EFFECTS OF WOOD PRESERVATIVES (CCA, CCB, CDDC, ACZA, ACQ AND CC) ON THE SETTLEMENT AND GROWTH OF MARINE BIO-FOULING ORGANISMS

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ABSTRACT

The effects of wood preservatives on settlement, abundance, growth and biomass development of fouling organisms (Non-target organisms) was studied on treated panels exposed at an Indian harbour, Krishnapatnam (Lat.13^{0}28' to13^{0}59' N; Long: 80^0 10' to 80^0 16'E). Panels of *Bombax ceiba* treated with copper chrome arsenic (CCA), copper chrome boric acid (CCB), ammoniacal copper zinc arsenate (ACZA), ammoniacal copper quaternary (ACQ), or ammoniacal copper citrate (CC) and hem-fir panels treated with copper dimethyldithiocarbamate (CDDC) were exposed over a 24 month period. There was considerable variation in abundance and biomass of fouling organisms among the preservatives. Algal and bryozoan settlement was common on all the treated and untreated panels during the initial stages (up to one month), but these communities were replaced due to heavy settlement of calcareous organisms such as barnacles, oysters and serpulids. A greater variety of fouling assemblages were recorded on control, CCB and CCA treated panels compared to CDDC, ACZA, CC and ACQ treated panels. CCA treated panels had heavier settlement of barnacles followed by oysters and bryozoans, while CCB treated panels had heavier settlement of oysters followed by barnacles and bryozoans. Serpulid settlement was negligible on treated panels, but heavy on control panels. The preservatives tested appeared to have a positive impact on settlement of barnacles, oysters and bryozoans. CDDC panels experienced heavier bryozoan settlement followed by barnacles, oysters and serpulids. The fouling assemblages that developed on ACZA, ACQ and CC panels consisted of fewer species that were less abundant than those found on other panels indicating that these preservatives negatively impacted settlement of fouling organisms. Biomass levels were found to be highest on CCB treated panels, while the lowest levels of fouling occurred on ACQ treated panels. The results illustrate the differential effects of wood preservatives on surface colonizers of wood in marine environments.

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INTRODUCTION:

While we no longer depend upon wood for our sailing vessels, wood continues to play an important role in the marine infrastructure supporting the many docks, wharves and other features of our harbors and ports. As in olden times, this wood remains susceptible to attack by fungi, insects and marine borers. This attack can be minimized by the use of naturally durable timbers, but the supplies of these materials are limited and their cost can be prohibitive. As a result, we often depend on the use of non-durable timbers that are supplementally protected with preservatives (Kumar and Morrell, 1993).

Marine wood preservation provided the impetus for our current wood treating practices and remains an important component of this industry. For decades, creosote was the preferred treatment, but a number of inorganic arsenicals (primarily chromated copper arsenate) have also emerged as important marine wood preservatives. Preservative treatment is an extremely effective method for extending the life of wood used in marine environments, however, the preservative levels required to achieve this protection are often 2 to 4 times those required for protecting wood in terrestrial environments. The use of such high chemical loadings was of little concern to users as ports sought to increase capacity and maximize their investment, but an ever-increasing environmental ethic has led to a reexamination of chemical use in sensitive environments including the use of chemically preserved wood.

While all chemicals have experienced increased public scrutiny, CCA, by virtue of its dominance in world commerce, has received the greatest attention (Solo-Gabriele et al., 2003; Townsend et al., 2001). One of the primary advantages of CCA as a preservative is its ability to react with and become fixed to the wood following treatment. The reactions by which this process occur have been extensively studied and these data have been used to develop procedures for minimizing the risk of metal loss into the surrounding environment as well as the creation of models that can be used to predict the relative risk of treated wood use under varying harbor conditions. Despite these efforts, it is virtually impossible to completely fix the CCA components or, for that matter, those of any other metal-based preservative to the wood. Metal-based preservatives appear to function, in part, by always having a small amount of each metal component in solution within the wood cell lumens. The rates of metal release into the surrounding environment have been studied in a number of applications, but the most intensively studied have been aquatic applications because of the sensitivity of many marine organisms to elevated copper levels. While there have been a number of studies examining the potential metal losses in aquatic environments, relatively few have examined the effects of these losses on marine fouling organisms and even fewer have examined the effects of the CCA replacement chemicals on these organisms. Marine fouling organisms should be especially sensitive to preservative leaching since these organisms must settle and grow on the chemically treated wood and since this wood is treated to such high chemical levels. Chemical losses create the potential for both acute and chronic effects on the settling organisms that may result in either reduced abundance of specific organisms or wholesale shifts in fauna and flora. These effects may be minimal in well-flushed areas, where any chemical releases are quickly diluted, but metals can buildup to high concentrations in more stagnant waters, especially when very large volumes of treated wood are employed (Weis and Weis, 1992a).

