

Biofouling: The Journal of Bioadhesion and Biofilm Research

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gbif20>

The control of calcification of antifouling paints in hard waters using a phosphonate inhibitor

Carolyn R Heath^a, B S C Leadbeater^a & Maureen E Callow^a

^a School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Published online: 09 Jan 2009.

To cite this article: Carolyn R Heath, B S C Leadbeater & Maureen E Callow (1996) The control of calcification of antifouling paints in hard waters using a phosphonate inhibitor, *Biofouling: The Journal of Bioadhesion and Biofilm Research*, 9:4, 317-325, DOI: [10.1080/08927019609378313](https://doi.org/10.1080/08927019609378313)

To link to this article: <http://dx.doi.org/10.1080/08927019609378313>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

THE CONTROL OF CALCIFICATION OF ANTIFOULING PAINTS IN HARD WATERS USING A PHOSPHONATE INHIBITOR

CAROLYN R HEATH, B S C LEADBEATER and MAUREEN E CALLOW[†]

School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

(Received 13 January 1996; in final form 26 February 1996)

The deposition of calcified biofilms on experimental "hard" antifouling paints was studied on panels immersed in a hard-water marina during the summer months of 1992. Various water analyses were performed and the water was supersaturated with respect to calcite during the time the panels were immersed. All paints contained cuprous oxide and Irgarol[®] 1051 (s-triazine herbicide) in combination with various loadings of an inhibitor of calcium carbonate formation, 1, hydroxyethylene 1,1 diphosphonic acid (HEDP). There was a reduction in the ratio of calcium carbonate to organic weight with increasing content of HEDP in the paints. The leaching rates of copper, Irgarol[®] 1051 and HEDP from paints were measured in laboratory experiments. The leaching results and the performance of the paints with respect to the formation of calcified deposits are discussed in relation to paint film characteristics and hard water chemistry.

KEYWORDS: calcium carbonate, calcite, algal biofilms, fresh water fouling, hard-water chemistry, antifouling paints, copper, phosphonate, HEDP, Irgarol[®] 1051

INTRODUCTION

Previous work has shown that deposition of calcium salts within algal biofilms on both antifouling paints and non-toxic surfaces in hard-waters is biologically mediated (Heath *et al.*, 1993). On boats, the calcified deposits are unsightly, rough and extremely difficult to remove, a process which may result in damage to the surface of the hull. The weight of biofilm on surfaces immersed in a hard water marina was found to be inversely related to depth; CaCO₃ accounted for approximately 56% of the dry weight of all biofilms and there was a positive correlation between the weight of CaCO₃ and organic weight at all depths (Heath *et al.*, 1993).

In laboratory experiments, precipitation of CaCO₃ in batch cultures of a range of axenic algae readily occurred when appropriate conditions were provided (Heath *et al.*, 1995). Precipitation of crystals occurred as a consequence of increased pH, and therefore the concentration of CO₃²⁻, resulting from photosynthesis, which caused the medium to become supersaturated with respect to CaCO₃ (Heath *et al.*, 1993; 1995). Precipitation was inhibited by a number of compounds known generally to inhibit crystal nucleation or crystal growth. The most effective compound out of fifteen tested, was a phosphonate, viz. 1, hydroxyethylene 1,1 diphosphonic acid (HEDP) (Heath *et al.*, 1995). HEDP (2.5 mg l⁻¹) prevented CaCO₃ precipitation by inhibiting crystal nucleation, in agreement with other studies (Reddy & Nancollas, 1973; Koutsoukos & Kontoyannis, 1984, Sikes & Wheeler, 1988). However, once nucleation was initiated, HEDP appeared to

[†] Corresponding author

have little effect on crystal growth although other workers have shown that phosphonates including HEDP also reduced the rate of crystal growth (Reddy & Nancollas, 1973; Robertson, 1973; Koutsoukos *et al.*, 1981; Xyla & Koutsoukos, 1987).

