Toxicity Stress of the Durah Power Plant Ash and its Effect on the Alga *Chlorococcum humicola* (Naeg) Rabenhorst 1868

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Abstract

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This study illustrates the acute toxic effect of ash released from Durah power plant (DPP) on the biology of the phytoplankton species *Chlorococcum humicola* in Iraq. The results showed that the median lethal concentration for killing 50% of the Alga population (LC_{50}) was 0.15 and 0.13 ppt (parts per thousand) for 24 and 48 hours exposure to crude ash concentrations, respectively. In contrast, no LC_{50} value was recorded for 72 and 96 hrs after exposure. The reduction in the optical density absorption value and the growth rate recorded was 0.083±0.121 cells for the highest ash concentration used, compared with 0.594±0.099 cells recorded for the control group. On the other hand, the doubling time for the control group was 1.16±0.652 an hour compared with 1.36±0.981 an hour recorded for 2 ppt ash exposure. The current study confirms that the crude ash concentrations tested had an adverse toxic effect on the biological parameters of the algal species *Chlorococcum humicola* in Iraq.

Keywords: Algae, fly ash, pollutants, power plant, *C. humicola*.

Introduction

Water is an essential element for all living organisms with a great ability to dissolve substances, and can be easily polluted (Cheepi, 2012). Pollutants are substances released to the water's environment as a result of anthropogenic activities which have a deleterious effect on living organisms (Moriarty, 1983).

Fly ash is a waste material resulting from coal combustion-based in thermal power plants, which continuously increased the source of pollution (ACAA, 2009). Due to its small particle size of ash (5 to 100 microns), it contaminates water in aerial and aquatic environments. This study aimed to assess the ecological risk of ash concentrations from Durah power plant (DPP) on one species of phytoplankton *Chlorococcum humicola*, the first item in the food chain. Any changes in the algal community composition have a clear effect on the food web dynamics, zooplankton diversity, total production, fish community, biochemical cycle and the global carbon cycle through the biological pump (Mousing, 2013).

Materials and Methods

Study Area Description

Durah power plant, located in southwest Baghdad on the right bank of Tigris River, releases their toxicants effluents directly to Tigris River without any treatment (Figure 1). The plant is located 5.5 Km to the west of the Durah refinery (Nashaat, 2010).

Algal material and culture conditions

All toxicity tests were conducted using the freshwater unicellular green algae *C. humicola*, obtained from the Department of Biotechnology, Ministry of Science and Technology. *Chlorococcum humicola* was isolated and purified in an axenic culture (media contain only one algae species without any bacteria, fungi or other contaminants (Jawad, 1982). The media used for preparing stocks and routine experimental studies was BG11. The culture was maintained at a constant temperature 25 ± 1 ◦C and a light intensity of 1000 Lux with 16 hr light/8 hr dark photoperiod. Media pH was adjusted to 7-7.5, and stock culture solution and media were prepared with distilled water under sterilized conditions (Kassim,1998).

Ash toxicity test

Different ash concentrations (0.1, 0.5, 1.0, 1.5 ppt) were prepared in BG11 algae medium in 100 ml conical flasks, then algal culture was added to reach a final concentration of 1.4×10^6 cells/ml in each test flask, whereas control group contained BG11 medium and algae without dissolved ash. Flasks were incubated under controlled laboratory conditions. Each ash concentration was examined after exposure to 24, 48, 72, and 96 hrs. Cells number was determined using a hemocytometer chamber, and using the across sectional method described by Martinez *et al*. (1975) and Fathi *et al*. (2012). Three replicates for each concentration were tested (Esmaeili, 2015).

In the same context, LC_{50} was calculated as an interpolated value based on the mortality rate percentages

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between the tested organisms. The experimental data was also plotted on a logarithmic probability graph paper with concentrations on the logarithmic scale and the mortality on the probability scale by using linear regression equation and curve estimation to get a X-axis curve that represents the log of concentration (Hamilton *et al*., 1978). On the other hand, Y-axis represented the probit units, then using a crossing between the number 5 from Y-axis (probit units) then descending the column to X-axis log concentration, and this represented the LC_{50} value after converting it to the inversion log (Goldstein, 1974; Goel, 2006).

