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THE RELEASE OF CADMIUM, CHROMIUM, COPPER, NICKEL AND ZINC

BY SEWAGE SLUDGE AND THE SUBSEGUENT UPTAKE BY MEMBERS

OF A TURTLE GRASS (IHALASSIA TESTUDINUM) ECosysTEM

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CENTER FOR ENERGY AND ENVIRONMENT RESEARCH

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ABSTRACT

This research was initiated to determine the rates of uptake, by a

Thalassia testudinum ecosystem, of Cd, Cry Cu, Ni, Pb and Zn which were

Jeached from sewage sludge by seawater. The experimental design used aerated

flowing seawater (8.4% min!), which passed over 2 0.1 m? bed of sewage

sludge before traversing the model ecosystem. The tanks, both control and experimental, were 9.2 mx 0.9.x 1.1 min size with a volune of 3.1 x 10? Titers. Each tank contained sand to a depth of 0.5 m for a total volume of 4.2 m3, The experiment ran for 125 days from March, 1975 to July, 1975 and was duplicated from Decenber 1975 to April, 1976.

?The largest net uptake of metals occurred in the "fouling organisns* where Cd, Pb, and Zn uptake closely paralleled the net loss of metals from ?the sewage sludge. Thalassia Teaves showed a net uptake for Cr, Pb, Ni, and

In for both experiments. The urchin (Lytechinus

ariegatus) a herbivore on
Thalassia leaves also demonstrated a net uptake of Cu, Cr, Pb, Zn, and Ni in

both experiments. The sea cucumber (Holothuria wexicana), in both experinents

showed a net uptake of Cr, Cu, Pb, and Zn, Net uptake of metals in mangroves (anizophorae mangle) was Vimited to the roots. In the First experiment Ni and 2n showed a significant uptake whereas in the second experiment only Cr demonstrated a net uptake. The uptake in mangrove roots appeared to be @ direct function of netal concentration in the sediment. The lack of apparent

rretal uptake in the sediment, except for Ni, Pby and Zn in the second experent, was probably due to the sampling technique rather than to a lack of

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uptake of metals by the sediment,

io consistent or significant metal uptake was found for the clam (codokia orbicularis), oyster (Crassosteia rhizophorae) or the snail (Ner{ta tessplata).

The lack of sufficient sanple mass, for these organisns, probably obscured any net uptake that may have occurred.

The results indicate that the dumping of sewage sTudge in coastal tropteal waters can lead to the uptake and concentration of toxic trace metals

by menbers of a turtle grass community.

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Mean net uptake of Cr between experimental and control, Thalassia leaves and urchin guts = Experiment IT... settee eee

Mean net uptake of Pb between experimental and control, Thalassia Teaves and urchin guts - Experinent Te se ss nner

Mean net uptake of Pb between experimental and control, Thalassia Teaves and urchin guts ~ Experiment TI... se asters creat

Mean net uptake of Ni between experimental and control, Thalassia Teaves and urchin guts ~ Experiment Toe ee ee ete eee

Mean net uptake of Ni between experimental and ?control, Thalassia leaves and urchin guts ~ Experiment IT... ss

Mean net uptake of Zn between experimental and contro}, Thalassia Teaves and urchin guts = Experiment Te... :

Mean net uptake of Zn between experinental and control, Thalassia Teaves and urchin guts ~

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SECTION T

INTRODUCTION

The deposition of sewage sludge in the marine environnent may adversely affect the resident organisms in three ways:(1) Siltation with resultant death of sessile organisms. (2) Production of an anoxic environment due to ?the oxidation of the large organic Toad in the sludge.(3) fy the release of toxic materials into water in forms that may be incorporated into the organ-isms. These materials usually include metals (zinc, cadeium, chromium, nickel, lead and copper), pesticides or organohalogens and hydrocarbons of petroleum origin, The mass dumping of treated sewage sludge into oceans and shallow inshore areas is occurring in a number of areas. In the New York Bight, an exanple of an area receiving large quantities of solid waste for almost a century (Carmody et al., 1973). Carmody, Pearce, and Yasso (1973) determined that there was a definite increase in the sediment of Cr, Cu, Pb, Ni and Zn due to the dunping of sewage sludge. The concentration of Cr, Cu, Cd, Pb, Ni, and Zn in sewage sludge is markedly elevated over anbient marine water concentrations (Salotto, B.V. and Farrell, J.B., 1971; Jacobs, S.A., 1973). Therefore, the deposit of this sludge in the marine environment could result in the uptake and concentration within the food web. The uptake of metals by marine organisms and concentration within the food web has been demonstrated numerous times (Jacobs, S.A-, 1973; Kerfoot and Jacobs, 19732,

1973p; Kerfoot, W.B., 1973; Shuster and Pringle, 1969; Valiela et al, 1974).

The most extensive compilation of metal concentrations in the tissues

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of marine organisms is found in Vinogradov (1953) and Goldberg (1965). However, the results were usually based on very smal] sample sizes so it is very Sifficult to assess the background levels of metals in marine organisms. Eisler (1973) compiled an annotated bibliography on biological effects of metals on aquatic organisms. Montgomery et al. (1976) found that in order

to obtain a valid sanple size for trace metal determination in the thread Fin herring (Opisthonema oglinum (Le Sueur))a pooled sample size of from 50 to 124 fish was necessary in order to detect a 15 to 25 percent difference

4m two populations. Other researchers have shown the effect of various metals on the marine food web or individual organisms both in laboratory

nodels and field experiments (Phelps, 0.K. et al., 1975; Nair, K.V. et a

1973; Eisler, R. et al., 19725 Eisler, R., 19715 Gardner, G.R. and G. LaRoche, 1973; Jackim, E., 1973; Frazier, J.M., 1976; Ferrell, RE. et ali, 1973; Huggett, R.J-, 19735 Hannan, P.J. and C. Patouillet, 1972). Schroeder (1975) demonstrated, using radioactive isotopes, that Thalassia testudinum

would concentrate cobalt and manganese in the leaves rather than the root structure (excluding rhizomes). The incorporation of cations in Thalassia testudinum was primarily accomplished through the leaves (Schroeder, 1975) The use of sewage sludge to anend agricultural soils has been extensive-

iy studied (Street, J.J. et al., 1977; Silviera, 0.J. and L.E. Sormers, 1977

Turner, R.I

. et ali, 1976). However, very little has been published re-
garding the rates of release of potentially toxic trace metals into complex tropical ecosystems. The necessity for this type of controlled field experiments was explained by Menzel (1977) for the CEPEX experiment. Inherently, it is impossible to duplicate exactly the marine ecosysten due to its spatial

and tenporal dimensions. Also, the isolation of organisms from their surrounding environment produces results that are anbiguous. Therefore, it 1s



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unitkely that any ?artificial? systen can duplicate conditions in the natural

environment.

