Environmental monitoring using bioassays

21

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21.1 Introduction

The degree and rapid deterioration of water quality in aquatic ecosystems has been in the focus for many decades since the growth of industrial activity and use of many environmental toxicants. The problems with public health caused by water pollution were already recognized in the early 1800s and at this time the first legislation was made as a consequence to water pollution [1]. Even during the 21st century it is one of the greatest challenges to achieve more sustainable water management, which is among many countries in Europe recognized by the Water Framework Directive (WFD). Until the 1990s in Sweden, untreated wastewater from the pulp and paper industries caused extensive pollutions in coastal aquatic ecosystems [2]. Today the situation has improved in developed countries indicating that less untreated wastewater is discharged into aquatic environments. In developing countries the amount of effluent or untreated wastewater into water sources is still very high. The large quantities of wastewater contain toxic metals, organic and inorganic pollutants, which all could be harmful for organisms in aquatic environments [3]. Even if acute lethality caused by pollutants and chemical compounds in aquatic ecosystems has become rare there is still a significant risk for negative effects on ecosystems even if the levels of the chemicals are low [4]. The low levels and high complexity of chemical compounds are nearly impossible to monitor and regulate which makes it even more important to develop methods to evaluate contamination to aquatic ecosystems [4,5].

One of the components that play a decisive role in the pollution of aquatic ecosystems is the ongoing urbanization. It is the change of land use and agricultural activities which increases the amount of fertilizers, pesticides, herbicides, and chemical compounds of different origin that contaminate water bodies. The use of herbicides in agriculture and forestry has increased during the last decades [6,7]. Due to the water solubility of herbicides the breakdown products are often found in aquatic ecosystems where they have the possibility to affect nontarget algal species [7]. In Portugal Primextra Gold TZ is one of the best-selling herbicides and for the synthesis of this pesticide copper is used [8]. The two main active ingredients in Primextra Gold TZ are terbuthylazine (TBA) and S-metolachlor where TBA inhibits photosynthesis in photosystem II (PS II) and S-metolachlor blocks cell division and cell enlargement [8]. The effects and damage on species and

ecosystems from pollutants like Primextra Gold TZ do not only show the complexity of the compound itself with different active ingredients and heavy metals but are also related to the use of herbicides in agricultural areas near aquatic systems. Impacts from pollution on aquatic systems differ depending on whether the outlet into the aquatic systems is from point sources or from nonpoint sources [9]. It is more straightforward and easier to use ecotoxicological test assessments of point sources than of nonpoint sources where the complexity is higher due to multiple stressor effects [10].

A group of compounds which are a part of all pollutants are heavy metals. The pollution of heavy metals is strongly related to industrial development and these compounds cannot be easily detoxified, which results in their persistence in the environment [11]. Even if most of the heavy metals are toxic they also act as essential elements in the living system and for cellular metabolism and growth [12]. Living organisms require trace amounts of some metals, although increased levels of these compounds may be highly toxic [13,14]. The group of heavy metals contains copper (Cu), zinc (Zn), lead (Pb), chrome (Cr), nickel (Ni), cobalt (Co), arsenic (As), and mercury (Hg). Some of these elements have also been found in the wood ash, which is derived from different biofuel sources in the forest industry [15]. Metal ions may also bind to soil particles but additionally to components of herbicides like glyphosate [16].

The contamination of aquatic ecosystems with heavy metals affects the organisms within this ecosystem in the way that heavy metals tend to be taken up by the organisms. Microalgae, which are the primary producers and the basis of the food chain in aquatic systems, are one of the first groups to be contaminated by the pollution. The effects of excess heavy metals on microalgae are suppression of photosynthesis, cell growth, chlorophyll synthesis, and motility [17]. Since microalgae are the first step in the food web, the contamination affects other organisms on a higher level in the food chain. A change in microalgae populations or in their chemical composition due to contamination may have a harmful impact on higher trophic levels in the environment [8]. As microalgae are very sensitive to toxic elements they could be used as an early warning system when monitoring pollution. The advantage of using microalgae is that there is no need to consider ethical constraints associated with higher organisms such as vertebrates when testing toxicity of different compounds [18]. The important role of microalgae is not only of interest for environmental monitoring but also within the fields of waste treatment, biodiesel production, and the use of biomass to obtain commercial products.

