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# THE EFFECTS OF THE POLLUTANT, SODIUM CYANIDE, ON THE MORPHOLOGY AND PHYSIOLOGY OF OEDOGONIUM CARDIACUM

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ABSTRACT

OEDOGONIUM cardiacum exposed to varying concentrations of sodium cyanide for 15 day periods exhibited both morphological and physiological alterations. Organisms were exposed to the pollutant in concentrations of 1, 10, 25, 50, and 100 parts per million. Exposure period for organisms in each concentration was 15 days. The 15 day period permitted assays of effects caused by the pollutant.

As the concentration of the pollutant increased fragmentation also increased. Exposure also caused organisms to lose chlorophyll. There is a direct proportion of chlorophyll loss to increased pollutant concentrations. This is true for chlorophyll "a" and "b". The third morphological alteration is the incidence of rupture. Cells in the higher concentrations rupture and organelles are lost.

Physiological effects altered by exposure include: reduced oxygen evolution, retardation of sexual and asexual reproduction, retardation of starch production and death. Death occurs when organisms are exposed to high concentrations over the total 15 day period.

## INTRODUCTION

Cyanide has a long history of being a poison. (Berry 1976). Many people have heard of cyanide as being the substance used in gas chamber executions. However, many are not aware of the fact that cyanide is a common water pollutant. (Berry 1974). Small quantities of the pollutant occur naturally in water, such as that produced by millipedes as a defense mechanisms against predators. (Berry 1974). Much of the cyanide in industrial use is commercially synthesized. Cyanide consists of carbon and nitrogen atoms joined by a triple bond. The joining of these atoms form the cyanide radical. The compound, referred to as the pollutant. Sodium cyanide is used in this study. Compounds containing the cyanide radical form a group of versatile reagents with many chemical and industrial applications. The cyanide

pollutant enter industrial waste streams from a variety of processing industries such as extracting gold and silver ores, synthetics manufacturing, coal cooking furnaces and electroplating.

The effect of cyanide on the morphology and physiology of the green algae Oedogonium cardiacum may give some indications of the effects on organisms dependent on the algal plant for food and oxygen. The effects could be traced to fish and ultimately man. Early studies indicate that concentrations of cyanide as low as 0.1 ppm in water can kill fish. (Doudoroff 56).

This study will assess the degree to which diseases rather than death will be produced in organisms utilizing the plant as a source of food. In addition, problems will be defined, hypotheses will be established and studies will be designed to assess the effects experienced by food chain organisms.

Materials and Methods

Oedogonium cardiacum, a filamentous freshwater green algae is the organism for study. It consists of several cells - per filament. Each cell has a nucleus and single large chloroplast of irregular net-like shape with many pyrenoids. The basal cell is modified as a holdfast. Growth of the filament occurs as various cells divide-mitotically.

Reproduction occurs both asexually and sexually. Asexual reproduction is by fragmentation or by the production of zoospores. Sexual reproduction is of the oogamous type. A filament may contain a large swollen cell, the oogonium, and small structures resembling zoospores in the antheridium. A sperm (zoospore) from the antheridium swims to the enlarged oogonium and enters through a pore in the wall of this cell and fuse with the single egg contained therein. The diploid zygote germinates producing four haploid zoospores each of which develops into a new filament.

The organism is grown in soil water medium or Bold's basal medium. Sodium cyanide is added to the medium in various concentrations. The control contains only the medium while concentrations of 1 ppm, 10 ppm, 25 ppm, and 100 ppm constituted the experimental groups. ppm dilutions indicate a weight colume ratio of milligrams of sodium cyanide per liter of solution e.g. mg/l.

Each concentration is grown in conical flasks for a period of fifteen (15) days. The growth period is conducted in twelve (12) hour light and dark periods. The light period coincide with the daylight period. The temperature for the growth period is $23^{o}C \pm 2$.

Daily observations are conducted with the light microscope. These observations permit assessment of fragmentation, cellular rupture, formation of reproductive structures and loss of cellular constituents.

Respiration measures are conducted by the use of Warburg apparatus. Each concentration is subjected to five (5) fifteen (15) minute respiratory measures daily for the fifteen (15) day study period.

Chlorophyll extraction techniques enable measures of chlorophyll loss for the fifteen (15) day period. Chlorophyll "a", "b" and total chlorophyll is measured by the spectrophotometer. Calculations based on spectrophotometric readings provide data indicating the amount of chlorophyll loss during the fifteen (15) day period.

Chromatographic assays are employed to determine the presence of carbohydrates produced and stored in the filaments at the end of the experimentation period. Carbohydrates are extracted and subjected to paper chromatography for analysis of the carbohydrates.

