072 \pm 0.108	8.724 \pm 0.225	26.393 \pm 1.554		
Station 6	3	Muscle	0.134 \pm 0.574	2.894 \pm 0.430	1.033 \pm 0.191	259.61 \pm 47.5	178.7 \pm 7.5
		Gill	0.303 \pm 0.157	3.188 \pm 0.493	2.146 \pm 1.669		
		Carapace	1.420 \pm 0.375	9.626 \pm 0.953	20.324 \pm 3.651		

level in crab muscle.

Oysters

Specimens of the oyster, Crassostrea virginica, were found at three stations in Calcasieu Estuary (Table 4). Chromium and lead were found in significantly greater concentrations in oyster shell than in oyster soft tissues at all three stations. Cadmium, however, was found to be significantly more concentrated in oyster soft tissues than in oyster shell at all stations tested.

Concentrations of all metals were found to be independent of the size, therefore age, of the oysters at the 95 percent confidence level.

In oyster shell analysis, lead was significantly more concentrated at all stations than chromium and cadmium. Chromium was found to be significantly more concentrated than cadmium in all oyster shells analyzed. Concentrations of metals in soft tissues showed a reversal in trends exhibited for chromium and lead concentrations in oyster shells. Lead was found to be significantly less concentrated than either chromium or cadmium at all stations in oyster soft tissues (Table 4). Cadmium and chromium concentrations were not found to be significantly different at the 95 percent confidence level in oyster soft tissues. Cadmium concentrations in both oyster soft tissues and oyster shells at station 3 were found to be significantly lower than concentrations of this metal at station 2. This difference was also noted for chromium and lead concentrations in oyster shells for both stations. Chromium and lead concentrations in oyster soft tissues were not found to be significantly different at any one station.

Table 4. *Crassostrea virginica*. Mean concentrations \pm standard deviations of cadmium, chromium and lead in whole soft tissues and shells of oysters collected from 3 locations in Calcasieu Estuary, Louisiana. Data for wet weights and shell length are also included.

Location	n	Tissue	Metal Concentration ($\mu\text{g/g}$. Wet Weight)			Whole Soft Tissue Wet Weights (g.)	Shell Length (cm.)
			Cadmium	Chromium	Lead		
Station 1	10	Soft Tissue	0.691 \pm 0.292	0.753 \pm 0.321	0.351 \pm 0.415	6.70 \pm 3.9	8.9 \pm 1.5
		Shell	0.457 \pm 0.875	2.390 \pm 0.313	11.341 \pm 1.880		
Station 2	10	Soft Tissue	0.793 \pm 0.183	0.611 \pm 0.141	0.216 \pm 0.213	7.81 \pm 2.5	10.1 \pm 1.1
		Shell	0.581 \pm 0.198	2.785 \pm 0.540	15.792 \pm 3.793		
Station 3	10	Soft Tissue	0.403 \pm 0.157	0.583 \pm 0.326	0.254 \pm 0.221	9.61 \pm 6.5	9.9 \pm 2.2
		Shell	0.376 \pm 0.101	2.307 \pm 0.311	10.193 \pm 1.572		

Clams

Rangia cuneata, a brackish water clam, was found at two stations in the estuary (Table 5). Cadmium and lead were found to be significantly more concentrated in clam shell than in soft tissues at both stations 4 and 6. Chromium concentrations in clam soft tissues and clam shell were not found to be significantly different at the 95 percent confidence level.

Concentrations of all metals tested in Rangia cuneata were found to be independent of body size at the 95 percent confidence level. All metals were more concentrated in shells at station 4 than concentration levels in shells at station 6. These differences were not found to be significant at the 95 percent confidence level. Analysis of clam soft tissues presented a different trend. Cadmium and lead were found in very low concentrations in clam soft tissues at both stations, in fact, all readings were below detection limits of the instrument (<0.01 ug/g wet weight) with the exception of one cadmium reading in soft tissues from station 4. Chromium was found to be significantly more concentrated at station 6 than at station 4 in clam soft tissues.

INTERSPECIFIC RELATIONSHIPS

Crabs and oysters

Callinectes sapidus and Crassostrea virginica were both found at stations 1 and 3 (Tables 3 and 4). Crab carapace contained significantly greater concentrations of all metals tested than oyster shells from both stations 1 and 3 at the 95 percent confidence level. Crab muscle was found to contain significantly greater concentrations of chromium and lead

Table 5. Rangia cuneata. Mean concentrations \pm standard deviations of cadmium, chromium and lead in whole soft tissues and shells of clams collected from two locations in Calcasieu Estuary, Louisiana. Data for wet weights of whole soft tissues and shell wet weights are also included.

