UNIVERSITY OF CALIFORNIA SAN DIEGO

Benthic macrofaunal invertebrates of the San Pedro Basin and the relationship to DDT waste barrels

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

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The Thesis of Angelica Marie Bradley is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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LIST OF ABBREVIATIONS

DDD	Dichloro-diphenyl-dichloroethane
DDE	Dichloro-diphenyl-dichloroethylene
DDMU	Chloro-chlorophenyl-ethene
DDT	Dichloro-diphenyl-trichloroethane
DDX	collective reference to DDT and its metabolites
MCC	Montrose Chemical Company
SCB	Southern California Bight
SE	Standard error
SPB	San Pedro Basin

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ABSTRACT OF THE THESIS

Benthic macrofaunal invertebrates of the San Pedro Basin and the relationship to DDT waste barrels

by

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Master of Science in Marine Biology

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The San Pedro Basin (SPB) contains thousands of DDT-production waste barrels dumped on the seafloor from 1947 to 1961. DDT, a harmful pesticide now banned, still impacts California marine life. This study evaluates the relationship between these waste barrels, DDX concentrations (DDT + related metabolites), and benthic macrofaunal assemblages. It analyzed

sediment samples from around six waste-barrels and three background sites to address (1) the DDX concentration and depth in relation to the barrels, (2) barrel-proximity effects on macrofaunal (> 300 μm) community structure, vertical distribution, or diversity of the macrofauna, (3) the potential relationship between DDX and macrofauna density, and (4) the potential role of the macrofauna to mix or mobilize DDX. DDX concentration was highest in the 2-6 cm fraction but showed no correlation with macrofaunal density. Macrofauna densities, relatively low (1553-11802 ind/m^2) compared to other low-oxygen areas of similar depth. Dominant among the macrofauna were Entoprocta (Barentsiidae), but nematodes were by far the most numerically abundant group. Paraonid, dorvilleid, and cirratulid polychaetes were present. Diversity was lowest within the bacterial halos surrounding the barrels and highest adjacent to the barrels. There is potential for the macrofauna to remobilize DDX into the water column and ultimately the SPB food web, but macrofaunal densities are low. Overall, the study suggests waste barrels have minimal impact on macrofauna communities. However, a broader regional survey, including megafauna and pelagic biota, is necessary for a more comprehensive understanding of how DDX is mobilized in the Southern California Borderland.

INTRODUCTION

Southern California Bight

The Southern California Bight (SCB) spans over 80,000 km^2 from Point Conception, California to Cape Colonet, Baja California, Mexico; it is an important ecological and economic resource, as it is home to numerous marine species (Stein & Cadien 2009). This area is characterized by a variety of ecosystems due to being located within a highly active tectonic area (Levin et al., 2016). The SCB overlays the continental borderland, which consists of several islands, as well as submarine banks, canyons, and deep basins (Schiff 2000).

San Pedro Basin

The San Pedro Basin (SPB), located between the Palos Verdes Peninsula and Santa Catalina Island, is one of several basins within the SCB. With a depth of around 850-900 m, the basin is located within the oxygen minimum zone with bottom water oxygen concentrations of around 2-10 μM (Collins et al., 2011).

The San Pedro Basin, the location of this study (Figure 1), measures 27 km in width, 36 km in length, and has an average depth of about 898m. The SPB undergoes flushing events, as observed between 1982-1984, in which cooler denser "new" water replaces the basin's warmer less dense water, resulting in a complete water turnover within six months to 2 years (Berelson,1991; Argonne National Laboratory, 2019). Conversely, the basin also experiences periods of stagnation, such as from 1984-1987, where the waters become anoxic (Berelson, 1991). These "renewal events" have been correlated with strong upwelling periods in the Santa Barbara Channel (Argonne National Laboratory, 2019). Estimates place the San Pedro Basin's sedimentation rate between 15-20 mg/cm^2y (Huh et al., 1990, as cited in Gorsline, 1992), a range that aligns with the findings of Christensen (1991). Several rivers discharge into the San Pedro

Basin including the Los Angeles River, Santa Ana River, and smaller rivers such as the Aliso Creek and Salt Creek (Argonne National Laboratory 2019). It is estimated that the SPB has higher pollutant inputs because of urban, industrial, and agricultural runoff (MMS 2001, 2005, Kaplan et al. 2010, Lyon and Stein 2010, as cited in Argonne National Laboratory). Gorsline (1992) concluded that the SPB acts as a natural sediment trap from which various sources of input can be traced. Sediments follow different pathways into the basins depending on particle size; larger sand particles are transported alongshore, finer sands are brought offshore by storms, and silts/clays are spread out to the central shelf in plumes (Karl, 1976).

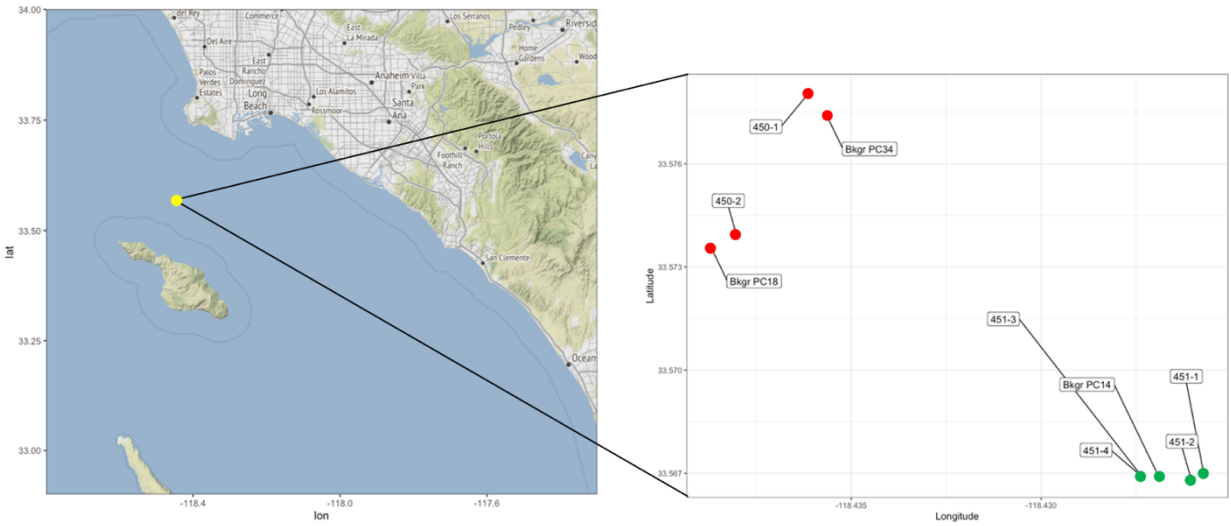

Figure 1. Overall map of DDT Dumpsite 2, within the San Pedro Basin. Red dots indicate barrels were cores taken on SuBastian Dive 450 (8/3/2021) and the green dots indicate barrels on Dive 451 (8/4/2021). The dives were about 1 km apart.

History of dumping and DDT use in Southern California

The Southern California Bight has a history of industrial dumping off its coasts, both near shore and in deeper waters, most of which has yet to be thoroughly quantified and understood. DDT (Dichloro-diphenyl-trichloroethane) is an organochlorine which was historically used in the United States as an agricultural pesticide. Although banned 50 years ago (1972), DDT and its metabolites persist in the environment. DDT breaks down into DDE and DDD and can have a

half-life of 2 to 15 years in soil and up to 150 years in aquatic environments (EPA 1979, ATSDR 2022)

Decades ago, the only existing manufacturer of DDT in California, Montrose Chemical Company (MCC) was responsible for the mass dumping of DDT-waste barrels into the SPB and the nearby waters. In 1947, Montrose began production of DDT and by 1956 had become the largest producer of DDT in the world, accounting for about 40 percent of global DDT production (Kehoe & Jacobson 2003). From the year MCC started production and the fourteen years following, it is estimated that over 10,000,000 gallons of waste was dumped into the ocean (Chartrand et al. 1985). The contents of the DDT-waste barrels were estimated to contain 0.5 – 2 percent DDT, as well as 72 % sulfuric acid, 25% *p*-chlorobenzene-sulfonic acid, and small amounts of water and hydrochloric acid (Hendel 1956, Chartrand et al. 1985, Kivenson et al. 2019). DDT waste was also delivered to San Pedro Basin uncontainerized in barges and bulk dumped (Chartrand et al. 1985, Chartrand and Hackney 2022). Commercial use of DDT was banned in the United States in 1972 due to its toxicity to wildlife, its tendency to bioaccumulate in the food web, and the danger to human health (Chartrand and Hackney 2022). By 1994, the EPA designated a 17-sq mile section of the Palos Verdes Shelf as a Superfund site; an area of marine sediments immensely contaminated with DDT and PCBs due to years of unregulated wastewater discharge by Los Angeles County and industries like Montrose Chemical Company using the sewer systems for DDT-waste discharge. But contamination of deep waters had been largely ignored. Recent reporting by the L.A. Times of ROV *Jason* dives made by David Valentine (UCSB) revealing waste barrels in the SPB, brought attention to DDT contamination to deeper waters (Rosanna Xia - LA Times April 2021, 2022).

A recent study by Merrified et al. (2023, in review) used sidescan sonar to assess the seafloor of the SPB. In addition to different size classes of waste barrels (found in previous surveys), they also classified other anthropogenic debris into two forms: cylindrical form factor and box shaped form factors. From observing debris spanning the entire width of their survey (74,117 debris-classified objects), they concluded that the seafloor of the SPB is significantly covered in debris. Additionally, they noted the linear distribution of the objects indicating that ships dumped these objects overboard for several kilometers. This further supports previous reports by Chartrand 1985, cited in Kivenson 2019. Merrifield (et al. in review) note that 55- or 42-gallon drums are fabricated from carbon steel sheet metal that has a finite life when submerged in seawater. At the low oxygen levels of the SPB (< 0.2 ml-O_2/L), they estimated a corrosion rate of 0.05 mm/y, leading to the implication that the barrels would have varying states of integrity in 2021.