The aim of this investigation was to determine whether the ability of HEDP to prevent or delay CaCO_3 precipitation in laboratory batch culture experiments could be reproduced in the field by incorporation of HEDP into antifouling paints. In view of the high water solubility of HEDP, a "hard" vinyl experimental antifouling paint base without rosin was chosen. The content of all other ingredients was the same as for the commercial vinyl-rosin antifouling paint used previously (Heath *et al.*, 1993).

MATERIALS AND METHODS

Field Site and Water Chemistry

Panels were immersed in a marina at Ely, Cambridgeshire, UK (OS Landranger 143, TL 546 798). On-site measurements of pH, alkalinity, temperature, soluble calcium, conductivity and chl *a* were made of water from 5 sampling points, at the surface and 60 cm below the surface, every 4 w throughout the summer months of 1992. From the measured data, saturation index (SI) and PCO_2 were calculated. Full details of the methods and calculations can be found in Heath *et al.* (1993).

Paint Formulations and Experimental Design

The paint vehicle was 100% vinyl resin. Cuprous oxide and Irgarol[®] 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine) (Ciba Geigy, Switzerland) were added to all paints at the concentrations found in the standard commercial antifouling paint used previously (Heath *et al.*, 1993). Sodium HEDP (1, hydroxy ethylene 1,1 diphosphonic acid) (Dequest[™] 2016D, Monsanto, UK) was added at 0 (control), 5, 10 or 15% of the dry film weight, replacing the zinc oxide filler.

Two coats of each paint formulation were applied to 7×10 cm rectangles on 8 mm primed marine-ply boards. Each board accommodated two rows of seven rectangles. The four formulations were randomised, being represented once on each board, the other rectangles being used for a different trial. There were four boards giving a total of four replicates per formulation. The boards were immersed for 16 w from June–September, 1992.

Biofilm Analysis

After 16 w immersion, the boards were removed and returned to the laboratory for analysis. Three strips (2×10 cm) of biofilm were removed into separate tubes for (a) chl *a* analysis, (b) identification of the most abundant species, (c) weight analysis (see Heath *et al.*, 1993). Dry weight (W1) was obtained by drying at 90°C to constant weight. CaCO_3 was removed by addition of excess 0.2 N HCl; the HCl was then decanted before drying at 90°C to constant weight. Weight of CaCO_3 was determined as the difference in dry weight (W1) and the weight (W2) after acid treatment. Inorganic (non- CaCO_3) weight (W3) was the residue following combustion for 5 h at 500°C . The various weights were calculated as follows: dry weight = W1; weight of CaCO_3 = W1-W2; organic weight = W2-W3. The data obtained from the various weight analyses are expressed as means \pm standard error of the mean and significance was tested by analysis of variance ($p < 0.05$).

Paint Leaching

1 cm tracks of paint, 200 μm dry film thickness, were applied to the middle of frosted glass microscope slides, size 76×40 mm. Slides were leached in sealed polythene boxes, $13 \times 12 \times 3.5$ cm, containing 100 ml hard water medium (ASTM, 1980) at 25°C , on an orbital shaker at 60 rpm for varying periods of time from 1–7 d depending on the amount of compound leached. ASTM medium contains NaHCO_3 (192 mg l^{-1}), $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (120 mg l^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (245.5 mg l^{-1}), KCl (8 mg l^{-1}). The pH is 7.6–8.0 and the hardness 160–180 $\text{mg l}^{-1} \text{ CaCO}_3$. Leachates were analysed from paints containing 0, 5 and 15% HEDP.

Analysis of Paint Leachates

Copper was analysed by atomic adsorption spectroscopy (Pye Unicam SP9) after acidification of 10 ml samples with $2 \mu\text{l ml}^{-1}$ concentrated nitric acid. Spectrosol copper nitrate solutions (BDH) were used for the standards.