Location	n	Tissue	Metal Concentration (ug/g wet weight)			Whole Soft Tissue Wet Weights (g.)	Whole Shell Wet Weights (g.)
			Cadmium	Chromium	Lead		
Station 4	10	soft tissues	0.113 \pm 0.230	1.786 \pm 0.374	0.100	2.15 \pm 0.3	17.7 \pm 2.5
		shell	0.603 \pm 0.102	3.248 \pm 0.262	15.055 \pm 1.924		
Station 6	10	soft tissues	0.100	4.296 \pm 1.013	0.100	2.47 \pm 0.5	23.5 \pm 3.4
		shell	0.526 \pm 0.153	2.963 \pm 0.257	13.352 \pm 1.676		

than oyster soft tissues from both stations. Cadmium was found in significantly greater concentrations in oyster soft tissues than in crab muscle at station 1, where the lowest cadmium concentrations were found in crab muscle. At station 3, where cadmium in crab muscle was in significant differences were found between crab muscle and oyster soft tissues at the 95 percent confidence level.

Crabs and Clams

Specimens of the crab, Callinectes sapidus, and of the clam Rangia cuneata, were both found at station 6 only (Tables 3 and 5). Crab carapace had significantly greater concentrations of all metals tested than clam shell. Cadmium and lead were both found in significantly greater concentrations in crab muscle than in clam soft tissues; however, chromium was significantly more concentrated in clam soft tissues than in crab muscle tissue at station 6.

Metal Interactions

In all oyster tissues analyzed, chromium and lead exhibited a significant correlation. High concentrations of chromium were present with high concentrations of lead and high lead concentrations were present when chromium was highly concentrated in oysters. In all clam tissues analyzed, cadmium and lead exhibited this same significant correlation. In all of the samples of crab tissue analyzed, levels of each of the three metals were found to have this same significant correlation, when one metal was highly concentrated, the others were also.

DISCUSSION

ENVIRONMENTAL RELATIONSHIPS

All water and sediment concentrations of all of the metals (cadmium, chromium, and lead) increased greatly between station 6 in Lake Charles, and station 5 in Prien Lake with one exception. Cadmium concentrations in the sediments decreased only slightly (Figures 2 and 3). There are several petroleum refineries and petrochemical plants located between Lake Charles and Prien Lake. It seems probable that effluents from these industries may be contributing to the increase in concentrations of heavy metals in the waters and sediments of station 5.

Station 4 is located in Lake Calcasieu about mid-lake off Commissary Point (Figure 1). Metal concentrations in the water decreased slightly between station 5 and 4 (Figure 2). This may be due to a dilution effect occurring as waters from the Calcasieu River enter Lake Calcasieu. The sediment metal concentrations continued to increase between stations 5 and 4 (Figure 3). This observation may be attributable to the adsorption properties of the suspended metals, causing them to be carried farther towards the Gulf of Mexico before being adsorbed by the sediments. More industries are also located between stations 4 and 5 and may be contributing more metals into the estuary.

Water and sediment concentrations of all metals greatly decreased between stations 4 and 3 (Figures 2 and 3). Station 3, located in the southeast portion of Lake Calcasieu, south of the old revetment, seems to be out of the main flow of the lake drainage and tidal currents (Figure 1).

Station 3, being somewhat isolated from regular mixing within the lake would be expected to have lower concentrations of metals in the sediments and water than the rest of the lake.

Station 2 is located in the south end of Lake Calcasieu near the west end of the old revetment (Figure 1). Water concentrations of all metals at station 2 were very similar to water concentrations at station 4. Since waters at station 2 seem to mix regularly with the main body of the lake, it would seem that concentrations of metals in the waters at this station would be similar to those in the rest of the lake. Metal concentrations in the sediments at station 2 were generally lower than in the rest of the estuary (Figure 3). It is not apparent why sediment concentrations of metals are so low at this station. Perhaps the salinity of this area is greater for longer periods of time than the rest of the lake because of its close proximity to the Calcasieu Pass, the connection between the Gulf of Mexico and Lake Calcasieu (Figure 1). Wolfe and Rice (1972) stated that salinity affects adsorption equilibria established between sediments and water. Bulk seawater components such as calcium and potassium may compete with trace components for adsorption sites. This salinity effect may explain the lower metal concentrations in the sediments at station 2 as compared to the metal concentrations in the water in the main body of the lake.