Discussion

Oedogonium c is grown in either soil water medium or Bold's basal medium containing sodium cyanide in concentrations of 0 ppm, 1 ppm, 10 ppm, 50 ppm, and 100 ppm for a period of fifteen (15) days. The growth period consists of twelve (12) hour dark and tweive (12) hour light periods. The temperature for the growth period is 23°C ± 2 degrees centrigrade. Daily observations with the light microscope indicates that as the concentrations of pollutant increased fragmentation of the filaments increased. Observations indicate that 1% of the filaments in the control (ppm) showed some fragmentation while 6% of the filaments fragmented in 100 ppm concentration. Figure One (1) indicates the percentage of fragmentation in the concentrations studied. Cell fragments occurred in all concentrations. However, the frequency of fragments increased with the increase of the pollutant. Fragmentation includes breaking of single cells of filaments, breaking of filaments, or abnormal twisting of cells causing the walls to rupture and break. The incidence of fragmentation is more apparent in the cells with the greatest exposure.

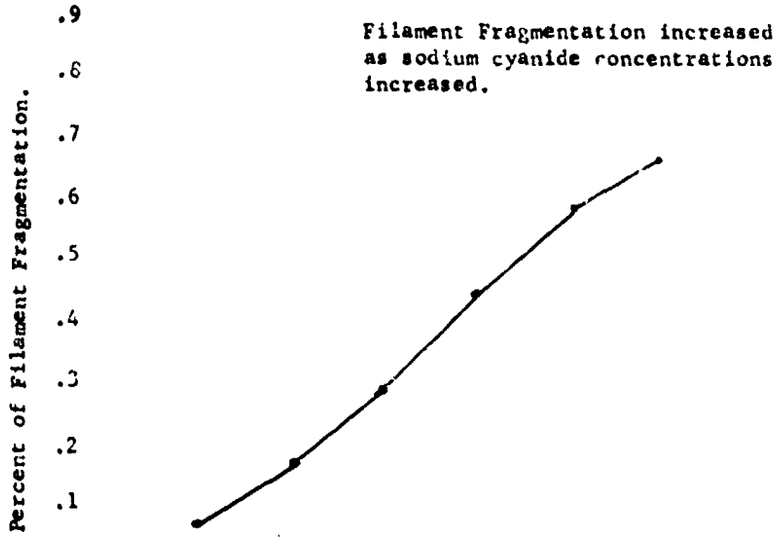


Fig. I- Filament Fragmentation

In addition to filament fragmentation the number of cells void of protoplast increased as the concentrations increased. Few cells in the control (0 ppm) flask are void of cellular constituents. However, an increase in cells without protoplasts were visible in concentrations of 50 ppm and 100 ppm.

Reproductive structures developing on filaments in the control were numerous. Both oogonia and antheridia are visible in the control. Sexual reproduction is apparent in the appearance of antherida and oogonia on filaments. Antheridia are indicated by short walls formed in the cell producing sperm cells. The number of cross walls increase as maturation of the antheridium takes place.

Also reproduction can be noted by the appearance of the oogonium. Increases in the reproduction structures indicate a tendency for increase in off spring.

As concentrations increase the number of oogonia and antheridia decreased. 7 % of the filaments in the control contained an antheridia or oogonia. In some instances filaments contained both antheridium and an oogonium. As the concentrations increased the incidence of reproductive structures decreased. The 100 ppm concentration showed 1% of the filaments with reproductive

structures.

As sodium cyanide concentrations are increased reproductive structures on filaments decrease

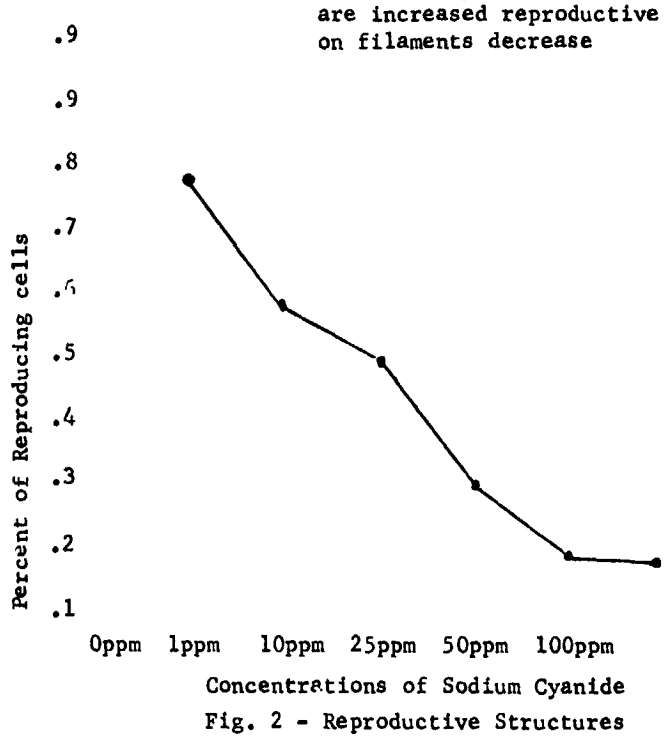


Fig. 2 - Reproductive Structures

The percentage of reproductive structures decreased from 8 % to 1 % in concentrations of 0 ppm to 100 ppm.

