Research Article

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Efficacy of antibiotics on bacterial contamination in outdoor cultures of *Spirulina platensis*

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ABSTRACT

Due to climatic effect outdoor cultures of *Spirulina* got contaminated with bacteria extensive growth of bacteria retorted the growth of *Spirulina*. To control the growth of these bacteria seven antibiotics i.e. Streptomycin, Gentamycin, Sodium benzoate, Sparfloxacin, Cephalaxin, Erythromycin and Tetracycline were applied and Streptomycin was found most suitable to control the growth of bacterial contamination.

Keywords : *Spirulina*, Outdoor Cultures, Antibiotics, Bacterial Contamination.

INTRODUCTION

In humid weather of rainy season *Spirulina* cultures in outdoor conditions get contaminated with bacteria. Though aquatic bacterial flora grew side by side with *Spirulina*, yet proliferate extensively in humid conditions. Due to the high rate of their multiplication in short span of time, these bacteria in the pond pose a problem. With the increase in bacterial flora, a faul smell prevailed in the atmosphere. The control of these microorganism becomes mandatory to maintain the food value of commercial alga. Common method of bacterial control is the use of antibiotics.

Edward *et al.* (1961) found that amisomylin, nystalin and actidione had little or no detectable effect on the Cyanophyceae and bacteria. Watanabe & Yamemota (1968) also used selected antibiotics to obtained pure culture of microalgae. Reddy (1977) found that streptomycin inhibited the growth and pigment synthesis in blue green algae. Cao *et al.* (1999) studied the sensitivity of *Spirulina platensis* to antibiotics and herbicides. In the present study, effect of seven different antibiotics i.e. Streptomycin, Gentamycin, Sodium benzoate, Sparfloxacin, Cephalaxin, Erythromycin, and Tetracycline were applied to control the bacterial contamination in outdoor cultivated *Spirulina platensis*.

MATERIAL AND METHODS

Effect of all these antibiotics was studied with reference to growth rate of *Spirulina* cultures in term of OD, turbidity, chlrophyll$_a$ and protein content of *Spirulina*. For measuring the turbidity, cultures were filtered through Whatman filter paper. Absorbance of filtrate was read on 560 nm. 1 mg/l concentration of antibiotics was added in each of one litre bacterial contaminated *Spirulina* cultures in separate plastic tubs. Initial optical density, turbidity, chlorophyll$_a$ and protein contents were recorded. Optical density and turbidity were recorded everyday, upto five days. 5th day, algal cultures were analysed for protein & chlorophyll$_a$ contents and cultures were transferred to fresh CFTRI (I) medium. On 5th day of subculturing, cultures were reanalysed for their chlorophyll$_a$ and protein contents. Contaminated culture, without antibiotics were treated as controls.

RESULT AND DISCUSSION

Initial day, optical density and turbidity of bacterial contaminated cultures were 1.70 and 0.30 respectively. Protein and chlorophyll$_a$ contents were 56 and 0.962% serially.

Controlled cultures

Controlled cultures showed continuous increasing trend in OD as well as in turbidity. From 1st to 5th day optical density was 1.74, 1.95, 2.36, 2.57 and 2.62 (Fig. 1). Similarly 0.35, 0.40, 0.44, 0.46 and 0.49 turbidity was recorded (Fig. 1). chlorophyll$_a$ and protein contents were 0.918 and 55% respectively. Cultures, when grown in fresh medium for 5 days, chlorophyll$_a$ and protein contents shot-up to an appreciable amount of 0.952 and 56% respectively (Fig. 9 & 10).

Fragmented and dead trichomes were observed in these cultures, while cultures were homogenous but dull green in colour. Faul smell was slightly reduced after subculturing.