Irgarol 1051 was measured semi-quantitatively using thin layer chromatography (TLC). Leachate samples (10 ml) were shaken with 0.5 ml isopropyl ether then placed on ice for 5–10 min to allow partition of the Irgarol 1051-containing solvent phase above the aqueous phase. A range of concentrations of samples and Irgarol 1051 standards (20 mg l^{-1}) were applied to TLC plastic sheets, silica gel 60-F₂₄₅ (Merck) using repeated loadings from 1 μl capillaries. The carrier solvent was 10% cyclohexane and 90% isopropyl ether. The concentration of Irgarol 1051 in samples was determined by comparing the intensity of spots viewed under UV light with the standards.

HEDP was measured as total dissolved phosphorus according to the method of Jurgans and Gude (1990). Nine ml of sample mixed with 1 ml 1.5% w/v potassium peroxodisulphate were digested by autoclaving for 1 h. Standards of NaHEDP (Dequest 2016D, Monsanto) in distilled water contained 0.1–3.0 $\text{mg l}^{-1} \text{ PO}_4\text{-P}$. The concentration of soluble reactive phosphate was measured using the method of Mackereth *et al.* (1978).

From the quantity of compounds measured in the leachates, leaching rates were calculated as $\mu\text{g compound cm}^{-2} \text{ d}^{-1}$. Data are plotted as the mean of four determinations \pm standard error of the mean.

RESULTS

Water Chemistry

None of the parameters measured varied with depth, so the results are the mean of the two depths sampled. The results are shown in Table 1. Since measurements were taken at monthly intervals, the data provide only a broad indication of conditions during the period of the trial. The soluble calcium concentration was fairly constant until Mid October when it rose sharply. Generally, alkalinity, pH, conductivity and saturation index (SI) fell from July to September. The water was always highly saturated with respect to CaCO_3 , and an extreme saturation ($\text{SI} = 2.0$) was observed in July. Low PCO_2 values (Table 1) coincided with high chl *a* levels and as these fell, the PCO_2 rose.

Analysis of Biofilms on Painted Panels

All of the paints formed good surface films before immersion but after 16 w immersion, paints containing the highest loading of HEDP (15%) exhibited some blistering. After

Table 1 Chl *a* and water chemistry of Ely marine during 1992

	Sample dates				
Parameter	1	2	3	4	5
chl <i>a</i> (µg ml ⁻¹)	0.06 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.03 ± 0.003	0.01 ± 0.002
pH	8.9 ± 0.04	8.9 ± 0.07	8.5 ± 0.08	7.9 ± 0.07	8.2 ± 0.1
Cond (µS cm ⁻¹)	938.4 ± 6.2	968.0 ± 10.5	887 ± 7.1	944.9 ± 12.7	1091.0 ± 6.4
Ca (mg l ⁻¹)	114.9 ± 0.5	112.2 ± 0.8	106.5 ± 0.5	113.6 ± 0.5	164.4 ± 0.5
Alk (meq l ⁻¹)	6.2 ± 0.05	5.2 ± 0.1	4.9 ± 0.1	5.0 ± 0.04	5.5 ± 0.01
PCo ₂ (atm)	0.4 ± 0.01	0.3 ± 0.03	0.8 ± 0.02	3.3 ± 0.2	1.5 ± 0.1
log Ω or SI	2.0 ± 0.01	1.9 ± 0.02	1.4 ± 0.01	0.6 ± 0.03	1.2 ± 0.03

Sample dates: 1, 2nd July; 2, 30th July; 3, 26th August; 4, 23rd September; 5, 21st October. Each value is the mean of 10 determinations ± SEM.

16 w immersion, all panels were covered with a green biofilm and there were no significant differences in the weight of chl *a* on panels from different treatments (data not shown). The addition of HEDP had a significant ($p = < 0.05$) effect on the dry, organic and inorganic weights but examination of Figure 1 reveals no trends with increased HEDP loading and the significant effect merely reflects variance in weight values. However, the increase in HEDP loading resulted in a highly significant ($p = < 0.01$) reduction in the amount of CaCO₃ present within the biofilm. Thus, as the loading of HEDP increased, the weight of CaCO₃ per unit organic weight decreased (Fig. 2), indicating that the amount of CaCO₃ present within biofilms was proportional to the concentration of HEDP in the paint.