21.2 Bioassay tests

During the last years the need for monitoring aquatic ecosystems in order to evaluate and manage the risk of environmental damage has led to the development of a number of different bioassay tests. These toxicity tests use a range of microorganisms, algae, invertebrates, and fish [19–21]. To detect biological effects often small

scaled biological systems on a molecular level, cellular (in vitro) or whole organism (in vitro) levels are being used [4]. The main aim is to generate protocols for toxicity, standards and limits imposed by regulators which are fast and economical. Bioassay methods are therefore based on the response of living organisms in order to analyze and evaluate potential effects of toxicants. In order to improve the ecological relevance of these tests it is of great importance to establish a scientific base when extrapolating from bioassay tests in the laboratory to field situations [22].

When using microalgae in bioassays the dominant parameters for study are physiological responses. Single-celled algae are very sensitive to toxic compounds and this makes it possible to identify the presence of a pollutant above a potentially harmful threshold, which is important when determining the suitability of the bioassays. In addition to the sensitivity of microalgae to toxicity, the cells respond more quickly than larger organisms, have a short generation time, and are easy to handle. The cells are small and only separated from the surrounding toxic medium by membranes or a cell wall, which enables rapid uptake of toxic compounds by their cells [23]. The high diversity of microalgae makes toxic effects of aquatic pollutants very heterogeneous due to the complexity and interactions between species, physiological responses, and environmental factors.

Physiological parameters such as photosynthesis, quantum yield, chlorophyll content, growth, motility, and fluorescence respond differently to pollutants and therefore it is of high value to set the criteria of the toxicity for the bioassays. The criteria include the standardization of culture medium and conditions and experimental parameters [24]. Factors which could affect the toxicity of a compound are physico-chemical properties such as pH, temperature, and light (irradiance and exposure time) [25]. Other biomarkers which have been proven to be excellent when studying environmental stressors are biomolecular and biochemical measures [8,9].

There is an urgent need for the development of early warning systems that can monitor, detect, and evaluate effects of toxicants and are rapid, reliable, and sensitive to a wide range of compounds. However, it must be pointed out that there are some problems concerning the evaluation of laboratory studies in relation to the effects of stressors on the health of aquatic ecosystems [9]. One important issue is to assess the ecological significance of laboratory early-warning indicators and to establish cause and effects relationships between specific stressors and the types and levels of environmental pollution and damage [9]. Fast responses could be observed either within hours or even during short-term incubation within minutes. Ahmed and Häder [26] defined end points for short tests after 0–24 h, and for long-term tests after 1–5 days. In a study by Pettersson and Ekelund [6] the short-term tests were 3 min and the long-term tests were 7 days when studying the motility in *Euglena gracilis*. Short-term and small-scaled bioassays are generally favored over long-term, large-scale bioassays [4].

A fast method is the noninvasive measurement of the in vivo fluorescence which facilitates monitoring of activity changes affecting PS II in microalgae. Fluorescence is one of three processes where the energy from light absorption by chlorophyll molecules is re-emitted as red fluorescence [7]. The two other processes

are photochemistry and dissipation of the excess energy as heat. The degree of fluorescence indicates the quantum yield of both photochemistry and heat emission from living cells. Brayner et al. [27] used biosensors when studying the effects of herbicides on three different green microalgae; the best results and most accurate responses were obtained with *Dictyosphaerium chlorelloides*.

One of the main advantages of quantifying fluorescence from single cells is that it does not require any preparation of the algae. Toxicity effects of the tested chemicals, even at low concentrations, can be obtained after 20 min of incubation [28]. The rapid techniques of pulse amplitude modulation (PAM) fluorometry [29] has a great advantage because it provides direct information on the photosynthetic activity of the algae, in comparison to measurements of cell numbers which requires several hours of incubation [7,26,30]. Other tests with microalgae and photosynthesis which are defined as short-term tests use 6 h to monitor and evaluate the toxic effects of metals [31].

In a study by Ahmed and Häder [17] PAM fluorescence was used for measurements of both photosynthetic yield and electron transport rate (ETR) when evaluating the effects of nickel and cadmium of photosynthetic parameters of *E. gracilis*. The duration of exposure to heavy metals at different concentrations varied from direct (20 min) exposure to long-term exposure of 5 days. The long-term monitoring using days was initiated because of the fact that heavy metals are nondegradable pollutants with an ability of bioaccumulation. Similar photosynthetic parameters were used in a study by Ekelund and Aronsson [32] where *E. gracilis* was shown to be more resilient to high pH than the green alga *Chlamydomonas reinhardtii* after 7 days at different concentrations of non-pH adjusted wood-ash solutions.