Streptomycin treated cultures

Continuous reducing trend in bacterial number was recorded in these cultures. From initial to 5th day, turbidity was 0.20, 0.12, 0.10, 0.8 and 0.4 (Fig. 2). On the other hand, algal growth was continuously increasing. However, growth rate was slower than in the controlled cultures. Optical density was 1.72, 1.79, 1.82, 1.95 and 2.10 from starting to 5th day respectively (Fig. 2). 5th day optical density was highest and turbidity was lowest in comparison to other antibiotic treated cultures. Analysis of algal cultures showed 0.776% chlorophyll$_a$ and 38% protein contents. 5th day after subculturing in fresh medium, the cultures possessed 0.910% chlorophyll$_a$ and 54% protein contents (Fig. 9 & 10). This antibiotic hampered the synthesis of chlorophyll$_a$ and protein both.

Streptomycin treated cultures in the tubs formed dark green layer on the surface. Cultures were comparatively healthier than the controlled set of cultures.

Gentamycin treated cultures

Gentamycin treated cultures showed continuous enhancing trend in algal growth. OD of the cultures was 1.76, 1.85, 1.98, 2.08 and 2.31 upto 5th day of the experiment (Fig. 3). Turbidity got reduced by 3rd day. It was 0.24, 0.23 and 0.22 sequentially on 4th day. The bacteria seemed to have acquired resistance against this antibiotic, as these proliferated rapidly. 4th day turbidity was 0.25 and 5th day it was 0.34 (Fig. 3). protein and chlorophyll$_a$ contents were 28 and 0.686%. These cultures were subcultured in fresh medium. On the 5th day of the subculturing, protein and chlorophyll$_a$ contents were 32 and 0.689%. That was too less, when compared with controlled cultures (Fig. 9 & 10).

Initially, cultures were green with healthy trichomes but soon chloresis started and trichomes turned yellowish.

Sodium benzoate treated cultures

Upto 4th day of the experiment, turbidity showed linear reduction in sodium benzoate treated cultures i.e. 0.27, 0.17, 0.15 and 0.13. On 5th day, sudden enhancement in turbidity was observed i.e. 0.28 (Fig. 4). Algal growth was continuously increasing, it was 1.74, 1.85, 1.90 and 1.96 at 1st, 2nd, 3rd, 4th and 5th day (Fig. 4). On 5th day, chlorophyll$_a$ and protein contents were 0.661 and 30% respectively. These cultures, on the 5th day of subculturing in the fresh minimal medium chlorophyll$_a$ and protein contents were 0.662 and 31%, while in control cultures these were 0.910 and 54% respectively (Fig. 9 & 10).

The cultures were dark green in colour but faul smell persisted. Few white patches and bubbles were seen on the algal surface layer.

Sparfloxacin treated cultures

Initially sparfloxacin had no effect on bacterial growth. Initial and 2nd day, turbidity was 0.25 and 0.31. From 3rd day onwards this antibiotic proved to be effective and continued reduction in bacterial flora was recorded. This went hand in hand with turbidity. It was 0.28, 0.24 and 0.22 at 3rd, 4th and 5th day (Fig. 5). Algal growth was continuously enhancing. OD from 1st to 5th day was 1.75, 1.88, 1.90, 1.92 and 2.04 (Fig. 5). 38 and 0.586% protein and chlorophyll$_a$ contents were on record at 5th day. When these cultures were grown in fresh nutrient medium after 5th day, chlorophyll$_a$ contents revised back, it was 0.893% but protein contents were still 48% only (Fig. 9 & 10).

Sparfloxacin treated cultures had mucilaginous patches of trichomes. Protozoan contamination was also seen in these culture, which were, however, green and healthy.

Cephalaxin treated cultures

Though cephalaxin treated cultures showed continuous decline in turbidity, but it was very insignificant i.e. 0.30, 0.28, 0.26, 0.24 and 0.22 from 1st to 5th day (Fig. 6). Simultaneously growth of the alga was continuously increasing. OD of these cultures was 1.76, 1.83, 1.85, 2.20 and 2.64 at 1st, 2nd, 3rd, 4th and 5th day respectively (Fig. 6). 5th day protein and chlorophyll$_a$ contents were 25 and 0.558% respectively. These were 40 and 0.689% when cultured in the fresh CFTRI(I) nutrient medium, after 5 days (Fig. 9 & 10).

