PILE SURFACE COVERAGE AND COPPER, CHROMIUM AND ARSENIC CONTENT OF BARNACLES GROWING ON EXPERIMENTAL MOORING PILES

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ABSTRACT

A marine test of commercially available timber piles was established in three ports in Queensland. For treated piles after two and four years' exposure, least barnacle growth (mean of 53% of surface) in the mid-tide zone was on double treated (CCA + PEC) *Pinus elliottii* piles, while PEC treated *Eucalyptus maculata* piles had highest mean barnacle coverage (71 and 75%). Untreated *Syncarpia glomulifera* piles had low barnacle coverage due to the presence of bark. Barnacles were collected from piles at Townsville and analysed for copper, chromium and arsenic. There was no statistically significant difference in the extractable arsenic content of barnacles between the different pile types at the two and four year inspections, but some significant differences occurred when results for the two inspections were combined. Barnacles from CCA-treated and double treated piles had higher chromium and particularly copper levels than barnacles from the untreated *S. glomulifera* piles and PEC-treated *E. maculata* piles. However, differences were not always statistically significant. Of the piles containing CCA, chromium levels were lowest in barnacles from double treated *E. maculata* piles. Arsenic and chromium levels in barnacles from each pile type were similar after two and four years, whereas copper levels had generally fallen.

INTRODUCTION

Through the collaboration of CSIRO, Queensland harbour authorities, Queensland DPI (Forestry), and Koppers Timber Preservation, a marine test of commercially available timber piles was established at Bundaberg, Townsville and Cairns, to represent a range of conditions found in Queensland. The piles were CCA salt (copper-chrome-arsenic) treated *Eucalyptus maculata* Hook. (spotted gum), CCA-treated *Pinus elliottii* Engelm. (slash pine), PEC (pigment emulsified creosote) treated *E. maculata*, and double treated (CCA + PEC) *E. maculata*, *P. elliottii* and *E. pilularis* Sm. (blackbutt). Untreated *Syncarpia glomulifera* (Sm.) Niedenzu (turpentine) is the major pile type in Australia and was included for comparison. The piles are inspected every two years for marine borer attack (Cookson and Barnacle, 1993; Cookson, 1996).

A number of studies have investigated the effect of CCA-treated timbers on marine organisms and the environment. Some studies indicate that the preservative can leach from timber at a rate that causes environmental problems (Weis and Weis, 1995), while others suggest leaching causes little change to the natural background levels of copper, chromium and arsenic (Albuquerque and Cragg, 1995; Baldwin *et al.*, 1996). The opportunity was therefore taken during inspections to assess the impact of the different commercial pile types on barnacles. The extent to which barnacles were able to colonise the piles was estimated at each site. Additionally at Townsville only, the

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copper, chromium and arsenic content of barnacles growing on the piles was determined. Barnacles are useful bioindicator species in studies of heavy metal pollution (Rainbow, 1987).

MATERIALS AND METHODS

Estimation of barnacle coverage on pile surfaces

There are forty mooring piles at each test site, comprising six replicates of each treated pile type, and four replicates of the untreated *S. glomulifera* piles (Table 1). The piles were examined during low tide for barnacle colonisation, two and four years after installation. All sites are located near the mouths of estuarine rivers. Inspections were completed during the dry season, and surface water salinities at the three sites ranged from 35-37. The salinity at Bundaberg during a wet season visit was 29, Townsville 12 and Cairns 29. The percentage of the pile surface area covered by barnacles near the mid-tide region was estimated. At first, the estimate was attempted by counting barnacles within a 200 mm square grid, however, it was soon realised that results depended greatly on exactly where the grid was placed on the pile surface. Therefore, estimation over the whole downstream face of the mid-tide area was made subjectively, and seemed more accurate.

Sampling barnacles for copper, chromium and arsenic at Townsville

Barnacles at Townsville were collected from five or six piles of each treatment, and four untreated *S. glomulifera* piles. Two days before inspection, the Townsville Port Authority scraped each pile in the tidal zone, except for the downstream face. During inspection and after estimating percentage barnacle growth on this downstream face, all remaining barnacles were scraped from the piles. Barnacles from the mid-tide region of the downstream face were collected and placed into 28 ml McCartney vials containing 70% ethanol. An average of 6 barnacles of diameter 11-16 mm, 10 barnacles 4-10 mm and 13 barnacles 2-3 mm were collected from each pile. Since all barnacles were removed during each inspection, barnacles collected at the four year inspection could not be more than two years old. Most barnacles collected were *Balanus* species (Poore, pers. comm.).

