

Copper-Chromium-Arsenic Levels in Barnacles Growing on Timber Marine Piles

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ABSTRACT

Barnacles collected from experimental marine piles that had been in service for 5.7 years at Townsville were analysed for copper, chromium and arsenic (CCA) content in both their tissue and shell. Compared to barnacles on untreated piles, copper levels in tissue and shell were significantly elevated in barnacles growing on CCA-treated *Pinus elliottii* Engelm. piles. Similarly, chromium levels in shell were higher in barnacles collected from CCA-treated *Corymbia maculata* (Hook.) K.D. Hill & L.A.S. Johnson piles. Although elevated, the copper and chromium concentrations in the tissue and shell of barnacles growing on CCA plus creosote-treated eucalypt piles were not significantly different from background levels. No elevation was evident in tissue arsenic concentrations. Comparison with CCA levels in barnacles collected from piles after two and four years service revealed that the rate of leaching of copper and chromium continues to decline. Barnacles growing 20-120 mm away from the CCA-treated *P. elliottii* piles did not have increased levels of copper or chromium.

INTRODUCTION

Wood used in a marine environment is prone to attack by a variety of marine borers. In some regions, particularly tropical waters, this hazard can be extreme. All but the most durable of timbers, for example, turpentine (*Syncarpia glomulifera* (Sm.) Niedenzu), require preservative treatment in order to provide useful service lives.

Much of the treated timber used in the sea around the world is treated with copper-chromium-arsenic preservative (CCA). Although predominantly fixed in the timber, it is widely recognised that some copper, chromium and arsenic will leach from CCA-treated timbers submerged in water (Brooks, 1996). The environmental impact of this leaching has been investigated in a number of studies, often with conflicting

conclusions. Weis & Weis (1992) and Weis *et al.* (1993a, 1993b) reported that metal levels in green algae (*Ulva lactuca* and *Enteromorpha intestinalis*), barnacles (*Balanus eburneus*) and oysters (*Crassostrea virginica*) found growing on CCA-treated timber structures were higher than those found in similar organisms gathered from other areas. On the other hand, Brown & Eaton (1997), Baldwin *et al.* (1996) and Albuquerque & Cragg (1995) suggest that, while leaching from treated timbers does occur, if the timber is properly seasoned and the preservative fixed, levels of metals leached are not great enough to have an adverse effect on the environment.

The majority of studies investigating the issue of metals leaching from CCA-treated timber in aquatic environments have concentrated on the introduction of freshly treated wood (Weis *et al.*, 1991; Hegarty & Curran, 1986). It is well documented that the degree of leaching from CCA-treated timber is greatest immediately after installation and decreases with time (Weis & Weis, 1996; Albuquerque & Cragg, 1995; Archer *et al.*, 1994). Little information is available regarding the leaching of copper, chromium and arsenic from treated wood that has been in service for an extended period of time. Such information would be more indicative of the long-term effects of the use of CCA-treated wood in the marine environment.

The ideal organism to monitor for evidence of contamination might be one actually growing on the pile itself. Barnacles are common fouling organisms in the tropical waters of northern Australia. Barnacles accumulate heavy metals from the surrounding environment, and their apparent inability to regulate metal levels make them useful as a biomonitor (Powell & White, 1990; Philips & Rainbow, 1988; Rainbow, 1987). A marine trial of commercially available timber mooring piles was established in three ports in Queensland in 1991 through the collaboration of CSIRO Forestry and Forest Products, Queensland harbour authorities, Queensland Department of Primary Industries (Forestry) and Koppers Timber Preservation. Our studies investigate the levels of copper, chromium and arsenic present in the tissue and shells of barnacles inhabiting the surface of these marine piles at the Townsville test site. Results after two and four years exposure were provided by Cookson *et al.* (1996), whereas the present article concentrates on the 5.7 year results.

MATERIALS AND METHODS

Timber piles

The Townsville test site contains 36 piles encompassing a range of different timber species and preservative treatments. Timber species and treatment combinations (number of replicates) include CCA-treated *Corymbia maculata* (Hook.) K.D. Hill & L.A.S. Johnson (spotted gum) [syn *Eucalyptus maculata* Hook.] (6), pigment emulsified creosote (PEC)-treated *C. maculata* (4), double treated (CCA + PEC) *C. maculata* (6), double treated *Eucalyptus pilularis* Sm. (blackbutt) (6), CCA-treated *Pinus elliottii* Engelm. (slash pine) (5), double treated *P. elliottii* (6) and untreated *S. glomulifera* (3).