1t may not be necessary to duplicate in a laboratory the real system, but rather to produce system that can duplicate itself (Menzel, D.W.. 4077), white at the same tine attenpting to duplicate the gross physical marine parameters, such as light, suspended sediment Toad, salinity and tenperature. In order to do this, a flow-through seawater system with replicate experiments in different seasons is mandatory:

our objectives in this research were to use a flow-through system of suffictent size and complexity so as to nearly duplicate a tropical marine ?ecosystem and 2150 allow repeatability over varying seasons. This system would allow us to determine the rates of uptake, by a tropical marine community, of Cuy Cé, Cry Zn, Ni, and Pb which were Teached from sewage sludge ty seawater. This community was comprised of turtle grass (Thalassia testudinum) with its associated sedimental infauna, spiny sea urchin (Lytechinus variegatus), sea cucunber (Volothures mexicana), attached ?foul-{ing organisns", plankton, and the red mangrove (Rhizophorae mangle).

?This ecosystem was chosen for three reasons: (1) the system comprised a tightly bound conmunt ty with strong interaction between the menbers, (2). the important role of the seagrass/mangrove ecosystem in the overall

tropical near shore environment (Jones, J.A., 19685 Odum, H.

< et al, 19595

Golley, F. et al., 1962), (3) the possibility of the active concentration of these trace metals in the higher trophic Tevels with passage of the materjals through the food webs.

The results from this research will aid in forming a basis for determin

{ing the potential effects of leached toxicants from sewage sludge on a trop~ical marine ecosystem. These problems are especially critical in Puerto Rico.



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The population density on the island is nearly 2000 people per square kiTorreters about ten times that of the continental United States. Only @ few towns ond cities have sewage treatment facilities and most of the sewage is dumped, mininally treated, into the rivers and bays.

With the increased awareness of the potential detrimental effects of raw sewage on the health of the inhabitants and the marine ecosysten, the construction of sewage plants will increase. This increase of modern plants wi11 produce large anounts of sewage sludge. The disposal of this sludge wiNl pose an especially critical problem in the densely populated, nountainous ?sland of Puerto Rico as the avatlable land for sludge disposal is very

Vimited and disposal poses its own set of ecological problems at least as

rious as marine disposal of sludge. Therefore, the judicious selection of ocean dunp sites for sludge, if this alternative 1s chosen, will require estimates on the leaching rates of toxic substances from sewage sludge by seawater. These substances can enter the, food web and becone concentrated relative to the concentration in the water column. The toxicity to marine organisms of Cd, Pb, Cr, Ni, Zn and Cu has been denonstrated (Efsler, Rs 1971; Eisler, R. et al., 1972; Frazier, J.M., 1976; Gardner,G. and LaRoche 1973; Ikuta, K., 1968). It is also possible that some of the Teachable substances could promote growth in portions of the ecosystem i.e., vitamins. chelators, organic growth substances (Vallentyne, J.R., 1957). However, the potential possible benefits of sewage sludge leachate will not be examined ?in this research.

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SECTION 2

METHODS

The seawater system and tanks were located near Branadero Bay, approximately 8 km from Mayaguez, Puerto Rico (Fig. 1). Two tanks (9.2mx 0.9mx 1.1 m) were constructed fron 1.9 cn exterior grade plywood with § om x 10 em external framing (see Fig. 2). These tanks were sealed with two coats of.

?Fibreglass epoxy resin and leached in running seawater for 2 weeks. The tanks were located in an open structure. The roof of the structure was fabricated of galvanized corrugated roofing. The underside of the roof was covered with plastic sheeting to prevent zinc contantnat fon.

Each tank was filled with 4.2 m® of calcareous beach sané (7x CaCO) fron Aguada, Puerto Rico. The mean depth of sand was 0.5 m with an addition a1 0.1 m of sand over the last 3 m of tank, The tanks were then Filled with seanater to a mean depth of 0.5 m and flushed for an additional week.

The seawater for the system was pumped from the dock of the Center for Eneray and Enviroment Research at a depth of 3 meters. The punp was a Sears Dynaglas "np (230 volt). A plastic minnow trap was used as a course pre-filter for a Filterchem ® combination filter and foot valve at the pump inlet. ANI piping used was grey schedule 40 P.V.C. The water traversed 73 m to a charcoal, sand and gravel filter (0.5 x 0.5 x 1.0 m), then to a 1000 Miter settling tank. The settling tank was constructed of 1.9 em thick plywood



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Lined with two coats of fibreglass ® and epoxy resin. The water was then gravity feé to the two seawater tanks with the flow split using P.V.C. "1" Joints. The flowrate in each tank was monitored and maintained at 8.2 to 8.7 4 min"! by PVC ballvalves. The total water volume was 3.1 x 10° Titre. The turnover time was 5.9 hrs per tank. The inflowing water was diverted

below the surface by submerging the inflow end using a perforated inflow pipe (Fig. 2). The seawater was continuously aerated with an air pump and air stones.

Sewage Sludge

Activated sludge was shipped from New York City in 208 £ polyethylene ined drums, The sludge was allowed to settle and the overlying liquid de-canted. In general, sludge is 5x solids. The black, "oily" sludge sturry remaining was mixed ina large fibreglass tank. The sludge was added to 2

depth of 7.6 on in an area 1 mwide by 2.3 m long (0.1 m)at the incurrent end

of the Experimenta) tank which had previously been sealed off from the rest of the tank. A PVC frame with smal] mesh screen attached was placed over the sludge to prevent direct contamination of the organisms. A duplicate screen was placed in the control tank. After 24 hrs, when the sludge had settled, the seal was removed and Experiment I was begun. At days 1, 5, 25, 50, 85 (Experiment IT only) and 125, after the sludge was added to the Experimental tank, samples of organisms, sediment, sludge (day 1, 125 EXPI) and water were taken from both tanks. A presample was taken prior to ad-dition of any sludge, in this study it is referred to as day 0.

Samp1 ing

Organisms

?The initial stocking quantity or density for the biological organisms,

used in both Experiment I and II, are shown in Table 1.

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sable 1. Physical disensions of organtsns with nusber of total

Sryesises stucles per tank ane nuber of orgsnisrs

Soupied per tent per day.

exrenavenT 1

Experimental Tank ?control Tank

| ow emertacted a/Sanctet | L__ WH detected #/Serple

Clans a trata es a oe Wy

sip 736: 8 2

Soatts

oysters

eehins

avenue 21

Experinental Tank Controt

ON estoctes_e/Sarplet ML fstockes ?W/Saeplet

Cans He 6.6 EO i.e los WO

sive ?63 S38 H

Ses Cucumbers i 2

si ?

seats.

oysters

fvsraite ust tien on day 125 control 2s none Tet

supper values are for gays 0 through 50, Experiment 1

over values for day 125. savers refer to naer of

frgsncass in each pooled sample

The organisms, except for the clans, were all collected using manual

techniques at Pt. Osteones, Puerto Rico. The organisms were allowed to

?equilibrate for 2 weeks in the flowing seawater system. The turtle grass

was replanted manually at 400 plants m2, after soaking in a 10x NAPDH sol~

ution (Kelly et al., 1971). The clans were collected at Pt. Viento on the Southeast coast of Puerto Rico.

