

Bioaccumulation of Trace Metals in Biofilms Formed on Aluminium and Steel in Aquatic Environment

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Knowledge on initial bioadhesion and composition of micro and macro nutrients within the biofilm matrix are recognised as essential for understanding the formation of biofilms. This communication highlights the heavy metal accumulation pattern in biofilms, influenced by the substrate and seasons. Natural biofilms were simultaneously grown on aluminium and steel panels for 72 hours by exposing them in the aquatic environment. Accumulation of toxic and nutrient metals in biofilms on different substrates and its seasonal variation were studied. Biofilms grown within 24 hours had shown significantly ($p < 0.05$) higher concentrations of trace metals in both the metal substrates. Trace metals accumulated over the substrates varied in different seasons. Among the metals, the accumulation of zinc was more during all the seasons. Biofilms grown on steel, concentrated more metals than on aluminium.

Key words: Biofilm, seasonal variation, heavy metals, aluminium, bioaccumulation.

Attachment of microbial communities on surfaces submerged in aqueous environment is termed as biofilm (Beech, 2004). The growth of biofilm is considered to be a complex process involving transport of organic and inorganic molecules and microbial cells to the surface for forming a conditioning layer. Studies on initial bioadhesion, factors influencing the initial formation of biofilms and composition of micro and macro nutrients within the biofilm matrix are recognised as essential in understanding the formation of biofilms. Microbial stratification was enhanced due to the formation of biofilm, which affects redox potential and pH within the matrix. This can alter the speciation of inorganic electrolytes as well as the development of mineral precipitates (Diels *et al.*, 1995; Beveridge *et al.*, 1997).

The formation of biofilm and subsequent biofouling have great environmental and economical implications. Shipping and fishing industry are experiencing substantial financial losses as a result of biofouling (Flemming, 1996). Microbially induced corrosion of metals occurs while in contact with microbial cells or with their metabolic products such as exopolymeric substances (EPS) (Gaylarde & Johnston, 1982; Gaylarde & Videla, 1987). The function of EPS is to enhance the ability of cells to adhere to the surfaces (Beech, 2004). Nutrients, which are scarce in aquatic environments, are concentrated on surfaces (Gaylarde & Beech, 1988). Bacteria require metal ions for their growth through binding of metal ions by EPS and/or lipopolysaccharides (Beech *et al.*, 1993). This phenomenon lead to the development of biofilm, often resulting in severe fouling

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and deterioration of metal. Some of the metal ions like chromium (Percival *et al.*, 1997) and copper inhibit the colonisation of bacteria on the surface which limit the degree of material damage. Trace metals in the aquatic environment bind in bacteria, flocculent particles and suspended organic materials. Bacteria have significant binding capacities for lead and the presence of iron increase the adsorption of lead (Harvey & Leckie, 1985; Lion *et al.*, 1988). Heavy metals in dry mass of biofilms were 100 to 60,000 fold higher than bulk phase (Friese *et al.*, 1997). The biomass in biofilms depends on the temperature, seasons, flow rate and lighting conditions (Scotto & Lai, 1998). Not much study has been conducted on the accumulation of heavy metals in biofilms formed over different metallic surfaces. To develop anti-fouling strategies in aquatic environments it is essential to know, the role of inorganic constituents on the accumulation pattern as trace metals are directly involved in large number of enzymatic reactions in microbes. The present study was aimed at the evaluation of the dynamics of heavy metal accumulation in biofilms formed over different metals like pure aluminium and stainless steel (SS304) in different seasons in the aquatic environment.