Metals in the waters at station 1 were less concentrated than similar concentrations at station 2 (Figure 2). Lead and chromium concentrations in the sediments increased slightly and cadmium concentrations decreased between stations 2 and 1 (Figure 3). Station 1 is located in the west cove of Lake Calcasieu (Figure 1). The west cove is largely isolated from the

rest of Lake Calcasieu by a partial "dike" formed from the dredgings of the ship channel that passes between the west cove and the main body of the lake. It seems probable that waters at station 1 would not mix regularly with the main body of the lake, therefore metal concentrations in the water may be expected to be different at station 1. Salinity was lower at station 1 than at any other station in the estuary. Perhaps this salinity difference would account for the slight increase of metal concentrations in the sediments at station 1 versus station 2.

BIOTIC-ENVIRONMENTAL RELATIONSHIPS

Body Size and Metal Concentration

Concentrations of cadmium, chromium and lead in each species of animal sampled and the wet weights of each individual animal were compared with an analysis of variance test. For Callinectes sapidus, Crassostrea virginica, and Rangia cuneata it was found that concentrations of cadmium, chromium, and lead in these animals were significantly independent of their wet weights at the 95 percent confidence level. Boyden (1974) found that clams, Mercenaria mercenaria, and mussels, Mytilus edulis, concentrated lead independently of body weight. Ayling (1974) in studies involving Crassostrea gigas, found that lead and chromium concentrations were independent of body size, however cadmium was found to be more concentrated in smaller individuals. Huggett et al. (1973) found no correlations between concentrations of zinc, copper and cadmium in Crassostrea virginica and the weight of the animal. It seems that no research has been conducted concerning metal concentration versus body weight differences in the blue crab, C. sapidus. According to Boyden (1974 and 1977), when species

demonstrate an independence in metal concentration and body size, sample means can be calculated and assumed to approximate population means. In the present study, sample means of metal concentrations in all species of animals are assumed to approximate population means at each station.

Crabs

For all metals tested in crab, Callinectes sapidus, tissues it appears that claw muscle contains the lowest concentrations of all metals, carapace contains the highest concentrations, and gill tissue contains intermediate concentrations of all metals. Hutcheson (1974) found similar tissue concentrations (using 0.11 ug/ml cadmium in the water) in C. sapidus. Wright (1977) in studies involving cadmium concentration in the shore crab, Carcinus maenas, also found these trends in tissue concentration to occur. The latter author also calculated cadmium concentrations in crab tissues relative to the water concentrations of cadmium. In water with salinity comparable to 50 percent seawater, essentially the same as estuarine salinities, at cadmium concentrations of 20 u-mole l^{-1} , carapace exhibited a concentration factor of 125X, gill tissue was 20X and muscle tissue was 3-5X the concentration in the external medium. Concentration factors in C. sapidus in the present study were calculated for each of the three metals tested. Cadmium in muscle tissue was 5X, gill tissue 11X, and carapace 58X the cadmium concentrations found in the water at stations 1, 3, 5, and 6 (mean 0.03 ug/ml). These concentration factors are not in strict accordance with those found by Wright (1977), however it should be noted that water concentrations of cadmium are much lower in the present study. It should also be recognized that C. maenas may not concentrate metals in exactly the same manner as C. sapidus. Chromium

concentrations in muscle tissue was 10X, gill 14X, and carapace 41X the concentration of this metal in the water at stations 1, 3, 5, and 6 (mean 0.25 ug/ml). Lead concentration factors in the tissues of C. sapidus were 6X for muscle, 11X for gill tissue, and for carapace, 120X greater than the concentration of this metal in the waters (mean 0.21 ug/ml).

Both crab muscle and gill tissues exhibited similar concentration patterns for all three metals tested. Chromium was found to be significantly more concentrated in muscle and gill tissues than lead or cadmium. Lead was found to be significantly more concentrated than cadmium in muscle and gill tissues at the 95 percent confidence level. These concentration patterns appear very similar to water concentration patterns occurring throughout the estuary (Cr>Pb>Cd). It seems that an absorptive process is occurring in these crab tissues in which metals are adsorbed onto the gills directly from the water, then transported into the body, probably after first being attached to organic ligands, and incorporated into the tissues. Hutcheson (1974) and Wright (1977) in studies involving C. sapidus and C. maenas, respectively, found this same absorptive mechanism to occur involving cadmium uptake.

