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# Detection and Toxicity of Titanium from Pulp and Paper Effluents

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Until now, little attention has been paid to the toxicity of titanium (Ti) in aquatic systems because Ti was considered to be biologically inert (Anon 1987). In fresh water, the concentration of titanium reported ranges from 2 to 107 μg/L (Berlin and Nordman 1979). In his classification of the pollution potential of metals, Wood (1974) placed Ti with a group of rare metals, i.e., Ta, Nb, Re, Ga, La, Os, Rh, Or, Ru and Ba. Yet, unlike rare metals, Ti is abundant in the earth's crust and has many industrial applications. It is used in the pulp and paper, paint, plastics and enamel industries as a whitener, in the chemical industry as a corrosion-resistant material, and in the tobacco industry as a clouding agent (Berlin and Nordman 1979). As a result, Ti could be abundant in the waste discharges from many of these industries. If the metal is harmful to fish eggs as reported by Oulasvirta (1990), the adverse effects on biotic communities downstream of an industrial discharge, particularly pulp and paper, should not be neglected.

Of the many inorganic and organic titanium compounds, titanium dioxide ($TiO_2$) is the most commonly used compound for whitening because of its extreme whiteness and brightness. Titanium dioxide interacts slowly with chemicals (Tengvall and Lundstrom 1992) and is reported to be poorly absorbed or retained by plants and animals (Berlin and Nordman 1979). Nevertheless, in our recent monitoring of metal toxicity in industrial wastewaters, it was observed that high concentrations of Ti accumulated in *Chlorella* cells (Wong et al. 1995). This raised the question as to whether Ti was a toxic metal, since it appeared to accumulate in algal cells. Therefore, the objective of this study was to examine, by means of algal assays, whether Ti was bioavailable and inhibited algal growth below pH 7.0, as most pulp and paper discharges are acidic.

## MATERIALS AND METHODS

The laboratory procedures used to analyze nutrients, metals and organic contaminants have been described by Poulton (1992). In the field, industrial wastewaters were sampled from the end of the effluent pipes. Nine metals

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were analyzed (i.e., Cu, Ni, Pb, Zn, Fe, Mn, Al, As, Cd, Cr and Hg) but not Ti. Metals in particulate form were scanned from particle surfaces of suspended colloids using energy dispersive X-ray microanalysis (SEM), and metals that were absorbed by algal cells were identified by X-ray microprobing (STEM) in the polyphosphate bodies of algal cells as described later in the section.

The two titanium chemicals used were: Ti standard solution (Plasma-Chem Associates Inc.) and potassium titanium oxalate, $K_2TiO(C_2O_4)_2$. The experiments were performed in four steps. The first step was to detect the presence of free Ti ions in the test solutions. The second step was to conduct toxicity tests. The third step was to examine fine structural alterations of affected *Chlorella* cells (the test species), and the fourth step was to identify metals accumulated in the polyphosphate bodies. Polyphosphate bodies are the organelles where absorbed metals are bound (Jensen et al. 1982).

Free Ti ions in 10% BBM algal growth medium were measured indirectly through the complexation process with EDTA, a chelating agent. A concentration of 5.0 $\mu$M/L EDTA was used in the tests. Since Ti ions complex with EDTA, the complexing capacity of EDTA added to the test solution decreases if there is an abundance of free Ti ions. In our preliminary titration, 5.0 $\mu$M/L EDTA was observed to complex 10.14 $\mu$M/L Cu. Using a Cu-ion specific electrode (Wong et al. 1995), the complexing capacity of the test water was titrated using small increments of Cu titrant (1.0 $\mu$M/L). The titration was terminated once the Nernst slope (a straight line) was obtained from the graph where electrode potential (mV) was plotted against the Cu titrant (Orion 1986). Free metal ions were calculated according to Guy and Chakrabarti (1976), and the complexing capacity of the test solution was estimated at the break point on the regression lines in a graph plotted between $(C_A - C_M)/C_A$ and $C_A$, where $C_A$ is the added metal concentration and $C_M$ is the measured Cu concentration. The concentrations of Ti standard solution used in the titration were 10, 15, and 25 $\mu$M/L (as Ti), and those of titanium oxalate were 25, 50 and 100 $\mu$M/L (as Ti).