In the laboratory, all soft tissue was removed from the barnacle shells under a dissecting microscope, using stainless steel forceps. Bryan *et al*. (1985) found no evidence that dissecting with stainless steel instruments caused metal contamination. A blank was created, where stainless steel forceps were rubbed together in 70% ethanol, the sample oven dried and 10% HCl added to the vial, but insignificant metal was obtained. During dissection, care was taken not to include any shell or treated wood fibres adhering to the basal plate of the barnacles. The soft tissue was placed on a tared watchglass, weighed, and oven dried overnight at 105^{o}C to determine dry mass. Mean wet mass of the tissue samples from each pile was 1410 mg (standard deviation 490), and mean dry mass was 204 mg (sd 79).

The dry flesh was pushed into a clean 25 ml vial with a glass rod, and 20 ml 10% aqueous HCl added. The tissue was left to soak for four weeks at room temperature. The solution from each vial was then drawn out with a plastic syringe, and passed through a 0.45 micron millipore filter into a clean vial. The filtered solution was analysed for copper, chromium and arsenic using a GBC Integra XM sequential ICP spectrophotometer. Standards were run every 4-6 samples to confirm the absence of any significant drift during the course of the analytical work. Detection limits in the extracts were 0.3 ppm for arsenic, 0.05 ppm for chromium and 0.05 ppm for copper. Replicates with readings below these values were considered to be zero in further calculations.

A number of factors were identified that could influence results. Firstly, it is important to note that the simple extraction procedure used in this study does not involve total dissolution of organic matter, and hence may not have removed all of the copper, chromium and arsenic from the barnacle tissue. Also, boats are often moored at these piles, so antifouling paint may have contributed to the copper content of the barnacles. All piles of course were subject to this variable. CCA traces could also have been extracted from the barnacles by the 70% ethanol in which they were stored. To determine if copper, chromium and arsenic was present in the ethanol solution, excess ethanol was collected after dissection of 14 different barnacle samples (9-10 ml), filtered through 0.45 micron millipore filter into a clean vial, and oven dried overnight at 105^{o}C. To each vial 10 ml 10% HCl was added and solution analysed. While chromium and arsenic were both below detectable levels in the ethanol solution, 22% of the copper found in both soft tissue and the ethanol was present in the ethanol. Also, only small quantities of crustacean soft tissue was extracted in this study, and no attempt was made to concentrate the analyte elements. Consequently, some of the barnacle extracts, particularly from the untreated and PEC-treated piles, contained arsenic and chromium levels below or close to the detection threshold.

The results were examined using an analysis of variance, and differences were considered significant when $p < 0.05$.

RESULTS

Barnacle coverage of piles

At Bundaberg, the piles were divided evenly amongst two sites (A and B) in the same river. Therefore, there were three replicates of each treated pile and two replicate *S. glomulifera* piles at each Bundaberg site. Barnacle cover was not estimated at site B near the river mouth, because the tidal zone surface of those piles was mostly devoid of fouling. Wave action at site B is often extreme, so that the abrasive action of mooring ropes and boats effectively keep the piles clean. Results from site A are presented, but were not statistically analysed on their own due to low replication (Tables 2 and 3).

Perhaps surprisingly, least barnacle coverage was found on the untreated *S. glomulifera* piles when the combined data from all sites were examined. Coverage was particularly low after two years (combined mean 38% cover) (Table 2). Barnacle cover was also low at the four year inspection (combined mean 50%) (Table 3). The reason for low coverage at the two year inspection is that *S. glomulifera* piles were installed with bark intact. While barnacles settled the bark free surface of *S. glomulifera* piles heavily (up to 90 % coverage), much less (about 5-10 %) coverage occurred on the fibrous bark. Mean results are given in Tables 2 and 3, so this difference produced a wide range of results reflected in the high standard deviations for *S. glomulifera* piles. Most of the bark was still present in the tidal zone at two years. After four years, however, *S. glomulifera* piles at all sites had lost bark in the tidal zone, so that settlement was higher. Counteracting the loss of bark, was the high degree of *Sphaeroma* attack in the mid-tide region at Townsville and Cairns. *Sphaeroma* competes for space with barnacles on timber piles, and heavily infested piles are often almost devoid of barnacles in the tidal zone.