Another rapid method based on photosynthetic activity which is used in environmental monitoring is the use of biosensors that reflect the real physiological impact of active compounds [33]. A biosensor can be defined as an integrated bioreceptor-physicochemical transducer device which consists of different elements: a bioreceptor or biological recognition element, which interacts with the pollutant molecules, a physicochemical transducer, which converts the biological response into a detectable physicochemical signal, and a microelectronic processor of this signal, which amplifies it and converts it into a numeric record [13]. Promising investigations using biosensors for photosynthetic activity and PS II could be attractive and sensitive enough when studying the effects of chemical pollutants [33]. The effects of pollutants or chemical compounds using biosensors will modify the signal from the photosynthetic activity [27].

To identify the toxicity of a substance in different organisms requires the determination of a predicted no-effects concentration (PNEC) [34,35]. The PNEC for a substance is regulated by the European Commission [5]. The PNEC data are obtained in short-term tests using common endpoints. Common endpoints for the assessment of toxicity for algal growth tests and photosynthetic activity in microalgae are the proportion of the dead algae or the photosynthetic activity after a defined exposure time to the toxic compound. This could either be described as an EC₅₀ value, which is the concentration of the test substance that results in a 50% inhibition [36], or by a the NOEC (No Observed Effect Concentration) value [37],

which is the highest tested concentration at which no significant inhibition occurs [6,22,38]. EC₅₀ and NOEC values are important values for the application of environmental quality standards.

Besides the common bioassay parameters of photosynthesis and growth of microalgae, swimming behavior of phytoplankton organisms is a reliable parameter for ecotoxicological tests when studying the effects of water quality. Swimming behavior has been observed in a broad context from ecological phenomena, phototactic orientation, physical stratification, avoidance, and sex [18]. Feng et al. [18] argue that the swimming behavior of microalgae can be used as a sensor for environmental signals and a link between biochemical processes and consequences on the ecosystem level. One method which has been used to identify toxicity effects on the motility of microalgae is the determination of toxicity by using different types of chips. A chip can be used to perform many parallel tests simultaneously [18].

A method based on image analysis that operates in real time and tracks the numbers of motile cells is ECOTOX [39–41]. The effects of wastewater, heavy metals, herbicides, and other pollutants are evaluated with the ECOTOX system by using the parameters motility and orientation of *E. gracilis* [3,6,42].

21.3 ECOTOX

Using the ECOTOX method allows for calculating the number of motile cells, the percentage of cells moving upwards (gravitaxis), the mean velocity, the compactness (form factor, which is the ratio of the circumference to the area normalized to a circle) of the cells, and the precision of orientation (*r*-value) (cf. Chapter 10: Ecotox, this volume). The system operates in real time and tracks up to 1500 cells in parallel. The cells of *E. gracilis* are pumped into an observation chamber. This chamber could hold up to 10^6 cells mL⁻¹ and the test sample of toxic compounds can be automatically diluted with increasing dilutions of 1:1, 1:3, 1:7, 1:15, and 1:31 [6].

An important factor before starting a measurement is the filling time of the cuvette. This time must be long enough that fresh cells of *E. gracilis* are transferred from the reservoir where the cells are located before the beginning of the measurement. The tracking time of the analysis shows variations but is often within the range of 3 to 5 min. Depending on the cells being used in the ECOTOX system there is a need to set minimum and maximum areas of the cells. The setup of areas makes it possible to exclude debris or other species which may be contained within the sample cuvette. The setup of a minimum speed of the organisms is of importance because it allows distinguishing between dead or nonmotile cells and active motile cells. In a study by Pettersson and Ekelund [6] cells were considered motile when they moved at $7 \mu\text{m s}^{-1}$ or faster but in a study by Azizullah et al. [3] the minimal velocity was chosen as $15 \mu\text{m s}^{-1}$. Within the system there is also a possibility to set different incubation times in darkness before measurements start.

21.4 Short-term vs long-term tests

ECOTOX measurements of the effects of herbicides using *E. gracilis* in short-term tests of 0, 30, or 60 s showed strong effects on the velocity and upward swimming at concentrations higher than 1.25 g L^{-1} [6] (Fig. 21.1). The results showed different effects dependent on what type of herbicide was used. Avans had a stronger effect than Roundup. Avans showed lower NOEC values than Roundup for the different parameters but after 7 days (long-term) the NOEC values were similar except for upward swimming.

