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# REDOX POTENTIAL TO MONITOR STRESS RESPONSE OF ALGAE TO HEAVY METAL POLLUTION

***Ecological indicators can be used to provide an early warning signal of environmental changes. Algae can be applied to biomonitoring marine and river water pollution due to the alteration of the two prevalent metabolic activities: breathing and photosynthesis. By measuring the redox potential, we evaluated the stress in algae system (genus Scenedesmus), due to the presence of heavy metals: lead, cadmium and chromium.***

## Introduction

The pollution of waters, marine and fluvial, is due to the incorrect, accidental or voluntary wastes dispersion. This entails a modification of the ecosystem, which can cause permanent or temporary damage, producing a problem of water scarcity for biodiversity [1] and a danger to human health [2].

The water pollution caused by the dispersion of heavy metals, found above all in industrial discharges due to the combustion processes, turns out to be one of the topics of greatest interest since it is increased with the industrial development of the territory influencing the health of the surrounding environment [3].

Heavy metals, whose specificity is high density (greater than 4.5 $g/cm^3$), particularly in our study chromium, lead, cadmium, are common toxicity agents, easily accumulated in nature (the last receptacles are represented by soil and sediment) and continuously more used due to their properties such as ductility, malleability, resistance to corrosion; very useful in various industries like these ones of automobiles, ceramics, paints and plastics. But they are considered potential hazardous substances by ATSDR (Agency for Toxic Substances and Disease Registry). One of their fundamental characteristics is that they are not degradable in non-toxic forms, they can at most be transformed into insoluble forms and therefore biologically unavailable, unless they are subsequently converted into more soluble substances.

The human organism does not metabolize heavy metals that are dangerous in their cationic form and above all they are very toxic when bound to short chains of carbon atoms. Their toxic function derives from the high affinity of metal cations for sulphur, since the sulfhydryl groups (-SH) normally present in the enzymes that control the rate of important metabolic reactions in the human body, easily bind to the cations of the heavy metals ingested or to molecules that contain these metals. The resulting metal-sulphur complex alters the whole enzyme which, since it cannot function normally, causes damage to health, sometimes causing death [4]. When the accumulation rate is greater than the detoxification one, toxic concentration levels are reached and a further phenomenon occurs, that of substitution: for example lead can substitute calcium in the bones (osteoporosis).

The consumption of water polluted by these metals is very dangerous for humans bringing to poisoning, both acute and chronic, related to following muscle pain, fatigue, headache, vomiting, coma. So, that using strict limits are established for all three by WHO (World Health Organization) **[5]**.

Conventional methods are applied to their determination such as absorption spectroscopy, ion chromatography, chemiluminescence, inductively coupled plasma mass spectroscopy. Today, in the scientific panorama, the methods constituted by a biological component are increasingly developed, allowing to monitor and report any pollution in progress **[6]** in an ecologically sustainable way.

The behaviour of microalgae of the genus Scenedesmus in the presence of pollutants **[7]** including heavy metals **[8]** has been studied.

This research intends to add a contribution to the water monitoring role of algae with the development of a bioindicator that allows to continuously monitor the health conditions of fresh water with low cost and quick detection **[9]**.

At the same time the paper wants to contribute to understand the response of algae to metal pollution in terms of redox potential related to their biological activities.

The monitoring mechanism is based on the two fundamental functions performed by microalgae for their survival: photosynthesis and breathing. These natural processes are negatively influenced by the presence of the heavy metal and their progress has been monitored through measures of redox potential.

The photosynthesis reaction involves the consumption of carbon dioxide and the production of oxygen:

$$CO_2 + H_2O \rightarrow \text{biomass} + O_2$$

while because of the breathing reaction oxygen is consumed:

$$\text{cells} + O_2 \rightarrow ATP + CO_2$$

The Nernst equation (1) used to calculate the redox potential explains why during the photosynthesis we have an increase of redox potential due to the increase of the concentration of oxygen, while we have the opposite case during the breathing, the redox potential decreases due to the decrease of the oxygen concentration:

$$E = E^0_{O_2/H_2O} + \frac{RT}{4F} \log \frac{Po_2 \cdot (H^+)^4}{a_{H_2O}} \qquad (1)$$

## Materials and methods

### *Cultivation of the algal species*

In 1 L glass cylindrical bottles (Fig. 1), the cultures of the microalgae of the genus Scenedesmus were carried out with mineral water Saguaro, specially chosen because it contained the main feeding species in amounts suitable for the proliferation and survival of the algae. The culture bottle is equipped with an aquarium aerator, Newair Aquarium Systems, which allows a homogeneous stirring of the entire solution. The bottle is placed on a magnetic stirrer activated when the microalgae begin to deposit on the bottom of the bottle.