For the treated piles, differences in the level of barnacle coverage were not great. However, results at the two year inspection suggest that double treated *P. elliottii* piles generally have lower initial coverage than the other treated piles (Table 2). This seemed to be due to the higher levels of crud (hardened exudate of creosote from PEC) found on the piles. Streaks of crud often extended into the tidal zone, and were not settled by barnacles. Conversely, crud was either absent or light on PEC and double treated eucalypt piles. Interestingly, PEC treated *E. maculata* piles generally had highest barnacle coverage.

Mean barnacle coverage on all piles was generally higher at Townsville on the four year inspection than the second year inspection, while at Cairns the reverse was observed. At Bundaberg the degree of coverage was mostly similar at both inspections.

Copper, chromium and arsenic content of barnacles from Townsville

The mean concentration of copper, chromium and arsenic found in barnacles collected from each pile type during the two and four year inspections are shown in Table 4. Results are expressed in terms of µg of metal or metalloid per gram of oven dry barnacle soft tissue. An analysis of variance was used to determine if the concentrations in barnacles was different between the two inspections (Table 4). A similar analysis was used to determine if there were any differences between pile types at each inspection, and at both inspections combined. Barnacles collected at the two and four year inspections were a maximum of two years old. For the comparison between different piling types, the results for untreated *S. glomulifera* and PEC treated *E. maculata* piles were combined to improve the replication for statistical analysis. The combined results for these two pile types are referred to as the ‘non-CCA’ piles.

Arsenic

The extractable arsenic content of barnacles between the different pile types at both the two and four year inspections was not statistically significant difference (Tables 5 & 6), although the results suggest a trend of higher arsenic content in barnacles from some of the CCA-treated pile types. Also, for each pile type there was no significant difference in arsenic levels in barnacles between the two and four year inspections (Table 4). However, when results for the two and four year inspections are combined (Table 7), some differences appear. Barnacles from both the CCA-treated *P. elliottii* piles and the CCA-treated *E. maculata* piles had significantly higher levels of arsenic in their soft tissues than barnacles from the other pile types.

Chromium

At the two year inspection, there was no significant difference in the levels of chromium found in barnacles from the various pile types (Table 5). However, at the four year inspection when no detectable chromium was found in barnacles from the non-CCA piles, only barnacles from double treated *E. maculata* piles were not significantly different to the control group (Table 6). Highest chromium levels were found in barnacles from the double treated *P. elliottii* piles and the CCA-treated *E. maculata* piles (Table 6). As for arsenic, chromium levels did not drop significantly over the two to four year inspections for those piles containing CCA (Table 4). When results for the two and four year inspections are combined (Table 7), results indicate that chromium levels in barnacles from both double treated eucalypt piles (*E. maculata* and *E. pilularis*) are not significantly different to those obtained from the non-CCA piles. However, barnacles collected from the remaining CCA-treated piles all have significantly higher levels of chromium in them than barnacles from the non-CCA piles.

Copper

Variations were more obvious in the levels of copper found in barnacles from each pile type and inspection. Only the double treated *E. pilularis* piles had barnacles with copper levels similar to barnacles from the non-CCA piles, at both the two and four year inspections (Tables 5 & 6). Up to 12 times higher copper levels were found in barnacles collected from the treated *P. elliottii* piles than were found on the non-CCA piles (Tables 5 and 6). The level of copper found in barnacles collected from double treated *E. maculata* piles was lower at the four year inspection. No other pile type showed a significant trend. However, if the results for all piles containing CCA (including double treated piles) are combined, the barnacles from the four year inspection have less copper in them than those from the two year inspection (Table 4). When the result of both inspections are combined, we find that all CCA-treated and double treated piles have significantly higher levels of copper in barnacles growing on their surfaces than on non-CCA piles (Table 7).

DISCUSSION

There are many environmental issues to consider when selecting a piling material for use in the sea. All materials have an environmental cost at some point. Timber wins over other materials such as steel and concrete in its manufacturing stage because it is renewable and requires less energy to produce. However, the use of treated timber may create a problem in the sea due to the leaching of toxic components. All heavy metals become toxic at some threshold availability, yet many play a vital role in metabolism (Rainbow, 1987). Antifouling paints come under particular scrutiny, because they are designed to release biocides into the environment at a rate that is toxic to adjacent fouling organisms. Therefore, antifouling paints on boats are usually replenished annually. For tributyltin oxide based paints, this strategy resulted in the build up in certain waters of tributyltin oxide to harmful levels for oysters and fish (Batley *et al.*, 1989). The strategy is different however in marine wood preservation.