Chemical analyses were performed on four samples from all piles to determine actual chemical loadings of both CCA and creosote wherever relevant. Samples were taken from both the outer five mm and a cross section of the full sapwood depth in the hardwood species, and from the outer five mm in treated *P. elliotii*. Timber species, treatment type and chemical loadings derived from analysis of samples collected are presented in Table 1.

Piles were installed at Townsville in September 1991 in the Ross River estuary. Piles were arranged randomly in a grid pattern covering an area 90 x 126 metres, the longer dimension being in the direction of water flow.

The piles were inspected after two, four and 5.7 years' exposure. The original purpose of the trial was to assess the efficacy of the various piles against marine borers. In order that an accurate appraisal of the performance of the piles could be made, inspections were conducted at low tide and all barnacles growing on the piles removed to expose timber surface. It follows that, since the four year inspection of the piles was performed in October 1995, the barnacles collected for this study would have been growing for no longer than 19 months.

Table 1. Mean preservative retentions in experimental mooring piles exposed at Townsville

Timber species	Treatment	CCA retention % m/m	Creosote retention % m/m	CCA retention kg/m ³	Creosote retention kg/m ³
<i>C. maculata</i>	Double (CCA/PEC)	1.4	10.7	13.3	101.9
<i>E. pilularis</i>	Double (CCA/PEC)	2.0	18.1	17.6	154.9
<i>P. elliotii</i>	Double (CCA/PEC)	8.3	26.2	63.7	201.0
<i>P. elliotii</i>	CCA	4.8	-	36.8	-
<i>C. maculata</i>	CCA	2.7	-	25.5	-
<i>C. maculata</i>	PEC	-	15.3	-	145.7
<i>S. glomulifera</i>	-	-	-	-	-

Barnacle analysis

Barnacles were collected from all experimental marine piles in April 1997. A metal scraper was used to dislodge barnacles from the piles, and care taken to ensure that all barnacles collected had the shell intact. As larval settlement occurs a number of times a year, the size distribution within barnacle populations was quite varied. No preference was given to barnacles of a particular size; rather, each sample consisted of upwards of 50 individuals encompassing the entire range of shell sizes present. Sizes ranged from about three to 15 mm diameter. Generally, the smallest individuals collected were adhering to the shell of larger individuals. No preference was given to particular barnacle species. Samples were packed in plastic bags and frozen on the day of collection.

Barnacles were stored in the laboratory at -5°C until required for analysis. Upon thawing, whole soft parts were removed using stainless steel instruments. Tissue from all barnacles removed from the same pile was combined, weighed and freeze-dried overnight. Pooling of tissue from such a large number of individuals was done in order to compensate for any influence of body size on metal loading, a phenomenon observed in a number of barnacle species (Powell & White, 1990; Philips & Rainbow, 1988). Dried tissue was digested in 250 ml conical flasks by initially soaking in 2.5 ml concentrated H_2SO_4 for 15 minutes before adding one ml aliquots of 30% w/v H_2O_2 . Hydrogen peroxide was initially added to the digest at room temperature, and subsequent additions were made once the reaction from that previous addition had subsided. Generally, after the addition of the third ml of H_2O_2 reactions diminished, at which point the flasks were transferred to a hot plate; heat was gradually increased and, as the solution darkened in colour, further one ml H_2O_2 aliquots added. Digestion was complete when the solution remained clear and acid began to reflux ten mm up the sides of the flasks. Digests were made up to 100 ml with deionised water. Copper, chromium and arsenic concentrations were determined using a GBC Integra XM sequential Inductively Coupled Plasma spectrophotometer (ICP). Standards were run every six samples to confirm the absence of any significant drift.

Technique integrity was assessed through the use of standard reference material, TORT-2 (lobster hepatopancreas) (National Research Council, Canada). While chromium levels were below limits of detection, recoveries of copper and arsenic averaged 96.8% and 102.6 %.

Barnacle shells were brushed thoroughly to remove dried algae and sediment. Since whole barnacles were removed from the marine pile, timber fibres remained attached to the basal plate. Inclusion of these fibres would obviously influence results. Thus shells were carefully removed from the basal plate using insulated pliers and the basal plate discarded. Shell fragments were placed in 125 ml conical flasks with 50 ml 1 N HCl at room temperature overnight, and heated to 60°C for three hours. Solutions were filtered and analysed using the ICP spectrophotometer. In addition to containing a known quantity of copper, chromium and arsenic, the standard solution used to calibrate the ICP also contained a quantity of CaCO_3 equivalent to that in the shell solutions.