Mangroves in Experiment 1 (230 seedlings) were planted as ungerminated

seedlings and as germinated seedlings in Experiment I (120 seedlings).

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tysters in Experiment I, were placed in tanks and suspended in one plastic wesh bag per tank. In Experinent 11 the oysters were placed 13 per vag, for a total of 10 bags per tark. The Tocatton of the bags was random: jzed except no bag was hung directly over the senage sTudge deposit.

Table 2 is a species, common nane list of plants and aninals used for

this study.

TABLE 2

Sone ee

Clam rbiculartS

oyster Crassostria rhizophorae

snail Nerita tessplate

Sea cucumber Holothurea sp. (predominently #. mexicana

Urchin ytechinus variegatus

Turtle grass Thalassia testudinum

Red mangrove Rnizophorae mangle

On

After equilibration, organisms were sampled at the prescribed inter-

vais, the samples were renoved, physical measurenents made, placed in double bags and frozen. SimuTtaneously, samples were coltected for hydrocarbon analyses. The sanples for hydrocarbon analyses were placed in glass bottles with aluninun foil seals, capped and frozen. The bottles were previously rinsed with 2N HCL, defonized water, CCxq, and air dried. The physical din-censions for the animals sampled and nunber of aninals sampled per day are shown tn Table 1.

overlying Water

Water samples were taken, for Experinent I and IT, prior to biological

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samples. There were three types of water samples taken: pore water for anal-

ysis of soluble trace metals and hydrocarbons, overlying water for soluble and particulate trace metals and hydrocarbons, and dissolved reactive phosphate (DRP). The DRP was sampled at weekly intervals. The trace metals and hydrocarbons in water and pore water were sampled at the same intervals as the organisms. Pore water samples were not taken in Experiment 11

Pore Waters

Samples were taken using one inch PVC pipes sealed at one end with 0.8 mm holes drilled in the lower 38 om of the pipe. The pipes were placed 46 cm from the sides at 1.2, 3.5, 5.8, and 8.1 m from the intake end of the tanks. Two liter water samples were withéravm using 6.4 mp Tygon ® tubing and 25 Tbs of vacuum pressure, fron a laboratory vacuum pump.

Sediment,

Sediment was collected using a 2.5 cm diameter x 25 cm PVC core. The sediment core was homogenized and ground after drying (105°C) and a 1g samples taken for trace metals. After day 25 in Experiment I and throughout Experiment II, 25g sample was taken for trace metals.

Dissolved and particulate trace metals

AIL water samples were placed in 2.5 £ glass bottles (which formerly contained conc. HNO3 or HCt) which were equilibrated in reagent grade seawater for 12 hrs prior to sampling (4 & samples for Experiment 11). The

sample bottles were drained, rinsed with the sample, filled, then filtered within 4 hrs. The filtering was accomplished using an all plastic filter system containing a .4 ym pore size, 142 mm diameter, Getnan ® fi1ters. The

filters were previously washed in 6 NHC and rinsed with copious anounts of R.0./D.1. water. The fraction retained on the filter was referred to as the ?particulate? portion. The filtrate was the ?soluble? fraction.



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Physical and Chemical Analyses

Genera

ANI reagents used are reagent ACS grade. AIT glassware was cleaned with detergent, rinsed with water from a Millipore * »Mi115-9" systen. AN? water referred to in this paper is R.0./D.1. water from the Millipore R ?Mi111-Q" system. This refers to the technique of reverse osmosis followed by fon-exchange. The final cleaning for glassware used to digest samples for trace eto! analysis involved refluxing the covered beakers with concentrated #03 followed by rinsing with R.0./0.1 water. ANI other equipment was washed with detergent, rinsed with R.0./D.I. water, followed with 6N HCE and rinsed with .0./0.1. water. Only non-netallic instruments and materials were used and all equipment, after cleaning, was stored covered with Parafilm R or plastic bags.

Oxygen, temperature, and salinity

A Yellow Springs Internatfonal YSI - 57 oxygen/tenperature meter was used. The oxygen output was both salinity and temperature compensated, The cnygen calibration was checked using the Winkler titration technique (steickiand and Parsons, 1965).

Dissolved reactive phosphate

(ne hundred mt samples were collected and imediately filtered through a Millipore Swin-Loc R adaptor containing an acid washed (6N HCL) 27 mm HiNlipore, 0.45 m pore size Filter using an acid washed 100 nt. polypropylene syringe. The sample was frozen and Tater DRP was determined using the single solution technique (Strfcklend and Parsons, 1965) with @ 10 en cell in a Becknan DU spectrophotoneter. & smal? nunber of samples were run on

Technicon autoanalyzer using standard Technicon procedures (Zimmermann, C. et al., 197).



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Particulate trace metals,

The 142 om diameter filters were placed in Pyrex r beakers, 6 mt of con-centrated HNO3: HCL (3:1) were added and the beaker covered with a ribbed watch glass. The solution was heated to a slow boil (less than 95°C), when the fiTters dissolved, the Tiquid was brought to a rapid boil and taken to

near dryness. Then, 4 mi concentrated HNO3 were added, followed by dropwise addition of 1 mt of iz, (308 by volune). The sample was then rebotled to near dryness and 3 drops of concentrated lzS04 were added. If the sample turned blacks more Hg0 was added. The samples were dried until S03 fumes disappeared. The sample was then brought to 10 mt volume with R.0./0.1. weter.

Soluble Trace Heta!

After 2.56 of sample was filtered for determination of ?particulate? trace metals (41 in Experiment IT), the effluent was run through two 1.2 cm diameter x 10 cn glass columns. The flow rate was maintained at 1 mi min?! cn?, The columns were in series and the first colunn contained 10 mt of chetex-100 "in the anmonium form (20 mt in Experiment I1) with 20 mt of Anver1ite xA0-2" resin (Rohm and Haas) in the second column. The Chelex resin was purified by 2 bed volumes (SV) of INHCE ({sothermally distilled) followed by 5 BV of D.1./R.0. water and then 2 BY TN NHgOH (isothermally distilled) with a final wash of D.1./R.0. water. The Anberlite XAD-2 resin was cleaned by washing for 10 minutes with 0.1./D.0. water. The water was decanted and the procedure repeated 5 times. The resin was washed four ?times with anhydrous methanol followed by four washes with 0.1./0.0. water. The Chelex resin was left covered with water prior to use. The Anberlite

was stored under methanol until just prior to use.