However, the effects on motility were less pronounced; this is probably due to the fact that cells of *E. gracilis* were still motile but that the orientation behavior was affected. In a study by Azizullah et al. [3] the gravitactic orientation of *E. gracilis* showed similar negative effects when exposed to wastewater. In comparison to cell shape and motility the gravitactic orientation was significantly impaired. The variation in sensitivity to pollutions of the different parameters in *E. gracilis* indicates that gravitactic orientation and cell shape are dependent of each other. Several studies show discrepancies between the effects of pollution on motility, cell shape, speed, and gravitactic orientation, which could be the result of changes in density between the cell body and the surrounding medium. Gravitactic orientation shows stronger negative effects when exposed to pollution which alter the density thus impairing the capacity to orient in the water column. In contrast to motility, velocity of *E. gracilis* showed a strong inhibition after short tests of only 30 and 60 s incubation with herbicides (Fig. 21.2) [6]. The effects were most pronounced with Avans and less with Roundup.

When testing the effects of industrial wastewater on *E. gracilis* the most sensitive parameters were gravitactic orientation and cell compactness [43]. The content of industrial wastewater is very complex depending on the large amount of different pollutants and toxic compounds. This complexity of wastewater makes it difficult to relate an impairment of motility parameters to a specific compound. Heavy metals which can occur in wastewater have different effects on motility parameters. After 24 h exposure to different heavy metals the motility and cell shape of *E. gracilis* showed no significant negative effects at the different concentrations (Table 21.1) [44].

Probably the concentrations were too low and the exposure time too short to induce significant effects of the different heavy metals. However, in a study with the heavy metals nickel (Ni) and cadmium (Cd) the effects were stronger after 24 h in comparison to tests immediately after exposure [17]. The EC_{50} values for upward swimming showed large differences, and the value after direct exposure was 292 mg L^{-1} and after 24 h incubation was 12.7 mg L^{-1} . The results from this study showed that longer incubation times did not show any significant difference for the different parameters, indicating that shorter incubation times are recommended for toxicological studies. However, it was concluded that acute toxicity is useful for rapid assessment but not enough to produce pronounced inhibition for all parameters [41].

Like wastewater, wood ash solutions contain toxic compounds [15]. Many different heavy metals are found in wood ash and the most common compound is cadmium (Cd). The potential release of these compounds into forest ecosystems

Figure 21.1 Upward swimming (%) of *Euglena gracilis* at different concentrations (g L^{-1}) of glyphosate with the herbicides Roundup (rdup) and Avans after different incubation times of 60 (A), 30 (B), and 0 s (C). Control (cont) tests are made without the addition of glyphosate. Columns indicate mean values with standard deviation, and asterisks indicate statistical significance between treatment and control.

Source: From Pettersson M, Ekelund NG. Effects of the herbicides Roundup and Avans on *Euglena gracilis*. Arch Env Contamin Toxicol 2006;50(2):175–181 with permission.

Figure 21.2 Velocity (μm s⁻¹) of *Euglena gracilis* at different concentrations (g L⁻¹) of glyphosate with the herbicides Roundup (rdup) and Avans. Short-term tests with different incubation times of 60 (A), 30 (B), and 0 (C). Control tests are made without the addition of glyphosate. Columns indicate mean values with standard deviation, and asterisks indicate statistical significance between treatment and control.

Source: From Pettersson M, Ekelund NG. Effects of the herbicides Roundup and Avans on *Euglena gracilis*. Arch Env Contamin Toxicol 2006;50(2):175–181 with permission.

Table 21.1 Effects of different concentrations of heavy metals and pentachlorophenol (PCP) on the cell shape (circularity) of *Euglena gracilis* after 24 h exposure.