Data were treated statistically by initially deriving mean metal concentrations for samples of barnacle populations collected from the same pile type. These means for each element were consequently compared using a Model I analysis of variance with each pile treatment considered as a fixed factor. When significant differences ($p < 0.05$) were identified, comparisons between pile types employed Tukey's test for multiple comparisons.

RESULTS

Data for copper, chromium and arsenic concentrations in dry tissue and shell from barnacle populations growing on various marine piles are presented in Figures 1-5 along with comparisons by displaying honestly significant difference (HSD) ($\alpha < 0.05$) as described by Andrews *et al.* (1980).

Copper

The mean copper concentration in the tissue of barnacles growing on CCA-treated *P. elliotii* was statistically higher ($\alpha < 0.001$) than in barnacles growing on all other pile types except double treated *P. elliotii* (Figure 1). The copper concentration in tissues from barnacles growing on the double treated *P. elliotii* piles was significantly greater than in barnacles from *S. glomulifera*, PEC-treated *C. maculata* and double treated *E. pilularis* piles.

There was no significant difference between mean copper concentrations in tissue from barnacles growing on the remaining marine pile types. However, there was an apparent trend suggesting that barnacles growing on piles with at least part CCA treatment had slightly higher tissue copper concentrations than those growing on non-CCA-treated piles. Indeed, barnacle populations growing on CCA-treated and double treated *C. maculata* piles had mean copper levels 1.3 times greater than those from barnacles growing on untreated piles. So too, metal levels in barnacles collected from double treated *E. pilularis* piles were elevated by comparison with those observed in barnacles from non-CCA-treated piles.

Copper concentrations in the shell of barnacles growing on both CCA-treated and double treated *P. elliotii* piles were significantly higher ($\alpha < 0.001$ and $\alpha < 0.01$ respectively) than those in barnacles growing on any of the other experimental marine piles (Figure 2). The only other significant difference ($\alpha < 0.05$) identified was that between the mean copper level in shells from barnacles growing on double treated *C. maculata* piles and those on the non-CCA-treated piles; *S. glomulifera* and PEC-treated *C. maculata*.

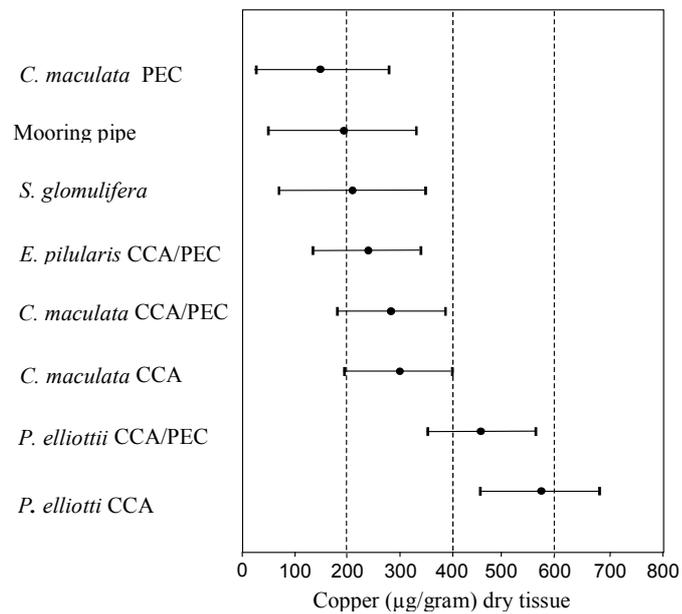


Figure 1 Mean concentration, showing honestly significant difference, of copper in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles.

Barnacles removed from the metal mooring pipe attached to the CCA-treated *P. elliotii* pile had mean tissue and shell copper concentrations similar to those in barnacle populations from the non-CCA-treated piles (Figures 1 and 2).