Chloresis in these cultures was very fast. Cultures were badly unhealthy and turned yellow in colour.

Erythromycin treated cultures

This much low i.e. 1mg/l concentration of erythromycin was lethal to algae. OD was continuously reducing. It was 1.71, 1.61, 1.27, 1.21 and 1.12 from 1st to 5th day (Fig. 7). Turbidity was continuously increasing in erythromycin treated cultures. 0.37, 0.38, 0.42, 0.44 and 0.47 turbidity was on record from 1-5th day respectively (Fig. 7). 5th day, protein and chlorophyll$_a$ contents were 24 and 0.265% respectively. 22% protein and 0.232% chlorophyll$_a$ was recorded after growing these bleached cultures in the fresh nutrient medium for 5days (Fig. 9 & 10).

Cultures were unhealthy, yellow and forming clumps and strong faul smell was experienced.

Tetracyclin treated cultures

Continuous growing trend in algal culturs was observed in tetracyclin added cultures. OD was 1.70, 1.74, 1.76, 1.82 and 1.86 from 1st to 5th day (Fig. 8). Initially, tetracyclin was effective in controlling the bacterial growth.

Turbidity on 1st and 2nd day was 0.22 and 0.17. Thereafter, bacteria seemed to have developed resistance against this antibiotic. 0.28, 0.34 and 0.36 turbidity was on record at 3rd, 4th and 5th day respectively (Fig. 8). 5th day 20% protein and 0.217% chlorophyll$_a$ were on record. These cultures were transferred to fresh nutrient medium and allowed to grow upto 5 days. Then the protein and chlorophyll$_a$ contents were 24 and 0.259% respectively (Fig. 9 & 10).

Cultures were healthy and dark green, yet white clumps were seen on algal surface layer.

Streptomycin reduced the turbidity and algal growth was continuously enhancing. Though protein and chlorophyll$_a$ contents were reduced during streptomycin treatment but after subculturing in fresh minimal medium without antibiotic these contents revived back. Similar results were observed by Kumar (1964) and Kumar & Kaushik (1971) with blue green algae. Eichenberger and Boschetti (1975) also found the light induced greening in streptomycin bleached cultures.

Gentamycin, Sodium benzoate, Tetracyclin and Sparfloxacin showed almost similar effects. Initially, these were effective on bacterial flora but later bacteria seemed to have developed resistance against these antibiotics. Protein and chlorophyll$_a$ were reduced. Even after subculturing into the fresh minimal medium cultures did not improve.

Erythromycin permanently bleached the cultures. Similar observations were recorded by Ebringer (1962) with this particular antibiotic.

Controlled cultures showed continuous growth of the alga. Protein and chlorophyll$_a$ contents were linearly enhanced. After subculturing growth and cellular metabolites were increased and came upto normal values of the alga. These results clearly showed that among these 7 antibiotics experimented upon presently, streptomycin can be used for the control of the bacterial contamination, if need be felt. The contaminated cultured which were untreated with antibiotics lead to the conclusion that cultures be left in open with additional aeration and supplementation of desired nutrients regularly. Antibiotics did reduce the bacterial flora of the cultures, enhanced due to humidity but the quality of the cultures was damaged by the antibiotics.

Fig. 1 : Outdoor cultivated *Spirulina* cultures with bacterial contamination (control)