Objectives and hypotheses

Benthic infauna can reflect levels of pollutant stresses in their environment due to being sedentary and constantly exposed to the pollutants accumulated in the sediments. Thus, they are valuable in determining the extent of contamination spatially as well as an overall measure of ecosystem conditions (Bilyard, 1987, Stein & Cadien 2009). This is significant because marine contamination has the potential to disrupt food supply and food quality for local and regional consumers, as well as altering ecosystem functions. Additionally, the benthic macrofauna is both a direct and indirect food source for many animals, including those harvested by local (commercial and recreational) fishers, which ultimately are consumed by humans. Any disruption to the density or diversity of these animals, which are near the bottom of the food chain for many other organisms, could result in a trophic cascade and alter the overall ecosystem.

Therefore, it is important for ecological and human health to monitor and manage pollution levels in marine ecosystems.

The SPB is about 16 km from the Superfund Site at Palos Verdes Shelf and is one of 14 known deep-sea dumpsites that contains industrial waste, military ordinance, radioactive waste, and chemical waste, among others (US EPA, 2022). Additionally, the SPB has been reported to have higher concentrations of DDX in some of the sediments than the Superfund Site itself (Kivenson, 2019; Almada et al., 2023).

This study intends to characterize the benthic macroinfaunal ecosystem communities of Dumpsite 2, including the macrofaunal taxonomic composition, density, and diversity, at a fine scale spatial resolution both horizontally across a gradient of distance to waste barrel, and vertically within the sediment column. This research is the first quantitative study focused on macrofaunal benthic communities associated with the contents of the DDT waste barrels and related compounds found in deep-sea sediments at the SPB dumpsite from around 880-890 m depth. This research extends previous work on benthos contamination to deep-sea sediments associated with DDT waste disposal. This research addressed the following questions:

A. Does the DDX concentration and depth distribution change with distance from the waste barrels?
B. Does proximity to the waste barrels determine macrofaunal community structure?
C. Does proximity to the waste barrels determine vertical distribution of macrofauna?
D. Do patterns of macrofauna distribution vary around different waste barrels?
E. Is there a relationship between sediment DDX concentration and the macrofaunal density, diversity, or taxonomic composition?

F. What is the potential role of the macrofauna in transferring DDX within sediments and the food web?

METHODS

Field Sampling

In early August of 2021 while aboard the R/V Falkor, members of the Levin, Jensen, and Rouse Labs sampled around six barrels in San Pedro Basin at DDT Dumpsite 2 using the ROV *SuBastian* (Figure 2). *SuBastian* Dive 450 took place at ~ 33.578066 ºN, -118.43624 ºW and *SuBastian* Dive 451 took place at ~ 33.567002 ºN, -118.42584 ºW. The team took paired pushcores; one for chemical analysis and sediment properties and the other for quantitative analysis of the macrofauna. These pushcore samples were taken in 5 m transects relative to the barrels in four zones: a) adjacent to the barrel – Zone A (when possible), b) on the bacterial mat halos about 1 m from the barrel – Zone B, c) outside the halo at 3 m from the barrel – Zone C, and d) about 5 meters from the barrel – Zone D (Figure 3). Three additional background samples were taken where no barrels were visible. Core diameter was 6.4 cm and samples were processed down to a depth of 10 cm. Cores are listed in Table 1.

Table 1. List of pushcores taken for macrofauna (>300 µm) from dives 450 and 451 for FK210726 and the respective depth, temperature, salinity, and oxygen concentration measurement. Each pushcore has a unique SCB-number and is listed in the order sampled.

Pushcore	SCB #	Barrel	Zone	Depth (m)	Temperature (C)	Salinity (ppt)	Oxygen (uM)
1	SCB-259d	450-1	D	884	5.261	34.76	0.63
3	SCB-261c	450-1	C	884	5.261	34.757	0.58
5	SBC-263b	450-1	B	884	5.258	34.76	0.59
7	SCB-265a	450-1	A	884	5.259	34.759	0.53
34	SCB-269	Background	E	883	5.258	34.763	0.46
10	SCB-271d	450-2	D	885	5.307	34.716	0.5
12	SCB-272c	450-2	C	885	5.26	34.76	0.5
14	SCB-275b	450-2	B	885	5.259	34.766	0.49
16	SCB-277a	450-2	A	885	5.263	34.759	0.34
18	SCB-281	Background	E	885	5.258	34.764	0.47
2	SCB-289d	451-1	D	885	5.261	34.759	0.63
4	SCB-291c	451-1	C	885	5.263	34.766	0.46
6	SCB-293b	451-1	B	885	5.263	34.757	0.43
8	SCB-297d	451-2	D	885	5.359	34.79	0.37
10	SCB-299c	451-2	C	885	5.258	34.764	0.37
12	SCB-301b	451-2	B	885	5.261	34.759	0.38
14	SCB-305	Background	E	885	5.261	34.764	0.35
16	SCB-307d	451-3	D	885	5.261	34.761	0.36
18	SCB-309c	451-3	C	885	5.262	34.756	0.36
20	SCB-311b	451-3	B	885	5.26	34.76	0.34
22	SCB-314d	451-4	D	885	5.263	34.757	0.32
24	SCB-316c	451-4	C	885	5.263	34.763	0.3
27	SCB-319b	451-4	B	885	5.263	34.761	0.39
32	SCB-321a	451-4	A	885	5.261	34.766	0.31

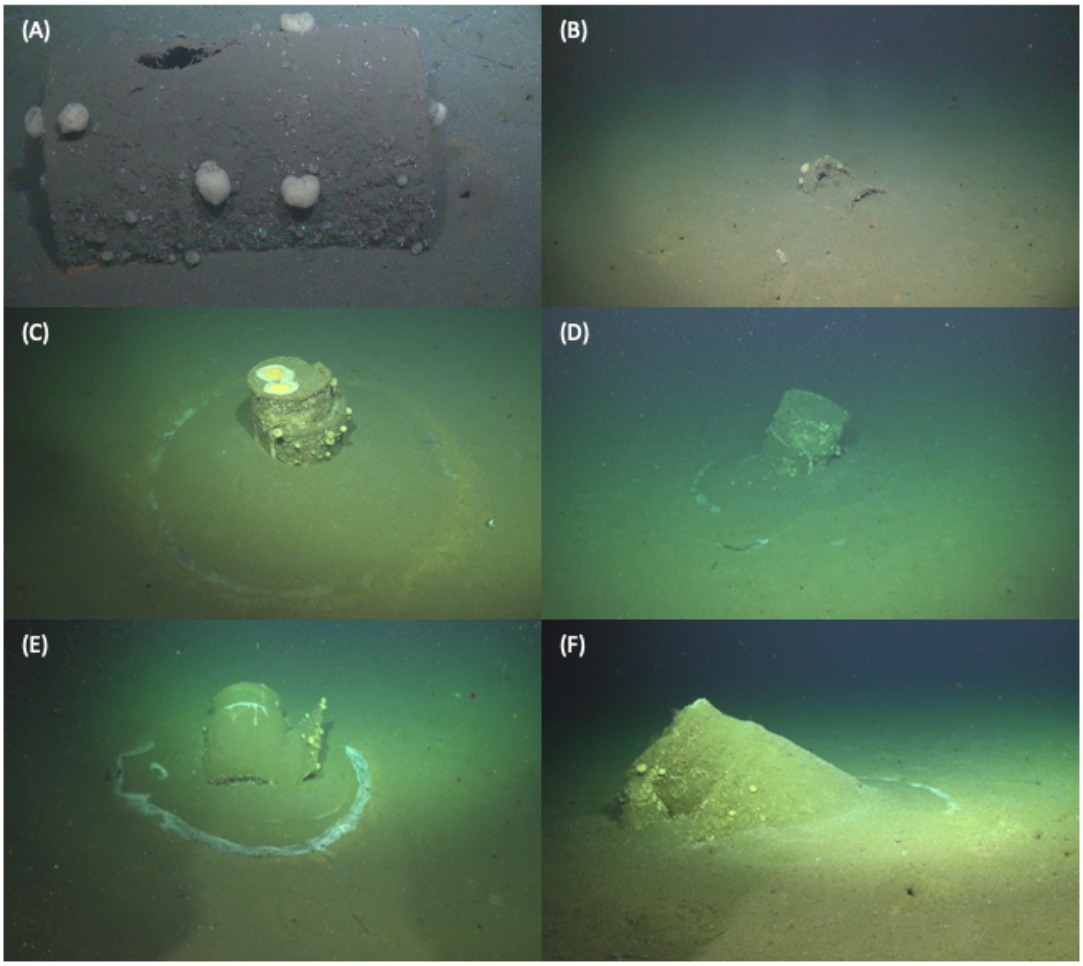

Figure 2. Photos of waste barrels captured by ROV SuBastian, from dives 450 (A, B) and 451 (C-F). Barrels are referred to by dive and sequence number. A. 450-1, B. 450-2, C. 451-1, D. 451-2, E. 451-3, F. 451-4.

