"The Release of Cadmium, Chromium, Copper, Nickel, and Zinc by Sewage Sludge and the Subsequent Uptake by Members of a Turtle Grass (Thalassia testudinum) Ecosystem" by John R. Montgomery, Mary Price, John Thurston, Gina Laite de Castro, Luz Laida Cruz, and Dominica DeCaro Zimmerman. 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, Cr, Cu, Ni, Pb, and Zn, which were leached from sewage sludge by seawater. The experimental design used aerated flowing seawater (8.4% min), which passed over a 0.1 m^2 bed of sewage sludge before traversing the model ecosystem. The tanks, both control and experimental, were 9.2 m x 0.9 m x 1.1 m in size with a volume of 3.1 x 10^3 liters. Each tank contained sand to a depth of 0.5 m for a total volume of 4.2 m^3. The experiment ran for 125 days from March, 1975 to July, 1975 and was duplicated from December 1975 to April, 1976. The largest net uptake of metals occurred in the "fouling organisms" where Cd, Pb, and Zn uptake closely paralleled the net loss of metals from the sewage sludge. Thalassia leaves showed a net uptake for Cr, Pb, Ni, and In for both experiments. The urchin (Lytechinus variegatus), a herbivore on Thalassia leaves, also demonstrated a net uptake of Cu, Cr, Pb, Zn, and Ni in both experiments. The sea cucumber (Holothuria mexicana), in both experiments showed a net

Uptake of Cr, Cu, Pb, and Zn, net uptake of metals in mangroves (Anizophorae Mangle) was limited to the roots. In the first experiment, Ni and Zn showed a significant uptake whereas in the second experiment, only Cr demonstrated a net uptake. The uptake in mangrove roots appeared to be a direct function of metal concentration in the sediment. The lack of apparent metal uptake in the sediment, except for Ni, Pb and Zn in the second experiment, was probably due to the sampling technique rather than to a lack of uptake of metals by the sediment.

No consistent or significant metal uptake was found for the clam (Codokia Orbicularis), oyster (Crassostrea Rhizophorae), or the snail (Nerita Tessellata). The lack of sufficient sample mass for these organisms probably obscured any net uptake that may have occurred. The results indicate that the dumping of sewage sludge in coastal tropical waters can lead to the uptake and concentration of toxic trace metals by members of a turtle grass community.

CONTENTS

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SECTION I INTRODUCTION

The deposition of sewage sludge in the marine environment may

The research aimed to use a flow-through system of sufficient size and complexity so as to nearly replicate a tropical marine ecosystem and allow repeatability over varying seasons. This system would enable us to determine the rates of uptake, by a tropical marine community, of Cu, Ce, Cr, Zn, Ni, and Pb which were leached from sewage sludge by seawater. This community was comprised of turtle grass (Thalassia testudinum) with its associated sedimental infauna, spiny sea urchin (Lytechinus variegatus), sea cucumber (Holothuria mexicana), attached "fouling organisms", plankton, and the red mangrove (Rhizophora mangle).

This ecosystem was chosen for three reasons: (1) the system comprised a tightly bound community with strong interaction between the members, (2) the important role of the seagrass/mangrove ecosystem in the overall tropical nearshore environment (Jones, J.A., 1968; Odum, H., et al., 1959; Golley, F., et al., 1962), (3) the possibility of the active concentration of these trace metals in the higher trophic levels with passage of the materials through the food webs. The results from this research will aid in forming a basis for determining the potential effects of leached toxicants from sewage sludge on a tropical marine ecosystem. These problems are especially critical in Puerto Rico.

The population density on the island is nearly 2000 people per square kilometer, about ten times that of the continental United States. Only a few towns and cities have sewage treatment facilities and most of the sewage is dumped, minimally 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 ecosystem, the construction of sewage plants will increase. This increase of modern plants will produce large amounts of sewage sludge. The disposal of this sludge will pose an especially critical problem in the densely populated, mountainous island of Puerto Rico due to the limited available land.

For sludge disposal, options are very limited and disposal itself poses its own set of ecological problems, at least as serious as marine disposal of sludge. Therefore, the judicious selection of ocean dump sites for sludge, if this alternative is chosen, will require estimates on the leaching rates of toxic substances from sewage sludge by seawater. These substances can enter the food web and become concentrated relative to the concentration in the water column. The toxicity to marine organisms of Cd, Pb, Cr, Ni, Zn, and Cu has been demonstrated (Eisler, 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 leachable 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.