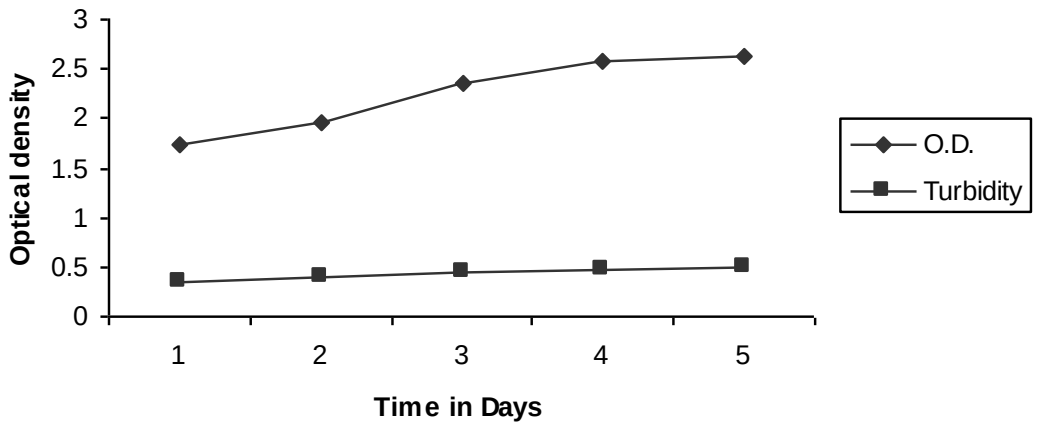


Fig. 2 : Effect of Streptomycin on bacterial contamination in outdoor cultivated *Spirulina* cultures

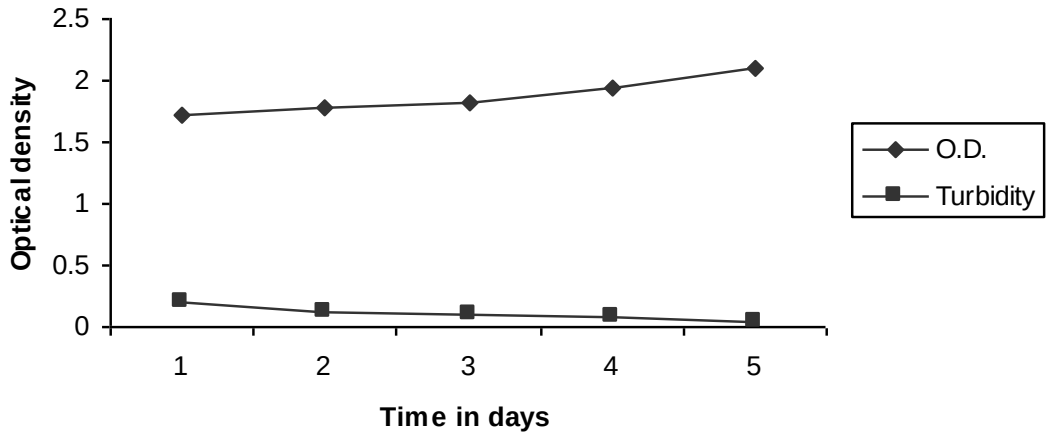


Fig. 3 : Effect of Gentamycin on bacterial contamination in outdoor cultivated *Spirulina* cultures

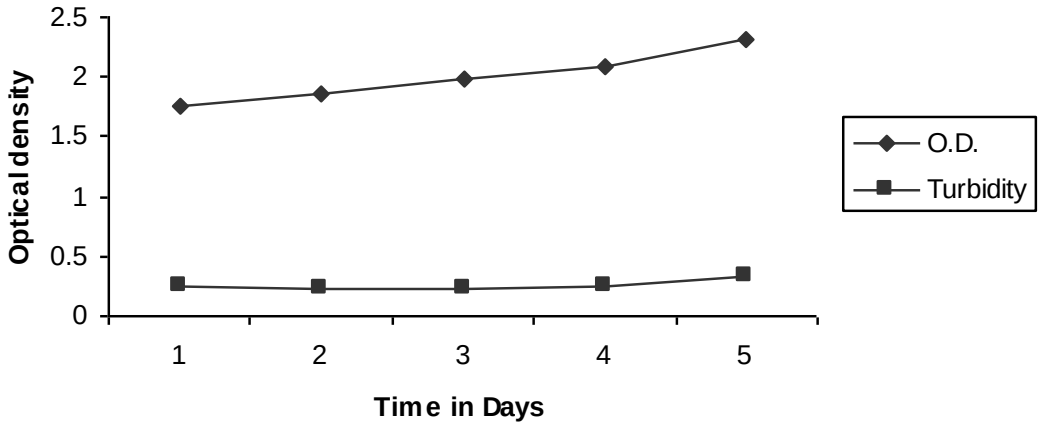


Fig. 4 : Effect of Sodium benzoate on bacterial contamination in outdoor cultivated *Spirulina* cultures

