J. Phytol. Res. 13 (2) : 117-122, 2000

KINETICS OF CADMIUM UPTAKE IN *STIGEOCLONIUM TENUE* KUTZ.

M. VANAJA*, N.V.N. CHARYULU and K.V.N. RAO
Central Research Institute for Dryland Agriculture, Santoshnagar, Saidabad, Hyderabad 500059 Andhra Pradesh, India.

The tolerance index concentration (TIC) and kinetics of cadmium uptake in *Stigeoclonium tenue* Kutz were studied. The TIC of Cd was 11.49 mg L^{-1} (10.26 μM Cd). The uptake of Cd was concentration and time dependent. At low levels (10 μM and 50 μM Cd), the absorbed Cd increased till the end of the experimental period. Whereas at higher concentrations of Cd (100 μM and 150 μM Cd), the absorbed amount of Cd at the end of the experiment was double when compared to the first hour absorption. Both the adsorption and absorption rates were higher during initial hours of incubation. At lower concentration (10 μM and 50 μM Cd), the amount of absorbed Cd was more than the adsorbed Cd at the end of the incubation period. At higher concentrations (100, 150 and 200 μM Cd) the amount of adsorbed Cd was more than the absorbed Cd. There seems to be a differential mechanism of adsorption and absorption is involved in the tolerance of *S. tenue* to higher concentrations of Cd.

Keywords: Cadmium; Heavy metals; *Stigeoclonium tenue* Kutz; Tolerance index concentation.

Introduction

Industrialization, urbanization and anthropogenic activities resulted in the environmental pollution by heavy metals[1]. The effect of a variety of metals on physiological and biochemical functions of different plant systems has been reviewed[2-7]. Algae growing in waters polluted by heavy metals have been shown to develop tolerance to higher concentration of heavy metals than those growing in unpolluted regions[8-10]. *Stigeoclonium tenue* has been reported as an important inhabitant of organically polluted rivers[11-13]. Several authors suggested methods to use *Stigeoclonium* as a bioassay organism for pollution[14-15]. After mercury and copper, cadmium has been reported to be the most toxic element to plants[18]. Cadmium has been shown to inhibit growth[19], photosynthesis[20], reduce the content of photosynthetic pigments[21], cause damage to the structure of chloroplast[22] and mitochondria[23].

Stigeoclonium tenue Kutz. isolated from the effluents of Ramagundam thermal power plant (Andhra Pradesh) has been used in the present investigation to study the tolerance and the kinetics of cadmium uptake. This alga has been used to understand various aspects of cadmium toxicity[24-25], tolerance and defense mechanisms developed by tolerant species. This would be useful for developing bio-treatment system for remedial of heavy metal pollution.

Material and Methods

Stigeoclonium tenue Kutz. isolated from the Ramagundam thermal power plant effluents was grown in pure anxenic cultures[25] on modified Bold's meium[26]. These cultures were maintained at 18 ± 1°C with cool white florescent light intensity of 6000 lux. Cadmium was given in the form of cadmium suphate (3 $CdSO_4$. $8H_2O$). Pure bacteria free inoculum of the alga was taken from the stock cultures. The inoculum consisted of an apparent homogenous suspension of the broken filaments of the alga to facilitate the transfer of equal amount of inoculum for all treatments.

Toxicity test and determination of tolerance index concentration (TIC): To study the toxic effect of Cd and to determine TIC, *Stigeoclonium* was grown on media with Cd concentration ranging from 1.0 mg l^{-1}, with an increment of 1.0^{-1}. The basal medium was used as control. For each treatment, ten tubes were maintained under standard culture conditions. The toxic and tolerance index concentration (TIC) for Cd was calculated following the semi-quantitative scale as per Harding and Whitton[27].

The growth of the alga at different

Cd concentrations was compared visually under microscope with control on 6^{th}, 8^{th} and 14^{th} day after re-inoculation. At the end of 14^{th} day(incubation period), the concentration of Cd at which the growth of the alga was as normal and vigorous as that of the control was categorized as "maximum concentration causing no inhibition of growth (1)". At the end of 8^{th} day, the concentration of Cd at which slight inhibition of growth occurred, the alga from that concentration was re-inoculated to fresh basal medium and grown up to 14^{th} day. The minimum concentration at which re-inoculated alga grow as fast as control was categorized as "minimum concentration causing the slight inhibition of growth (2)". The concentration of Cd at which the re-inoculated alga showed even slight recovery by 14^{th} day was recorded as the "maximum concentration at which the alga is alive (3)". The concentration of Cd where there was no recovery of the alga and death of the cells at the end of 14^{th} day was considered as the "minimum concentration at which the alga was killed (4)". While determining these concentrations for different categories, the identified concentration range was further narrowed by taking 0.1 to 0.9 mg l^{-1} of that particular concentration.