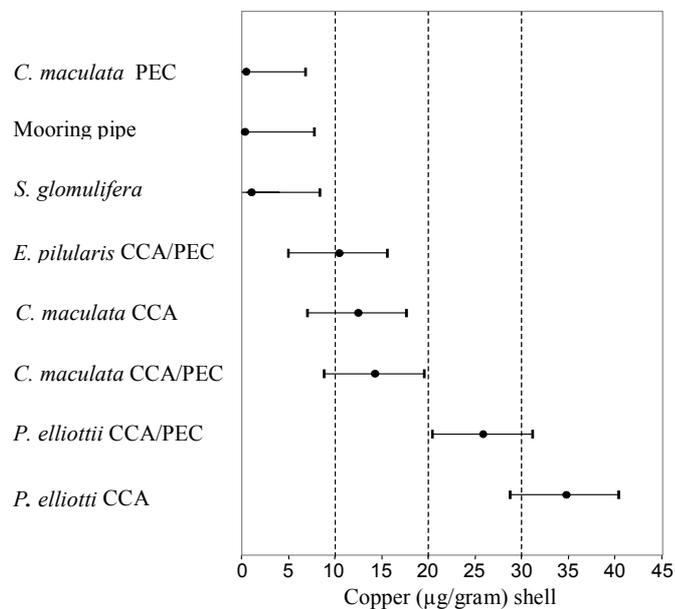


Figure 2 Mean concentration, showing honestly significant difference, of copper in shell of barnacles inhabiting 5.7 year old experimental marine piles.

Chromium

There was no significant difference between chromium concentrations in the tissues of barnacles collected from any of the experimental pile types (Figure 3). However, the ranked order of mean concentrations suggests that there is some minor correlation between chromium levels in barnacle tissue and the type of pile from which they were collected. Tissue from barnacles growing on CCA-treated *P. elliotii* piles had a mean concentration of chromium roughly 3.5 times that found in barnacles collected from non-CCA-treated piles. Barnacles growing on CCA-treated *C. maculata*, double treated *C. maculata* and double treated *P. elliotii* contained similar levels of chromium, about 2.5 times that in barnacles from non-CCA-treated piles. Chromium concentrations in the tissues of barnacles growing on double treated *E. pilularis* piles were moderately lower at 1.5 times the mean concentrations for non-CCA-treated piles.

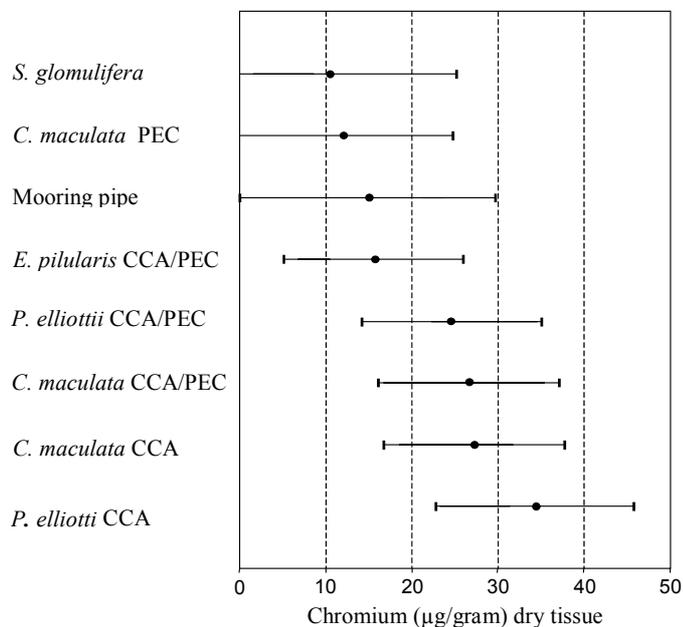


Figure 3 Mean concentration, showing honestly significant difference, of chromium in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles

Mean chromium concentrations in shells from barnacles growing on both *S. glomulifera* and PEC-treated *C. maculata* piles were minimal (Figure 4). Shells of barnacles growing on CCA-treated *C. maculata* piles had a mean chromium content of 20.6 µg/g, which is significantly higher ($\alpha < 0.01$) than the mean chromium level in barnacle shells collected from any other pile type except CCA-treated *P. elliotii*. Barnacles growing on CCA-treated *P. elliotii* piles had a mean chromium concentration in their shells of 15.0 µg/g, which is significantly higher than corresponding values in barnacles collected from both non-CCA-treated pile types ($\alpha < 0.01$). Although mean chromium concentrations in barnacle shells collected from double treated *P. elliotii*, *C. maculata* and *E. pilularis* were

slightly elevated, they were not significantly different from those in barnacles from the non-CCA-treated piles.

Mean chromium levels in the tissue and shells of barnacles gathered from the metal mooring pipe were negligible, as they were in barnacles collected from non-CCA-treated piles.