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In Experiment I and IT, the metals were extracted from the Chelex-100 ® resin after the resin colunn was first washed with 100 mt of water. The ?trace metals, in EXP I, were eluted with 40 mt of boiling concentrated HCL. The resin was rinsed with 20 mt of water and this water was combined with the acid eluate. The eluate was botled down (95°C) to 1 mt and then brought to 10 me volume with water. This was referred to as the soluble inorganically complexed fraction. In Experiment I1, the Chelex 100 was eluted batchwise Using 6 mt concentrated HNO3 per 10 mt resin (Davey, E.W. and A.E. Soper, 1975) rather than eluting with concentrated HCL. The Chelex resin was filtered and the acid eluate dried to 1 mt followed by the addition of 6 mi 2NNCR (isothermally distilled) and dried down to 1 mt. This was repeated and the final product was diluted to 10 mt with distilled water. The anbertite ® resin was first washed with 100 mi water then the resin was placed in an open glass Petri dish, rinsed with methanol, and the resin jgnited. The ignited resin wes placed in a muffle oven and the tenperature raised in 100°C increnents until 400°C. The resin was maintained at 400% for 1 additional hour then the tenperature was raised to 450°C for 24 hours. The cooled resin was dissolved in § mt INHCL, then boiled down to 1 mt and brought to the desired final volume. This fraction was referred to as the soluble, organically complexed trace metal (Montgomery, J.R. and J.E. Echevarria, 1975). The trace metals were determined by atomic absorption spectrophotonetry (A.A.S.) on a Perkin-flner Model 303 A.A.S. with a

deuterium background corrector. The output was recorded using a Model 56 Perkin-Elmer recorder and DCR-1 digital readout. The flow chart for sample collection and processing 1s shown in Fig. 3.

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Analysis of National Bureau of Standards (NBS) bovine liver and orchard eaves were performed along with the regular samples. One gran samples were used, when available, for all standards and samples. The flow chart for sample coltection 4s shown in Figure 3.

The number of organtsns sampled per day per experiment are shown in Table 1. The samples were defrosted after collection and dissected into the foVlowing tissues using Pyrex glass shards and plastic gloves.

Urchins = shell and internal tissues.

Sea cucumbers - body, muscle bands and internal tissues. In Experiment TL, muscle bands were combined with internal tissues

Snails, clans, oysters - shell and internal organs.

Fouling organisms - total mass scraped fron glass collecting plates (23 x 30 cn). In Experiment 1, all plates were sampled at each sanpling

tine, In Experiment 11, cunulative results were obtained by sanpling

plates which had remained in the tanks for 25,50,85,125 days after

additon of studge.

Thalass'

= Roots and leaves. The roots and rhizones were combined

and are referred to as roots.

Mangroves - The lower 3.8 cm of the hypocoty! not including the roots.

This ?is referred to in this paper as the botton, Growing stem includes

a] of the primary meristanatic tissue from the hypocotyl excluding the

eaves and the roots/rhizones.

After dissection into these various parts, the wet weight was obtained.

The samples were dried in covered, cleaned Pyrex beakers at 105*C. The dried

sample was weighed, then homogenized, using a ceramic mortar and pestle.



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TABLE 3

Results in ug g"! dry weight for determination of metals

in NBS bovine liver and orchard leaf standards. The re-

sults for Experinent I and II are shown with statistics

and our computed minimum detectable concentration (MDC).

EXPERIMENT 1

Bovine Liver

ca cu cr Pb In Ni

oc 0.3 8.9 3.4 0.5 28 09

R 0.56 7 2.5 132 -

so 0.26 20.2 98 a7

N 9 10 10 i

EXPERIMENT IT

co cu cr Pb an Ni

x 0.55 187.8 WC OTL.

so 0.45 15.50 17.59 0.69

N 49 4a 53 48

Nes 0.27 4.04 193810 = 0.383 0.08 1304 10

values

EXPERIMENT I

Orchard Leaves

i 0c 2.300 26 44.8 26.1 3.9

3.9 (0632.82 54 0.6

n 6 6 n 3

EXPERIMENT 11

Orchard Leaves

x 0.66 u - 5.0 25.6 4.0

so 0.34 15 3.6 7.64 13

N 7. 20 23 26 24

fos COMER EET (ES) BET ET TST Oe

Va

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The ground sample, not used for analysis, was stored in plastic, screw cap potties. The dried samples were placed in cleaned Pyrex beakers, at Teast 1g where possible, and were digested using the following procedure.

Twenty-five mi of a concentrated lNOg: HCE mixture (3:1) was added to the dried, weighed sample in the covered clean beaker, The sample wes slow ly boiled and refluxed in the covered beaker at 90 to 95°C untiT 1 mt of solution renained. The sample was allowed to cool then 30 mt of 30% Hy0) was added Slowly and reheated to 90-95°C until 1 mt of the solution remained.

A further 10 mt of HNO3:HCE mixture was added and reheated. The mixture was alowed to cool and 10 mt of 30% Hp02 was added. This was allowed to boi again (90-95°C). The 10 mi additions were repeated, if necessary, a maximum of three times, Six mt of 2N HCE were added, boiled at 90-95°C, until 3 mt vere left. The sample was allowed to cool then centrifuged in a clean 50 mt

plastic capped centrifuged tubes at 1700 rpm for 20 minutes. The liquid was carefully decanted and brought to 10.0 mt with R.0./D.1. water. This sanple

was placed in clean, 25 mt polyethylene vials with caps.



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SECTION 3

RESULTS

The raw data, expressed as ug metal/g dry weight for al? samples, are found in Appendix A. The conputed net uptake for each metal is located in Appendix B and 1s expressed as mean experimental results (9/9 dry weight) rninus the mean control results for each sample, with their respective stan= dard deviations, and degree of freedom. The ?t" value for the null hypothe~ sis, Hot Mj-Mp = 0 (P<0.06) where My 4s experimental and N is control is, aso shown in Appendix 8. The analyses for the NES biological standards are shown in Table 3. The results for all fractions of dissolved and particulate trace metals in the water for both control and experimental tanks (Experinent 11) are in Appendix C. The wet/dry weight ratios with statistics for al the sarples in Experinent I and 11 are in Appendix 0.

?The experinents were replicated, Experiment I began on 11 March, 1975 and ended 15 July 1975; Experiment 11 began on Decenber 12, 1975 and ended Aprit 26, 1976. The experinental tank vefers to the tank with the sludge

added and the contro! tank was identical, in experimental design, to the experinental tank except no sludge was added.

Temperature, salinity, and oxygen

In Experiment I the temperature ranged from 21 to 26°C with a salinity range from 32 to 34 °/oo. In Experiment II the temperature ranged from 21 to 26.5 with a salinity range from 28 to 35 %00 (¥, 30 °/00, $.0. 4.2 °/00. N= 18).



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Dissolved reactive phosphate (DRP)

The results are shown in Figs. 4 and § for control, Experiment 1 and 1! and experimental, Experiment I and 11, respectively. The plotted points are the mean values for a five day period.