OSRAM Ultra-Vitalux 300W sun lamps were used to illuminate the algal culture in the bottle.

When it was necessary to make a new algal culture in an empty bottle, 500 mL of algal solution from a previous culture, 1500 mL of Saguaro water and 1 mL of Bayfolan universal fertilizer from Bayer Garden were mixed together. Before inserting the aerator tube into the solution, it was washed with aqueous solution containing 1:10 hypochlorite for one day and then dried.

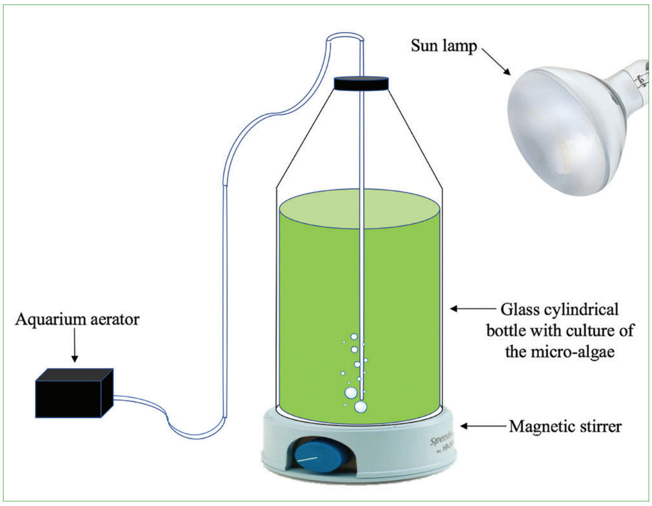

Fig. 1 - Equipment for algal cultivation

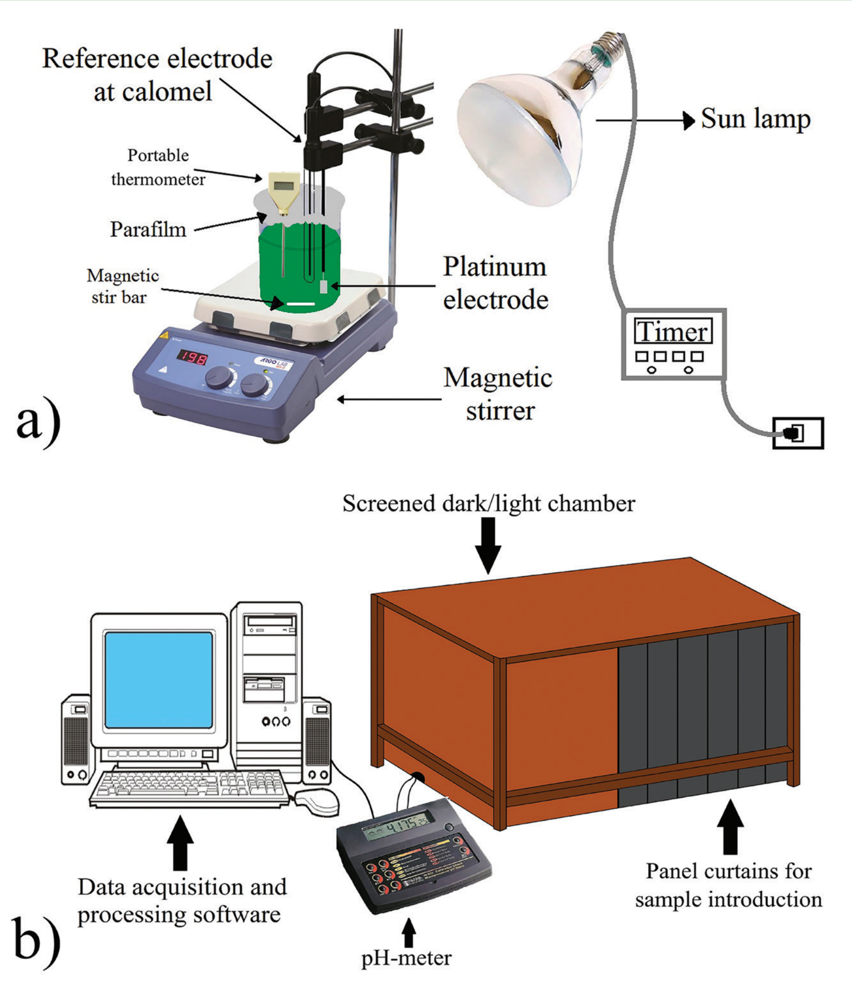

Fig. 2 - a) Components of the experimental system inside the chamber; b) equipment for monitoring and recording

### *Experimental equipment*

Fig. 2 shows the system for making the experiments repeatable: 200 mL of algal aqueous solution are contained in a 250 mL beaker, closed with Parafilm. Throughout the duration of the experiment, the solution is magnetically stirred at a speed of 250 rpm sufficient to keep the algae well in suspension.

The Osram Ultra-Vitalux lamp, which simulates sunlight, is connected to a digital timer programmed to alternate on/off power, subjecting the microalgae to dark/light cycles of 5 mins each.

The instrumentation is enclosed in a chamber consisting of plywood walls held together by wooden boards, to the right of which there is a black plastic window. The chamber prevents the outside light from penetrating inside and reaching the algal solution.

The reference calomel electrode and the working electrode, a platinum plate, are connected to the Microprocessor pH meter model 223 of Hanna Instruments, which in turn is connected via a serial cable to a PC, where the HI 92000 software receives and records the redox potential values read by the instrument every minute for 120 minutes.

The temperature of the solution was monitored by a Hanna Instruments portable thermometer. While the pH was measured only for the algal solution without pollutants (blank), the addition of the pollutants does not significantly change pH of the algal solution.