Crab carapace was found to have a different concentration pattern for the three metals tested (Table 3). In carapace, lead was found to be significantly more concentrated than chromium. Chromium was found to be significantly more concentrated than cadmium at the 95 percent confidence level. This concentration scheme is very similar to the pattern exhibited by metal concentrations in the sediments throughout the estuary (Pb>Cr>Cd). Most authors agree that the concentration of metals in the exoskeleton is due largely to the process of adsorption (Hutcheson 1974). It is known

that concentration of metals in sediments is due to the process of adsorption. It seems reasonable, therefore, that concentrations of metals in the sediments, and those found in the carapace should show similar concentration patterns.

Clams

Cadmium and lead were found in very low concentrations in clam soft tissues at both stations 4 and 6 (Table 5). Clam shell was found to have significantly greater concentrations of cadmium and lead than clam soft tissues. These results seem to suggest an active excretion of cadmium and lead from the soft tissues into the shell, perhaps as a method of detoxifying the metals. Sturesson (1976) in studies involving lead concentrations in Mytilus edulis, a bay mussel, found this active deposition of metals in the shell to occur, as well as adsorption of metals onto the exterior of shells. Anderson (1977) found concentrations of cadmium, copper, lead and zinc to be higher in the soft tissues of freshwater clams than similar concentrations in the shells. These observations were made using dry weights of the tissues for analyses. Since soft tissues are 80-90 percent water, a gram-for-gram comparison of metal concentrations between soft tissues and shells using dry weights would not be expected to agree with the same comparison using wet weights of the tissues.

Chromium concentrations in clam shell tissue were not found to be significantly different from soft tissue concentrations. This seems to suggest a different excretory process occurring for chromium in clams versus cadmium and lead excretion, which seems largely to be a deposition to shell tissue. Brooks and Rumsby (1964) found that clams concentrated chromium in soft tissues to a greater extent than did oysters, results which are

similar to those of this study.

Clam shell analyses revealed lead to be significantly more concentrated than chromium and chromium to be significantly more concentrated than cadmium at both stations 4 and 6. These results seem to correlate with sediment concentrations of all metals from stations 4 and 6 (Pb>Cr>Cd). This similarity in orders in shell and sediment metal concentration seems to suggest that an adsorptive process is occurring in addition to the active absorptive process previously mentioned. In soft tissues, chromium was found to be significantly more concentrated than cadmium or lead. This concentration order fits that of the water more closely (Cr>Pb>Cd), perhaps more evidence suggesting an absorptive process occurring in the soft tissues.

Oysters

Analysis of metal concentrations of oyster shell showed that at all stations, lead was significantly more concentrated than chromium or cadmium and chromium was significantly more concentrated than cadmium. These results were found to be significant at the 95 percent confidence level (Table 4). The sediments of stations 1, 2, and 3 were found to have the same concentration order (Pb>Cr>Cd). This similarity in concentration order seems to indicate a similar concentration mechanism between sediments and oyster shells. The same shell concentrating mechanisms seem to be present in oyster shells. There is probably an adsorptive process occurring along with an active absorptive concentration of metals in oyster shells.

Oyster soft tissue analysis revealed a completely different concentration order of all metals considered. Lead was in significantly lower concentrations in oyster soft tissues than cadmium or chromium. Cadmium and

chromium concentrations in oyster soft tissues were not found to be significantly different from each other at the 95 percent confidence level. However, cadmium generally was more concentrated. The concentration order for metals exhibited for oyster soft tissues ($Cd \approx Cr > Pb$) was not similar to water or sediment metal concentration orders. Brooks and Rumsby (1964) found similar concentration orders of metals in the oyster Ostrea sinuata ($Cd > Cr > Pb$). Kopfler and Mayer (1973) also found little or no correlation between lead or cadmium in Crassostrea virginica and water concentrations of these metals. Frazier (1976) found some correlations in ratios of metal content between stations in the soft tissues of C. virginica and similar ratios for sediment concentrations of cadmium, copper, zinc, iron, and manganese. It was stressed that the sediments must be in equilibrium with the overlying waters for these correlations to exist. It is not known whether the sediments and waters in Calcasieu estuary are in equilibrium. Wolfe and Rice (1972) indicate that tidal flushing and wind dominated currents in a shallow estuary may promote the exchange of elements between the water and sediments creating a very fluctuating system.