Fouling Organisms

tn Experiment 1. Cé, Cu, Pb, and Zn showed a significant net uptake after 125 days (Figs. 6, 8 9, 11), whereas in Experiment II, Pb (Fig. 10) showed a steady uptake to the end of the sampling period (85 days). For Experiment II, Cé and Ni denonstrated a steady uptake until day 50 (Figs. 7 and 13) then a decline in the net uptake. Zinc and chrontum indicated a net

uptake after day 50 (Figs. 12 and 15) and Cu shoved 1ittle significant up-
take for the sampling period (Appendix B).

The initial values for trace metals in sewage sludge are shown sn
able 4.

A rapid decline was noted in the concentration of C#, Cry Cu, Ni. and
In in the sewage sludge for Experiment I (Figs. 6, 15, 8, 14, 11). The Pb
results are inconclusive for both experiments (Fig. 9 and Appendix B) due
to the Toss of lead as the easily volatilized PbS04 during the digestion of
the sludge. Because of the shorter sampling interval for sTudge in Expert=
went 11, a tho stage decline for Cd, Ni, and Zn was shown (Figs. 7. 13, 12).
?he first decrease in metal concentration was nearly instantaneous with a

further, smaller decrease after day 50.

Thalassia

Leaves

The results of EXP I were biased due to the ?die off? of the plants by

---Page Break---

day 50. This was probably due to insuffictent light. The plants survived for the full 125 day period in Experiment II after the Tight Tevel was increased.

Copper, Crs Pb, Nf and Zn showed rapid increases in net uptake in Experiment I (Figs. 16,18,20,22,24). In Experiment II the same overall trend of increasing net uptake was shown for Cu, Cr, Pb, and Cd for Experiment 11 (Figs. 17,19,21,26). Mekel and zinc, in Experiment 11 did not show @ significant uptake up to day 50 but the net uptake was signficant at day 85 and 125 (Figs. 23, 25).

Roots

The only significant net uptake of trace metals for Thalassia roots and rhizomes in Experiment Iwas for nickel, Cr, Cu, and Pb (Figs. 27.29.31, 33).

The repeat experiment also showed a signficant net uptake for Cr and In (Figs. 30, 36) with Pb, Ni, and Cu showing peaks of significant net uptake values at day 5 and/or 25 days (Figs. 34,28,32). Cadmium uptake was significant only at day 25 (Fig. 37)

Urchin, internal organs

To facilitate later discussion, the results of the net uptake for urchins

are shown with the results for Thalassia leaves.

Copper, Cry and Pb show a steady and rapid increase in net uptake for EXP I and 11 (Figs. 16,17,18,19,20,21). The net uptake for Cd was not significant in Experiment I (Appendix 8), however in Experiment II (Fig. 26) ?the net uptake increased after day 50. The results for nickel and Zn uptake are very similar in Experiment I and 11 (Figs. 22,23,24,25,26). Zinc shows

a steady increase with a decrease,in net uptake,at day 50 followed by

v

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rapid increase in both Experiment I and 11

Sea cucumber, internal organs

In both Experiment I and II, Cr, Cu, Pb and Zn showed very similar net uptakes over the 125 day period (Figs. 38,39,40,41,42,43). Ni showed a net uptake spike at day 5 in Experiment 1,and in Experiment I! a spike showing uptake of nickel occurred at day 5, 50 and a smaller spike at day 125 (Figs. 38, 41).

Mangrove Roots

Nickel, in Experiment I, shows an initial decrease in net uptake before day 50 with a gradual increase to day 125 (Fig. 27). The opposite occurs in Experiment I1 where the net uptake increases rapidly up to day 5 (Appendix 8). The net uptake for Cr was not significant for Experiment 1 (Appendix 8), but indicated a linear net uptake from day 50 to day 125 for Experiment 11 (Fig. 30). Lead uptake in Experiment I and 11 (Figs. 33 and 34) exhibits similar trends. However, Pb. uptake is much higher in Experi~

ment 1 (Fig. 33). Zinc, in Experiment 1,

1s0 demonstrated @ rapid linear

uptake curve (Fig. 35) which was very similar to the uptake in Experiment IT (Fig. 36). However, the net uptake was greater for 2n in Experiment 1. The net uptake for Cu was not significant in Experiment 1 (Appendix 8), but a

peared to show a gradual uptake after day 50 followed by a decline to origin-at levels (Fig. 32).

other

No stontficant net uptake was shown for the oysters, clams, mangrove body, stem or leaves, snaTs, holothurian body and muscle parts or sediment

for Experiment I or II. The results shown are in Appendix 8. The significant results for net uptake of metals are shown in Figs. 6-43 and are indicated by

open rectangles. Dark rectangles indicate non-significant results.



---Page Break---

TABLE 4.

Concentration of C8, Cry Cus Niy Pb and 2n in uo/9 dry weight, in sewage sludge used in this expert nent. The results are for Experiment 1 and It. Results for the sane metals as determined by Salotto and Farrel) (1971) for activated sludge

are included.

Experiment

cé cr cu Poe Ni Zn

x 109 2120. 2860. 603192926

SD 6.70 115. 124.0 5511.3 388.4

Experiment 11

C4 Gr cu Poe Wi Fy

% 166. 36672445. 21.0 376, 2508

SD 85.5 338.5 266.4 © 3.29 12.7 122.1

Salotto and Farrel] (1971)

ctr cu POF ONE Zn

350 4330 11001500 3803300

Lead values are low due to digestion technique:

rt

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Addition of sewage

Collection of sedinent/ Collection of water

Sludge/organtsms (0,1, samples for trace

5,25,50,85%, 125 days) metal and hydro-

carbon analysis,

{ES

sample taken*

Frozen in plastic bags for analysis Filtered

of hydrocarbons

Defrost Frozen

sample for* hydro- R

Dissection carbon analysis Anberlite XAD-2

resin columns and

Sample pooted by tissue Extracted with CCL, Chelex-100 * resin

??J Extract stored in * colunn

?Subsample* freezer |.

mercury

analysis Elution, dilution

wet weight |

o Analysis, atomic

Dry Toss absorption

Dry weight

Grind by

mortar/pestle

Digest/dtution

1

Analysis,

atomic absorption

samples were collected at 0,1,5,25,50,85** and 125 days after addition of

sewage sludge.

?Samples for mercury, and hydrocarbons in both water, sedinent, sludge and organisns were stored frozen for future analyses.

san additional sampling was made for Experiment II, Day 0 refers to the san~ ple taken before addition of sewage sludge.

FIGURE 3. Flow chart of trace metal and hydrocarbon sampling from collection processing into final sample form.



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SECTION 4

piscussron

oysters and clans have been extensively studied regarding trace metal uptake because of their comercial significance, hardiness and feeding rethod. Bivalves, in general, show uptake and concentration of most trace retals (Kerfoot, W.B., 19733 Kerfoot, W.B. and S.A. Jacobs, 1973 a and bs Shuster, C.N. and B.H. Pringle, 1969; Huggett, RJ. et al., 19735 and Frazier, J.N., 1976). However, oysters and clans in our research showed no consistent nor significant net uptake of metal except possibly zinc and

copper. In Experiment I

ail the oysters were placed in a single net bag.

