UTILITY OF THE BROWN ALGA, *PADINA BORYANA*, AS A BIOMONITOR FOR POLYCHLORINATED BIPHENYLS (PCBS) IN TROPICAL MARINE WATERS: A PRELIMINARY ASSESSMENT

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OF THE WESTERN PACIFIC
UNIVERSITY OF GUAM

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ABSTRACT

A feasibility study was conducted to determine if *Padina boyana*, a pan-tropical brown seaweed, could be cultured and transplanted into areas where it does not normally occur, for pollution monitoring and assessment purposes. To this end, *P. boryana* propagules were successfully recruited on newly commissioned polypropylene ropes and allowed to grow *in situ* for 80 days before translocation to a PCB contaminated site for 13 days. Representative plants were subsequently harvested from the ropes at 2-3 day intervals for PCB analysis. Nine PCB congeners were consistently detected in all samples and were accumulated at rates that generally increased with decreasing chlorine content. PCB uptake in the alga was clearly biphasic and believed to reflect two passive processes: a) the rapid adsorption of chlorobiphenyls on external components of *P. boryana* cell walls, and b) the much slower absorption of congeners across cellular membranes into the plant's cytoplasmic interior. The equilibration time and mean steady state PCB (Σ_9PCBs) concentration in the 'fast compartment' was estimated at 4 days and ~11 ng/g dry weight (ppb) respectively. Uptake in the 'slow compartment' was assumed to follow first order kinetics with equilibration time and steady state estimates of >150 days and >200 ppb respectively. The implications of these and other findings of the study are discussed from a biomonitoring perspective. Recommendations for future studies are also presented.

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INTRODUCTION

The Stockholm Convention, signed in 2001, calls for increased global efforts to properly manage and dispose of man-made persistent organic pollutants (POPs) and to ultimately eliminate them from the environment at some point in the not too distant future. As a result, monitoring programs designed to track POPs across international boundaries have become more sophisticated in recent years. Much of this effort has evolved in temperate regions of the world, however, and there remains an urgent need to develop low-cost, reliable and readily implemented methods of evaluating POPs in tropical areas, where many of the world's developing nations exist, and where economic advancement continues to take precedence over environmental concerns. Coral reef communities in such areas are particularly prone to deteriorating water quality conditions associated with inappropriate waste disposal, unregulated sewage discharges and urban runoff. Biomonitoring strategies designed to protect and preserve these fragile ecosystems continue to be hampered by inadequate bioindicator databases, inadequate laboratory facilities, and poorly trained personnel. The work described herein is, therefore, of particular importance because it describes a relatively simple, cheap and effective way of monitoring spatial and temporal changes in PCBs in tropical marine waters using a common algal representative.

The idea of using living organisms as sentinels of POPs, like PCBs, came about in the1960's following the discovery of methyl-mercury, DDT (and related compounds) and PCBs in UK and North American wildlife, and the realization that contaminant concentrations in these organisms were reflective of environmental amounts available to them. Such indicator species are now formerly referred to as 'biomonitors' and, to be of any real value in aquatic POPs monitoring programs, should meet the following requirements put forward by Butler *et al.* (1971), Haug *et al.* (1974) and Phillips (1977):

1. *They should be sedentary in order to be representative of the area from which they are collected*
2. *They should be widely distributed and abundant throughout the study area*
3. *They should be easily recognized, and readily sampled*
4. *They should be of sufficient size to provide adequate tissue for chemical analysis*
5. *They should be relatively long-lived to permit sampling over several months or years*
6. *They should accumulate the pollutant without being killed by the levels encountered in their immediate environment*
7. *They should be amenable to translocation into areas where they do not normally occur*
8. *They should demonstrate a simple correlation between pollutant levels accumulated in their tissues and the average pollutant concentration in the surrounding water*

Biotic representatives commonly employed in POPs marine monitoring programs are fish, crustaceans and bivalve mollusks. This is due in part to the fact that these organisms demonstrate reasonable bioaccumulation capacities for POPs. They are also frequently harvested for human consumption and their contaminant concentrations are therefore important from a human health perspective. Marine algae, by way of contrast, have received relatively little attention from a POPs biomonitoring standpoint, although they have been widely used to determine the distribution and abundance of heavy metals in aquatic environments (Phillips

1980, Burdon-Jones 1982, Levine 1984, Lytle and Lytle 2001). The reason for the current lack of interest in algae as biomonitors of POPs is not immediately obvious despite them satisfying the first six prerequisites listed above. Several algal species are also an economical important food resource in certain countries which would be more than sufficient stimulus to learn more about the affinities of this group for potentially toxic contaminants.

According to some researchers, the passive uptake of PCBs across algal cell membranes may be hampered by the external matrix of the cell wall (Levine 1984, Spacie and Hamelink 1985, Moy and Walday 1996). What little data exists in the literature, however, indicates that at least some algal species have reasonable bioaccumulation capacities for PCBs with reported levels ranging from <10 ng/g dry weight, in representatives from relatively clean waters, to >1000 ng/g dry weight in specimens from highly polluted environments (Amico *et al.* 1979, Maroli *et al.* 1993, Ogden 1997, Hope *et al.* 1998, Denton *et al*. 1999, Schaible 2010).

In Guam, several algal species are commonly found around the island although densities vary according to season, degree of exposure and distance offshore. Nearshore representatives are clearly candidates of choice for further investigation on this subject because the impacts of land-based sources of marine pollution are typically higher in this particular zone.

Padina is one of the more common brown algae found on Guam and extends from low intertidal to shallow subtidal regions in protected back-reef areas and embayments around the island. It has been used fairly extensively for monitoring heavy metals in Guam (Denton *et al* 2006a, 2009a) and elsewhere in the world (Burdon-Jones *et al*., 1982, Denton and Burdon-Jones 1986, Levine 1984, Phillips 1994, Lytle and Lytle 2001, Denton *et al.* 2009b). PCB data for local *Padina* species also exists (Ogden 1987, Denton *et al.* 2006b, Schaible 2010). Examining the suitability of *Padina* spp. for monitoring PCBs in local waters during the current study was therefore seen as a logical extension of the work already completed.

Padina is an easily recognizable genus of benthic algae (Figure 1) and is commonly referred to as Mermaids Fan, Mermaid's Ear, Peacocks Tail or Funnel Weed. The genus belongs to the family: Dictyotaceae and is pantropical, extending as far north as the English Channel (51°N) and as far south as the Bass Strait in Australia (Figure 2). Close to three dozen species of *Padina* currently occur world-wide (Guiry and Guiry 2011) and at least seven of these are found on Guam (Lobban and Tsuda 2003).

A general morphological characterization of several *Padina* species is provided by Geraldino *et al.* (2005). In general, the thallus is 2-cell layers thick at the blade and 3 or more cell layers thick at the stipe, although other strata-morphologies exist. The alga gains sturdy purchase on reef flat substrate by means of a strong rhizoidal holdfast. The frond grows by generating concentric bands of tissue from within the curled collar of the apical edge of the blade. Across the blade, wide rows of sporangial tissue are present as dark circumferential bands and occur parallel to less noticeable rows of hair-cells evident as distinct stripes along the blade.

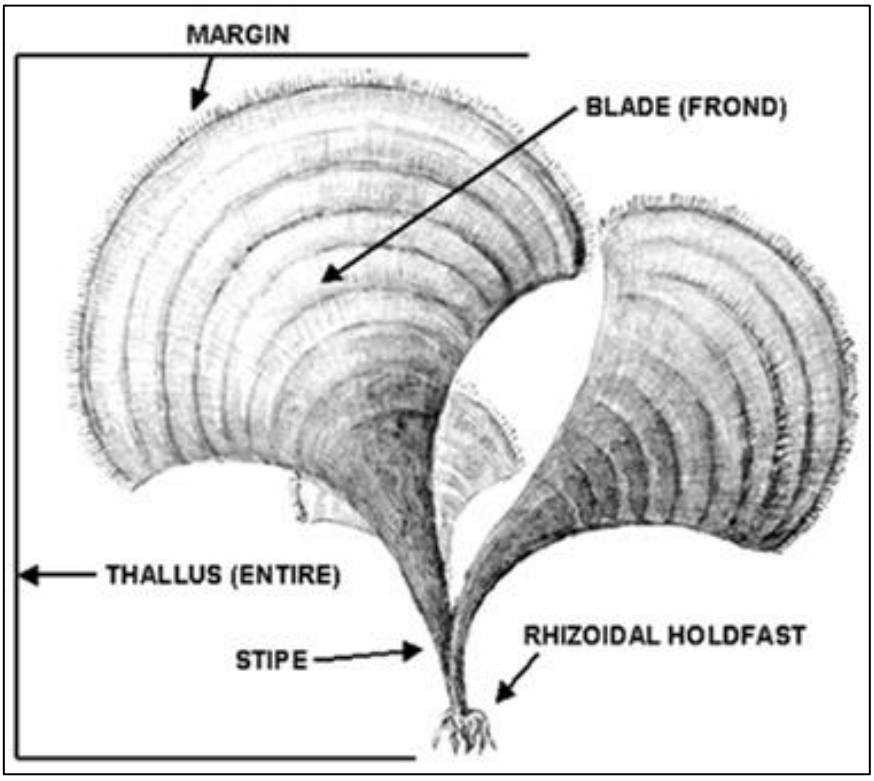

Figure 1: Morphology of *Padina* species (courtesy: http://cutinfo.ru)

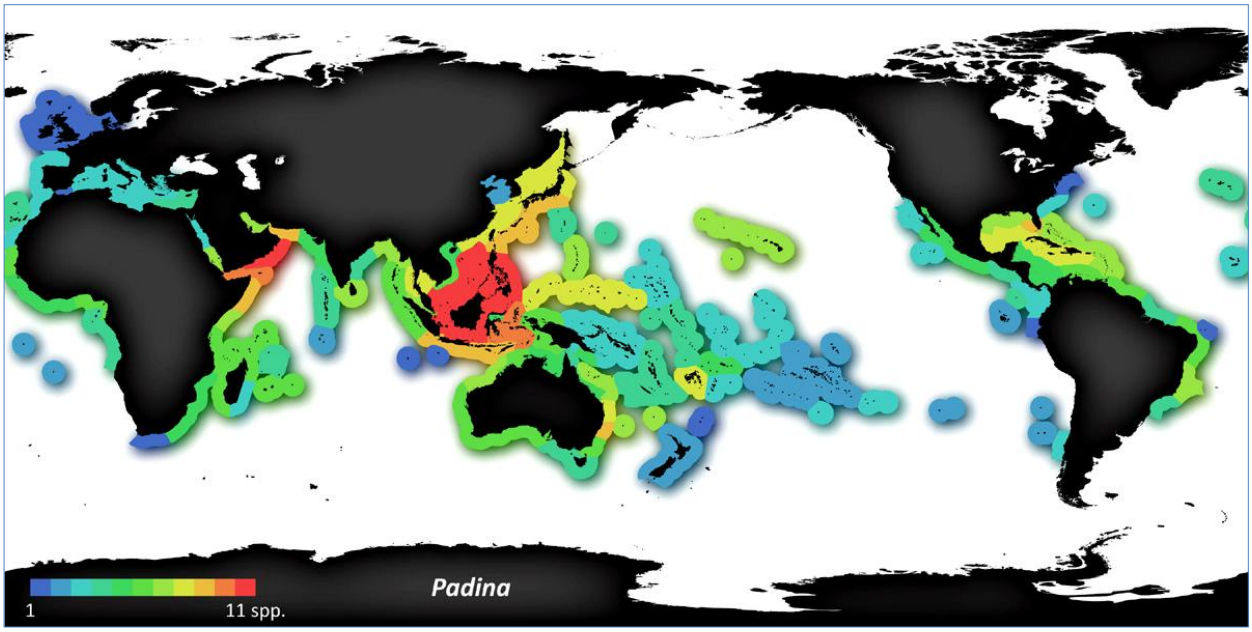

Figure 2: *Padina* species richness and global distribution (courtesy Dr. Tom Schils, Marine Laboratory, University of Guam).

Padina is identified at the species level using light microscopy to observe morphological features including but not limited to reproductive sori, the presence or absence of an indusium (membrane) covering the sori, hair-line positioning relative to sori, and number of cell layers (Abbott and Huisman 2003). The alga has an inner cell wall composed of cellulose and secretes an outer mucilaginous layer consisting of the polysaccharide fucoidans, ascophyllans and fucogalactans (Kloareg and Quatrano 1988). This outer layer provides a medium for the settlement of minerals, microorganisms and sediment (Nassar and Yoneshigue-Valentin 2006). Epibionts including crustose coralline algae, tube dwelling worms, mollusks and micro-crustaceans frequently occur on the blade. While *Padina* spp. can be found on Guam year round, the peak growth seasons occur in the spring (March-May) and again in the early fall (August-October) (T. Schils, pers. comm.).

As candidates for monitoring PCBs and other POPs on Guam, *Padina* spp. have considerable potential, as noted earlier by Denton *et al.* (1999). However, whether these algae have application as a long- or short-term integrator of PCBs in the water column (see Phillips 1980), remains to be determined. Likewise, culturing techniques suitable for their convenient translocation into coastal areas where they do not normally occur, or at least not in sufficient densities for contaminant analysis, have yet to be perfected. Certainly one current draw-back of incorporating these algal representatives into any POPs biomonitoring program for Guam (or anywhere else, for that matter) is that their use is somewhat constrained by their patchy and fluctuating seasonal abundance. Additionally, their growth stages are often very different between sites, depending on water quality and the timing of propagation, and this may be a problem if PCB levels in the algae are accumulated in an age-dependent fashion.

For these reasons the following investigation was undertaken to determine: a) a simple, cheap and reliable means of culturing *Padina boryana* in a convenient form suitable for transplantation studies, and b) the sensitivity and time-integration capacity of *P. boryana* transplants to specific PCB congeners under field conditions.

MATERIALS AND METHODS

STUDY OUTLINE

The experimental work described herein was essentially divided into two discrete phases: Phase 1 focused on developing a simple, convenient and cost-effective means of recruiting *Padina boryana* in a readily transportable form. This part of the study was conducted at Dadi Beach on the western side of central Guam (Figure 3). Phase 2 explored the feasibility of transplanting *P. boryana* recruits into areas where it does not normally occur for pollution and monitoring assessment purposes. To this end, the *P. boryana* recruits were relocated from Dadi beach to the PCB-polluted waters adjacent to Orote Dump, approximately 2.4 km further north. Transplanted samples were periodically removed for PCB analysis in order to evaluate the effectiveness of the species as a bioindicator of these ubiquitous contaminants.