Respiration of oxygen evolution is a measure of the physiological activities of the cells. Measures were conducted for five (5) fifteen (15) minute periods per day. The measures are averaged to provide a measure of the oxygen evolved for that day. The fifteen (15) day measures for 0 to 100 ppm are recorded in Figure 3. The data indicate that the control organisms were producing six (6) micro liters of oxygen on the tenth (10) day and the 1 ppm and the 10 ppm had ceased to evolve any measurable amounts of oxygen on the tenth (10) day.

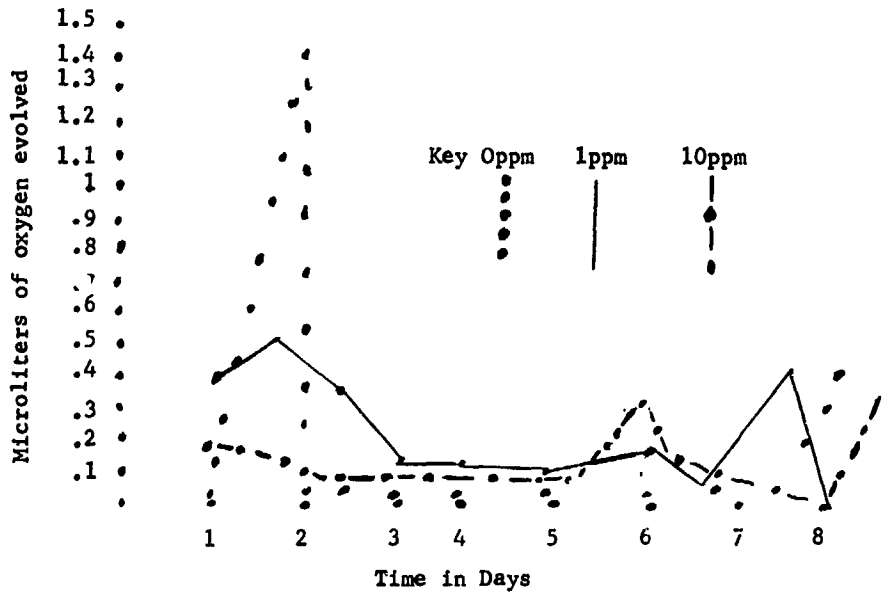


Fig. 3 - Respiration Measures

Figure 4 contains data for 25 ppm, 50 ppm, and 100 ppm. The 50 ppm concentration indicates practically no oxygen evolved about nine (9) days. On the nineth and tenth days almost five (5) microliters of oxygen are evolved. This measure is not indicative of the 25 ppm and 100 ppm. Oxygen is produced on two (2) days and then decreases through the tenth (10) days measures.

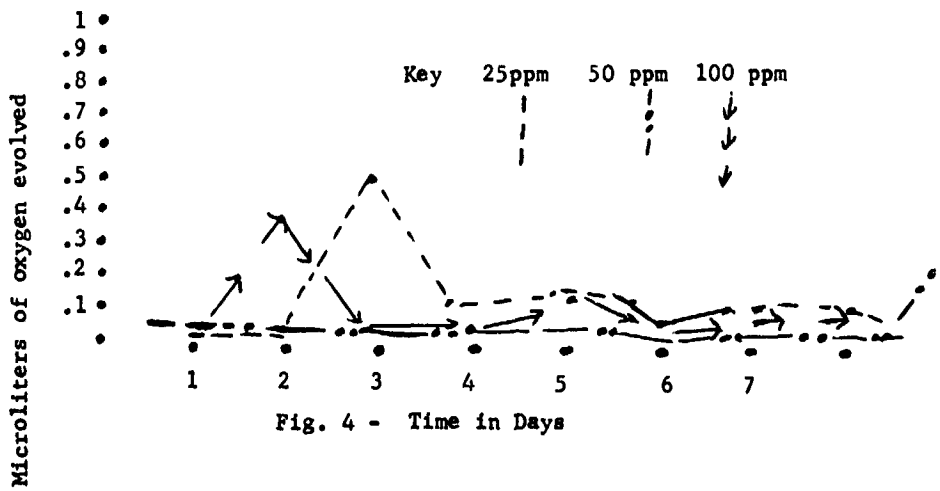


Fig. 4 - Time in Days

The data indicate that oxygen production decreases as the concentrations of the pollutant increase.