This procedure was changed to ten net bags per tank in Experiment IT. This drastically reduced the crowding and increased the survival of the oysters beyond day 50 in Experiment IT. The wet weight of the clam and oyster internal organs was too smal} to allow an adequate sample size for the trace etal analyses. This sane problem was encountered in the analyses for the snails, Holothurtan muscle parts in Experinent 1, and zooplankton samples for both Experiment I and 11.

?The results for 1ead in the sewage sludge were very low compared to other investigators (Table 4) and 4s due to the addition of HS0q to aid in the digestion of the sludge organic matter. When the sanple was heated, the lead was Tost as volatile PbSOg. The HyS04 was only used for the digestion of the sludge.

The results for the analyses on the NBS bovine liver standards (Table 3)

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fare in good agreement for Cu and Zn and high for Cd. Our results for Cr ?and Pb in bovine Tiver were below our detection limits. The results for NBS orchard Teaves were in good agreement for Cu, Pb and Zn. Cadmium, in NBS orchard leaves, was too high as was nickel. The coefficient of varia

tion (CV) for Ni was Tower than the CV for Cd. The results for Cr, in orchard Veaves, were in good agreenent with the non-certified value for Cr published by NBS.

Our results for the concentration of trace metals in water for Experihment I were at or below the detection limits for nearly all metals and were therefore not shown. This was due to the 2.5 2 sample size. When we used 4£ sample in Experiment II, the results for most of the metals were within our detection limits. However, no significant difference was noted between etal concentrations in the experimental tank and those in the control tank. Obviously, metals were released from the sludge, as shown by the results in Figs. 6 through 15 and, also, by the significant net uptake of metals by Thalassia leaves and roots, urchin and sea cucumber internal parts, and fouling organisms. This net uptake in organisms was hard to explain when no net change was seen in the water of the experimental tank. The problem in interpretation of the water results was due to the ?point sampling? of ?the water versus the "integrated" sampling for the organisms, sediment, and sludge. The ?point? concentration of the water was very low at any one time (Appendix C). These trace concentrations, coupled with the high blanks and matrix problens in trace metal analyses of seawater by flane atomic absorption spectrophotonetry, produced results with very high within-sample variance. This high variance effectively obscured any differences between

?the experimental and control tanks in Experiment II. Schuster and Pringle

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(1969) demonstrated that the concentration of metal in the water, at any one time, may not be the best indicator of the total contamination by metals.

?The net uptake of metal by "fouling organisms" was clearly shown in both Experiment I and II. This net uptake was replicated in Experiment I and IT for Cd, Pb, and Zn. The uptake of copper by fouling organisms was only significant in Experiment I and for Ni and Cr in Experiment Il. The fact that Ni and Cr didn't show a net uptake in Experiment I was probably due to 2 change in sample technique for fouling organisms initiated in Experinent 11. In Experiment 1, when a settling plate was sampled for fouling organisms, clean plates were added for the next sampling period, whereas, in Experiment IL, a plate which was sampled at day 125 had remained in the system from day 0 to day 125. The fouling organisms sampled in Experiment II are, therefore, integrated samples over the sampling time. The net uptake by ?the fouling organisms of Cd, Pb, and Zn closely follows the loss of these same metals fron the sewage sludge for both Experiment I and If. This same correlation, between fouling organisms and sludge, holds true for Cd and Ni uptake in Experiment I.

?The net uptake by Thalassia leaves and urchin internal organs closely parallel each other for Cu, Cr, Pb, Ni and Zn in both Experiment I and 11. Significant cadnium uptake by Thalassia leaves and urchin internal organs was seen only in Experiment II. Schroeder (1975), using radioactive 57co, 60¢0, S4¥n and ©2n in seawater, showed that, in Thalassia testudinun, the

rhizomes had the highest uptake followed by leaves then roots. In both Experiments our results for Thalassia, show significant net uptake for Cu,

Cr, Ni and Pb in roots. The net uptake was higher in Experiment I for Pb



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land Cu and higher in Experiment 11 for Ni and Cr. Cadwiun and zinc had a net uptake in Thalassia roots only in Experiment I1, This uptake of metals by the urchin and the close correlation between the urchin's uptake and the uptake in Thalassia leaves was not surprising as the urchins were voracious foragers on Thalassia leaves and epiphytes. This was also shown in the field by Camp et al., (1973). The net uptake of some metals in leaves was closely coupled to the uptake in the roots of Thalassia. The results for Cd, Ni, and Cr in Experiment II indicate a translocation of these two metals from roots to leaves. The translocation of metal was also indicated for Zn ?in Experiment II from day 85, where the net uptake decreases in the roots with an increase in the leaves and vice versa at day 125. In general, the maximum net uptake in Thalassia leaves was slightly greater than the uptake in roots. This 1s in apparent contradiction of Schroeder's results (1975) where roots were found to have the greatest uptake. However, our root sanple consisted of roots plus rhizones and, as rhizomes showed the highest net uptake in Schroeder's paper (1975), the contraction between our results and Schroeder's 1s cleared up.

?The interpretation of the significant net uptake for mangrove roots indicates that only Ni and Zn in Experiment I show a near linear increase over time, In Experiment 11, only Cr showed a linear response over time, ?whereas, Zn and Cu Showed an increase in net uptake followed by attainment, of apparent equilibrium then Toss of the metal. Lead shows no net uptake until day 85, These results appear to be loosely correlated with the fact that the sediment never showed a net uptake unti} at day 8 in Experiment I] ?when Ni, Pb and Zn begin to show a slight net uptake (Appendix 6) in the

sediment. Since no other part of the mangroves sampled indicated a signifi-



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cant uptake of metal (Appendix 8), then the only source for the metals in ?the mangrove roots must be the sediments. If that is so, then only Ni, Pb ?and Zn should show net uptake in mangrove roots and then only after day 85 in Experiment II, This lack of significant uptake of metals by the sediments could be due to the pooling of whole sediment samples from a 2.5 cm diam. x 25 cm core. If the gradient of trace metal concentration in the sediment was very steep, then the pooling of the core could have diluted the higher surface metal concentrations with the much lower metal concentrations in the bottom of the core.

There was similarities in net uptake by Holothurian guts for Experiments I and 11. Although there are similar patterns of uptake in the Holothurian

gut, the maximum values for net yptake were always higher in Experiment 1 for Ni, Cu, Pb and Zn. Chromium showed a higher maximum net uptake in Exper iment II for Holothurian guts. In Experiment IT, the sewage sludge showed a two stage release. The first stage occurred at day 1 followed by @ slow constant release until day 50 when another rapid release occurred. A slow but steady release would more likely result ina higher maximum uptake, whereas, a two stage release, as shown by the sewage sludge in Experiment II would, due to the estimated 6 hour residence time for the water in the tanks and 8.5 2 min"! flow rate, produce a lower maximum net uptake. The data for the net loss of metals from the sewage sludge was only available for two sampling times in Experiment I, so it is difficult to determine the true in-stantaneous net loss of metals from the sludge.