Previous studies indicate that communities which develop on CCA treated wood have lower species diversity, abundance of certain species and biomass levels compared to untreated panels (Weis and Weis, 1992a, b; 1996) and adverse impacts have been found on the physiology of some organisms in close proximity to CCA treated surfaces (Weis et al., 1992). These studies quantitatively examined the effects of CCA treated wood on the early successional stages of epi-biotic development.

A number of studies have examined the effects of wood preservatives on settlement patterns, growth and biomass development of epi-biota in temperate and sub-tropical environments (Albuquerque and Cragg, 1995a, b; Baldwin et al., 1996; Brown, 1998; Brown and Eaton, 1997; 2001; Brown et al., 2000, 2001, 2002, Cookson et al., 1996; Marchall and Manton, 1997; 1998; Weis and Weis, 1999), but there is little data on the effects of these treatments in tropical waters. Since temperature can influence the toxicity of some metals (Bryer et al., 1985), examining treatments under a variety of environmental conditions should be any important component of any environmental assessment.

In this report, we describe the effects of preservative treatment on settlement of fouling organisms on hardwood and softwood panels treated with various metallic wood preservatives and exposed in tropical waters in India.

MATERIALS AND METHODS:

Bombax ceiba panels (37.5 by 37.5 by 150 mm long) were pressure treated with copper chrome arsenic (CCA), copper chrome boric acid (CCB), ammoniacal copper zinc arsenate (ACZA), ammoniacal copper quaternary (ACQ) or ammoniacal copper citrate (CC) to a target retention of 40 kg/m^3. Actual retentions ranged from 28 to 35 kg/m^3. Similarly, Hem-fir (a mixture of species of *Abies* and *Tsuga* sold commercially in the U.S.) panels (19 by 87.5 by 300 mm long) were treated with copper dimethyldithiocarbamate (CDDC) to a target retention of 10.9 kg/m^3. Treatment procedures can be found elsewhere (Tarakanadha et al., 2003; Narayanappa et al., 1999). All panels were labeled and holes were drilled at both ends then attached to ropes for immersion at the test site. Treated panels along with untreated controls were exposed one meter below the low tide level from a jetty at Krishnapatnam harbor.

Panels were examined for barnacles, serpulids, bryozoans and oysters (dominant fouling organisms on test panels) after 1, 3, 6, 12, 18 and 24 months. Barnacles, serpulids and oysters were counted individually, while levels of encrusting bryozoans were estimated by counting the total number of colonies and measuring colony spread (diameter) on each panel. Fouling species were identified to species wherever possible. At each observation, fouling settlement on each preservative treated panel was removed and placed into a tared plastic container. The panels were then resubmerged. While fouling removal at each time point disrupted the normal colonization patterns on the wood, it also created an open surface that provided a measure of the suitability of the panel at any given time to settlement and establishment by fouling organisms. The fouling organisms collected from each panel surface at each time point were weighed and the container weight subtracted to provide total wet biomass. The containers were oven-dried at 60^{0}C to constant weight then weighed again to estimate total dry biomass on each panel.