### *Chemicals*

The characteristics of the selected heavy metal salts and the amounts used to prepare 0.1 M solutions are listed in Tab. 1.

We have chosen to use nitrates for their high solubility.

20 minutes after the beginning of the data acquisition, 20 mL of polluting solution containing the heavy metal are added to the beaker containing 200 mL of algal solution, by means of a 20 mL BD Plastipak syringe puncturing the Parafilm. Each experiment was carried out in triplicates; the curves presented in the next paragraph are obtained from the average values.

The selected concentration of polluting compounds does not fall within the range of environmental interest (1-10 μM) or clinical (0.01-1 μM). It was chosen specifically higher because the aim in this work is to verify if there was a significant response from the microalgae to the presence of the pollutant. Lower concentrations will be investigated successively.

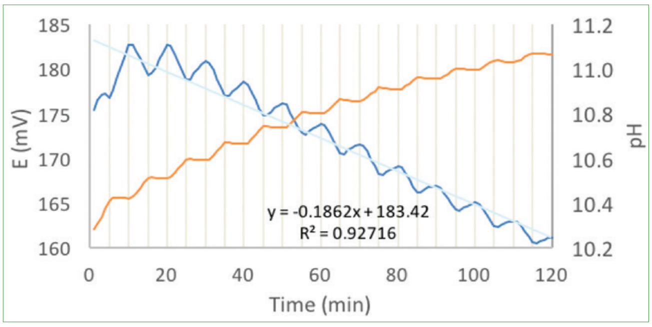

Fig. 3 - Redox potential and pH curves of algal solution

## Results and discussion

### *Algal solution*

The two metabolic processes of the algae, photosynthesis and breathing, should produce and respectively consume a stoichiometric amount of $O_2$ and $CO_2$, so their alternated values should be constant unless there is a stop or a decrease of one or both the two processes. During the photosynthetic process, the redox potential curve increases, $O_2$ is produced and $CO_2$ consumed, while breathing has the opposite behaviour. The trend of redox potential of the algal solution, Fig. 3, decreases, with a slope of -0.19, over time meaning that algae consume more $O_2$ than they can produce. The standard deviation increases with the increase of the experiment time, excluding the first 15 minutes during which the electrode goes to equilibrium. In the algal solution, it passes from a minimum of 2.4 mV to a maximum of 20.1 mV. Being a biological system, algae due to unknown factors can respond differently so repetitions are unavoidable.