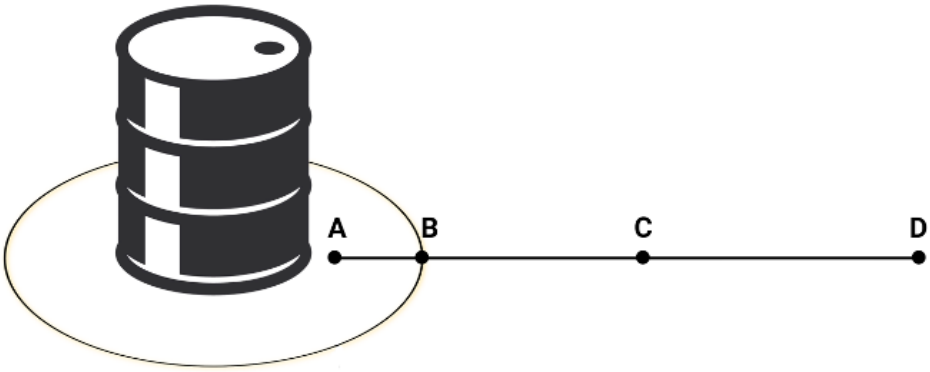

Figure 3. A schematic showing how barrels were sampled with a 10 cm – deep pushcore at zones A (adjacent), B (1m), C (3m), and D (5m) from the barrel.

Shipboard processing

The paired cores were sectioned in vertical increments within a few hours of collection. The cores for chemical analysis and sediment properties were vertically sectioned at 0-2, 2-4, 4-6, 6-8, and 8-10 cm and the quantitative faunal analysis cores were sectioned at 0-1, 1-2, 2-5, and 5-10 cm. The faunal analysis sections were preserved, unsieved, in 8% buffered formaldehyde and then stained with Rose Bengal. The sediment core fractions were frozen at -20°C.

Laboratory processing

To extract the fauna from the sediment, each section was sieved in the laboratory through three mesh screens to separate animals by size: >500, 300-500, and 45-300 μm. The fauna retained on the screens were then sorted, counted, and identified to the lowest possible taxon using a dissecting microscope. The fauna larger than 300 μm is reported here and includes traditional macrofaunal taxa along with large meiofauna (mainly nematodes and copepods).

Analysis of chemical contaminants, including DDX (i.e. DDT and derivatives DDE, DDD, and DDMU) was conducted at Physis Environmental Laboratories, Inc. Briefly, each aliquot of frozen sediment, representing every vertical and horizontal sampling sections, was extracted from sediments using Soxhlet with methylene chloride. Sample extracts were cleaned up using alumina/silica chromatography. Quantification and identification of DDX was performed using a high-resolution GC (Aglient 7980A) interfaced with a quadrupole MS (Aglient 5985C).

Data and Statistical Analysis

The surface area of one pushcore was 32.2 cm^2. Density of macrofaunal communities was standardized to 10,000 cm^2 (= 1 m^2). Densities were compared between barrel samples and background samples (Wilcoxon Rank Test); and among the zones (Kruskal Wallis and One-way ANOVA) both with macrofaunal density data only and again with the addition of macro-sized meiofauna (>300 μm). Diversity indices such as species richness (S), Pielou's evenness (J') and Shannon-Wiener diversity index were calculated per sediment core and per zone. Multivariate analysis of macrofaunal communities (no meiofaunal taxa) and diversity analyses were conducted using Primer software (PRIMERv7, Clark and Gorely, 2015). The ordination method used was the non-metric multidimensional scaling (nMDS) and differences between dives and zones were examined. The species density matrix was transformed by the fourth root and the similarity matrix was made with the Bray-Curtis index; analysis of similarity (ANOSIM) and similarity percentage (SIMPER) were then conducted.

RESULTS

DDX

DDX concentrations were assessed across the six barrels (4 zones) and the three background sites (Zone E - sites with no barrel visible) by analyzing sediment from 2-cm increments down to 10 cm in 24 unique pushcores (Figure 4). The barrels and background samples, assigned with distinct SCB identifiers, were collected over two days with barrels 450-1, 450-2, and background samples SCB-268 and SCB-280 collected on August 3, 2021 (*SuBastian* Dive 450) and the remaining samples collected on August 4, 2021 (*SuBastian* Dive 451).

In barrels 450-1 and 450-2 (Fig. 2A, B), DDX concentration peaked in the 2-4 cm fractions across all zones, with the highest concentrations in zone D (Fig. 4). Notably, barrel 450-2 (Fig 2D) exhibited a maximum DDX concentration of 1205.5 ng/g, which is more than double the highest concentration in 450-1. Barrels 451-1, 451-2, and 451-3 (Fig. 2C, D, E) lack a zone A core due to the presence of a hard precipitate (brucite) adjacent to the barrel, preventing the use of a pushcore. The highest DDX concentrations for these three barrels were observed in 2-4 and 4-6 cm fractions, with the highest concentration of 1283.9 ng/g of DDX at barrel 451-3 (Fig. 2E). The final barrel, 451-4, deviated from the pattern of the previous five barrels with the greatest DDX concentrations in the 6-8 and 8-10 cm fractions of zone A. However, in zones B-D, the greatest concentrations were found in the 4-6 cm fractions, which aligns with previous barrels. This pattern suggests a higher sediment accumulation rate directly adjacent to barrel 451-4 (Fig. 2F).

The background samples revealed higher concentrations of DDX than the barrels with a peak measurement of 1404 ng/g at 2-4 cm. Some sediment samples were unavailable for analysis including for zone C for barrel 450-1 and in the 4-10 cm fractions of zone B for barrel 451-3.

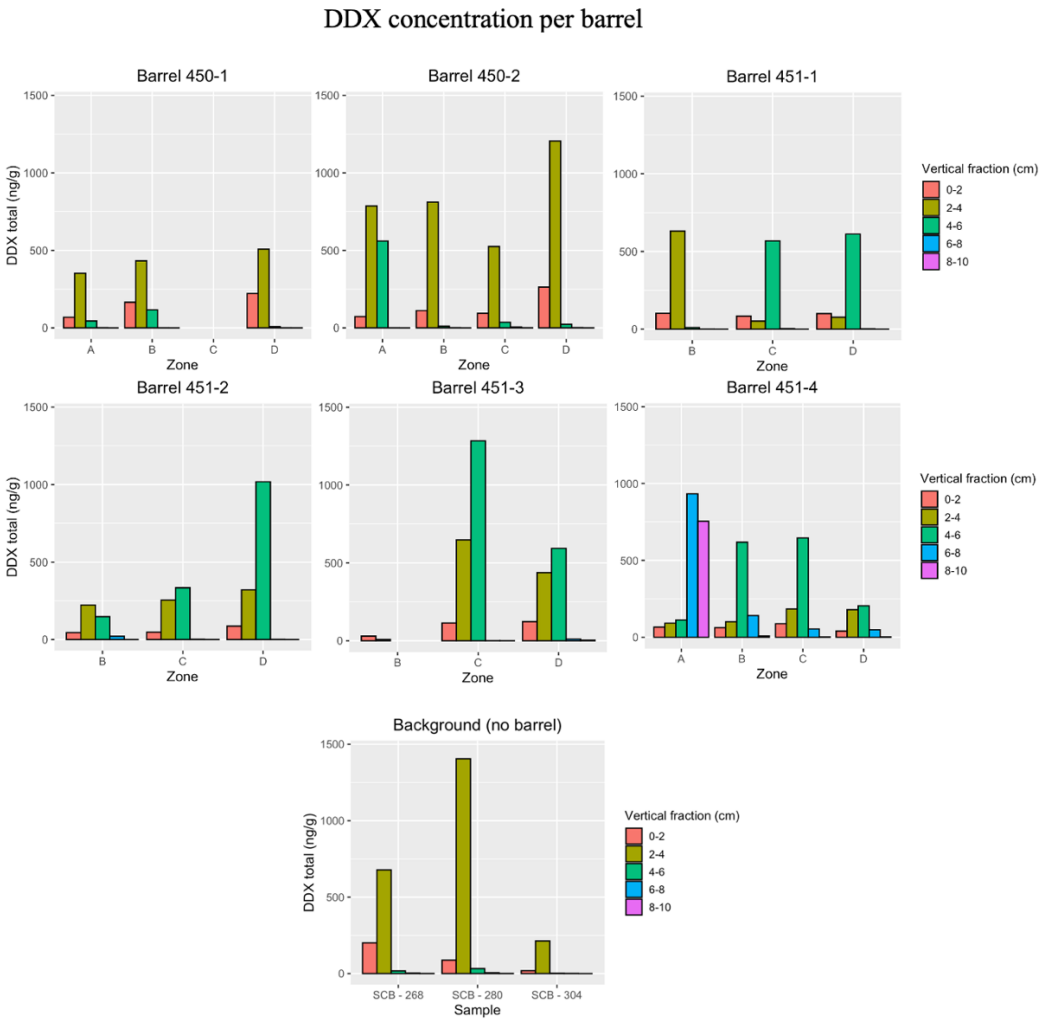

Figure 4. Average DDX concentration is shown for each zone of the six barrels and the three background samples; every zone is represented by five vertical fractions from 0-10 cm. There is no sediment data for Barrel 450-1 zone C, and 451-3 zone B, fractions 4-10.

Macrofauna >300 µm

Density - Results are presented for both traditional macrofaunal taxa only, and for macrofauna plus meiofaunal taxa such as nematodes and copepods that were retained on a 300-µm mesh.

The average densities of macrofauna ranged from 1,553 ind/m^2 to 11,802 ind/m^2 across the zones for all barrels. No significant difference was apparent when comparing macrofauna

densities among the barrel pushcores to background samples (Fig. 5A) [Wilcoxon Rank test, W = 45.5, p = 0.238].

However, with the addition of nematode and copepod densities, the mean densities increased significantly - by two orders of magnitude - (46,745 ind/m^2 - 176, 938 ind/m^2) (Fig. 5B). There was no statistical difference between barrel and background densities [Wilcoxon Rank Test, W = 38, p = 0.601].