SITE DESCRIPTIONS

Background Site:

Dadi Beach is a relatively sheltered, shallow-water bay adjacent to the US Navy base at the southern end of the Orote Peninsula. The bay has a well-developed fringing reef and is a popular recreational dive and fishing site for civilians and military personnel alike. *P. boryana* is well represented in the nearshore waters attached primarily to coral rubble, boulders and reef pavement. The chosen culturing site was located in the shoreward shallows near the sand bar of the Neye Island Channel at the western end of Dadi Beach, near South Tipalao (Figure 3).

Contaminated Site:

Orote Dump is situated midway along the length of the western Orote Peninsula coastline (Figure 3) and contains a variety of residential, industrial and construction wastes. It is owned by the United States Navy and was operated by them from 1944 to 1969. The waste consolidated inside the dump is protected from surface water infiltration by a low-permeability cover (cap). The cliff-line portion of the dump, consisting of contaminated soil and buried waste, is protected from erosion by a low permeability cap and a concrete seawall. Sub-marine groundwater springs are located to the north and south of the seawall (EarthTech 2004). *P. boryana* transplants were deployed in shallow water towards the southern end of the seawall, near one of the largest dissolution features in the area (Figure 3).

FIELD RECRUITMENT TRIALS

Initial attempts at recruiting *P. boryana* focused on coral rubble, small boulders, cement blocks and ceramic tiles as suitable settlement substrates. This was not particularly successful and moving the bulky substrates from one location to another proved too cumbersome to be of any real value from a transplant perspective. Far greater recruitment success was subsequently achieved with polypropylene rope as follows: ten 3-m lengths of newly commissioned, 13-mm thick, polypropylene rope were anchored above a patch of actively propagating *P. boryana*, with heavy gauge copper wire. The ropes were supported at their center with a small buoy (water displacement volume ~100 ml) and were readily colonized by *P. boryana* propagules from the parent patch. In order to limit competitive colonization by other types of algae and biofouling organisms, rope deployment was timed to coincide with the arrival of new *P. boryana* recruits on nearby pre-cleaned coral boulders. Newly recruited *P. boryana* propagules were inspected on a weekly basis and culled of additional recruits and other unwanted organisms that settled on the

ropes. Earlier experiments indicated an *in situ* growth period of at least 2-months was necessary to provide sufficient *P. boryana* tissue for PCB analysis with adequate replication over the intended experimental period (Schaible, unpublished data). Mean algal densities on the ropes were determined to be approximately 150 plants per meter (Figs. 4 & 5).

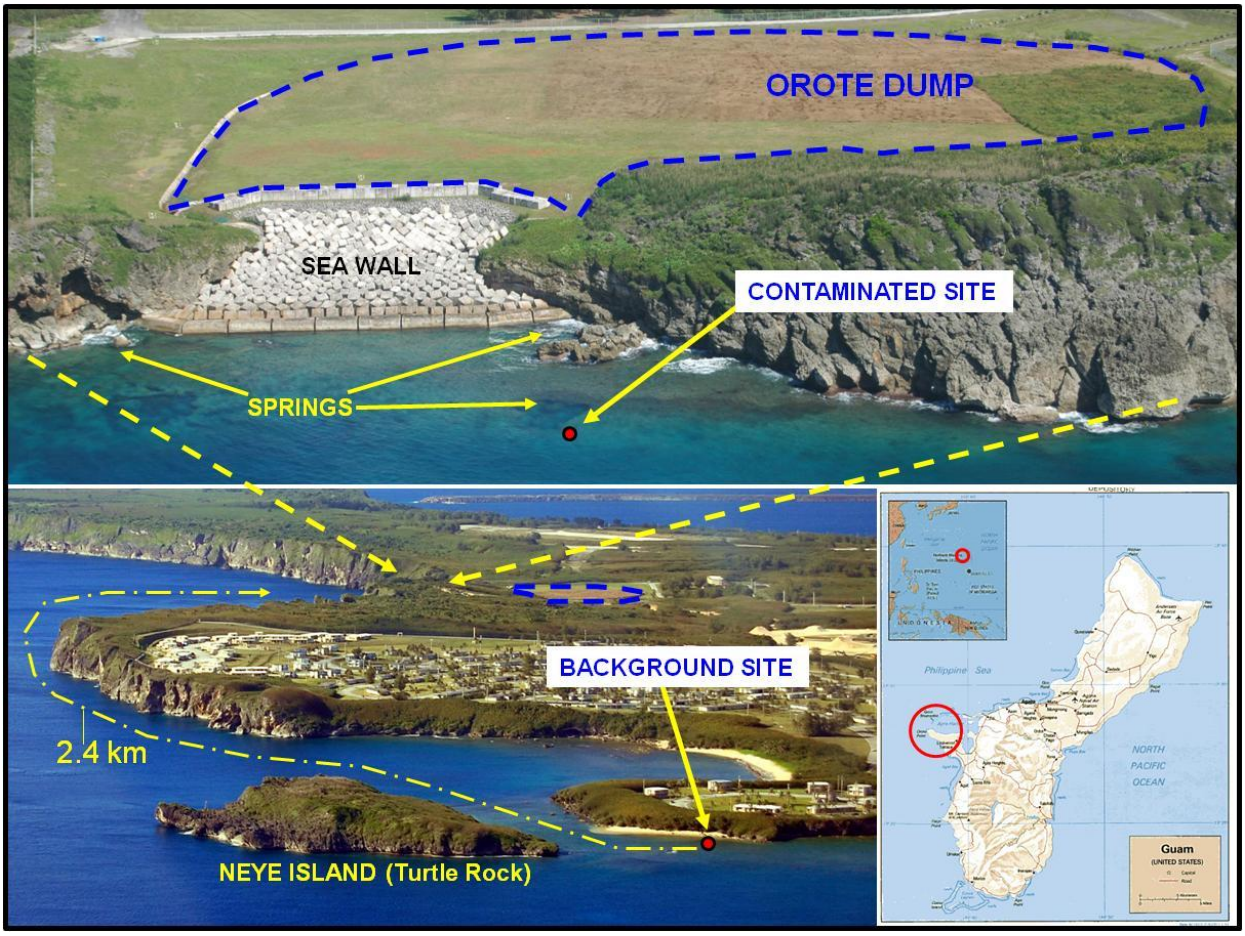

Figure 3: Location of experimental sites along the Orote Peninsula on the western side of the central Guam (insert). Lower and upper frames are high resolution photographs of Dadi Beach (13º24'52"N, 144º35'55"E) and Orote Dump (13º25'47"N, 144º38'17"E) respectively. Position of *P. boryana* recruits (Dadi Beach) and transplants (Orote Dump) relative to the shoreline are indicated by the red dots.

TRANSPLANT FEASIBILITY STUDY

The *P. boryana* transplanted by sea kayak to the Orote Dump seawall were approximately 80 days old, having developed on the culturing ropes at Dadi Beach between late February and early May, 2009. At the contaminated site the algae ropes were suspended inside a buoyant PVC cage floating within the water column approximately 0.5 meters below mean tide level (Figure 6).

The cage was partially pre-fabricated for easy assembly in the field. Joints and cross-bars were pre-glued and pre-drilled. Joints and cross-bars were aligned and stitched with copper wire

through pre-drilled holes. The entire cage was enclosed with 1-cm square nylon mesh to minimize algal losses due to fish herbivory. Weekly cleaning of the nylon mesh was required to remove epiphytes and silt deposits and maintain adequate light for the algal transplants.

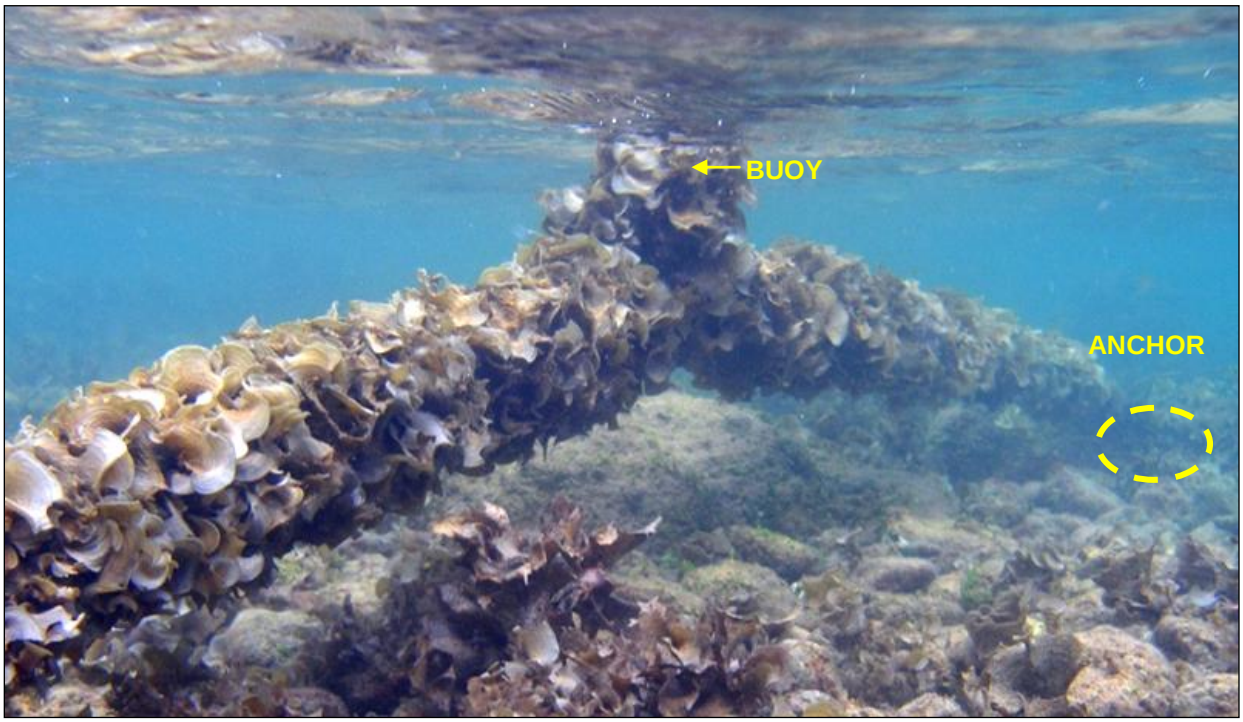

Figure 4: Two-month old *P. boryana* recruits on newly commissioned polypropylene rope deployed at Dadi Beach

Figure 5: Close-up of colonized rope showing young *P. boryana* recruits competing for space with other algal species. Mature *P. boryana* in parent patch shown in background.

SAMPLE COLLECTION AND PREPARATION

P. boryana representatives were sampled for PCB analysis from Dadi Beach immediately before transplanting to the Orote Dump site and thereafter at 2-3 day intervals for 13 days at the contaminated site, as weather and wave conditions permitted. Sampling was conducted between May 11 and May 24, 2009. Unfortunately, the PVC cage was not strong enough to withstand heavy storm surge and suffered irreparable damage with loss of all transplants during inclement weather conditions following day 14.

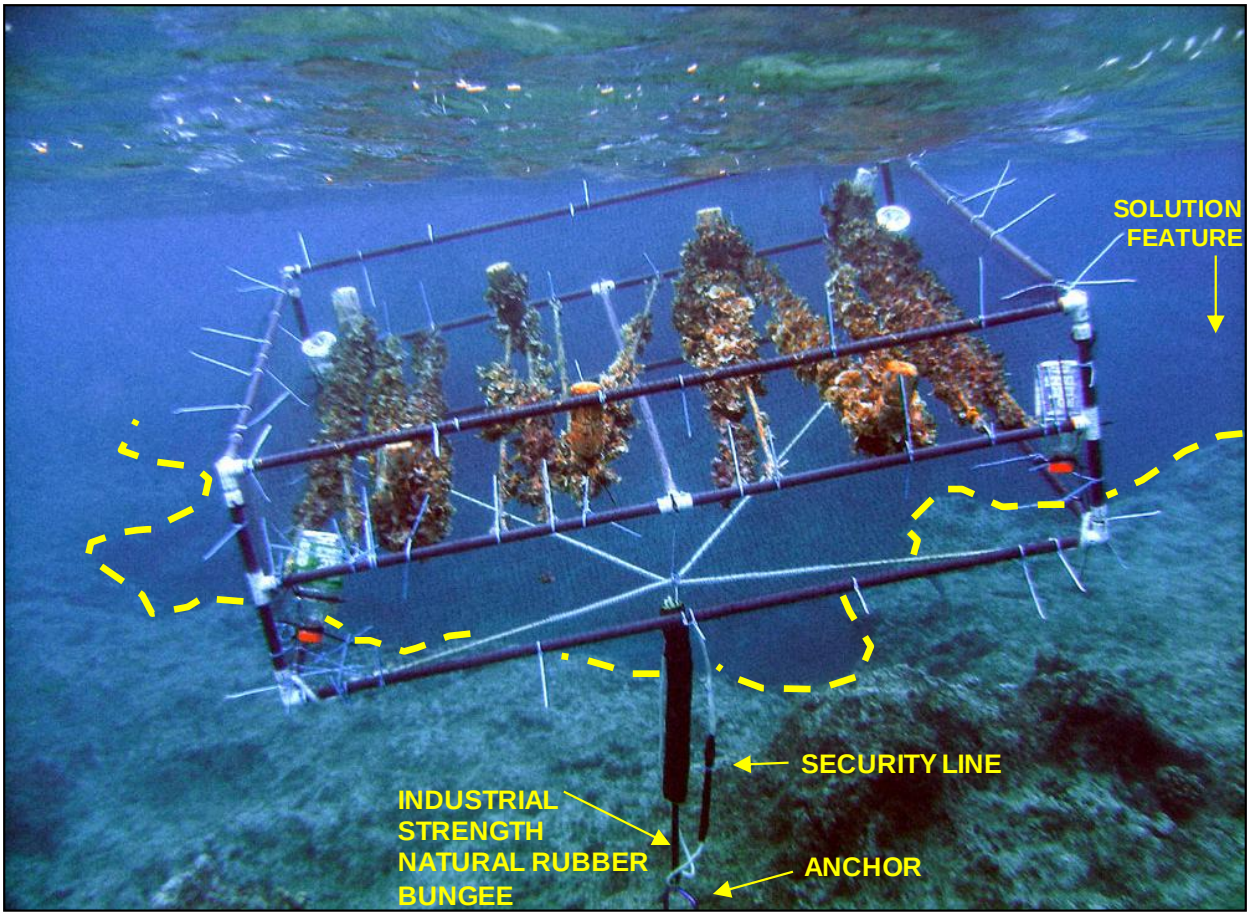

Figure 6: *P. boryana* transplants inside protective Schedule-80 PVC cage covered with 1-cm square nylon mesh to prevent fish herbivory.