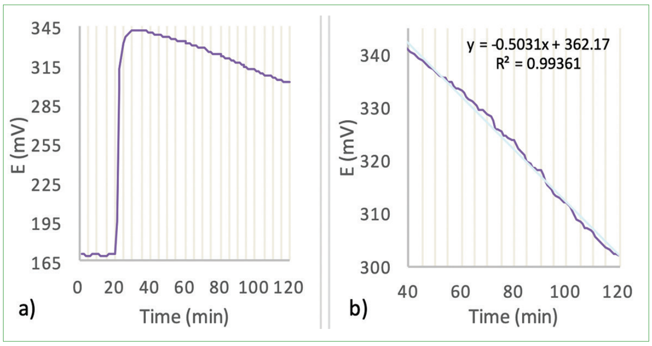

Fig. 4 - a) Variation of the redox potential during the addition of chromium nitrate. b) Segment investigated after electrode stabilization

The temperature of the solution increases of up 2 °C, due to the heat emitted by the lamp. The same increase occurs in all the other experiments. Due to this low variation the temperature term in Nernst equation was not considered.

### *Chromium nitrate*

Fig. 4a shows the trend of the redox potential curve of the entire experiment with chromium nitrate; while Fig. 4b shows the trend of the redox potential after the sudden increase due to the addition of the pollutant.

From 1st minute to 20th the trend is similar to that of the algal solution, with positive and negative peaks due to the switching on and off of the lamp and indicating the normal metabolic activities of photosynthesis and breathing.

When the pollutant solution is added, the redox potential increases for 12 minutes, passing from

| Chemicals | Molar Mass (mg/mol) | Purity (%) | Producer | Solubility (g/L at 20 °C) | Weighted quantity (g) | Hazard Statements |
|---|---|---|---|---|---|---|
| $Cr(NO_3)_3$ | 400.15 | >97 | FLUKA | 810 | 8.003 | H315, H317, H319, H332, H335, H511 |
| $Pb(NO_3)_2$ | 331.20 | 99.5 | Carlo Erba | 525 | 6.624 | H302, H317, H318, H332, H351, H360, H372, H373, H400, H410 |
| $Cd(NO_3)_2{\cdot}4H_20$ | 308.47 | >99 | FLUKA | 1100 | 6.169 | H301, H302, H312, H330, H332, H340, H350, H360, H372, H400, H410 |

Tab. 1 - List of characteristics of the compounds used to prepare 200 mL polluting solutions

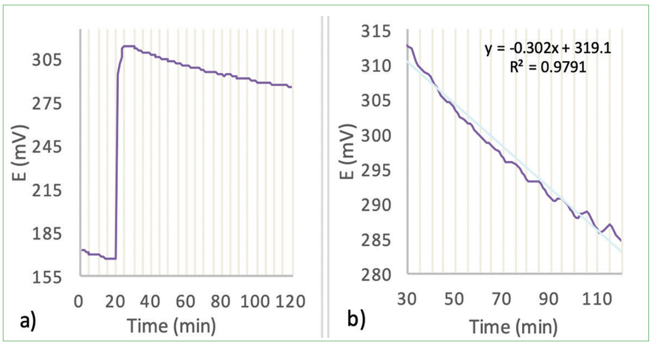

Fig. 5 - a) Variation of the redox potential during the addition of lead nitrate. b) Segment investigated after electrode stabilization

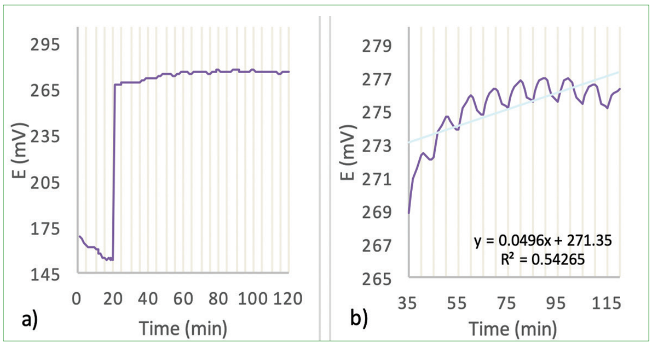

Fig. 6 - a) Variation of the redox potential during the addition of cadmium nitrate. b) Segment investigated after electrode stabilization

the value 170.7±7.6 mV to 341.9±24.1 mV, thus increasing by 171.2 mV. The higher values of standard deviation are present in this part of the experiment because the pollutant strongly destabilizes the algal solution that requires a time, different depending on the nature and concentration of the pollutant its, to re-establish itself.