Based on these observations the non-inhibitory, lethal and tolerance index concentration (TIC) were calculated. The "non-inhibitory concentration" is the geometric mean of the maximum concentration causing no inhibition (1) and minimum concentration causing slight inhibition (2). The "lethal concentration" is the geometric mean of the maximum concentration at which the alga was alive (3) and the minimum concentration at which the alga was killed (4). The tolerance index concetration (TIC) is th geometric mean of non-inhibitory and lethal concentrations of Cd.

Determination of the cellular cadmium in the algal mat was carried out as per Singh and Yadava[28] by atomic absorption spectrophotometer (AAS- Prekin-Elmer 2380) at 228.8 nm. To measure the adsorbed cadmium, the algal mat was washed with 0.2 M cysteine solution. The washings were pooled and analysed for Cd by AAS. The amount of cellular (absorbed) Cd and adsorbed cadmium was expressed as μg of Cd per gram dry weight. Each value is a mean of three replicates and all the results were statistically analysed.

Results and Discussion

Studies on Cd toxicity and TIC have shown that *S. tenue* showed no inhibition of growth upto 6.2 mg l^{-1} (55.18 μM) Cd wherein it grew as fast as the control. At 6.8 mgl^{-i} (60.52 μM) and above Cd inhibited the growth of the alga. The alga taken out form this concentration (i.e.6.8 mg l^{-1} or 60.52 μM) on 8^{th} day and re-inoculated in the basal medium showed a lag phase in the beginning, but recovered and showed better growth up to 14^{th} day. The alga grown at 18.2 mgl^{-1} (161.98μM) and taken out at 8^{th} day recovered slightly after re-inoculation in the basal medium. But the growth was not as good as the control. When the alga was grown at 22.7 mg l^{-1} (202.03 μM) Cd the growth was completely inhibited and finally resulted in the death of the alga. From the toxicity and TIC studies the following observations were also made.

i. Non- inhibitory concentration of Cd	**6.49mgl^{-1} (57.76 μM Cd)**
ii. Lethal concentration of Cd	**20.33 mgl^{-1} (180.94 μM Cd)**
iii. Tolerance index concentration (TIC)	**11.49mg l^{-1} (10.26 μM Cd)**

The present investigation has shown that *S. tenue* can withstand high Cd concentration and can grow normally up to about 55 μM Cd. This observation is supportive of the classification of this alga as a chemobiont by Fjerdingstad[29]. However there are no reports regarding the resistance of this alga to Cd.Harding and Whitton[27] showed *Stigeoclonium* to be tolerant to high zinc levels. *S. tenue* tolerance to zinc and lead[30], copper and chromium [29-31] have been reported. Based on the present study on tolerance index concentration (TIC) for cadmium, *Stigeoclonium* can be categorized as resistant to high levels of cadmium.

The amount of Cd absorbed or cellular Cd increased with the increase in Cd concentration (Fig.1) and the uptake of Cd are time dependent. The rate of absorption

