

# ECOTOXICOLOGIA

## AULA TP 1

random] [plasmid

Chromosomes and plasmids are both DNA molecules, but they are not identical. Chromosomes are large, circular DNA molecules that contain the genetic information of an organism. Plasmids are smaller, circular DNA molecules that are not essential for the survival of the organism. Plasmids can replicate independently of the chromosome and can be transferred between cells. Plasmids often carry genes that confer antibiotic resistance or other beneficial traits to the host cell.

Chemically, DNA consists of two strands of sugar-phosphate backbone joined by hydrogen bonds between the nitrogenous bases. The two strands are antiparallel to each other and are twisted into a right-handed helix. The sequence of these two strands is the genetic code that provides information. The information is stored in the sequence of the nitrogenous bases. The sequence of the bases is determined by the sequence of the nitrogenous bases. The sequence of the bases is determined by the sequence of the nitrogenous bases.

Water-soluble DNA is organized into very specific higher-order structures. These structures are called nucleosomes. Nucleosomes are formed by the wrapping of DNA around a core of histone proteins. The histone core is composed of eight histone proteins. The DNA is wrapped around the core in a regular, repeating pattern. The nucleosome is the basic unit of DNA packaging. The nucleosome is the basic unit of DNA packaging.





## PLANEAMENTO DAS AULAS

**AULA 1 – INTRODUÇÃO AOS TESTES DE ECOTOXICOLOGIA. BIOMARCADORES E DELINEAMENTO EXPERIMENTAL**

AULA 2 – EFEITOS DO GLIFOSATO NO CRESCIMENTO DOS ORGANISMOS TESTE (TAXAS DE INIBIÇÃO E CONSTANTES DE INIBIÇÃO IC50). MARCADORES BIOFÍSICOS DE TOXICIDADE I

AULA 3 – MARCADORES BIOFÍSICOS DE TOXICIDADE II

AULA 4 – MARCADORES BIOQUÍMICOS DE TOXICIDADE I – PIGMENTOS VEGETAIS

AULA 5 – MARCADORES BIOQUÍMICOS DE TOXICIDADE II – PIGMENTOS VEGETAIS II

AULA 6 - MARCADORES BIOQUÍMICOS DE TOXICIDADE III – DANO MEMBRANAR

AULA 7 – MARCADORES BIOQUÍMICOS DE TOXICIDADE IV – DANO MEMBRANAR E ÁCIDOS GORDOS

AULA 8 - MARCADORES BIOQUÍMICOS DE TOXICIDADE III – SOD

AULA 9 – TÉCNICAS DE EXTRAÇÃO E ANÁLISE DE METAIS PESADOS

AULA 10 - NANOTOXICOLOGIA



## INTRODUÇÃO AOS TESTES DE ECOTOXICOLOGIA

**BIO-INDICADORES:** ORGANISMOS QUE EXPRESSAM SINTOMAS PARTICULARES OU RESPOSTAS QUE INDIQUEM MUDANÇAS EM ALGUMA INFLUÊNCIA AMBIENTAL, GERALMENTE DE FORMA QUALITATIVA (HAWKSWORTH, 1992). DÃO INFORMAÇÃO QUALITATIVA (WOLTERBEEK *ET AL.*, 1995).

**BIO-MONITORES:** ORGANISMOS, CUJA DISTRIBUIÇÃO E POPULAÇÕES SÃO ESTUDADOS DURANTE UM CERTO ESPAÇO DE TEMPO, E COMPARADOS A UM MODELO, ONDE OS DESVIOS DO ESPERADO SÃO AVALIADOS. DÃO INFORMAÇÃO QUANTITATIVA (WOLTERBEEK *ET AL.*, 1995).

**BIO-MARCADORES:** SÃO ENTIDADES QUE PODEM SER MEDIDAS EXPERIMENTALMENTE E INDICAM A OCORRÊNCIA DE UMA DETERMINADA FUNÇÃO NORMAL OU PATOLÓGICA DE UM ORGANISMO OU UMA RESPOSTA A UM AGENTE STRESSOR.



## INTRODUÇÃO AOS TESTES DE ECOTOXICOLOGIA

### TESTES DE TOXICIDADE AGUDA

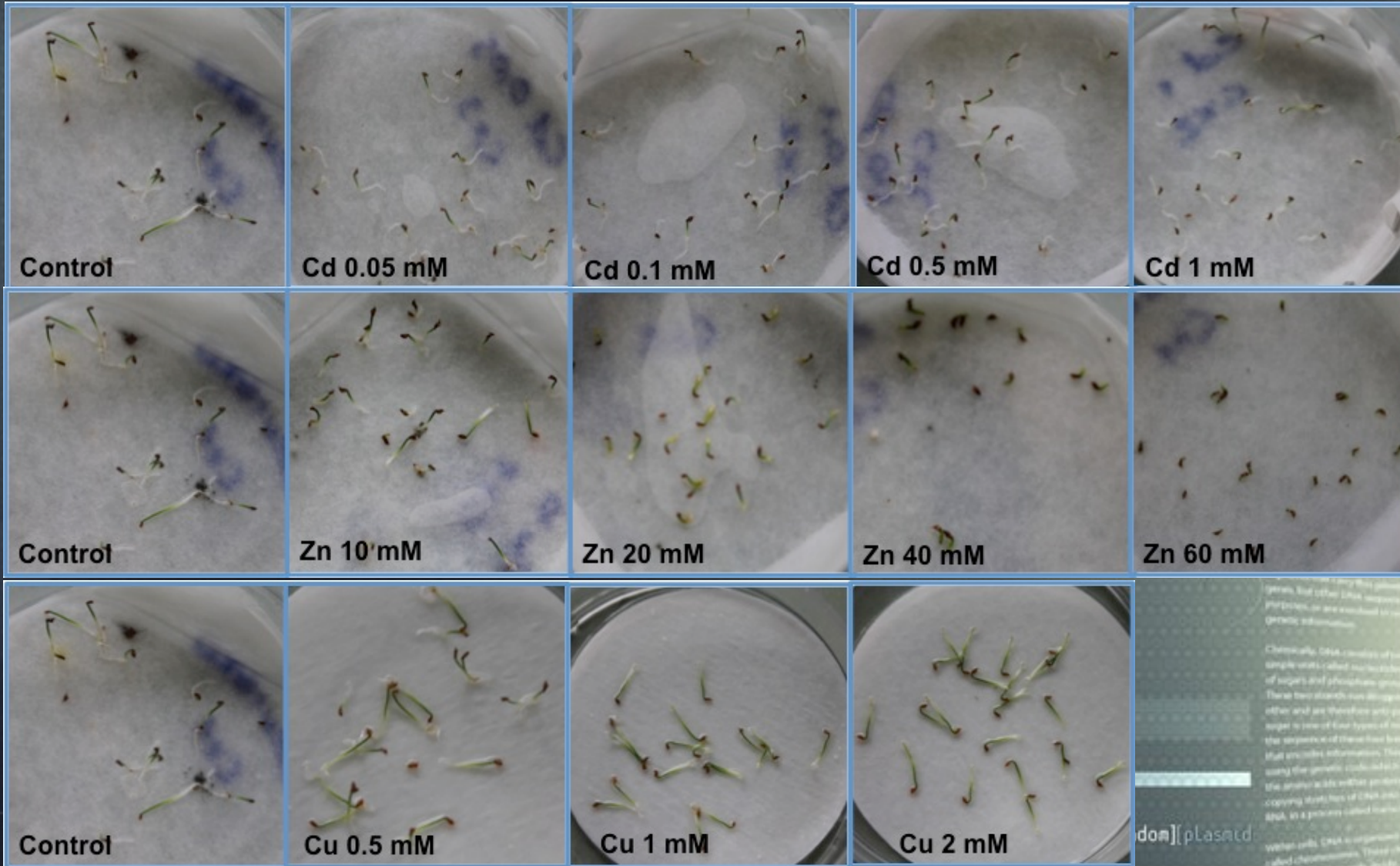
ACUTE TOXICITY DESCRIBES THE ADVERSE EFFECTS OF A SUBSTANCE THAT RESULT EITHER FROM A SINGLE EXPOSURE OR FROM MULTIPLE EXPOSURES IN A SHORT PERIOD OF TIME. TO BE DESCRIBED AS ACUTE TOXICITY, THE ADVERSE EFFECTS SHOULD OCCUR WITHIN 14 DAYS OF THE ADMINISTRATION OF THE SUBSTANCE.