From 32nd to 40th minute there are no significant variations. Beyond this minute the redox potential decreases almost linearly, (Fig. 5b) only after the 75th minute it starts again to show a hint of sinusoidal trend with positive and negative peaks although much flattened. In this part of the trend the reduction of redox potential compared to that of the blank is faster due alternatively to a greater activation of the breathing process or to inhibition of the photosynthetic process.

The difference between the blank and the presence of chromium nitrate is also detected by the slope of the two curves, respectively -0.19 and -0.50. The increased negative slope indicates that the heavy metal produces an imbalance of the two metabolic processes of the algal system.

### *Lead nitrate*

Fig. 5a shows the trend of the redox potential over the entire duration of the experiment, while Fig. 5b shows its decrease after the jump due to the addition of the lead nitrate solution.

As in the previous case, during the first 20 minutes a sinusoidal trend similar to that one of the blank is observed. Once the polluting solution has been added from the 20th minute to the 25th the redox potential passes from 167.0±5.2 to 313.1±4.8 mV, with an increase of 146.1 mV. Subsequently there are no significant changes until 28 minutes, beyond which the redox potential decreases. In this last stretch from the 71st minute the sinusoidal trend begins to be re-established so that at the end of the experiment it is restored but the balance between the breathing and photosynthesis is compromised with respect to its value. The slope of the curve is more negative than that one of the blank, respectively -0.30 and -0.19, therefore the addition of lead nitrate can be alternatively hypothesized has favouring the breathing process or hindering the photosynthesis.

With respect to chromium nitrate, in this case a shorter time is needed to the electrode to be stabilized once the pollutant has been added, as well as a lower jump is observed.

### *Cadmium nitrate*

Fig. 6a shows the trend of the redox potential during the entire duration of the experiment, while Fig. 6b shows the part of the curve after the potential jump due to the addition of the cadmium nitrate polluting solution.

From the 1st to the 20th minute the trend is the same observed in the previous cases. With the addition of the pollutant solution, in just one minute a potential jump from 154.0±4.1 mV to 267.2±2.0 mV, varying by 113.2 mV occurs. From 21st minute to 35th there are no significant variations. Over the 35th minute the sinusoidal pattern stabilizes with well-defined positive and negative peaks and the

Fig. 7 - Algal aggregation of contrast to the toxicity of cadmium nitrate

redox potential increases till to 70 minutes, from 268.9±5.4 to 276.4±5.7 mV, beyond which the sinusoidal trend remains constant without unbalance of the two processes, in a range of values between 277.0 (positive peak, lamp on) and 275.1 (negative peak, lamp off).

In this case, given the nature of the trend, the slope is slightly positive, 0.05 and this important difference compared to the blank and to the experiments with chromium nitrate and lead nitrate is not the only one. It is important to underline that the potential jump, in this case is very rapid, but lower compared to the addition of the other pollutants. In addition, the sinusoidal pattern reappears in a well-defined manner well in advance: 15 minutes for cadmium nitrate, 71 minutes for lead nitrate and 75 minutes (although hinted and difficult to be observed for chromium nitrate).

All these differences of cadmium nitrate, which apparently would conflict with its toxic nature due to heavy metal, are explained by a behaviour of the microalgae Scenedesmus observed only during experiments with this chemical compound (Fig. 7). When microalgae were added to the polluting solution, they produced a protein substance that allowed them to be aggregated counteracting in a unitary way the pollutant toxicity. Due to this effect, the necessary response for survival, cadmium nitrate should be considered the most polluting among the tested three salts. The aggregation has thus improved the process of photosynthesis by balancing it with that one of breathing.

To confirm the importance of this phenomenon, further tests were carried out by modifying the volume of polluting solution added to the algal solution.

Fig. 8 shows the trends of the redox potential at the addition of the pollutant after 20 minutes, only in the third case (red curve) after another 20 minutes a further addition of the polluting solution was carried out.