All samples taken for analysis were immediately placed in a chilled container and transported by sea kayak back to the lab. Here they were cleaned, weighed and prepared for analysis. *P. boryana* collected at each sampling event were pooled and subsequently divided on a haphazard basis into six replicate groups for analysis. Individual fronds were cleaned of epibionts and sediment under a stream of filtered seawater. All stipe and holdfast materials were discarded. Thus prepared, the samples were stored in pre-cleaned labeled polyethylene bags at –10° C prior to freeze drying for 48 hours.

The dried samples were homogenized within their respective polyethylene bag by gently crushing between finger and thumb. The resulting algae flakes were subsequently sieved through a 1 mm mesh stainless steel screen. The fraction passing through the sieve was assumed to contain residual sedimentary material and discarded. The fraction retained by the sieve was further pulverized and re-sieved. On this occasion, the fraction passing through the screen was retained for analysis and generally weighed between 1.5 and 2.5 g.

SAMPLE ANALYSIS

PCB Extraction:

PCBs were extracted from the dried *P. boryana* samples using an accelerated solvent extraction unit (*Dionex: Model ASE 200*). Each stainless steel extraction cell was pre-loaded with a cellulose fiber filter, 6 g of activated alumina and a glass fiber filter in that order. This combination of filters and alumina provided an initial separation of extracted PCBs from interfering coextractants in the algal tissue. Approximately 2.5 g of pulverized algal tissue was then weighed into each extraction cell followed by a 10 µl spike of the surrogate standard PCB 103 (2,2',4,5',6-pentachlorobiphenyl, final concentration 100 ng/ml) to allow corrections for final recovery. The cells were then topped off with pre-baked (400°C) diatomaceous earth and sealed. Accelerated solvent extraction was performed with 30 ml of analytical grade n-hexane at a pressure of 1500 psi and 150^{o}C.

The sample extracts were concentrated to 0.7 ml under a gentle stream of purified air (molecular sieve) using the *Zymark Turbo Vap II* set to 35°C and twice extracted with an equal volume of concentrated sulfuric acid to remove any remaining coextractants. Following gentle centrifugation to separate the two immiscible layers, the lower-density, upper solvent fraction was quantitatively removed to pre-calibrated blow-down vials using a disposable glass Pasteur pipette. The extracts were reduced to a final volume of 0.20 ml prior to spiking with 10 µl of the 2,000 ng/ml internal standard, pentachloronitrobenzene (final concentration 100 ng/ml) to correct for losses associated with instrument variability. The final extracts were then loaded into 200 µl glass inserts of gas chromatographic septum vials ready for analysis.

PCB Quantification

All PCB analyses were conducted at the Water and Environmental Research Institute of the Western Pacific (WERI) laboratory using the Varian 3800 GC equipped with ECD and outfitted with a *TRACE® TR5-ms* fused-silica capillary column (60 m long, 0.25 mm internal diameter, 0.25 µm film thickness). The procedures outlined herein essentially followed USEPA Method 8082.

Each batch of samples prepared for analysis consisted of seven *P. boryana* samples and one method blank (pre-baked diatomaceous earth). A series of *P. boryana* matrix PCB spikes (100 µg) and mussel tissue (*Perna perna*) standard reference material (SRM 2977, National Institute of Standards and Technology) replicates were also analyzed periodically to ensure method accuracy and precision. PCBs were quantified against a 20-PCB conger calibration-check solution (AccuStandard®). These are listed in Table 1 and represent a range of PCB homologues (dichloro- through decachloro-biphenyls), and a range of environmentally relevant congeners both from an abundance and toxicological perspective (NOAA 1993a,b).

Table 1: PCBs in Calibration Standard Solution (AccuStandard® C-CCSEC).

IUPAC PCB Congener Number[a]	Environmental Prevelance (%)[b]	Dominant in these Commercial Aroclors[c]	Chlorine Atoms/Mol	Structural Arrangement
8	7	1221, 1242	2	2,4’
18[d]	22	1016, 1242	3	2,2’,5
28[d]	17	1016, 1242	3	2,4,4’
44[d]	29	1242, 1254	4	2,2’,3,5’
52[d]	31	1242, 1254	4	2,2’,5,5’
66[d]	10	1254	4	2,3’,4,4’
77[e]	10	none	4	3,3’,4,4’
101[d]	36	1254, 1260	5	2,2’,4,5,5’
105[d,e]	15	none	5	2,3,3’,4,4’
118[d,e]	27	1254, 1260	5	2,3’,4,4’,5
126[e]	5	none	5	3,3’,4,4’,5
128[d]	22	none	6	2,2’,3,3’,4,4’
138[d]	31	1254, 1260	6	2,2’,3,4,4’,5’
153[d]	37	1254, 1260	6	2,2’4,4’,5,5’
170[d]	27	1260	7	2,2’,3,3’,4,4’,5
180[d]	25	1260	7	2,2’,3,4,4’,5,5’
187[d]	22	none	7	2,2’,3,4’,5,5’,6
195	15	none	8	2,2’,3,3’,4,4’,5,6
206	10	none	9	2,2’,3,3’,4,4’,5,5’,6
209	9	none	10	2,2’,3,3’,4,4’,5,5’,6,6’

[a]Numbering system for each PCB congener developed by the International Union of Pure and Applied Chemistry (IUPAC); [b]Prevalence data from McFarland and Clarke (1989); [c]Aroclor data from De Voogt *et al.* (1990); [d]Major component of environmental mixtures (NOAA 1993); [e]Congeners are considered significantly dioxin-like(dl) by the World Health Organization, and are therefore potentially very toxic (Van den Berg *et al.* 2005); [f]grey highlight indicates congeners readily isolated as major stand-alone peaks in *P. boryana* during the present study.

Nine of the 20 congeners present on the calibration standard were readily isolated as stand-alone peaks exhibiting little to no overlap with neighboring peaks and were selected for study here. They are also identified in Table 1 and represent Cl-3, Cl-4, Cl-5, Cl-6, and Cl-7 homolog groups.

Congener recoveries from matrix spikes ranged from 72-106% and were considered acceptable (Table 2). Recoveries from the marine mussel homogenate (NIST 2977), while within acceptable limits for the lower molecular weight PCBs (43%-173%), were relatively low for some of the higher molecular weight congeners (25-62%). Professional analysts in environmental laboratories typically achieve recoveries >40% using EPA Method 8082. However, it is not uncommon for matrix effects to lower PCB recoveries in certain types of

environmental materials, particularly sediments and biota (R. Burpee, Owner Manager of MEE Environmental Laboratory, pers. comm.).

Table 2: PCB Recoveries (ng/g dry weight) from Matrix Spikes and Marine Mussel Standard Reference Material (NIST SRM 2977)

IUPAC PCB Congener Number	Matrix Spike[a]	NIST-SRM 2977	
	Mean (Range)	Certified Mean (± 95% C.L.)	This Study Mean (Range)
8	82.8 (80.5-85.2)	1.99 (1.85-2.13)	0.86 (0.74-0.99)
18	80.9 (67.2-94.6)	-	-
28	77.3 (71.5-83.1)	5.17 (4.81-5.53)	8.92 (8.83-9.29)
52	89.9 (73.0-106)	8.02 (7.46-8.58)	7.76 (7.49-7.96)
44	85.4 (72.3-98.5)	3.22 (3.01-3.53)	2.88 (2.84-2.92)
66	104 (87.4-120)	3.55 (3.37-3.73)	2.87 (2.81-2.92)
101	97.0 (79.7-114)	10.6 (9.70-11.5)	6.10 (6.06-6.15)
77	83.9 (69.1-98.7)	-	-
118	88.5 (79.5-97.4)	10.0 (9.56-10.4)	6.15 (6.08-6.22)
153	90.3 (76.9-103)	14.1 (12.8-15.4)	6.20 (6.07-6.27)
105	98.4 (84.5-112)	-	-
138	106 (88.8-123)	7.94 (7.31-8.57)	3.74 (3.67-3.77)
187	83.9 (72.8-94.9)	4.47 (4.15-4.79)	1.52 (1.45-1.61)
128	94.5 (79.3-109)	2.38 (2.10-2.66)	0.60 (0.53-0.70)
180	76.1 (64.8-87.4)	6.32 (5.61-7.03)	1.87 (1.83-1.91)
170	78.9 (64.8-92.9)	2.74 (2.39-3.09)	0.78 (0.76-0.80)
195	74.3 (61.2-87.4)	-	-
206	72.3 (57.2-87.4)	-	-
209	73.2 (64.2-82.1)	-	-

[a]10 µL of 2 µg/mL C-CCSEC Standard (20 ng) in final extract volume of 200 µL. Dashes indicate no data.

Analytical Detection Limits

Analysis of chromatograms of algae samples spiked with low level of PCB congeners yielded the following analytical detection limit: 0.03 ng/g (PCBs 170 and 187), 0.05 ng/g (PCB 180), 0.06 ng/g (PCBs 52 and 101), 0.07 ng/g (PCB 44), 0.10 ng/g (PCBs 18, 28, and 153). These limits are an order of magnitude lower than those reported by NOAA NS&T and U.S.EPA Method 8082 for sediments and biota.

STATISTICAL ANALYSIS

Statistical analysis was conducted on the 'total' PCB data set (Σ_9PCBs) to determine significant differences in accumulation over time. The Levene's test found general equality of variance

($p>0.05$) amongst all groups analyzed. Based on this finding, parametric tests were employed. A *One-way Analysis of Variance* (ANOVA) was performed separately on the data for each PCB congener to determine significant differences between concentration means in *P. boryana* tissues over time (i.e., 0, 2, 4, 7, 9, 11 and 13 days). The *R-Stat* software (Version 2.13.1) developed by the *R-Project* was used for the ANOVA. The *Bonferroni-Holm Posthoc Multiple Comparisons Test* (MCT) was used to determine which time groups differed significantly from one another.

RESULTS

Concentrations of the nine PCB congeners consistently found and measured in *Padina boryana* transplants at the Orote site over the 13 day exposure period are listed in Table 3 and expressed as ng/g dry weight. Zero-time data were taken from cultured specimens at Dadi beach immediately before deployment to the contaminated site. All data are graphically presented for individual congeners in Figures 8 through 16 and for the sum of all nine congeners (Σ_9PCBs) in Figure 17. A typical *P. boryana* chromatogram obtained during the latter half of the exposure period is shown in Figure 7 and is compared with the 20 congener reference standard containing the surrogate standard, PCB-103, and the internal standard, PCNB.

It is clear that PCB levels increased in *P. boryana* over time despite some considerable variability between replicates. However, the ANOVA and MCT tests revealed a significant difference ($p \leq 0.05$) between 0 time and day 13 data-sets only.

Accumulation rates varied appreciably between congeners over the exposure period with lower chlorinated members generally exhibiting higher maxima than the heavier congeners. Based on mean values alone, PCBs 28 (Cl_3), 52 (Cl_4) and 101 (Cl_5) were almost always the three most dominant congeners detected in the *P. boryana* transplants. These were generally followed by PCBs 18 (Cl_3), 44 (Cl_4) and 153 (Cl_6). The three heptachloro congeners, PCBs 170, 180 and 187 consistently ranked among the least accumulated chlorobiphenyls.

It is noteworthy that the shape of the polynomial regression lines fitted to the data-sets was similar for all congeners (Figures 8 through 16). The general pattern can be described as an initial rapid accumulation phase between day 0 and day 2, followed by a general leveling off between day 2 and day 7. Then a second uptake phase is observed between days 7 and 13 (Figure 17).

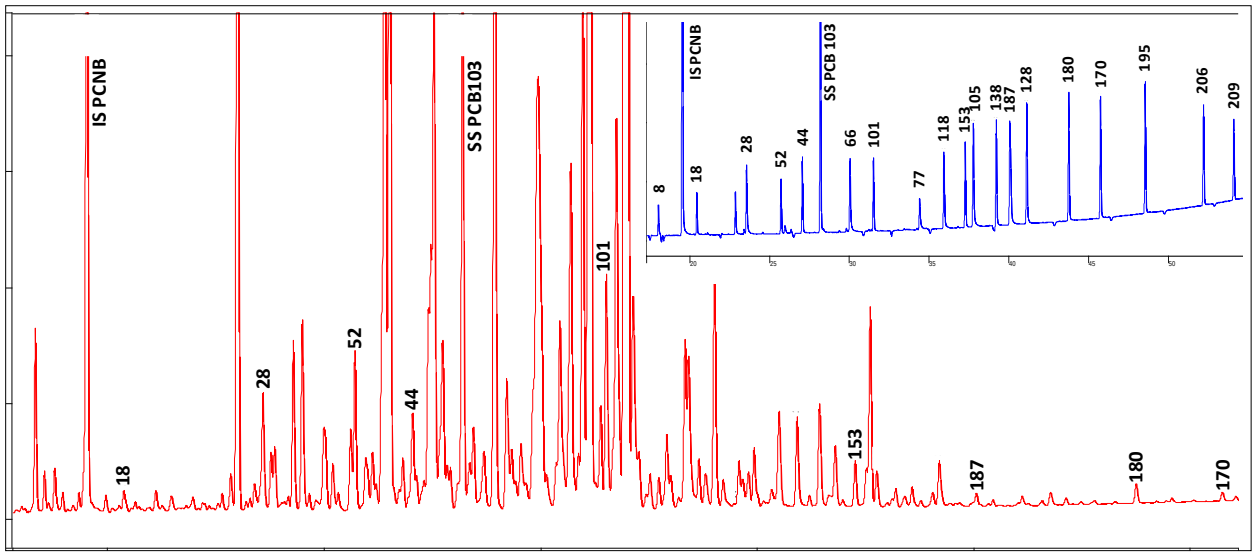

Figure 7: Typical chromatograph of PCBs extracted from *P. boryana* during the present study. Inset shows AccuStandard® (C-CCSEC) calibration standard chromatogram

Table 3: PCB Congener Concentrations (ng/g dry weight) in *P. boryana* Over the 13-Day Exposure Period