Service lives of at least 20 to 30 years are sought from the one application (or double application) of preservative. After this time, at least half to three quarters of the original retention of preservative must generally remain in the wood for it to have been an effective treatment. For example, four *E. macrorhyncha* F. Muell. ex Benth. natural rounds treated for a small specimen test with low temperature creosote (Barnacle and Cookson, 1990) to an average retention of 255 kg/m^3 were recently analysed. After 22 years exposure at Kwinana near Perth the specimens were still serviceable, and 68% of the original creosote could be removed from the samples by Dean and Stark soxhlet extraction (Cookson, unpublished data).

The presumed low rate of preservative leaching from treated wood in our tests is consistent with the rapid colonisation of the treated piles after installation. Piles were examined within three weeks of installation, and barnacles and algae were found on most. This rate of colonisation compares with that of a surface coated with antifouling paint that is designed to release at least nine micrograms of copper per cubic centimetre per day to control *Balanus* sp. (Cristie and Dalley, 1987). Our comparison of barnacle growth on piles was made two and four years after installation, when differences in coverage between the different piles was small, though sometimes significant. Comparisons made after shorter exposure times might have revealed greater differences. Weis and Weis (1996) found reduced diversity of fouling organisms on CCA-treated wood compared to untreated wood after one and two month exposures. After three months' exposure there was no significant difference in population, although growth of some fouling species appeared stunted. Sturgess and Pitman (1996) also found that CCA-treated wood inhibited the early colonisation of surface wood by marine microorganisms. The reduced colonisation on CCA-treated surfaces may be due to leaching of CCA. Our results suggest that copper is the main element that is accumulated by barnacles on the surface of CCA-treated piles. This is consistent with the findings of Warner and Solomon (1990) and Weis *et al.* (1991). Another factor that might reduce fouling on CCA-treated timbers compared to untreated controls, is that the preservative would make wood fibres less palatable to some microorganisms that might otherwise be a part of the fouling community of untreated wood. Corroding steel piles, and mooring pile runner pipes attached to the Townsville piles, often have reduced barnacle and fouling growth, presumably due to the leaching of iron.

When barnacles from Townsville were analysed for copper, chromium and arsenic content, perhaps not surprisingly, the results seem to relate closely to the original CCA retention of the pile. Both species of double treated eucalypt had lowest CCA retentions, and the copper, chromium and arsenic content of barnacles collected from their surfaces was often not significantly different to that of the non-CCA piles. Higher CCA retentions occured in both the *E. maculata* piles and *P. elliottii* piles treated with CCA alone, and these piles also often had significantly higher levels of copper, chromium and arsenic in their associated barnacles than the non-CCA pile controls. Eucalypts treated with CCA alone have not been recommended as marine piles in the tropics, based on their widespread poor performance against teredinid borers (Cookson, 1987). Similarly, CCA-treated softwood piles generally fail to give adequate service lives in the tropics due to attack by species of *Sphaeroma*. It is possible that for double treated timbers, the creosote component reduces leaching of CCA, however, this was difficult to determine from the pile types used in this study.

The results suggest that the leaching of arsenic from the CCA and double treated piles did not unduly enhance the arsenic content of barnacles after two and four years' exposure. Some marine organisms have a high background level of arsenic. Mackay *et al.* (1975) found just 1.14 µg/g wet weight in oysters from NSW, while at Townsville we found about 19 µg/g dry mass or about 130 µg/g wet mass on non-CCA piles. Stalker and Cornwell (1975) give figures for marine crustaceans of 30 to 130 µg/g dry mass arsenic. The mean range of arsenic found in a variety of crustaceans range from about 8 to 179 µg/g dry mass (Phillips, 1990). The highest arsenic level found in barnacles on the treated piles was 94 µg/g dry mass.

Higher levels of chromium were found in barnacles from some of the piles, although this difference was not statistically significant at the two year inspection. Of the active ingredients in CCA, chromium appears to leach least (Leightley, 1987). Weis *et al.* (1993) found higher chromium levels in barnacles collected from CCA-treated pine, while mussels collected from the same timbers displayed no significant increase from background detectable levels.