The addition of 0.4 mL produced a jump of potential, lasting one minute, of 15.6 mV while in the two cases where 2 mL were added, the jump, still lasted one minute, is of 78.0 and 80.5 mV. These last two values confirm the possibility of connecting the amplitude of the jump to the amount of

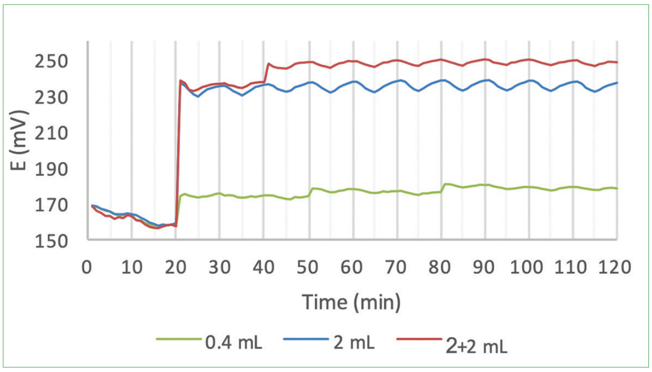

Fig. 8 - Variation of the redox potential during the addition of 0.4, 2 and 4 mL of chromium nitrate

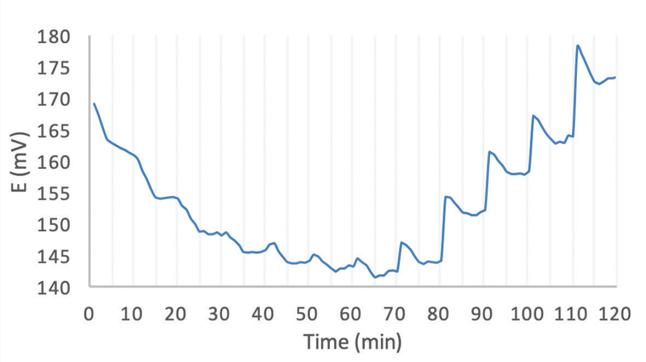

Fig. 9 - Variation of redox potential during the addition of polluting solution

pollutant. The second addition of pollutant (red curve) at the 40th minute produced a jump of 10.1 mV, lower than the first addition because the electrode is already in equilibrium with the polluting solution. In all the three experiments the sinusoidal trend is similar to observed with the addition of 20 mL of pollutant; this indicates that already at low concentration of cadmium nitrate the microalgae respond to counteract it.

By trying to add increasing volumes up to 20 mL in the same algal solution, a descending trend was initially observed (Fig. 9) followed by a rising behaviour. The addition of 0.2 mL after 20 minutes from the beginning of the experiment has no effect on the trend, while additions from the 30th to the 60th minute lead to a reduction of the negative slope and a flattening of the peaks. The potential jumps are not present for the small quantities added as the electrodes stabilize rapidly. Since the addition of the 70th minute, in which the total amount of polluted solution is 4 mL, the trend starts with a positive slope, further confirming the protective response activity performed by microalgae for their survival.

## Conclusions

Given the results obtained, it is possible to state that microalgae of the genus Scenedesmus can act as a good bioindicator for the presence of heavy metals in an aquatic environment. Comparing the characteristics of redox potential trends (potential jump, sinusoidal recurrence time and slope) it was possible to evaluate the toxicity to algae from the three tested pollutants: cadmium nitrate is the most toxic as it is the only case in which the algae respond to the stressful condition by aggregating. The cadmium nitrate follows in which the sinusoidal trend is only hinted and finally the lead nitrate for which in the middle of the experiment the sinusoidal pattern reappears. However, the slope indicates that the presence of the toxic substance changes the normal metabolic processes of photosynthesis and breathing.

The advantage of using microalgae as bioindicators is that they are simple to be cultivated and to grow and easy to be managed, do not require large containers for cultivation and transport and are species easily found in sea, rivers and lakes.

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### Potenziale redox per monitorare la risposta dello stress delle alghe all'inquinamento da metalli pesanti

Gli indicatori ecologici possono essere utilizzati per fornire un rapido segnale di allerta dei cambiamenti ambientali. Le alghe possono essere applicate al biomonitoraggio dell'inquinamento marino e fluviale a causa dell'alterazione delle due attività metaboliche prevalenti: la respirazione e la fotosintesi. Misurando il potenziale redox, abbiamo valutato lo stress nel sistema algale (genere Scenedesmus) dovuto alla presenza di metalli pesanti: piombo, cadmio e cromo.