Chromium and lead concentrations were significantly lower in oyster soft tissues than similar concentrations in oyster shell. Concentration factors of these metals in soft tissues of oysters over water concentrations of metals were 2.4X for chromium and 1.3X for lead. Oyster shell concentration factors were 9.1X for chromium and for lead, 57X the water concentrations of these metals. These findings seem to suggest either an active excretion of chromium and lead from soft tissues into the shell, perhaps as a detoxification mechanism, or an active excretion of these metals directly into the water and an adsorptive accumulation of metals onto the

shell.

Brooks and Rumsby (1964) and Segar (1971) both found that metals are concentrated to a greater extent in oyster soft tissues than in oyster shells. These two studies used dry weights and concentrations when comparing the shells and soft tissues. In the present study wet weights and concentrations were used. For reasons previously mentioned, the results of the earlier studies and of the present study should not be expected to be similar. The wet weights were used in the present study because such values are more nearly representative of values in the living organisms.

Cadmium was found in significantly lower concentrations in the shell as compared to the soft tissues in oysters. Concentration factors in oyster soft tissues for cadmium were 18X, and for shell tissue 13X, the concentration of cadmium found in the water. Although absolute cadmium concentrations in oyster soft tissues aren't significantly greater than soft tissue chromium concentrations, cadmium is concentrated to a much greater extent above the levels of cadmium in the water than is chromium (cadmium = 13X, chromium = 2.4X). Valiela (1974) found that C. virginica concentrated cadmium to a much greater extent than they concentrated lead. This phenomenon was attributed to cadmium being less well bound in the sediments than lead and therefore being more available to the oysters. Cadmium would also have been found to be highly concentrated in clams in the present study if this were the only reason for cadmium concentrations being elevated in oyster soft tissues. As in this study, Brooks and Rumsby (1964) reported that cadmium was found in higher concentrations in oyster soft tissues than in clam soft tissues. They suggested that cadmium is used in the enzyme systems of oysters, and is actively absorbed. Leland et al.

(1974) state that cadmium may be found in higher concentrations in most benthic organisms. These authors seem to support the hypothesis that cadmium isn't as tightly bound in the sediments, and is therefore more available to benthic organisms.

INTERSTATION COMPARISONS

Crabs

Cadmium, chromium and lead was found in significantly greater concentrations at the 95 percent confidence level in crab muscle and carapace at station 3 than at station 1. Crab gill tissue concentrations of these three metals were not found to be significantly different at these two stations. Sediment concentrations of cadmium and lead were found to be greater at station 3 than at station 1. Chromium concentrations in the sediments at stations 1 and 3 were found to be very similar. The metal concentration dynamics in the sediments seem to be similar to the metal concentration dynamics in the blue crabs. Station 1 was located in the west cove of Lake Calcasieu, and as previously mentioned is largely isolated from the main body of the lake. Station 3 is located in the southeast portion of Lake Calcasieu (Figure 1). The metal concentrations in the sediments and water at station 3 are not particularly high when comparing station 3 with the other stations in the estuary. Blue crabs, unlike clams and oysters, are not restricted to a confined area, they may undertake extensive seasonal and less extensive daily migrations (Tagatz 1969). Crabs at station 3 probably feed in the southern end of the main body of Lake Calcasieu and are thus exposed to higher metal concentrations

Clams

Cadmium and lead were found in very low concentrations in clam soft tissues at both stations 4 and 6 (Table 5). As a result of these low concentrations, no significant differences were noted between these two stations in clam soft tissues. Chromium concentrations in clam soft tissues were significantly higher at station 5 than similar concentrations at station 4. Chromium concentrations in the water were greater at station 5 than at station 4. Perhaps the soft tissue concentrations of chromium in clams are determined by water concentrations of the metal. Concentrations of cadmium, chromium, and lead were all greater in clam shell at station 4 than concentrations in shell from station 5, however these differences were not found to be significant at the 95 percent confidence level. The concentrations of all three metals in the sediments were greater at station 4 than at station 5 (Figure 3). It seems apparent that concentration dynamics of metals in clam shells and sediments are very similar. Clam shell analyses would seem to be a good indicator of the concentrations of cadmium, chromium, and lead in the estuarine environment available to benthic organisms.