IUPAC PCB #	Chlorine Atoms/mol	Replicate Number	Dadi Beach	Orote Seawall					
			Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 13
18	3	1	0.76	1.07	1.30	1.08	0.93	1.19	1.49
18	3	2	1.23	1.20	1.82	1.30	1.14	1.13	1.48
18	3	3	0.72	0.83	1.03	1.59	1.30	1.33	1.56
18	3	4	0.05	0.03	0.77	1.33	1.14	1.15	1.39
18	3	5	0.99	1.11	1.18	1.24	1.37	1.54	1.21
18	3	6	0.03	ND	ND	ND	ND	ND	1.78
28	3	1	1.30	2.13	1.94	2.00	1.55	2.11	2.32
28	3	2	1.10	0.86	1.57	0.78	0.73	1.11	1.55
28	3	3	2.08	2.81	3.30	3.48	3.45	3.21	3.46
28	3	4	1.27	1.87	2.00	2.23	1.96	2.28	2.39
28	3	5	1.46	1.91	1.54	1.12	1.72	1.76	2.97
28	3	6	2.22	2.61	3.39	3.39	4.07	3.88	4.59
52	4	1	1.40	2.08	2.50	2.98	2.92	2.57	2.81
52	4	2	1.52	1.41	2.09	1.48	1.25	1.84	2.19
52	4	3	2.25	2.94	3.59	5.56	4.01	4.50	5.25
52	4	4	1.45	1.90	2.16	3.16	2.25	2.75	2.90
52	4	5	1.62	2.11	1.72	1.43	1.86	1.75	4.37
52	4	6	2.28	4.41	2.95	2.95	4.24	5.18	4.68
44	4	1	0.59	0.87	0.96	1.05	0.98	0.92	0.99
44	4	2	0.96	0.80	1.20	0.86	0.62	1.03	1.12
44	4	3	0.95	1.32	1.47	2.07	1.70	1.82	2.06
44	4	4	0.53	0.69	0.81	1.08	0.84	0.98	0.98
44	4	5	1.12	1.40	1.15	1.38	1.34	1.19	1.44
44	4	6	0.78	1.40	0.94	0.94	1.60	1.66	1.74
101	5	1	2.19	3.43	3.88	2.87	3.48	2.84	2.93
101	5	2	1.80	1.75	2.60	1.52	1.78	2.17	2.96
101	5	3	3.65	4.77	4.66	5.64	4.68	5.48	5.86
101	5	4	2.19	2.50	2.66	2.90	2.40	2.83	2.50
101	5	5	1.87	2.70	1.68	1.52	2.18	1.91	3.32
101	5	6	2.36	4.16	2.38	2.38	5.10	3.16	3.20

Table 3 (continued): PCB Congener Concentrations (ng/g dry weight) in *P. boryana* over the 13-Day Exposure Period

IUPAC PCB #	Chlorine Atoms/mol	Replicate Number	Dadi Beach	Orote Seawall					
			Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 13
153	6	1	0.43	0.67	0.88	0.67	0.74	0.83	0.84
153	6	2	0.38	0.61	0.81	0.67	0.91	1.21	1.48
153	6	3	0.65	1.05	0.70	0.78	0.74	1.29	1.05
153	6	4	0.53	0.55	0.46	0.56	0.57	0.79	0.61
153	6	5	0.42	0.76	0.54	0.70	0.75	0.74	0.58
153	6	6	0.28	0.73	0.33	0.33	1.20	0.48	0.51
187	7	1	0.06	0.04	0.10	0.14	0.13	0.22	0.19
187	7	2	0.03	0.14	0.19	0.22	0.32	0.51	0.41
187	7	3	0.02	0.03	0.05	0.05	0.06	0.18	0.11
187	7	4	0.03	0.04	0.04	0.09	0.10	0.19	0.11
187	7	5	0.03	0.09	0.11	0.29	0.16	0.17	0.09
187	7	6	ND	0.11	0.04	0.04	0.18	0.10	0.11
180	7	1	0.09	0.11	0.23	0.35	0.23	0.48	0.44
180	7	2	0.07	0.24	0.31	0.40	0.55	0.88	0.76
180	7	3	0.08	0.09	0.12	0.19	0.17	0.49	0.31
180	7	4	0.09	0.09	0.10	0.21	0.24	0.34	0.24
180	7	5	0.03	0.19	0.17	0.36	0.30	0.28	0.16
180	7	6	ND	0.24	0.08	0.08	0.43	0.21	0.22
170	7	1	0.03	0.06	0.11	0.16	0.12	0.22	0.20
170	7	2	0.02	0.11	0.15	0.18	0.27	0.44	0.34
170	7	3	0.04	0.05	0.05	0.10	0.08	0.23	0.14
170	7	4	0.04	0.04	0.04	0.08	0.09	0.14	0.10
170	7	5	0.03	0.12	0.10	0.18	0.15	0.15	0.09
170	7	6	ND	0.07	0.04	0.04	0.21	0.10	0.10
ΣPCB 18			0.63	0.85	1.22	1.31	1.18	1.27	1.49
ΣPCB 28			1.57	2.03	2.29	2.17	2.25	2.39	2.88
ΣPCB 52			1.75	2.47	2.50	2.93	2.75	3.10	3.70
ΣPCB 44			0.82	1.08	1.09	1.23	1.18	1.27	1.39
ΣPCB 101			2.34	3.22	2.98	2.80	3.27	3.07	3.46
ΣPCB 153			0.45	0.73	0.62	0.62	0.82	0.89	0.85
ΣPCB 187			0.03	0.08	0.09	0.14	0.16	0.23	0.17
ΣPCB 180			0.07	0.16	0.17	0.26	0.32	0.45	0.35
ΣPCB 170			0.03	0.07	0.08	0.12	0.15	0.21	0.16
Σ_9PCBs			7.71	10.7	11.0	11.6	12.1	12.9	14.5

ND = PCB congener concentration in *P. boryana tissues* were below the analytical detection limit

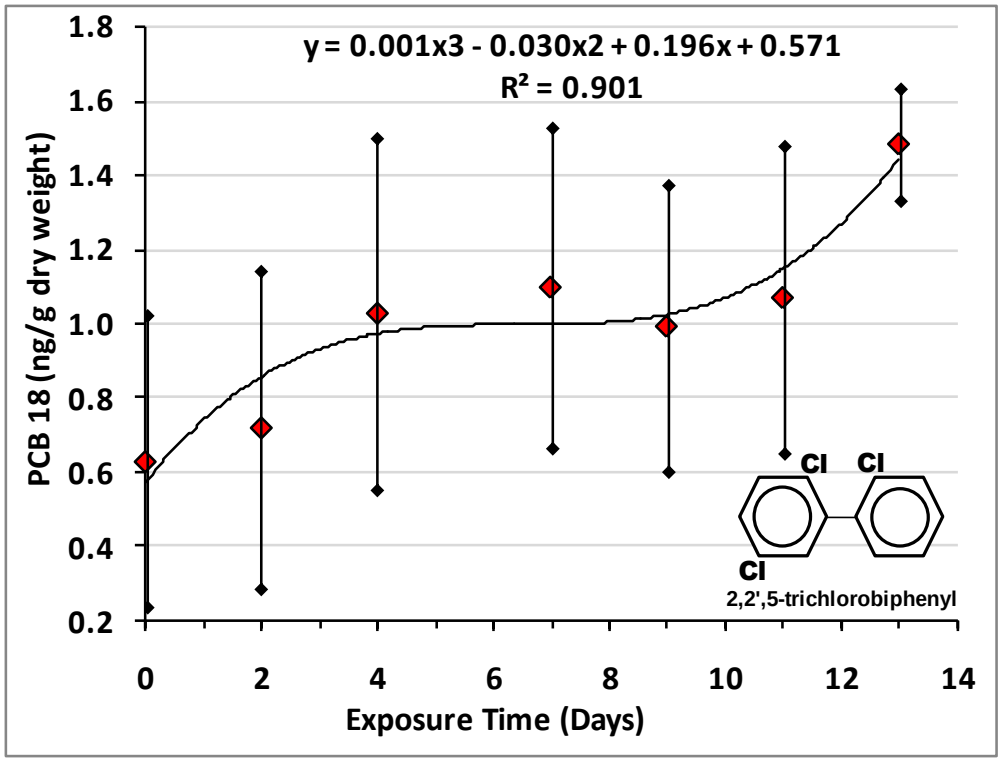

Figure 8: Uptake of PCB 18 in *P. boryana*. Error bars represent 95% C.L.

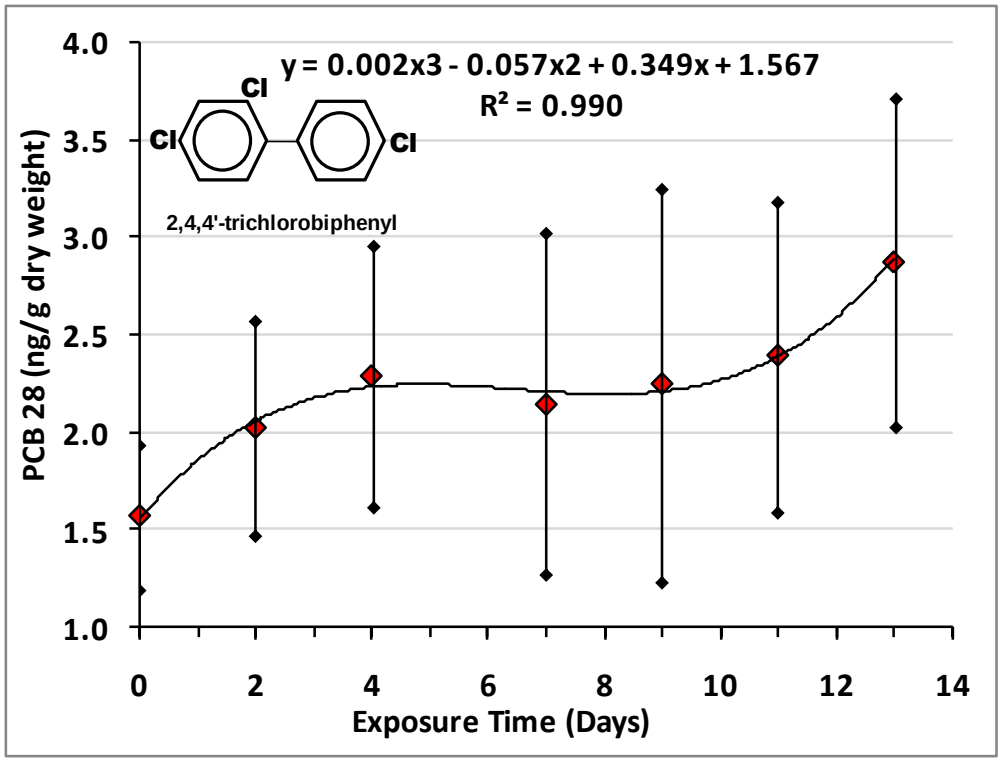

Figure 9: Uptake of PCB 28 in *P. boryana.* Error bars represent 95% C.L.

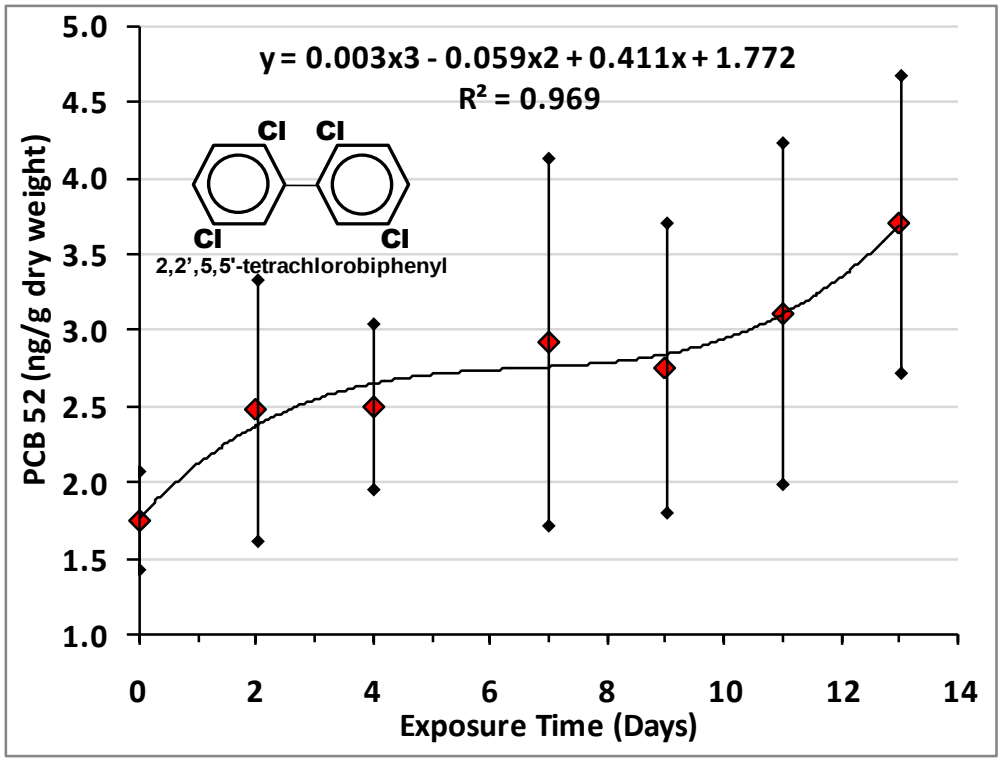

Figure 10: Uptake of PCB 52 in *P. boryna.* Error bars represent 95% C.L.