SECTION 2 METHODS

The seawater system and tanks were located near Branadero Bay, approximately 8 km from Mayaguez, Puerto Rico (Fig. 1). Two tanks (9.2m x 0.9m x 1.1m) were constructed from 1.9 cm exterior grade plywood with 5 cm x 10 cm external framing (see Fig. 2). These tanks were sealed with two coats of fiberglass 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 contamination. Each tank was filled with 4.2 m^3 of calcareous beach sand (70% $CaCO_3$) from Aguada, Puerto Rico. The mean depth of sand was 0.5 m with an additional 0.1 m of sand over the last 3 m of the tank. The tanks were then filled with seawater 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 Energy and Environment Research at a depth of 3 meters. The pump was a Sears Dynaglas pump (230 volt). A plastic minnow trap was used as

A course pre-filter for a Filterchem® combination filter and foot valve was used at the pump inlet. The ANI piping used was grey, schedule 40 P.V.C. The water traversed 73 meters to a charcoal, sand, and gravel filter (0.5 x 0.5 x 1.0 meters), then to a 1000 liter settling tank. The settling tank was constructed of 1.9 cm thick plywood.

The settling tank was lined with two coats of Fibreglass® and epoxy resin. The water was then gravity fed to two seawater tanks with the flow split using P.V.C. "T" joints. The flow rate in each tank was monitored and maintained at 8.2 to 8.7 liters per minute by PVC ball valves. The total water volume was 3100 liters. The turnover time was 5.9 hours 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 was shipped from New York City in 208-liter polyethylene lined drums. The sludge was allowed to settle and the overlying liquid decanted. In general, sludge is 5x solids. The black, "oily" sludge slurry remaining was mixed in a large fiberglass tank.

The sludge was added to a depth of 7.6 cm in an area 1 meter wide by 2.3 meters long (0.1 m) at the incurrent end of the experimental tank which had previously been sealed off from the rest of the tank. A PVC frame with a small 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 hours, when the sludge had settled, the seal was removed and Experiment I was begun. On days 1, 5, 25, 50, 85 (Experiment II only), and 125, after the sludge was added to the experimental tank, samples of organisms, sediment, sludge (day 1, 125 EXPER) and water were taken from both tanks.

A pre-sample was taken prior to the addition of any sludge, in this study, it is referred to as day 0.

Sampling Organisms: The initial stocking quantity or density for the biological organisms used in

both Experiment I and II are shown in Table 1.

Table 1. Physical dimensions of organisms with number of total synthesis studies per tank and number of organisms occupied per tent per day.

Experiment 1. Experimental Tank: Control Tank | Oil Extracted Sample | WH Detected Sample Count | Total Echinus and Oyster Samples: 736 | 82 Sea Cucumbers and Oysters: Experimental Tank Control on Day 21

Experiment 2. Experimental Tank: Control Tank | Oil Extracted Sample | WH Detected Sample Count | Total Echinus Samples: 6.6 | 63 Sea Cucumbers: 2 |

Note: Sea cucumber and oyster data are based on day 125 control. Supper values are for days 0 through 50, Experiment 1 over values for day 125.

Savers refer to the number of fragments in each pooled sample. The organisms, except for the clams, were all collected using manual techniques at Pt. Ostiones, 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 solution (Kelly et al., 1971).

The clams 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 2 (120 seedlings).

Oysters in Experiment 1, were placed in tanks and suspended in one plastic mesh bag per tank. In Experiment 2, the oysters were placed 13 per bag, for a total of 10 bags per tank. The location of the bags was randomized, except no bag was hung directly over the sewage sludge deposit.

Table 2 is a species, common name list of plants and animals used for this study.

TABLE 2
Clam - Rbiculartis
Oyster - Crassostria Rhizophorae
Snail - Nerita Tessellate
Sea Cucumber - Holothuria sp. (predominantly H. Mexicana)
Urchin - Lytechinus Variegatus
Turtle Grass - Thalassia Testudinum
Red Mangrove - Rhizophorae Mangle

After equilibration, organisms were sampled at the prescribed intervals, the samples were removed, physical measurements made, placed in double bags and frozen. Simultaneously, samples were collected for hydrocarbon analyses.