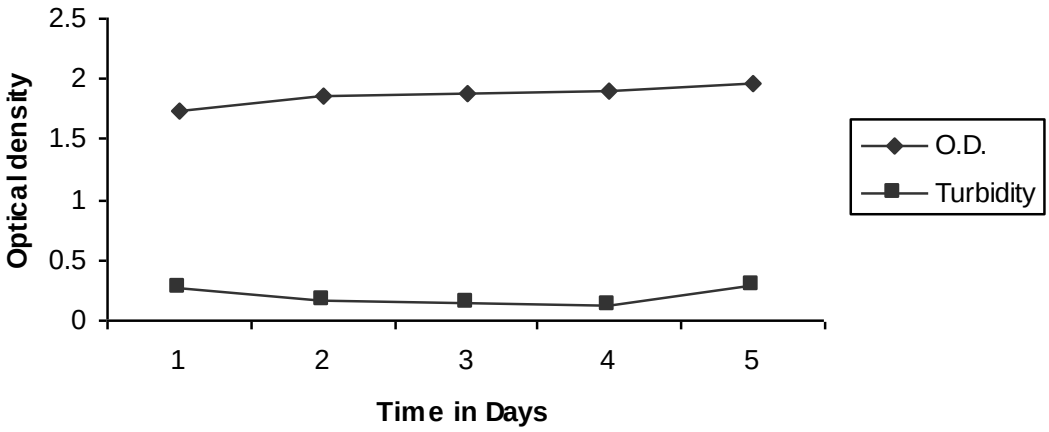


Fig. 5 : Effect of Sparfloxacin on bacterial contamination in outdoor cultivated *Spirulina* cultures

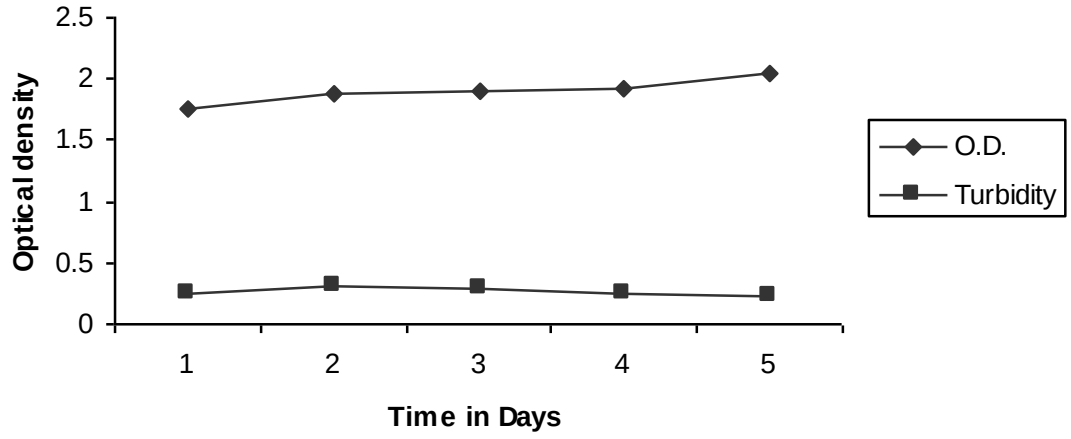


Fig. 6 : Effect of Cephalaxin on bacterial contamination in outdoor cultivated *Spirulina* cultures