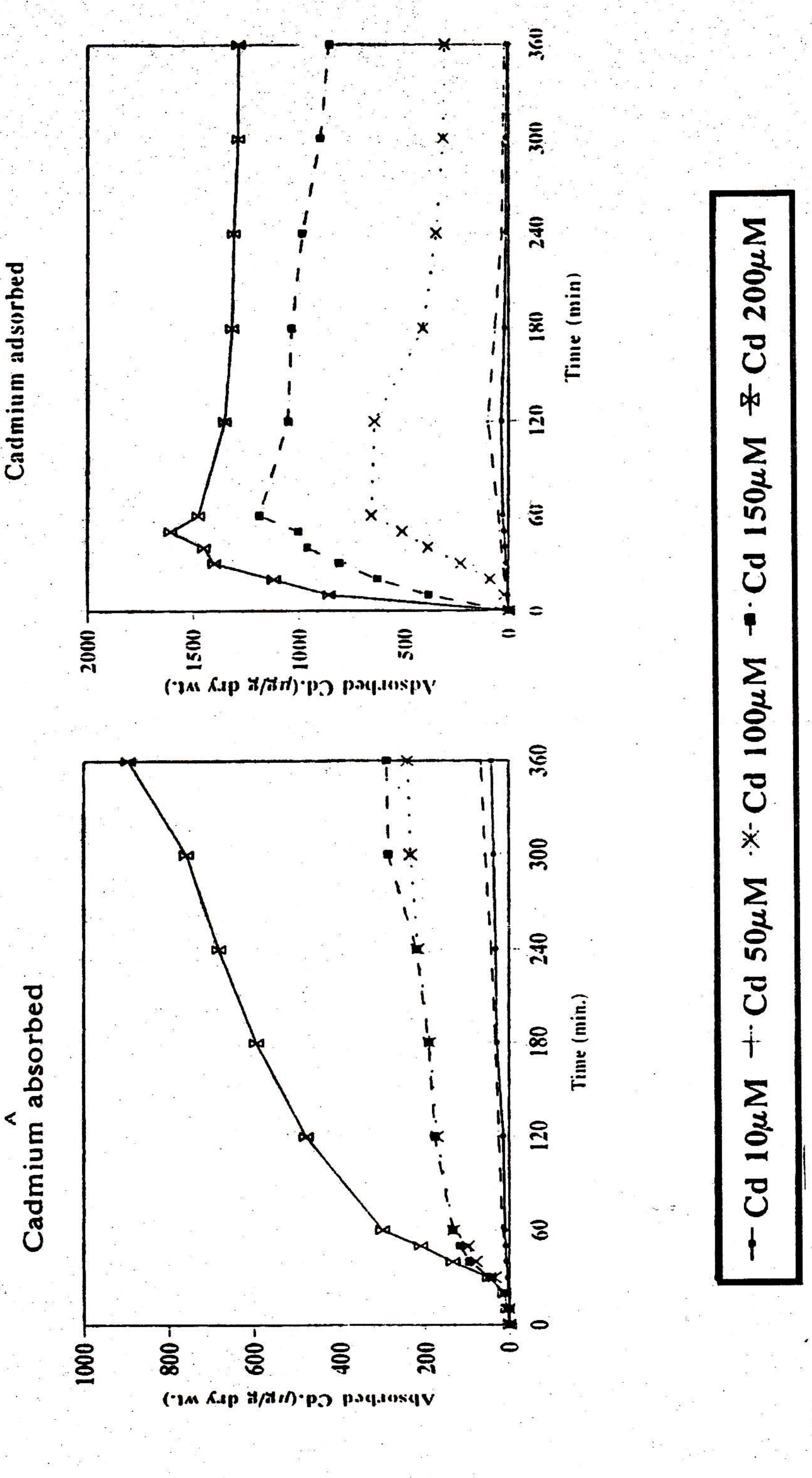

Fig.1. Pattern of Cadmium absorption and adsorption in *Stigeoclonium tenue*.