The original objectives for this research, as stated in the Introduction, have been obtained. A definite net uptake was found in members of a tropical

marine comunity. These results were replicated at two different seasons

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ang the results, considering the complexity of the system, agree quite well. Originally, the fish, Diodon helacanthus (puffer) mas to be added to the system. However, after collecting over 360 specimens we found that they were too sensitive to environmental factors other than sewage sludge and were easily killed. Therefore, fish were deleted from the ecosystem in

order to expedite the completion of the experiment.

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SECTION 5

CONCLUSIONS AND RECOMMENDATIONS

Net uptake of trace metals by menbers of a Thalas

cia testudinun/

Rhizophorae mangle community was demonstrated in two 125 day replicated experinents over the period from March 1975 to April 1976. The net uptake was especially significant in: Thalassia testudinum leaves and roots/

rhizomes, internal organs of the urchin Lytechinus variegatus, "fouling

organisms", internal organs of Holothures sp., and for some metals in Rhizophorae mangle roots. In general, the net uptake pathways followed the trophic Tevels in the food webs. This pathway was especially noticeable

?from the water to the fouling organisms and fron the water to the Thalassia testudinum Teaves and thence to the urchin herbivore Lytechinus varieaatus. The net uptake in the Holothurea sp. were closely related to the net uptake in the fouling organisms and the trace metal loss rate of the sludge. The food web in this artificial systen wes doninated by the fouling organisms as would be expected. The uptake of trace metals, leached from the sludge, was always greatest in this complex group.

The results of Tong term exposure, in a simulated tropical ecosystem, to toxic trace metals more closely approximates the situation in nature then laboratory experiments. In this respect we are in agreement with the state-rent by Phelps et al. (1975) "Chronic exposures to elevated metal Tevels through the food web should be used as a nore realistic test for possible deleterious effects from such metals as Cr. No artificial system can ever

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reproduce a natural ecosysten. However, it is more important to closely approximate the natural system with a system which can duplicate itself (Wenzel, 0.M. 1977). Our results show that chronic exposure to toxic metals ?can lead to uptake and concentration of these metals in marine organisms. We were able to duplicate our results using this complex simulation of a marine ecosysten.

Nore detailed work on the net uptake of toxic metals, using this conplex

simulated ecosystem, and the roles of the ?fouling organisms" and plankton {in this systen 1s needed. This must involve integrated sanpling of the organisns as well as the water. The advent of more precise methods of direct trace metal determination in seawater using anodic stripping voltammetry and ronflane atomic absorption spectrophotonetry, in Meu of flane AAS, should ?allow a reasonable sanple size for both organisms and water.

The authors would not recomend, based on the results of this research, the dumping of sevage sludge in shallow, tropical marine environments. The potential exists for rapid concentration of toxic trace metals by menbers of the food web high on the trophic scale. These toxic levels could then affect organisns used as food by man.

The next logical step in future research would be to add vertebrate organisms to this systen to see if a herbivore, which normally consumes, turtle grass, would show a net uptake of toxic trace metals.



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Figure 1. Study site location

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Figure 2. Diagram of experimental design, tank

configuation, and seawater systen

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Addition of sewage

CoNtection of sedinent/

Sludge/organisms (0,1,

5,25,50,85°%, 125 days)

sample taken*

Frozen in plastic bags

Defrost,

Dissection

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Sample pooled by tissue

Subsanpie

morcury

analysis

wet weight,

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Dry 105°C

Dry weight

Grind by

mortar/pestle

Digest/ditution

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Analysis.

atomic absorption

for analysis

of hydrocarbons

sample for* hydro~

carbon analysis

Extracted with CCL,

Extract stored in

freezer

Collection of water

samples for trace

metal and hydro~

carbon analysis,

Filtered

Frozen

os

Anberlite XAD-2

resin columns and

Chelex-100 R

see

Elution, dilution

Analysis, atomic

absorption

Samples were collected at 0,1,5,25,50,85** and 128 days after addition of

Sewage sludge.

?Samples for wercury, and hydrocarbons in both water, sedinent, sludge and

forganisns were stored frozen for future analyses.

?*#An additional sampling was made for Experiment 11, Day 0 refers to the san-

ple taken before addition of sewage sludge.

FIGURE 3. Flow chart of trace metal end hydrocarbon sampling from collection

processing into final saople form.

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Figure 4.

ORR. CONTROL Exe. 1

AIG-AT/L

ORP- CONTROL Exe. 2

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Dissolved reactive phosphate concentration Experinent I and I],

control. Data points are mean values for 5 cay period,

excluding day 1.

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Figure 5.

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AIG-RT/L

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AIG-AT/L.

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EXPERIMENTAL Exe 1

EXPERIMENTAL Exe. 2

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Dissolved reactive phosphate concentration Experiment I and I,

experimental. Data points are mean values for 5 day period,

excluding day 1.

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<o SEWAGE- Exe. |

NERN PPM

Figure 6. Mean net uptake of Cd between experimental and control, fouling organisms and sewage - Experiment I.



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MERN PPM

Figure 7. Mean net uptake of Cé between experimental and control, fouling organisms and sewage - Experiment 1.

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Figure 8.

<U FOULING ORGANISMS- Exe. 1

MERN (E-C> / PPM

<u SewAGe- exe. |

MERN PPM

Hean net uptake of Cu between experinenta} and con

organisms and sewage - Experiment I. eral touting

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Pe FOULING ORGANI SHS.

MERN (E-C) /PPH.

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Figure 9. Mean net uptake of Pb between experimental and control, fouling

organiss and sewage - Experiment 1



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MERN (E-C) /PPM

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Figure 11. Mean net uptake of Zn between experimental and contro}, fouling

?organisms and sewage - Experiment 1.



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ZN FRULING ORGANISMS? Exe. 2

MERN (E-?) /PPM

ZN SEWRGE- Exe. 2

MERN PPM

Figure 12. Mean net uptake of Zn between experimental and control, fouling

organisns and sewage - Experiment IT.



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Figure 13.

MERN <E-¢) PPM

Ni Sewee- exe. 2

Mean net uptake of Ni between experimental and control, fouling organisms and sewage ~ Experiment II.

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MERN PH

MERN PPM

Figure 14.

Mean net uptake of Cr and Ni between experinental

and control, sewage ~ Experiment I.

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MEAN CE-C) PPM

NERN PPM

Figure 15. Mean net uptake of Cr between experimental and control,

fouling organisms and sewage - Experiment 11

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cu URCHIN GUT- Exe.

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Figure 16.

Mean net uptake of Cu between experimental and controls

Thalassia leaves and urchin guts - Experinent 1.

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Figure 17.

CU THRLASSIA LERVES- Exe. 2

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Mean net uptake of Cu between experimental and control ,

Thalassia leaves and urchin guts - Experiment II

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Figure 18.