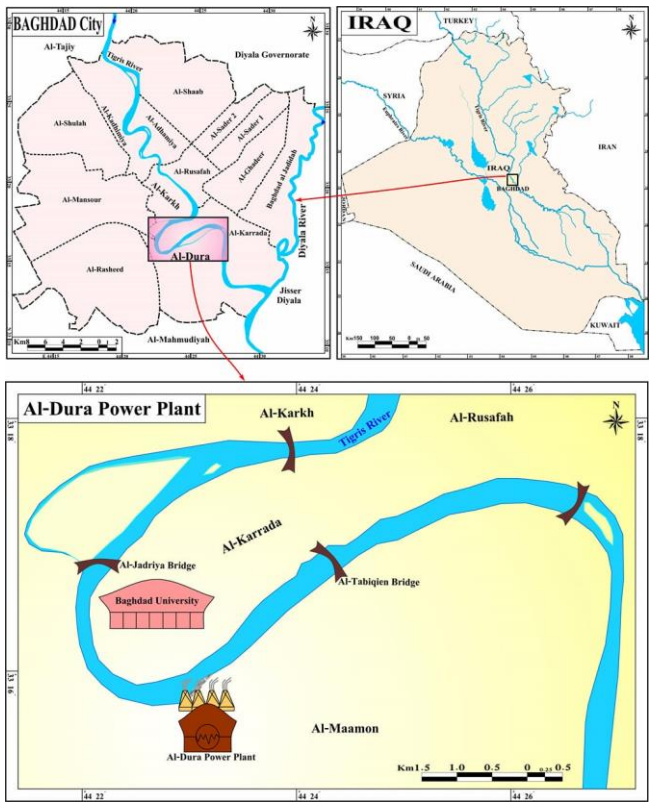

Figure 1. Maps of Iraq and Baghdad which show Durah power plant location on the Tigris River, central Iraq (Ministry of Water Resources, 2007. Scale 1/100000).

The growth rate and the doubling time were calculated according to Reynolds (1984) as in the following equations:

$$M = \frac{\ln\left(\frac{X1}{X0}\right)}{T}$$

where: M = Growth rate, X0= Cell numbers at the beginning of the experiment, X1= Cell numbers at the end of the experiment, T = Time (hours), and

$$G = \frac{ln2}{\mu}$$

where: G = Doubling time, Ln2= 0.693, μ = Growth rate.
The inhibition rate (%) was calculated according to Nyholm (1985) as in the following equation:

$$\text{Inhibition rate \%} = \frac{1 - \text{XT}}{\text{XE}} \times 100$$

where: XT =Cell number/ml for each treatment, XE = Cell number/ml for each control.

During the experiment period, the optical density at 450 nm wavelength was also measured every 24 hrs using a Spectrophotometer (PD-303 model by APEL CO), after calibration with the media only without algae treatment (Battah *et al*., 2015; Tam, 1989).

Results and Discussion

The LC_{50} values of *C. humicola* exposed to crude ash concentrations were 0.152 and 0.13 ppt during 24 and 48 hours. In contrast, the LC_{50} didn't show any value after 72 and 96 hrs. of exposure, and this may be related to an increasing in the mortality rate among the exposure groups (Figures 2 and 3).

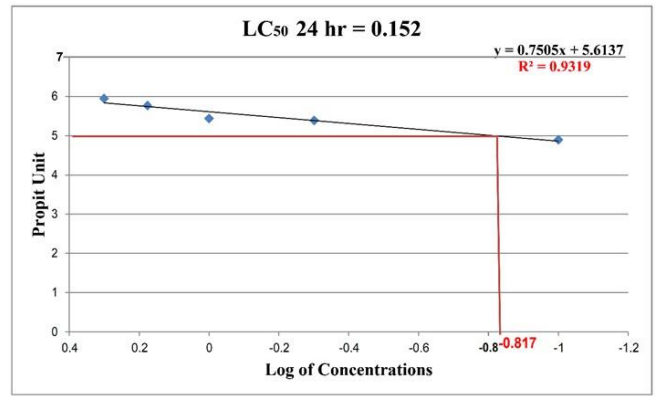

Figure 2. Median lethal concentration (LC_{50}) of *C. humicola* 24 hr. after exposure.

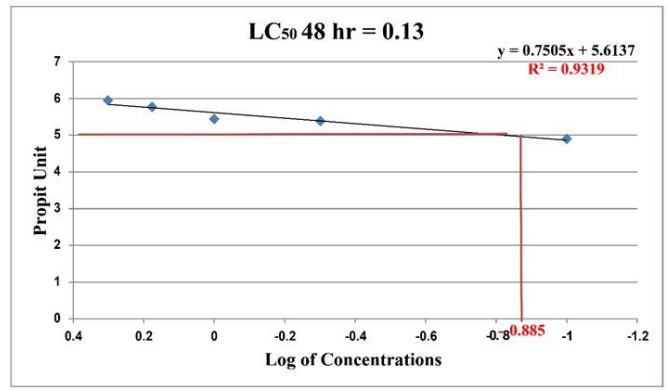

Figure 3. Median lethal concentration (LC_{50}) of *C. humicola* 48 hrs after exposure.

Toxicity symptoms were observed on exposed algal cells, which included a change in the size and number of the chloroplasts, disorganization of the cells components, the appearance of granules, in addition to the occurrence of dead of cells with the increase in ash concentration.