RESULT AND DISCUSSION

A variety of organisms settled on the various panels over the test period including: algae (*Enteromorpha intestinalis, E. compressa, Ulva lactuca);* serpulids *(Serpula vermicularis, Hydroides elegans, Mercierella enigmatica, Pomatoceros triquetor);* oysters *(Crassostrea madrasensis, Saccostrea cucullata);* barnacles *(Balanus amphitrite, Megabalanus tintinnabulum);* bryozoans *(Membranipora amoyensis, Hippoporina americana, Alderina arabianensis)* and bivalves *(Modiolus striatulus, Perna indica* and *P. viridis)* (Table 1). Control panels were colonized by a wide array of organisms in the short time before they were destroyed. Of the 17 species found on the panels at the site, only *M. amoyensis, A. arabianensis*, and *P. indica* were absent from the control panels. These results illustrate the role of woody debris as habitat in the marine environment. CCB treated panels contained all but one of the 17 species (*P. viridis*) over the 24 month period, while CCA treated panels were colonized by all but *S. vermicularis* and *P. triquetor*. These results suggest that CCB and CCA treatments did not adversely affect settlement or establishment of most fouling organisms. Panels treated with CDDC had a somewhat reduced diversity of organisms, with only 11 of the 17 organisms present over the test period; however, even fewer species were present on the panels treated with ACZA, ACQ or CC. These results suggest that surface conditions were unsuitable for settlement on panels treated with these chemicals. It is unclear how this diminished flora might affect the surrounding environment; however, it is clear that ammoniacal based treatments produce a different, less diverse species mixture on the wood. This reduced diversity is of relatively little consequence when the wood is used in isolated structures, but could become important where large amounts of timber treated with one chemical were employed.

Biomass levels on the panels increased steadily over the exposure period for all of the treated panels (Figure 1). Panels treated with ammonia-based systems (ACZA, ACQ and CC) had the lowest biomass levels while CCB treated panels had the highest. Panels treated with CCA also developed high levels of total biomass at each inspection. Biomass level serves as an indicator of receptivity of the panel surface to settle and colonization. Leaching of chemical from the panels would be expected to play a major role in this process. Ammoniacal-copper treated (ACZA, ACQ, CC) panels will tend to lose copper at a faster rate than acid-based systems (CCA, CCB, CDDC) and contain much higher loadings of copper (Archer et al.,1994). This combination would be expected to produce higher metal losses that would affect settlement to a greater degree and this trend was evident in our biomass results.

Settlement of specific groups of organisms varied with treatment. Barnacle levels were highest on CCA treated panels followed by CCB and CDDC treated panels (Figure 2a). ACZA, ACQ and CC treated panels all had uniformly low levels of barnacle colonization, suggesting that some component of the treatments had inhibited settlement. Oysters exhibited a similar trend to the barnacles for the first 3 months of exposure, then the levels on CCA treated panels declined to levels similar to those found with the other treatments (Figure 2b). Oyster levels on CCB treated panels declined between 12 and 24 months. Oyster levels on the other treatments were similar, although ACQ and CC treatments tended to have lower levels of colonization at all time points.

Serpulid levels were extremely high on the untreated panels, but were uniformly low on all treated panels. These results suggest that treatment will inhibit settlement or colony establishment by these organisms (Figure 2c). Bryozoan levels on control, CCA and CDDC treated panels tended to be elevated for the first 3 months of exposure, then levels in CCA treated samples declined to level similar to those found on panels treated with the other systems (Figure 2d). Panels treated with CDDC continued to have the highest levels of bryozoans after 24 months of exposure. The reasons for these differences are unclear. Similar results have been demonstrated in earlier studies from temperate waters (9) where bryozoan settlement, especially by *Bugula turrita,* was enhanced on CCA treated panels. Aggregation of the bryozoan *B. turrita* on CCA treated panels (Weis and Weis, 1992) and of *B. neritina* on copper treated panels (Wisely, 1962) have also been observed. Aggregation was apparently initiated by copper ions progressively restricting the swimming movements of the larvae passing over the painted surfaces. Larvae that are unable to swim descend on the surfaces, resulting in greater accumulation of larvae on treated surfaces. Brown *et al*., (2001) reported heavy settlement of bryozoans, *Electra pilosa* and *Bugula fulva* on CCA treated wood after 6 months of submergence, but bryozoan abundance was reduced after 12 months of immersion.