### TESTES DE TOXICIDADE CRÓNICA

CHRONIC TOXICITY, THE DEVELOPMENT OF ADVERSE EFFECTS AS A RESULT OF LONG TERM EXPOSURE TO A CONTAMINANT OR OTHER STRESSOR, IS AN IMPORTANT ASPECT OF AQUATIC TOXICOLOGY. ADVERSE EFFECTS ASSOCIATED WITH CHRONIC TOXICITY CAN BE DIRECTLY LETHAL BUT ARE MORE COMMONLY SUBLETHAL, INCLUDING CHANGES IN GROWTH, REPRODUCTION, OR BEHAVIOR. CHRONIC TOXICITY IS IN CONTRAST TO ACUTE TOXICITY, WHICH OCCURS OVER A SHORTER PERIOD OF TIME TO HIGHER CONCENTRATIONS. VARIOUS TOXICITY TESTS CAN BE PERFORMED TO ASSESS THE CHRONIC TOXICITY OF DIFFERENT CONTAMINANTS, AND USUALLY LAST AT LEAST 10% OF AN ORGANISM'S LIFESPAN.

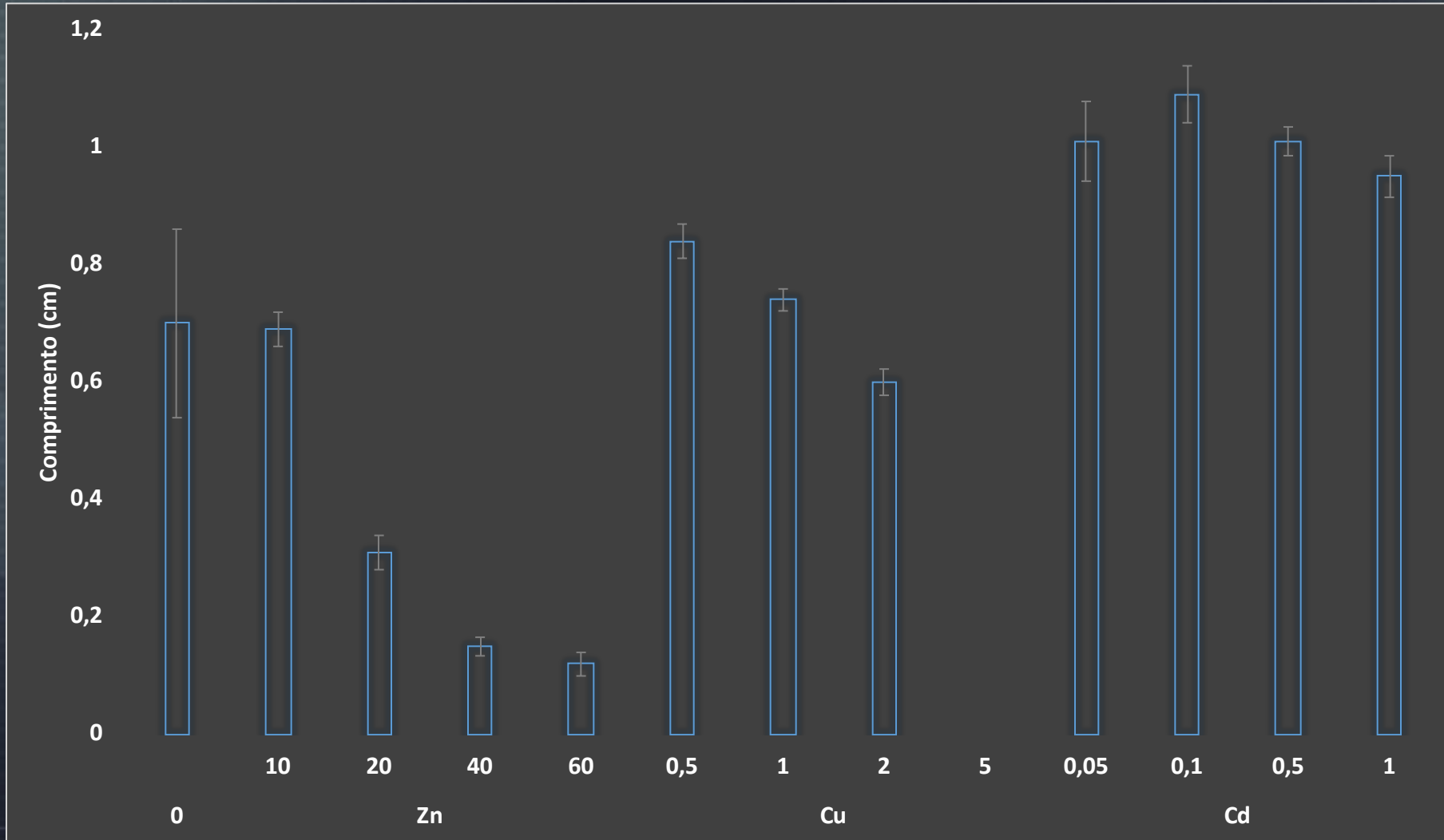


EXEMPLOS



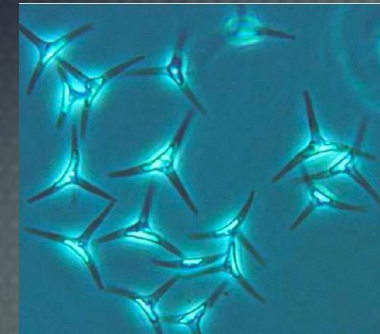
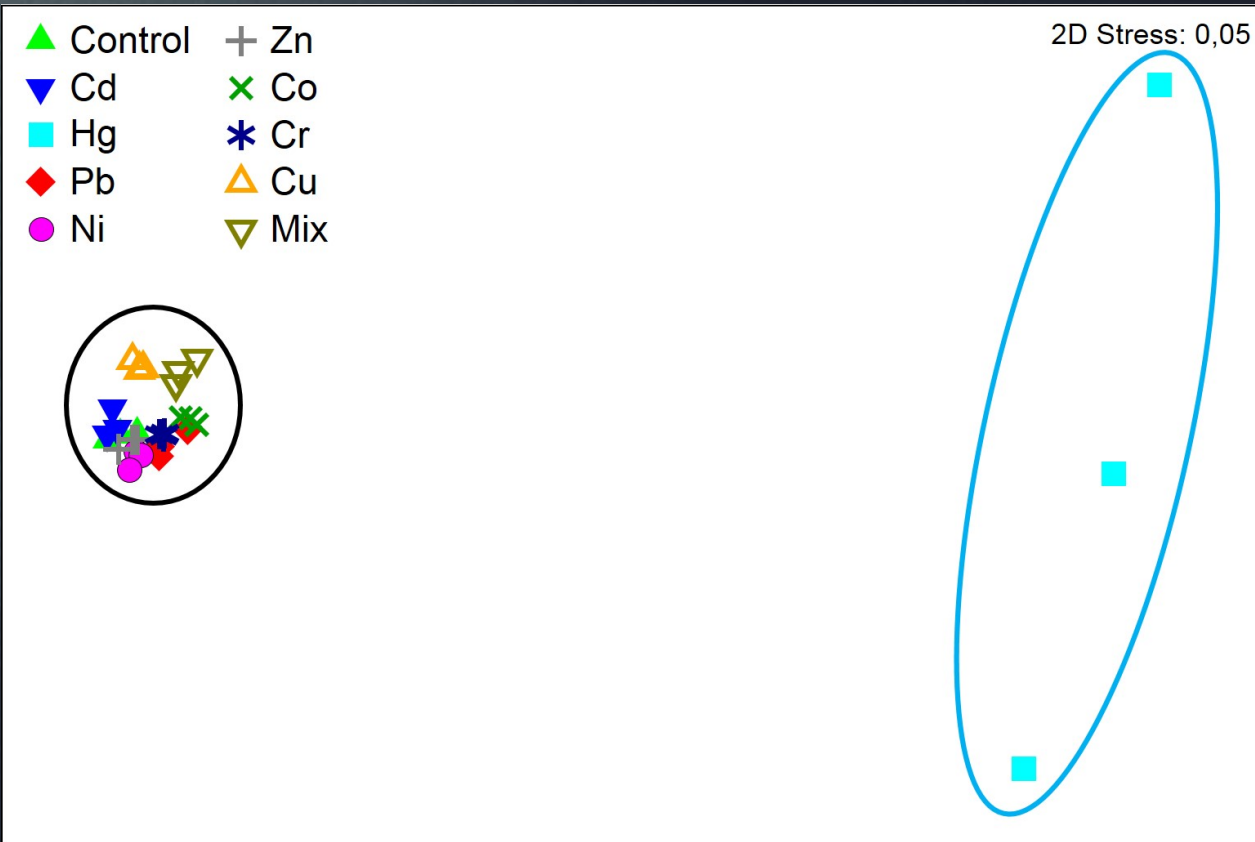