Oysters

In considering differences in concentrations of metals in oyster shells among stations, it becomes apparent that station 2 contained the highest concentrations of all metals. The concentrations of chromium and lead in shells at station 2 were significantly greater than concentrations of these metals at either station 1 or 3. Cadmium in oyster shells was not significantly more concentrated at station 3 than at station 1. These concentration dynamics for metals in the oyster shells are similar to the

concentration dynamics for metals in the water samples, with station 2 having higher concentrations of all metals than either stations 1 or 3. These results indicate that oyster shells are a good indicator of the amount of metals in the waters at the various stations.

Station 2 is situated nearer to Calcasieu pass than either stations 1 or 3 (Figure 1). It is possible that this station would be subject to more tidal currents than would station 3 and especially station 1. Wolfe and Rice (1972) observe that the tidal flushing effect in estuaries may make particulate materials, containing adsorbed metals, more available for adsorption and absorption to the organisms in that estuary. This effect could be occurring in the oysters at station 2, making concentrations of metals in their shells higher than at neighboring stations.

Concentrations of chromium and lead in oyster soft tissues were not found to be significantly different among any of the stations. Cadmium was found to be significantly less concentrated at station 3 than at either stations 1 or 2. The lower levels of cadmium in oyster soft tissues may be attributable to lower concentrations of this metal in the water at this station. In the present study oyster soft tissues were not as good an indicator of existing conditions concerning metal concentrations as were oyster shells.

Concentration factors of metals in oyster shells and soft tissues over water concentrations of the metals for cadmium, chromium and lead were calculated for each station. Concentration factors of metals in oyster shells and in oyster soft tissues over absolute sediment metal concentrations ($\mu\text{g metal/gm sediment}$) were also calculated. No correlations were found among concentrations of metals in the waters and oyster tissue

concentration factors over water concentrations at each station. When considering absolute concentrations of metals in the sediments (ug/gm) and oyster tissue concentration factors over the absolute sediment metal concentrations, some correlations become apparent. Absolute sediment metal concentrations increased almost linearly between stations 3 and 1 (Station 3>Station 2>Station 1). Metal concentration factors for both oyster soft tissues and oyster shells over absolute sediment metal concentrations decreased between stations 3 and 1 (Station 3>Station 2>Station 1), Table 6. It would appear that where the absolute sediment metal concentrations are the highest, the oysters are able to concentrate the metals to a lesser extent. Perhaps the sediments are adsorbing more metals, making them less available to oysters.

TROPHIC CONCENTRATION

Crabs, Callinectes sapidus, and oysters, Crassostrea virginica, were both found at stations 1 and 3 in the estuary. All crab carapace concentrations of the three metals considered were significantly greater than all oyster shell concentrations of these metals at both stations. Oyster soft tissue contained significantly less chromium and lead than crab muscle tissue from both stations. Cadmium concentrations were significantly greater in oyster soft tissues at station 1 than were concentrations of this metal in crab muscle tissue. At station 3 no significant differences between crab muscle and oyster soft tissues existed concerning cadmium concentrations. Brooks and Rumsby (1964) found that cadmium concentrations were higher in oyster soft tissues than in clam soft tissues, however, no

Table 6. Concentration factors in Crassostrea virginica. Metal concentrations in shells and whole soft tissues (ug/g weight)/absolute metal concentrations in sediments (ug/g wet weight).

Tissue	Metal	Location		
		Station 1	Station 2	Station 3
Whole Soft Tissues	Cadmium	3.6	0.3	0.08
	Chromium	0.3	0.2	0.1
	Lead	0.07	0.02	0.01
Shell	Cadmium	2.4	0.2	0.08
	Chromium	1.0	0.8	0.5
	Lead	2.1	1.7	0.5

comparison was made with crab tissue concentrations of this metal. As previously mentioned, it is thought that cadmium may be a significant factor in oyster enzyme systems causing the elevated levels of this metal (Brooks and Rumsby 1964). Leland et al. (1974) found that cadmium levels seemed to be elevated in benthic organisms.

Crabs, C. sapidus and clams, Rangia cuneata, were both found at station 6. Crab carapace contained significantly greater concentrations of all three metals than did clam shell. Clam soft tissues contained significantly lower concentrations of cadmium and lead than crab muscle tissue, however chromium was found to be significantly more concentrated in clam soft tissues than in crab muscle tissues. Brooks and Rumsby (1964) attributed high chromium concentrations in clams to sediment particles still in the digestive tract when analyzed.