The samples for hydrocarbon analyses were placed in glass bottles with aluminum foil seals, capped and frozen. The bottles were previously rinsed with 2N HCL, deionized water, CCxq, and air-dried. The physical dimensions for the animals sampled and the number of animals sampled per

day are shown in Table 1. Overlying Water samples were taken for Experiment I and II, prior to biological samples. There were three types of water samples taken: pore water for analysis 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 II. Pore Water samples were taken using one-inch PVC pipes sealed at one end with 0.8 mm holes drilled in the lower 38 cm 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 withdrawn using 6.4 mm Tygon ® tubing and 25 Tbs of vacuum pressure, from a laboratory vacuum pump. 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 sample was taken for trace metals. After day 25 in Experiment I and throughout Experiment II, a 25g sample was taken for trace metals. Dissolved and particulate trace metals were placed in 2.5 L glass bottles (which formerly contained conc. HNO3 or HCl) which were equilibrated in reagent-grade seawater for 12 hrs prior to sampling (4 & samples for Experiment II). 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 µm pore size, 142 mm diameter, German ® filters. The filters were previously washed in 6 NHC and rinsed with copious

Amounts of R.O./D.I. water were used. The fraction retained on the filter was referred to as the "particulate" portion, while the filtrate was known as the "soluble" fraction.

Physical and Chemical Analyses

General reagents used are of reagent ACS grade. All glassware was cleaned with detergent, rinsed with water from a Millipore "Mi115-9" system. Water referred to in this paper is R.O./D.I. water from the Millipore "Mi111-Q" system. This refers to the technique of reverse osmosis followed by ion-exchange. The final cleaning for glassware used to digest samples for trace metal analysis involved refluxing the covered beakers with concentrated HNO3 followed by rinsing with R.O./D.I. water. All other equipment was washed with detergent, rinsed with R.O./D.I. water, followed with 6N HCl and rinsed with R.O./D.I. water. Only non-metallic instruments and materials were used and all equipment, after cleaning, was stored covered with Parafilm or plastic bags.

Oxygen, temperature, and salinity were measured using a Yellow Springs International YSI - 57 oxygen/temperature meter. The oxygen output was both salinity and temperature compensated. The oxygen calibration was checked using the Winkler titration technique (Strickland and Parsons, 1965).

Dissolved reactive phosphate (100 ml samples) were collected and immediately filtered through a Millipore Swin-Loc adaptor containing an acid-washed (6N HCl) 27 mm Millipore, 0.45 µm pore size filter using an acid washed 100 ml polypropylene syringe. The sample was frozen and later DRP was determined using the single solution technique (Strickland and Parsons, 1965) with a 10 cm cell in a Beckman DU spectrophotometer. A small number of samples were run on a Technicon autoanalyzer using standard Technicon procedures (Zimmermann, C. et al., 1970).

Particulate trace metals were collected. The 142 mm diameter filters were placed in Pyrex beakers,

6 ml of concentrated 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 filters dissolved, the liquid was brought to a rapid boil and taken to near dryness. Then, 4 ml concentrated HNO3 were added, followed by dropwise addition of 1 ml of I2 (30% by volume). The sample was then reboiled to near dryness and 3 drops of concentrated H2SO4 were added. If the sample turned black, more HgO was added. The samples were dried until SO3 fumes disappeared. The sample was then brought to a 10 ml volume with H2O/0.1. water. Soluble Trace Metal: After 2.56g of the sample was filtered for determination of "particulate" trace metals (41 in Experiment II), the effluent was run through two 1.2 cm diameter x 10 cm glass columns. The flow rate was maintained at 1 ml min per cm2. The columns were in series and the first column contained 10 ml of Chelex-100 "in the ammonium form" (20 ml in Experiment II) with 20 ml of Amberlite XAD-2 resin (Rohm and Haas) in the second column. The Chelex resin was purified by 2 bed volumes (BV) of 1N HCl (isothermally distilled) followed by 5 BV of DI/H2O water and then 2 BV 1N NH4OH (isothermally distilled) with a final wash of DI/H2O water. The Amberlite XAD-2 resin was cleaned by washing for 10 minutes with 0.1/D2O 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 Amberlite was stored under methanol until just prior to use.