EXEMPLOS





EXEMPLOS



*Phaeodactylum tricornutum*

random][pLasncd

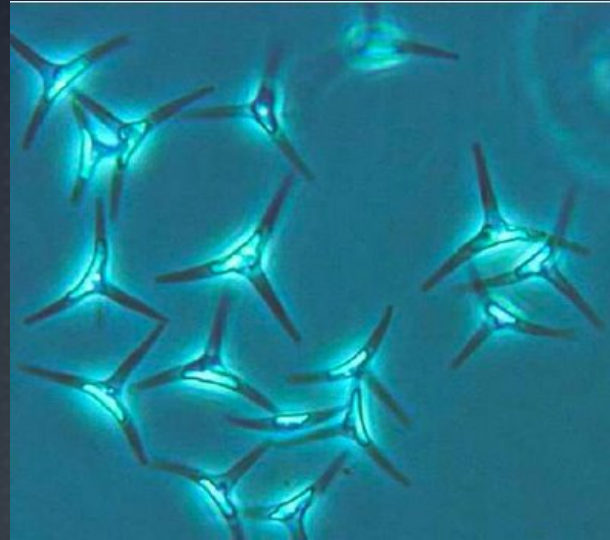
Chemically, DNA consists of long, unbranched, double-stranded molecules called nucleotides. The backbone of DNA is composed of alternating phosphate groups and deoxyribose sugars. These two strands run antiparallel to each other and are therefore anti-parallel. The sequence of these two strands is complementary. This information is used to synthesize proteins using the genetic code. The process of copying DNA into the messenger RNA is called transcription.

When cells, DNA is organized into long, thin, thread-like structures called chromosomes. These chromosomes are duplicated before cells divide. In a process called cell replication, eukaryotic organisms undergo meiosis, which produces gametes. In prokaryotes, the DNA is organized into a single circular chromosome. The DNA is organized into a single circular chromosome. The DNA is organized into a single circular chromosome.





*Phaeodactylum tricornutum*



*Phaeodactylum tricornutum*  
Diatomácea modelo da Ecotoxicologia

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Chemically, DNA consists of long, single strands of simple units called nucleotides. Each nucleotide is made of sugar and phosphate groups. The sugar and phosphate groups are attached to each other and are therefore not free. Attached to each sugar is one of four types of bases, called nucleobases. The sequence of these bases in the DNA molecule that encodes information. This information is used using the genetic code to build proteins. The process of copying a strand of DNA into the strand of RNA is called transcription.

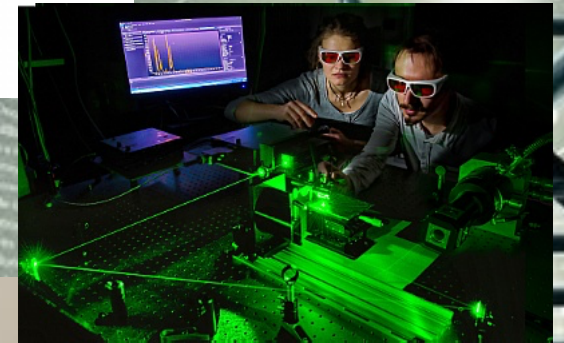
In eukaryotic cells, DNA is organized into very dense structures called chromosomes. These chromosomes are duplicated before cell division in a process called DNA replication. Eukaryotic organisms contain their DNA inside the cell nucleus and some of their DNA is organized into mitochondria or chloroplasts. In prokaryotes, the DNA is organized into a single circular chromosome. The DNA is organized into a single circular chromosome. The DNA is organized into a single circular chromosome.





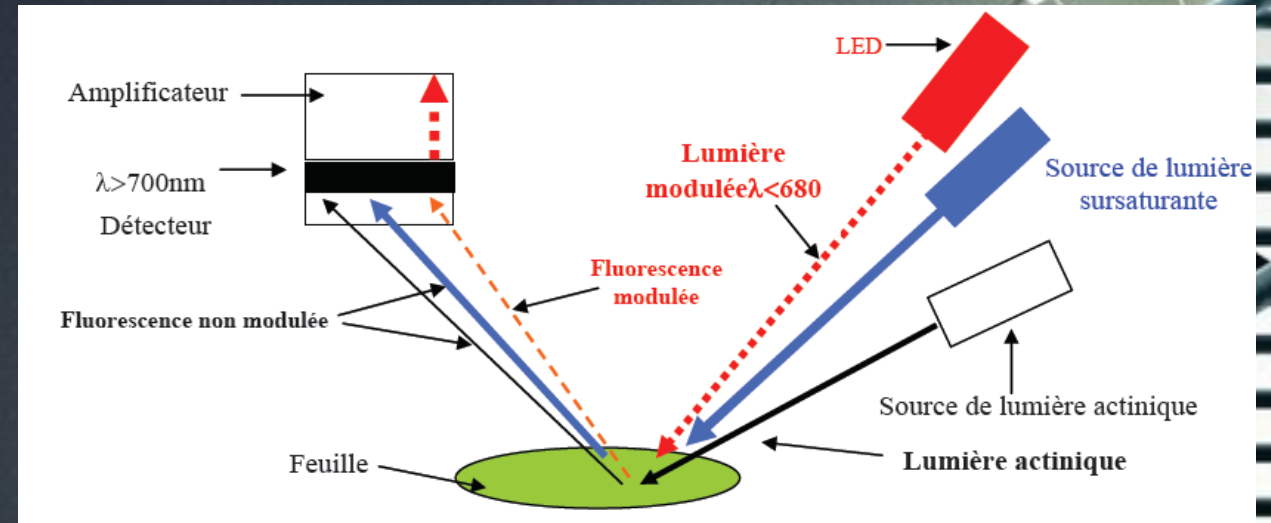
## TÉCNICAS ÓPTICAS

- **Aplicação de técnicas ópticas para detecção não invasiva de sinais de toxicidade**
  - a) Fluorimetria de pulso modulado (PAM)
  - b) Fluorimetria de Imagem (Imaging-PAM)
  - c) Fluorescência Induzida por Laser (LIF)
  - d) Reflectância por Espectrorradiometria



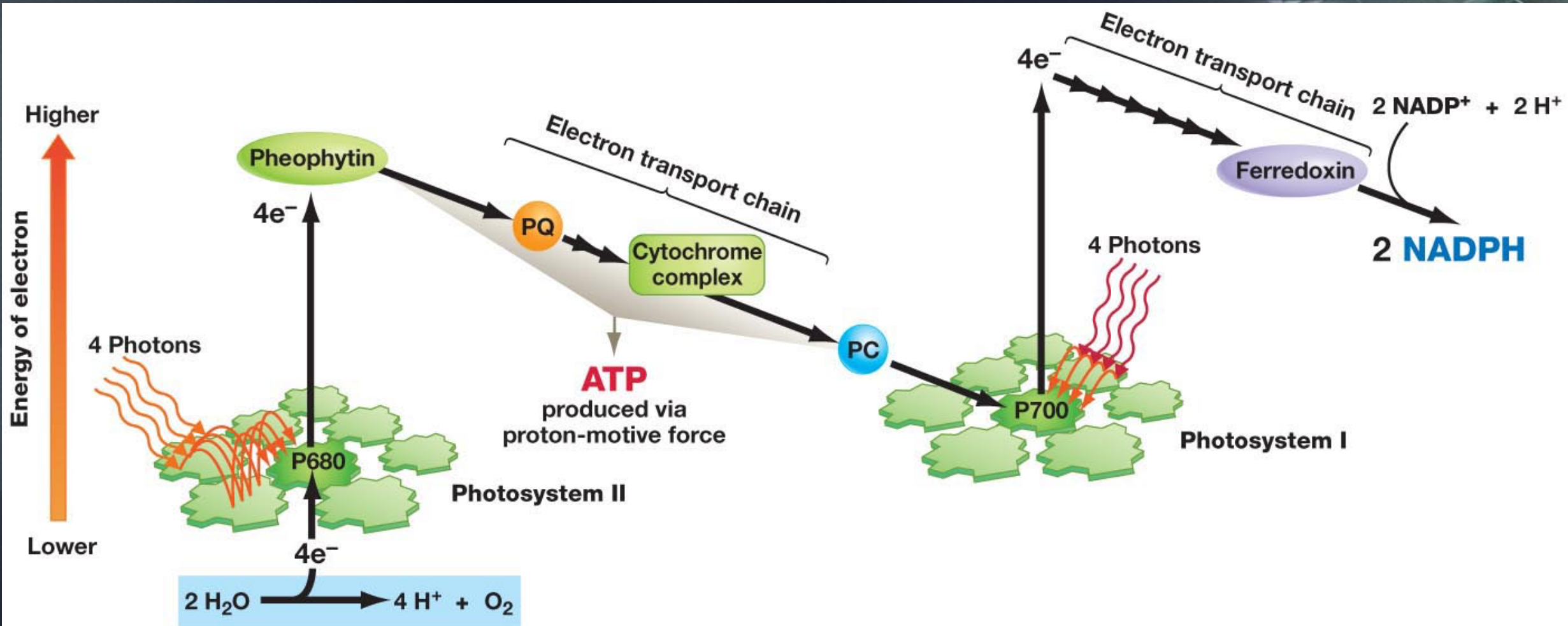


PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY



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PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY



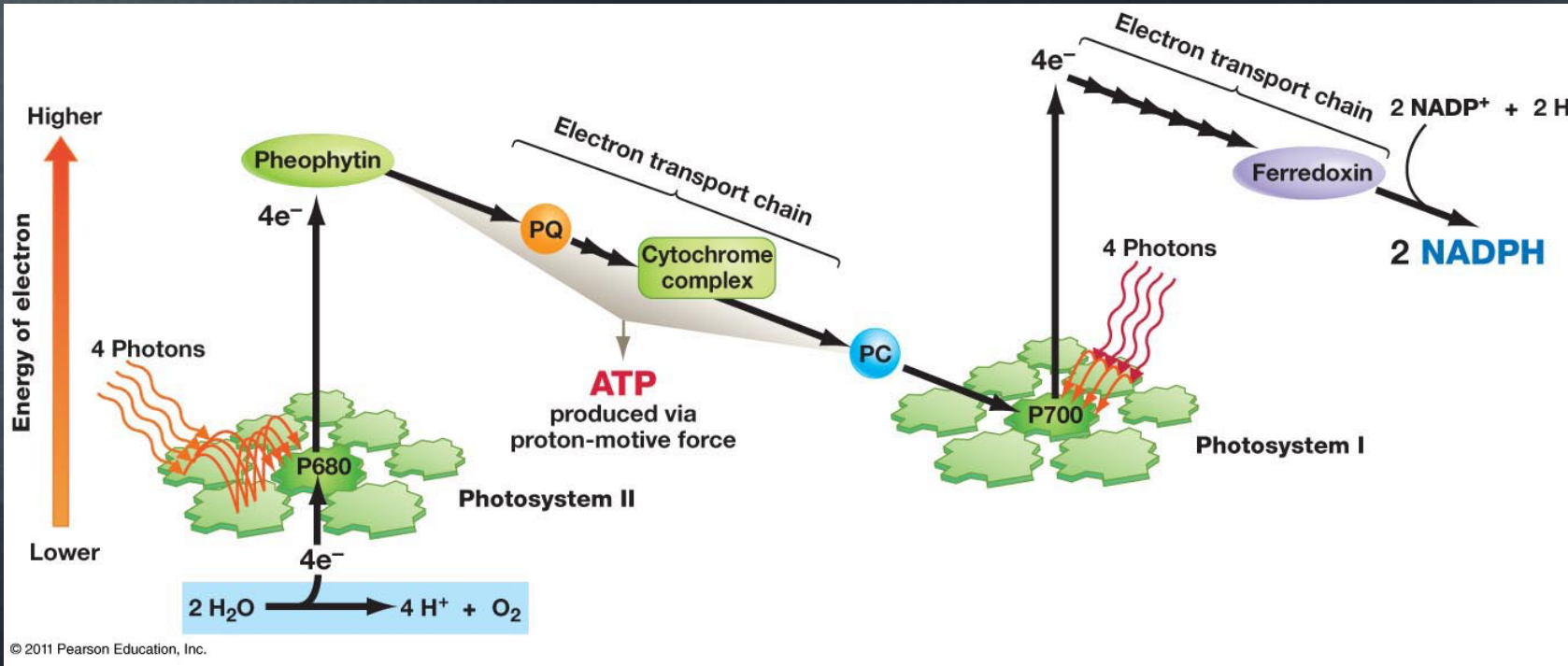
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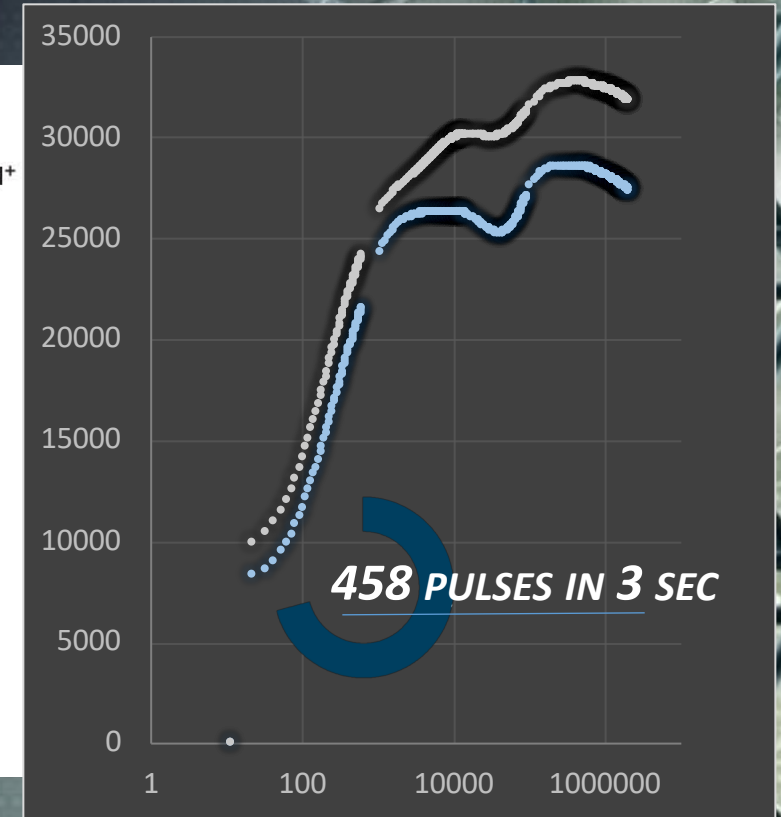




PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY



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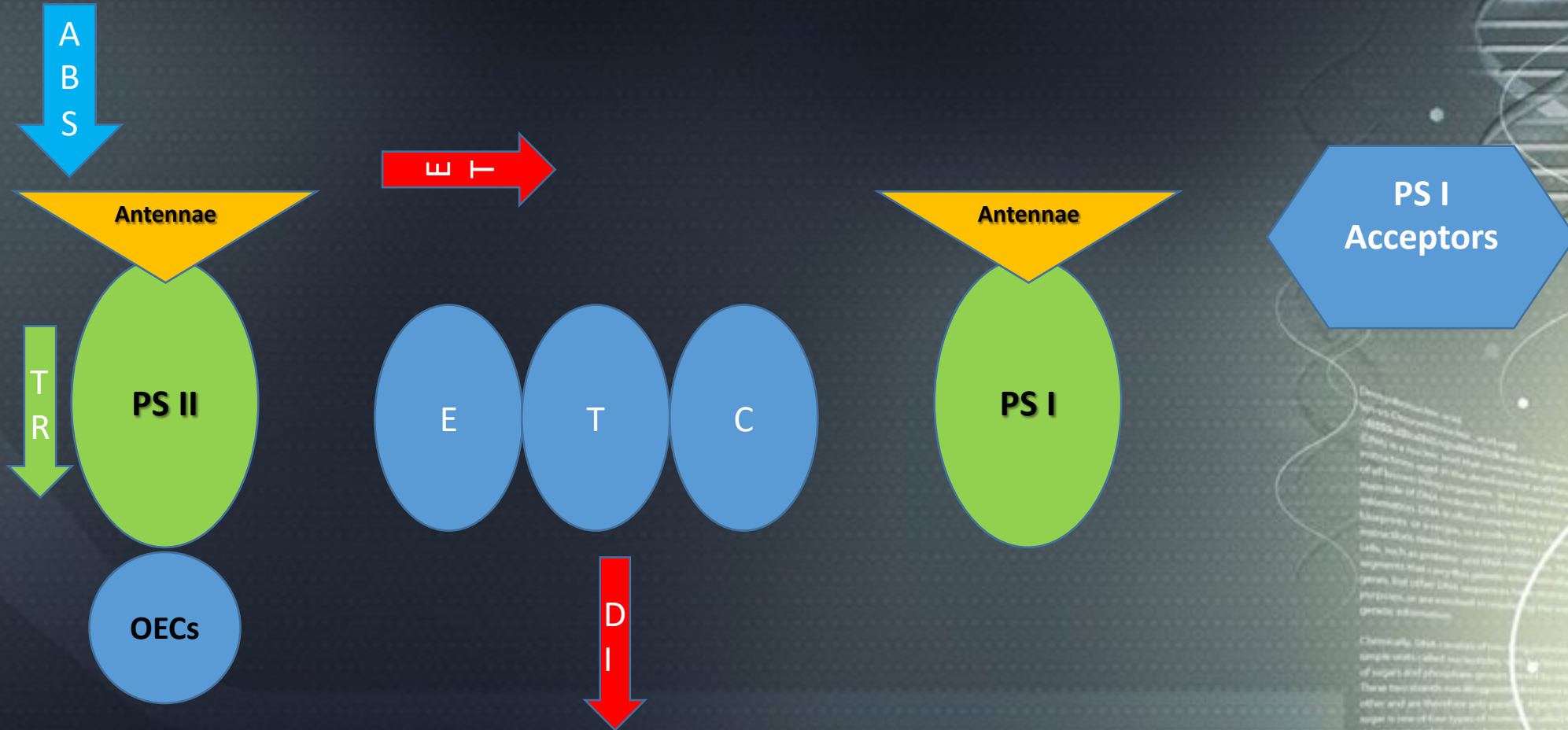