There was a general trend in macrofaunal taxon density across the different zones surrounding the barrels. The mean density of macrofauna increased with increasing distance from the barrel, from zone A to zone D (Fig. 5C). However, zone E (background samples) demonstrated even lower densities than those near the barrel (zone A) suggesting that barrel presence might enhance macrofaunal density. However, density did not differ statistically across zones [Kruskal Wallis, $\chi^2 = 7.2055$, df = 4, p = 0.125].

Upon incorporating data for meiofaunal taxa (mainly nematodes), the distribution of average densities across the zones shifted. The highest average densities were found in zones B and C, while zone A exhibited the next highest average density. In contrast, zones D and E displayed the lowest average densities. However, the mean densities were not statistically different among the zones [One-way ANOVA, F = 1.221, df = 4, p = 0.335].

The potential relationship max DDX concentrations and macrofaunal density per pushcore was assessed and no apparent effect was found [$R^2 = 0.1002$, p = 0.077] (Fig. 6).

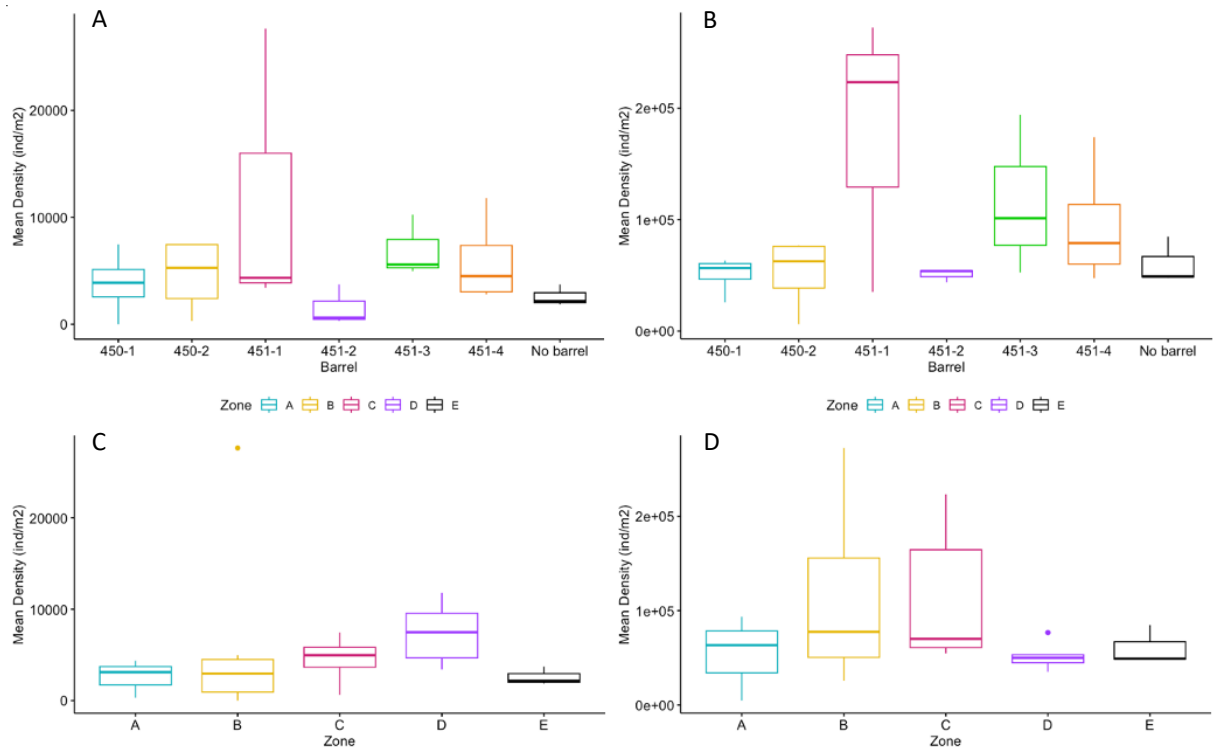

Figure 5. Average ± one standard error density of macrofaunal communities by barrel with only traditional benthic macrofauna (A), traditional macrofauna and macrofauna-sized nematodes and copepods (B), average ± one standard error density of macrofauna communities by zone with only traditional benthic macrofauna (C), and traditional macrofauna and macrofauna-sized nematodes and copepods (D).

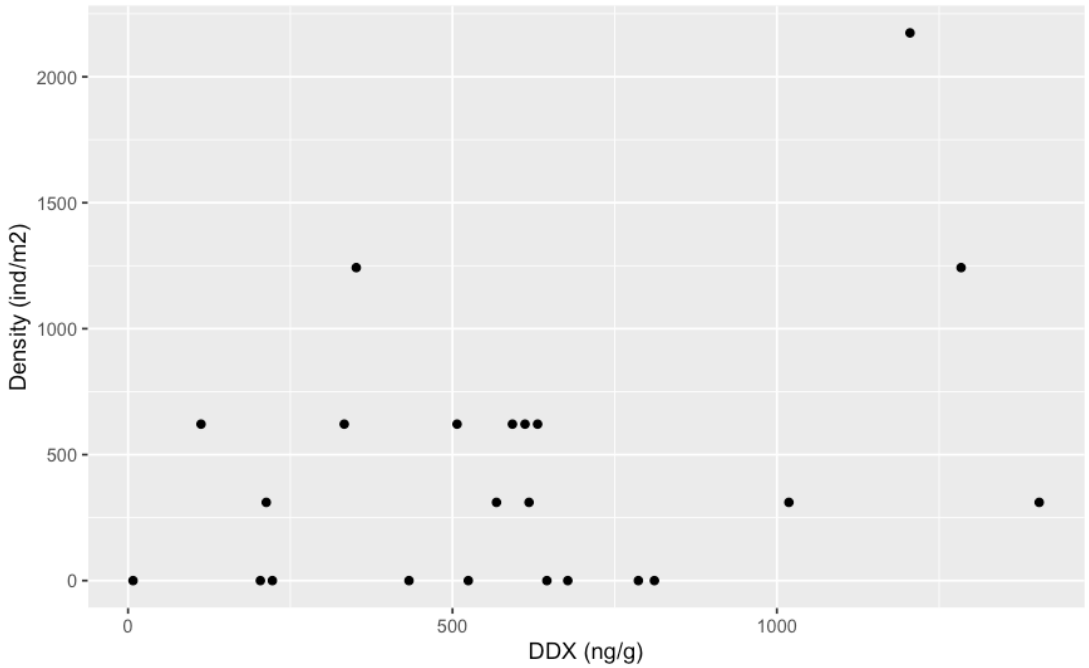

Figure 6. Macrofaunal density per pushcore sample as a function of DDX concentrations (highest concentration measured within 2-6 cm).

Taxonomic composition - In this study I identified twenty different taxa within the benthic invertebrate community, which were grouped into the following higher taxonomic classifications: Polychaete families - Chaetopteridae, Chrysopetalidae, Cirratulidae, Dorvilleidae, Hesionidae, Paraonidae, Polynoidae, Serpulidae, Syllidae, Entoprocta, Mollusca, Nemertina, Porifera, Nematoda, and Copepoda.

When analyzing the taxonomic composition across zones (Fig. 8), Entoprocta represented the highest percentage (63.21 %) of the total invertebrate community across all zones (34 - 71.8%). However, due to difficulties in distinguishing individuals in these colonial animals while sorting, their quantification was not consistent across all samples. Considering all Annelida taxa as a single group, they constituted the next highest percentage of the total invertebrate community (28.6 % of total composition, 13.6 - 56 % across the zones). The dominant polychaete families present were Dorvilleidae (10.37%), Cirratulidae (6.42%), and Paraonidae (5.43%). When nematodes were incorporated into the taxonomic composition, they dominated every zone, representing the largest percentage of the total invertebrate community (91.9 % of total, 80.9% - 95.6% across the zones).

The taxonomic composition (excluding Nematoda and Copepoda) across barrel zones revealed no discernable patterns (Fig. 8) (ANOSIM $R = 0.09284$, $P = 0.122$). However, the near significance of the zone effect led to investigation of pairwise differences via ANOSIM. Zones B and C communities were found to be different ($p = 0.045$).

There were also no higher composition differences between cores taken on Dive 450 and 451 (Fig. 8). However, Nemertina were only found at dive 450 and no polychaetes were present at dive 450, zone B.

To understand the differences in composition of the macrofaunal community across samples, nMDS plots were used to examine community composition data by dive and by zone (Fig. 9). Composition in cores taken during the two dives, located ~ 1km apart (450 - blue, 451 - red), were not significantly different (ANOSIM R = -0.05456, P= 0.75). Similarly, no significant effect of barrel proximity (zone) was detected on community composition (R = 0.09284, P = 0.1215). These composition metrics only describe the traditional macrofauna community, excluding nematodes and copepods, as species-level identifications were not available for the meiofauna.

Diversity – A total of 18 macrofaunal morphospecies were collected, not including nematodes and copepods. Species richness ranged from 0-7 species per core and the average (± 1SE) across the total 24 sediment cores was 3.46 ± 0.42, evenness (J') averaged 0.65 ± 0.06 (Table 2). Five of the cores only had 1 or 0 species. Barrel 450-1, zone A has relatively high values for species richness, evenness, and Shannon index. Barrel 450-2 exhibited a gradual decrease in evenness from zone B to D. Overall, zone A exhibited the highest average for species richness, evenness, and Shannon index (Table 3). Conversely, zone B had the lowest averages for all three diversity metrics (Table 3). The rarefaction index ES (20), calculated for data pooled by zone, also exhibited highest diversity in Zone A and lowest in Zone B (Table 3). Despite these general trends, the diversity did not differ significantly across the zones [One-way ANOVA. Richness (S): $F = 0.553$, $df_1 = 4$, $df_2 = 19$, $p = 0.713$; Evenness (J'): $F = 1.604$, $df_1 = 4$, $df_2 = 19$, $p = 0.225$; Shannon-Wiener (H'): $F = 0.912$, $df_1 = 4$, $df_2 = 19$, $p = 0.477$].