In Experiment I and II, the metals were extracted from the Chelex-100 resin after the resin column was first washed with 100 ml of water. The trace metals, in EXP I, were eluted with 40 ml of boiling concentrated HCl. The resin was rinsed with 20 ml of water and this water was combined with the acid eluate. The eluate was boiled down (95°C) to 1 ml and then brought to 10 ml volume with water. This was referred to as the soluble inorganically complexed fraction. In Experiment II, the Chelex 100 was eluted batchwise using...

(23 x 30 cm). In Experiment 1, all plates were sampled at each sampling time. In Experiment 2, cumulative results were obtained by sampling plates which had remained in the tanks for 25, 50, 85, 125 days after addition of sludge. Thalass' = Roots and leaves. The roots and rhizomes were combined and are referred to as roots. Mangroves - The lower 3.8 cm of the hypocotyl, not including the roots. This is referred to in this paper as the bottom. Growing stem includes all of the primary meristematic tissue from the hypocotyl excluding the leaves and the roots/rhizomes. 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.

TABLE 3 Results in µg/g dry weight for determination of metals in NBS bovine liver and orchard leaf standards. The results for Experiment 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 II 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 II 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

The ground sample, not used for analysis, was stored in plastic, screw cap bottles. The dried samples were placed in cleaned Pyrex beakers, at least 1g where possible, and were digested using the following procedure. Twenty-five ml of a concentrated HNO3: HCl mixture (3:1) was added to the dried, weighed sample in the covered clean beaker. The sample was slowly boiled and refluxed in the covered beaker at 90 to 95°C until 1 ml of solution remained. The sample was allowed to cool then 30 ml of 30% H2O2 was

Slowly added 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 allowed to cool and 10 mt of 30% Hp02 was added. This was allowed to boil again (90-95°C). The 10 mt 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 were left. The sample was allowed to cool then centrifuged in a clean 50 mt plastic capped centrifuge 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 sample was placed in clean, 25 mt polyethylene vials with caps.

SECTION 3 RESULTS

The raw data, expressed as ug metal/g dry weight for all samples, are found in Appendix A. The computed net uptake for each metal is located in Appendix B and is expressed as mean experimental results (9/9 dry weight) minus the mean control results for each sample, with their respective standard deviations, and degree of freedom. The “t" value for the null hypothesis, Not Mj-Mp = 0 ($P<0.06$) where My is experimental and N is control is also shown in Appendix B. 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 (Experiment II) are in Appendix C. The wet/dry weight ratios with statistics for all the samples in Experiment I and II are in Appendix D.

The experiments were replicated. Experiment I began on March 11, 1975, and ended July 15, 1975; Experiment II began on December 12, 1975, and ended April 26, 1976. The experimental tank refers to the tank with the sludge added and the control tank was identical, in experimental design, to the experimental tank except no sludge was added.

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% (¥30, $0.42, N=18).

Dissolved Reactive Phosphate (DRP): The results are shown in Figures 4 and 5 for control, Experiment 1 and 2, and experimental, Experiment 1 and 2, respectively. The plotted points are the mean values for a five day period.

In Experiment 1, fouling organisms Ce, Cu, Pb, and Zn showed a significant net uptake after 125 days (Figures 6, 8, 9, 11), whereas in Experiment 2, Pb (Figure 10) showed a steady uptake to the end of the sampling period (85 days). For Experiment 2, Ce and Ni demonstrated a steady uptake until day 50 (Figures 7 and 13), then a decline in the net uptake. Zinc and Chromium indicated a net uptake after day 50 (Figures 12 and 15), and Cu showed little significant uptake for the sampling period (Appendix B).

The initial values for trace metals in sewage sludge are shown in Table 4. A rapid decline was noted in the concentration of Ce, Cr, Cu, Ni, and In in the sewage sludge for Experiment 1 (Figures 6, 15, 8, 14, 11). The Pb results are inconclusive for both experiments (Figure 9 and Appendix B) due to the loss of lead as the easily volatilized $PbSO_4$ during the digestion of the sludge.

For Experiment 2, a two-stage decline for Cd, Ni, and Zn was shown (Figures 7, 13, 12). The first decrease in metal concentration was nearly instantaneous with a further, smaller decrease after day 50.