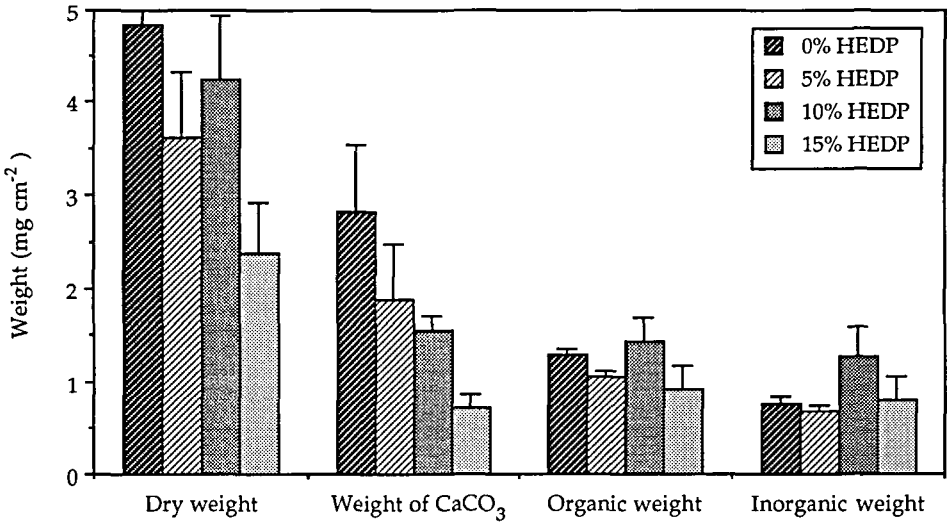


Fig. 1 Weight components of biofilms formed on paints containing HEDP, immersed for 16 w in Ely Marina. Each point is the mean of 4 replicates. Bars show SEM.

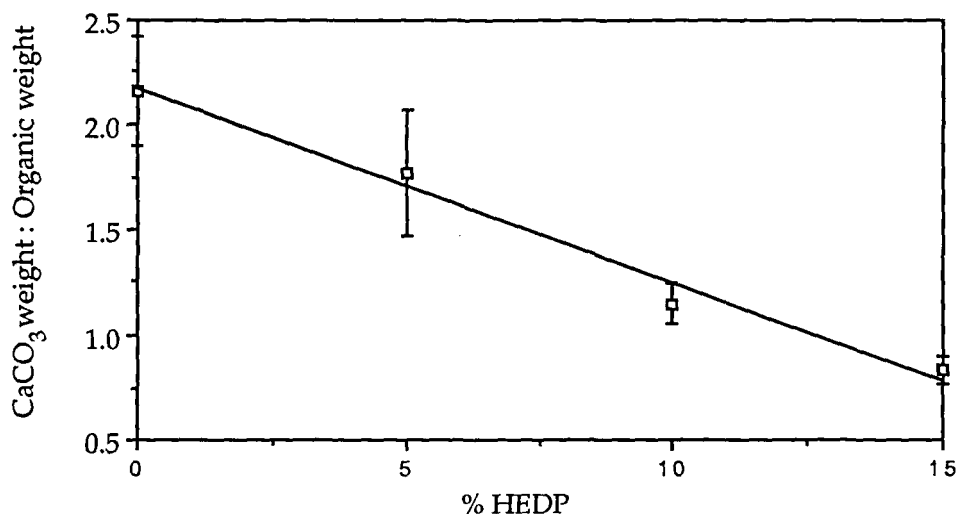


Fig. 2 The proportion of CaCO_3 per unit organic weight present in biofilms formed on paints containing different loadings of HEDP. Each point is the mean of 4 replicates. Bars show SEM.

The addition of HEDP had no effect on the species diversity of the biofilms growing on the surface. Diatoms and green algae accounted for approximately 60% and 40% respectively of the total mass of fouling organisms. The most commonly encountered genera of diatoms were *Navicula*, *Synedra*, *Cymbella* and *Fragilaria* whilst *Chaetophora* and *Chlorococcum* were the dominant genera of green algae.