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the sequence of these base pairs...  
 that encodes information. This information is used  
 using the genetic code to build a specific sequence of  
 the protein as its amino sequence. The process of  
 copying this flow of DNA into the messenger RNA  
 step is a process called transcription.

Within cells, DNA is organized into very condensed  
 called chromosomes. These chromosomes are  
 duplicated before cells divide. In a process called  
 replication, eukaryotic organisms undergo several  
 rounds, and produce three most of their cells with the  
 cell nucleus and some of their DNA organization, such  
 mitochondria or chloroplasts. [1] In contrast,

PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY



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Chemically, DNA consists of long, unbranched chains of nucleotides called nucleic acids. Each nucleotide is composed of a phosphate group, a five-carbon sugar, and a nitrogenous base. The phosphate group is attached to the sugar, and the sugar is attached to the nitrogenous base. The phosphate group is attached to the sugar, and the sugar is attached to the nitrogenous base. The phosphate group is attached to the sugar, and the sugar is attached to the nitrogenous base.





# PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY

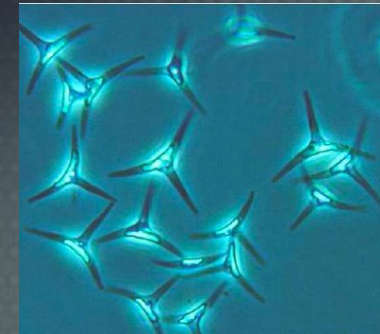
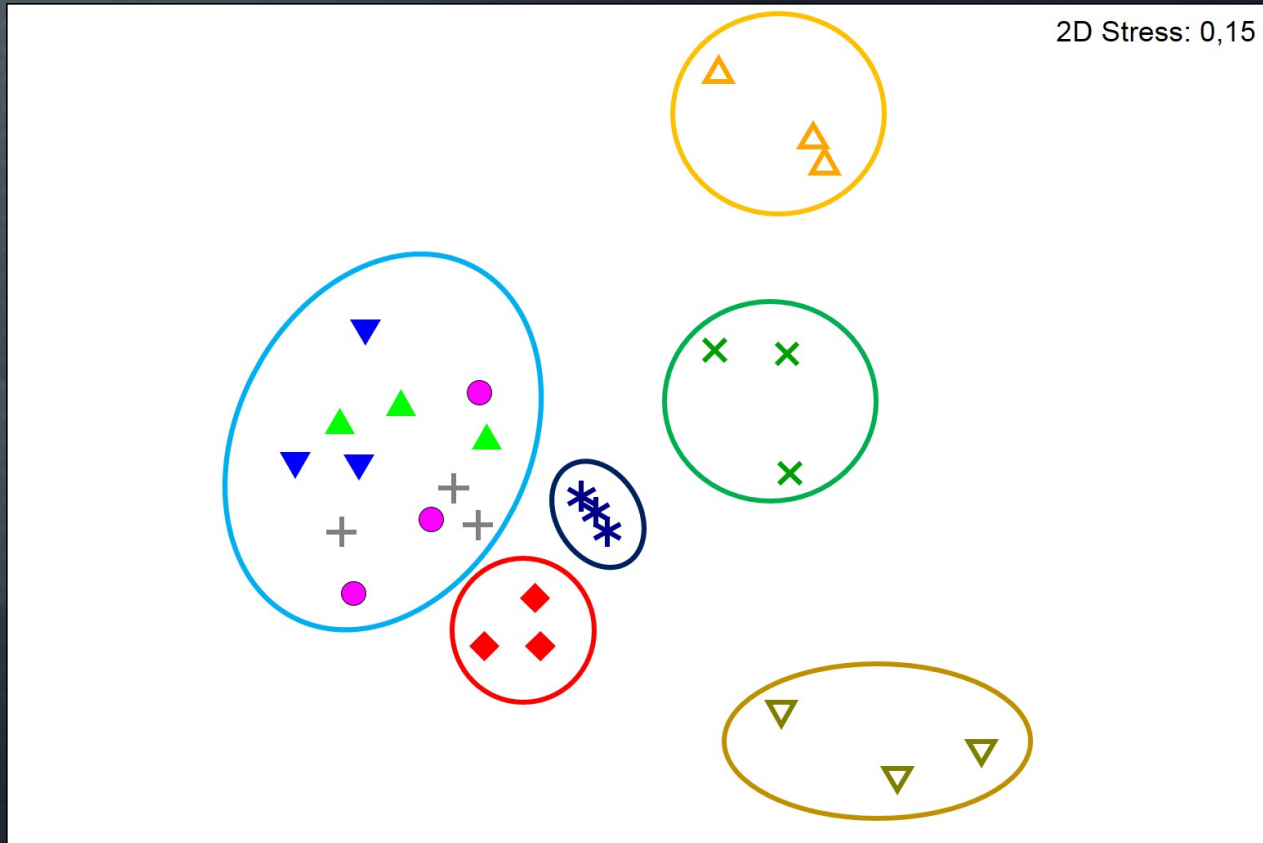


Chemically, DNA consists of two complementary strands of simple units called nucleotides. Each nucleotide is composed of a phosphate group, a five-carbon sugar, and a nitrogenous base. The two strands are held together by hydrogen bonds between the nitrogenous bases. The sequence of these bases determines the genetic information. This information is used to synthesize proteins, which are the building blocks of life.

When cells, DNA is organized into long molecules called chromosomes. These chromosomes are duplicated before cells divide. In a process called cell replication, eukaryotic organisms produce pairs of homologous chromosomes. Each pair consists of one chromosome from each parent. The chromosomes are then passed on to the next generation.