Materials and Methods

Pure aluminium and stainless steel (SS304) sheets were purchased from commercial source. The concentrations of chromium, manganese and nickel in SS304 were 16.87, 1.56 and 11.38% respectively. In aluminium, the concentrations of iron, magnesium, manganese and titanium were 0.71, 0.017, 0.020 and 0.02% respectively; the rest being aluminium. Pure aluminium and

SS304 were cut in to coupons of 15x10 cm size, cleaned with water, acetone, ultrasonic cleaner and finally rinsed with MilliQ Gradient water. Coupons were fitted in a steel rack by using polyethylene washers and screws. Two sets of coupons were immersed every month in the Cochin estuary, South India, one set retrieved after 24 hours and the other after 72 hours. The experiment was carried out during June 2005 to May 2006 covering monsoon, post-monsoon and pre-monsoon seasons. Coupons retrieved after 24 and 72 h were wiped carefully with preheated and preweighed GF/F filter paper, avoiding all dirt, if any attached to it. The glass fiber filter paper was then folded and kept in whatman No. 1 filter paper and stored in clean polyethylene bags. The samples kept in polythene bags were transported to the laboratory and were dried in an air oven at $55 \pm 2^\circ\text{C}$. Dried filter paper was weighed and digested using 9:4 HNO_3 : HClO_4 by Milestone Ethos Plus Microwave digestion system with a heating programme of heating from room temperature to 150°C , in 10 min and maintaining at 150°C for 15 min. After cooling, the samples were processed for analysis. Digested samples were made up to 100 ml and the metallic constituents *viz.*, cadmium, chromium, copper, cobalt, nickel, arsenic, selenium, manganese, lead, vanadium and zinc were analysed. All metals in the sample were analysed using Perkin Elmer Optima 2000DV ICP AES and hydride generator was used for measuring arsenic and selenium. Calibration standard curve was drawn using NIST traceable Perkin Elmer 21 multi element standard solution and blank solution analysed along with the samples. A minimum of three replicate samples were analysed along with the blank solution.

Water samples were collected from the estuary throughout the exposure period and were analysed voltammetrically for zinc, cadmium, lead and copper using Autolab 797 VA Computrace with HMDE, Pt and Ag/AgCl (reference) system as per DIN 38406 Part 16 method. Statistical analysis was carried out using Microsoft Excel.

Results and Discussion

Microscopic evaluation of aluminium and stainless steel coupons immersed in the estuary for 72 h revealed that there was considerable variation in the nature of biofilm attached to the substrates. Debeer *et al.* (1994) proposed that the diffusion of nutrients and oxygen entrained was transported through inter connected water channels from the bulk phase. These biofilms are water rich aggregates of microorganisms such as algae, bacteria and protozoa and their EPS. They cover interfaces in natural systems and are able to concentrate and bind ions from the passing water (Neu *et al.*, 1992; Bremer & Geesey, 1993; Neu, 1996)

Cadmium, cobalt and vanadium were not detected in any of the biofilm samples. Chromium and nickel accumulation in biofilm was maximum during pre-monsoon season and these metals were not detected in any of the samples collected during post-monsoon (Fig. 1). The biofilm formed over aluminium accumulated higher concentration of chromium initially but in SS304 during the same period it was only about 50% compared to aluminium. Chromium mainly reaches the estuary through fertilizers and anthropogenic inputs from heavy industrial units located nearby. Decreased accumulation of chromium initially by the biofilm in SS304 was due to the inhibition caused by the presence of chromium in the

SS304 substrate, as chromium is toxic to microorganisms. Pre-monsoon season exhibited higher salinity and low inflow of water from the rivers. This might have influenced the concentration of more chromium, manganese and nickel in water leading to higher amounts of accumulation by the microorganisms in the biofilm.

Lead was detected throughout the year in biofilms formed on aluminium and SS304 coupons and both showed similar pattern of accumulation (Fig. 1). Increased concentration of lead was detected during post-monsoon and the biofilms accumulated over SS304 had more lead than on aluminium. In both aluminium and SS304 coupons, the 24 h