Thalassia Leaves: The results of Experiment 1 were biased due to the "die off" of the plants by day 50, probably due to insufficient light. The plants survived for the full 125 day period in Experiment 2 after the light level was increased. Copper, Cr, Pb, Ni and Zn showed rapid increases in net uptake in Experiment 1 (Figures 16, 18, 20, 22, 24). In Experiment 2, the same overall trend of increasing net uptake was shown for Cu, Cr, Pb, and Cd (Figures 17, 19, 21, 26). Nickel and zinc in Experiment 2 did not show a significant uptake up to day 50, but the net uptake was significant thereafter.

On days 85 and 125, significant net uptake of trace metals was observed for Thalassia roots and rhizomes in Experiment I, especially for nickel, Cr, Cu, and Pb (Figs. 23, 25). The repeated experiment also demonstrated a significant net uptake for Cr and In (Figs. 30, 36), with Pb, Ni, and Cu showing peaks of significant net uptake values on day 5 and/or 25 (Figs. 34, 28, 32). Cadmium uptake was significant only on day 25 (Fig. 37).

To facilitate subsequent discussions, the results of the net uptake for urchins are presented alongside the results for Thalassia leaves. Copper, Cry, and Pb exhibited a steady and rapid increase in net uptake for Experiments I and II (Figs. 16, 17, 18, 19, 20, 21). The net uptake for Cd was not significant in Experiment I (Appendix 8), but in Experiment II (Fig. 26), the net uptake increased after day 50. The results for nickel and Zn uptake were very similar in both experiments (Figs. 22, 23, 24, 25, 26). Zinc showed a steady increase with a decrease in net uptake at day 50, followed by a rapid increase in both experiments.

The internal organs of the sea cucumber, in both experiments, showed very similar net uptakes of Cr, Cu, Pb, and Zn over the 125-day period (Figs. 38, 39, 40, 41, 42, 43). Ni showed a net uptake spike at day 5 in Experiment I, and in Experiment II, spikes in uptake of nickel occurred at days 5, 50, and a smaller spike at day 125 (Figs. 38, 41).

For mangrove roots, nickel in Experiment I showed an initial decrease in net uptake before day 50, followed by a gradual increase to day 125 (Fig. 27). The opposite was observed in Experiment II, where the net uptake increased rapidly up to day 5 (Appendix 8). The net uptake for Cr was not significant for Experiment I (Appendix 8), but indicated a linear net uptake from day 50 to day 125 for Experiment II (Fig. 30). Lead uptake in both experiments (Figs. 33 and 34) exhibited similar trends. However, Pb uptake was much higher in Experiment I (Fig. 33). Zinc, in Experiment I, demonstrated a rapid linear uptake curve (Fig. 50).

35) which was very similar to the uptake in Experiment II (Fig. 36). However, the net uptake was greater for Zn in Experiment 1. The net uptake for Cu was not significant in Experiment 1 (Appendix 8), but appeared to show a gradual uptake after day 50 followed by a decline to original levels (Fig. 32). No significant net uptake was shown for the oysters, clams, mangrove body, stem or leaves, snails, 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.

TABLE 4. Concentration of C8, Cr, Cu, Ni, Pb, and Zn in µg/g dry weight, in sewage sludge used in this experiment. The results are for Experiment 1 and II. Results for the same metals as determined by Salotto and Farrell (1971) for activated sludge are included.

Experiment C8 Cr Cu Ni Zn
x 109 2120. 2860. 603192926
SD 6.70 115. 124.0 5511.3 388.4

Experiment II
C8 Cr Cu Pb Ni Zn
% 166. 36672445. 21.0 376, 2508
SD 85.5 338.5 266.4 © 3.29 12.7 122.1

Salotto and Farrell (1971)
C8 Cr Cu Pb Ni Zn
350 4330 11001500 3803300

Lead values are low due to digestion technique.

Addition of sewage Collection of sediment/ Collection of water Sludge/organisms (0,1, samples for trace 5,25,50,85%, 125 days) metal and hydrocarbon analysis, ES sample taken. Frozen in plastic bags for analysis. Filtered of hydrocarbons. Defrost Frozen sample for hydrocarbon analysis. Amberlite XAD-2 resin columns and Sample posted by tissue. Extracted with CCl4. Chelex-100 resin. Extract stored in column ‘Subsample’ freezer. Mercury analysis. Elution, dilution wet weight.