The inhibition rate of *C. humicola* was increased with an increase in ash concentration during 96 hrs of exposure. The highest ash inhibition effect was 94.7%, which occurred in 2 ppt concentration after 48 hrs, whereas ash concentration

at 0.1 ppt caused 49.09% inhibition after same period of exposure, and this value was increased to reach 92.72% after 96 hrs. exposure with the same concentration (Figure 4).

On the other hand, the results obtained showed that LC_{100} was 1, 1.5 and 2 ppt of ash concentration after 72 hrs exposure.

The optical density of *C. humicola* suspension showed a reduction in the absorption values with an increase in ash concentrations during exposure compared with the control group (Figure 5).

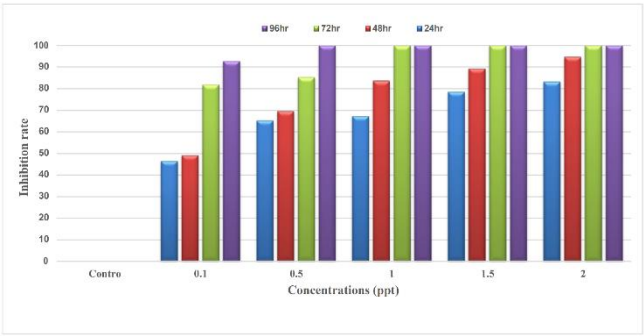

Figure 4. Inhibition rate (%) of *C. humicola* during different exposure periods.

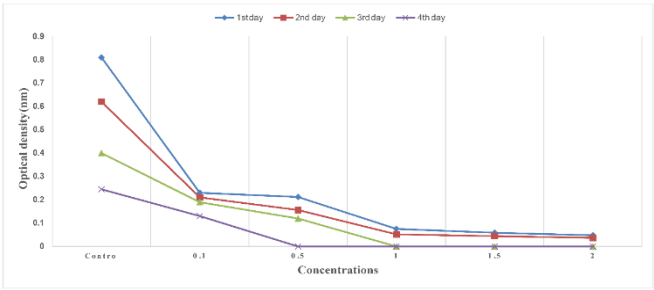

Figure 5. The optical density for *C. humicola* in 450 nm during 4 days of exposure.

Figure 6 shows the decrease in the growth rate when the period of different ash concentrations exposure was increased. The growth rate was 0.55±0.097 cells in ash concentration of 0.1 ppt after 24 hrs exposure, and was the highest. Whereas, the lowest growth rate of cells was 0.083±0.121 cells in the 2 ppt ash concentrations after 48 hrs. exposure compared to the control, which was 0.594±0.099 cells. Nevertheless, *C. humicola* did not show any growth rate 72 hrs. after exposure to ash concentrations of 1.0, 1.5 and 2 ppt, likewise 96 hrs. after exposure to ash concentrations 0.5, 1.0, 1.5, 2.0 ppt, and this may be attributed to the death of the algal cells at the higher ash

When the relation between the doubling time and growth rate was studied, it was found that the highest growth rate was reached when the lowest doubling time was recorded. The doubling time for the control group was 1.16±0.652 an hour. This value was increased to reach 1.36±0.981 an hour in the highest ash concentration (Figure 7).

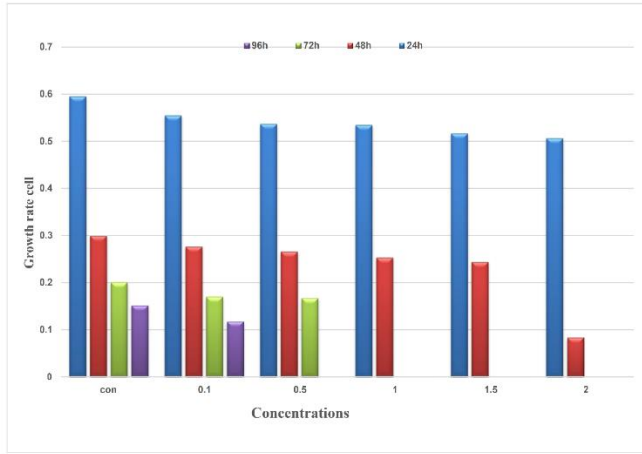

Figure 6. Growth rate of *C.humicola* during the exposure period.

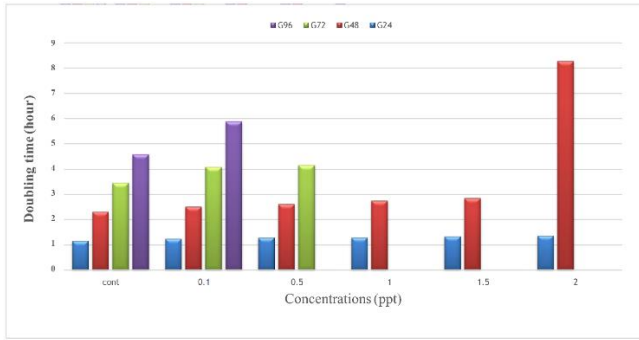

Figure 7. Doubling time of *C. humicola* during the different exposure periods to different ash concentrations.