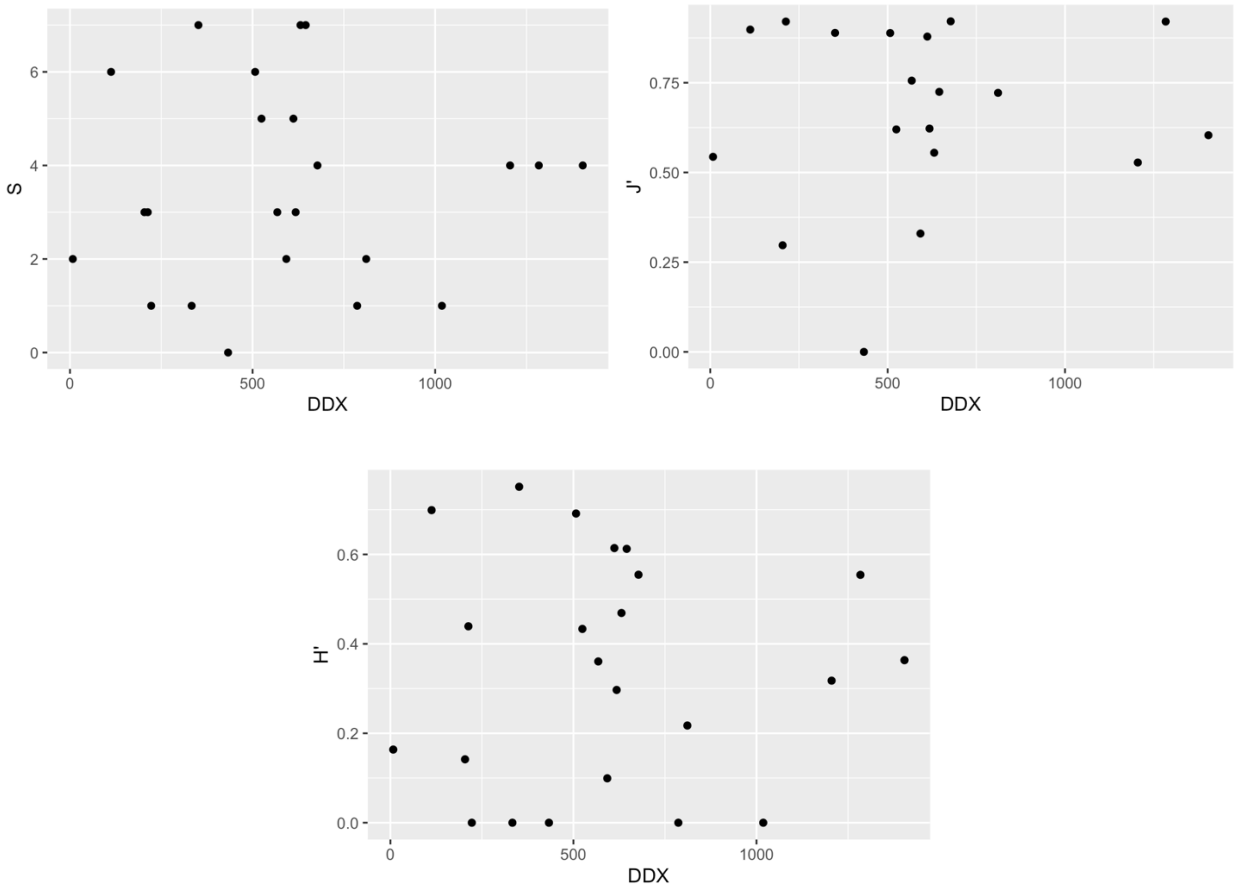

Figure 7. Relationship between macrofaunal diversity indices and max DDX concentration. Top-left: Species richness ($R^2 = 0.0012$, $p = 0.874$); top-right: Pielou's evenness ($R^2 = 0.0048$, $p = 0.779$); bottom: Shannon-Wiener Index ($R^2 = 0.0009$, $p = 0.894$).

Table 2.Diversity metrics for macrofauna (>300 µm) representing each sediment core sample (0-10 cm). Species richness (S), number of individuals (N), Pielou's evenness (J'), Shannon-Wiener (H'), Standard error (SE)

Dive	Pushcore	Barrel	Zone	S	N	Pielou J'	H'(log10)
450	7	450-1	A	7	14	0.889	0.7513
450	5	450-1	B	0	0	0	0
450	3	450-1	C	2	11	0.4359	0.1323
450	1	450-1	D	6	24	0.8886	0.6914
450	16	450-2	A	1	1	***	0
450	14	450-2	B	2	10	0.7219	0.2173
450	12	450-2	C	5	24	0.62	0.4334
450	10	450-2	D	4	24	0.5277	0.3177
450	34	Background	E	4	7	0.9212	0.5546
450	18	Background	E	4	12	0.6038	0.3635
451	6	451-1	B	7	89	0.555	0.469
451	4	451-1	C	3	14	0.7559	0.3607
451	2	451-1	D	5	11	0.8787	0.6142
451	12	451-2	B	1	1	***	0
451	10	451-2	C	1	2	***	0
451	8	451-2	D	1	12	***	0
451	20	451-3	B	2	16	0.5436	0.1636
451	18	451-3	C	4	18	0.9206	0.5543
451	16	451-3	D	2	33	0.3298	0.09929
451	32	451-4	A	6	10	0.8982	0.699
451	27	451-4	B	3	9	0.6224	0.2969
451	24	451-4	C	7	19	0.7247	0.6125
451	22	451-4	D	3	38	0.2971	0.1418
451	14	Background	E	3	6	0.9206	0.4392
			Mean	3.46		0.65	0.33
			SE	0.42		0.06	0.05

Table 3. Diversity metrics by zone; mean and standard error (SE)

Zone	A	B	C	D	E
S					
mean	4.67	2.5	3.67	3.5	3.67
SE	1.86	0.91	0.81	0.70	0.27
J’					
mean	0.89	0.49	0.69	0.58	0.82
SE	0.003	0.11	0.07	0.11	0.08
H’ (log10)					
mean	0.48	0.19	0.35	0.31	0.45
SE	0.20	0.07	0.09	0.11	0.05
ES(20)	9.07	4.37	6.16	5.21	5.56

Table 4. Total infauna counts from 24 pushcores. Totals are presented for traditional macrofauna only and with addition of macrofaunal-size meiofauna.

Macrofauna (>300) Collected at ~ 885 m from San Pedro Basin					
Taxon	**0-1 cm**	**1-2 cm**	**2-5 cm**	**5-10 cm**	**Total**
Annelida					
Ampharetidae		3			3
Chaetopteridae	2				2
Chrysopetalidae	5		2		7
Cirratulidae	7	5	6	8	26
Dorvilleidae	28	12	1	1	42
Hesionidae	3	1	2		6
Paraonidae	10	5	7		22
Polynoidae	4				4
Serpulidae	1		1	1	3
Syllidae		1			1
Entoprocta					
Barentsiidae	190	42	7	17	256
Mollusca					
Bivalvia	1		1		2
Parvaplustrum sp.	11	11	3		25
Porifera	1				1
Nemertina	2		2	1	5
Total	265	80	32	28	405
% of total	65.4	19.8	7.9	6.9	
Nematoda	3276	1296	1178	175	5925
Arthropoda					
Copepoda	104	6	6	1	117
Total	3645	1382	1216	204	6447
% of total	56.5	21.4	18.9	3.2	