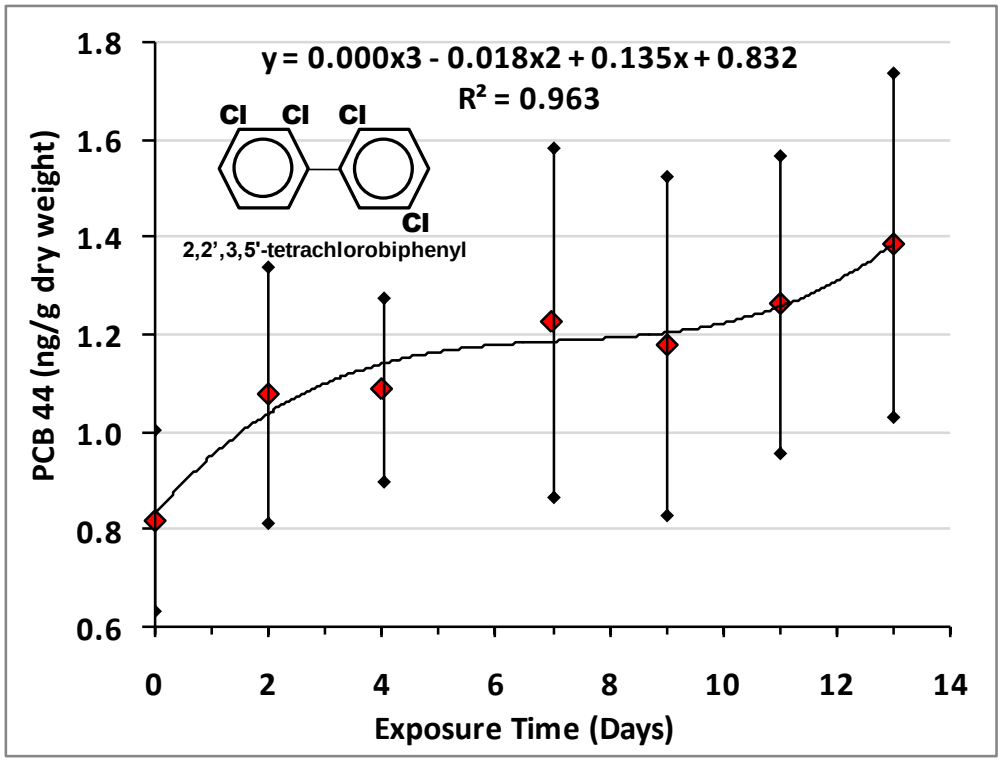

Figure 11: Uptake of PCB 44 in *P. boryana.* Error bars represent 95% C.L.

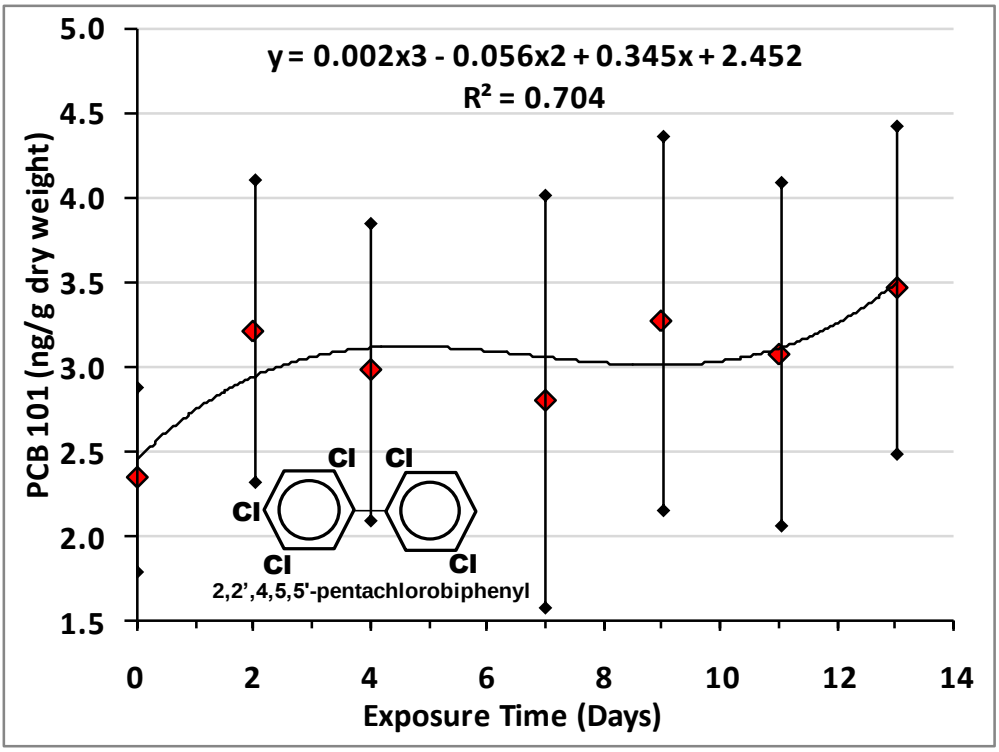

Figure 12: Uptake of PCB 101 in *P. boryana.* Error bars represent 95% C.L.

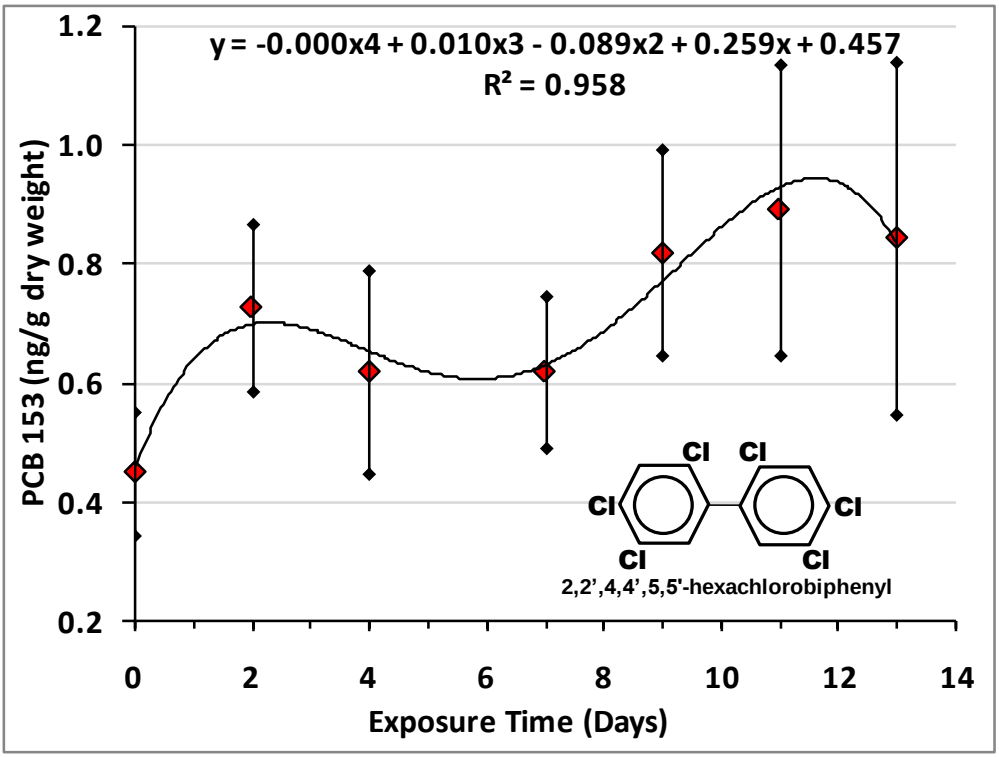

Figure 13: Uptake of PCB 153 in *P. boryana.* Error bars represent 95% C.L.

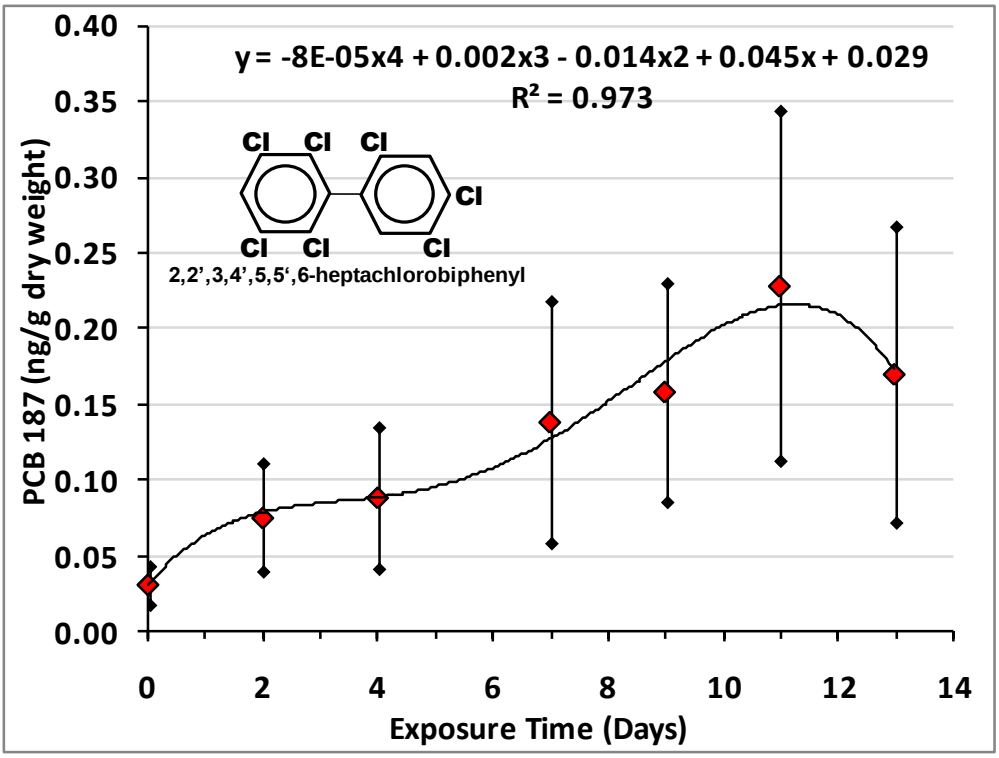

Figure 14: Uptake of PCB 187 in *P. boryana.* Error bars represent 95% C.L.

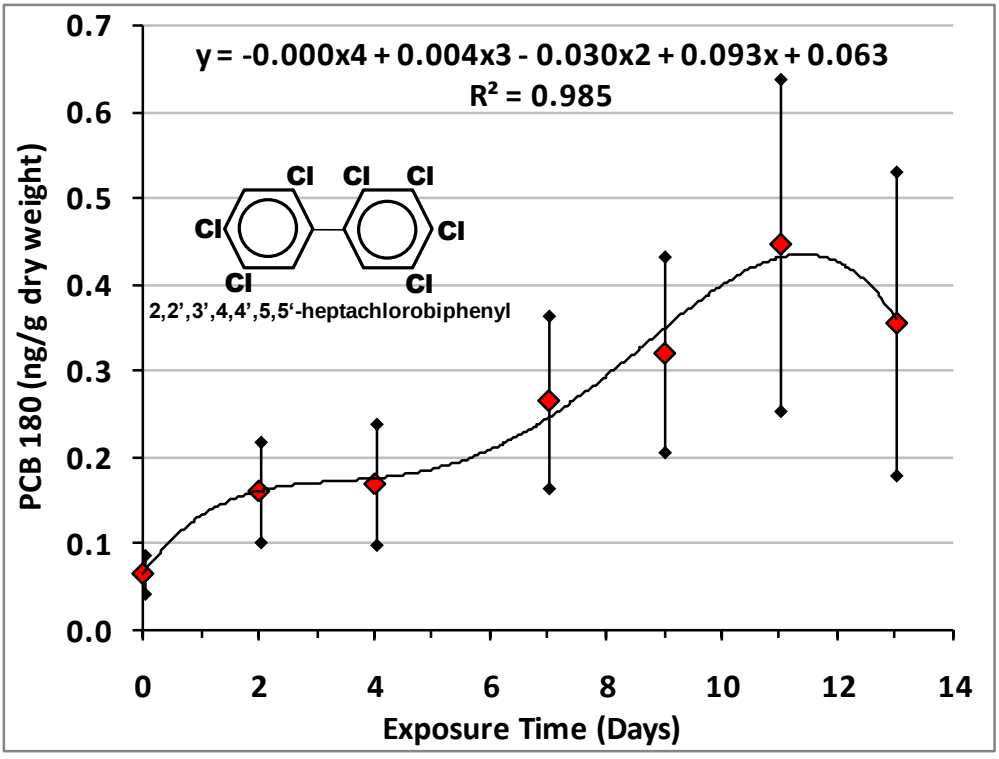

Figure 15: Uptake of PCB 180 in *P. boryana.* Error bars represent 95% CL.

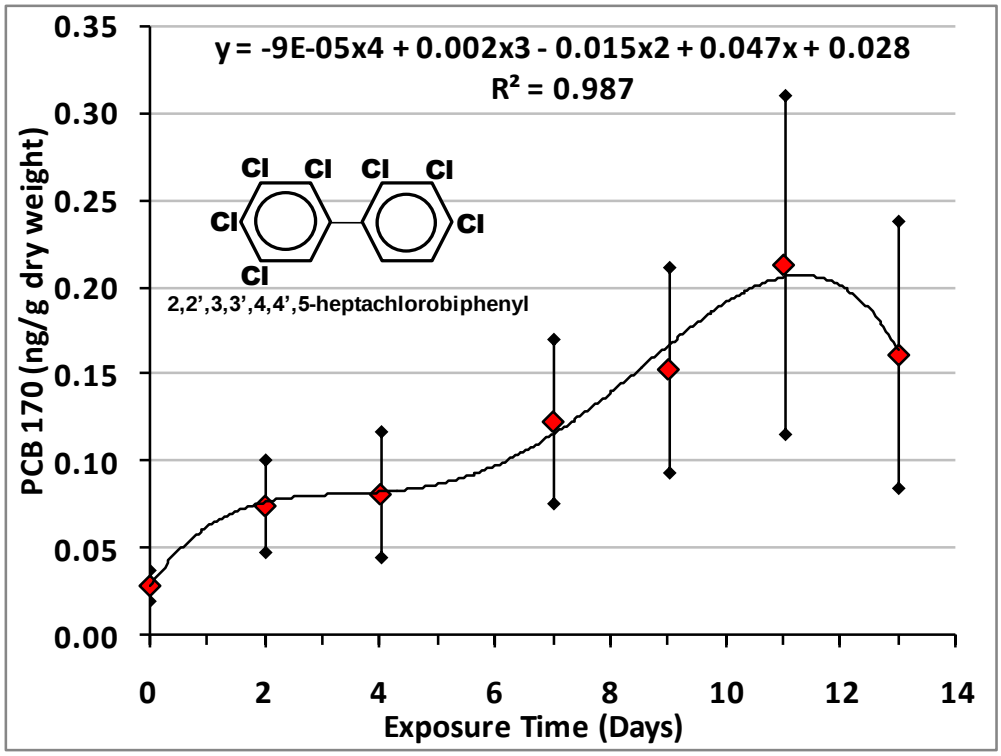

Figure 16: Uptake of PCB 170 in *P. boryana.* Error bars represent 95% CL.