*Chlorella fusca* Shihara & Krauss (UTEX 343), a unicellular green alga, was used as the test species in the algal assays. Ten percent BBM (Bold's Basal Medium) (Hutchinson and Stokes 1975) was the growth medium. The initial pH of the medium was 6.5. In duplicate, dilution Ti concentrations were prepared at 1, 2.5, 5, 10, 25, 50, 100, 200 and 300 $\mu$M/L (i.e., for both the Ti standard solution and titanium oxalate solution), with zero Ti concentration as the control. The test solutions were inoculated with $40 \times 10^3$ /mL *Chlorella* cells. The cultures were incubated for six days with the light energy in the growth chamber measured at 65 $\mu$Ein/$m^2$/sec. The temperature was 20° C and the photoperiod was 12 hr light and 12 hr dark.

At the end of the experiment, the algal cells were counted. Cell numbers

were plotted against Ti concentration for the interpretation of Ti toxicity. To prepare cells for electron microscopic examination, *Chlorella* cells were fixed overnight with 4% glutaraldehyde (0.1 M cacodylate buffer) at 4°C and post-fixed with 2% osmium tetroxide (same buffer) for 1 hr the next day. After dehydration through a graded ethanol series, the cells were embedded in 100% Spurr's medium. Thin sections were cut and stained with uranyl acetate and lead citrate. Fine structure was examined using a Joel JEM 100 CXII transmission electron microscope at 80 KV (Wong and Wainwright 1994).

The accumulation of metals in the poly-P bodies was probed by energy dispersive X-ray microanalysis (STEM). Thin sections were collected on carbon-coated nylon grids and stained with uranyl acetate. The poly-P bodies located under the electron microscope were subjected to spot analysis. For each effluent dilution concentration or Ti concentration, five to ten poly-P bodies were analyzed. The data were processed by a Tracor Northern 5500 pulse-height analysis system with an acquisition time of 100 sec.

## RESULTS AND DISCUSSION

Most pulp and paper wastewaters are acidic. In the test waters, the pH was measured at between 5 and 6. Both organic contaminants (e.g., PCP, tetrachlorobenzene, hexachloroethane, trichlorotoluene, DDT, chlordane) and metals (e.g., Cu, Pb, Zn, Mn and Al) were encountered in the test waters (Wong 1995). From observations of ultrastructural damage and bioassays using the EDTA complexation approach (Wong et al. 1994, 1995), both metal and organic toxicity were confirmed. Although Ti was not included in the chemical analysis, its abundance could be seen from X-ray scans of suspended particles (Fig. 1a), and its bioavailability from X-ray microprobing of the polyphosphate bodies (Fig. 1b).

Of the many species of metals, free metal ions are reported to be the most toxic and most bioavailable (Steeman Neilson and Laursen 1976). Therefore, for metals such as Ti, free metal ions or ion complexes (e.g., $Ti(H_2O)_6^{3+}$ and $TiO^{2+}$) must be present to cause damage to algal cells.

Potassium titanium oxalate was water soluble. Titanium in the standard stock solution containing 5% HF was, in fact, a solution of titanium halides. In both the titanium oxalate and Ti standard solutions, free metal ions were monitored through the complexation of EDTA. Free metal ions were not detected in the titanium oxalate solutions since the complexing capacity in the solutions remained unaltered. However, in the case of the Ti standard solutions, the complexing capacity was observed to decrease as Ti standard solution concentration increased (Fig. 2a). As more and more EDTA molecules were tied up with the increase in free Ti ions, the complexing capacity remaining in the solution to be titrated by Cu in the Cu-titration process decreased (inset, Fig. 2a). It appeared that titanium halides