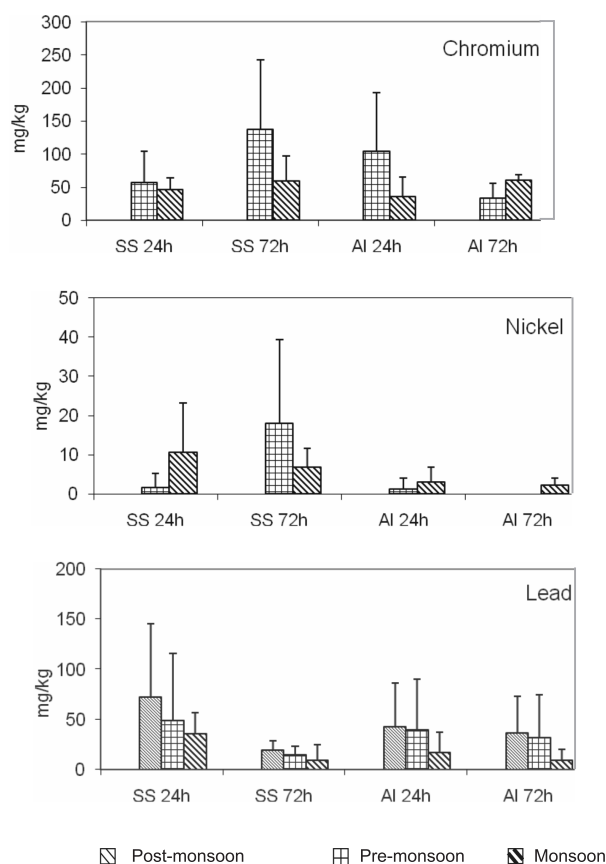


Fig. 1. Seasonal variation of chromium, nickel and lead concentration in biofilms accumulated over aluminium and SS304 after 24 and 72 h of immersion in the estuary; SS = SS304; Al = Aluminium. All values are mean $\pm$ standard deviation

biofilm sample showed higher concentration of lead in all the seasons and its concentration decreased in 72 h biofilm. Lead fraction in natural aquatic system is mainly associated with organic materials. Laboratory experiments of Lion & Rochlin (1989) revealed that the bacteria have significant binding capacities for lead.

Increased concentrations of selenium and arsenic were exhibited in biofilm of SS304 coupons exposed during post-monsoon season and the concentration decreased in 72 h samples (Fig. 2) while in the case of aluminium, maximum concentration of the elements was in 72 h coupons.

Biofilms collected from both the aluminium and SS304 coupons showed significantly ($p < 0.05$) higher concentration of zinc during monsoon (Fig 3). Zinc was identified as the most abundant metal in the biofilm and zinc constituted 76% of the total metals

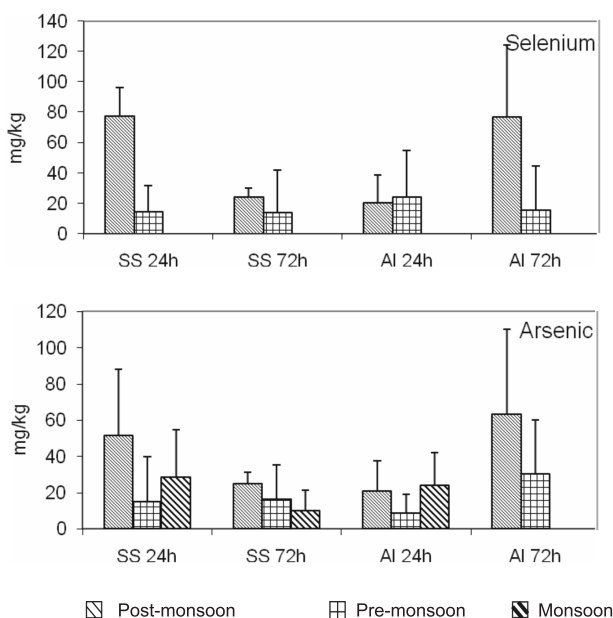


Fig. 2. Seasonal variation of selenium and arsenic concentration in biofilms accumulated over aluminium and SS304 after 24 and 72 h of immersion in the estuary; SS=SS304; Al=Aluminium. All values are mean $\pm$ standard deviation