Paint Leaching

HEDP. Initial leaching rates were extremely high, with paints containing 15% HEDP displaying rates in excess of $300 \mu\text{g HEDP cm}^{-2} \text{d}^{-1}$ during the first day following immersion (Fig. 3a). However, the leaching rate declined rapidly and after 20 d, the leaching rate was similar from both paints. After 40 d, the leaching rate was constant and leaching rates of $0.35 \mu\text{g cm}^{-2} \text{d}^{-1}$ were recorded up to the end of the experiment (60 d). At 60 d, 18% of the total HEDP in the paint had been lost.

Copper. The initial leaching rate (up to 5 d) was relatively high from the paint containing 15% HEDP (Fig. 3b). There was no difference between the leaching rate of the control paint (0% HEDP) and the paint containing 5% HEDP. After 16 d, a constant leaching rate of $0.09 \mu\text{g cm}^{-2} \text{d}^{-1}$ was recorded for all paints.

Irgarol 1051. Irgarol 1051 was not recorded in leachates from control paints (0% HEDP) until 4–5 d after immersion. Leaching rates of Irgarol 1051 from control paints were extremely low ($0.25 \mu\text{g cm}^{-2} \text{d}^{-1}$) throughout the experiment (Fig. 3c). The addition of HEDP increased the rate of Irgarol 1051 leaching and a higher leaching rate was recorded over the first 40 d for paints containing 15% HEDP. After 30 d, the paints containing 5% and 15% HEDP had similar steady leaching rates of $2 \mu\text{g cm}^{-2} \text{d}^{-1}$.