DISCUSSION

CCA treatment had a negligible impact on epi-biotic community. The total number of individuals, biomass and growth were actually higher on CCA, CCB and CDDC treated panels compared to controls although the loss of untreated control after 3 months limits direct comparisons. . This suggests that leached components of these preservatives had no adverse effect on fouling organisms. These results support earlier field studies conducted in temperate regions (Albuquerque, 1998; Alburquerque and Cragg, 1995a; Baldwin et al., 1996; Brown and Eaton, 2001; Brown et al., 2000, 2001, 2002; Cragg et al., 1998).

Generally, the rates of metal loss from treated structures are highest soon after submersion in the sea water, then gradually decrease with time. The impact of leachate is higher on fouling organisms in confined conditions, while leached metals may be rapidly diluted by the surrounding seawater in natural environments, minimizing the effects of the initial leaching surge. Brooks (1996) reported that the leaching of metals was most rapid during the first 5 or 6 days after installation in an aquatic environment, then leaching rates halved on each successive day after immersion. A number of studies have reported that chromium and arsenic emissions were low and always below the toxic levels (Brown and Eaton, 2001; Beslin and Ivanbrook, 1998; Lebow et al., 1999). On the other hand, Weis and Weis (1992a, b; 1996) suggested that leached metals from treated wood were a source of both chronic and acute pollution to marine biota which resulted in significantly lower numbers of species, diversity indices and biomasses. These experiments were performed in poorly flushed, relatively stagnant waters where concentrations of leachate could build up to high levels. These tests also used relatively short exposures (one month). The absence of marked effects on biomass and species diversity after 3 months in our tests suggest that any biological effect of leachate from CCA treated wood was short term relative to the service life of timber (Albuquerque, 1998).

Heavy settlement of barnacles, bryozoans, oysters and moderate settlement of serpulids on CCA and CCB treated panels indicates that these preservatives had minimal impact on fouling organisms or that the organisms may be somewhat tolerant of these components.

The heavy settlement of bryozoans and barnacles on CDDC treated panels implies that the material had little effect against fouling organisms. Moreover, Bryozoans tolerate a wide range of toxicants and were among the major organisms that initially settled on the treated panels. Oysters, appeared to be unaffected by treatment, although large quantities of metals, especially copper, can accumulate in body tissues with no adverse effect (Tarakanadha and Rao, 2002).

The lower intensity, biomass and growth of fouling organisms on ACZA, ACQ and ACC treated panels compared to CCA and CCB suggests that panels treated with ammoniacal preservatives probably release higher quantities of metals, that may deter or repel fouling larvae. The consistently lower levels of fouling on the ACZA treated panels may be either due to higher levels of metal loadings in these panels or greater bio availability of metals on panel surfaces (Tarakanadha et al., 2003).

Albuquerque (1998) studied mussel settlement and biomass on panels treated with CCA, ACQ or creosote and found the lowest values on ACQ treated panels. Mussel biomass was similar for all chemical types, suggesting that settlement was affected by the type of preservative, but that growth of successful settlers was less affected by the chemicals.

A lower abundance of barnacles on control panels compared to CCA treated panels indicates that the surface of control panels may be unattractive to barnacle larvae or reduced recruitment might have occurred in the early stages of surface colonization. Lower barnacle level may also reflect the relatively rapid degradation of the untreated panels, which created limited opportunities for settlement. Cragg *et al.* (1998) found that recruitment of barnacle larvae after 3 weeks of exposure was much higher on CCA treated panels than on untreated panels and suggested that high levels of recruitment could ultimately result in greater abundance of foulers. It is unclear why the treated wood allows for more successful colonization, although changes in the chemical environment near the wood surface may stimulate settlement (Brown and Eaton, 1997).

Settlement of serpulids tended to be lower on treated panels compared to the controls. Albuquerque *et al*., (2001) also reported significant reductions in *Pamatoceros triquetor* on CCA treated panels in Portugal. Heavy settlement of serpulids on untreated panels suggests that these tubeworms select surfaces based upon nutritional potential much in the same way that polychaete larvae show species specific settlement responses to particular surface polysaccharides or glycoproteins in the bacterial films on the wood surface (Kirchman et al., 1992). Treatments may have altered this bacterial flora to produce a different nutritional potential that was less attractive to the serpulids in our test site..