Analysis, atomic absorption. Dry Toss. Dry weight. Grind by mortar/pestle. Digest/dilution. 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, sediment, sludge, and organisms were stored frozen for future analyses. An additional sampling was made for Experiment II. Day 0 refers to the sample taken before the addition of sewage sludge. Figure 3 represents the flow chart of trace metal and hydrocarbon sampling from collection processing into final sample form.

Section 4 discusses oysters and clams which have been extensively studied regarding trace metal uptake because of their commercial significance, hardiness, and feeding method. Bivalves, in general, show uptake and concentration of most trace metals (Kerfoot, W.B., 1973; Kerfoot, W.B. and S.A. Jacobs, 1973 a and b; Shuster, C.N. and B.H. Pringle, 1969; Huggett, R.J. et al., 1973; and Frazier, J.N., 1976). However, oysters and clams in our research showed no consistent nor significant net uptake of metal except possibly zinc and copper.

In Experiment I, all the oysters were placed in a single net bag. This procedure was changed to ten net bags per tank in Experiment II. This drastically reduced the crowding and increased the survival of the oysters beyond day 50 in Experiment II. The wet weight of the clam and oyster internal organs was too small to allow an adequate sample size for the trace metal analyses. This same problem was encountered in the analyses for the snails, Holothurian muscle parts in Experiment 1, and zooplankton samples for both Experiment I and II.

The results for lead in the sewage sludge were very low compared to other investigators (Table 4) and is due to the addition of H_2SO_4 to aid in the digestion of the sludge organic matter. When the sample was heated, the lead was lost as volatile $PbSO_4$. The H_2SO_4 was only used for the digestion of the sludge.

The results for the analyses on the NBS bovine liver standards (Table 3) are in good agreement for Cu and Zn and high for Cd. Our results for Cr and Pb in bovine liver were below our detection limits. The results for NBS orchard leaves were in good agreement for Cu, Pb, and Zn. Cadmium, in NBS orchard

The levels of leaves and nickel were too high. The coefficient of variation (CV) for Ni was lower than the CV for Cd. The results for Cr in orchard leaves were in good agreement with the non-certified value for Cr published by NBS. Our results for the concentration of trace metals in water for Experiment I were at or below the detection limits for nearly all metals and were therefore not shown. This was due to the 2.5 sample size. When we used a 4 sample in Experiment II, the results for most of the metals were within our detection limits. However, no significant difference was noted between metal concentrations in the experimental tank and those in the control tank.

Obviously, metals were released from the sludge, as shown by the results in Figures 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 problems in trace metal analyses of seawater by flame atomic absorption spectrophotometry, 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 (1969) demonstrated that the concentration of metal in the water, at any one time, may not be the best indicator of total contamination by metals. The net uptake of metal by "fouling organisms" was clearly shown in both Experiments I and II. This net uptake was replicated in Experiments I and II 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 II. The fact that Ni and Cr didn't show a net uptake in Experiment I was probably due to a change in sample technique for fouling organisms initiated in Experiment II. In Experiment I, when a settling plate was sampled for fouling organisms, clean plates were added for the next sampling period. Whereas, in Experiment II, 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 from the sewage sludge for both Experiment I and II. 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 II. Significant cadmium uptake by Thalassia leaves and urchin internal organs was seen only in Experiment II. Schroeder (1975), using radioactive 57co, 60co, S4n and O2n in seawater, showed that in Thalassia testudinum, 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 and Cu and higher in Experiment II for Ni and Cr. Cadmium and zinc had a net uptake in Thalassia roots only in Experiment II. 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 is in apparent contradiction with Schroeder's results (1975) where roots were found to have the greatest uptake. However, our root sample consisted of roots plus rhizomes and, as rhizomes showed the highest net uptake in Schroeder's paper (1975), the contradiction between our results and Schroeder's is 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 II, 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 loss 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 until 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 significant 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 were similarities in net uptake by Holothurian guts for Experiments I and II. Although there are similar patterns of

In the Holothurian gut uptake, the maximum values for net uptake were always higher in Experiment 1 for Ni, Cu, Pb, and Zn. Chromium showed a higher maximum net uptake in Experiment II for Holothurian guts. In Experiment II, the sewage sludge showed a two-stage release. The first stage occurred on day 1 followed by a slow, constant release until day 50 when another rapid release occurred. A slow but steady release would more likely result in a 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 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 instantaneous 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 community. These results were replicated at two different seasons.