These findings seem to suggest a trophic concentration, since both R. cuneata and spat of C. virginica are known to be a major food source of C. sapidus (Tagatz 1969). Since concentrations of metals are generally higher in crab muscle tissue, as well as crab carapace, than in the bivalves considered, the process of absorption as well as adsorption seems to be occurring. These findings could possibly be attributable to a more efficient excretory mechanism for metals in the bivalves, however no literature has been encountered that would support this theory.

SYNERGISTIC EFFECTS OF METALS

Bryan (1971) has suggested that synergistic effects occur among metals which facilitate their uptake by aquatic organisms. In all crab tissues there was a significant correlation, all metals were mutually concentrated.

In oyster shell and soft tissues, chromium and lead exhibited a significant positive correlation in concentrations. Zook et al. (1976) found the same results for chromium and lead in Crassostrea virginica. It was not indicated that this was a synergistic effect, only that it was a metal-metal interaction. In clam tissues analyzed in the present study it was found that lead and cadmium have the same correlation.

When considering all of the above results, it is apparent that lead is one of the metals being found highly concentrated when other metals are highly concentrated in each of the species. Perhaps lead is a metal that facilitates the concentration of other metals by aquatic organisms. More research in the synergistic effects of metals is needed before any firm conclusions can be made.

CONCLUSIONS

1) Many estuarine organisms do not concentrate cadmium, chromium or lead uniformly in all anatomical body regions. Blue crabs, Callinectes sapidus, were found to concentrate all three of these metals in greater concentrations in the carapace than in the gill and muscle tissues, and to greater concentrations in gill tissue than in claw muscle tissue. The eastern oyster, Crassostrea virginica, was found to concentrate lead and chromium to greater concentrations in the shell than in whole soft tissues. Cadmium was found in slightly higher concentrations in oyster whole soft tissues than in the shells. Rangia cuneata, an estuarine clam, concentrated cadmium and lead to much greater levels in the shell than in whole soft tissues. Clam shell and whole soft tissues were found to have similar concentrations of chromium. These results seemed to be contingent upon the use of wet weight concentrations were used in all tissue determinations.

2) The extent to which each metal was concentrated in biological tissues, referred to as concentration orders in biological tissues, were compared to the concentration orders in water and sediments. It was felt that observance of similarities in concentration orders may aid in the determination of concentrating mechanisms. Concentration orders of chromium, cadmium and lead in the sediments were similar to concentration orders of these metals in crab carapace, and oyster and clam shells (Pb>Cr>Cd). These comparisons seemed to denote that shell and carapace tissues were indicative of long term conditions and/or an adsorptive concentrating

mechanism was being used. Concentration orders of the metals in water samples and in crab claw muscle and gill tissues were similar (Cr>Pb>Cd). Clam whole soft tissues had similar concentration orders, however cadmium and lead were found in concentrations too low to be determined as statistically significant. These findings suggest that soft tissues of clams and of the crabs seemed to be indicative of the short-term availability of these metals to biota and/or an absorptive concentration mechanism is at work in the soft tissues. It was expected that oyster soft tissues would provide similar results, however, they did not.

3) A trophic concentration of metals in the estuarine food chain seems to be evident. Crab carapace was found to contain cadmium, chromium, and lead in greater concentrations than did either oyster or clam shells. Metals were concentrated to higher levels in crab claw muscle than in clam and oyster whole soft tissues with the exceptions of cadmium in oysters and chromium in clams. Cadmium was more concentrated in oyster whole soft tissues and chromium was more concentrated in clam whole soft tissues than in crab claw muscle tissue.

4) Cadmium, chromium and lead were not found to be concentrated in a manner that was dependent on body weight in any of the animals tested.

5) In blue crabs, cadmium, chromium and lead seemed to be synergistically concentrated. In oysters, chromium and lead seemed to have synergistic concentrating properties. In clams, cadmium and lead were found to be mutually concentrated.

6) Concentrations of cadmium, chromium and lead, as monitored in the animals analyzed, seemed to increase south of Lake Charles in Prien Lake, eventually reaching highest concentrations in the main body of Lake

Calcasieu. The west cove of Lake Calcasieu and the area of the lake south of the old revetment are to some extent physically isolated from the rest of the lake. As a result concentrations of metals in the animals from these areas were found to be lower than in the main body of the lake.

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