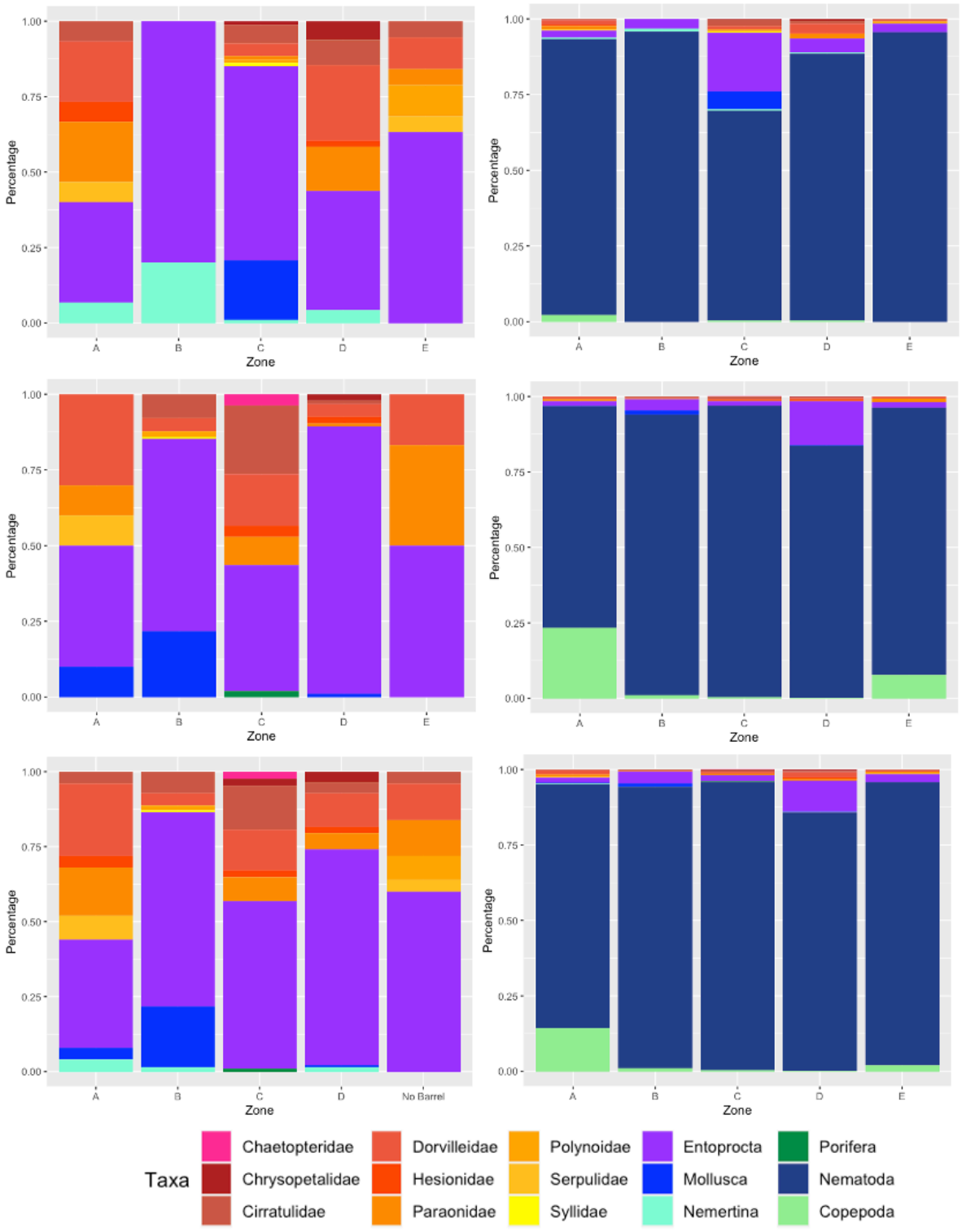

Figure 8. Taxonomic composition (%) of invertebrate communities (> 300 µm) at different zones in San Pedro Basin Dumpsite 2 (~900 m), with traditional macrofauna taxa (left panels) and with the addition of macrofauna-size nematodes and copepods (right panels). Taxon composition is displayed separately for dive 450, dive 451, and both dives combined from top to bottom, respectively.

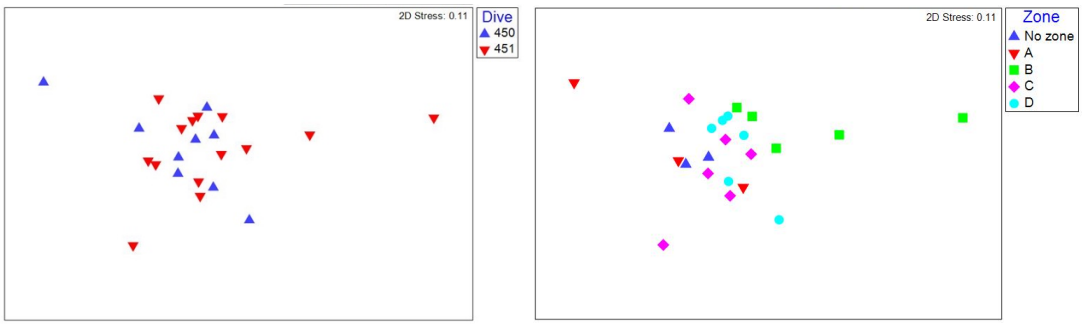

Figure 9. Multidimensional scaling ordination plots of macrofaunal community structure (0-10 cm), by dive (left) and by zone (right) in sediments associated with DDT waste barrels at San Pedro Basin Dumpsite 2 (water depth: ~900 m). Each point represents the macrofaunal community in a single core and proximity of points reflects similarity.

Vertical distribution - The vertical distribution of macrofauna revealed a predominance of taxa residing in the top 1-cm fraction of the sediment samples across all zones (41.7 - 76.0 %) (Fig. 10). In zones B, C, D, and E, the macrofauna in the top 1 cm account for over 50% of all taxa found in those zones. A low percentage of macrofauna was found from 2-10 cm. Upon incorporating nematodes and copepods into the analysis of vertical distribution (Fig. 10), the dominance within the top 1-cm layer appears less pronounced (except in zone B (60.9 %)), though still prominent. Additionally, a more substantial presence of meiofaunal individuals was observed deeper in the sediments, down to 2-5cm (Fig. 11), compared to the distribution observed when nematodes and copepods were excluded (Fig. 10).

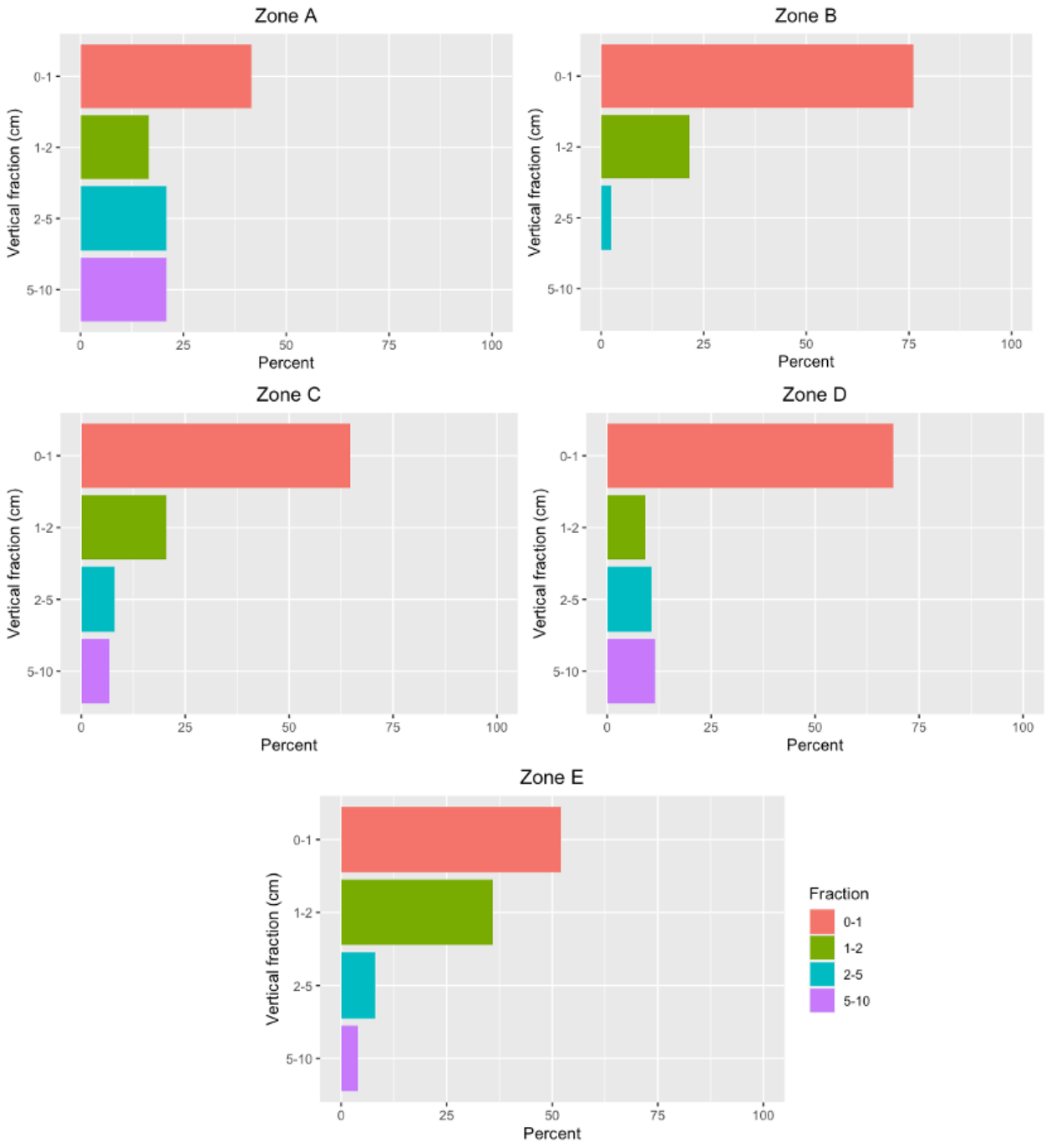

Figure 10. Vertical distribution (%) of total macrofaunal individuals per vertical fraction for each zone. Zone E represents background samples where no barrel was visible.