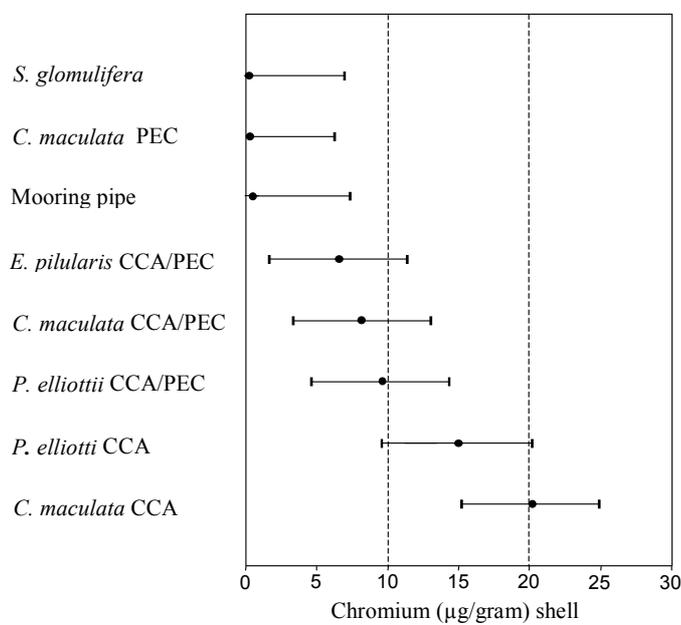


Figure 4 Mean concentration, showing honestly significant difference, of chromium in shell of barnacles inhabiting 5.7 year old experimental marine piles.

Arsenic

Our arsenic analyses using the ICP were associated with a high-percentage relative standard deviation. This was primarily due to alterations made to the ICP since earlier analyses, which were more accurate (Cookson *et al.*, 1996). While arsenic levels in barnacle tissue digests are presented, concentrations in shell solutions were below the limits of detection using the current system.

Statistical analysis of arsenic levels within the tissue of barnacles growing on experimental piles failed to reveal any significant differences (Figure 5). Barnacles collected from double treated *C. maculata* piles had the highest mean concentration, 36.7 µg/g, which is similar to that of barnacles growing on CCA-treated *C. maculata* (34.0 µg/g). Tissue from barnacles growing on *S. glomulifera* piles had a mean arsenic level of 28.4 µg/g. Barnacles collected from all other experimental pile types; CCA-treated *P. elliotii*, double treated *P. elliotii*, PEC-treated *C. maculata* and double treated *E. pilularis*, all had very similar mean tissue arsenic concentrations at 23.8, 23.5, 23.4 and 23.2 µg/g respectively. The mean arsenic concentration in the tissue of barnacles collected from the mooring pipes was 24.2 µg/g, similar to this group.

Comparison of copper and chromium levels in tissue and shell

Figure 6 compares the copper and chromium levels identified in tissue and shell. The majority of copper accumulated in the bodies of barnacles is retained in the tissue. Chromium levels in shell were up to 70% of the content measured in oven dry tissue.

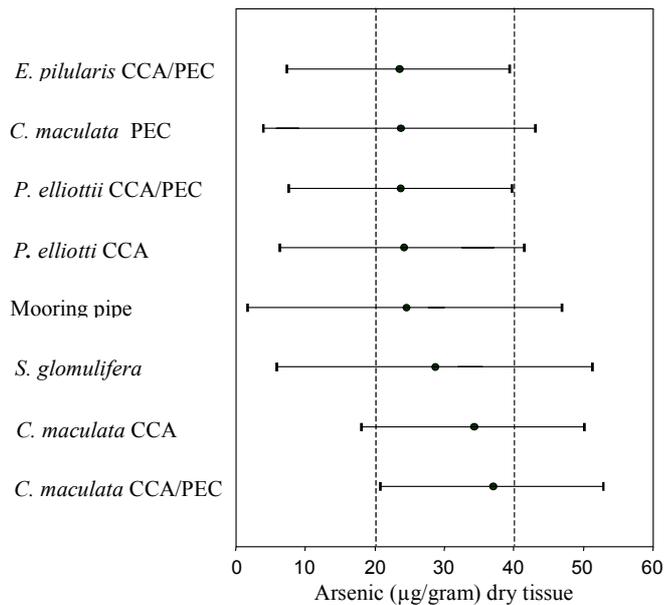


Figure 5 Mean concentration, showing honestly significant difference, of arsenic in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles.

CCA losses over time

Metal levels found in barnacle tissue in this study were compared to levels identified in a previous study by Cookson *et al.* (1996) of the same piles after two and four years service. This earlier study employed a different method of extraction. The procedure was compared using the same standard reference material (lobster hepatopancreas). Copper and arsenic recoveries of 94% and 101.5% respectively were obtained. These values were similar to those achieved using the present method of tissue digestion (96.8% and 102.6% respectively). Therefore, comparison between the studies seems warranted.