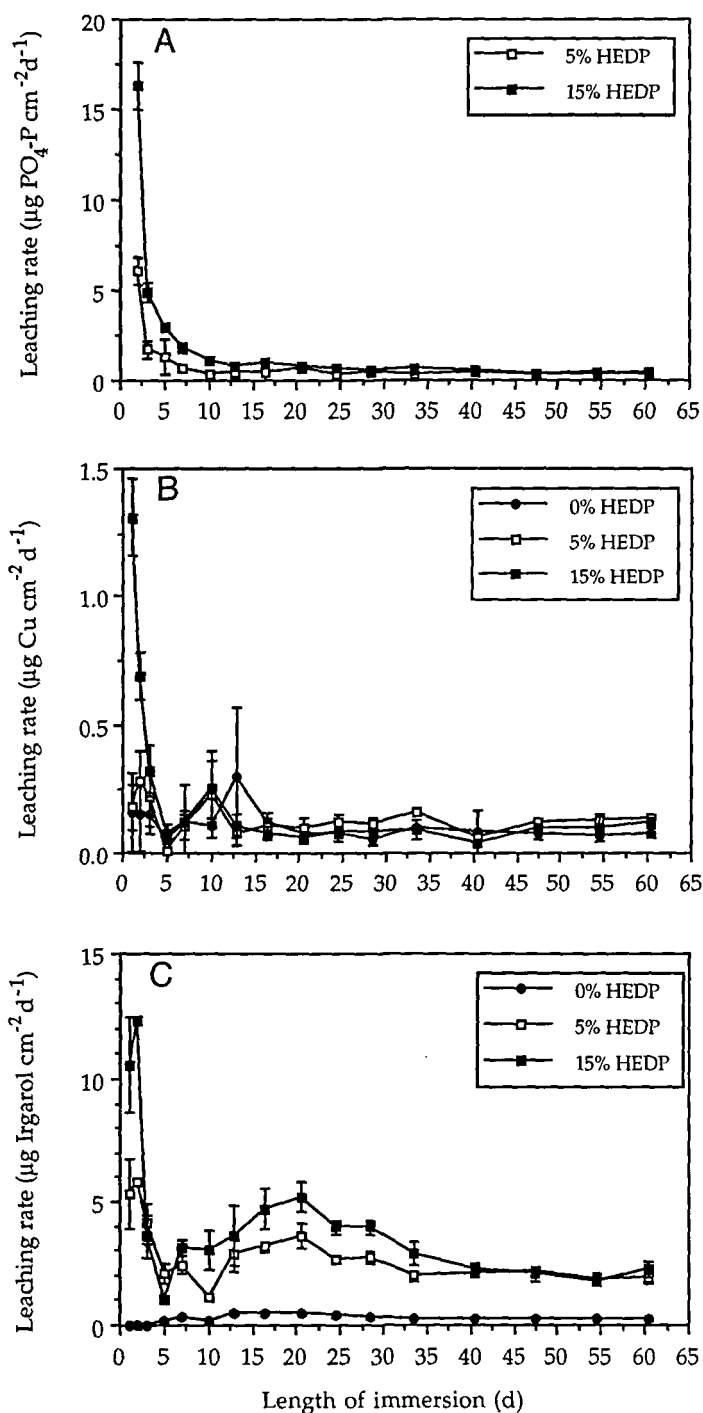


Fig. 3 Leaching rate of HEDP (A), copper (B) and Irgarol 1051 (C). The leaching rate of HEDP at 1d (not shown) from 5% and 15% HEDP paints was 73.4 ± 6.3 and $313 \pm 7.9 \mu\text{g cm}^{-2}\text{d}^{-1}$ respectively. Each point is the mean of 4 replicates. Bars show SEM.

DISCUSSION

The water chemistry was similar to that found during the previous summer (Heath *et al.*, 1993), and the major fluctuations were probably governed mainly by the activity of photosynthetic organisms. The removal of CO_2 by algae resulted in the water being saturated with respect to calcite, thereby providing conditions favourable for precipitation.

The decrease in the quantity of CaCO_3 within biofilms was proportional to the loading of HEDP in the paint suggesting that HEDP has the potential to control precipitation of CaCO_3 in the natural environment, a process which depends on effective release of this compound from anti-fouling paints.

The anti-fouling performance of paints and its correlation with the leaching rates of active ingredients has been the subject of a number of detailed studies in the marine environment (*e.g.* Hunter & Evans, 1991). Indeed, almost all studies on anti-fouling paints are concerned with their performance in seawater and anti-fouling paint technology has been driven by the need to produce coatings which prevent all types of fouling on moving vessels at sea (Callow, 1990). There has been little attention to fouling in freshwater environments where the number vessels is small and the fouling challenge is minor compared to that in the marine environment (see Callow, 1993). Release of active ingredients (toxins) from anti-fouling paints depends on paint composition and on factors such as the speed of passage through the water and water chemistry, especially with respect to ionic strength, pH and temperature (Anon, 1952; de Wolf & van Londen, 1966).

The leaching studies showed that the leaching rates of copper and Irgarol 1051 from the control paint were extremely low. These data were not unexpected since the 100% vinyl matrix used would be impermeable and non-erodable. A commercial paint containing cuprous oxide and Irgarol 1051 in the same proportions but based on a standard rosin-vinyl matrix, exhibited much better antifouling performance when immersed at the same test site (Heath *et al.*, 1993) and markedly higher leaching rates (Callow, unpublished). Conventional, commercial paints are always formulated using a combination of "film former" such as vinyl and "solubilizer" such as rosin (Anderson, 1993). Earlier preliminary trials showed that inclusion of HEDP in vinyl-rosin paints resulted in blistering and detachment due to ingress of water. Thus, 100% vinyl paints were used in this study since it was expected that the highly water soluble HEDP would take over the role of rosin and act as a "solubilizer".

Leaching, and hence antifouling performance, is related to speed of water movement, necessary to remove the biocide depleted leached layer, thereby exposing fresh paint at the surface. Agitation during leaching was gentle, in order to provide similar conditions to those present in the marina where the water is largely static, movement being dependent on wind and turbulence from the manoeuvring of boats. However, the conditions employed for leaching would have provided adequate mixing and prevented the build up of a saturated boundary layer which would have reduced the rate at which compounds were leached from the paint.