MERN ¢E-C) / PPM

eR URCHIN GUT- EXP. 1

> /PPM

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NERN «

Mean net uptake of Cr between experimental and control,

Thalassia leaves and urchin guts - Experiment 1.

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?R THRLASSIE LERVES- Exe. 2

MERN CEC) PPM

MERN (E-C) PPM

Figure 19. Mean net uptake of Cr between experinental and control,

Thalassia leaves and urchin guts ~ Experinent. 11.

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Figure 20.

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Hean net uptake of Pb between experimental and controls

Thalassia leaves and urchin guts - Experiment 1.

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NERN (E-?) /PPH

Figure 21. Mean net uptake of Pb between experimental and control,

Thalassia leaves and urchin guts ~ Experiment 11.



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Figure 22.

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Ni URCHIN GUT- Exe. 1

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Mean net uptake of Ni between experimental and control,

Thalassia leaves and urchin guts = Experiment: I.

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Figure 23.

NIOTHICRSSIR Cemves. exe =

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MEAN (E-C) PPM

Mean net uptake of Ni between experimental and control ,

Thalassia leaves and urchin guts = Experinent I]

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Figure 24. Mean net uptake of 2n between experimental and control.

?Thalassia leaves and urchin guts - Experinent I.



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??2m URCHIN GUT- Exe. 2

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Figure 25. Mean net uptake of 2n between experinenta? and control.

Thalassia leaves and urchin guts ~ Experinent 11.



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Figure 26. Mean net uptake of Cd between experimental and control,

Thalassia leaves and urchin guts - Experiment 1

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Figure 27. Mean net uptake of Ni between experimental and control,

Thalassia roots and mangrove roots ~ Experiment I.

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Nl THALASSIA RODTS? EXP 2

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Figure 28. Hean net uptate of Ni between experimental and control.

Thalassia roots and mangrove roots - Experiment 11.

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Days

Fioure 30. Heap net uptake of Cr between experimental and control,

Thalassia roots and mangrove roots - Experiment {1

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+1 quauysadxg - s7004 eLssejeys *{0s3u02 pue eqUaWladxa uaaMaq ng so ayeydn yOu UE ?Le 24nd

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Figure 32.

cu MANGREVE ROOT- Exe. 2

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cu THRURSS I ROOTS? EXP. 2

MEAN ¢E-C) / PPM

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Hean net uptake of Cu between experimental and control,

Thalassia roots and mangrove roots - Experiment 17

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Figure 34. Mean net uptake of Pb between experimental and control,

Thelassia roots and mangrove roots - Experiment I!



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MERN (E-C) /PPM

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gn THALASSIA ROOTS EXP 2

MEAN ¢E-C) PPI

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Figure 36. Mean net uptake of Zn between experimental 200 control.

wean re ta roots and mangrove roots - Experiment TT.

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NERN CE-C) PEM

NI MDLOTHURIAN GUT- EXP. 1

HERN <E-C) PPM

Figure 38. Mean net uptake of Cr and Ni between experimental and

control, sea cucumber guts - Experiment I



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Figure 39.

?o HOLOTHURIAN SUT- Exe. 2

MEAN (E-C> / PPM

MEAN «E-C) / PPM

Mean net uptake of Cd and Cr between experimental and

control, sea cucumber guts - Experiment 11.

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Figure 41. Mean net uptake of Cu and Ni between experimental and

contro}, sea cucumber guts ~ Experiment IT.



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Figure 43.

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Mean net uptake of Pb and Zn between experimental and

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Appendix A

Sorted Raw Data Experiment I and 11

There are eleven(11) columns shown which represent the following:

colunn

1 = Animal type

Clam

Holathurian

Nangrove

T= Thalassia

P = Fouling Organisms

Urchin

S = Sediment

2 - Tissue type

Muscle

internal contents (guts)

Shell

Body/sotton

Leaves

Roots and rhizomes

G = Upper Heristem

= Solids

3. Experiment munber ~ 1(1) or 11(2)

4 ~ Sample type - whether from experimental tank (0) or contro! tank (1)

5 = Represents sample time

0 = presanple

1 = 1 day after sewage added

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6 = Concentration (ug/g dry weight) Cd

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Multiple values for the same sample represent replicate samples. The value -999.9 is an indication of a value that could not be detected. All manipulation of the data used one half minimum detectable concentration (MDC) when a value was Tess than or equal to the MDC. The MOC values are listed inv the Results (Table 4).

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APPENDIX 8

Results for net uptake for Experiments I and IT

A Students t test (P<0.05) was eleulated for the null hypothesis:

Ho: uy * uz where uy 1s experimental and up ts control for each metal for each combination of Experiment I or Il, at each sample tine, with n-2 dearees of freedom shown (Deg). Also shown are the values of ? mean-

C mean), the individual means and standard deviations.



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[APPENDIX C

Experiment 11, Mater Data

The colum headings are defined as follows:

HC E is data from experimental tank

ch control .

METAL concentration (ug/2) shown are for the defined

metal, either Cd, Cr, Cu, Ni, Pb or Zn

3. MEAN PART values shown represent mean (19/2) concentration

for particulate fraction

SD PART represents the standard deviation of the mean

particulate function

5. MEAN SOC values shown represent mean concentration (19/2)

for the soluble organic fraction (Retained on

Anberlite resin)

6. sp soc represents the standard deviation of the mean

soluble organic fraction

7. MEAN SIC values shown represent the mean concentration

(ug/t) of the soluble inorganic fraction

(Retained on Cheiex-100 ® resin)

8. sp sic represents the standard deviation of the soluble

inorganic fraction



---Page Break---

MEAN SOC SD SOC MEAN SIC SD SIC

?SD PART

METAL MEAN PART

cd

ec

?8

cu

Ni

140

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[APPENDIX D

Wet/Ory Ratios

Wet/dry ratios are shown for each sample and tissue type for Experiments

Land 11. These values may be multiplied by the metal concentration (19/9

dry weight) values to obtain concentrations in ug/g wet weight.

441

---Page Break---

Mean Wet/Dry

deviation($b)

Clan

Gut

Shel

Sea cucumber

Body

Gut

Muscle

Urchin

Gut

She1t

Snail

Gut.

?She

oysters

Gut

Shev)

Thalassia

Leaves

root/

Rhizomes

"Fouling

organisms"

Senage

Sediment

Mangroves

Bottom of

hypocoty?

Meristem

above

hypocotyl

Leaves

Roots

10

Ratio (3) with n (nunber of sanples) and Standard

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78

5.2

14.8

14

33

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45

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0.60

1a

1.0

142

N x 8.0

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12 Va 0°00

12 45 0.40

13 10.6 3.20

Not determined, combined

with out

12 10.7 2.30

13 2.0 0°20

7 3.9 1.0

7 i 0:0

le nes 2.10

13 13 0:20

12 lee 2.90

3 9.9 22

7 5.0 1a

7 8.7 4a

13 14 0.10

7 3.4 0.60

7 4.0 0.40

5.3 1.3

5.6 0.50

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