Regarding the results, considering the complexity of the system, they agree quite well. Originally, the fish, Diodon helacanthus (puffer), was 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.

SECTION 5 CONCLUSIONS AND RECOMMENDATIONS

Net uptake of trace metals by members of a Thalassia testudinum/Rhizophorae mangle community was demonstrated in two 125-day replicated experiments 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.

Rhizophora mangle roots. In general, the net uptake pathways followed the trophic levels in the food webs. This pathway was especially noticeable from the water to the fouling organisms and from the water to the Thalassia testudinum leaves and then to the urchin herbivore Lytechinus

variegatus. The net uptake in the Holothuria 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 system was dominated 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 long-term exposure, in a simulated tropical ecosystem, to toxic trace metals more closely approximates the situation in nature than laboratory experiments. In this respect, we are in agreement with the statement by Phelps et al. (1975) "Chronic exposures to elevated metal levels through the food web should be used as a more realistic test for possible deleterious effects from such metals as Cr. No artificial system can ever reproduce a natural ecosystem. However, it is more important to closely approximate the natural system with a system which can duplicate itself (Wenzel, O.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 ecosystem. More detailed work on the net uptake of toxic metals, using this complex simulated ecosystem, and the roles of the "fouling organisms" and plankton in this system is needed. This must involve integrated sampling of the organisms as well as the water. The advent of more precise methods of direct trace metal determination in seawater using anodic stripping voltammetry and non-flame atomic absorption spectrophotometry, in lieu of flame AAS, should allow a reasonable sample size for both organisms and water. The authors would not recommend, based on the

Results of this research concern the dumping of sewage sludge in shallow, tropical marine environments. There is potential for rapid concentration of toxic trace metals by members of the food web high on the trophic scale. These toxic levels could then affect organisms used as food by humans. The next logical step in future research would be to add vertebrate organisms to this system to see if a herbivore, which normally consumes turtle grass, would show a net uptake of toxic trace metals.

30KM MONA PASS. "18°N e7'"w

Figure 1. Study site location

Figure 2. Diagram of experimental design, tank configuration, and seawater system

Addition of sewage, collection of sediment/ sludge/organisms (0,1, 5,25,50,85%, 125 days), sample taken. Frozen in plastic bags, defrost, dissection, sample pooled by tissue, subsample mercury analysis, wet weight, dry 105°C, dry weight, grind by mortar/pestle, digest/dilution 1 analysis. Atomic absorption for analysis of hydrocarbons, sample for hydrocarbon analysis extracted with CCL4, extract stored in freezer.

Collection of water samples for trace metal and hydrocarbon analysis, filtered, frozen on Amberlite XAD-2 resin columns and Chelex-100 R, elution, dilution, analysis, and atomic absorption.

Samples were collected at 0,1,5,25,50,85 and 128 days after addition of sewage sludge. Samples for mercury, and hydrocarbons in both water, sediment, sludge and organisms were stored frozen for future analyses. An additional sampling was made for Experiment II, Day 0 refers to the sample

taken before addition of sewage sludge.

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

Figure 4. ORR. CONTROL Exe. 1 AIG-AT/L ORP- CONTROL Exe. 2 25 crys

Dissolved reactive phosphate concentration Experiment I and II, control. Data points are mean values for 5 day period, excluding day 1.

Cu Thalassia Leaves- Exp. 1 & 2. Figure 16. Mean net uptake of Cu between experimental and control Thalassia leaves and urchin guts - Experiment 1.

Figure 17. Cu Thalassia Leaves- Exp. 2. Figure 17. Mean net uptake of Cu between experimental and control, Thalassia leaves and urchin guts - Experiment 2.

Figure 18. Mean net uptake of Cr between experimental and control, Thalassia leaves and urchin guts - Experiment 1.