The effect of ammoniacal-based systems on settlement is particularly important since many of these chemicals have been developed as CCA replacements. It is clear that these systems have a much greater effect on the fouling community and merit further study. Albuquerque and Cragg 1995a) reported lower species richness, abundance and biomass on ACQ treated panels compared to CCA and creosote. The present study indicates that the effect of conventional preservatives on non-target organisms are moderate or negligible compared to ammoniacal preservatives like ACZA, ACC and ACQ.

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Table 1. Settlement of various fouling organisms on test panels treated with different wood preservatives and exposed for 24 months in India.

Fouling species	GROUP	INCIDENCE OF SPECIES (+/-)						
		NONE	CCA	CCB	CDDC	ACZA	ACQ	CC
Enteromorpha intestinalis	Algae	+	+	+	+	+	+	+
E. compressa	Algae	+	+	+	+	-	+	+
Ulva lactuca	Algae	+	+	+	+	+	-	+
Serpula vermicularis	Serpulid	+	-	+	-	-	-	+
Hydroides elegans	Serpulid	+	+	+	+	+	-	-
Mercierella enigmatica	Serpulid	+	+	+	+	+	+	-
Pomatoceros triquetor	Serpulid	+	-	+	+	-	-	-
Crassostrea madrasensis	Oyster	+	+	+	+	-	+	+
Saccostrea cucullata	Oyster	+	+	+	-	-	-	-
Balanus amphitrite	Barnacle	+	+	+	+	+	+	+
Megabalanus tintinnabulum	Barnacle	+	+	+	+	+	-	-
Membranipora amoyensis	Bryozoan	-	+	+	-	-	-	-
Hippoporina americana	Bryozoan	+	+	+	+	+	+	-
Alderina arabianensis	Bryozoan	-	+	+	+	+	-	+
Modiolus striatulus	Bivalve	+	+	+	-	-	-	-
Perna indica	Bivalve	-	+	+	-	-	-	-
P. viridis	Bivalve	+	+	-	-	-	-	-

Figure 1. Relative levels of biomass (dry weight basis) produced on panels treated with selected preservatives and exposed in an Indian harbor for 24 months.

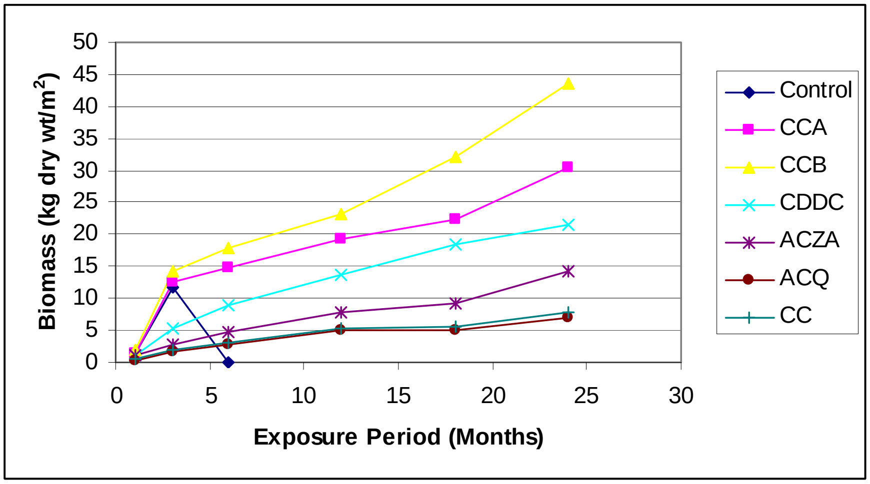

Figure 2. Incidence of a.) barnacles, b.) oysters, c.) serpulids, or d) bryozoans on panels treated with various preservatives and exposed in an Indian harbor for 1 to 24 months.