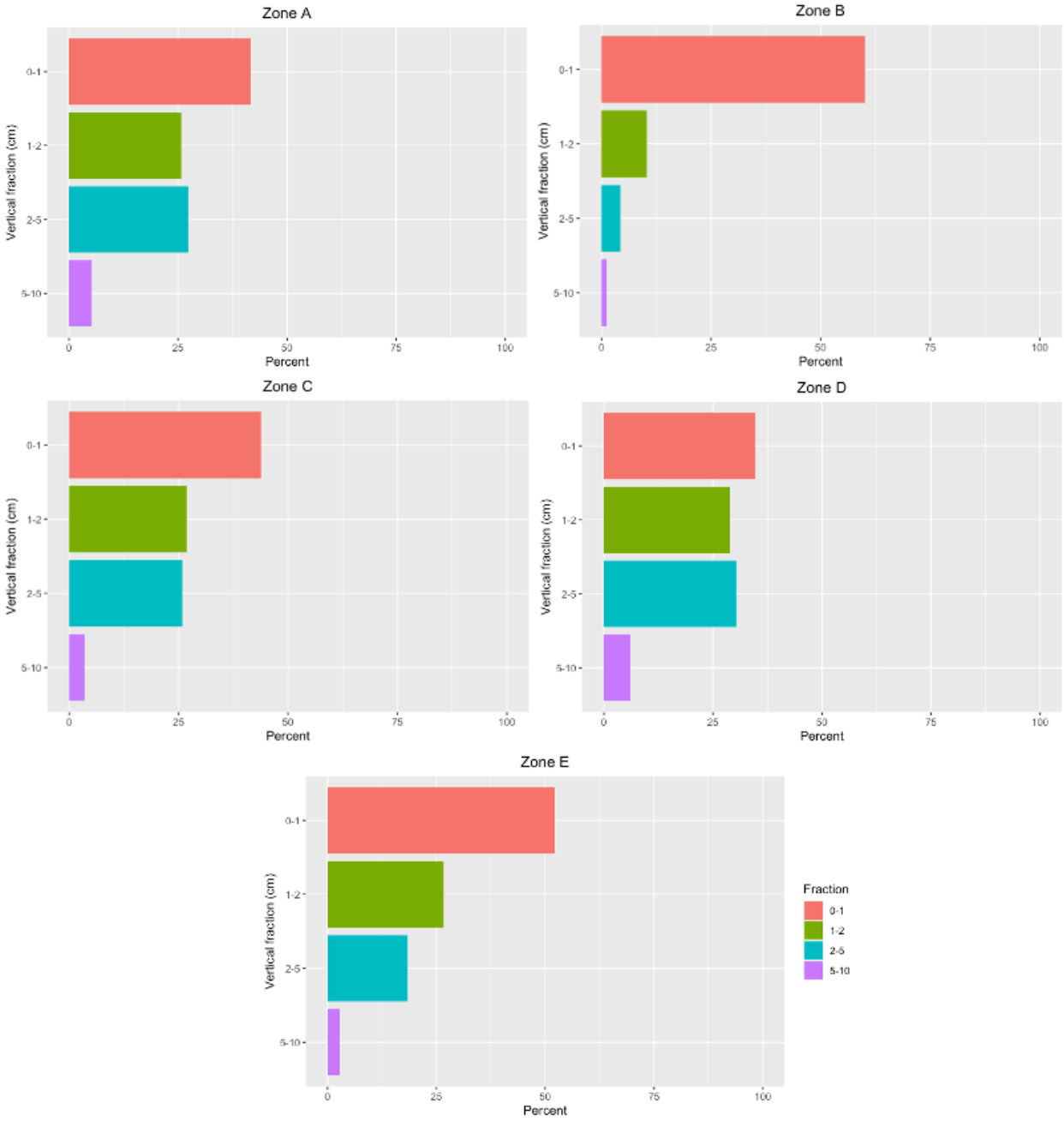

Figure 11. Vertical distribution (%) of total macrofaunal individuals, including macrofaunal-size nematodes and copepods, per vertical fraction for each zone. Zone E represents background samples where no barrel was visible.

DISCUSSION

In this study I set out to determine the extent to which the San Pedro Basin benthic macrofaunal community structure and diversity are impacted by the DDX pollution introduced by waste-barrels.

DDX

Analyzing DDX concentrations in the sediment for each of the barrels and background sites revealed a pattern with peak values typically at the 2-4 cm fraction for Dive 450 or the 4-6 cm fraction for Dive 451. Kivenson et al. (2019) suggests the period of dumping corresponds with the 4-6 cm fraction. This could imply limited bioturbation from the benthic infauna in the 50 years since the barrels were dumped; decades of consistent bioturbation would have disrupted the DDX concentrations in sediment profiles, eliminating the pattern we observe. However, the lower concentrations at the surface could be evidence that bioturbation has been occurring; "diluting" the contaminants with new sediment which can then be resuspended in the water column and eventually transported by currents. Sedimentation rates for this region have been reported to be 15-20 mg cm^2/yr (Gorsline, 1992). It is likely that the presence of barrels created small-scale currents, scour, or sedimentation that typically alters local sedimentation. The depth distribution of DDX appears consistent within each barrel profile (across the zones) aside from Barrel 451-4, where peak DDX concentrations are deeper within the sediment, at 6-10 cm.

Background samples exhibiting similar or higher concentrations of DDX suggests a widespread presence of DDX at the study site, highlighting the other known sources of dumping, including direct dumping to the site and contaminants leaking from punctured barrels on their path to the seafloor. The overall greater decay and destruction seen in barrels during Dive 450 further support this. However, in barrels 450-2 and 451-3, significantly higher maximum

concentrations of DDX could suggest a localized source of contamination, resulting from the nonuniform methods of dumping wastes (Kivenson et al., 2019). These barrels could have contained more waste when they contacted and became lodged in the seafloor decades ago. Additionally, barrels sampled during dive 450 were observed to be significantly more degraded than those seen on dive 451, indicating that they were dumped at different times (Merrifield et al., in review).

Macrofauna >300 μm

Density and vertical distribution - Macrofaunal densities ranged from 1553 ind/m^2 to 11802 ind/m^2 and no significant difference among barrel and background pushcore samples suggests that the DDT-waste barrels do not directly influence macrofaunal density in any considerable way. However, when incorporating meiofauna (>300 μm), total densities increased by an order of magnitude. Despite substantial increase in total density with the addition of meiofauna, there remained no statistical difference between barrel and background densities. This suggests that the DDT-waste barrels do not significantly affect densities of macrofaunal sized meiofauna taxa. The mean macrofauna densities generally increased, with increasing distance from the barrels. In zone E, densities were lower than the zones around the barrels. However, since they were not statistically different, further investigation could reveal the underlying cause of such patterns. The intermediate mean densities observed in zones B and C suggest a possible gradient effect from either the pollutants localized from the barrel or an influence from other environmental factors.

The SPB, at 885 m, technically sits within the Eastern Pacific oxygen minimum zone. The bottom-water oxygen concentration is exceptionally low (< 0.1 ml/l O_2) in the basin

because it's isolated from consistent flushing by a sill and acts as a trap for organic matter, which consumes oxygen through decay. The oxygen concentrations in the SPB are lower than true oxygen minimum zones because of the sill.

A comparison of the SPB macrofauna with the macrofauna of other oxygen minimum zones at equivalent depth reveals that densities are generally lower in the SPB (Fig. 12). For example, in the California, Oregon, Peru, Chile, Oman, and Pakistan margins (800-900 m), densities ranged from approximately 4,100 ind/m^2 - 19,100 ind/m^2, whereas the SPB densities ranged from 1,553 - 11,802 ind/m^2 (Fig. 12). Another difference is the dominance by Entoprocta (63% of total fauna) in the San Pedro Basin (Fig. 8) whereas Annelida typically comprise more than 50% of the macrofauna at the open margin OMZs (Levin et al., 2000; 2002; 2009; 2010; Palma et al., 2005; Gallardo et al., 2005). The vertical distribution of the San Pedro Basin macrofauna (Fig. 10) is similar to the open-margin OMZ macrofauna in being concentrated within the upper 2 cm. There is no clear influence of DDT-waste barrels on vertical distribution of macrofauna. Regardless of the zone, a majority of macrofauna live in the top 2cm which is consistent with previous literature on benthic macro-infauna (Stull, 1996)**.**

Oxygen minimum zones are sometimes considered chemosynthetic ecosystems (Levin, 2003). The presence of the bacterial halo around the barrels sampled in Dive 451 (Fig. 8) raises the question whether the barrels induce chemosynthetic processes. Zone B pushcores in Dive 451 sampled within the bacterial mat halos around the barrels exhibited faunal differences from those sampled in zone B from Dive 450 (Fig. 8). The gastropod *Parvaplustrum* was only found within the zone B bacterial mats. This genus is usually found at methane seeps and whale falls (Valdez, 2019). This raises the possibility that the barrels could be "stepping stones" for chemosynthetic species.

Taxonomic composition – There is no clear influence of DDT-waste barrels on taxonomic composition, however, zones B and C differed from each other (ANOSIM, $p = 0.045$). The presence of sulfide-oxidizing bacterial halos in zone B could be the cause for spatial variation**.** Previous research has shown that the presence of hydrogen sulfide in the sediments can cause lower species diversity and a variation in taxonomic composition compared to sites that lack hydrogen sulfide (Levin et al. 2003). Though no significant differences between the dives were evident, the dive-specific occurrence of nemerteans and the absence of polychaetes in dive 450, zone B could suggest small-scale environmental heterogeneity or just sampling variability.

Diversity- Zone A had the highest diversity among the zones. This could be attributed to the animals living on the barrels, transiting from barrel to seafloor. We hypothesize that zone B has low diversity due to hydrogen sulfide; the bacterial mats in the halos of most zone Bs (Fig. 2) appear to be formed of bacteria that oxidize hydrogen sulfide, which is toxic to aerobic macrofauna. There seems to be no consistent trend across the barrels. Overall, the diversity in the SPB is extremely low. However, the study only sampled (24 cores x 32.2 cm^2). When investigating the possible effects of DDX, the macrofaunal diversity does not seem to be correlated with the DDX concentration ($p < 0.05$) (Fig. 7).