In Dulas Bay where there is a pollution problem from copper, barnacles can have 3000 µg copper/g dry mass (Rainbow, 1987). At most other sites examined, copper levels ranged from 0.4 to 913 µg/g (Rainbow, 1987). In Hong Kong waters, copper in *Balanus* ranged from 116 to 3472 µg/g (Phillips and Rainbow, 1988). The highest levels found on piles at Townsville were 1247 µg/g for double treated *P. elliottii* piles and the lowest 265 µg/g for double treated *E. pilularis* piles.

Creosote may also cause some environmental hazards. The main problem occurs shortly after pile installation. As wood absorbs moisture and swells, creosote can be forced from the piles, and indeed small oil slicks were noticed arising from some piles (mainly double treated *P. elliottii* piles) within one to two weeks of installation. Once wood swelling has stabilised, the piles do not produce an oil slick. Creosote can be degraded by certain bacteria (Belas *et al.*, 1979; Drisko *et al.*, 1962). On aged piles, these bacteria may exist within the fouling community, and degrade excess creosote that nears the surface of the pile.

Of the treated pile types examined that contain CCA, double treated eucalypts cause least alteration to the natural background copper, chromium and arsenic content of barnacles.

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Table 1.
Timber species and preservative pile types in mooring pile study at three ports. Mean retentions are for the outer 5 mm case of the piling sapwood, and are given as % m/m of oven dry wood, and as kg/m^3 of air dry wood. PEC retentions are based upon the creosote component of the PEC.

Timber species	CCA retention % m/m	CCA retention kg/m^3	Creosote ret'n % m/m	Creosote ret'n kg/m^3
E. maculata	1.6	42	11.4	108
E. pilularis	2.0	49	17.3	153
P. elliottii	8.0	170	26.1	200
P. elliottii	5.1	108	-	-
E. maculata	2.5	66	-	-
E. maculata	-	-	17.5	167
S. glomulifera	-	-	-	-

Table 2.
Mean percentage of pile surface covered with barnacles on mid-tide down stream face at three ports after two years. Means with similar letters within a column are not significantly different ($p < 0.05$).

Timber species	Preservatives	Bundaberg, Site A	Townsville	Cairns	Mean (sd) for all sites
E. maculata	CCA + PEC	50	58 bc	75 b	64 (13) bc
E. pilularis	CCA + PEC	65	68 cd	82 b	73 (13) c
P. elliottii	CCA + PEC	42	47 b	65 ab	53 (14) b
P. elliottii	CCA	52	65 cd	82 b	69 (15) c
E. maculata	CCA	50	77 d	77 b	72 (12) c
E. maculata	PEC	68	62 c	82 b	71 (13) c
S. glomulifera	Nil	55	20 a	48 a	38 (37) a

Table 3.
Mean percentage of pile surface covered with barnacles on mid-tide down stream face at three ports at the four year inspection. Means with similar letters within a column are not significantly different ($p < 0.05$).

Timber species	Preservatives	Bundaberg, Site A	Townsville	Cairns	Mean (sd) for all sites
E. maculata	CCA + PEC	60	73 a	43 ab	55 (18) ab
E. pilularis	CCA + PEC	67	82 a	61 bc	68 (14) bc
P. elliottii	CCA + PEC	50	80 a	42 ab	53 (20) ab
P. elliottii	CCA	72	80 a	55 bc	67 (16) bc
E. maculata	CCA	62	70 a	38 a	53 (17) ab
E. maculata	PEC	80	83 a	68 c	75 (15) c
S. glomulifera	Nil	70	83 a	24 a	50 (31) a

Table 4.
Mean level of copper, chromium and arsenic present in barnacles collected from timber piling on the 2 and 4 year inspections at Townsville. Also shows the probability that results for year 2 and 4 are not significantly different. Results given in µg/gm dry mass.