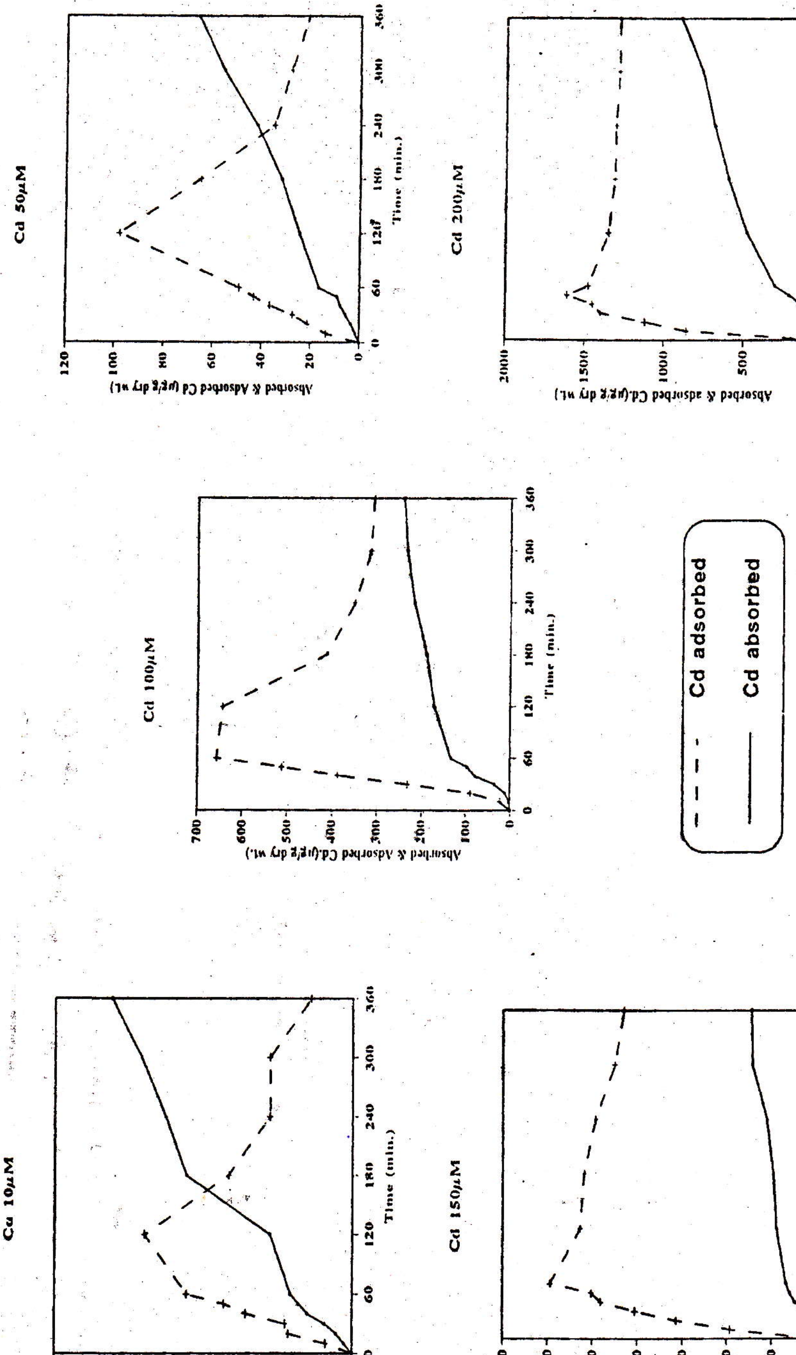

Fig.2. Relation between Cd absorption and adsorption in *Stigeoclonium tenue.*

in the first one hour was more at all levels of Cd, when compared with later absorption rates (Fig.1). At 10and 50 µM Cd, the absorbed Cd increased till the end of the experimental period, and it was four fold when compared to the absorption at the end of first one hour. Whereas at 100 and 150 µM Cd, the amount of absorbed Cd was doubled when compared to the first hour absorption. At the end of the experiment the algal cells absorbed 40.42 $\mu g\ g^{-1}$ dry weight at 10 µM Cd, and it increased by about 6 fold at 100 µM and by 22 fold at 200 µM Cd (Fig.1).

The amount of adsorbed Cd at initial periods of incubation was more than absorbed Cd at all concentrations of cadmium. In the first two hours of incubation, the adsorbed Cd at 10 and 50 µM Cd level was much higher than absorbed Cd. However, at later hours of incubation (as time lapsed), the content of adsorbed cadmium decreased to less than absorbed Cd (Fig.2). With the increase in the Cd concentration in the medium (100,150 and 200 µMCd), maximum amount of Cd adsorption reached within 50 to 60 minutes (Fig.2). The amount of adsorbed Cd at the end of the experimental period remained higher than the absorbed Cd at these higher concentrations of cadmium. The studies on the kinetics of Cd uptake have shown that:

(i) Both absorption and adsorption rates were high during the initial hours of incubation.

(ii) At low Cd levels (10and 50 µM Cd), the amount of absorbed Cd was more than the adsorbed Cd at the end of incubation period.

(iii) At higher Cd levels (100, 150 and 200 µM Cd) the amount of adsorbed Cd was more than absorbed Cd at the end of incubation period.

The metal accumulation by microorganisms generally comprises of two phases; a rapid binding of cations to the negatively charged groups on the cell surface and the subsequent metabolism dependent intra-cellular uptake[32]. The present study has shown that *Stigeoclonium* cells removed cadmium ions from the medium in two possible ways. Firstly, the metal was adsorbed on the surface of the algal cells, mostly through passive uptake as shown in the cysteine washings. Secondly, a large part of Cd was absorbed through energy dependent active uptake. This pattern of Cd absorption and adsorption was dependent on the amount of Cd in the external medium and also on the length and/or duration of exposure of the algal cells to metal ions (Fig.1). At low levels of Cd, the adsorption of Cd was more at initial period than the absorbed Cd. At later stages of incubation, the amount of Cd absorbed has over taken the amount of Cd adsorbed (Fig.2). Similar biphasic pattern of metal uptake has also been reported in other microbes [32-34] and higher plants[35].

Acknowledgements

We thank Head, Botany Department, Osmania University for providing facilities. M. Vanaja is grateful to CSIR for the award of fellowship during the tenure of this work.

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