Concentration (mg L ⁻¹)	Heavy metal		Lead	Zinc	PCP
	Copper	Nickel			
0	4.5 ± 0.8	4.5 ± 0.8	4.5 ± 0.8	4.5 ± 0.8	4.5 ± 0.8
0.1	3.7 ± 0.6	3.9 ± 0.7	3.9 ± 1.1	3.0 ± 0.5	4.4 ± 0.7
0.5	3.4 ± 0.5	4.2 ± 0.6	3.8 ± 0.9	3.1 ± 0.6	3.6 ± 0.8
1.0	2.7 ± 0.6	4.2 ± 0.6	3.6 ± 1.0	3.3 ± 0.6	3.7 ± 1.1
1.5	3.7 ± 0.8	4.3 ± 1.1	2.9 ± 0.7	3.7 ± 0.7	
2.0	3.4 ± 0.8	4.3 ± 0.7	3.4 ± 0.7	4.0 ± 0.8	
5.0					4.3 ± 1.2
10.0					1.7 ± 0.2

Mean values and standard deviation are shown.

could have negative effects on microorganisms. In contrast to the content of heavy metals, wood ash is also enriched with nutrients, which may have positive effects for the forest ecosystems. The most pronounced effects from wood ash application are an increased pH and an elevated content of cations. In order to study the effects of wood ash *E. gracilis* was used in both short- and long-term tests.

The tests were performed with ECOTOX and the time intervals for short-term tests were 0, 24, and 48 h. In the experiments the effects of pH on cells of *E. gracilis* were tested which ranged from 7.0 to 11.2. The effects of wood ash on motility parameters, when no adjustment of pH was made, showed strong inhibition at higher concentrations (above 10 g L⁻¹, Fig. 21.3).

Especially upwards swimming and velocity after 48 h showed significant inhibition. Under natural conditions *E. gracilis* is limited to habitats with low acidity and has growth rates between 0.33 to 1.12 at pH values ranging from 2.0–6.0 [45]. Above pH 6 the growth rate shows an inhibition which looks similar to the effects of high pH values on the motility parameters of *E. gracilis*. Similar results were shown in a study by Danilov and Ekelund [46]; cells of *E. gracilis* below pH 4 and above pH 8 did not survive.

In the tests with wood ash and no adjustments of pH it is difficult to distinguish between effects from either high pH or from toxic compounds in the wood ash. However, when the pH of the wood ash solution was adjusted to pH 7 the results showed a stimulation of the motility of *E. gracilis* (Fig. 21.4) [15]. Higher motility of *E. gracilis* in the presence of wood ash at an adjusted pH is probably an effect of the availability of nutrients in wood ash such as calcium (Ca), potassium (K), and magnesium (Mg) [15].

The observed differences in the motility parameters and growth rate are a combination of the addition of nutrients, organic compounds, and pH changes [45]. A long-term (7 days) growth test using *E. gracilis* with both adjusted pH 7 and no

Figure 21.3 Inhibition of motility parameters in *Euglena gracilis* after 0 h (white bars), 24 h (gray bars), and 48 h (black bars) exposure to wood ash solution (2.5, 5, 10, 15, 25 g L⁻¹); pH was not adjusted. Graphs show motility (A), upwards swimming (B), compactness (C), and velocity (D). Inhibition values are shown to those of the controls of each test.

Figure 21.4 Mean values and standard deviation of *Euglena gracilis* motility tests in wood ash solution with pH adjusted. Controls were prepared with distilled water.

adjustment of pH showed only a strong inhibition at the highest concentration of wood ash (15 and 25 g L⁻¹) and a pH value of around 11 [15]. This indicates that *E. gracilis* has a high sensitivity at high pH values.

Copper (Cu) is an essential microelement and has also been shown to have toxic effects at higher concentrations on different parameters in *E. gracilis* [26,46]. The immediate response to copper was rapid when studying motility parameters with ECOTOX [26]. Already after 3 min copper showed a disturbance of the different motility parameters. The sensitivity of the different parameters was highest for motility followed by *r*-value, velocity, and upward swimming. The same order of sensitivity was found after 24 h but the EC₅₀ values changed for motility and upward swimming in comparison to the short-term tests. The EC₅₀ values for the short-term tests in this study were in the concentration range of from 19 to 50 mg L⁻¹ which is much higher than the values found for copper in industrial wastewater in Pakistan where copper concentration varied from 0.01 to 0.18 mg L⁻¹ [43]. In contrast, the results from the effects of wastewater in Pakistan on the different motility parameters of *E. gracilis* showed strong inhibition on cell compactness and gravitactic orientation. These negative effects of wastewater were not only due to copper but also to a large mixture of heavy metals including Cd, Fe, Ni, Cr, Zn, and Pb [43].