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EXEMPLOS

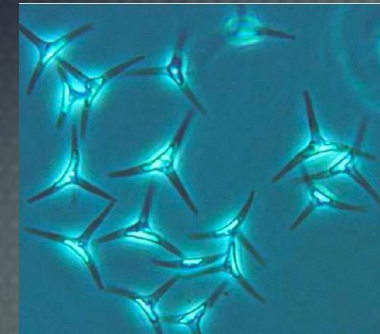
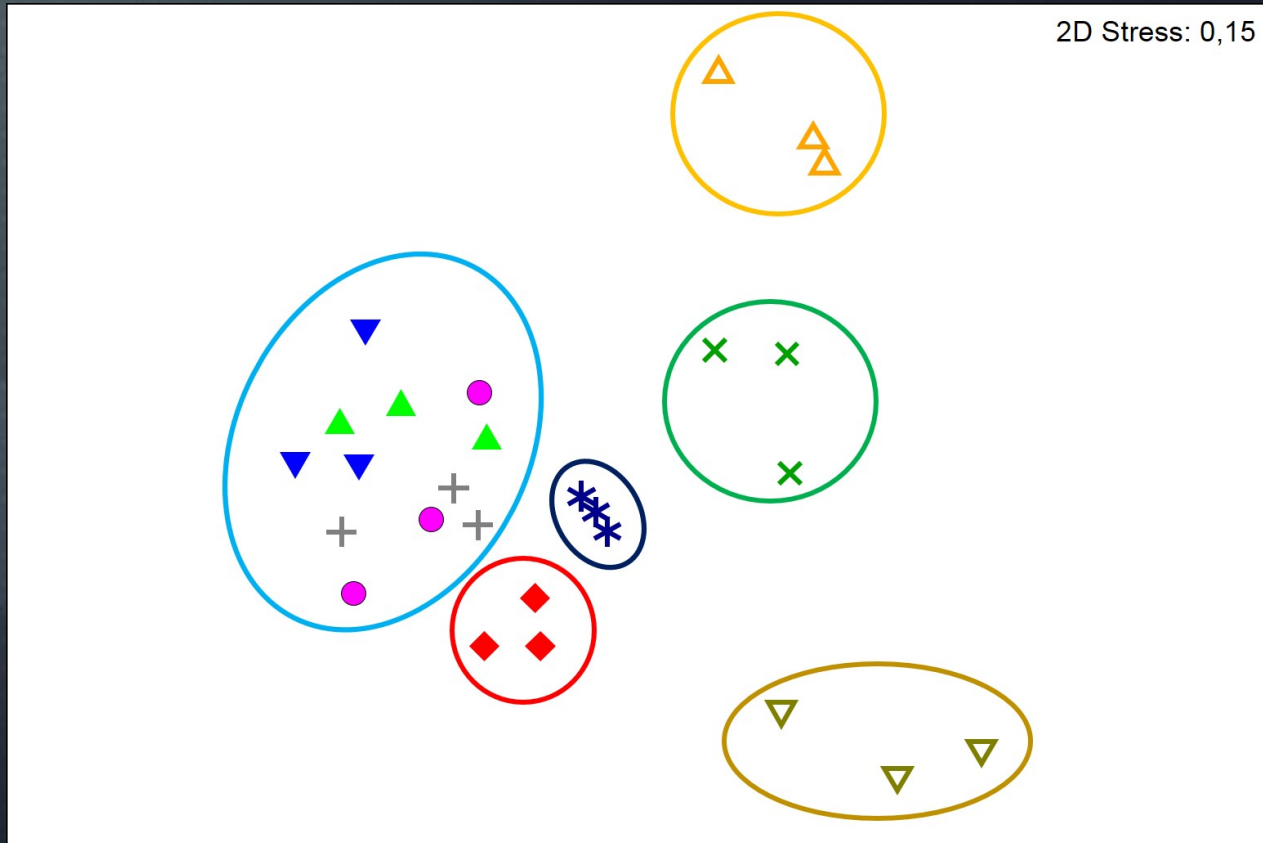


*Phaeodactylum tricornutum*

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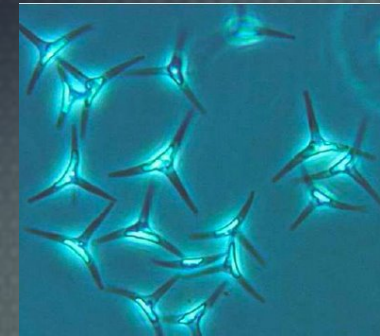
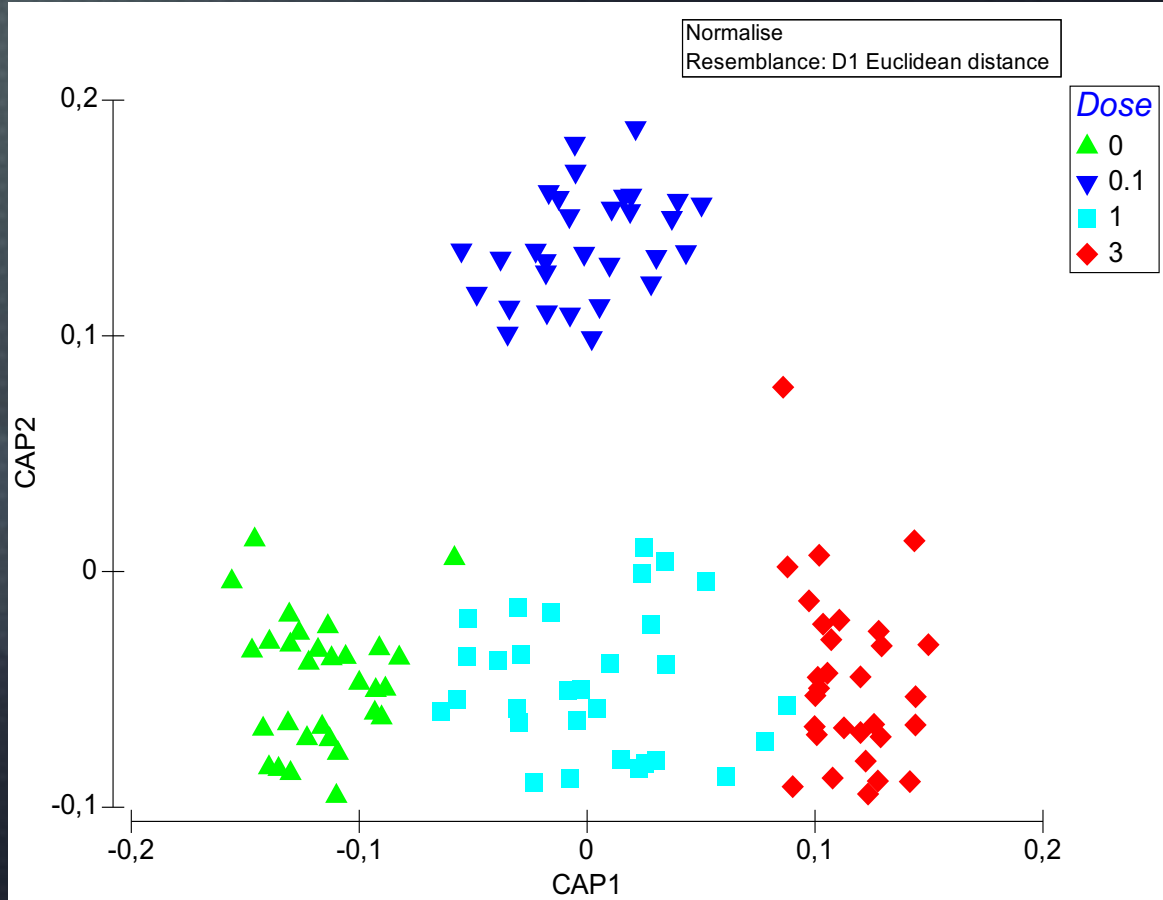
EXEMPLOS