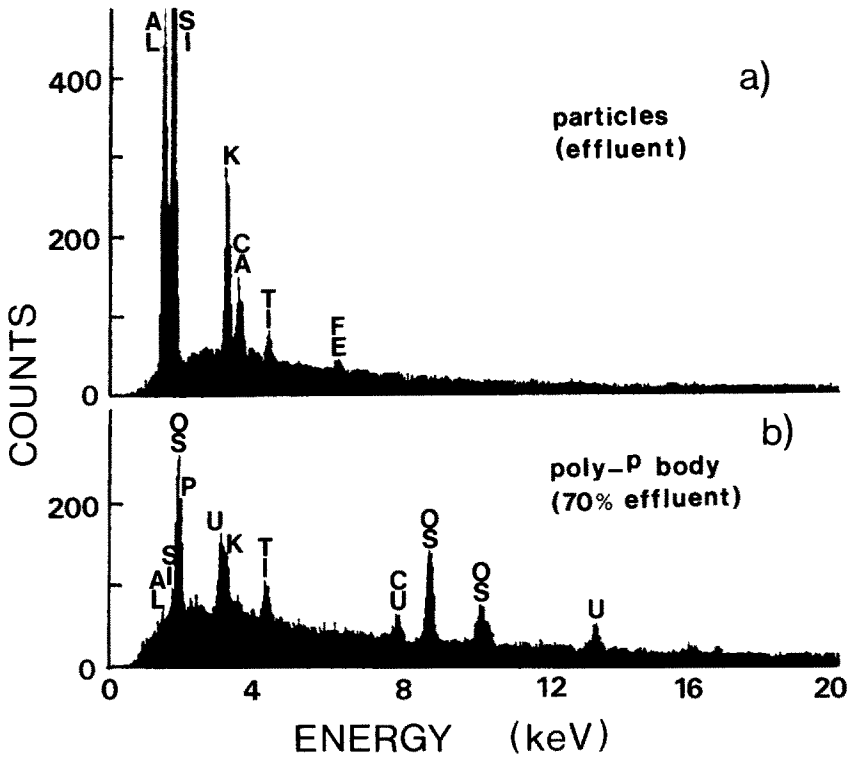


**Figure 1a).** X-ray scan of particles from the pulp and paper wastewater showing a significant amount of Ti.

**1b).** X-ray spectrum of a polyphosphate body from an affected *Chlorella* cell after assays with 70% pulp and paper effluent. The bioavailability of Ti and Cu is identified by the pulse heights in the spectrum. Osmium (Os) is the fixative, and Uranium (U) is the stain.

(i.e. fluorides and chlorides) at low pH could dissociate into ionic forms.

Quite unexpectedly, in the algal assays, *Chlorella* biomass was observed to decline at 5 $\mu$M/L titanium oxalate concentration, and even more surprisingly, levelled off at 25 $\mu$M/L (Fig. 2b). Because algal biomass remained at about the same level even at a concentration of 300 $\mu$M/L, titanium oxalate could not be considered lethal. Since there was no detection of free Ti ions from the compound, and no fine structural damage observed in the algal cells, titanium oxalate in excess quantities, like metal-EDTA complexes (Wong et al. 1995), interfered with metabolic processes rather than destroying them.

On the other hand, Ti was very toxic when supplied as a halide solution (Fig. 2). Algal biomass declined sharply at a concentration as low as 1 $\mu$M/L Ti. Algal growth was not observed above 50 $\mu$M/L Ti. Like other harmful metals, fine structural damage was confined to the chloroplast