The addition of highly water-soluble HEDP increased the rate of leaching of both copper and Irgarol 1051 and initial leaching rates were relatively high. High initial leaching rates are usually seen when paints are immersed in water, a phenomenon exaggerated if the paints have been applied by a brush (de Wolf & van Londen, 1966). The steady state leaching rate of copper was very low (approximately $0.1 \mu\text{g cm}^{-2} \text{d}^{-1}$) and was probably ineffective in controlling algal fouling as $10\text{--}20 \mu\text{g Cu cm}^{-2} \text{d}^{-1}$ is required to control fouling by algae in the marine environment (de la Court, 1987). The

toxicity of copper depends on speciation (see Fleming & Trevors, 1989) and several studies have shown that toxicity is related to the concentration of Cu^{2+} ions rather than total copper concentration (for a discussion, see Tubbing *et al.*, 1994). However, many factors control the toxicity of copper in hard freshwaters and the responses of organisms vary. Many workers have shown a decrease in toxicity to algae at high pH values (Starodub *et al.*, 1987 Luderitz & Nicklish, 1989; Swartzman *et al.*, 1990) whilst others have shown reduced toxicity at low pH values (Peterson *et al.*, 1984). Alkalinity may also affect toxicity (Jin *et al.*, 1991), but water hardness exerts the greatest influence on copper toxicity and as the concentration of Ca^{2+} and Mg^{2+} ions are increased, copper becomes less toxic (Hoffman *et al.*, 1982; Mance *et al.*, 1984). Thus, in hard fresh waters such as in Ely marina, it might be expected that copper is of limited use in controlling algal fouling. Irgarol 1051 is a member of a group of *s*-triazine herbicides whose activity is unaffected by hard water chemistry (Hoffman *et al.*, 1982). Although the addition of HEDP significantly increased the leaching rate, it appeared that Irgarol 1051 had little effect on algal colonization since the organic weight and species diversity were similar on all of the paints irrespective of HEDP loading. There are no published data on the leaching rates of Irgarol 1051 required to control algal fouling but these experiments would suggest that leaching rates in excess of $2 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ are required.

The steady state leaching rate of HEDP from both the 5% and 15% paints was $0.35 \mu\text{g}\cdot\text{cm}^{-1}\cdot\text{d}^{-1}$ and although the rate of leaching from paints in the marina cannot be assumed to be the same, it is likely to be similar. Thus, it would appear that HEDP leaching at $0.35 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ is capable of reducing the precipitation of CaCO_3 . This rate is much lower than that of $5 \mu\text{g}\cdot\text{HEDP}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$, said to be necessary to prevent the deposition of calcium salts on subcutaneous bioprosthetic cusps placed in rats (Levy *et al.*, 1985), but the amount of phosphonate required to prevent deposition of CaCO_3 depends entirely on the precipitating environment.

Although 15% HEDP paints released the crystal inhibitor at a faster rate than paints containing 5% HEDP for only 20 d, this period was long enough to influence the total amount of CaCO_3 precipitated within the biofilm. HEDP did not substitute for rosin satisfactorily although the data obtained using a 100% vinyl matrix were sufficiently encouraging to justify a further paint trial in which barium salt of HEDP was used in a vinyl-rosin matrix.

Acknowledgements

This study was carried out during the tenure (CRH) of a NERC CASE studentship in conjunction with International Paint Ltd.