*Phaeodactylum tricornutum*

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EXEMPLOS - SDS



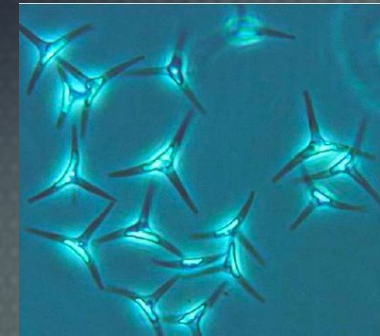
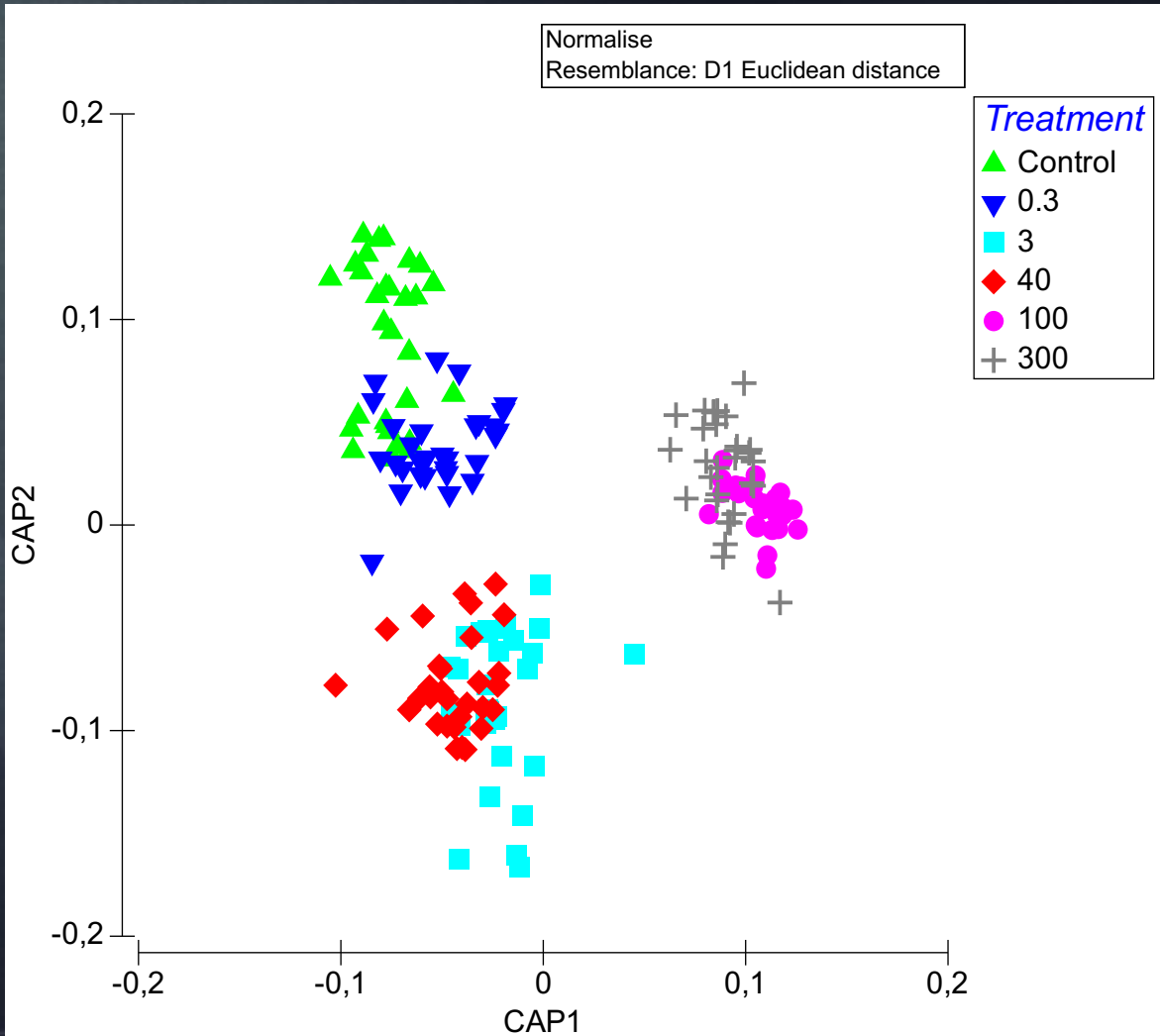
*Phaeodactylum tricornutum*

random][pLasatd

Chemically, DNA consists of two complementary strands of simple units called nucleotides. Each nucleotide consists of a phosphate group, a five-carbon sugar, and a nitrogenous base. These two strands run diagonally opposite to each other and are therefore anti-parallel. Attached to each sugar is one of four types of nitrogenous bases, in the sequence of these has been called the genetic code. This information is used to synthesize proteins using the genetic code. In prokaryotes, the DNA is organized into a single circular chromosome. The DNA is also organized into smaller units called plasmids. In eukaryotes, the DNA is organized into multiple chromosomes. Each chromosome is a long, thin, thread-like structure that contains many genes. The DNA is also organized into smaller units called plasmids. In eukaryotes, the DNA is organized into multiple chromosomes. Each chromosome is a long, thin, thread-like structure that contains many genes. The DNA is also organized into smaller units called plasmids.



EXEMPLOS - IBUPROFENO

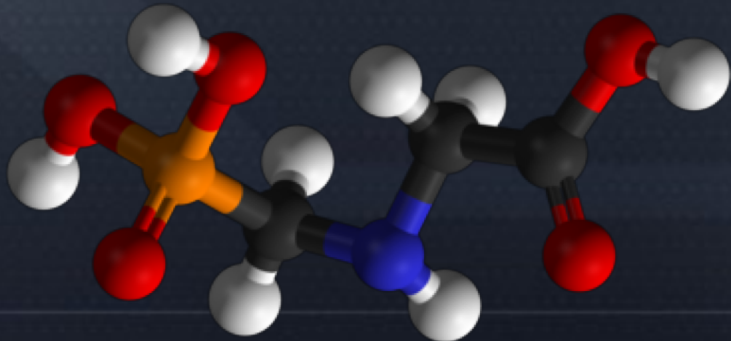
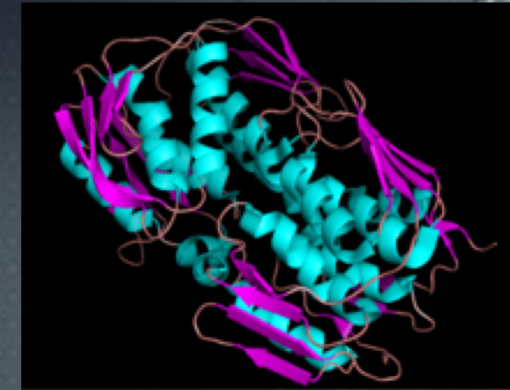


*Phaeodactylum tricornutum*

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## CASO PRÁTICO DE ESTUDO: GLIFOSATO

**GLYPHOSATE (IUPAC NAME: N-(PHOSPHONOMETHYL)GLYCINE) IS A BROAD-SPECTRUM SYSTEMIC HERBICIDE AND CROP DESICCANT. IT IS AN ORGANOPHOSPHORUS COMPOUND, SPECIFICALLY A PHOSPHONATE, WHICH ACTS BY INHIBITING THE PLANT ENZYME 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE. IT IS USED TO KILL WEEDS, ESPECIALLY ANNUAL BROADLEAF WEEDS AND GRASSES THAT COMPETE WITH CROPS. IT WAS DISCOVERED TO BE AN HERBICIDE BY MONSANTO CHEMIST JOHN E. FRANZ IN 1970. MONSANTO BROUGHT IT TO MARKET FOR AGRICULTURAL USE IN 1974 UNDER THE TRADE NAME ROUNDUP. MONSANTO'S LAST COMMERCIALY RELEVANT UNITED STATES PATENT EXPIRED IN 2000.**



**EPSP SYNTHASE IS THE BIOLOGICAL TARGET FOR THE HERBICIDE GLYPHOSATE. GLYPHOSATE IS A COMPETITIVE INHIBITOR OF PEP, ACTING AS A TRANSITION STATE ANALOG THAT BINDS MORE TIGHTLY TO THE EPSPS-S3P COMPLEX THAN PEP AND INHIBITS THE SHIKIMATE PATHWAY. THIS BINDING LEADS TO INHIBITION OF THE ENZYME'S CATALYSIS AND SHUTS DOWN THE PATHWAY. EVENTUALLY THIS RESULTS IN ORGANISM DEATH FROM LACK OF AROMATIC AMINO ACIDS THE ORGANISM REQUIRES TO SURVIVE.**



## DELINEAMENTO EXPERIMENTAL



Control



Low GLYPH



Medium GLYPH



High GLYPH

**EFEITOS NO CRESCIMENTO (EC50, IC50)**

**BIOMARCADORES BIOFÍSICOS**

**FLUORESCENCIA DE PULSO MODULADO DA CLOROFILA A**

**BIOMARCADORES BIOQUÍMICOS**

**PEROXIDAÇÃO LIPÍDICA (TBARS)**

**PREFIL PIGMENTAR**

**SUPERÓXIDO DISMUTASE**

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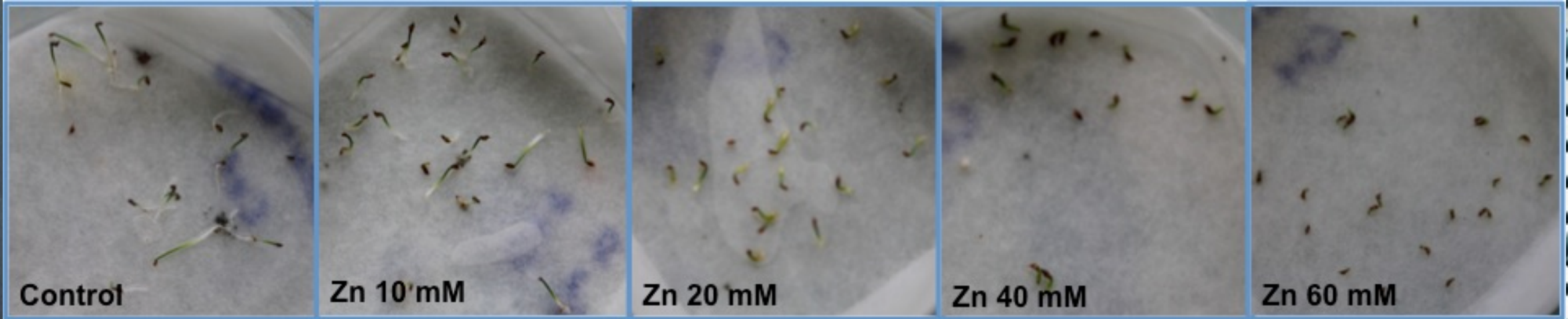
## INHIBITORY CONCENTRATION (IC50)

**THE HALF MAXIMAL INHIBITORY CONCENTRATION (IC50) VALUE DETERMINATION: THE HALF MAXIMAL INHIBITORY CONCENTRATION IS A MEASURE OF THE EFFECTIVENESS OF A COMPOUND IN INHIBITING BIOCHEMICAL PROCESSES AND BIOLOGICAL FUNCTIONS.**

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INHIBITORY CONCENTRATION (IC50)



...nucleotides, or a string, is a code, since it contains the instructions needed to construct other molecules of cells, such as proteins, and that contains the instructions that carry this genetic information. The DNA genes that offer this information have different purposes, or are involved in making the exact copy of genetic information.