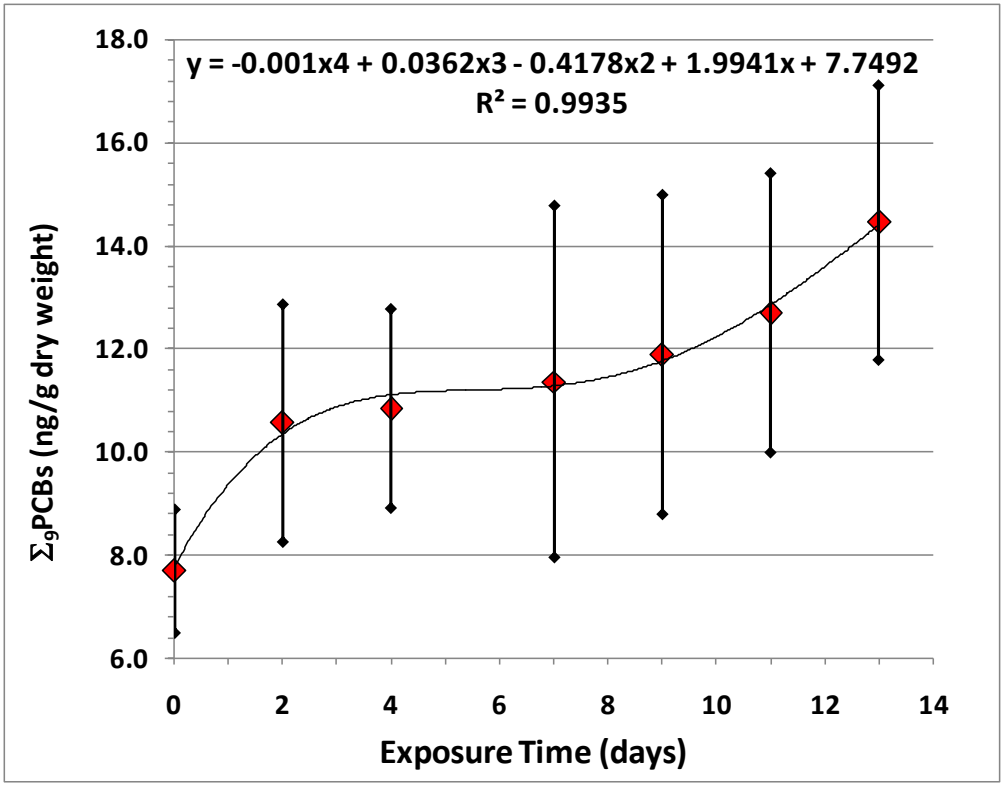

Figure 17: Total Σ_9PCBs uptake in *P. boryana.* Error bars represent 95% CL.

DISCUSSION

TOTAL PCBS IN MARINE ALGAE

While macroalgae have been used extensively as aquatic bioindicators of heavy metal pollution (Phillips 1980, Burdon-Jones 1982, Levine 1984, Lytle and Lytle 2001), their ability to reflect ambient changes in PCB concentrations has received relatively little attention (Denton *et al.* 1999). What limited information exists, certainly suggests that marine macroalgae have the ability to accumulate PCBs to levels several orders of magnitude above background (Hope *et al*. 1998). Selected studies are summarized in Table 4 for comparative purposes. From these data, it may be inferred that PCB concentrations in algae from relatively clean environments are less than 10 ng/g dry weight (ppb) when expressed as total Aroclor. In sharp contrast, specimens from highly contaminated waters can yield values in excess of 1000 ppb. It is noteworthy that one of the highest levels recorded in Table 4 is for *Padina sanctae-crucis*, from Guam. This particular sample was taken adjacent to the Orote dump site several months before remediation efforts were implemented (Ogden 1997). Total PCB concentrations (Σ_{20}PCBs) in the alga approached 600 ppb. This is approximately equivalent to 1200 ppb total Aroclor (NOAA 1989). Interestingly, Schaible (2010) reported values an order of magnitude lower in the same species collected from the same location shortly after the dumped had been capped in 2000. In the current study, the maximum Aroclor equivalent concentration found in *Padina boryana* transplants at this site was almost an order of magnitude lower again (Table 4). Whether or not these findings reflect a marked attenuation of PCBs in the area over the last decade or so, remains a matter of some debate and will be referred to again later in the discussion.

CONGENER ABUNDANCE IN MARINE ALGAE

With the advent of high resolution gas chromatography in the 1980s, researchers focused more on quantifying individual PCB congeners in environmental matrices and less on reporting levels as Aroclor equivalent concentrations. This analytical improvement has provided a much better understanding of the dynamics of PCB transport in the environment (McFarland and Clarke 1989). PCBs released into the environment, partition between environmental compartments (air, water, soil and biota) at rates determined largely by the vapor pressures and water solubilities of the individual congeners. As a general rule, water solubility and volatility decreases with increased chlorine content of the PCB molecule. Because of this, the lower chlorinated congeners are generally more abundant in air and surface waters (Tanabe and Tatsukawa 1986, Bright *et al.* 1995), while the less mobile, higher chlorinated members tend to preferentially accumulate in soils and sediments (De Voogt 1990, Thompson 1996). This fractionation process becomes more apparent over time and distance from PCB sources.

Marine algae, by virtue of their morphological and physiology characteristics, reflect levels of dissolved PCBs available to them in the water column (Amico *et al.* 1979, Maroli *et al.* 1993). As a consequence, they tend to preferentially accumulate the lower chlorinated homologues (Amico *et al.* 1982, Denton *et al.* 1999). Notable exceptions to this general rule apply when algae are collected close to active PCB sources. For example, the earlier work of Schaible (2010) noted congener profiles identical to that of Aroclor 1260 in *P. sanctae-crucis* collected near the Orote dump site. His data provided clear evidence of continued PCB discharges in that particular area and emphasized the utility of marine algae in differentiating between weathered and fresh PCB inputs.

In the current study, the higher chlorinated congeners (Cl_6-Cl_7) consistently ranked among the least abundant PCBs (Table 5) in *P. boryana* before and after translocation to the Orote site. There was, however, a marginal increase in their relative abundance at the end of the 13-day exposure period, which is suggestive of continuing PCB inputs at this site. Nevertheless, a comparative analysis of congener abundance with values calculated from the earlier Ogden study (Ogden 1997) provides strong evidence for PCB attenuation at the Orote site, at least over the experimental exposure period (Table 5). Whether this picture persists during wet season conditions, when groundwater intrusion from the Orote peninsula is maximal, remains to be determined.

INTERPRETATION OF UPTAKE DATA

It is generally believed that PCB uptake in marine algae is principally driven by surface adsorption kinetics with congeners attaching to extracellular binding sites (alginic acids and polysaccharides) on exposed parts of the plant (Levine 1984, Moy and Walday 1996). According to Spacie and Hamelink (1985), algal cell structure does not allow the passive transport of organic molecules much larger than 100 g/mol. If this holds true for all algae, then PCBs, which have molecular weights ranging from 200 to 500 g/mol, are effectively excluded from intracellular storage compartments within algal cells.

The findings of the current study certainly support the surface adsorption hypothesis, which is typically characterized by rapid accumulation to steady state conditions within 1-2 days (Levine 1984, Moy and Walday 1996). Interpreting the second rise in PCB concentrations noted in *P. boryana* during the latter part of the exposure period (Figure 17) is a little more difficult and requires consideration of a range of physical changes in the environment as well as biological processes at work in seaweed itself. Some possibilities are considered below.

Climatic Factors:

Since the source of PCB contamination at the Orote site is land-based, it is reasonable to assume that groundwater flows provide the primary transportation mechanism to adjacent coastal waters. Groundwater intrusion into the ocean occurs all along the Orote peninsula although flow rates vary substantially between wet and dry seasons. Be that as it may, there was no significant rainfall immediately before or during the exposure period. Thus, the chance of any net increase in PCB delivery rates to the exposure site over the experimental period seems most unlikely.

Wave and wind conditions certainly affect suspended solid loadings in the water column and this in turn can affect the soluble PCB fraction both qualitatively and quantitatively. In this regard, it is noteworthy that high winds and rough seas were observed at Orote on days 5 and 6, while on either side of this window, conditions were relatively calm. Because of the differential partitioning processes described earlier, any net increases in PCB availability associated with water turbulence would have produced congener profiles in the water column dominated by higher chlorinated biphenyls. An analysis of relative congener abundance in *P. boryana* harvested at each sampling interval showed no evidence of this. On the contrary relative abundances of all 9 congeners varied by less than 10% over the entire exposure period with the great majority varying by less than 5%. These data strongly suggest ambient congener concentrations were stable over the entire exposure period.

Table 4: Published PCB Concentrations in Marine Macroalgae from Guam and Other Parts in the World

Species	Location	PCBs (ng/g dry wt)	Relative Environmental Enrichment	Quantification Standard	Reference
Chlorophyceae					
Codium tomentosum	Priolo, Cicily	591[a]	Highly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Ulva intestinalis	Priolo, Cicily	107[a]	Highly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Ulva intestinalis	Castelluccio, Cicily	78[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Ulva sp.	Alberoni, Venice	3[a]	Relatively Clean	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Ulva rigida	Castelluccio, Cicily	36[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Ulva sp.	San Giuliano, Venice	14 - 25	Mildly Contaminated	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Ulva sp.	Alberoni, Venice	1[a]	Relatively Clean	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Ulva sp.	Sacca Sessola, Venice	3 - 8	Relatively Clean	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Phaeophyceae					
Colpomenia sinuosa	Priolo, Cicily	279[a]	Highly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Cystoseira fimbriata	Priolo, Cicily	105[a]	Highly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Cystoseira fimbriata	Castelluccio, Cicily	58[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Desmarestia sp.	Antarctica	1 - 4	Relatively Clean	Σ_{12}PCBs	Montone *et al.* 2001
Dictyota acutiloba	Midway Atoll	47[a]	Mildly Contaminated	Σ_{20}PCBs	Hope *et al.* 1998
Dictyota sp.	Alberoni, Venice	13[a]	Relatively Clean	Aroclor 1242:1254 (1:1)	Maroli *et al.* 1993
Asteronema breviarticulatum	Midway Atoll	30[a]	Mildly Contaminated	Σ_{20}PCBs	Hope *et al.* 1998
Halopteris scoparia	Catania, Cicily	70[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Padina boryana	Orote Dump Seawall, Guam	8 - 14	Mildly Contaminated	Σ_{9}PCBs	This Study
Padina pavonica	Castelluccio, Cicily	100[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Padina sanctae-crucis	Orote Dump Seawall, Guam	570[a]	Highly Contaminated	Σ_{20}PCBs	Ogden 1997
Padina sanctae-crucis	Orote Dump Seawall, Guam	2 - 101[b]	Mildly Contaminated	Aroclor 1260	Schaible 2010
Padina sp.	Guam Harbors	2 - 16[b]	Relatively Clean	Σ_{20}PCBs	Denton *et al.* 2006
Rhodophyceae					
Gracilaria sp.	San Giuliano, Venice	11 - 20	Mildly Contaminated	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Gracilaria sp.	Alberoni, Venice	7[a]	Relatively Clean	A.1242:A.1254 (1:1)	Maroli *et al.* 1993
Hypnea musciformis	Castelluccio, Cicily	105[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Liagora viscida	Catania, Cicily	37[a]	Mildly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Pterocladiella capillacea	Priolo, Cicily	110[a]	Highly Contaminated	A.1248:A.1254 (1:1)	Amico *et al.* 1979
Porphryra sp.	Alberoni, Venice	5[a]	Relatively Clean	Aroclor 1242:1254 (1:1)	Maroli *et al.* 1993

[a]Mean data only; [b]Original data converted to dry weight assuming 90% water content; [c]Σ_9PCBs and Σ_{20}PCBs may be converted to total Aroclor equivalent concentrations using multipliers of 4 and 2 respectively (Valoppi et al. 1998, NOAA 1989, ACE-EPA 1992).

Table 5: Relative Abundances of PCB Congeners in *Padina* species from Orote Dump Site

PCB No	Chlorine Atoms/Mol	% of Σ_9PCBs (Rank Order of Abundance)[a]		
		June 1995 (Ogden 1997)[b]	May 2009 (This Study)[c]	
		Orote Dump Seawall	Orote Dump Seawall	Dadi Beach
		pre remediation	post remediation	background
101	5	3.5 (6)	27.0 (1)	30.5 (1)
52	4	1.5 (7)	24.1 (2)	22.8 (2)
28	3	0.8 (8)	19.7 (3)	20.5 (3)
44	4	0.7 (9)	10.2 (4)	10.7 (4)
18	3	18.4 (4)	8.7 (5)	8.2 (5)
153	6	19.0 (3)	6.2 (6)	5.8 (6)
180	7	25.3 (1)	2.1 (7)	0.8 (7)
187	7	19.6 (2)	1.1 (8)	0.4 (8)
170	7	11.3 (5)	1.0 (9)	0.3 (9)

[a]Average abundance = [Σ (% of Σ_9PCBs represented by each congener in each sample)]/total number of samples;
[b]*P. sanctae-crucis* analyzed in 1997; [c]*P. boryana* examined during the current study.

Higher suspended solid loads in the water column on days 5-6 could also have increased the amount of sediment adhering to *P. boryana* during the latter half of the experiment. Given the nature of the rigorous cleaning and preparatory process, however, it is doubtful that this additional sediment would have been carried forward to the extraction phase of the analysis. And if it had, it is most unlikely that it would have been sufficient to raise tissue PCB concentrations by the amount determined. For example, based on the dry weight of algal samples analyzed, Σ_{20}PCBs levels in 5 mg of residual sediment remaining on the processed fronds would have had to exceed 2,500 ppm, well above levels recorded in sediments during the original Ogden survey (Ogden 1997).

Biological Factors

Age-related changes in algal wet to dry weight ratios will influence PCB concentrations if there is no concomitant loss or gain in absolute conger weight. For example, the loss of cytoplasmic components during algal senescence may well accentuate PCB concentrations in whole plants, whereas growth could have the opposite effect. Unfortunately, wet to dry weight ratios were not obtained during the present work. However, the fact that there were no visible signs of algal senescence, or appreciable losses of fresh weight in whole plants weighed at each sampling interval suggest that changes in algal wet to dry weight ratios were insignificant.

The final and most plausible explanation for the sinusoidal uptake curve shown in Figure 17 is that PCB uptake by *P. boryana* is a dual mechanistic process reflecting both the surface adsorption of congeners to external binding sites as well as their passive absorption through cellular membranes. Although such a biphasic process has never been identified in marine macroalgae before, it is difficult to deny its existence given the high lipophilicity of PCBs and their propensity for accumulating in lipid pools of biological systems.