Bartlett *et al.* (1974) when exposed *Selenastrum capricornutum* to multiple concentrations of heavy metals collected from power plants reported similar adverse effects. Similar results were reported by Kassim (1998), who worked with the algae *Scenedesmus acutus.* In contrast to our study, Al-Akailly (2006), who investigated the effect of Ni and Zn on *Ankistrodesmus bibraianus*, detected reduction in the growth rate to reach 0.07 cell with an increase in doubling time to 5.56 an hour compared with the control group which gave a growth rate of 0.23 cells with a doubling time of 3.230 an hour.

The relative growth rate with the lowest doubling time observed in this study was in agreement with the results reported by Shrivastava *et al.* (2012) when exposing *Spirogyra decimina* to fly ash compared with the control group Likewise, these adverse effects were also observed by Al-Naymi (2019), who reported that the LC_{50} values were 0.60, 0.34 and 0.17 ppt after 24, 48 and 72 hours exposure of *Chlorella vulgaris* to different concentrations of ash which were collected from Durah power plant, and were close to the findings reported in the present study.

The highest relative algae growth rate with lowest doubling time in this study may be related to the effect of the toxic metals in ash on the algal cells' metabolic function and effect on the protein/enzymes synthesis (Sundo, 1989). The adverse effects may be attributed to toxic metals which

inhibited oxygen production. This led to the loss in the photochemical activity of the reaction center, thus resulting in functional and structural damages to the photosynthetic apparatus, leading to inhibition in the growth rate and caused the algal cells death (Esmaeili, 2015). Whether or not similar effect can happen to the photosynthetic system in higher plants need to be investigated.

Even though zinc in the fly ash is an essential microelement (Kabata–Pendias, 2011), when in excess, it has phytotoxic effect on algae, and possibly higher plants, which may affect the photosynthetic apparatus such as light capture stomata conductance, pigment biosynthesis, electron transfer, CO_2 assimilation and enzymes support (Vassilev *et al*., 2011; Verbruggen *et al*., 2013). The impact of the elements found in the fly ash on photosynthesis and the measurement of gas exchange parameters, like the photosynthetic rate, depended on chlorophyll content and CO_2 assimilation (Arshad *et al*., 2016; Rusinowski *et al*., 2019).

The results obtained in this study showed that ash concentrations caused stress and harmful conditions to algae. Their highly toxic and severe effect may explain damage in cytoskeleton and cause disorganization of the cytoskeletal structure (Al-Naymi, 2019).

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الملخص

النعيمي، نور عدنان شاكر، حنان عدنان شاكر النعيمي ومهند رمزي نشأت. 2022. الإجهاد السُمّي وتأثيرات رماد محطة كهرباء الدورة على الطحلب *Chlorococcum humicola* (Naeg) Rabenhorst 1868. مجلة وقاية النبات العربية، 40(2): 188–192.
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أوضحت هذه الدراسة التأثيرت السُميّة الحادة للرماد المنبعث من محطة كهرباء الدورة (DPP) والتغيرات الأحيائية لأحد أنواع الطحالب النباتية *Chlorococcum humicola* في العراق. أظهرت النتائج أن متوسط التركيز المميت لقتل 50% من الأعداد (LC_{50}) كان 0.15 و0.13 جزء بالألف بعد 24 و48 ساعة من التعرض لتركيزات الرماد الخام، على التوالي، بينما لم تسجل أيّ قيمة LC_{50} بعد 72 و96 ساعة. كان الانخفاض في قيمة امتصاص الكثافة الضوئية ومعدّل النمو المُسجَّل 0.121±0.083 خلية عند أعلى تركيز مستخدم للرماد مقارنةً مع 0.594±0.099 خلية في معاملة الشاهد؛ ومن ناحيةٍ أخرى، كان زمن التضاعف 1.16±0.652/الساعة في مجموعة الشاهد مقارنةً مع 1.36±0.981/الساعة الذي تمّ تسجيله للرماد بتركيز 2 جزء بالألف. خلصت الدراسة إلى أنّ لتراكيز الرماد الخام المختبرة تأثيرٌ سمّيٌّ سلبي، فضلاً عن تسبّبها بتغيراتٍ خطيرة في المعايير الأحيائية للطحلب *Chlorococcum humicola* في العراق.

كلمات مفتاحية: الطحالب، الرماد المتطاير، الملوثات، محطات الكهرباء، *C. humicola*.

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