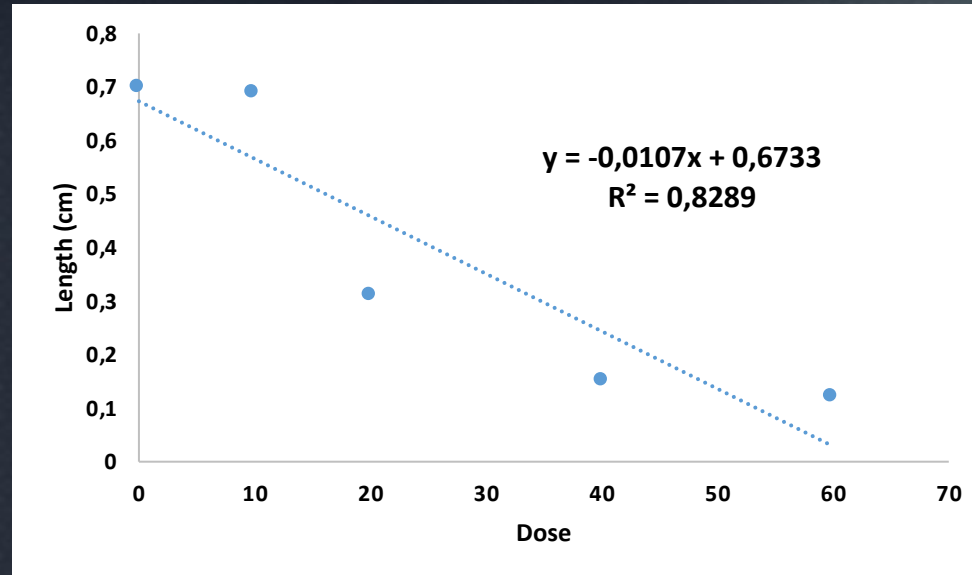
Chemically, DNA consists of two long polymers of simple units called nucleotides, with a backbone of sugars and phosphate groups joined by ester bonds. These two strands run antiparallel to each other and are therefore anti-parallel. Attached to each sugar is one of four types of nitrogenous bases, which in the sequence of these bases is the code for the amino acid that provides information. This information is read using the genetic code, which is the relationship of the genetic code with proteins. The process of copying these bits of DNA into the internal code is called transcription.

When cells, DNA is organized very tightly into structures called chromosomes. These chromosomes are duplicated before cells divide, in a process called cell replication. Eukaryotic organisms possess linear chromosomes, and prokaryotes have most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. [1] In contrast, prokaryotes do not have a nucleus and their DNA is organized into a single circular chromosome.

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INHIBITORY CONCENTRATION (IC50)

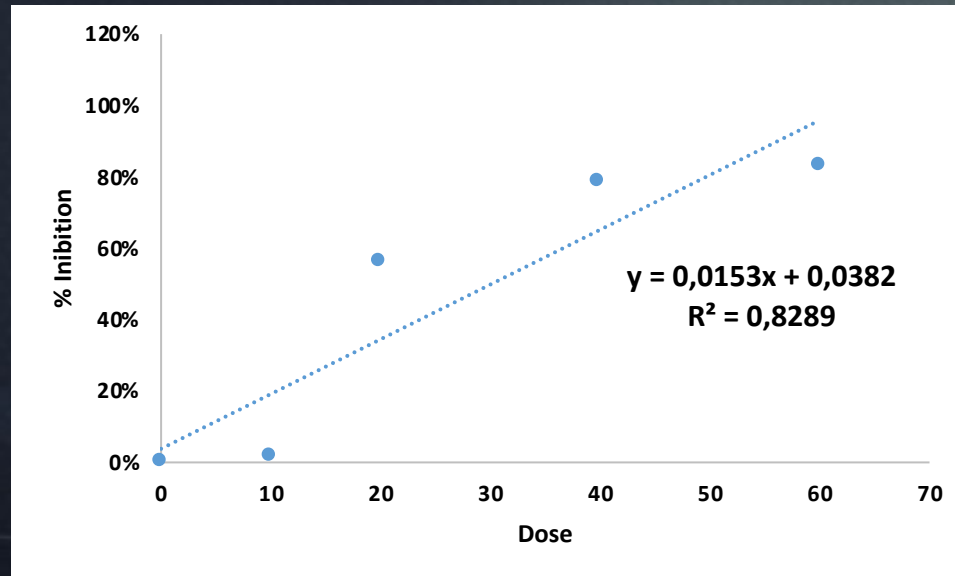
Dose	Length (cm)
0	0,7
10	0,69
20	0,31
40	0,15
60	0,12





## INHIBITORY CONCENTRATION (IC50)

Dose	Length (cm)	% Inibition
0	0,7	0%
10	0,69	1%
20	0,31	56%
40	0,15	79%
60	0,12	83%

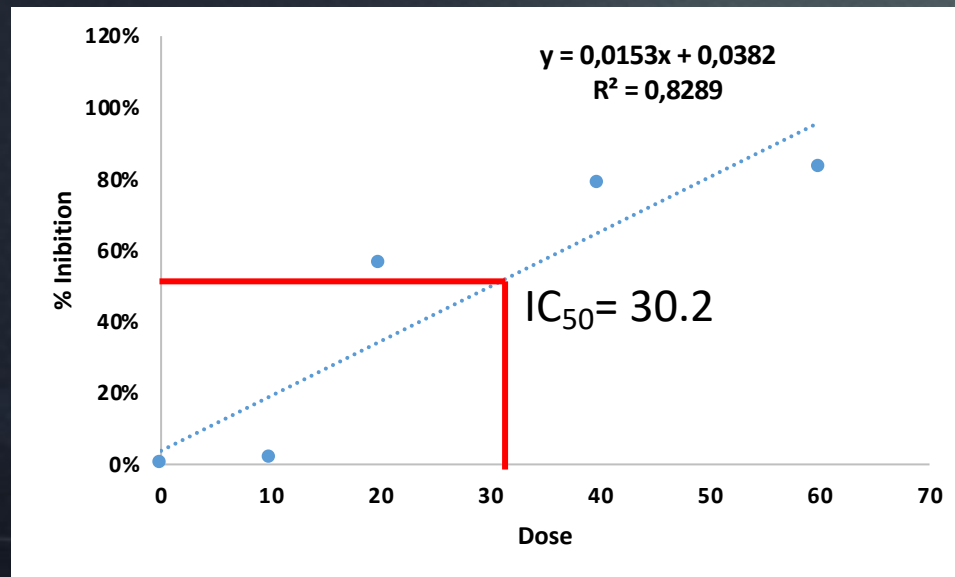


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## INHIBITORY CONCENTRATION (IC50)

Dose	Length (cm)	% Inhibition
0	0,7	0%
10	0,69	1%
20	0,31	56%
40	0,15	79%
60	0,12	83%



random][pLasatd





## HALF MAXIMAL EFFECTIVE CONCENTRATION (EC50)

**HALF MAXIMAL EFFECTIVE CONCENTRATION (EC50) REFERS TO THE CONCENTRATION OF A DRUG, ANTIBODY OR TOXICANT WHICH INDUCES A RESPONSE HALFWAY BETWEEN THE BASELINE AND MAXIMUM AFTER A SPECIFIED EXPOSURE TIME.**

random][pLasatd

*TRABALHOS EM CURSO E PERSPETIVAS DE MESTRADO*

*CLIMATE CHANGE IMPACTS IN MARINE ORGANISMS*

*NANOTOXICOLOGY*

*PHARMACO-TOXICOLOGY*

*MULTI-GENERATIONAL EVOLUTION*

random][pLasncd