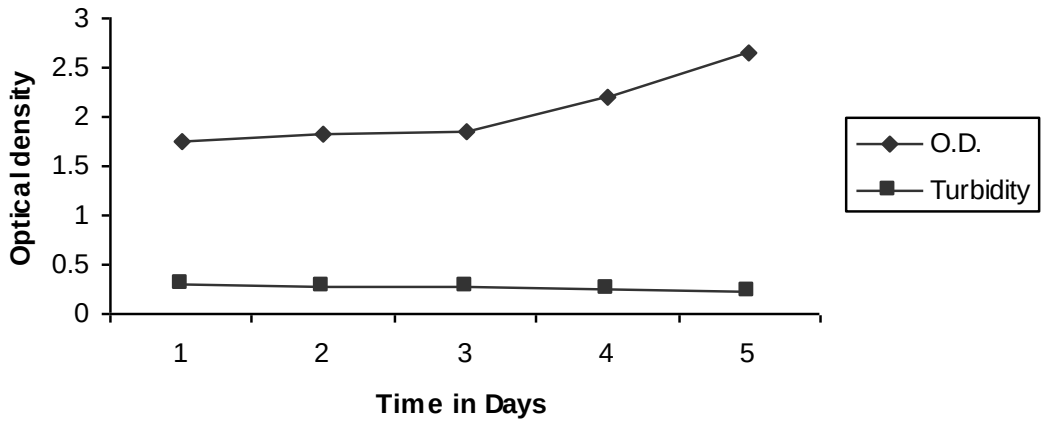


Fig. 7 : Effect of Erythromycin on bacterial contamination in outdoor cultivated *Spirulina* cultures

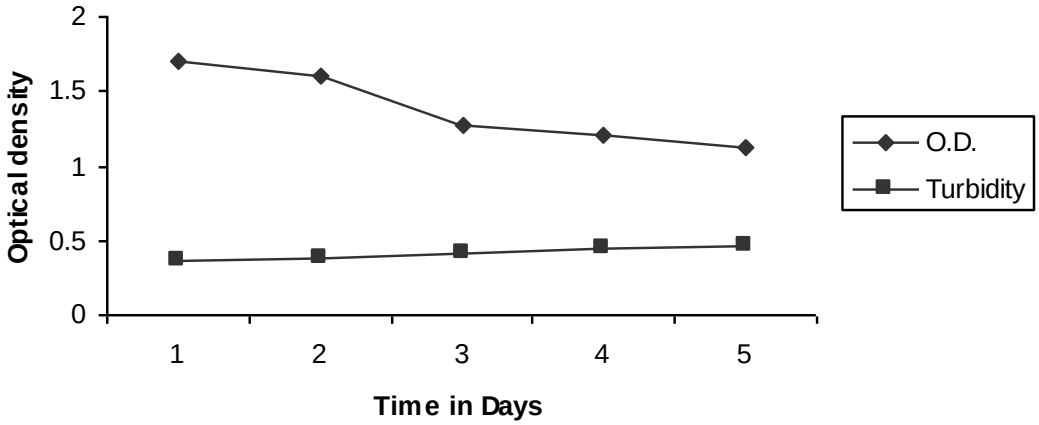


Fig. 8 : Effect of Tetracyclin on bacterial contamination in outdoor cultivated *Spirulina* cultures

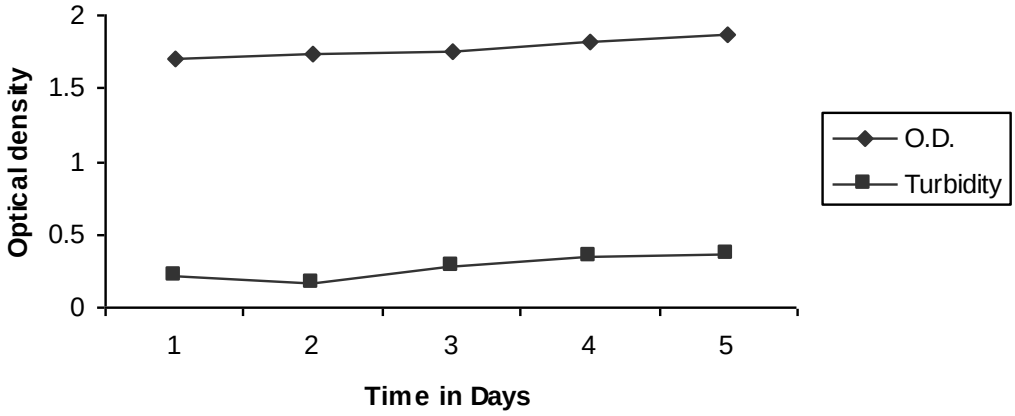


Fig. 9 : Impact of antibiotics on protein contents of outdoor cultivated *Spirulina* cultures

