Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. IV, 2015
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

THE ASSESSMENT OF THE ECO-TOXICITY IN THE WATERS OF SOMESUL MIC RIVER BY USING SCENEDESMUS OPOLIENSIS ALGAE CULTURES

Sebastian PLUGARU, Mihaela ORBAN, Tiberiu RUSU

Technical University of Cluj Napoca, Romania, Faculty of Materials and Environmental Engineering, Bd. Muncii 103-105, Cod 400641 Cluj-Napoca, Romania,
Phone: +40264.401.622, Fax: +0264.415.054

Corresponding author email: sebastian.plugaru@yahoo.com

Abstract

The algae population is the key factor in determining the biological productivity of the river basins. The fish production depends a great deal on the quantity and quality of the phytoplankton, as well as on the benthic macrophyte, shelters and ideal spawning places for numerous species of fish.
The ecotoxicological analyses carried out on algae and certain small sized spineless species have a very high precision. The algae and the spineless species make up biological indicators of polluted waters.
The bioindication can be regarded as an anthropogenically induced biochemical and molecular response displayed by the modification of the physiological parameters and the effects are seen at one or more levels of the biological system.

Key words*: green algae, ecotoxicology,bioindicators.*

INTRODUCTION

The algae constitute in the immensity of the plant kingdom a special group with numerous species, most of which microscopic and unicellular, most of the time invisible for the eye of the passer-by and so they often remain unknown and ignored. It is only on the summer days we spend at the beach that they surface from within the sea carried by the waves as if to be seen, allowing us to see them on the sand where they find their demise (Dragos, 1997).
At the same time, the ecotoxicology analyses carried out on algae and some very small species of invertebrates have a very high accuracy. The algae and the species of invertebrates represent biological indicators of polluted waters.
The level of water pollution has increased impermissibly over the last decades, especially in those parts of the world where the population and the industry developed intensively and rapidly without any measures concerning the protection of the water quality being taken. It is important to point out that the areas from which the contaminated waters were sampled, the municipalities of Gherla and Dej, Somesul Mic Basin respectively, are known as excessively industrialized areas with very polluting industries, especially around Gherla and Dej, which spill their polluting substances in Somes and of course its tributaries.
The need for a larger and larger quantity of water and mineral substances for the algae cultures has lead in the last few decades to thorough research on the possibility of using natural fresh water or residual water for algae cultivation.
Experiments with the culture of microscopic algae have begun to spread rapidly in all highly developed countries interested in preserving the biodiversity. Microscopic algae multiply and grow very fast and that allows for a larger assimilation surface to be obtained in a matter of a few days (Momeu, 2008).

MATERIALS AND METHODS

The present paper aims at identifying and demonstrating the action of the risk factors on freshwater algae (*Scenedesmus opliensis)* tested in different concentrations of the contaminated waters in Somesului Basin by observing their physiological growth inhibition response depending on the action of the risk factors and nutrients.

The algae species originate from pure cultures and are inoculated in a well-defined environment obtained by combining different quantities of the utilized growing environment with various concentrations of the contaminated water. The used recipients are then incubated in constant temperature and luminosity with the purpose of determining the cellular density in each recipient at a certain time span.
The purpose is to observe the way in which every concentration of contaminated water affects the exponential growth of the algae within 72 hours in comparison with the control samples. The inhibition is measured as a decrease in the growing rate as compared to the witness samples.
The tested organism used is the Scenedesmus Opoliensis freshwater planktonic algae.
Classification: phylum Chlorophyta, class Chlorophyceae, order Chlorococcales, family Scenedesmaceae (Peterfi, 1979; Parvu, 2003).
Scenedesmus species are associated in coenobia which normally contain 2 to 5 individuals. They can have two cytoplasmic extensions at their ends. Scenedesmus opoliensis belongs to a four individual coenobium having two cytoplasmic extensions at each end (Ionescu, 1972).

Figure 1. Scenedesmus opoliensis

The growing environment used for the incubation of the algae is a standard environment containing a wide range of nutrients to ensure the natural growing conditions of the organism.
I prepared 4 stock solutions for the preparation of the growing environment according to the recommendations of the European Standard ISO 8692 concerning water quality.