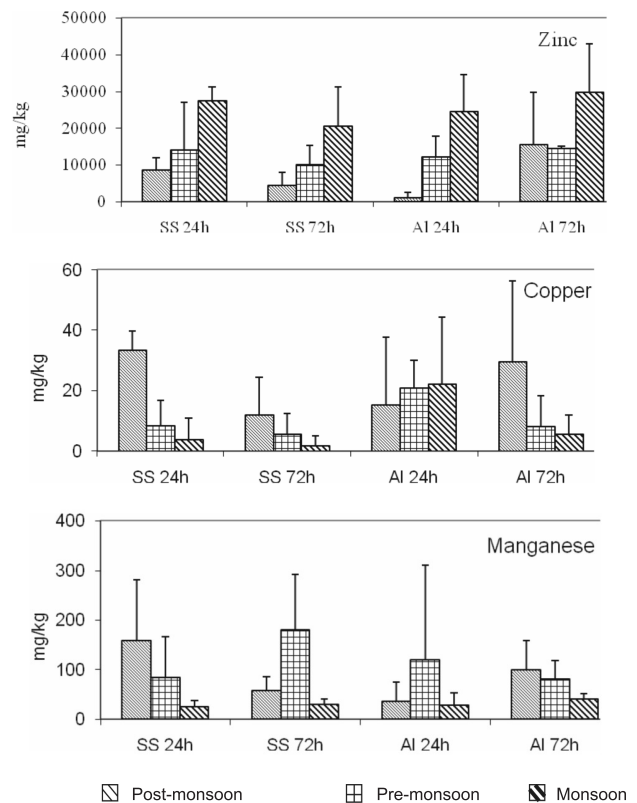


Fig. 3. Seasonal variation of zinc, copper and manganese concentration in biofilms accumulated on aluminium and SS304 after 24 and 72 h of immersion in the estuary; SS=SS304; Al=Aluminium. All values are mean $\pm$ standard deviation

in biofilm (Lion *et al.*, 1988). The present study also revealed that zinc content was the highest among the metals analysed. Michelbach & Wöhrle (1993), Friese *et al.* (1997) and Rocher *et al.* (2004) reported presence of high concentration of zinc in biofilms.

High concentrations of copper were recorded from biofilms formed on SS304 during the post-monsoon while on aluminium, 24 h biofilm accumulated maximum copper in all the three seasons. Though copper is an essential trace element for different biological reactions, it being toxic to microorganisms, its accumulation was expected to be very minimum. Post-mon-

soon is known to be the most productive season in this area and hence hectic biological activity in the aquatic system influenced the accumulation of more copper during this season. Copper was accumulated in the biofilm during the initial growth stages and its concentration decreased after 24 h.

Maximum manganese concentration was detected during the pre-monsoon season except in biofilms formed on 72 h aluminium panels. Probable source of manganese in the study area is mainly from the shipping industries and corrosion products from the marine structures.

The heavy metal accumulation in the biofilm formed over aluminium and stainless steel showed significant ($p < 0.05$) variation in different seasons. Increased levels of chromium, nickel, lead and copper were accumulated initially (within 24 h) in biofilms formed over aluminium. Arsenic, selenium and manganese were detected in 72 h biofilm formed over aluminium coupons. The accumulation pattern of chromium and nickel was distinctly different in aluminium and SS304 during the year. The biofilm formed over SS304 had maximum concentration of metals within 24 h except nickel, chromium and manganese (Fig. 4). Chromium concentration in biofilms formed on SS304 coupons was highest in 72 h samples but on aluminium it was in 24 h samples. The zinc concentration in the biofilm was higher compared to the other elements analysed (Fig. 4).

Scotto & Lai (1998) suggested that the corrosivity of natural seawater on stainless steels and similar alloys was higher and it was due to the depolarization of oxygen