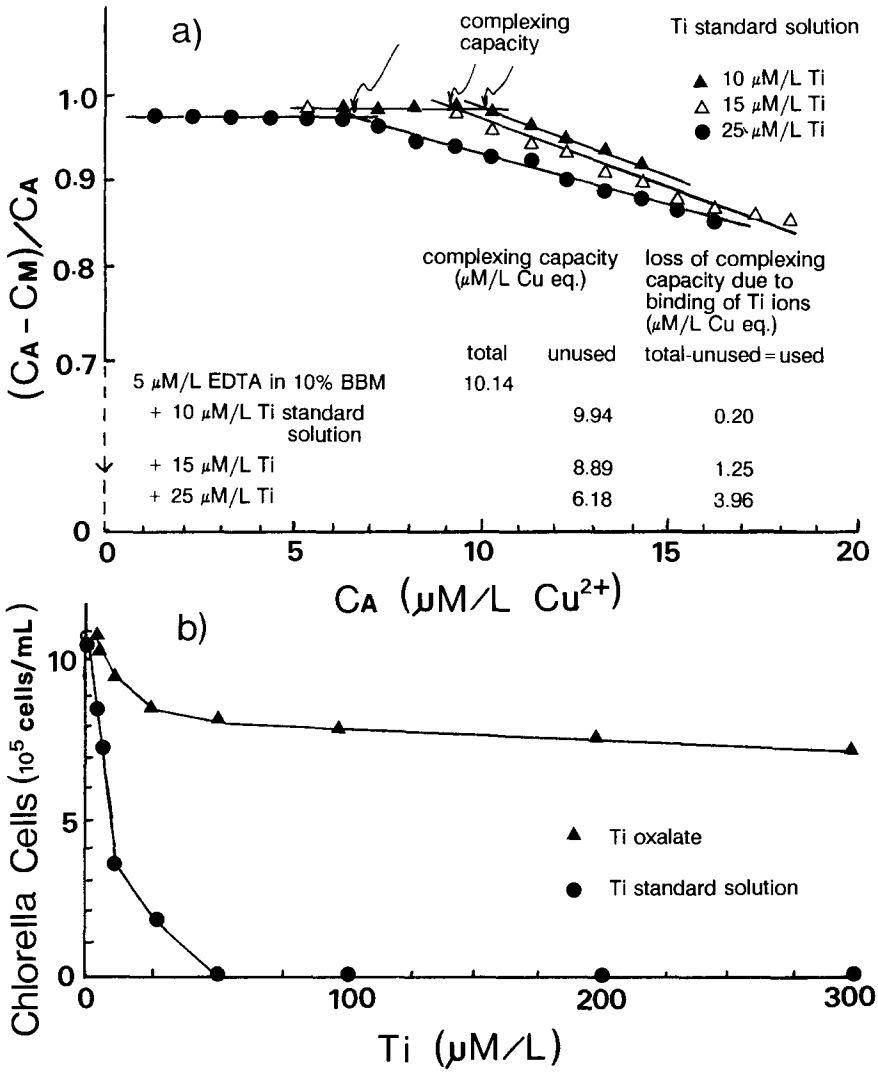


**Figure 2a).** Complexing capacity of the test solution containing 5 μM/L EDTA and 10, 15 or 25 μM/L Ti (from the Ti standard solution) was estimated at the break point of the regression curves. The changes in complexing capacity as Ti concentration increased are presented in the Table, inset.

2b). Final populations of *Chlorella* cells in assays of Ti standard solution and titanium oxalate solution.

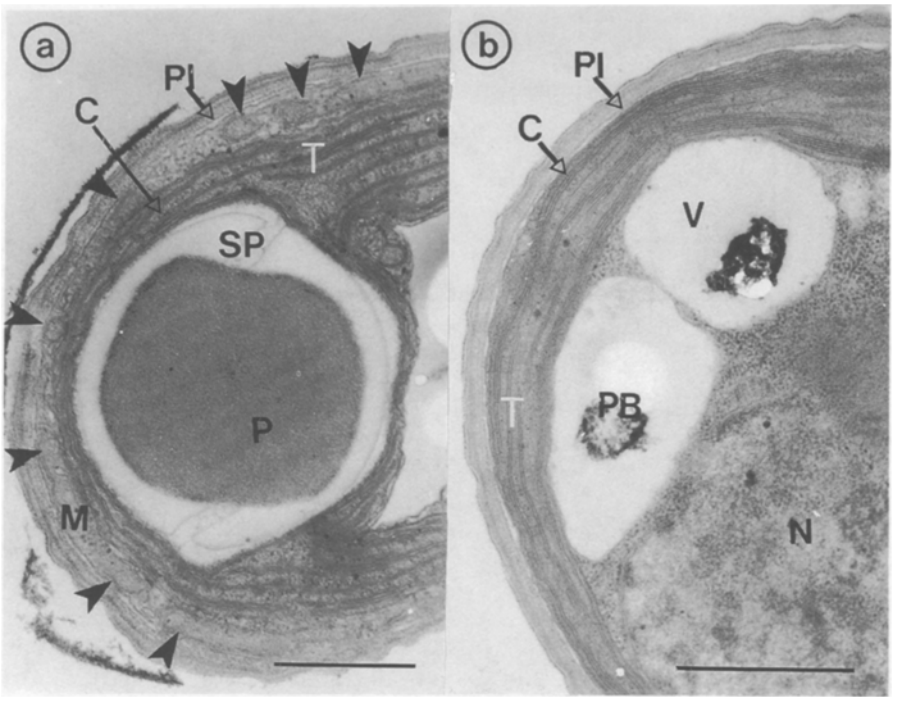


**Figure 3a).** Electron micrograph of an affected *Chlorella* cell exposed to 10 $\mu$M/L Ti. The chloroplast (C) was reduced in size. A thin layer of peripheral cytoplasm containing many mitochondria (M) (pointers) separates the chloroplast and the plasmalemma (Pl). Scale bar = 0.5 $\mu$m.