The following table displays the macronutrients and micronutrients used in the preparation of the stock solutions (Table 1).

Table 1. Micro and macronutrients

Stock Solution	Nutrients	The mass concen. in the stock solution	Final mass concen. in the test solution
Stock Solution 1: macronutrients	NH_4Cl	1.5 g/l	15 mg/l
	$MgCl_2*6H_2O$	1.2 g/l	12 mg/l
	$MgCl_2*2H_2O$	1.8 g/l	18 mg/l
	$MgSO_4*7H_2O$	1.5 g/l	15 mg/l
	KH_2PO_4	0.16 g/l	1.6 mg/l
Stock Solution 2: Fe-EDTA	$FeCl_3*6H_2O$	64 mg/l	64 µg/l
	$Na_2EDTA*2H_2O$	100 mg/l	100 µg/l
Stock Solution 3: trace elements	H_3BO_3	185 mg/l	185 µg/l
	$MnCl_2*2H_2O$	415 mg/l	415 µg/l
	$ZnCl_2$	3 mg/l	3 µg/l
	$CoCl2*6H_2O$	1.5 mg/l	1.5 µg/l
	$CuCl_2*2H_2O$	0.01 mg/l	0.01 µg/l
	$Na_2MoO_4*2H_2O$	7 mg/l	7 µg/l
Stock Solution 4	Na HCO_3	50 g/l	50 mg/l

European standard ISO 8696-water quality

All recipients used in the test are made of glass because this material is well known for being inert.
Used laboratory equipment: modern incubator insuring a white fluorescent light, providing a constant and uniform illumination in accordance with the requirements of the test. The Thoma chamber used for calculating the cellular density has a 0.1 mm depth and the area of the smallest square is 0.05 mm. Erlenmeyer flasks which can store up to 250 ml of liquid, pH meter and a conductivity meter. Other instruments used: Berzelius beakers, graduated cylinder, adjustable mechanic pipettes with plastic or glass tips (1 ml, 5 and 10 ml), razor blade, disks and of course an optical microscope with 20x, 40x, 100x objective and 10x ocular, oven.
The preparation of the four stock solutions was followed by the preparation of the growing environment.
For 500 ml of growing environment 10 ml from the stock solution 1 and 1 ml from each the stock solutions 2, 3 and 4 were used.
After the preparation of the growing environment the concentrations of the contaminated water to which the algae would be exposed was chosen.

Two contaminated waters were used: one concentration of 50% from the tested volume and one from 100% in which the algae would be tested. According to these concentrations the reaction of the algae will be observed.
The test was carried out in four weeks during which two types of residual water from two different areas where toxic substances are said to exist were tested.
The preparation of the test samples was carried out by mixing the volumes set by the nutritive environment with the volumes of residual water necessary for each recipient of the volume indicated by the algae culture so that the cellular density in each recipient did not exceed 10^4cells/ml.
After the inoculation the pH in each recipient was calculated in order for it to be compared with the pH at the end of the test.
The recipients were incubated after the preparation of the solutions.
Each recipient was covered with aluminum foil in order to avoid the evaporation; a small hole was made in the foil with a pin so that the CO_2 can enter the recipient during the incubation.
In order for the algae to grow optimally a certain amount of CO_2 is needed. Atmospheric CO_2 can provide a concentration of CO_2 in an open test, but this is not possible in a closed space. That is why it is recommended that in closed systems a small hole should be made so that the concentration of CO_2 is maintained constant.
The recipients were incubated at the temperature of 23°C under continuous white light and agitation. The intensity of the light ranged between 60 µmol/(m^2*s) and 100 µmol/(m^2*s).
For the inoculation, a necessary quantity for obtaining an initial cellular density of 10^4 cells/ml was calculated and used and it proved to be the best for the growing of the cultures experimented on.
During the 72 hour test the daily density of the algae suspensions in each recipient was measured using the Thoma chamber and the microscope. The results then underwent the statistical analysis.
The residual water from Somesul Mic River originates from two different possibly polluted locations.

RESULTS AND DISCUSSIONS

Municipality of Gherla

The water collected in the municipality of Gherla has pH 7.20 and conductivity 512 µs/cm.
The pH had an increase from the beginning to the end of the test. The pH was measured both on the inoculation day and at the end of the test.