The rationale behind limited absorption of PCBs across algal cell membranes is based on the results of experiments conducted with temperate, intertidal algae that have thickened and gelatinous outer cell walls (Levine 1984, Moy and Walday 1996). The density and complexity of this matrix essentially provides an effective barrier against water loss during periods of emersion and limits the absorption of hydrophobic organic chemicals with molecular weights generally exceeding 100 g/mol (Spacie and Hamelink 1985). Since this exclusionary mechanism is purely a physical process, it seems reasonable to assume that it is less effective in algal species with flimsier outer cell wall structures, like *Padina* spp. Based on this assumption, the Σ_9PCBs accumulation data derived from the current work was redrawn to illustrate what is now believed to be a reasonable representation of both uptake processes acting in concert (Figure 18). A stylized rendition of the two separate processes is also presented in Figure 19.

Evidence in support of intracellular PCB absorption in delicate algal species is provided by the work of Swackhamer *et al.* (1993) and Koelmans *et al.* (1999). These authors examined PCB uptake in the unicellular green algae, *Selenastrum* and *Scenedesmus* and demonstrated slow rates of intracellular accumulation that required 20-30 days to reach equilibrium. They further observed that the accumulated PCBs were associated not only with the lipid fraction, but also with non lipid intracellular components, including a range of complex polysaccharides.

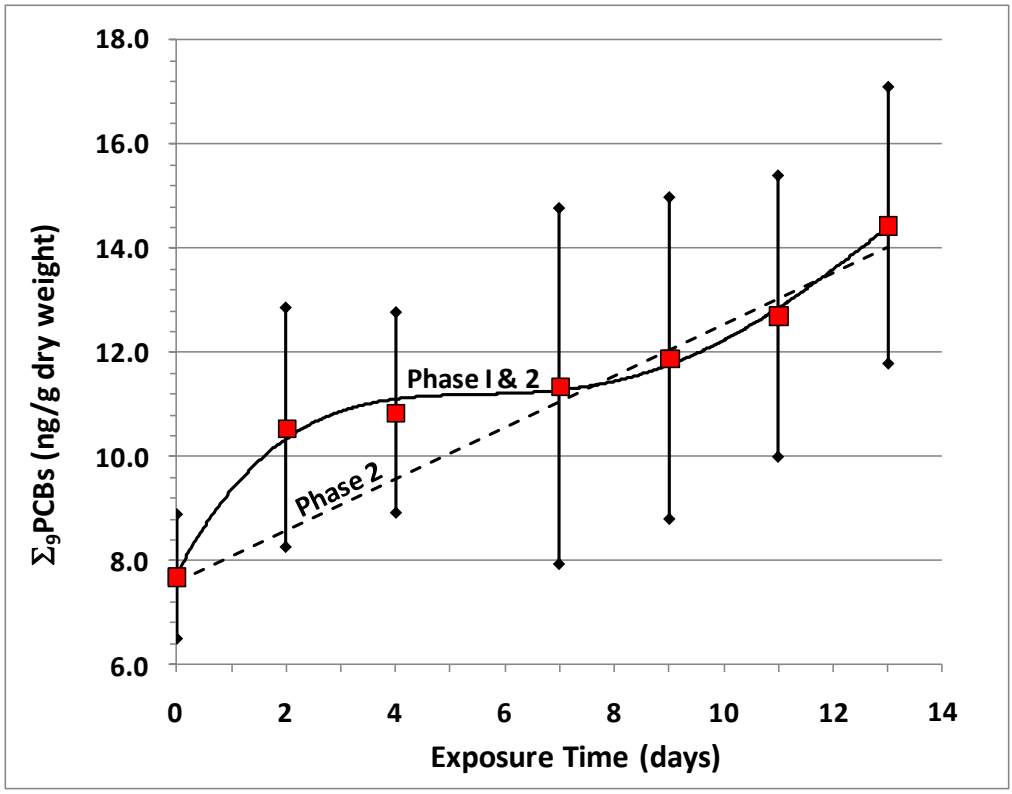

Figure 18: Total uptake of Σ_9PCBs in *P. boryana*. Error bars represent the 95% C.L. The solid line represents the net effect of adsorptive (Phase 1) and absorptive (Phase 2) processes acting in concert. The broken line is a theoretical representation of absorption (Phase 2) only with an estimated slope value of 0.4962 ng/g dry weight/day

INFLUENCE OF BIPHASIC UPTAKE ON SAMPLE VARIABILITY

The fact that *P. boryana* accumulates PCBs by two very distinct and separate processes, readily explains the unusually high degree of replicate variability encountered during this study. Clearly, PCB residues associated with intracellular algal components will not be as efficiently extracted from course material as they are from finely ground material. Thus, simply crushing dried algal fronds and passing through a 1-mm screen is insufficient to yield a truly homogenous sample. What we have instead are disproportionate amounts coarse and fine material distributed between each replicate. Samples proportionately high in course material will of course yield PCBs predominantly accumulated by adsorption processes whereas samples high in finer material will reflect greater amounts of the absorbed fraction. To test this hypothesis, an archived *P. boryana* sample retrieved from the Orote site on day 13, was crushed and separated into coarse (>1 mm) and fine (<1 mm) fractions. Subsequent mean PCB recoveries were 4 ng/g and 14 ng/g, with 100% and 37% variability between replicates, for each fraction respectively. Thus it is necessary to render *P. boryana* samples to a fine powder prior to extraction in order maximize total PCBs recoveries and minimize replicate variability.

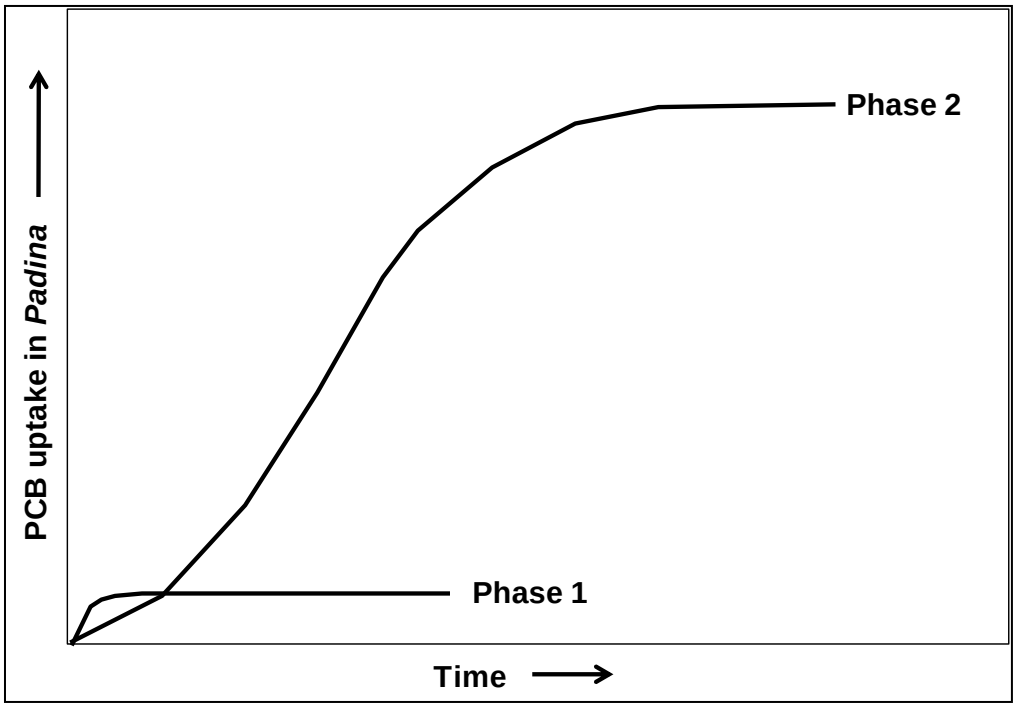

Figure 19: Stylized diagram illustrating biphasic uptake of PCBs in *P. boryana.* Phase 1 depicts rapid accumulation associated with surface adsorption of PCB congeners to extracellular binding sites; Phase 2 depicts slower rate of accumulation associated with passive absorption of PCBs across cell membranes. Note initial lag in uptake due to size-dependent differential partitioning rates of PCBs congeners through algal cell wall.

The implications of these findings are very clear in situations where *Padina* spp. are used for future PCB monitoring and assessment purposes. Failure to adequately homogenize algal representatives compromises both accuracy and precision in the determination of spatial and temporal differences in ambient PCB concentrations. Statistical power and resolution of

significant difference between datasets is consequently affected. At best, such an impaired study would provide underestimates of actual field values and lack the statistical sensitivity to distinguish between sites. At worst, it could produce conflicting and contradictory data that are indefensible and totally misleading.

OTHER MONITORING CONSIDERATIONS

One of the earliest and simplest modeling tools to be used in biomonitoring surveys was the bioconcentration factor or BCF. This partition coefficient is the ratio between the concentration of a contaminant in the biomonitor (C_B) of choice and the surrounding water (C_w). It is typically written as

$$BCF = C_B/C_w \quad (1)$$

The model assumes steady state conditions have been reached in the biomonitor (i.e., $C_B = C_{ss}$) and that the resulting coefficient remains constant over contaminant ranges normally encountered in the environment. BCFs are usually empirically derived and are fairly consistent between members of the same organistic group. Once known, they can be used to provide first order approximations of dissolved contaminant levels in the water column by simply rearranging Equation (1) to solve for C_w.

PCB data provided by Hope *et al.* (1998) for seawater and two species of algae from Midway Atoll, revealed an average BCF of 3.65×10^3 for the nine PCB congeners examined here. Applying this value to the data reported by Ogden (1997) for PCBs in *P. sanctae-crucis,* from the Orote site before mitigation strategies were implemented (Table 4), gives an Aroclor equivalent concentration in seawater of 312 ng/L which certainly ranks among the highest values reported in the literature. By the same means, data from the Schaible (2001) study conducted shortly after remediation yielded a value of 28 ng/L. Likewise, *P. boryana* cultured at Dadi Beach during the current study and analyzed immediately before transplanting to Orote, gave an Aroclor equivalent concentration in seawater of 8 ng/L. The latter value is indicative of a moderately contaminated environment and marks the upper limit of a range of PCB values reported by Tanabe and Tatsukawa (1986) for North Atlantic Ocean waters. Levels typically encountered in relatively clean oceanic waters are in the low pg/L range or less (Gioia *et al.* 2008).

Monitoring capabilities associated with biomonitor use are considerably enhanced if the uptake and depuration kinetics for the contaminant in question are understood and the appropriate rate constants identified. Such information is useful in determining steady state concentrations of contaminants in the biomonitor, as well as contaminant half-lives and sampling frequencies necessary to provide continuous records of contaminant availability in trend monitoring studies (Phillips 1980).

The simplest and most frequently used model developed for such purposes is the single compartment, first order kinetics model (Spacie and Hamelink 1985). This model assumes contaminant levels are derived exclusively from water and that uptake rates by organisms are directly proportional to ambient seawater concentrations. It likewise assumes that contaminant losses from the organism are proportional to concentrations accumulated by the organism. The equation is typically written as:

$$dC_B/dt = \text{uptake} - \text{loss} = k_1 C_w - k_2 C_B \quad (2)$$

where k_1 and k_2 are the rate constants for uptake and loss. Under steady state conditions, the rate of uptake balances the rate of loss so that:

$$dC_B/dt = 0 = k_1 C_w - k_2 C_{ss} \quad (3)$$

By this means, the bioconcentration factor is conveniently defined as:

$$\text{BCF} = C_{ss}/C_w = k_1/k_2 \quad (4)$$

The integrated form of Equation (2) is:

$$C_B = k_1/k_2 \, x \, C_w \, [1\text{-}\exp(-k_2 t)] \quad (5)$$

and assumes that contaminant concentrations in the organism are zero at zero time. In instances where this not the case Equation (5) is modified as follows:

$$C_B = k_1/k_2 \, x \, C_w \, [1\text{-}\exp(-k_2 t)] + C_{start} \exp(-k_2 t) \quad (6)$$

where C_{start} is the starting contaminant concentration in the organism at zero time. Equivalent forms combining equations (4) and (6) are:

$$C_B = C_{ss} \, [1\text{-}\exp(-k_2 t)] + C_{start} \exp(-k_2 t) \quad (7)$$

$$C_B = \text{BCF} \, x \, C_w \, [1\text{-}\exp(-k_2 t)] + C_{start} \exp(-k_2 t) \quad (8)$$

Once the rate constants are known, the model may be used to derive time-integrated values for C_w regardless of whether steady state concentrations have occurred in the biomonitor or not. Equation (6), for example, is rearranged as follows:

$$C_w = \{[C_B\text{-}C_{start} \exp(-k_2 t)]/[1\text{-}\exp(-k_2 t)]\} \text{ x } k_2/k_1 \quad (9)$$

At the simplest level, the relationship between contaminant concentrations in water and in organisms at steady state can be calculated by the rearrangement of Equation (4) as shown below:

$$C_w = C_{ss} \text{ x } k_2/k_1 \quad (10)$$

In the current study, a value for k_1 was approximated from the slope of the Phase II regression line (0.4962 ng/g/day) in Figure 18. The assumptions here were that uptake was linear and that there was no loss of PCBs in *P. boryana* over the 13-day exposure period. Under such conditions, Equation (2) reduces to:

$$\triangle C_B/\triangle t = k_1 C_w \quad (11)$$

and was rearranged to solve for k_1 using a C_w value of 28 ng/L derived from Schaible's data as described earlier:

$$k_1 = \text{Slope}/C_w = 72\ d^{-1} \quad (12)$$

Equation (4) was then rearranged to solve for k_2, with Schaible's 2001 value for PCBs in *P. sanctae crucis* (Table 4) serving as a proxy for C_{ss}:

$$k_2 = C_w \times k_1/C_{ss} = 0.0198\ d^{-1} \quad (13)$$

The time required to approach steady state is determined solely by the loss rate constant (k_2). For example, 95% of steady state concentrations will be reached when $C_B = 0.95\ C_{ss}$. Substituting this into Equation (7), the time required may be found from:

$$t_{ss95} = -\ln 0.05/k_2 = 2.996/k_2 = 151\ d \quad (14)$$

By adjusting the decimal fraction in Equation 14, the mean PCB concentration determined in *P. boryana* at the end of the 13-day exposure period (14.4 ppm) was shown to account for ~23% of estimated steady state concentration. Thus, after 151 days, the transplants would have accumulated an Aroclor equivalent concentration of 239 ppb, had they survived that long. This value, although only a very rough approximation, compares reasonably well with levels determined in *P. sanctae-crucis* collected near the Orote dump seawall ten years earlier (Schaible 2010). The fact that it is substantially lower than the Aroclor equivalent concentration found in this species by Ogden (1997), however, strongly suggests that some attenuation of PCBs has occurred at the Orote site since remediation activities took place.