21.5 ECOTOX measurements in Egyptian lakes

In the Northern part of Egypt near the Mediterranean coast several lakes and lagoons are located. Alexandria is the second largest city in Egypt and has about 4.1 million inhabitants (<http://en.wikipedia.org/wiki/Alexandria>). The lakes are polluted by industrial and domestic waste from the city and the surrounding areas. These wastes undergo only primary treatment, which removes suspended solid particles by filtration. Solubilized pollutants and toxic substances are not removed from the wastewater. Samples were taken from the wastewater treatment plant west of Alexandria before and after treatment and analyzed using the ECOTOX system.

Located to the Southeast of Alexandria is a large brackish lake (Lake Mariout), which has an area of ca. 250 km². This lake is the source of fish (mainly *Tilapia*) for the local market in Alexandria. This economically important fish is heavily affected by pollution since about two thirds of the city sewage water is drained into the lake. Only half of this wastewater undergoes primary treatment before it is discarded into the lake. Over the years the input of almost untreated domestic waste and industrial effluents has resulted in elevated concentrations of heavy metals such as Cd, Pb, and Cu, which accumulate in the water and sediments of lake Mariout [47].

The wastewater samples were collected in sterile 50-mL tubes and transferred rapidly to the laboratory where they were frozen until subsequent analysis. Prior to the ECOTOX measurements the samples were thawed at room temperature and subsequently centrifuged at 1000 × g for 5 min. When necessary, the samples were diluted with deionized water before key chemical parameters were determined such as pH, nitrate (NO₃⁻), nitrite (NO₂⁻), total and carbonate hardness using

colorimetric tests (Merck, Germany). Dissolved oxygen was measured (DO-5509, Lutron electronics enterprise Co., LTD Taiwan).

Analysis of the input wastewater to the treatment plant using the ECOTOX instrument shows a significant pollution from mainly domestic effluents. As also found in other pollution analyses [48], gravitactic orientation (r -value) shows the highest inhibition of up to 30% at a 1:1 dilution of the sample (Fig. 21.5A).

Figure 21.5 Inhibition (ordinate, %) of movement and orientation parameters in *Euglena gracilis* by samples from a wastewater treatment plant in Alexandria at decreasing dilution comparing input (A) and output wastewater (B): compactness (red), motility (yellow), alignment (black), upward swimming (green), r -value (blue). Negative values indicate an augmentation in the parameter. Each data point represents the mean of three independent measurements with standard deviation.

Source: redrawn after Ahmed H. Biomonitoring of aquatic ecosystems [PhD thesis]. Erlangen-Nürnberg: Friedrich-Alexander-Universität; 2010 [49].

The other parameters (compactness, motility, alignment and upward swimming) are less affected and some are not significant. The comparison with the samples from the output from the wastewater plant after mechanical filtration of solid particles reveals that the toxicity is not reduced by the treatment (Fig. 21.5B). In fact, most of the inhibition values are even higher and the *r*-value shows an inhibition of 37% at the lowest dilution of 1:1. Among the lakes in the Nile Delta, Lake Mariout is the most polluted one [47]. It can be subdivided into four basins—the main basin, the fish farm basin, the Southwest basin, and the Southeast basin [50]. The main basin, located to the Northwest, contains the most polluted water. In addition to domestic wastewater, it receives industrial effluents and agricultural runoff. This combined pollution increases the sulfur concentration in the water and reduces the dissolved oxygen concentration. In contrast, the Southeast basin contains the least polluted water which enters from the El-Umoum drain and the Noubaria canal [50–52].

Even though the domestic and industrial wastewaters from Alexandria are being strongly diluted in the lake, water samples taken from the Southeast basin of Lake Mariout resulted in a significant inhibition of the tested endpoints. E.g., the *r*-value was inhibited by 11%–24% at the lowest and highest concentration, respectively, and also alignment and upward swimming were significantly impaired by increasing sample concentrations (Fig. 21.6A).

Samples from the main basin even resulted in a higher inhibition with the *r*-value being reduced by 15%–57.5% at the highest and lowest dilution, respectively (Fig. 21.6B). Also the motility, upward swimming, compactness, and alignment were strongly inhibited with increasing concentration of the polluted water.

For comparison, a pond with the name Rakta situated east of Alexandria was analyzed. This pond is strongly polluted by untreated effluents from nearby industries. At the highest dilution factors (17 to 5) no significant inhibition could be detected in all movement parameters (Fig. 21.7). At higher concentrations (lower dilution factors 2 and 3) motility was not significantly affected or even enhanced, while compactness, alignment, and upward swimming showed an inhibition of about 11%. As in other tests of aquatic ecosystems, the *r*-value showed the highest inhibition of up to 29% by water samples of pond Rakta.