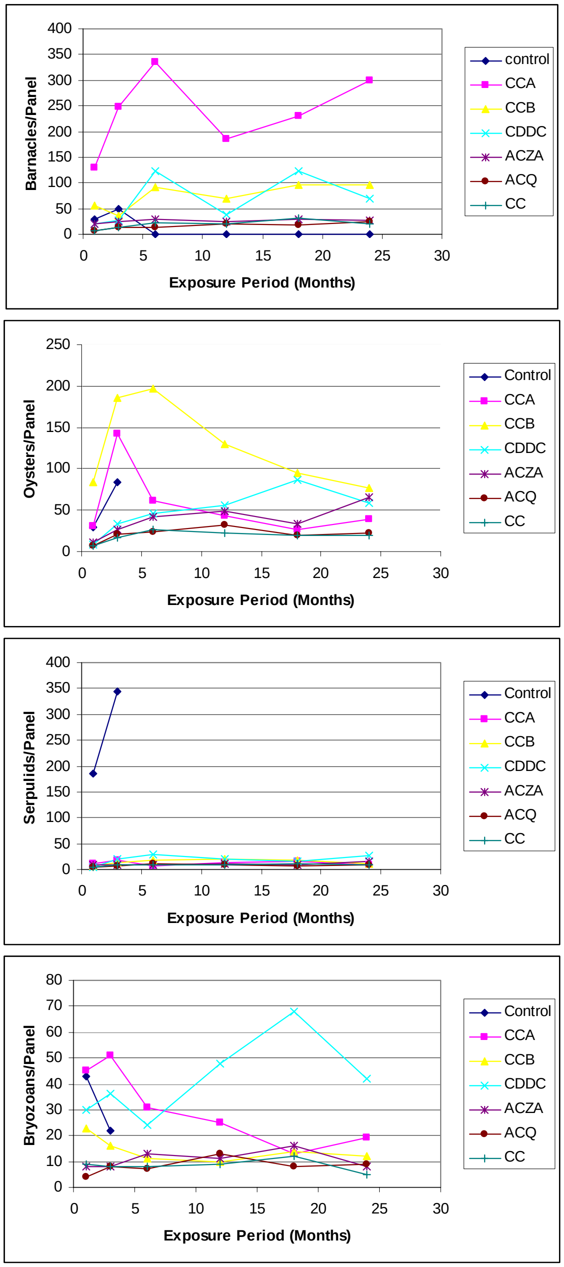

Table 2 Incidence of selected fouling organisms as assessed by settlement, growth and biomass accumulation on control and treated panels exposed for 1 to 24 months in a harbor near Krishnapatnam, India.