Figure 7 presents the levels of CCA in the tissue of barnacles collected from the experimental piles after two and four years service (Cookson *et al.*, 1996) and from the present study (5.7 years service). There is a distinct trend of decreasing copper loadings

in those barnacles growing on CCA-treated piles over time. The trend was similar for chromium though less pronounced. No trend was apparent for arsenic, as elevated levels were not detected in either study.

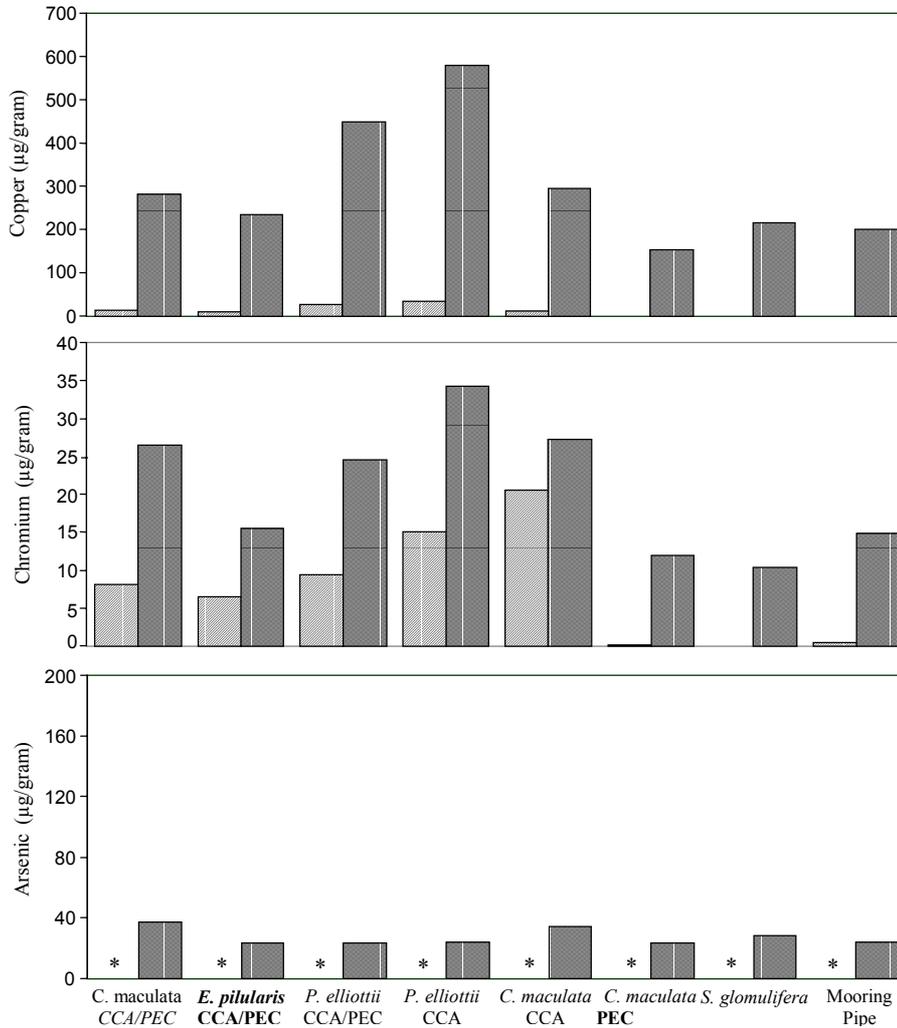


Figure 6 Comparison of the mean concentration of metals in shell and dry tissue, of barnacle populations growing on 5.7 year old experimental marine piles.

▨ shell ■ dry tissue * indicates levels at or below detection limits.

DISCUSSION

Relatively few significant differences were found in the copper, chromium and arsenic contents of barnacles growing on various treated and untreated piles that had been in service for 5.7 years. No significant difference in tissue arsenic content was found, which

is consistent with the results obtained from the two and four year inspections (Cookson *et al.*, 1996). When differences were found for copper and chromium, elevated levels were more common in barnacles on treated softwood than hardwood. The contamination profiles of copper and chromium in barnacle tissue were similar. Highest levels of accumulation for both elements were in barnacles collected from CCA-treated *P. elliotii*. These levels were both around three times that found in barnacles growing on non-CCA-treated piles. Treatment of hardwood with CCA generally results in much lower quantities of total preservative retained, as compared to softwood, because of thinner sapwood bands. Accordingly, levels of copper and chromium in tissues of barnacles growing on *C. maculata* piles treated at least in part with CCA were much lower than those from *P. elliotii* piles, and roughly 1.3 times those in barnacles from non-CCA-treated piles. Likewise, levels of copper and chromium in the tissue of barnacles taken from double treated *E. pilularis* piles were lower than from *C. maculata*. Once again, *E. pilularis* generally has a narrower sapwood band than does *C. maculata*. Therefore, while retentions of preservative within the sapwood region of both pile types are comparable (indeed that in *E. pilularis* piles was greater), overall there is substantially more CCA retained by *C. maculata* piles upon treatment.