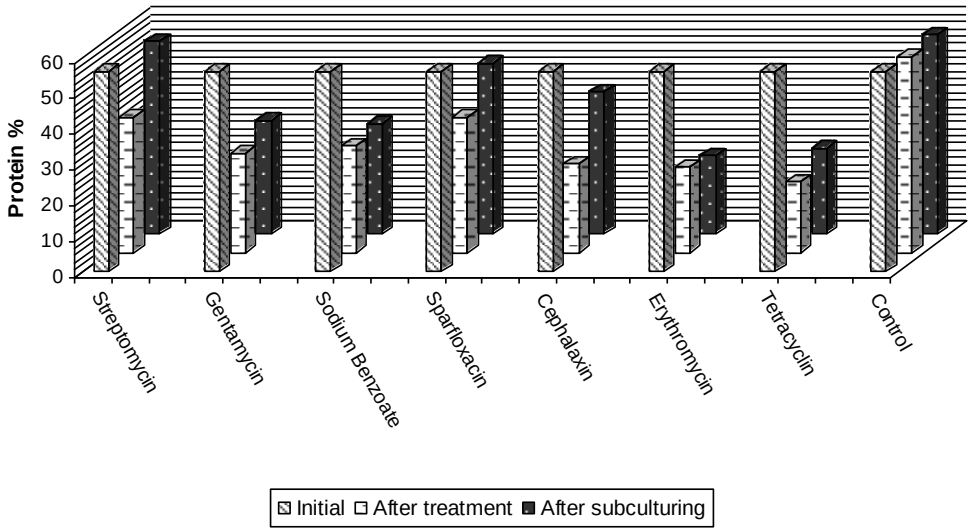
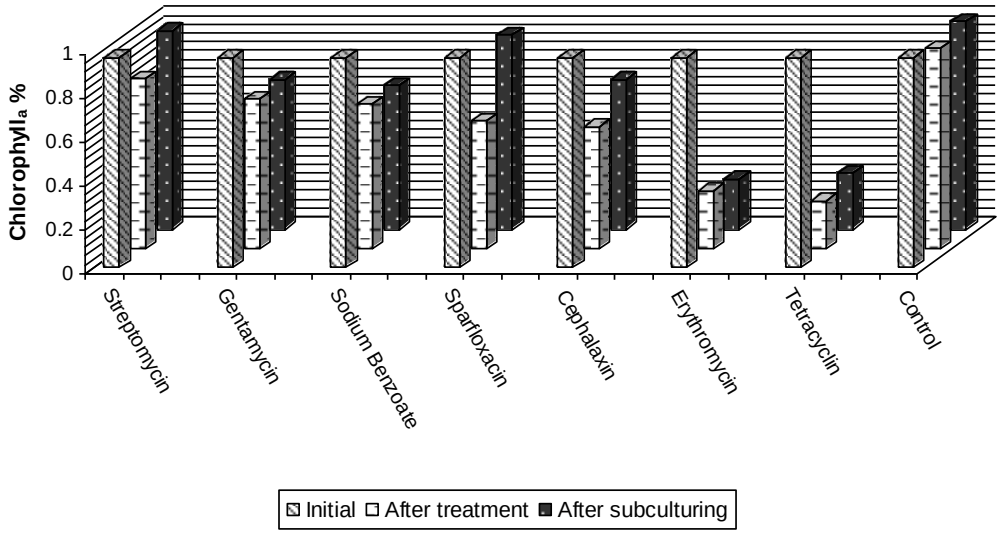


Fig. 10 : Impact of antibiotics on chlorophyll$_a$ contents of outdoor cultivated *Spirulina* cultures



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