EXPOSURE TIME (Months)	Treatment	ORGANISM FREQUENCY/PANEL				Average Colony Diameter (mm.)				Biomass DRY WT. (Kg/m^2)
		Barnacles	*Oysters*	*Serpulids*	*Bryozoans*	*Barnacles*	*Oysters*	*Serpulids*	*Bryozoans*	
1	Control	29 (15)	29 (7)	185 (11)	43 (12)	-	-	-	-	1.51
	CCA	129 (39)	31 (10)	12 (4)	45 (12)	-	-	-	-	1.32
	CCB	55 (12)	84 (19)	7 (12)	23 (6)	-	-	-	-	1.91
	CDDC	21 (8)	9 (5)	5 (3)	30 (4)	-	-	-	-	1.17
	ACZA	21 (14)	11 (4)	9 (2)	8 (3)	-	-	-	-	1.10
	ACQ	6 (2)	7 (3)	5 (2)	4 (0)	-	-	-	-	0.40
	CC	6 (1)	7 (3)	4 (1)	9 (3)	-	-	-	-	0.44
3	Control	49 (2)	84 (30)	344 (79)	22 (8)	12 (2)	12 (1)	27 (59)	17 (4)	11.60
	CCA	248 (43)	143 (27)	17 (3)	51 (16)	13 (3)	22 (3)	18 (47)	20 (2)	12.70
	CCB	35 (16)	186 (23)	13 (5)	16 (7)	13 (2)	22 (3)	20 (64)	17 (2)	14.20
	CDDC	26 (12)	34 (19)	21 (6)	36 (6)	11 (3)	14 (1)	12 (42)	27 (4)	5.20
	ACZA	24 (6)	27 (8)	8 (2)	8 (3)	10 (3)	17 (3)	18 (21)	15 (3)	2.90
	ACQ	13 (2)	21 (6)	6 (1)	8 (3)	12 (3)	18 (4)	15 (33)	21 (2)	1.71
	CC	13 (5)	17 (4)	8 (2)	8 (2)	15 (4)	20 (2)	18 (53)	19 (2)	1.87
6	CCA	336 (39)	61 (11)	7 (2)	31 (13)	11 (4)	23 (3)	16 (33)	24 (3)	14.90
	CCB	91 (28)	197 (7)	17 (3)	11 (5)	15 (4)	24 (5)	17 (43)	23 (3)	17.80
	CDDC	123 (32)	46 (6)	28 (3)	24 (4)	13 (3)	18 (3)	18 (32)	34 (5)	8.80
	ACZA	30 (16)	42 (1)	9 (2)	13 (3)	15 (4)	29 (5)	15 (40)	21 (2)	4.76
	ACQ	14 (4)	24 (6)	12 (3)	7 (2)	13 (2)	21 (3)	16 (35)	18 (4)	2.93
	CC	22 (8)	26 (7)	8 (1)	8 (2)	14 (3)	21 (3)	10 (25)	22 (5)	3.12
12	CCA	185 (55)	43 (13)	14 (3)	25 (6)	21 (4)	22 (4)	17 (30)	30 (8)	19.40
	CCB	70 (20)	130 (16)	19 (3)	10 (2)	19 (3)	35 (5)	18 (25)	21 (4)	23.10
	CDDC	37 (13)	56 (7)	21 (3)	48 (3)	13 (3)	12 (3)	16 (24)	41 (4)	13.60
	ACZA	25 (15)	49 (10)	11 (3)	11 (2)	16 (2)	22 (3)	16 (31)	25 (5)	7.82
	ACQ	20 (4)	32 (9)	10 (2)	13 (5)	14 (1)	18 (3)	14 (24)	16 4)	4.90
	CC	19 (5)	22 (6)	10 (4)	9 (2)	17 (4)	21 (4)	15 (49)	24 (2)	5.17
18	CCA	231 (39)	27 (5)	15 (2)	13 (3)	24 (1)	33 (5)	18 (38)	22 (5)	22.40
	CCB	97 (24)	95 (35)	17 (8)	14 (4)	18 (3)	44 (9)	17 (34)	26 (4)	32.10
	CDDC	122 (12)	86 (24)	16 (3)	68 (4)	14 (3)	14 (4)	12 (23)	42 (4)	18.30
	ACZA	28 (12)	34 (5)	9 (4)	16 (3)	17 (3)	40 (7)	12 (22)	20 (4)	9.20
	ACQ	18 (3)	20 (4)	6 (1)	8 (2)	16 (2)	23 (4)	12 (1)	24 (6)	5.11
	CC	31 (9)	19 (5)	12 (4)	12 (2)	19 (2)	22 (3)	14 (27)	21 (2)	5.70
24	CCA	299 (55)	39 (8)	10 (4)	19 (5)	27 (4)	39 (10)	18 (38)	28 (8)	30.50
	CCB	95 (35)	77 (15)	11 (3)	12 (4)	21 (2)	47 (7)	17 (36)	32 (8)	43.60
	CDDC	69 (28)	59 95)	27 (9)	42 (6)	11 (2)	19 (3)	18 (17)	67 (26)	21.60
	ACZA	27 (11)	66 (13)	15 (6)	8 (4)	19 (4)	23 (4)	13 (24)	23 (2)	14.20
	ACQ	24 (4)	23 (6)	9 (1)	9 (2)	19 (5)	30 (5)	14 (16)	25 (5)	7.10
	CC	20 (6)	21 (8)	10 (4)	5 (1)	13 (1)	27 (5)	12 (3)	27 (5)	7.90