Sampling frequencies necessary to provide a continuous record of pollutant availability are usually geared to contaminant turnover rates in the biomonitor of choice (Phillips 1980). Sampling weekly for contaminants with half-lives of several months, for example, would be totally inappropriate as would the opposite scenario. Assuming PCB elimination rates from the intracellular pool of *P. boryana* follow first-order kinetics, the half-time (t_{50}) for these contaminants may be calculated as follows:

$$t_{50} = \ln 2/k_2 = 0.693/k_2 = 35\ \text{days} \quad (15)$$

Thus the most cost effective sampling frequency necessary to provide a continuous record of PCB availability at any particular site, using *P. boryana* as the sentinel species, would be in the order of one month. Further work is necessary to verify this, however. Likewise the significance and kinetic characteristics of the PCBs adsorbed to the outer cell wall merits further evaluation from a monitoring perspective. While this fraction would seem to represent only a small percentage of total PCB residues in *P. boryana* growing naturally in an area, the fact that it reaches steady state within 4 days lends itself particularly well to short-term monitoring studies using transplanted individuals. By this means, inter-site difference in PCB availability could conceivably be identified very rapidly by differential extraction of the intact frond.

CONCLUSIONS AND GENERAL RECOMMENDATIONS

The study reported here is the first of its kind to be conducted anywhere in the world. It clearly shows that *Padina* spp. have great potential as biomonitors of PCB contamination in tropical marine waters providing certain conditions are met. The biphasic uptake mechanisms that collectively accumulate PCBs in this alga reflect both short- and long-term time-integrating processes that may be separated analytically, if necessary, to determine short- and long-term changes in the ambient availability of these contaminants in the water column. The development of a simple, cheap and highly effective means of cultivating *Padina* sporelings on clean polypropylene ropes also adds greatly to the power of this organism as a monitoring tool by facilitating its deployment to sites where it is not normally encountered.

Suggested refinements to the deployment technique adopted here would be to use smaller tubular mesh cages each containing a single rope of *Padina* recruits. The plastic mesh should be of sufficient gauge and size to protect the alga against herbivorous fish and permit adequate light to reach the recruits. These lightweight yet robust structures, maintained vertically in the water column by float and anchor, would be better able to withstand rough seas and inclement weather conditions than the more cumbersome PVC cradle used in the current investigation.

The age/size of algal transplants depends upon the monitoring objectives. For long-term assessments extending over periods several months, the recruits may be moved to sites of interest as soon as they are sufficiently well established to withstand the physical trauma associated with the relocation process. This will allow them sufficient time to approach steady-state conditions before being sampled for analysis. For short-term assessments lasting only a few days, the algal representatives need to be of sufficient size to provide adequate tissue for analysis before being transplanted. In the latter instance representatives will be sampled only once whereas repeat sampling at monthly intervals may be more desirable for long-term, trend monitoring purposes.

Differential extraction process designed to identify contribution to the total PCB pool from both adsorption and absorption processes would involve accelerated solvent extraction of intact, fresh frond replicates (A) and finely ground, freeze dried frond replicates (B). After converting all wet weight data sets to dry weight, contributions from the latter compartment are derived by difference, i.e., by subtracting A (PCBs accumulated by surface adsorption) from B (the total PCB pool).

FUTURE DIRECTIVES

On the basis of the work already completed, the following recommendations are made for further study in order to refine the use of *Padina* for monitoring PCBs in Guam's coastal waters:

- Design laboratory and field experiments to provide a better understanding of the adsorption and absorption processes governing PCB uptake in both extracellular and intracellular compartments of *Padina*, and fully evaluate the relative importance of these from a pollution monitoring and assessment perspective.
- Determine the depuration characteristics of PCBs in each of the above compartments and establish whether first-order kinetics is approximated or not.

- Identify appropriate rate constants for the uptake and loss of PCBs in each compartment and consider the effects of age/growth on these parameters. Passive absorption of PCBs across cell membranes may well be influenced by any changes in the thickness and complexity of the outer cell walls over time.
- Accurately determine *Padina's* time-integration capacity for PCBs and establish appropriate sampling frequencies for the long-term monitoring of these contaminants in the water column.
- Determine growth related changes in the chemical composition and abundance of intracellular lipids in *Padina* and determine how such qualitative and quantitative changes might influence PCB uptake over the life-span of the alga.
- Explore cage design alternatives and improvements for future *Padina* transplant initiatives, with emphasis on developing effective ways to eliminate or, at least, minimize herbivory and bio-fouling without compromising water circulation or light penetrability.

LITERATURE CITED

Abbott, I. and J. Huisman. 2003. New species, observations, and a list of new records of brown algae (Phaeophyceae) from the Hawaiian Islands. *Phycological Research,* 51: 173–185.

ACE [U.S. Army Corps of Engineers] - EPA [U.S. Environmental Protection Agency]. 1992. *Guidance for Performing Tests on Dredged Material Proposed for Ocean Disposal. Draft Report*. New York, NY: ACE - New York District, and, EPA - Region II; 81 pp.

Amico, V., G. Oriente, M. Piattelli and C. Tringali. 1979. Concentrations of PCBs, BHCs and DDTs residues in seaweeds of the east coast of Sicily. *Marine Pollution Bulletin,* 10: 177-179.

Amico, V., R. Chillemi, G. Impellizzeri, G. Oriente, M. Piattelli, S. Sciuto and C. Tringali. 1982. Levels of chlorinated hydrocarbons in sediments from the central Mediterranean. *The Science of the Total Environment*, 24: 91-99.

Bright, D.A., S.L. Grundy and K.J. Reimer, 1995. Differential bioacumulation of non-ortho substituted and other PCB congeners in coastal Arctic invertebrates and fish (*Myoxocephalus quadricornis, M. scorpius, Gadus ogac and Salvelinus alpinus*). *Environmental Science and Technology*, 29: 2504-2512.

Burdon-Jones, C., G.R.W. Denton, G.B. Jones and K.A. McPhie. 1982. Regional and seasonal variation of trace metals in tropical Phaeophyceae from north Queensland. *Marine Environmental Research*, 7:13-30.

Butler, P.A., L. Andren, G.J. Bond, A. Jernlov and D.J. Reish. 1971. Monitoring organisms. In *FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing*, Rome 1970. *Supplement 1: Report of the Seminar on Methods of Detection, Measurement and Monitoring of Pollutants in the Marine Environment. FAO Fisheries Report No. 99*, Supplement 1: 101-112.

Denton, G.R.W. and Burdon-Jones, C. 1986. Trace metals in algae from the Great Barrier Reef. *Marine Pollution Bulletin*, 17: 98-107.

Denton, G.R.W., Concepcion, L.P., Wood, H.R., Eflin V.S., and Pangelinan G.T. 1999. Heavy metals, PCBs and PAHs in marine organisms from four harbor locations on Guam. A pilot study. *WERI Technical Report No. 87*, 154 pp.

Denton, G.R.W., Concepcion, L.P., Wood, H.R. and Morrison R.J. 2006a. Trace metals in marine organisms from four harbours in Guam. *Marine Pollution Bulletin*, 52: 1786-1804.

Denton, G.R.W., Concepcion, L.P., Wood, H.R. and Morrison R.J. 2006b. Polychlorinated biphenyls (PCBs) in marine organisms from four harbors in Guam. *Marine Pollution Bulletin*, 52: 214-225.

Denton, G.R.W. and Morrison, R.J. 2009a. Impact of a rudimentary landfill on the trace metal status of Pago Bay, Guam. *Marine Pollution Bulletin*, 58: 150-162.

Denton, G.R.W., Morrison, R.J., Bearden, B.G., Houk, P., Starmer, J.A. and Wood H.R. 2009b. Impact of a coastal dump in a tropical lagoon on trace metal levels in surrounding marine biota: a case study from Saipan, Commonwealth of the Northern Mariana Islands (CNMI). *Marine Pollution Bulletin*, 58: 424-455.

De Voogt, P., D.E. Wells, L. Reutergardh and U.A.Th. Brinkman. 1990. Biological activity, determination and occurrence of planar, mono- and di-ortho PCBs. *International Journal of Environmental Analytical Chemistry,* 40: 1-46.

EarthTech, Inc. 2004. Phase I investigation summary report, Orote landfill: south Orote Peninsula, marine operable unit, Guam. Guam: EarthTech, Inc. (2001 seawater analytical data).

Geraldino, P, L. Liao and S. Boo. 2005. Morphological study of the marine algal genus *Padina* (Dictyotales, Phaeophyceae) from southern Philippines: Three species new to Philippines. *Algae,* 20: 99-112.

Gioia R., L. Nizzetto, R. Lohmann, J. Dachs, C. Temme and K.C. Jones. 2008. Polychlorinated biphenyls (PCBs) in air and seawater of the Atlantic Ocean: sources, trends and processes. *Environmental Science & Technology*, 42: 1416–1422.

Guiry, M.D. and G.M. Guiry. 2011. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 02 August 2011. (search term *Padina*)

Haug, A., S. Melsom and S. Omang. 1974. Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga, *Ascophyllum nodosum. Environmental Pollution*, 7: 179-192.

Hope, B., S. Scatolini and E. Titus. 1998. Bioconcentration of chlorinated biphenyls in biota from the North Pacific Ocean. *Chemosphere*, 36: 1247-1261.

Kloareg, B., and R. Quatrano. 1988. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanography and Marine Biology, Annual Review,* 26: 259-315.

Koelmans, A.A., H. Van der Woude, J. Hattink, and D.J.M. Niesten. 1999. Long-term bioconcentration kinetics of hydrophobic chemicals in *Selenastrum capricornutum* and *Microcystis aeruginosa*. *Environmental Toxicology and Chemistry*, 18: 1164–1172.

Levine, H.G. 1984. The use of seaweeds for monitoring coastal waters. **In**: *Algae as Ecological Indicators,* Shubert, L.E. (ed), London: Academic Press, pp. 189-210.

Lobban, C.S. and Tsuda, R.T. 2003. Revised checklist of benthic marine macroalgae and seagrasses of Guam and Micronesia. *Micronesica,* 35-36: 54-99.

Lytle, J., and T. Lytle. 2001. Use of plants for toxicity assessment of estuarine ecosystems. *Journal of Environmental Toxicology and Chemistry*, 20: 68-83.

Maroli, L., B. Pavoni, A. Sfriso and S. Raccanelli. 1993. Concentrations of polychlorinated biphenyls and pesticides in different species of macroalgae from the Venice Lagoon. *Marine Pollution Bulletin*, 26: 553-558.

McFarland, V.A. and J.U. Clark. 1989. Environmental occurrence, abundance and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener specific analysis. *Environmental Health Perspectives*, 81: 225-239.

Montone, R.C., S. Taniguchi, J. Sericano, R.R. Weber and W.H. Lara. 2001. Determination of polychlorinated biphenyls in Antarctic macroalgae *Desmarestia* sp. *The Science of the Total Environment,* 277: 181-186.

Moy, F.E. and M. Walday. 1996. Accumulation and depuration of organic micro-pollutants in marine hard bottom organisms. *Marine Pollution Bulletin,* 33: 56-63.

Nassar, C., and Y. Yoneshigue-Valentin. 2006. Iron ore particles on four seaweed species from camburi beach (Espirito Santo State, Brazil). *Brazilian Journal of Oceanography,* 54: 155-159.

NOAA 1989. A summary of data on tissue contamination from the first three years (1986-1988) of the Mussel Watch Program. *National Oceanic and Atmospheric Administration Technical Memorandum NOS OMA 49*. 154 pp.

NOAA 1993a. National Status and Trends Program for Marine Environmental Quality. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992. Volume I. Overview and summary of methods. *National Oceanographic and Atmospheric Administration Technical Memorandum NOS ORCA 71*. July 1993. 117 pp.

NOAA 1993b. National Status and Trends Program for Marine Environmental Quality. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992. Volume II. Comprehensive descriptions of complementary measurements. *National Oceanographic and Atmospheric Administration Technical Memorandum NOS ORCA 71*. July 1993. 101 pp.

Ogden, Inc. 1997. Final removal site evaluation report, Orote landfill site. *Comprehensive Long Term Environmental Action Navy.* Ogden Inc. (*Padina* tissue analytical data 1995).

Phillips, D.J.H. 1977. Use of biological indicator organisms to quantitate organochlorine pollutants in aquatic environments – a review. *Environmental Pollution*, 16: 167-229.

Phillips, D.J.H. 1980. *Quantitative Aquatic Biological Indicators*. CRC Press, Boca Raton, FL.

Phillips, D.J.H. 1994. Macrophytes as biomonitors of trace metals. **In**: *Biomonitoring of Coastal Waters and Estuaries*, Kramer, K.J.M. (ed.), pp. 85-106

Schaible, B.C. 2010. Profile of polychlorinated biphenyls in the brown alga *Padina sanctae-crucis* along the Orote Dump coastline, Orote Peninsula, Western Guam. *Journal of Toxicology and Environmental Health, Part A: Current Issues*, 73: 1349-52.

Spacie A. and J.L. Hamelink. 1985. Bioaccumulation. **In**: *Fundamentals of Aquatic Toxicology: Methods and Applications*. Rand, G.M. and S.R. Petrocelli (eds.), Hemisphere Publishing Corporation, Washington. Chapter 17, pp. 495-525.

Swackhamer, D.L. and R.S. Skoglund. 1993. Bioaccumulation of PCBs by phytoplankton: kinetics vs. equilibrium. *Environmental Toxicology Chemistry*, 12: 831-838.

Tanabe, S. and R. Tatsukawa 1986. Distribution, behavior and load of PCBs in the ocean. **In**: *PCBs and the Environment*, Vol. 1, J.S. Waid (ed.), CRC Press Inc., pp 143-161.

Thompson, M., S.L.R. Ellison, A. Fajgelj, P. Willett and R. Wood. 1999. Harmonized guidelines for the use of recovery information in analytical measurement. *Pure & Applied Chemistry*, 71: 337–348.

Valoppi, L., M. Petreas, R. Donohoe, L. Sullivan, and C. Callahan. 1998. Use of PCB congener and homologue analysis in ecological risk assessments. *USEPA, Region 9, Biological Technical Advisory Group, San Francisco, CA.* January 1998. 33 pp.

Van den Berg, M., L.S. Birnbaum, M. Denison, M. DeVito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker and R.E. Peterson. 2006. The 2005 World Health Organization re-evaluation of human Toxic Equivalency Factors for dioxins and dioxin-like compounds. *Toxicological Sciences*, 93: 223-241.