Timber species	Preservative	Element	Year 2	Year 4	Prob.
E. maculata	CCA + PEC	As	28	46	0.52
E. pilularis	CCA + PEC	As	46	45	0.99
P. elliottii	CCA + PEC	As	34	58	0.14
P. elliotti	CCA	As	67	53	0.75
E. maculata	CCA	As	93	94	0.98
E. maculata	PEC	As	26	26	0.99
S. glomulifera	none	As	17	20	0.91
E. maculata	CCA + PEC	Cr	24	12	0.31
E. pilularis	CCA + PEC	Cr	43	41	0.94
P. elliottii	CCA + PEC	Cr	77	91	0.75
P. elliottii	CCA	Cr	87	39	0.56
E. maculata	CCA	Cr	94	88	0.88
E. maculata	PEC	Cr	5	0	0.08
S. glomulifera	none	Cr	16	0	0.36
E. maculata	CCA + PEC	Cu	822	345	0.03*
E. pilularis	CCA + PEC	Cu	404	265	0.08
P. elliottii	CCA + PEC	Cu	1247	692	0.09
P. elliottii	CCA	Cu	1137	1057	0.74
E. maculata	CCA	Cu	759	535	0.25
E. maculata	PEC	Cu	95	71	0.22
S. glomulifera	none	Cu	111	69	0.14
All timbers	CCA or CCA + PEC	Cu	876	567	0.01*

*Statistically significant difference ($p < 0.05$)

Table 5.
Difference between barnacles on pile types after two years' exposure. Means joined by vertical line are not significantly different. Results given in µg/gm dry mass.
*Non-CCA piles combined.

Pile type	Mean
Arsenic	
Untreated *S. glomulifera** + PEC *E. maculata**	22
CCA + PEC *E. maculata*	28
CCA + PEC *P. elliottii*	30
CCA + PEC *E. pilularis*	46
CCA *P. elliottii*	67
CCA *E. maculata*	93
Chromium	
Untreated *S. glomulifera* + PEC *E. maculata*	9
CCA + PEC *E. maculata*	24
CCA + PEC *E. pilularis*	43
CCA + PEC *P. elliottii*	77
CCA *P. elliottii*	87
CCA *E. maculata*	94
Copper	
Untreated *S. glomulifera* + PEC *E. maculata*	101
CCA + PEC *E. pilularis*	404
CCA *E. maculata*	759
CCA + PEC *E. maculata*	822
CCA *P. elliottii*	1137
CCA + PEC *P. elliottii*	1247

Table 6.
Difference between barnacles on pile types at four year inspection. Means joined by vertical line are not significantly different. Results given in µg/gm dry mass.
*Non-CCA piles combined. **Below detectable limits

Pile type	Mean
Arsenic	
Untreated *S. glomulifera** + PEC *E. maculata**	23
CCA + PEC *E. maculata*	43
CCA + PEC *E. pilularis*	45
CCA *P. elliottii*	53
CCA + PEC *P. elliottii*	58
CCA *E. maculata*	94
Chromium	
Untreated *S. glomulifera* + PEC *E. maculata*	0**
CCA + PEC *E. maculata*	12
CCA *P. elliottii*	39
CCA + PEC *E. pilularis*	41
CCA *E. maculata*	88
CCA + PEC *P. elliottii*	91
Copper	
Untreated *S. glomulifera* + PEC *E. maculata*	71
CCA + PEC *E. pilularis*	265
CCA + PEC *E. maculata*	345
CCA *E. maculata*	535
CCA + PEC *P. elliottii*	692
CCA *P. elliottii*	1057

Table 7.
Difference between barnacles on pile types determined when results for both two and four year inspections are combined. Number = combined number of piles analysed. Means joined by vertical line are not significantly different. Results given in µg/gm dry mass. *Non-CCA piles combined.

Pile type	Number	Mean
Arsenic		
Untreated *S. glomulifera** + PEC *E. maculata**	20	19
CCA + PEC *E. maculata*	10	36
CCA + PEC *P. elliottii*	11	43
CCA + PEC *E. pilularis*	12	45
CCA *P. elliottii*	11	61
CCA *E. maculata*	11	93
Chromium		
Untreated *S. glomulifera* + PEC *E. maculata*	20	5
CCA + PEC *E. maculata*	10	18
CCA + PEC *E. pilularis*	12	42
CCA *P. elliottii*	11	65
CCA + PEC *P. elliottii*	11	84
CCA *E. maculata*	11	91
Copper		
Untreated *S. glomulifera* + PEC *E. maculata*	20	86
CCA + PEC *E. pilularis*	12	334
CCA + PEC *E. maculata*	10	584
CCA *E. maculata*	11	657
CCA + PEC *P. elliottii*	11	995
CCA *P. elliottii*	11	1101