In addition to toxic pollutants such as heavy metals, organic and inorganic toxins, the physicochemical parameters of the water samples affect the inhibition of the movement, orientation and form parameters of the test organism *E. gracilis* in the ECOTOX measurements. These are summarized in Table 21.2. Especially the pH and total and carbonate hardness influence the water quality and affect the toxicity and thereby modify the biological risk [53]. In the analyzed samples pH and nitrite were in the normal range for freshwater lakes and drinking water. The samples from the input and output of the wastewater treatment plant as well as pond Rakta and the main basin of Lake Mariout had decreased dissolved oxygen (D.O.) values ($4.1\text{--}4.6\text{ mg L}^{-1}$) as compared with the recommended values ($\geq 5\text{ mg L}^{-1}$). In addition, the analyzed samples had moderate to increased water hardness.

Figure 21.6 Inhibition (ordinate, %) of movement parameters in *Euglena gracilis* by samples from the Southeast basin (A) and the main basin (B) of Lake Mariout at decreasing dilution: compactness (red), motility (yellow), alignment (black), upward swimming (green), *r*-value (blue). Negative values indicate an augmentation in the parameter. Each data point represents the mean of three independent measurements with standard deviation.
 Source: redrawn after Ahmed H. Biomonitoring of aquatic ecosystems [PhD thesis]. Erlangen-Nürnberg: Friedrich-Alexander-Universität; 2010.

21.6 Conclusions

Aquatic ecosystems are still affected by the contamination of industrial and municipal wastes. The pathways for pollutants and wastes to enter aquatic ecosystems are often via nonpoint sources, runoff from land or direct discharges from industries. Toxic compounds associated with these wastes are heavy metals, organic and inorganic compounds, pesticides and herbicides, which all may have negative impacts on aquatic organisms and function of aquatic ecosystems. Therefore it is of importance to establish monitoring systems which have the possibility and sensitivity to get an early warning signal of environmental damage. For testing the water quality the OECD guidelines promote microalgae and cyanobacteria as bioindicators [54].

Figure 21.7 Inhibition (ordinate, %) of the movement and orientation parameters of *Euglena gracilis* by samples from the Rakta pond at decreasing dilution: compactness (red), motility (yellow), alignment (black), upward swimming (green), r-value (blue). Negative values indicate an augmentation in the parameter. Each data point represents the mean of three independent measurements with standard deviation.

Source: redrawn after Ahmed H. Biomonitoring of aquatic ecosystems [PhD thesis]. Erlangen-Nürnberg: Friedrich-Alexander-Universität; 2010.

Table 21.2 Chemical parameters of wastewater samples from different sources near Alexandria, Egypt.

Source of water samples	pH	CH (mg L ⁻¹)	TH (mg L ⁻¹ Ca)	NO ₃ – (mg L ⁻¹)	NO ₂ – (mg L ⁻¹)	D.O. (mg L ⁻¹)
<i>Lake Mariout</i>						
Main basin	8	160	143	0	0	4.6
South east basin	8	160	143	10	0.5	6.6
Rakta Pond	8	160	143	10	0	4.5
<i>Wastewater treatment plant in Alexandria</i>						
Input	8	160	143	0	0.5	4.1
Output	8	160	143	0	0.5	4.1

TH, total hardness (mg L⁻¹ Ca); CH, carbonate hardness CaCO₃ (mg L⁻¹); D.O., dissolved oxygen (mg L⁻¹).

In the OECD tests monitoring growth over a period of normally 72 h is recommended. As a complement to toxicological growth studies swimming behavior of motile microorganisms could be used. The present study shows that by using the ECOTOX method there is a possibility to calculate the number of motile cells,

gravitaxis, the mean velocity, the compactness, and the precision of orientation of the cells. In most of the studies with the ECOTOX method the freshwater flagellate *E. gracilis* is used. This flagellate is very appropriate for this method due to its well-developed motility and precise gravitactic and phototactic orientation. Especially gravitactic orientation seems to be the most sensitive parameter when testing the effects of wastewater and heavy metals. The results indicate that ECOTOX, with its possibility of performing measurements within short periods (min), could be very useful in the future as an early warning biomonitoring system.

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