References

- Anderson C D (1993) Self-polishing antifouling; a scientific perspective. *Ship Repair and Conversion* 93
- Anon (1952) *Marine Fouling and its Prevention*. US Naval Institute. Annapolis MD
- ASTM (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Report E729-80. American Standards Testing and Materials, Philadelphia
- Callow M E (1990) Ship-fouling: problems and solutions. *Chem Ind* 5 March: 123–127
- Callow M E (1993) A review of fouling in freshwaters. *Biofouling* 7: 313–327
- de la Court F H (1987) The minimum leaching rate of some toxins from antifouling paints required to prevent settlement of fouling organisms. *Biodeterioration* 7: 305–314
- Fleming C A, Trevors J T (1989) Copper toxicity and chemistry in the environment: a review. *Water Air Soil Pollut* 44: 143–158
- Heath C R, Leadbeater B S C, Callow M E (1993) Formation and calcification of biofilms on anti-fouling paints in hard waters. *Biofouling* 7: 29–57

- Heath C R, Leadbeater B S C, Callow M E (1995) Effect of inhibitors on calcium carbonate deposition mediated by freshwater algae. *J Appl Phycol* **7**: 367–380
- Hoffman R W, Bills G, Rae J (1982) An *in situ* comparison of the effectiveness of four algicides. *Water Res Bull* **18**: 921–926
- Hunter J E, Evans L V (1991) Raft trial experiments on antifouling paints containing zineb and copper. *Biofouling* **3**: 113–137
- Jin H, Zhang Y, Yang R (1991) Toxicity and distribution of copper in an aquatic microcosm under alkalinity and hardness. *Chemosphere* **22**: 577–596
- Jurgens K, Gude K (1990) Incorporation and release of phosphorus by planktonic bacteria and phagotrophic flagellates. *Mar Ecol Prog Ser* **59**: 271–284
- Koutsoukos P G, Kontoyannis C G (1984) Prevention and inhibition of calcium carbonate scale. *J Cryst Growth* **69**: 367–376
- Koutsoukos P G, Amjad Z, Nancollas G H (1981) The influence of phylate and phosphonate on the crystal growth of fluorapatite and hydroxyapatite. *J. Colloid Interface Sci* **83**: 599–605
- Levy R J, Wolfrum J, Schoen F J, Howley M A, Lund S A, Langer R (1985) Inhibition of calcification of bioprosthetic heart valves by local controlled release of diphosphonate. *Science* **228**: 190–192
- Luderitz V, Nicklish A (1989) The effect of pH on copper toxicity to blue-green algae. *Int Rev Gesamten Hydrobiol* **74**: 283–291
- Mackereth F J H, Heron J, Talling J F (1978) In: *Water Analysis: some Revised Methods for Limnologists*. Freshwater Biological Association
- Mance G, Brown V M, Yates J (1984) Proposed environment quality for list II substances in water. Copper. WRC Technical Report
- Peterson H G, Healey F P, Wagermann R (1984) Metal toxicity to algae: a highly pH-dependent phenomenon. *Can J Fish Aquat Sci* **41**: 974–979
- Reddy M M, Nancollas G H (1973) Calcite crystal growth inhibition by phosphonates. *Desalination* **12**: 61–73
- Robertson W G (1973) Factors affecting the precipitation of calcium phosphate *in vitro*. *Calcif Tissue Res* **11**: 311–322
- Sikes C S, Wheeler A P (1988) Regulators of biomineralization. *Chemtech* October: 620–626
- Starodub M E, Wong P T S, Mayfield C I, Chau Y K (1987) Influence of complexation and pH and individual and combined heavy metal toxicity to a freshwater green alga. *Can J Fish Aquat Sci* **44**: 1173–1180
- Swartzman G L, Taub F B, Maedor J, Huang C, Kindig A (1990) Modelling and effect of algal biomass on multispecies microcosms response to copper toxicity. *Aquat Toxicol* **17**: 93–118
- Tubbing D M J, Admiral W, Cleven R F, Iqbal M, van de Meent D, Verweij W (1994) The contribution of complexed copper to the metabolic inhibition of algae and bacteria in synthetic media and river water. *Water Res* **28**: 37–44
- de Wolf P, van Londen A M (1966) Antifouling compositions. *Nature* **209**: 272–274
- Xyla A G, Koutsoukos P G (1987) Effect of diphosphonate on the precipitation of calcium carbonate in aqueous solutions. *J Chem Soc Faraday Trans* **83**: 1477–1484