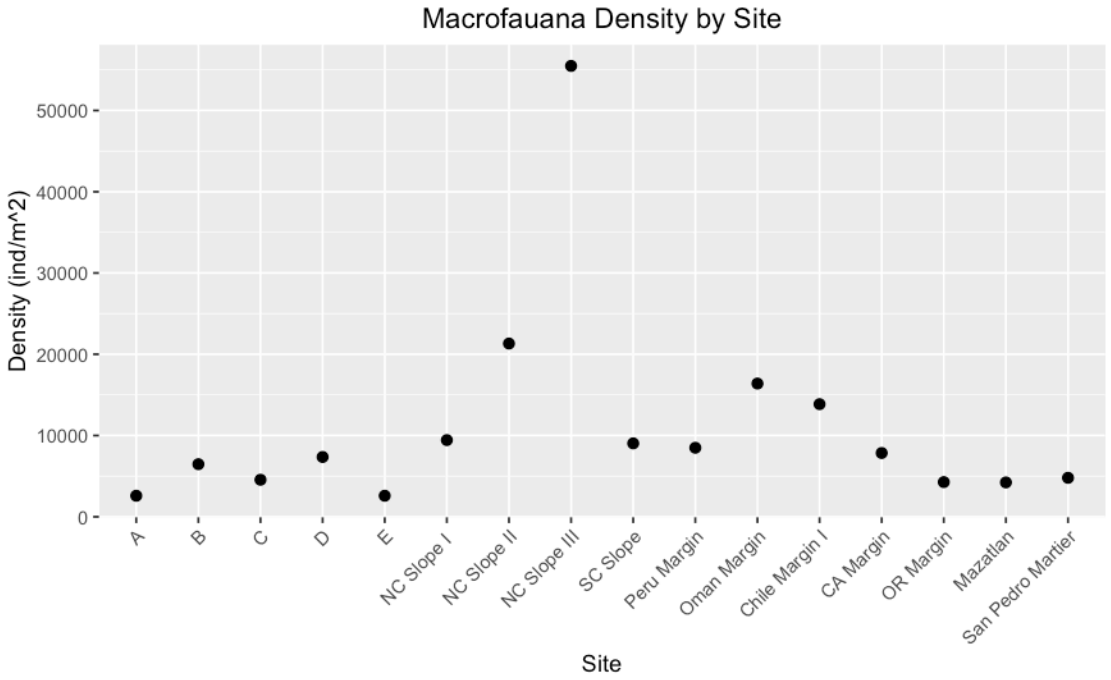

Figure 12. Macrofauna densities of DDT Dumpsite 2 (represented by zones A - E) and other areas of similar depth located within an oxygen-minimum zone. NC: North Carolina (Schaff 1992), SC: South Carolina (Blake and Grasse, 1998), Peru (Levin et al. 2002), Oman (Levin et al. 2000), Chile (Gallardo et al. 2004), CA: California, OR: Oregon (Levin et al. 2009), Mazatlán and San Pedro Martier located in Mexico (Levin, unpublished 2005).

DDX transfer by benthic infauna

Due to the persistence of chemical pollutants in marine sediments and the potential for bioturbation by the benthic macro-infauna, it is important to analyze the macrofaunal community at DDT Dumpsite 2 and their potential transfer of DDX throughout the sediment. Most of the fauna collected for this study are tiny and as such will have limited effects on the movement of DDX throughout the sediment, given their feeding and dwelling modes (Table 5). These animals can alter DDX distribution in several ways including sediment mixing, localizing the contaminant in their tissues, and passing the contaminant through the food chain (Stull et al. 1996). Of the taxa collected, the burrowers have the potential to mix DDX vertically within the sediment. These animals include Paraonidae, Cirratulidae, and Nematoda (Table 5). Notably, the presence of Aphelochaeta (Cirratulidae) in the deep sediments (53.9% were found in 2-10 cm)

suggest they have the highest likelihood among the taxa collected to mix the sediment and alter DDX. The animals that have the greatest potential to localize the DDX within their tissues are those that are sessile suspension feeders such as Porifera, Chaetopteridae, and Serpulidae. Preliminary data of tissue sample chemical analysis (for Serpulidae and Porifera) indicate they possess detectable levels of DDX (C. Neira, personal communications). This implies the source of DDX is in the water (mobilized from sediment resuspension), from the barrel, or from sediment bacteria that enter the water column. Lastly, the motile taxa (Chrysopetalidae, Dorvilleidae, Hesionidae, Polynoidae, Syllidae, Mollusca, and Nemertina), which represent 1.43% of the macrofaunal taxa, are likely to transfer DDX to subsequent trophic levels.

Through bioturbation, nematodes and other small fauna can lead to transport of particulate material and absorbed pollutants from deeper sediment horizons to the surfaces facilitating the loss of contaminants into the water column. Inversely, their reworking activity by feeding and burrowing contributes to the transport of oxygen into the deeper sediment layers, which enhances the microbial-mediated degradation of persistent contaminants (Bradshaw et al., 2006; Schratzberger & Ingels, 2017). Nematodes are by far the most abundant taxa and likely to be the most significant in trophic transfer. Nematodes and the motile macrofauna could be subjected to predation near the surface of the sediments by demersal predators, however, macrofaunal abundance is low at this depth (~ 885 m) due to significantly low oxygen concentrations.

Table 5. List of San Pedro Basin macrofauna life habits, motility, and feeding guilds.

Phylum	Family	Life habit	Motility	Feeding mode
Annelida	Ampharetidae	Tube dweller; burrower	Motile	Deposit feeder
Annelida	Chaetopteridae	Tube dweller	Sessile	Suspension feeder
Annelida	Chrysopetalidae	Free living	Motile	Carnivore; scavenger
Annelida	Cirratulidae	Burrower	Motile	Deposit feeder - subsurface; detritivore
Annelida	Dorvilleidae	Free living; sometimes mucous tube-dwelling	Motile	Microphagous feeder; Bacterivore, facultative carnivore
Annelida	Hesionidae	Free living	Motile; discreetly motile	Bacterivore, Carnivore; sit and wait predator
Annelida	Paraonidae	Burrower	Burrower	Deposit feeder - subsurface; detritivore
Annelida	Polynoidae	Free living	Motile	Carnivore; sit and wait predator
Annelida	Serpulidae	Tube dweller	Sessile	Suspension feeder; some garden methanotrophic symbionts in their tentacles
Annelida	Syllidae	Free living	Motile; discreetly motile	Macrophagous/microphagous feeder; possibly omnivore
Entoprocta	Barentsiidae	Colonial	Sessile	Ciliary sieving; downstream collecting
Mollusca	Bivalvia	Free living	Motile	Filter feeder
Mollusca	Parvamplustrum	Free living	Motile	Predator
Porifera	Porifera	Free living or colonial	Sessile	Filter feeder; possibly carnivorous; possess symbionts
Nemertina	Nemertina	Free living	Motile	Sit and wait predator
Nematoda	Nematoda	Free living	Burrower	Scavenger; deposit feeder
Arthropoda	Copepoda	Free living	Motile; swimmer	Detritivore; bacterivore; herbivore

CONCLUSION

This study contributes to the risk assessment of DDT exposure in humans and ocean life. It is an important step toward understanding the complex interactions between anthropogenic chemical contaminants, specifically DDT and its metabolites, on the benthic macroinvertebrates of the San Pedro Basin. Our findings reveal that DDX, primarily DDE, is widespread within the sediment and not localized solely around the barrels. We found that the barrels have a limited effect on the biota aside from diversity, with patterns of overall high diversity in Zone A and low diversity in Zone B. By assessing the condition of the benthos, these findings highlight the potential pathways for chemical contaminants to enter the food web, which could ultimately affect human life. However, this study was limited in scope. Specifically, our analysis was based on a small sample of barrels, and we know there are thousands present at DDT Dumpsite 2. Despite the limitations, our findings suggest that the barrels are not a major influence on the taxonomic composition and density of the macrofauna. Yet, the slight, but noticeable impact on

diversity as well as the pervasiveness of DDE in the sediment emphasize the importance of continuous monitoring and further research.

Table 6. Macrofaunal counts per sediment core (> 300 μm)

451	451	451	451	451	451	451	450	450	451
451-3	451-3	451-3	451-4	451-4	451-4	451-4	Backgrou	Backgrou	Backgrou
B	C	D	A	B	C	D	E	E	E
SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-269	SCB-281	SCB-305
0	3	0	1	0	0	0	0	1	2
0	0	0	0	0	0	0	0	0	0
0	0	0	2	0	1	2	1	1	1
0	7	0	1	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	6	0	0	1	0	1	0	1	0
0	0	0	1	0	0	0	1	0	0
0	0	0	0	0	2	0	0	0	0
0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	2	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	2	0	0	0	0
0	0	2	0	0	0	0	0	0	0
14	2	31	4	0	11	35	3	9	3
0	0	0	1	0	0	0	0	0	0
2	0	0	0	7	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
307	607	136	221	551	184	115	148	261	140
3	0	0	70	0	4	0	0	0	12

Table 6. (continued)

450	450	450	450	450	451	451	451	451	451	451
450-1	450-2	450-2	450-2	450-2	451-1	451-1	451-1	451-2	451-2	451-2
D	A	B	C	D	B	C	D	B	C	D
SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-	SCB-
0	0	0	2	0	0	0	1	0	2	0
7	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
7	0	0	2	0	1	0	2	0	0	0
5	0	0	0	0	4	0	0	0	0	0
2	0	0	0	2	8	4	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	1	0	0
1	0	0	0	0	0	1	2	0	0	0
0	0	0	1	0	0	0	0	0	0	0
2	0	0	2	1	0	0	0	0	0	0
0	0	8	17	19	59	9	5	0	0	12
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	15	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	2	0	2	0	0	0	0	0	0
148	19	149	220	222	770	704	101	172	174	129
0	0	0	0	1	18	1	1	0	0	0

Table 6. (continued)

Dive	450	450	450
Barrel	450-1	450-1	450-1
Zone	A	B	C
SCB #	SCB-	SCB-	SCB-
Aricidea lopezi	1	0	0
Aricidea antennata	2	0	0
Dorvillea sp.	0	0	0
Parougia sp.	3	0	1
Ophryotrocha sp.	0	0	0
Aphelochaeta sp.	1	0	0
Serpulidae	0	0	0
Chaetopteridae	0	0	0
Syllidae	0	0	0
Polynoidae	0	0	0
Hesionidae	1	0	0
Ampharetidae	0	0	0
Chrysopetalidae	0	0	0
Barentsiidae	5	0	10
Bivalvia (juvenile)	0	0	0
Parvaplustrum sp.	0	0	0
Porifera	0	0	0
Nemertina	1	0	0
Nematoda	185	83	179
Copepoda	5	0	2

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