Analysis for traces of copper and chromium in shell has revealed, in accordance with conclusions from the tissue analysis, that a distinct relationship occurs between accumulated levels in barnacles and the amount of CCA preservative retained in the timber substrate. Copper and chromium concentrations were almost undetectable in barnacles growing on non-CCA-treated marine piles, whereas populations taken from piles treated at least in part with CCA had elevated levels of both metals. Although the highest levels of chromium were recorded in barnacles from double treated *C. maculata* piles, in general the degree of copper and chromium contamination in shell reflected the initial retention of CCA in the corresponding pile type.

It is interesting to note that although the double treated *P. elliotii* piles had a CCA retention almost twice that of the CCA-treated *P. elliotii* piles, the mean levels of copper and chromium in the tissues were lower in barnacles from the double treated piles. While further studies are required to confirm this, it would seem likely that the second treatment with PEC slows the leaching of CCA from *P. elliotii* piles. The same does not appear to be true for the double treatment of *C. maculata*, as mean tissue levels of copper and chromium were similar whether the barnacles were growing on CCA-treated piles or double treated piles, even though the mean CCA retention was higher in the former. There was, however, substantially more chromium in the shell of barnacles collected from CCA-treated *C. maculata* piles than from similar double treated piles.

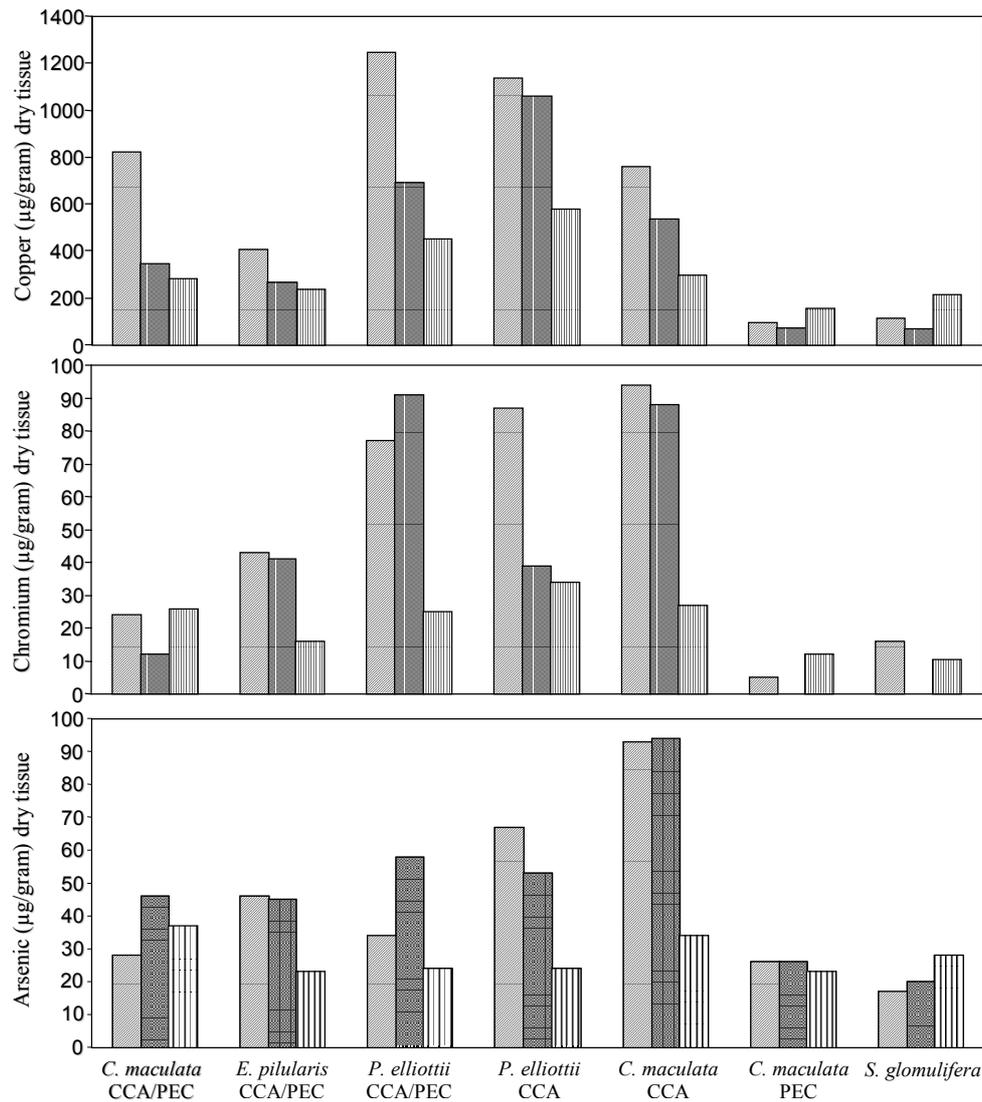


Figure 7 Mean concentration of copper, chromium and arsenic in barnacle tissue samples taken from experimental marine piles after 2, 4 and 5.7 years service life.

▨ 2 yrs ▩ 4 yrs ▮ 5.7 yrs

A number of studies have investigated the reliability of barnacle shells as a chemical record of trace elements in the environment (Watson *et al.*, 1995; Bourget, 1974), mostly with inconclusive results. Such studies found that, while barnacles had higher shell metal-concentrations from environments with higher metal loadings, there were a large number of variables that could also influence shell metal concentration such as growth rate (Bourget, 1974), seasonal change and shell size (Watson *et al.*, 1995). These studies have focussed on trace elements in the environment being the primary source of contamination, while the possible source in this study is far more direct. Mean levels of copper identified in this study, up to 35 ppm, were much greater than those

described in other reports, at up to around 4 ppm (Watson *et al.*, 1995). Further work is desirable in order to determine the process that contaminates barnacle shells to such an extent, e.g. by filter feeding or direct diffusion from the timber surface. Monitoring levels of chromium in the shell of barnacles could prove particularly useful, as the concentrations identified in the shell are similar to those in dry tissue and would exceed that in wet tissue. That is to say, the majority of the chromium burden in an individual organism may be present in the shell.