Table 2. Variations of the algae densities – Gherla

Days	Witness	Conc. 50%	Conc. 100%
Day Inoculation	10^4	10^4	10^4
Day 1-24h	$2.3*10^6$	$2.2*10^6$	$0.32*10^6$
Day 2-48h	$3*10^6$	$2.9*10^6$	$0.9*10^6$
Day 3-72h	$4*10^6$	$3*10^6$	$1.5*10^6$

There was a significant increase in the samples up to 3 and 4 cel/ml.
At the 100% concentration which contains only residual water from the Gherla region downstream the algae grew at a normal rate reaching 1.5 cel/ml. However, in comparison with the previous concentrations a decrease can be observed.

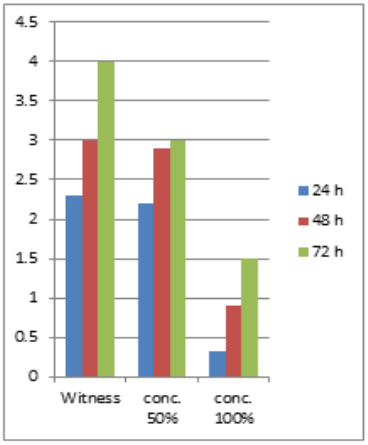

Figure 2. The variations of the algae densities – Gherla

Municipality of Dej

The water in this region is characterized by a high degree of pollution. After the measurements the results were the following: 6.66 pH and 942 µs/cm conductivity.

The pH value increased from the beginning to the end of the test and it was measured on the inoculation day as well as at the end of the test.

Table 3. Variations of the algae densities – Dej

Days	Witness	Conc. 50%	Conc. 100%
Day Inoculation	10^4	10^4	10^4
Day 1-24h	$2.42*10^6$	$0.2*10^6$	$0.3*10^6$
Day 2-48h	$3*10^6$	$0.7*10^6$	$0.9*10^6$
Day 3-72h	$3.6*10^6$	$1.7*10^6$	$1.3*10^6$

In samples of different concentrations (50% and 100%) the density increased. However, the increase was lower than in that in the previous region. The yellowish colour of the water and the specific smell are two notable characteristics of this water.

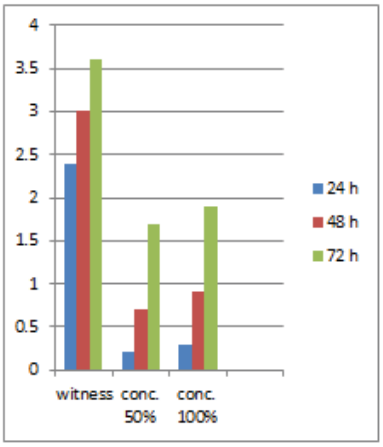

Figure 3. The variations of the algae densities – Dej

CONCLUSIONS

Bio-indicators can give accurate information regarding the quality of the water.

The degree of pollution of the water as well as the type of pollution can be analysed and identified by analysing the algae species from the perspective of the biological and physiological reactions.

The preservation of the water quality is something permanent and each member of the society has to bring his own contribution to it in a very conscious and responsible way.

The toxicity tests involve using certain algae cultures, carefully chosen, in which the tested organism is exposed to various concentrations of the toxic agent.

It would be ideal that the tested organism does not produce elements which could influence the structure of the metal ions, but it is well known that microscopic algae eliminate such chemical compounds altering the speciation of the metals, their accessibility and their reaction to algae.

REFERENCES

Dragos N., An introduction to the algae; The culture collection of algae at the Institute of Biological Research Cluj-Napoca, University Press, Cluj-Napoca.

Ionescu A., 1972, Lumea algelor, Editura Stiintifica, Bucuresti.

Momeu L., 2008, Algele ca bioindicatori, Editura Universitara "Lucian Blaga", Sibiu.

Parvu M., 2003, Botanica Sistematica I, Gloria, Cluj-Napoca.

Peterfi S., 1979, Tratat de algologie. Vol. 3: Euglenophyta, Chlorophyta, Xanthophyta, Editura Academiei Republicii Socialiste Romania, Bucuresti.