Chlorophyll is extracted at the close of ten (10) day study period. The extraction procedure provides data for chlorophyll "a" and "b" and total chlorophyll. The measure does not indicate the amount of chlorophyll lost per day. The measure is the amount lost over ten (10) day study period. Visible observations indicate that chlorophyll is lost to some degree. The control organisms are quite green while the organisms in the 100ppm concentration are a white to milky white color. Indicating chlorophyll has been lost during the experimentation period.

The chlorophyll is extracted and measures are made using the B & L spectrophotometer. Calculations show that the control organisms contain .4 mg of chlorophyll "a" per gram of tissue, .5 mg of chlorophyll "b" per gram of tissue, and 8 mg of total chlorophyll per gram of tissue. Organisms in the 100ppm lost 1.6 mg of chlorophyll "a" per gram of tissue, 1.9 mg of chlorophyll "b" per gram of tissue and 2.1 mg total chlorophyll per gram of tissue. Figure 5 include calculations for each concentration for the study period.

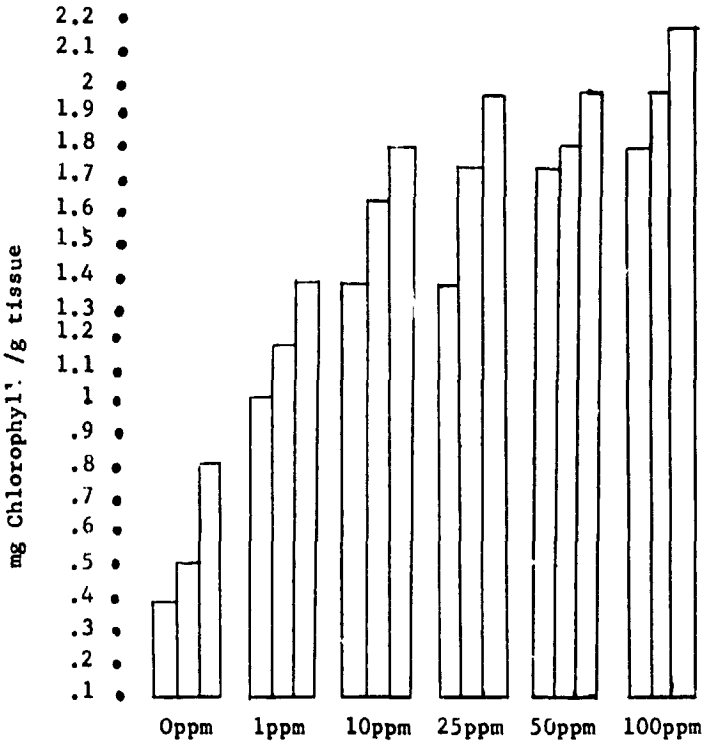


Fig. 5 - Chlorophyll Loss

Spectrophometric measures are based on the percentage transmittance. Therefore, the higher the bar graph the smaller the amount of chlorophyll present. As a result more chlorophyll was lost from the organisms exposed to higher concentrations of sodium cyanide. Chlorophyll extraction did not occur until the study was completed. However, visible observations on a daily basis indicated that the 100 ppm concentration organisms were void of much of their chlorophyll two (2) to three (3) days before the other concentrations.

Findings

O. cardiacum exposed to sodium cyanide concentrations of 0 ppm, 1 ppm, 10 ppm, 50 ppm, and 100 ppm for fifteen (15) days showed a variety of morphological and physiological changes. Morphological changes that occurred include an increase in the number of cells fragmenting as the concentration of the pollutant is increased. Along with increased fregmentation, a larger number of cells rupture and all cytoplasmic material and organells are lost. Rupturing increases as the pollutant concentration decreases.

Reproductive structures occur in large numbers in the growth medium containing 0 ppm of sodium cyanide. However, increase in concentration causes a decrease in the number of reproductive structures forming.

Physiologically, chlorophyll is lost from organisms exposed to concentration of pollutants from 1 ppm to 100 ppm. The largest amount of chlorophyll is lost by the organisms in the 100 ppm concentration.

Carbohydrates are in smaller quantities as measured by paper chromatography. The extraction procedure and analysis technique yielded only small traces carbonhydrate material in the control organisms. In concentrations of 10 to 100 ppm no assessment of carbohydrate was apparent in chromatographis analysis

Sodium cyanide in 1 ppm to 100 ppm cause a wide variety of morphological and physiological effects on O. cardiacum.

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