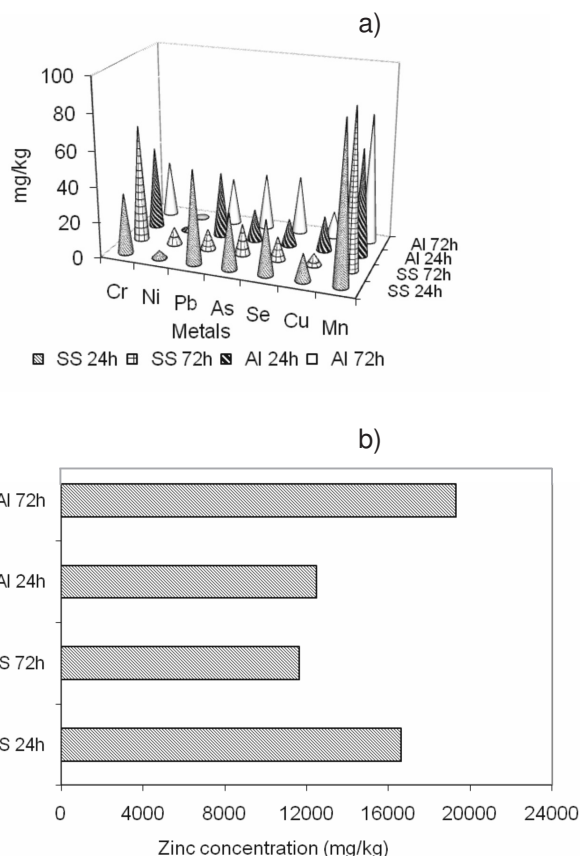


Fig. 4. Annual concentrations of metals accumulated in biofilms formed on aluminium and SS304 after 24 and 72 h of immersion in the estuary (a) metals Cr, Ni, Pb, As, Se, Cu and Mn (b) Zn; SS=SS304; Al=Aluminium

reduction reaction induced by the settlement of biofilms on surfaces. The present study shows increased concentrations of metals in biofilms formed on steel than on aluminium. These metallic concentrations also may influence the corrosion of steel under marine condition. Metal oxides formed due to corrosion on the surfaces of substrates can help to adsorb organic molecules from aquatic environments and this molecule will alter subsequent trace metal adsorption. The surface films can mask the properties of the underlying metal by changing the surface charge or by occupying adsorption sites of the oxide surface (Tipping & Cooke, 1982). Metal oxides bound to surfaces could

influence subsequent cell adhesion and the formation of biofilms by altering the surface charge, nutrient concentration or the micro environmental factors affecting colonization by bacteria. Microorganisms are usually embedded in anionic exo polymeric materials which influence the mineralization process (Beveridge & Doyle, 1989). Hence microorganisms in biofilms produce minerals that are not predicted by chemical or thermodynamic arguments (Little *et al.*, 1997).

Copper was significantly ($p < 0.05$) higher in water samples collected from the estuary during the year and pre-monsoon season recorded the highest value while cadmium, lead and zinc were recorded in trace levels only (Fig. 5). Water samples collected during monsoon showed higher concentrations of zinc and the result correlates with the zinc content in biofilm.

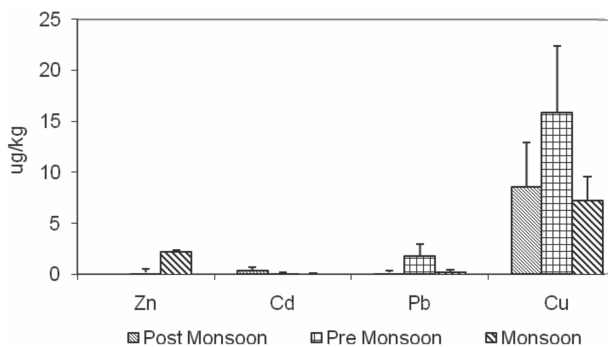


Fig. 5. Concentration of Cd, Zn, Cu and Pb in the estuarine water during the study period

The heavy metal accumulation in the biofilm depends on the substrate and seasons. Zinc accumulation was significantly higher among the metals analysed. Lead and copper were detected throughout the year and the level in the biofilm was comparatively lower than reported by other

researchers. Cadmium, cobalt and vanadium were not detected in any of the biofilm samples. There was an inhibition of absorption of copper and cadmium since they are toxic even though the former was abundant in the aquatic environment. Arsenic and selenium were accumulated more within 24 h in SS304 whereas in aluminium it was 72 h. Chromium, manganese and nickel concentrations were comparatively higher in biofilms collected from steel, since these metals were active constituents of the substrate. Further investigations on the fractions of zinc accumulated in biofilms, the forms of zinc absorbed by the biofilm and to which part of the microorganism it was accumulated are to be carried out.

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