Cr Thalassia Leaves- Exp. 2. Figure 19. Mean net uptake of Cr between experimental and control, Thalassia leaves and urchin guts - Experiment 2.

Figure 20. Mean net uptake of Pb between experimental and control, Thalassia leaves and urchin guts - Experiment 1.

Figure 21. Mean net uptake of Pb between experimental and control, Thalassia leaves and urchin guts - Experiment 2.

Figure 22. Ni Thalassia Leaves- Exp. 1. Mean net uptake of Ni between experimental and control, Thalassia leaves and urchin guts - Experiment 1.

Figure 23. Ni Thalassia Leaves- Exp. 2. Mean net uptake of Ni between experimental and control, Thalassia leaves and urchin guts - Experiment 2.

Figure 24. Mean net uptake of Zn between experimental and control, Thalassia leaves and urchin guts - Experiment 1.

Zn Thalassia Leaves- Exp. 2. Figure 25. Mean net uptake of Zn between experimental and control, Thalassia leaves and urchin guts - Experiment 2.

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

The text appears to be a series of excerpts from an academic paper or report, possibly related to a scientific experiment involving Thalassia leaves, urchin guts, mangrove roots, and various elements like Ni, Cr, Cu, Pb, and Zn. It's difficult to provide a corrected version without more context and clarity on the original intent of the author. The text also includes several potential page breaks and figures, which suggests it may be a draft or a corrupted document. I would recommend reaching out to the author for a more accurate and complete version of the document.

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Appendix A Sorted Raw Data Experiment I and II

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

Column 1 = Animal type
Clam
Holothurian
Mangrove
T= Thalassia
P = Fouling Organisms
Urchin
S = Sediment

2 - Tissue type
Muscle
Internal contents (guts)
Shell
Body/cotton
Leaves
Roots and rhizomes
G = Upper Meristem
= Solids

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

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

5 = Represents sample time
0 = presample
1 = 1 day after sewage added
5=5 days

6 = Concentration (ug/g dry weight)

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 less than or equal to the MDC. The MDC values are listed in the Results (Table 4).

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I'm sorry, but the provided text appears to be a mixture of random characters, numbers, and words. It's not clear what it is supposed to say, making it impossible for me to correct it. If you could provide more context or a clearer text, I would be able to assist you better.

I'm sorry, but the text provided seems to contain a mix of random characters, numbers, and words. It doesn't appear to form coherent sentences or paragraphs in any language. Could you please provide more context or a clearer version of the text you want to be fixed?

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I'm sorry, but the text you've provided is largely nonsensical and seems to contain a mix of random characters, numbers, and words. It's hard to fix it without understanding what it's supposed to mean. Could you provide more context or a more coherent message?

[APPENDIX C]
Experiment 11, Master Data

The column headings are defined as follows:

1. HC E is data from the experimental tank control.

2. 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 the particulate fraction.
4. SD PART represents the standard deviation of the particulate fraction.

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Mean Particulate Function 5: The MEAN SOC values shown represent the 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: The MEAN SIC values shown represent the mean concentration (ug/t) of the soluble inorganic fraction (retained on Chelex-100 ® resin).
8: SP SIC represents the standard deviation of the soluble inorganic fraction.

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MEAN SOC
SD SOC
MEAN SIC
SD SIC
SD PART METAL
MEAN PART cd ec
8 cu Ni

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APPENDIX D: Wet/Dry Ratios
Wet/dry ratios are shown for each sample and tissue type for Experiments 1 and 11. These values may be multiplied by the metal concentration (19/9 dry weight) values to obtain concentrations in ug/g wet weight.

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Mean Wet/Dry deviation($b): Clan Gut Shell, Sea cucumber Body Gut Muscle, Urchin Gut Shell, Snail Gut, Shell oysters Gut Shell, Thalassia Leaves root/Rhizomes, "Fouling organisms", Senage Sediment, Mangroves Bottom of hypocotyl, Meristem above hypocotyl, Leaves, Roots.
Ratio (3) with n (number of samples) and Standard for all organisms sampled in Experiment 1 and 10.2, 78, 5.2, 14.8, 14, 33, 3.4, 45, 5.4, 1.90, aa, 2.2, 7.10, 0.00, 0.60, 0.60, 1a, 1.0, 142, N x 8.0, 13, 10.7, 1.20, 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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