**3b).** In control cells, the chloroplasts were attached to the plasmalemma, and polyphosphate bodies (PB) were conspicuous in the vacuoles (V). Scale bar = 0.5 $\mu$m.

(Wong et al. 1994). At 25 $\mu$M/L Ti standard solution, the thylakoidal membranes were badly distorted as the *Chlorella* cells began to disintegrate. At a weaker Ti concentration of 10 $\mu$M/L, the chloroplast in most of the algal cells decreased in size, creating a layer of peripheral cytoplasm dotted with many mitochondria (pointers in Fig. 3a) between the chloroplast and the plasmalemma. On the other hand, with control cells, the chloroplast and the plasmalemma were attached to each other, sometimes with small vesicles visible between them (Fig. 3b).

Despite the disturbance in the chloroplast, polyphosphate bodies remained conspicuous in the vacuoles of affected *Chlorella* cells. In cells exposed to titanium oxalate, there was no Ti detected in the organelles. Conversely, when the culture was treated with Ti standard solutions, noticeable amounts of Ti were detected from the microprobing of the polyphosphate bodies of affected cells (Fig. 4). While the identification of Ti in the polyphosphate

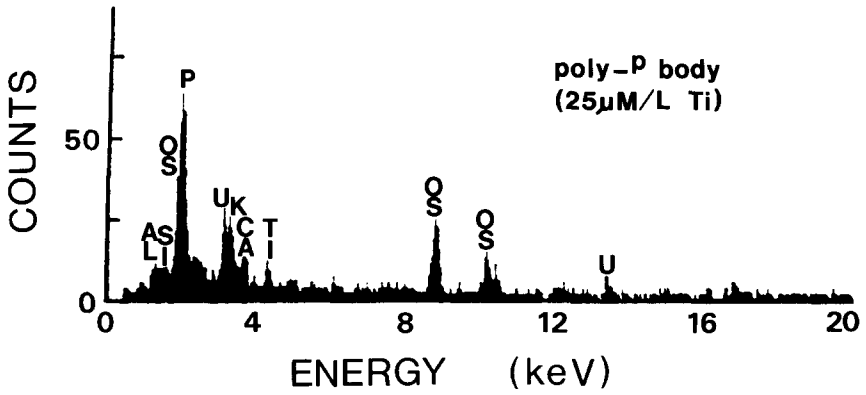


**Figure 4.** An example illustrating Ti accumulation in the polyphosphate body of an affected *Chlorella* cell exposed to 25 μM/L Ti standard solution.

bodies suggested absorption, damage to the chloroplasts of the *Chlorella* cells signified Ti toxicity.

In studies of the contamination of metals in the outfall of $TiO_2$ industry effluents, Oulasvirta (1990) and Ballan-Dufrançais et al. (1990) reported possible harmful effects of Ti on Baltic herring eggs and mussels, respectively. As the effluents were acidic, Ti ions were observed to be abundant. Returning to the pulp and paper effluent, where the pH mostly remained below 6.5, mixtures of Ti and titanium halide ions were to be expected in the discharge, since chlorine, as well as $TiO_2$, was used in the processes of bleaching and whitening. Although under mixed-metal conditions, Ti ions were unlikely to be identified by traditional chemical analysis, EDX-ray microprobing of polyphosphate bodies provided information on which metals were being absorbed. As seen in Fig. 1b, the pulse height of Ti confirms its prevalence. If Ti is as toxic as suggested by the assays, this metal could be the major factor in the metal toxicity of pulp and paper and other industrial effluents. Also, in view of a groundwater study by Kaplan et al. (1994) that some well waters could be contaminated by Ti, perhaps a closer look at the toxicity of Ti, e.g., establishing a toxicity guideline for the metal, is needed for industrial wastewaters.

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