Comparison of copper and chromium levels identified in this study with those from previous work reveals an apparent trend suggesting a decline in the level of leachates being accumulated. From this it can be proposed that the degree of copper and chromium leaching from CCA-treated marine piles continues to decline during service.

The mean levels of copper and chromium detected in the tissue and shell of barnacles collected from the metal mooring pipes strongly suggests that contamination by leachates from CCA-treated timber occurs only in individuals within very close proximity of the timber/water interface. These barnacle populations were between 20-120 mm from the surface of CCA-treated *P. elliotii* piles and yet had metal levels very similar to those found in barnacles collected from untreated piles. Indeed, this can be taken further to suggest that the nature of a particular organism can make it more susceptible to acquiring leachates from treated timber. Barnacles grow with a basal plate of substantial surface area in intimate contact with the timber surface. Alder-Ivanbrook & Breslin (1999) found that the blue mussel, *Mytilus edulis*, accumulated little copper and chromium when exposed to CCA type C treated timber. A fouling organism such as *M. edulis* is secured by use of byssal threads, which provide far less intimate contact with the substrate and consequently are less likely to accumulate leachates from treated timber.

Our results from the analysis of barnacles suggest that there is no significant long-term contamination of the water column with CCA arising from the use of treated piles. However, barnacles growing directly on the surface of some pile types may have significantly elevated copper and chromium levels. Highest contamination was in barnacles growing on the surface of CCA-treated *P. elliotii* piles. A second treatment with creosote appeared to reduce the loss of CCA from treated *P. elliotii* piles. Of those pile types containing CCA preservative, least and generally insignificant contamination was found in barnacles growing on double treated hardwood piles of *E. pilularis* and *C. maculata*.

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Additional Papers

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ABSTRACT

This volume contains nineteen papers from the 10th International Congress on Marine Corrosion and Fouling, held at the University of Melbourne in Melbourne, Australia, in February 1999. The scope of the congress was to enhance scientific understanding of the processes and prevention of chemical and biological degradation of materials in the sea. Papers in this volume range across the themes of marine biofilms and bioadhesion, macrofouling processes and effects, methods for prevention of marine fouling, biocides in the marine environment, biodeterioration of wood in the sea, and marine corrosion.

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